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Classification and Clinical Management of Variants of Uncertain Significance in High Penetrance Cancer Predisposition Genes

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ABSTRACT

In 2008, the International Agency for Research on Cancer (IARC) proposed a system for classifying sequence variants in highly penetrant breast and colon cancer susceptibility genes, linked to clinical actions. This system uses a multifactorial likelihood model to calculate the posterior probability that an altered DNA sequence is pathogenic. Variants between 5%–94.9% (class 3) are categorized as variants of uncertain significance (VUS). This interval is wide and might include variants with a substantial difference in pathogenicity at either end of the spectrum. We think that carriers of class 3 variants would benefit from a fine-tuning of this classification. Classification of VUS to a category with a defined clinical significance is very important because for carriers of a pathogenic mutation full surveillance and risk-reducing surgery can reduce cancer incidence. Counselees who are not carriers of a pathogenic mutation can be discharged from intensive follow-up and avoid unnecessary risk-reducing surgery. By means of examples, we show how, in selected cases, additional data can lead to reclassification of some variants to a different class with different recommendations for surveillance and therapy. To improve the clinical utility of this classification system, we suggest a pragmatic adaptation to clinical practice.

BACKGROUND

Evaluation of the Pathogenicity of Variants of Uncertain Significance

Besides classical pathogenic mutations that truncate or inactivate the protein, the continuous development of various sequence-based technologies in DNA diagnostic laboratories is resulting in the detection of an increasing number of variants for which the clinical significance is unknown. These variants, also referred to as variants of uncertain significance (VUS), include missense variants, small in-frame deletions or insertions, synonymous nucleotide substitutions, certain truncating mutations (such as mutations in the last exons of genes), as well as alterations in noncoding sequences or in untranslated regions.

In silico approaches predict the consequences of DNA sequence changes in an indirect manner based on evolutionary nucleotide and amino acid conservation, the possible effect of amino acid substitutions on protein structure^{1, 2} or the predicted effect on messenger RNA splicing.³ Some other methods measure the direct association of the variant with disease, and include cosegregation of the variant with disease in a family,^{4, 5} family history,⁶⁻⁸ co-occurrence of the variant with pathogenic mutations on the second allele,^{9, 10} tumor pathology,^{2, 11, 12} and analysis of the tumor DNA (e.g., array comparative genomic hybridization and genomic methylation).¹³⁻¹⁶ There are also functional studies that assess the impact of genetic variants on the activity of the protein *in vitro*.¹⁷⁻²⁰

In 2004, Goldgar et al introduced a multifactorial likelihood model (MLM) for the classification of the VUS in *BRCA1* (MIM #113705) and *BRCA2* (MIM #600185) in which the odds of causality, obtained from different methods under the assumption of independence, could be combined. In general, when a VUS reached odds higher than 1,000:1 in favor of pathogenicity, it could be classified as pathogenic, and when it was lower than 1:100 against pathogenicity, the variant could be classified as neutral.²¹ This model was improved in 2008 by the addition of the prior probability of pathogenicity of a variant based on its position and function.^{1, 22}

In 2007, the UK Clinical Molecular Genetics Society and the Dutch Society of Clinical Genetics Laboratory Specialists proposed "Good Practice Guidelines for the Interpretation and Reporting of Unclassified Variants in Clinical Molecular Genetics Laboratories".²³ It proposed reporting variants in four classes: (I) certainly not pathogenic, (II) unlikely to be pathogenic, (III) likely to be pathogenic, and (IV) certainly pathogenic. In 2008, the American College of Medical Genetics (ACMG) proposed a six-class system for interpretation and reporting of sequence variants, with an emphasis on the importance of appropriate reporting of sequence variations using standardized terminology and established databases: (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 which 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.²⁴ However, neither of these two systems recommended using quantitative information for the classification and clinical management of variants. An expert working group, convened at IARC (<http://www.iarc.fr>) in 2008, proposed a standardized classification system applicable to sequence-based results in highly penetrant cancer predisposition genes such as *BRCA1*, *BRCA2*, *MLH1* (MIM #120436), and *MSH2* (MIM #609309). This classification system interprets results from the MLM and translates these to recommendations for clinical practice.²⁵

Current Clinical Management of the VUSs

According to the IARC classification (Table 1), the counselees who carry a variant in class 1 should be counselled as if no mutation was detected for this disorder. The carriers in class 5 should be counselled as those who are carriers of the conventional pathogenic mutations. Variants in class 2 and 4 should be clinically managed as variants in class 1 and 5, respectively²⁵. The DNA alterations that are in class 3 are classified as VUS, which means that the laboratory interpreted the DNA alteration based on standard evidence at the time of the test (mostly *in silico* and literature review) and found that there was insufficient evidence to classify the alteration as either pathogenic (deleterious) or neutral. Within this classification, a VUS should not be used for predictive testing in at-risk individuals and the surveillance should be based on family history. The authors suggested that the research testing of the family members might be helpful to further classify variants (Table 1).²⁵

Options for Communication of the VUSs

Based on this classification, we think there are currently broadly two approaches in clinical practice for communication of an identified VUS to the tested individual and their family members: communicating all the VUSs or communicating none of the VUSs.

1. Communicating all the VUSs

From a research point of view, collecting as much evidence from all sources as possible for all the VUS will allow the reclassification of the maximum number of variants. As summarized by Spurdle et al the majority of *BRCA1* and *BRCA2* variants submitted to ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles, <http://www.enigmaconsortium.org>) as of September 2010 were missense variants (61% and 64%, respectively).²⁶ In the study of Easton et al, 1,177 out of 1,433 (82%) variants were either missense variants or in-frame deletions or insertions. Of all the missense variants and in-frame deletions or insertions in *BRCA1/2*, about 12% are estimated to be pathogenic (based on combined likelihood ratios [LR])⁶. Furthermore, several studies have shown that counselees and the family members of those who know themselves to be a carrier of a VUS experience considerable distress due to the possibility that they face a high cancer risk and due to the uncertainty surrounding this risk^{27, 28}. When *BRCA* VUS reports

Table 1. IARC classification system for sequence variants identified by genetic testing and recommendations associated with each class of variant. This table is adapted with permission from authors¹

Class and description	Probability of pathogenicity	Clinical testing	Surveillance recommendations if at-risk relative is positive	Research testing of family members
1 Not pathogenic or of no clinical significance	<0.001	Do not use for predictive testing in at-risk individuals	Treat as "no mutation detected" for this disorder	Not indicated
2 Likely not pathogenic or of little clinical significance	0.001-0.049	Do not use for predictive testing in at-risk individuals	Treat as "no mutation detected" for this disorder	Maybe helpful to further classify variant
3 Uncertain	0.05-0.949	Do not use for predictive testing in at-risk individuals	Based on family history (and other risk factors)	Maybe helpful to further classify variant
4 Likely pathogenic	0.95-0.99	Test at risk relatives for variant	Full high risk surveillance	Maybe helpful to further classify variant
5 Definitely pathogenic	>0.99	Test at risk relatives for variant	Full high risk surveillance	Not indicated

are interpreted by clinicians with minimal training in genetics, misunderstandings are compounded²⁹. Moreover, for a variant in class 3 with a prior probability of for example, 0.05 (lower end of probability of pathogenicity in class 3), a likelihood ratio of 361.2 (19/0.0526) toward pathogenicity is needed to ascend to a posterior probability of 0.95 (lower end of probability of pathogenicity in class 4) (Figure 1A), with a clinical consequence for the patients. To achieve this LR, a lot of additional data such as histopathological information and extensive segregation data are necessary. The same variant in class 3 with a prior probability of 0.05 can easily descend to class 2, but this does not have any clinical consequence for the carriers (Table 1).

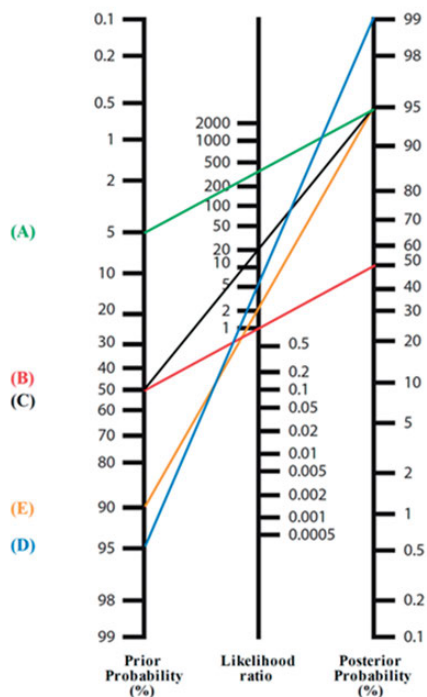


Figure 1. Nomogram for Bayes theorem.³⁰ Copyright© (1975) Massachusetts Medical Society. Reproduced from Fagan³⁰ with permission from Massachusetts Medical Society. A line drawn from prior probability on the left of Figure 1 through the likelihood ratio in the center of the figure gives the posterior probability on the right side of the figure (explanation of 1A-1E in the text). Likelihood ratio (LR) is a measure of accuracy of a diagnostic test. The LR of any clinical finding is the probability of that finding when a condition is present divided by the probability of the same finding when the condition is absent.³¹ Posterior probability of pathogenicity can be calculated as: posterior odds/ (posterior odds + 1) and the posterior odds are calculated as: $LR \times (\text{prior probability}/[1-\text{prior probability}])$.³² For example, for a variant in class 3 with a prior probability of 0.05, to ascend to a posterior probability of 0.95 (class 4), a LR of 361.2 is needed. [Posterior probability = 0.95 = [posterior odds/(posterior odds + 1)] → posterior odds = 19; and posterior odds = $LR \times [\text{prior probability}/(1-\text{prior probability})]$ → $19 = LR \times [0.05/(1-0.05)]$ → $19 = LR \times 0.0526$ → $LR = 19/0.0526 = 361.2$].

2. Communicating none of the VUSs

Previous studies have shown that VUS may be recategorized when additional information becomes available, and although basic *in silico* categorization is fairly robust, it has also been shown that additional information is central to an accurate appraisal³³. Communication of a VUS test result provides the opportunity to discuss collection of additional information and material with the counselee. A consequence of not communicating the variants is that potentially pathogenic variants will go unrecognized and remain categorized as class 3 VUS, patients and their family members are then advised based on the family history. Some may choose risk-reducing surgery that could be avoided if a genetic test can be offered and they are shown not to have inherited a clearly pathogenic variant.

Recommendations for the Communication of a Variant and Examples

Since, (1) the odds are low that a random VUS in class 3 is pathogenic,⁶ (2) most of the variants after inclusion of additional data will be classified as likely not pathogenic, (3) communication of any VUS can lead to psychological distress,^{27, 28} (4) misinterpretation of a VUS may have significant adverse sequelae in terms of inappropriate decisions,³⁴ and as a consequence (5) an increase in overall costs to the health care system and the individual,³⁵ we believe that communicating all class 3 variants in a health care setting is unhelpful and may be harmful. However, communication within a research setting is clearly a different and potentially useful option.

When a VUS is identified in a high-risk cancer gene, a molecular geneticist in the DNA diagnostic laboratory, in collaboration with national and international colleagues, provides the classification (Figure 2). For a better clinical management of the VUS, our suggestion is that the laboratory specialists divide VUS class 3 into two subgroups: class 3A with a posterior probability of 0.05–0.499 and class 3B with a posterior probability of 0.5–0.949. We put forward these recommendations for the classification of high penetrance cancer predisposition genes because these genes are most commonly and completely analyzed and a lot of clinical data about these genes are available that can be used in the statistical classification of their variants. In principle, any high penetrance cancer susceptibility gene can be classified by this model. However, the model needs to be adapted to quantify the posterior probability based on different lines of evidence that are used to classify the variant.²⁵ Since its introduction for *BRCA1/2* in 2008, convening expert panels such as ENIGMA have continuously updated and fine-tuned the MLM. Members of the InSiGHT committee (International Society for Gastrointestinal Hereditary Tumours, <http://insight-group.org>) reviewed the types of data available for each mismatch repair (MMR) gene and developed quantitative scores for these different types of data. As a result, MLM was used for the classification of VUS in MMR genes in 2013.² It is expected that, in the future, other international groups adapt the model for use in the classification of other cancer predisposition genes.

We suggest communication and testing of family members when the posterior probability of pathogenicity of a VUS is higher than 0.5 (i.e., category 3B) but no

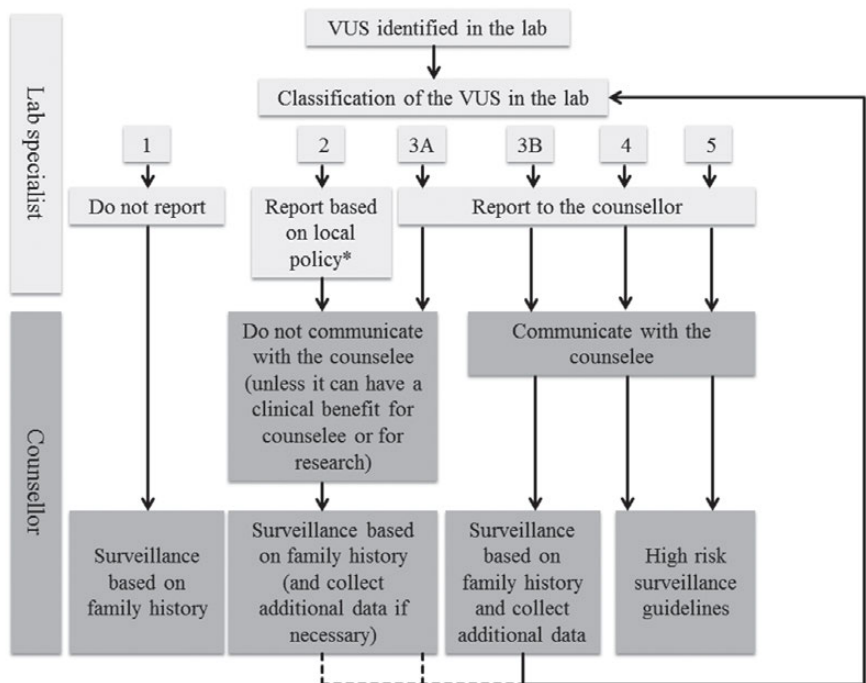


Figure 2. Schematic view for the laboratory and clinical management of the variants. *According to the “Practice Guidelines for the Evaluation of Pathogenicity and the Reporting of Sequence Variants in Clinical Molecular Genetics” by the UK Association for Clinical Genetic and the Dutch Society of Clinical Genetic Laboratory Specialists Science, local policy will determine whether class 2 variants are reported to the counsellors.

communication of variants in class 3A, unless the counsellor has a reason to expect a clinical benefit for the counselee or, for example, when there is an opportunity for research among many affected family members. Furthermore, the counsellor should inform recipients of any inconclusive genetic test result to seek contact with the cancer genetics center within a few years so that the pedigree can be reassessed and (additional) DNA testing can be offered, should there be new insights into cancer genetics or new DNA sequencing technologies available.

Based on estimations in previous studies, only about 20% of all the variants in *BRCA1* and *BRCA2* are pathogenic.⁶ Therefore we believe that the number of variants in classes 3A and 3B is not equal and rather few variants have a posterior probability above 50% (3B). We chose 50% as threshold because for this probability there is an equal chance that a variant is pathogenic or neutral (odds 1:1) (Figure 1B). For a variant with a 50% risk of pathogenicity, a LR of 19:1 is sufficient to reach a posterior probability of 95% (class 4) (Figure 1C). This can be obtained by addition of some pathological data from a few tumors and evidence of cosegregation of the variant with cancer (assuming that most of the additional data are in favor of pathogenicity). For example, it is estimated that

the LR increases 4.41-fold for every carrier of a *BRCA1* VUS who is diagnosed with breast cancer at the age of 50 years or older with a triple negative tumor (negative estrogen and progesterone receptor status and no amplification of *HER2*).¹² LR of cosegregation is highly dependent on the exact family information such as number of affected and unaffected individuals in the family, age of diagnosis, and the degree of kinship. For example, if the index is a female who has breast cancer at the age of 29 years, and carries a specific variant in *BRCA1*, and her sister also carries the same variant and is affected with ovarian cancer at the age of 41 years, and there are two healthy untested siblings at about the age of 50 years with healthy parents, then the LR of cosegregation for this family will be about 2.⁵ In general, genotypes of distantly related individuals with very early onset of cancer or old healthy individuals give the strongest LRs in favor of or against pathogenicity⁵. Also, each MSI high tumor with a VUS in one of the MMR genes increases the LR 6.96-fold toward pathogenicity.² An example is c.1852_1854delAAG, p.Lys618del in the *MLH1* gene for which the prior probability of being pathogenic was 0.5. After addition of LR for cosegregation and tumor characteristics, the variant was classified as pathogenic with posterior probability of 1.0.² Another example is c.5066T>G, p.Met1689Arg in *BRCA1* that had a prior probability of pathogenicity of 0.66. After addition of other information such as family history and co-occurrence data, the probability of pathogenicity reached 0.989 that led to reclassification of this variant to class 4 (likely pathogenic) and allowed family members to be offered meaningful predictive genetic testing.^{6, 32}

Caveats

The examples given above are only to illustrate how additional information can change the classification. The thresholds for classification are carefully set by IARC.²⁵ Because reclassification of a variant from class 3 to class 4 or class 5 can have serious clinical consequences for the carriers of the variant, the upper range of class 3 in the IARC classification is set very high (0.95). However, if prior is 0.5, to ascend from posterior probability of 0.95–0.99, a 5.3-fold (99/18.6) increase in LR is needed (Figure 1D), whereas from 0.90 to 0.95 only a 2.1-fold (18.6/9) increase in the LR is sufficient (Figure 1E). So, for the same increase of about 5% in the posterior probability, much less information is needed and the classification can in some cases easily change from one class to another.

It is important to emphasize that collection of information by the counsellors should not be selective, which means that the counsellor needs to collect all available evidence, not just evidence that supports the pathogenic status of the variant or just to the point at which a high posterior probability is reached. Failure to do this may lead to an overestimation of the LR through selection bias. Furthermore, all the collected information, when not strictly confidential, should be shared with the molecular geneticists who are responsible for classification. Also, it is important to appreciate that confidence in a posterior probability increases as multiple additional data sources from diverse resources increase. Moreover, probabilities might be based on misinterpretation due to

incorrect underlying assumptions in the model, exceptions to certain rules, incomplete knowledge of some underlying biophysical property of the gene or protein, or to many other factors.³⁶ For example, *BRCA1* c.594-2A>C also known as *BRCA1* IVS9-2A>C that was presumed to be pathogenic based on predicted impact of base change on splicing and biochemical evidence but eventually is proven to be benign based on other biological evidences.³⁷ Lindor et al also suggested keeping these possibilities in mind and integrating them into discussions with the counselees who are actually involved in making personal medical decisions.³⁶

DISCUSSION AND CONCLUSION

As previously mentioned, there is still no universally accepted international guideline for genetic counsellors regarding the communication and research testing of the family members of the carriers of VUS.³⁴

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Communication of a VUS test result provides the opportunity to discuss collection of additional information and material with the counselee that can eventually lead to a better assessment of the variants. However, there is a fine balance between on the one hand causing additional stress for the counselees and extra costs for the health care system and on the other hand a reduction in morbidity and mortality through better screening, possibility for prophylactic surgery, and personalized chemotherapy (such as, if proven effective, treatment with platinum and PARP inhibitors in *BRCA1/2* mutation carriers)^{38, 39} when a variant can be classified as pathogenic.

If, after collection of additional information, a variant is downgraded from class 3 to 2, this will not change the clinical management of the carriers. For these reasons, we propose that in a primarily clinical setting, counsellors are not obliged to communicate all VUS. Since reclassification of a VUS with posterior probability >0.5 has a realistic chance of leading to a change in clinical management, we consider that communication of information to counselees who are carriers of class 3B variants would encourage the collection of additional information in the family and would thus represent a worthwhile investment of resources given the potential gains in clinical utility.

It is important to mention that the assumption that is valid in the MLM for the classification of the variants is that the variant under study is either neutral with respect to cancer risk or has the same risk as known highly penetrant pathogenic mutations. The IARC system was developed for highly penetrant risk genes and therefore it is probably not suited for classifying low or intermediate penetrance variants either in known genes such as *BRCA1* (e.g., c.5096G>A, p.Arg1699Gln)⁴⁰ or moderate risk genes such as *CHEK2* (MIM #604373). For such cases, there is still no clear guideline for communication or clinical management of the counselees and their family members. More insight into the exact cancer risk associated with such variants is needed to determine a suitable approach to classification of lower risk variants.

In this paper, we propose an extension to the existing classification system,²⁵ currently used for VUS in the high-risk cancer predisposing genes, and we suggest a new

communication protocol. The purpose of these recommendations is to improve the clinical management of the counselees by a more precise classification of the variants without causing unnecessary stress for the counselees or additional costs for the health care system, while minimizing the risk of missing pathogenic mutations in clinical practice.

National and international collaborative research consortia such as the HEBON (HEreditary Breast and Ovarian cancer research in the Netherlands, <http://www.hebon.nl>), InSiGHT, and ENIGMA play an extremely valuable role in improving cancer risk estimates by assisting definitive classification through collection of all available information on variants and associated phenotypes, and by working closely with clinical groups in many countries to further enhance the value of genetic testing for patients.

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