BRCA1/2 Sequence Variants of Uncertain Significance: A Primer for Providers to Assist in Discussions and in Medical Management

NORALANE M. LINDOR,^a DAVID E. GOLDFAR,^b SEAN V. TAVTIGIAN,^c SHARON E. PLON,^d FERGUS J. COUCH^e

^aDepartment of Health Sciences Research, Mayo Clinic, Scottsdale, Arizona, USA; Departments of ^bDermatology and ^cOncological Sciences, Huntsman Cancer Institute, University of Utah School of Medicine, Salt Lake City, Utah, USA; ^dDepartments of Pediatrics and Hematology-Oncology, Baylor College of Medicine, Houston, Texas, USA; ^eDepartment of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA

Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. BRCA1 • BRCA2 • Counseling • Likelihood • Management • Multifactorial

ABSTRACT

Introduction. DNA variants of uncertain significance (VUS) are common outcomes of clinical genetic testing for susceptibility to cancer. A statistically rigorous model that provides a pathogenicity score for each variant has been developed to aid in the clinical management of patients undergoing genetic testing.

Methods. The information in this article is derived from multiple publications on VUS in *BRCA* genes, distilled for communicating with clinicians who may encounter VUS in their practice.

Results. The posterior probability scores for *BRCA1* or *BRCA2* VUS, calculated from a multifactorial likelihood model, are explained, and links for looking up specific VUS are provided. The International Agency on Cancer Research (IARC) of the World Health Organization has proposed a simple five-tier

system for clinical management that is not widely known to clinicians. Classes 1 and 2 in this system are managed as neutral variants, classes 4 and 5 are managed as pathogenic variants, and class 3 variants still have insufficient evidence to move to either end of this scale and, thus, cannot be used in medical management.

Conclusions. Development of models that integrate multiple independent lines of evidence has allowed classification of a growing number of VUS in the *BRCA1* and *BRCA2* genes. The pathogenicity score that is generated by this model maps to the IARC system for clinical management, which will assist clinicians in the medical management of those patients who obtain a VUS result upon testing. *The Oncologist* 2013;18:518–524

Implications for Practice: DNA variants of uncertain clinical significance (VUS) are encountered for all types of genetic testing. No generally recognized standard exists for how to advise those who are found to have these variants. Using the *BRCA* genes as examples, this article explains what is involved in attempting to assign significance to a VUS and how to find and use new information on *BRCA* VUS specifically, aimed at assisting clinicians in handling these results in their practices.

INTRODUCTION

Variants of uncertain significance (VUS) are alterations in the DNA sequence of a gene that have unknown effects on the function of the gene product or disease risk. The genetic code for the *BRCA1* gene contains around 6,000 base pairs of coding DNA and the *BRCA2* gene is even larger, with over 10,000 base pairs of coding DNA. Because of the size of these genes, many alterations in the DNA sequence have been identified that may be unique to one or only a few patients and whose clinical relevance has never been determined. Despite the testing of hundreds of thousands of patients, laboratories analyzing the *BRCA* genes continue to encounter new or rare variants in genetic sequences. In individuals of European ancestry in the United States, VUS results account for approximately 5%–6% of alterations reported from clinical testing, and among individuals of African-American ancestry, up to 21% of tests will show a VUS

in *BRCA1* or *BRCA2* [1]. Testing laboratories in Europe estimate that up to 15% of alterations in *BRCA1* and *BRCA2* are VUS.

“Pathogenic” refers to a mutation causing or contributing to disease. The term “deleterious” is often substituted in laboratory reports, though the terms are not technically interchangeable (see the glossary at the end of Lindor et al. [2]). Pathogenic/deleterious mutations are DNA variants that, by virtue of their position or type, can be confidently predicted to disrupt the function of the genes that have been associated with disease, even if never seen by the laboratory before. Alternatively, DNA sequence variants can be shown to be pathogenic using rigorous statistical analysis of relevant genetic data.

Neutral variants (also called “nonpathogenic,” “of no clinical significance,” or “benign”) are DNA alterations that are

Correspondence: Noralane M. Lindor, M.D., Mayo Clinic, 13400 East Shea Blvd., Scottsdale, Arizona 85259, USA. Telephone: 480-301-6817; Fax: 480-301-8387; E-Mail: nlindor@mayo.edu Received November 28, 2012; accepted for publication February 13, 2013; first published online in *The Oncologist Express* on April 24, 2013. ©AlphaMed Press 1083-7159/2012/\$20.00/0 <http://dx.doi.org/10.1634/theoncologist.2012-0452>

known or inferred not to significantly affect the function of the gene or to be associated with clinically important increased risk of disease. The term “polymorphism” usually refers to a neutral variant that occurs in at least 1% of the population. Some polymorphisms could have subtle effects on gene function but are still not considered pathogenic/deleterious mutations. It is generally accepted that clinical laboratories do not need to encumber their reports by detailing all the clearly neutral variants seen in any given patient because these details have no clinical importance.

VUS are the focus of this paper. These DNA changes cannot be presently considered pathogenic or neutral. It is an inconvenient truth that most gene alterations are not interpretable by merely discovering a deviation from the published DNA sequence. VUS do not introduce a “stop” signal into the transcribed gene. Instead, there may be more subtle changes; for example, a single base change that results in a missense mutation (substitution of one amino acid for another) in the protein encoded by the gene. They may also be in positions in the gene that would have uncertain biologic effects, such as being close to but not immediately in an intron-exon splice site region. It is standard for clinical testing labs to report VUS, causing consternation and uncertainty for patients and providers alike. Unfortunately, VUS are not rare. At present, there are thousands of unique VUS recorded in the *BRCA1* and *BRCA2* genes with new variants being added every day.

The American College of Medical Genetics recommended standards for interpreting and reporting sequence variations for any gene, and proposed six descriptive categories [3]. However, these categories provide no guidance as to how to apply these categories in clinical decision making.

This manuscript aims to provide an overview of VUS-related issues and options for nongeneticists and to provide information on a classification system for *BRCA1* or *BRCA2* VUS that applies methods that integrate multiple independent lines of evidence [4–7] and links the new scores for specific VUS classifications to clinical utility [8].

Table 1. Example of a test report for *BRCA* gene testing

Test performed	Results	Interpretation
<i>BRCA1</i> sequencing	No mutation detected	No mutation detected
Rearrangement	No mutation detected	No mutation detected
<i>BRCA2</i> sequencing	D381N	Uncertain significance

A PRIMER ON VUS FOR HEALTH CARE PROVIDERS

Case

A 54-year-old woman presents with newly diagnosed invasive breast cancer and a family history of breast cancer in her mother at age 56 years. Genetic testing for mutation in *BRCA1* and *BRCA2* genes was conducted and a VUS in *BRCA2*, shown in Table 1, was reported.

A VUS result means that the laboratory interpreted the DNA alteration based on standard evidence at the time of the test and found that there was insufficient evidence to classify the alteration as either pathogenic (deleterious) or neutral. A VUS should be considered not clinically useful, and it should not be factored into decision making. This should remain true until further evidence emerges from scientific studies to shift the interpretation toward either a neutral or pathogenic interpretation. It may be easiest to proceed as if the test had not been done at all and to base medical advice on the family history or a patient’s own medical presentation alone. Figure 1 illustrates the concept of a continuum of interpretation of DNA variants.

Why Can’t Certain DNA Variants Be Interpreted?

VUS are most often “missense” changes that alter the DNA sequence, making a different codon of three nucleotides, which results in a different amino acid in the final protein. VUS can also be DNA changes around the regions of the gene that demarcate coding from noncoding regions (splice sites between exons and introns), which affect proper assembly of the final protein product. By definition, for most cancer susceptibility genes that function similarly to *BRCA1* and *BRCA2* (i.e., as tumor suppressor genes), VUS do not appear to disrupt translation of the gene by introduction of a “stop” signal or clearly affecting the gene product production or assembly. There are an almost endless number of theoretical possibilities in which a gene, with a typical coding region of several thousand base pairs, could be altered. Thus, many VUS that are reported have never been seen before or have been seen only a handful of times. If a variant is found that does not truncate the protein and has not previously been classified, the DNA variant will be classified as a VUS. Note that as *BRCA* testing in the United States has been conducted predominantly on Caucasians, knowledge of the normal DNA variants in individuals of different ethnic backgrounds is deficient. Higher rates of VUS in *BRCA* genes have been reported consistently in non-Caucasian populations [9–11].

Figure 1. Outcomes of mutation testing. Table 2 defines classes.

Would Repeating the Study in a Different Lab Give New Information?

Repeating this test is not recommended. A VUS is not veiled language for a questionable laboratory call. There clearly is a DNA alteration detected, but the clinical interpretation of that DNA alteration is unclear. Because different laboratories use different reporting categories, the terminology in the report may vary somewhat.

What Can Be Done to Help Figure out Whether a VUS Is Pathogenic or Neutral?

Multiple methods are now being applied to move interpretation of a VUS toward a conclusion. As explained below, individually, these methods are neither 100% reliable nor infallible, and often are not available even on a research basis. However, when combined, these methods may lead to a more certain clinical interpretation for many VUS. The different lines of evidence that can be brought to bear on VUS interpretation are presented below.

Segregation Analysis Within the Family

Segregation analysis focuses on whether the VUS cosegregates (is inherited together) with the phenotype (breast or ovarian cancer) in a manner consistent with that expected for pathogenic mutations in the gene of interest (in this case, *BRCA1* or *BRCA2*). Because first-degree relatives have a 50% chance of sharing any specific DNA change, this analysis requires large numbers of individuals with the VUS to build a circumstantial evidence case for causation. For some diseases, segregation analysis can be quite powerful. For example, if one was studying a distinctive phenotype such as albinism, then seeing albinism in a relative who did not carry the suspected DNA VUS being investigated in that family would be powerful evidence that the VUS was not the cause of albinism in that family. In reality, most genetic disorders do not have such a striking phenotype. Because breast cancer is so very common, and ovarian cancer is not uncommon either, the information derived from segregation analysis for *BRCA* VUS interpretation is seldom conclusive. Nevertheless, it can be of great value in selected families. Take the example of a woman with a VUS result whose father's side of the family has many individuals with breast/ovarian cancer but no cancer on her mother's side of the family. The finding that she inherited the VUS from her mother (the unaffected side) would provide strong evidence that this VUS is not the cause of the breast/ovarian predisposition in her father's family (the affected lineage). In general, evaluation of cosegregation requires complex statistical analysis under a specific model of the disease inheritance [12, 13].

More powerful than single family analysis, combining segregation analysis from several families can be done, if the identical VUS is observed in multiple families. Myriad Genetics (<http://www.myriad.com/products/braanalysis.php>), the main testing laboratory in the United States, does offer limited testing of carefully chosen family members at no charge for *BRCA* VUS found in their laboratory to help amass this sort of data for the future.

Summary of Personal and Family History Analysis

Given a large dataset of individuals who had testing, including their personal cancer histories (age[s] at diagnosis and tumor

type[s]) and family histories (number of affected first- and second-degree relatives, their age[s] at diagnosis and their tumor type[s]), the ensemble of these personal and family histories will naturally form a distribution from quite innocuous to severe. The subset of personal and family histories from individuals who were found to carry a pathogenic variant will show a distribution of histories shifted toward the severe end of the spectrum. In contrast, if we look at the subset of personal and family histories from individuals who were not found to carry either a pathogenic variant or a VUS, the distribution of their histories will be shifted toward the innocuous end of the spectrum. Given the histories from the group of subjects who carry a specific VUS and these two distributions (innocuous and pathogenic), it is possible to calculate the probability that the VUS of interest is pathogenic [4, 14]. Unfortunately, this sort of analysis requires access to very large datasets. ENIGMA (Evidence-Based Network for the Interpretation of Germline Mutant Alleles), an international consortium of over 100 scientists and clinicians from over 19 countries, is presently engaged in trying to piece together this sort of evidence for VUS in the *BRCA* genes [15].

Co-Occurrence in Trans

Like all autosomal genes, humans have two copies of each of the *BRCA* genes, one inherited from each parent. Both human and mouse laboratory data suggest that carrying the same *BRCA1* pathogenic mutation on both chromosomes (being homozygous) is embryonic lethal. Homozygosity for *BRCA2* mutations results in a rare genetic condition called Fanconi anemia associated with growth failure, multiple congenital anomalies, and often diagnosis of cancer in childhood. These observations have provided a powerful way to specifically reclassify VUS in *BRCA1* and *BRCA2* as neutral DNA changes, if perchance the VUS has been seen in the same patient who carries a definite pathogenic mutation in the same gene. It is not enough, however, to see a laboratory report of a pathogenic mutation plus a VUS to be able to make this interpretation, because one must also demonstrate that the mutation and the VUS occur on different copies of the gene (that they are in *trans*) rather than both being within the same copy of the gene (being in *cis*). If they are in *cis*, then that person still has a normal copy of the *BRCA* gene and the VUS still cannot be interpreted. Determining the phase (*trans* vs. *cis*) can be demonstrated most directly by conducting *BRCA* analyses of the proband's parents or other family members. Note that the use of co-occurrence data has been muddled by demonstration of DNA variants that have more subtle perturbations of gene function than the classical pathogenic mutations [16]. These "hypomorphic alleles" are only recently being studied and are not currently part of most discussions on VUS.

Evolutionary Conservation Across Species

A number of computer programs (in silico methods) exist to allow geneticists to look at whether specific functional domains, individual amino acids, or even nucleotide base pairs in a gene sequence are conserved through evolution (phylogenetic analysis). These programs are often available online and are used by many testing laboratories to help interpret variants. Examples of these programs include Sorting Intolerant from Tolerant (SIFT, <http://sift.jcvi.org/>), Polymorphism Phenotyp-

Table 2. Proposed classification for DNA sequence variants and correlation of clinical recommendation with probability that any given alteration is deleterious

DNA alteration class	Definition	Probability of being deleterious	Clinical testing	Surveillance recommendations
5	Definitely pathogenic	>0.99	Test at-risk relatives for the variant	Full high-risk surveillance
4	Likely pathogenic	0.95–0.99	Test at-risk relatives for the variant	Full high-risk surveillance
3	Uncertain	0.05–0.949	Do not use as predictive testing in at-risk relatives	Counsel based on family history and other risk factors
2	Likely not pathogenic	0.001–0.049	Do not use as predictive testing in at-risk relatives	Counsel as if no mutation detected
1	Not pathogenic	<0.001	Do not use as predictive testing in at-risk relatives	Counsel as if no mutation detected

Table adapted from Plon et al. 2008 [8]. Note that for most variants, a quantitative probability is not yet available, as insufficient lines of evidence exist to generate the probability.

ing v2 (PolyPhen-2.1, <http://genetics.bwh.harvard.edu/pph2>), and Align-Grantham Variation Grantham Deviation (Align-GVGD, <http://agvgd.iarc.fr>) [7, 17, 18]. Tools for in silico analysis have been reviewed [19]. A high level of conservation of gene sequence through evolution indicates that a specific functional domain of the gene must be maintained with a specific DNA sequence to function adequately. Areas of a gene that have acquired substantial DNA sequence changes in the evolution to humans are thought to be less essential or non-critical to the function of that gene. Conservation is a reflection of evolutionary fitness. If the VUS is in a highly conserved portion of the gene, then logic would suggest that deviation of almost any type might be harmful; moreover, the more severe the change is, the more likely it is to be harmful. On the other hand, if the VUS was in an area that was not highly conserved, then perhaps this VUS was not clinically relevant. A comparison across these different computer programs is often conducted to determine whether they result in similar conclusions. Unfortunately, this is not always the case [20, 21], underscoring again that no single method is currently adequate for reinterpreting VUS.

Protein conformational modeling provides another method for attempting to understand whether a specific alteration in an amino acid might alter the function of a protein molecule; but interpretation for BRCA molecules is limited by the incomplete knowledge regarding all the functions of the BRCA proteins within the cell.

Functional Assays

For the BRCA genes, several different types of functional assays have been developed. These include assays for cellular survival and viability, DNA recombination repair, and genomic instability, all of which are modulated by the BRCA genes [22]. In addition, expression of RNA and protein, allelic imbalance (differential RNA expression of the two gene copies), transcriptional activation, intracellular localization, and centrosome amplification can be compared with wild-type and known deleterious standards [23]. There is no gold standard by which to compare these results, as pathogenic mutations do not affect all these functional endpoints in the same way, so multiple assays may be needed to identify a specific loss of functionality. Furthermore, validation work is necessary to be sure that an observed laboratory deviation actually correlates

with other methods of distinguishing pathogenic from neutral variants. For example, Guidugli et al. recently used known neutral and pathogenic BRCA2 variants from the DNA binding region of BRCA2 to calibrate a BRCA2 homologous recombination DNA repair assay and to establish high sensitivity and specificity for pathogenic mutations [24]. Because a specific region with a known function was studied and because known neutral and pathogenic mutations were used for calibration, the suggestion is that this assay can be used to classify all VUS in this commonly mutated region of BRCA2, even in the absence of other types of information. Unfortunately, calibrated assays do not exist for most domains or functions of most genes at present.

Pathology Profile

The histopathologic characteristics of tumors from VUS carriers can be compared to published data that compared sporadic breast tumors with tumors from those with deleterious BRCA-gene mutations. For example, Spearman et al. estimated an odds ratio of 8 in favor of pathogenicity for BRCA1 if medullary histology was found in a breast cancer of a person with a VUS [25]. This histology is not associated with pathogenic BRCA2-related tumors. Conversely, HER2-positive tumors were associated with an odds ratio of 0.15 for both BRCA1 and BRCA2 (because mutation carriers are less likely to demonstrate HER2-positive tumors). Chenevix-Trench et al. used published norms to establish odds ratios favoring pathogenicity interpretations using estrogen receptors, progesterone receptors, and the status of several cytokeratins [26]. Similar odds were tabulated for each clinical pathology feature and combination of features that might help differentiate a BRCA-related tumor from a sporadic tumor. Groups of characteristics such as triple-negative status were treated as an independent variable, and because the data was based on empirical data, the likelihood ratios (LRs) can be combined to produce a net probability for pathogenicity versus neutrality. The fact that tumors from carriers of deleterious BRCA1 mutations are not uniform in their characteristics does limit the power of this approach. Furthermore, the assignment of the odds figure is inherently inexact, as publications vary considerably in their observations across histopathologic features.

Parameters	Deffenbaugh 2002 [29]	Abkevich 2004 [30]	Goldgar 2004 [4]	Judkins 2005 [31]	Phelan 2005 [32]	Tesoriero 2005 [33]	Wu 2005[22]	Bonatti 2006 [34]	Chenevix-Trench 2006 [26]	Tavtigian 2006 [7]	Carvalho 2007 [35]	Easton 2007 [14]	Lovelock 2007 [23]	Osorio 2007 [36]	Farrugia 2008 [37]	Giannini 2008 [38]	Hansen 2008 [39]	Malacrida 2008 [40]	Spearman 2008 [25]	Spurdle 2008 [41]	Tavtigian 2008 [5]	Tavtigian 2008 [6]	Caux-Moncoutier 2009 [42]	Chang 2009 [43]	Lee 2010 [44]	Sweet 2010 [45]	Walker 2010 [46]	Whiley 2010 [47]	Biswa 2011 [48]	Whiley 2011 [49]	Thomassen 2012 [50]	
Cosegregation in families; segregation analysis	X		X		X	X	X	X				X	X	X	X	X		X		X									X	X	X	
Co-occurrence in trans with deleterious mutations	X		X	X	X		X		X	X		X	X		X				X	X	X	X							X		X	X
Evolutionary conservation/ Grantham analysis	X	X	X		X				X	X			X		X	X		X	X	X	X	X					X	X	X		X	X
Tumor pathology markers									X									X	X								X		X		X	
Loss of wild-type allele in tumor									X					X		X		X	X								X					
ER/PR status									X				X	X				X	X	X												
Grade									X				X	X					X													
Her2 status																			X													
Triple-negative status																			X													
Histology									X				X						X													
Age at diagnosis																			X													
Cytokeratin 5/6 and 14													X							X												
Tubule formation (BRCA2 only)									X											X												
Logistic regression of deleterious vs. wild-type with proband and family cancer history												X			X																	
Mutation in functional domain									X						X					X												
RNA-based splicing studies or protein truncation or allelic imbalance								X	X								X		X				X					X				X
Functional assays					X		X				X		X		X									X	X		X	X	X	X		
Analysis of disruption of specific protein domains	X	X			X					X		X		X						X									X		X	
Frequency among controls	X													X																		

Figure 2. Overview of studies that have attempted reclassification of *BRCA* VUS and listing of which parameters were evaluated in each study.

Therefore, this process alone cannot be expected to provide definitive data, but it can contribute to a combined analysis as described below.

Putting It All Together: *BRCA1* and *BRCA2* VUS Interpretation Methods

Progress in computational methods in the last 10 years has moved away from simple binary classification of VUS as pathogenic or neutral, providing instead a numerical probability of pathogenicity. In 2000, the Breast Cancer Information Core working group (<http://research.nhgri.nih.gov/bic/>) laid plans to develop a system that could combine multiple independent sorts of analyses by combining LRs to predict pathogenicity of VUS results. An LR compares the probability of the observed data under a hypothesis of pathogenicity (comparing VUS data to cases with truncating mutations) versus a hypothesis that the VUS was neutral. LRs from all available sources were multiplied to obtain an “integrated LR.” To be conservative, the working group considered $LR > 1,000$ to be pathogenic and $LR < 0.01$ to be benign/neutral. DNA variants scoring between those numbers remained classified as VUS. A collection of papers by scientists and physicians who attended a workshop sponsored by the International Agency for Research on Cancer (IARC, World Health Organization) explain all facets of this approach [5, 8, 19, 27].

Using a Bayesian model for those VUS that are missense changes (the largest group), a prior probability of pathogenicity can be determined for each VUS by evaluating biophysical characteristics of amino acids and evolutionary conservation of protein multiple sequence alignments to predict where missense substitutions fall in a spectrum from enriched deleterious to enriched neutral using the Align-GVGD program (<http://agvgd.iarc.fr>) [6]. All other independent lines of evidence, such as those described above (e.g., segregation in families or functional assays that can be expressed mathematically as LRs) can then be integrated with the prior probability to generate a posterior probability of pathogenicity for each variant. Thus, the original assignment of pathogenicity by Align-GVGD, based only on the specific amino acid change, can be refined.

On the basis of the numerical value of the posterior probability, the IARC group then introduced a clinical translation step, summarized in Table 2, in which a five-tier classification scheme for DNA findings is provided [8]. The authors have proposed how these levels of predicted pathogenicity might be used to counsel patients about cancer surveillance and when it is reasonable to use a DNA variant as a marker for predictive testing in at-risk relatives. Levels 1 and 2 are likely neutral; level 3 remains a true VUS; levels 4 and 5 are treated as pathogenic for clinical purposes.

Posterior Probabilities of Pathogenicity in *BRCA* VUS

On the basis of the methods described above, *BRCA* variants that were originally called uncertain can be assigned new posterior probabilities and can be assigned to one of the five classes. Tables that show the result of reclassification as either neutral or deleterious have been published [2, 28]; 113 *BRCA1* variants analyzed in this fashion are posted on the IARC website for *BRCA1* (http://brca.iarc.fr/LOVD/variants.php?action=view_unique&select_db=BRCA1), and 104 scored variants for *BRCA2* are also posted (http://brca.iarc.fr/LOVD/variants.php?action=view_unique&select_db=BRCA2). The same information can be found in a more patient-friendly format at http://mayoresearch.mayo.edu/mayo/research/womens_cancer/projects.cfm.

Although this information represents the newest way to reclassify VUS, it is important to remember that these are probabilities and there remains a possibility that the interpretation is wrong, that nature has brought together a number of events coincidentally rather than causally, that some underlying assumptions in the models are wrong, that there are exceptions to rules, that some underlying biophysical property of the gene or proteins is unrecognized, etc. For example, a DNA change that appears to encode a change in protein sequence may have a larger impact on the change by impacting splicing. This is one reason that it is preferable to combine several different types of analyses to yield the final score. All providers using these tables need to bear in mind this possibility and integrate that into discussions with their patients who are trying to make medical decisions.

DISCUSSION

Professional societies certainly recognize the clinical dilemmas presented by discovery of VUS in genetic testing. The American College of Medical Genetics (2008) issued a statement on reporting of DNA sequence alterations in six categories, including two categories relevant to VUS. The six categories are (1) sequence variation is previously reported and is a recognized cause of the disorder; (2) sequence variation is previously unreported and is of the type that is expected to cause the disorder; (3) sequence variation is previously unreported and is of the type that may or may not be causative of the disorder; (4) sequence variation is previously unreported and is probably not causative of disease; (5) sequence variation is previously reported and is a recognized neutral variant; and (6) sequence variation is previously not known or expected to be causative of disease, but is found to be associated with a clinical presentation. This recommendation did not address how to move a VUS from category 3 to category 2 or 4 or how to apply these categories to clinical issues such as predictive testing of relatives.

The Clinical Molecular Genetics Society in the UK (2008; <http://www.cmgs.org/>) ratified a detailed practice guideline regarding use of each line of evidence to bring interpretation to VUS, including all the methods described previously. Like the IARC method, they state that overall conclusions on the clinical significance of a variant should be based on more than one line of evidence. This group recommended use of four classes to describe DNA sequence alteration: Class 1 is “certainly nonpathogenic,” class 2 is “unlikely to be pathogenic but

cannot be formally proven,” class 3 is “likely to be pathogenic but cannot be formally proven,” and class 4 is “certainly pathogenic.” The guidelines do not attempt to define “formally proven.”

In contrast, the IARC Unclassified Genetic Variant Working Group was the first group to tie clinical recommendations with a numerical probability of a DNA alteration being pathogenic [8], as shown in Figure 2. If such odds ratios become more readily available, this system may prove to be a turning point in clinical practice. Research continues to refine the data on which the LRs are calculated. Table 2 reviews the studies that have contributed to the knowledge base on which scoring may be attained. Additional studies are underway, which will allow additional reclassification of today’s VUS (IARC category 3) into tomorrow’s IARC category 1, 2, 4, or 5.

FUTURE STEPS

As genetic testing becomes more common and as panels with multiple genes replace single gene testing, VUS will become increasingly common in clinical practice and those ordering the test will be responsible for explaining what the results mean for each patient and their relatives. In addition, direct-to-consumer genetic testing of single genes, gene panels, or sequencing of essentially the entire genome will lead to the unearthing of massive numbers of VUS in all kinds of genes. Physicians who had nothing to do with the ordering of these tests may be called upon to interpret and advise patients, so a general approach to these conversations will be invaluable. There may be a temptation to act on genetic information that is truly uninterpretable, which runs a significant risk of doing active harm. The information about methods to reclassify VUS presented in this overview is applicable to all genes, though the database links provided here are specific to *BRCA1* and *BRCA2* genes, for which reclassification efforts are more advanced than for most other genes. By understanding more about the issues and remedies for VUS interpretation, clinicians can help navigate medical decision-making using the best available information and become comfortable with accepting that many DNA results cannot be interpreted with the tools and data available today.

ACKNOWLEDGMENTS

This study is supported by a Mayo Clinic Specialized Program of Research Excellence (SPORE) in breast cancer (P50 CA116201) and National Institutes of Health grant CA116167.

AUTHOR CONTRIBUTIONS

Conception/design: Noralane M. Lindor, David E. Goldgar, Sean V. Tavtigian, Fergus J. Couch

Collection and/or assembly of data: Noralane M. Lindor, David E. Goldgar, Sean V. Tavtigian, Fergus J. Couch

Data analysis and interpretation: David E. Goldgar, Sean V. Tavtigian, Sharon E. Plon, Fergus J. Couch

Manuscript writing: Noralane M. Lindor, David E. Goldgar, Sean V. Tavtigian, Sharon E. Plon, Fergus J. Couch

Final approval of manuscript: Noralane M. Lindor, David E. Goldgar, Sean V. Tavtigian, Sharon E. Plon, Fergus J. Couch

DISCLOSURES

David E. Goldgar: Royalties on *BRCA* testing (IP); **Fergus J. Couch:** Myriad Genetics Laboratories Inc. (IP). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

REFERENCES

1. Ready K, Gutierrez-Barrera AM, Amos C et al. Cancer risk management decisions of women with BRCA1 or BRCA2 variants of uncertain significance. *Breast J* 2011;17:210–212.
2. Lindor NM, Guidugli L, Wang X et al. A review of a multifactorial probability-based model for classification of BRCA1 and BRCA2 variants of uncertain significance (VUS). *Hum Mutat* 2012;33:8–21.
3. Richards CS, Bale S, Bellissimo DB et al. ACMG recommendations for standards for interpretation and reporting of sequence variations: Revisions 2007. *Genet Med* 2008;10:294–300.
4. Goldgar DE, Easton DF, Deffenbaugh AM et al. Integrated evaluation of DNA sequence variants of unknown clinical significance: Application to BRCA1 and BRCA2. *Am J Hum Genet* 2004;75:535–544.
5. Tavtigian SV, Greenblatt MS, Goldgar DE et al. Assessing pathogenicity: Overview of results from the IARC Unclassified Genetic Variants Working Group. *Hum Mutat* 2008;29:1261–1264.
6. Tavtigian SV, Byrnes GB, Goldgar DE et al. Classification of rare missense substitutions, using risk surfaces, with genetic- and molecular-epidemiology applications. *Hum Mutat* 2008;29:1342–1354.
7. Tavtigian SV, Deffenbaugh AM, Yin L et al. Comprehensive statistical study of 452 BRCA1 missense substitutions with classification of eight recurrent substitutions as neutral. *J Med Genet* 2006;43:295–305.
8. 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* 2008;29:1282–1291.
9. Nanda R, Schumm LP, Cummings S et al. Genetic testing in an ethnically diverse cohort of high-risk women: A comparative analysis of BRCA1 and BRCA2 mutations in American families of European and African ancestry. *JAMA* 2005;294:1925–1933.
10. Kurian AW. BRCA1 and BRCA2 mutations across race and ethnicity: Distribution and clinical implications. *Curr Opin Obstet Gynecol* 2010;22:72–78.
11. Haffty BG, Silber A, Matloff E et al. Racial differences in the incidence of BRCA1 and BRCA2 mutations in a cohort of early onset breast cancer patients: African American compared to white women. *J Med Genet* 2006;43:133–137.
12. Thompson D, Easton DF, Goldgar DE. A full-likelihood method for the evaluation of causality of sequence variants from family data. *Am J Hum Genet* 2003;73:652–655.
13. Mohammadi L, Vreeswijk MP, Oldenburg R et al. A simple method for co-segregation analysis to evaluate the pathogenicity of unclassified variants; BRCA1 and BRCA2 as an example. *BMC Cancer* 2009;9:211.
14. Easton DF, Deffenbaugh AM, Pruss D et al. A systematic genetic assessment of 1,433 sequence variants of unknown clinical significance in the BRCA1 and BRCA2 breast cancer-predisposition genes. *Am J Hum Genet* 2007;81:873–883.
15. Spurdle AB, Healey S, Devereau A et al. ENIGMA—evidence-based network for the interpretation of germline mutant alleles: An international initiative to evaluate risk and clinical significance associated with sequence variation in BRCA1 and BRCA2 genes. *Hum Mutat* 2012;33:2–7.
16. Spurdle AB, Whitley PJ, Thompson B et al. BRCA1 R1699Q variant displaying ambiguous functional abrogation confers intermediate breast and ovarian cancer risk. *J Med Genet* 2012;49:525–532.
17. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. *Genome Res* 2001;11:863–874.
18. Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: Server and survey. *Nucleic Acids Res* 2002;30:3894–3900.
19. Tavtigian SV, Greenblatt MS, Lesueur F et al. In silico analysis of missense substitutions using sequence-alignment based methods. *Hum Mutat* 2008;29:1327–1336.
20. Dorfman R, Nalpathamkalam T, Taylor C et al. Do common in silico tools predict the clinical consequences of amino-acid substitutions in the CFTR gene? *Clin Genet* 2010;77:464–473.
21. Hicks S, Wheeler DA, Plon SE et al. Prediction of missense mutation functionality depends on both the algorithm and sequence alignment employed. *Hum Mutat* 2011;32:661–668.
22. Wu K, Hinson SR, Ohashi A et al. Functional evaluation and cancer risk assessment of BRCA2 unclassified variants. *Cancer Res* 2005;65:417–426.
23. Lovelock PK, Spurdle AB, Mok MT et al. Identification of BRCA1 missense substitutions that confer partial functional activity: Potential moderate risk variants? *Breast Cancer Res* 2007;9:R82.
24. Guidugli L, Pankratz VS, Singh N et al. A classification model for BRCA2 DNA binding domain missense variants based on homology directed repair activity. *Cancer Res* 2013;73:265–275.
25. Spearman AD, Sweet K, Zhou XP et al. Clinically applicable models to characterize BRCA1 and BRCA2 variants of uncertain significance. *J Clin Oncol* 2008;26:5393–5400.
26. Chenevix-Trench G, Healey S, Lakhani S et al. Genetic and histopathologic evaluation of BRCA1 and BRCA2 DNA sequence variants of unknown clinical significance. *Cancer Res* 2006;66:2019–2027.
27. Goldgar DE, Easton DF, Byrnes GB et al. Genetic evidence and integration of various data sources for classifying uncertain variants into a single model. *Hum Mutat* 2008;29:1265–1272.
28. Vallee MP, Francy TC, Judkins MK et al. Classification of missense substitutions in the BRCA genes: A database dedicated to Ex-UVs. *Hum Mutat* 2012;33:22–28.
29. Deffenbaugh AM, Frank TS, Hoffman M et al. Characterization of common BRCA1 and BRCA2 variants. *Genet Test* 2002;6:119–121.
30. Abkevich V, Zharkikh A, Deffenbaugh AM et al. Analysis of missense variation in human BRCA1 in the context of interspecific sequence variation. *J Med Genet* 2004;41:492–507.
31. Judkins T, Hendrickson BC, Deffenbaugh AM et al. Application of embryonic lethal or other obvious phenotypes to characterize the clinical significance of genetic variants found in *trans* with known deleterious mutations. *Cancer Res* 2005;65:10096–10103.
32. Phelan CM, Dapic V, Tice B et al. Classification of BRCA1 missense variants of unknown clinical significance. *J Med Genet* 2005;42:138–146.
33. Tesoriero AA, Wong EM, Jenkins MA et al. Molecular characterization and cancer risk associated with BRCA1 and BRCA2 splice site variants identified in multiple-case breast cancer families. *Hum Mutat* 2005;26:495.
34. Bonatti F, Pepe C, Tancredi M et al. RNA-based analysis of BRCA1 and BRCA2 gene alterations. *Cancer Genet Cytogenet* 2006;170:93–101.
35. Carvalho MA, Marsillac SM, Karchin R et al. Determination of cancer risk associated with germline BRCA1 missense variants by functional analysis. *Cancer Res* 2007;67:1494–1501.
36. Osorio A, Milne RL, Honrado E et al. Classification of missense variants of unknown significance in BRCA1 based on clinical and tumor information. *Hum Mutat* 2007;28:477–485.
37. Farrugia DJ, Agarwal MK, Pankratz VS et al. Functional assays for classification of BRCA2 variants of uncertain significance. *Cancer Res* 2008;68:3523–3531.
38. Giannini G, Capalbo C, Ottini L et al. Clinical classification of BRCA1 DNA missense variants: H1686Q is a novel pathogenic mutation occurring in the ontogenetically invariant THV motif of the N-terminal BRCT domain. *J Clin Oncol* 2008;26:4212–4214; author reply 4214–4215.
39. Hansen TV, Bisgaard ML, Jonson L et al. Novel de novo BRCA2 mutation in a patient with a family history of breast cancer. *BMC Med Genet* 2008;9:58.
40. Malacrida S, Agata S, Callegaro M et al. BRCA1 p.Val1688del is a deleterious mutation that recurs in breast and ovarian cancer families from North-east Italy. *J Clin Oncol* 2008;26:26–31.
41. Spurdle AB, Couch FJ, Hogervorst FB et al. Prediction and assessment of splicing alterations: Implications for clinical testing. *Hum Mutat* 2008;29:1304–1313.
42. Caux-Moncoutier V, Pages-Berhouet S, Michaux D et al. Impact of BRCA1 and BRCA2 variants on splicing: Clues from an allelic imbalance study. *Eur J Hum Genet* 2009;17:1471–1480.
43. Chang S, Biswas K, Martin BK et al. Expression of human BRCA1 variants in mouse ES cells allows functional analysis of BRCA1 mutations. *J Clin Invest* 2009;119:3160–3171.
44. Lee MS, Green R, Marsillac SM et al. Comprehensive analysis of missense variations in the BRCT domain of BRCA1 by structural and functional assays. *Cancer Res* 2010;70:4880–4890.
45. Sweet K, Senter L, Pilarski R et al. Characterization of BRCA1 ring finger variants of uncertain significance. *Breast Cancer Res Treat* 2010;119:737–743.
46. Walker LC, Whitley PJ, Couch FJ et al. Detection of splicing aberrations caused by BRCA1 and BRCA2 sequence variants encoding missense substitutions: Implications for prediction of pathogenicity. *Hum Mutat* 2010;31:E1484–E1505.
47. Whitley PJ, Pettigrew CA, Brewster BL et al. Effect of BRCA2 sequence variants predicted to disrupt exonic splice enhancers on BRCA2 transcripts. *BMC Med Genet* 2010;11:80.
48. Biswas K, Das R, Alter BP et al. A comprehensive functional characterization of BRCA2 variants associated with Fanconi anemia using mouse ES cell-based assay. *Blood* 2011;118:2430–2442.
49. Whitley PJ, Guidugli L, Walker LC et al. Splicing and multifactorial analysis of intronic BRCA1 and BRCA2 sequence variants identifies clinically significant splicing aberrations up to 12 nucleotides from the intron/exon boundary. *Hum Mutat* 2011;32:678–687.
50. Thomassen M, Blanco A, Montagna M et al. Characterization of BRCA1 and BRCA2 splicing variants: A collaborative report by ENIGMA consortium members. *Breast Cancer Res Treat* 2012;132:1009–1023.