A review of a multifactorial probability based model for classification of *BRCA1* and *BRCA2* variants of uncertain significance (VUS)

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Abstract

Clinical mutation screening of the *BRCA1* and *BRCA2* genes for the presence of germline inactivating mutations is used to identify individuals at elevated risk of breast and ovarian cancer. Variants identified during screening are usually classified as pathogenic (increased risk of cancer) or not pathogenic (no increased risk of cancer). However, a significant proportion of genetic tests yield variants of uncertain significance (VUS) that have undefined risk of cancer. Individuals carrying these VUS cannot benefit from individualized cancer risk assessment. Recently a quantitative "posterior probability model" for assessing the clinical relevance of VUS in *BRCA1* or *BRCA2* that integrates multiple forms of genetic evidence has been developed. Here we provide a detailed review of this model. We describe the components of the model and explain how these can be combined to calculate a posterior probability of pathogenicity for each VUS. We explain how the model can be applied to public data and provide Tables that list the VUS that have been classified as not pathogenic or pathogenic using this method. While we use *BRCA1* and *BRCA2* VUS as examples, the method can be used as a framework for classification of the pathogenicity of VUS in other cancer genes.

Keywords

BRCA1; BRCA2; VUS classification; missense mutations

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Introduction

Clinical mutation screening of the *BRCA1* and *BRCA2* breast and ovarian cancer predisposition genes (MIM#s 113705, 600185, respectively) for the presence of germline inactivating mutations is a widely used method for identifying individuals at elevated risk of breast and ovarian cancer. The criteria for genetic testing of these genes vary, but generally women with a significant family history of these cancers are offered testing. The following are possible outcomes of genetic testing:

Pathogenic mutations: Based on testing criteria, between 10% and 15% of all genetic tests yield known pathogenic mutations, often called "deleterious" mutations (See Definitions of Vocabulary) that truncate and/or inactivate *BRCA1* or *BRCA2* and predispose to an elevated age-specific risk of breast and ovarian cancer. These are DNA variants that take the form of nonsense mutations, small out-of-frame insertion or deletion mutations, larger gene rearrangements and splicing alterations that all truncate or remove important domains of the BRCA proteins. In addition, certain missense substitutions are considered "pathogenic" because they inactivate protein function. These mutations can be confidently predicted to disrupt the function of the *BRCA1* or *BRCA2* protein leading to increased risk of breast or ovarian cancer. Individuals who carry inherited pathogenic mutations in their DNA have, on average, substantially elevated age-dependent risks of breast and ovarian cancer compared to those without mutations in the general population. Women carrying pathogenic *BRCA1* mutations have a 59% risk of breast cancer and a 34% risk of ovarian cancer by age 70, whereas women with *BRCA2* pathogenic mutations have a 51% breast cancer and 11% ovarian cancer risk by age 80 (Antoniou, et al., 2008). These risks may vary by population but are always significantly elevated over population risks. Importantly, individuals with *BRCA1* and *BRCA2* mutations and their family members can benefit from risk assessment, enhanced cancer surveillance, risk-reducing prophylactic mastectomy and oophorectomy, and counseling. Specifically, those carrying pathogenic mutations are known to be at substantially elevated risk of cancer, whereas individuals from the same families who do not carry the pathogenic mutation benefit from the knowledge that they are not at increased risk of cancer.

Not pathogenic or low clinical significance (LCS) variants: A further 80% of tests either fail to identify alterations in the *BRCA1* and *BRCA2* genes or identify variants that are considered to be "not pathogenic", often termed "neutral", or of "low clinical significance" low clinical significance" (See Sources of Data). This group of variants includes common polymorphisms, seen in greater than 1% of alleles in the general population, and rare variants that display little or no association with breast cancer risk in families. These variants are predicted or have been shown to have no significant influence on the normal function of the *BRCA1* or *BRCA2* proteins.

Variants of **u**ncertain **s**ignificance (VUS): In many countries as many as 30% to 50% of variants identified during BRCA1 and BRCA2 gene testing are Variants of Uncertain Significance (VUS), also referred to as unclassified variants (UVs) (Hofstra, et al., 2008). In the USA these variants may only account for 5% to 10% of alterations because of ongoing classification efforts by Myriad Genetics Laboratories Inc. VUS are mainly missense substitutions that result in single amino acid changes, but also include in-frame small deletions or insertions that change only small numbers of amino acids, silent coding alterations that may influence splicing or translation, or intronic changes of unknown influence on gene splicing. These alterations have unknown functional effects on *BRCA1* and *BRCA2* and cannot at this time be classified as either "Pathogenic" or "Not Pathogenic/ low clinical significance". As a result individuals found to carry these variants in their DNA and members of their families cannot benefit from risk assessment measures offered to

members of families known to carry *BRCA1* or *BRCA2* deleterious mutations. Unfortunately, even though individual VUS are rare, the finding of a VUS is not a rare event. At present, there are hundreds of unique VUS recorded in the BRCA genes (Breast Cancer Information Core Database: http://research.nhgri.nih.gov/bic/).

Interpretation of a VUS in a patient sample

A VUS result issued by a testing laboratory means that that there was insufficient evidence to classify the alteration as either pathogenic or not pathogenic at the time of the test. Many individuals diagnosed with a VUS ask whether the finding of a VUS results from a questionable laboratory call or from technical limitations. Given the quality control measures of most diagnostic laboratories it should be made clear that a DNA alteration exists, but the clinical interpretation of that DNA alteration is unclear. A VUS finding should be considered not clinically useful and should not be factored into clinical decision making. This should remain so until further evidence emerges to shift the interpretation toward either a not pathogenic or pathogenic interpretation. Since additional information can result in reclassification of VUS as "pathogenic" or "not pathogenic", as described below, continuous evaluation of the literature is an important component of VUS interpretation.

Information about VUS that is often not provided as part of clinical testing reports is stored in a number of different locations. The Breast Cancer Information Core (BIC) (http://research.nhgri.nih.gov/bic/) lists many of the VUS that have been identified through clinical testing or research studies and have been voluntarily reported to the BIC. Efforts are underway to update the BIC with results from testing laboratories around the world. More recently an LOVD database has been developed at Leiden University in The Netherlands (http://chromium.liacs.nl/LOVD2/cancer/home.php?select_db=BRCA1) that summarizes the findings in the scientific literature for many VUS. While this database does not attempt to interpret the results of the studies, it is particularly useful for understanding the range of functional and genetic studies that may have been applied to certain VUS for the purpose of clinical classification.

The Posterior Probability model for classification of *BRCA1* and *BRCA2* VUS

Multiple methods are now being applied to move interpretation of a VUS toward a conclusion. The method that has made the greatest contribution to classification of individual VUS is termed the "posterior probability model". This method combines prior probabilities of causality derived from an evolutionary sequence conservation model (Align-GVGD) (http://agvgd.iarc.fr/) with likelihoods of causality (Easton, et al., 2007) derived from measures of associations between the VUS and cancer. Specifically, the likelihood component uses information on a) how the VUS segregates with cancer in families, b) whether the VUS is seen in combination with a known pathogenic mutation (which should be lethal for *BRCA1* or cause Fanconi Anemia for *BRCA2* if the VUS is pathogenic), c) the personal and family history of cancer (age of onset and cancer type) associated with the VUS, and d) the histopathology of the associated breast tumors. By combining the prior probability with the likelihood component a final or posterior probability of causality for each VUS can be calculated. While a number of assumptions are made in these calculations, and there is a degree of error associated with each component of the model, this approach has been used to classify several *BRCA1* and *BRCA2* VUS as pathogenic and not pathogenic. Here we explain the basis of the model, we outline how it can be applied to public datasets and provide current lists of *BRCA1* and *BRCA2* VUS that have been classified as pathogenic or not pathogenic by this method. This information should allow the research and clinical community to better understand and critically evaluate the

interpretation of results in the literature. This should also bring greater transparency and clarity to the provision of information about VUS to patients.

A. The Multifactorial (Combined) Likelihood Model

"Segregation Analysis" within the family—Segregation analysis results in an odds or likelihood ratio that a VUS is linked to breast and/or ovarian cancer in families more than expected by chance. If most individuals in families carrying a particular VUS develop breast cancer then there is a strong likelihood that the VUS may be causing the cancer phenotype. Unfortunately, the power of this analysis is dependent on access to genotype data from large numbers of individuals, which is rarely available for *BRCA1* and *BRCA2* VUS. Thus, the information derived from segregation analysis for BRCA VUS interpretation is seldom conclusive. Nevertheless, it can be of great value in selected large families with multiple living affected individuals.

Co-occurrence in "trans"—Homozygosity for *BRCA1* or *BRCA2* pathogenic mutations leads to embryonic lethality or Fanconi anemia, respectively Therefore, an odds or likelihood against the VUS being pathogenic can be calculated when a VUS is found to co-occur with a pathogenic mutation, if it can be demonstrated that the mutation and the VUS occur on different copies of the gene (*in trans*) rather than within the same copy of the gene (*in cis*) (Easton, et al., 2007). The use of co-occurrence data alone for classification of VUS is complicated by the existence of "hypomorphic variants" with subtle effects on protein function and on risk that may not result in embryonic lethality or Fanconi anemia.

Personal and family history—Classical cancer predisposing truncating mutations in *BRCA1* and *BRCA2* are associated with a distinct phenotype, which includes early onset of cancer in individuals and multiple cases of breast and/or ovarian cancer in families. When individuals and families with pathogenic mutations were compared to individuals and families without mutations, specific aspects of the *BRCA1/BRCA2* phenotype, such as age of onset of cancer and number of cancers of different types, were associated with specific likelihoods of a pathogenic mutation being present (Goldgar, et al., 2004). This was subsequently applied to families with VUS so that each specific age of onset and cancer history combination in a family with a VUS is associated with a specific likelihood that the VUS is pathogenic (Easton, et al., 2007). Importantly, these original calculations were based on mutation data provided by Myriad Genetic Laboratories Inc. Thus, these likelihoods need to be recalibrated when family history data is derived from other testing centers that use different testing criteria.

Pathology profile—Statistical weighting can be applied to histopathologic characteristics of tumors from VUS carriers based on characteristics commonly observed in tumors containing known pathogenic BRCA-gene mutations. Specifically, a high proportion of breast tumors with pathogenic *BRCA1* mutations are high grade and are negative for estrogen receptor, progesterone receptor, and HER2/neu expression. Similarly, *BRCA2* mutant tumors are moderate to high grade and are more likely to display tubule formation (Bane, et al., 2009; Lakhani, et al., 2002). Ovarian tumors in both *BRCA1* and *BRCA2* mutation carriers tend to be ER positive and high grade with serous histology (Chenevix-Trench, et al., 2006; Spurdle, et al., 2008). By comparing the frequency of these and other characteristics in tumors from carriers and non-carriers of *BRCA1* or *BRCA2* pathogenic mutations, a series of likelihoods in favor of the variant being pathogenic have been estimated (Chenevix-Trench, et al., 2006; Spurdle, et al., 2008). Here we show the odds associated with a combination of ER status, tumor grade and cytokeratin status for *BRCA1* breast tumors (Table 1). For example, while breast tumors in a *BRCA1* VUS carrier that are ER negative and grade 2 or 3 provide evidence in favor of the variant being pathogenic, all

other combinations of ER and Grade provide evidence against the variant being cancer-associated. In contrast, ER status and grade offer little predictive power for *BRCA2* VUS. Only tubule formation has proven useful for classification of VUS, with a tubule formation score of three associated with odds of 1.2:1 in favor of pathogenicity and a score <3 associated with odds of 2:1 against pathogenicity (Chenevix-Trench, et al., 2006; Spurdle, et al., 2008). Importantly, because individual tumor characteristics may not be conditionally independent, LRs for each individual tumor characteristic should not be combined in an integrated evaluation of a particular VUS (Goldgar, et al., 2008). Instead a single estimate based on the known tumor characteristics, such as that shown for ER status and grade above, should be used. Because some of these estimates are based on small numbers of tumors, efforts at refining and/or establishing the likelihood estimates associated with these and other characteristics of *BRCA1* mutant breast tumors such as cytokeratin 5/6, EGFR and TP53 status are underway using tumor information from large numbers of known *BRCA1* carriers and non-carriers from the Breast Cancer Association Consortium (BCAC) and Consortium for Investigators of Modifiers of *BRCA1/2* (CIMBA). In the future, mutation, expression, miRNA or methylation profiles associated with *BRCA1* or *BRCA2* status may also prove useful for VUS classification.

Calculation of multifactorial likelihood estimates—To determine the overall likelihood ratio (or odds ratio) for pathogenicity vs. non-pathogenicity for a particular VUS, the LRs for the VUS from each independent component of the model (which may be composed of multiple families, tumors, etc., each contributing to the overall LR for that component) are multiplied together. These calculations are also often conducted on a log scale where Log odds for each category are added. Using this approach, such odds/likelihood ratios from manuscripts or separate studies can be combined to generate updated likelihood ratios, provided that the datasets are independent.

> **Likelihood of pathogenicity = LR Co-occurrence × LR Pathology × LR Segregation × LR cancer history**
>
> *Where LR is likelihood ratio or odds of pathogenicity

B. The sequence-based Prior Probability model

A number of "in silico" methods based on orthologous protein sequence alignments are available for predicting the influence of variants on protein activity. Examples include Sorting Intolerant From Tolerant (SIFT) (available at: http://blocks.fhcrc.org/sift/SIFT.html), and Polymorphism Phenotyping (PolyPhen). These methods are based on the notion that phylogenetic conservation of protein sequence throughout evolution reflects the requirement for certain amino acids for protein activity.

Recently, the Align-GVGD algorithm (http://agvgd.iarc.fr/agvgd_input.php) was developed to account for the extent of the physicochemical change in an amino acid residue in a VUS and the extent of the evolutionary conservation of the amino acid residue in that position (Tavtigian, et al.). Using this method, variants within specific functional domains of *BRCA1* (N-terminal RING finger domain (amino acids 1-102) and C-terminal BRCT domains and coiled coil domain (amino acids 1396-1863)) and *BRCA2* DNA binding domain (amino acids 2400-3190) are graded by the structural/chemical impact of the variants and the evolutionary conservation of the mutated amino acid residues. These Align-GVGD grades (C0, C15, C25, C35, C45, C55, and C65; in which a C0 variant occurs in a poorly conserved amino acid and a C65 variant occurs in an amino acid fully conserved from pufferfish through humans) have been correlated with specific probabilities of cancer causality (prior probabilities) and validated relative to the personal and family cancer history LR estimates

described above (Tavtigian, et al., 2008). Thus, a variant in a defined functional domain with a C65 grade is assigned a prior probability of 0.81 (81% chance of being pathogenic) (Table 2). In contrast, the estimated prior probability for a variant in a key domain with a C0 grade was originally 0.00 (95%CI 0.00-0.06) (Tavtigian, et al., 2008). However, because a prior of 0.00 cannot be used in a Bayesian calculation, and in the interest of making the prior probabilities slightly more conservative, these VUS are assigned a prior probability of 0.03 (3% chance of being deleterious), which is the midpoint of the confidence interval. For example, a query for the *BRCA2* VUS D2723H on the AlignGVGD website yields a Prediction Grade of C65 equating to a prior probability of pathogenicity of 0.81, meaning that the variant is fully conserved from sea urchin to human, is predicted to have a substantial functional impact and has a high probability of being pathogenic (Table 2) (Tavtigian, et al., 2008). VUS located outside of the key functional domains are all attributed a grade of C0 and a lower probability of pathogenicity of 0.02. Originally the prior probabilities of these variants were estimated at 0.00 (95%CI 0.00-0.04) (Easton, et al., 2007), but for the reasons given above we have again assigned the prior probability at the midpoint of the confidence interval.

VUS located in consensus dinucleotides at exon/intron boundaries (nucleotide +1/+2 or −1/−2 relative to an exon) are attributed specific prior probabilities of 1.0 (95%CI 0.91-1.0). However, a prior probability of 0.96 reflecting the midpoint of the estimate is more commonly applied. These variants are expected to alter the consensus splice site activity and to uniformly disrupt protein function either through truncation or in-frame deletion of large regions of the encoded proteins (Easton, et al., 2007). Intronic variants outside the donor or acceptor dinucleotide have been attributed a prior probability of 0.26 (0.15–0.39 95% CI), which is the midpoint of the estimate derived from available data (Easton, et al., 2007). Efforts are ongoing to assign prior probabilities to other variants in exons and introns that may have influence on splicing. This will be somewhat dependent on standards defined by detailed laboratory based splicing studies. An added complication is that intronic and exonic variants may also influence gene expression through effects on splicing regulation that lead to partial effects on splicing of alleles. Whether partial effects on splicing have influences on cancer risk equivalent to truncating mutations remains to be determined In addition, VUS in other domains that become functionally characterized over time will be attributed higher prior probability scores.

Importantly, each probability shown in Table 2 has associated wide 95% confidence intervals. Because these confidence intervals allow for substantial variation in prior probability within each Align-GVGD grade, an IARC Working Group on VUS (Plon, et al., 2008) noted that the probabilities should not be used in isolation to predict the causality or pathogenicity of a VUS. Instead these prior probabilities should be combined with likelihood estimates to derive posterior probabilities of pathogenicity for VUS.

C. Posterior Probability model

The sequence based "prior probability" and the "combined likelihood" estimates can be used to calculate the "posterior probability" of a VUS being pathogenic. Similarly, all other independent lines of evidence that can be expressed mathematically as likelihood ratios can be integrated with the prior probability to generate a posterior probability of pathogenicity for each variant. The posterior probability on a scale of 0 to 1.00 is <u>calculated using Bayes theorem by first</u> determining the "Posterior Odds of pathogenicity" and then generating the final "Posterior probability of pathogenicity" as shown in the box below.

Posterior Odds = Likelihood ratio × [prior probability/(1-prior probability)]

Posterior Probability of Pathogenicity = Posterior Odds / (Posterior Odds + 1)

Essentially, the term prior probability/(1-prior probability) represents the initial odds ratio in favor of a given VUS being pathogenic vs. not pathogenic before any of the model components are added. For example, if the prior probability from Align-GVGD is 0.8 and the combined LR from all other sources is 100 (odds of 100:1 in favor of pathogenicity) then the Posterior odds are $100 \times 0.8/0.2 = 400:1$ in favor of the VUS being pathogenic and the Posterior Probability of causality is: $400/401 = 1.00$. Using this approach, publically available multifactorial likelihood estimates (Easton, et al., 2007) can be readily combined with the prior probabilities available through the Align-GVGD website (http://agvgd.iarc.fr/) (Tavtigian, et al.) to calculate "posterior probabilities" for *BRCA1* and *BRCA2* VUS.

Based upon the numerical value of the posterior probability calculations, The IARC Working Group on VUS introduced a clinical translation step, summarized in Table 3, involving a five-tier classification scheme (Plon, et al., 2008). Class 1 and 2 are likely not pathogenic; Class 3 remains a true VUS; Class 4 and 5 are treated as pathogenic for clinical purposes. A Clinical Working Group affiliated with the IARC group further proposed how these levels of predicted pathogenicity might be used to counsel patients about cancer surveillance and when it is reasonable to use a VUS as a marker for predictive testing in at-risk relatives. In brief, when a Class 1 or 2 variant is identified, the variant can be excluded from further consideration, but the proband and family members must be counseled on the basis of family history of cancer which could result from a pathogenic mutation in another predisposition gene. When a Class 3 variant is identified, the same approach should be taken, and the variant cannot be used for determining whether additional family members are at risk. In cases where an effect on protein function in a functional assay, or high prior probabilities based on Align-GVGD are known, counseling may lean towards a more aggressive approach to risk management. When a Class 4 or Class 5 variant is identified the individual must be considered a carrier of a fully pathogenic mutation. High-risk surveillance involving frequent screening for breast and ovarian cancer or prevention options such as prophylactic oophorectomy and/or mastectomy, while accounting for the age of the individual, should be recommended. In addition, genetic testing of all at-risk relatives for the Class 4 or 5 variant should be recommended.

Application of the Posterior Probability Model

The multifactorial model has been applied to many *BRCA1* and *BRCA2* VUS for which family and/or pathology data are available. Some of the results from these analyses can be found scattered across a large number of publications. However, it is quite challenging to screen the scientific and medical literature in order to determine whether a specific VUS has actually been classified. In addition, some results from posterior probability model analyses and/or the information needed for assessment of VUS using the posterior probability model are often accessible to only a small number of researchers actively working to classify VUS and are not available to other researchers, patients and clinical care providers.

To simplify this process we have screened the current literature for results derived from correct applications of the multifactorial likelihood or posterior probability models to VUS in the *BRCA1* and *BRCA2* genes. Tables 4 and 5 display the current lists of *BRCA1* and *BRCA2* missense variants and small insertions and deletions that have been classified as Class 1 and 2 (Not pathogenic/likely not pathogenic) or Class 4 and 5 (likely pathogenic/pathogenic) using the posterior probability model. We do not show Class 3 or VUS.

In Table 4 a total of 24 *BRCA1* missense variants considered pathogenic and 96 considered not pathogenic are shown. Similarly in Table 5, 15 *BRCA2* missense variants considered

pathogenic and 98 considered not pathogenic are presented. We also collected data on classification of intervening sequence variants (IVS) or intronic splicing variants using the posterior probability model. In Table 6, we display 20 IVS considered Class1/2 or neutral and 20 IVS considered Class 4/5 or pathogenic. The variants classified as Class 4 or 5 in Table 6 likely result in splicing aberrations. The most recent reference for the likelihood estimates for each variant is provided in the Tables since results from older manuscripts may have been updated through the availability of additional data. Where possible, modifications have been made in the likelihood estimates to correct for inaccuracies. This is particularly important when considering the use of Pathology Data. Until recently likelihood estimates included data from loss of heterozygosity (LOH) studies in tumors carrying VUS (Chenevix-Trench, et al., 2006; Spearman, et al., 2008). However, it is now appreciated that tumors containing known pathogenic mutations can exhibit loss of the mutant instead of the wildtype allele (Beristain, et al.; Hofstra, et al.; Spurdle, et al.). For these reasons, LOH data are not included in Likelihood and posterior probability model calculations.

The data are also presented in an online *BRCA1/BRCA2* variant LOVD classification database (http://brca.iarc.fr/LOVD). This database lists all missense variants that have been classified as pathogenic or likely pathogenic (Class 4/5) and not pathogenic or likely not pathogenic (Class 1/2) using the posterior probability classification model and the same nomenclature as described here. The LOVD database will be updated and expanded as additional data become available from family studies. Here we add to the data shown in the LOVD database by presenting results for intronic variants, a subset of which disrupt splicing of the *BRCA1* or *BRCA2* genes (Table 6).

Limitations of the Posterior Probability Model

It is important to realize that there are a number of assumptions incorporated into the posterior probability model. Because of this there is the possibility that a VUS can be misclassified. For example, under the prior probability model derived from evolutionary sequence comparisons, mutations in residues conserved throughout orthologs from sea urchin to human have a much greater probability of cancer causality than mutations in poorly conserved residues. Thus, mutations in amino acids that appear to be poorly conserved, but actually have been specifically selected in certain species (gain of function), may be inappropriately assigned low prior probabilities, which often results in classification as Class 3 VUS due to limiting data from the likelihood components of the model. For this reason, the IARC working group required that other data (family, pathology or functional) had to be combined with the prior probability derived from sequence analysis before a VUS could be assigned a specific Class. Likewise, the likelihood estimates based on personal and family history are currently specific to the population screened by Myriad Genetic Laboratories in the USA before 2006. Inclusion of personal and family history data from other centers or countries depend upon re-estimation of the likelihood ratios for each phenotypic category. The posterior probability model is also limited by the availability of family data. Many VUS are found in a very small number of families, which do not generate sufficient genetic information to allow these VUS to be assigned to Class 1/2 or Class 4/5. To address this problem ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles) was formed (www://enigmaconsortium.org). This worldwide consortium is focused on combining information from around the world to classify VUS. In particular the focus is on collecting information on families with specific VUS and on tumor pathology with the intent of evaluating these data and potentially classifying VUS using the posterior probability model.

Other methods for characterizing VUS

Alternative family and pathology based likelihood models

A number of other family and pathology based methods for classification of VUS are being developed. For instance other groups have developed independent likelihood-based models for evaluation of VUS identified in specific countries (Gomez Garcia, et al., 2009; Mohammadi, et al., 2009). These approaches are promising but for the moment are based on limited amounts of data. Others have used pathology data to assign pathology-based likelihood estimates. These estimates are based on very small numbers of tumors and should be applied with caution (Spearman, et al., 2008; Sweet, et al., 2010). Importantly ENIGMA is undertaking a very large study focused on re-evaluation of pathology characteristics for *BRCA1* and *BRCA2* tumors, which should lead to more robust likelihood estimates.

Functional assays

The development of functional assays for classification of *BRCA1* and *BRCA2* VUS is an area of intense activity. One approach that is focused on the BRCT domains of *BRCA1* involves measuring the transcriptional activity of the domains when containing VUS. This method has been extensively cross validated and exhibits high sensitivity and specificity (Lee, et al., 2010). Recently, a computational model was developed to derive likelihood estimates for the results of this functional assay which allow the incorporation of functional assay data into the overall multifactorial likelihood and the posterior probability models (Iversen, et al., 2011). Likewise, a DNA repair assay that measures the influence of VUS located in the *BRCA2* DNA binding domain on the homologous recombination activity of *BRCA2* has been developed (Farrugia, et al., 2008). This assays exhibits 100% sensitivity and specificity for known pathogenic and not pathogenic variants in the DNA binding domain and may be useful for classification of VUS. Another interesting approach is the development of mouse embryonic stem cells (ES) cells that are deficient in *Brca1* or *Brca2* and can be used as a host to express human *BRCA1* and *BRCA2* VUS. Using this approach cells containing functionally proficient VUS survive while cells expressing VUS that have defective function either do not survive or survive but exhibit characteristics of *BRCA1* and *BRCA2* deficient cells such as sensitivity to DNA damaging agents, chromosomal instability and centrosome amplification (Chang, et al., 2009; Kuznetsov, et al., 2008).

Each of these approaches will provide important information for the classification of *BRCA1* or *BRCA2* VUS. It is important to note that it is still unclear how different activities of *BRCA1* and *BRCA2* measured *in vitro* are related to their tumor suppressive functions *in vivo*. Thus, extensive validation relative to a panel of variants classified using the posterior probability model, as was done for the *BRCA1* transcriptional assay and the *BRCA2* DNA repair assays, is critical for establishing the clinical utility of the results obtained. Furthermore, integration of multiple functional data sources will require detailed analysis to determine whether results from different assays are independent. Interestingly, both assays have identified variants with partial functional effects. Whether the reduced activity associated with these VUS is directly correlated with intermediate risk of cancer remains to be determined.

The increased availability of data on genetic variation in human populations has been the impetus behind the development of a large field of research activity directed at assigning pathogenicity to different alleles of cancer genes. The method detailed here emerged in this context. Ultimately, models such as the ones presented here may incorporate data from large scale genome-wide association studies (GWAS) and thus be able to assess risk contribution in a continuous, rather than discrete (high versus low risk), manner. This continuum may include frequent variants with small effect, low frequency variants with moderate effect and

even rarer variants with large effects. We anticipate that the extension of this model to other genes will be gradual. The main impetus for evaluating variants in additional genes will be when variants become relevant in clinical practice, likely as results from a multi-gene predisposition panels. However, when extending to other genes, the inability of the model to account for high *de novo* mutation rates will have to be considered.

Summary

VUS will continue to be a challenge for the medical community. The multiplicity of publications focused on efforts to classify VUS highlights the lack of universally accepted reporting standards for VUS and the challenges presented by trying to establish pathogenicity or absence of pathogenicity with current methodology. Reclassification of any VUS as neutral or pathogenic will require ongoing integration of new data and modeling that incorporates multiple independent lines of evidence. Here we describe a posterior probability model that utilizes several sources of information for the purpose of VUS classification and provide Tables showing 59 pathogenic *BRCA1* and *BRCA2* variants and 214 non-pathogenic *BRCA1* and *BRCA2* variants that were classified by this method. In addition we describe ongoing efforts to classify VUS using data collected through ENIGMA and by functional assays. Systematic application of the posterior probability method is expected to increase the number of classified deleterious and neutral variants in *BRCA1* and *BRCA2* in the near future. This article was written primarily to assist health care providers in understanding the challenges and issues in dealing with VUS and to collate new information for the *BRCA1* and *BRCA2* VUS for which there is emerging evidence for or against pathogenicity. It is our hope that the lists of classified VUS may assist health care providers and patients in medical decision making when faced with a VUS finding.

Acknowledgments

Appendix

Sources of Data

Mutation nomenclature website http://www.lovd.nl/mutalyzer/

The Breast Cancer Information Core (BIC) listing of *BRCA1* and *BRCA2* mutations http://research.nhgri.nih.gov/bic/

Literature database summarizing information on UV/VUS classification http://chromium.liacs.nl/LOVD2/cancer/home.php?select_db=BRCA1

Align-GVGD sequence based analysis identifying the Classification Class of each VUS http://agvgd.iarc.fr/

Results of multifactorial analyses of unclassified variants http://brca.iarc.fr/LOVD/

Leiden Open Variant Database (LOVD) displaying classified *BRCA1* and *BRCA2* VUS http://brca.iarc.fr/LOVD

ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles) www://enigmaconsortium.org

Definitions of vocabulary

1. "deleterious" is short for "evolutionarily deleterious" and is most applicable to the output from alignment-based missense substitution prediction algorithms such as PolyPhen and Align-GVGD.

   "damaging" is short for "damages protein function" and is most applicable to the output from functional assays.

   "pathogenic" refers to disease causality.

   These three terms should NOT be used interchangeably!

2. In Table 3 from Plon et al (Human Mutation 29: 1282-1291, 2008), The following definitions are used:

   IARC Class 2 is "likely not pathogenic of little clinical significance"

   IARC Class 1 is "not pathogenic or of no clinical significance"

   Thus "not pathogenic" should be used instead of words like "neutral" or "benign" or "polymorphism".

3. (3) Uncertain vs. unclassified

   "Uncertain" implies that an analysis has been done, and the variant has a posterior probability between 5% and 95%.

   "Unclassified" is the state of a variant before any effort at classification.

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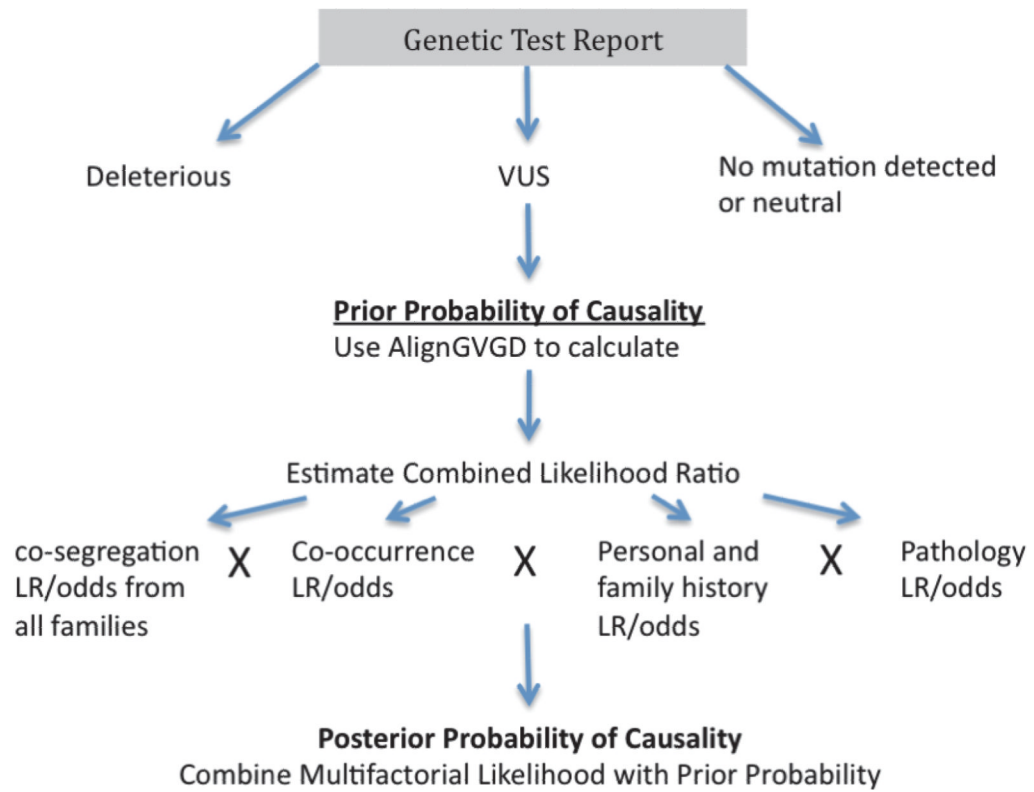
Figure 1.
Method for Determination of Posterior Probability of Causality for each VUS.

Table 1

Frequencies of pathology characteristics in breast tumors from 600 *BRCA1* carriers diagnosed under age 60 and 258 tumors from sporadic cases diagnosed under age 60

Characteristic	*BRCA1* tumors (%)	Control Tumors (%)	LR (*BRCA1* path)
ER +ve	9.6	67.2	0.14
ER +ve Grade 1	0.6	13.0	0.05
ER +ve Grade 2	3.5	25.0	0.14
ER +ve Grade 3	5.1	28.0	0.18
ER −ve, Grade 1	1.5	1.6	0.97
ER −ve, Grade 2	9.0	4.7	1.93
ER −ve, Grade 3	80.0	27.0	2.95
ER−ve, CK5/6−ve, CK14 −ve	20.9	24.0	0.87
ER−ve, CK5/6+ve, CK14 −ve	13.4	2.4	5.58
ER−ve, CK5/6−ve, CK14 +ve	12.4	4.8	2.58
ER−ve, CK5/6+ve, CK14 +ve	43.8	1.6	27.38

Table 2

Prior probabilities associated with VUS from defined functional domains[#] graded by Align-GVGD

Align-GVGD grade	Prior Probability	95% CI
C65	0.81	(0.61-0.95)
C35-C55	0.66	(0.34-0.93)
C15-C25	0.29	(0.09-0.56)
C0	0.03	(0.00-0.06)
Outside functional domains	0.02	(0.00-0.04)
Splicing consensus site alteration	0.96	(0.91-1.00)
Intronic variants outside the consensus dinucleotides	0.26	(0.15-0.39)

[#]*BRCA1* BARD1 binding (amino acids 1-102) and BRCT domains and coiled coil domain (amino acids 1396-1863), *BRCA2* DNA binding domain (amino acids 2400-3190). CI: Confidence Interval

Table 3

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

Class	Definition	Posterior Probability	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

Adapted from Plon et al., 2008.

Table 4

***BRCA1* VUS Classified as Class 1, 2 or Class 4, 5**

BRCA1 exon	Codon	HGVS: protein level	BIC: DNA level	HGVS: DNA level	Odds in favor of causality	Reference	Prior Probability of being deleterious	Posterior Probability of being deleterious	IARC Class
2	M18T	p.Met18Thr	172T>C	c.53T>C	31.61	Easton et al., 2007	0.66	0.98	4
2	L22S	p.Leu22Ser	184T>C	c.65T>C	38.88	Sweet et al., 2010	0.81	0.99	5
3	T37K	p.Thr37K	229C>A	c.110C>A	634.47	Sweet et al., 2010	0.81	1.00	5
3	C39R	p.Cys39Arg	234T>C	c.115T>C	34.60	Sweet et al., 2010	0.81	0.99	5
3	C44S	p.Cys44Ser	249T>A	c.130T>A	149.60	Sweet et al., 2010	0.81	1.00	5
3	C44Y	p.Cys44Tyr	250G>A	c.131G>A	83.48	Sweet et al., 2010	0.81	1.00	5
3	K45Q	p.Lys45Gln	252A>C	c.133A>C	0.03	Sweet et al., 2010	0.03	8.00×10^{-4}	1
5	C61G	p.Cys61Gly	300T>G	c.181T>G	1285.43	Spearman et al., 2008	0.81	1.00	5
5	D67Y	p.Asp67Tyr	318G>T	c.199G>T	9.38×10^{-5}	Easton et al., 2007	0.03	2.90×10^{-6}	1
7	Y105C	p.Tyr105Cys	433A>G	c.314A>G	5.15×10^{-6}	Easton et al., 2007	0.02	1.05×10^{-7}	1
7	I124V	p.Ile124Val	489A>G	c.370A>G	6.80×10^{-3}	Easton et al., 2007	0.02	1.39×10^{-4}	1
7	N132K	p.Asn132Lys	515C>A	c.396C>A	8.13×10^{-3}	Easton et al., 2007	0.02	1.66×10^{-4}	1
7	P142H	p.Pro142His	544C>A	c.425C>A	5.04×10^{-5}	Chenevix-Trench et al., 2006	0.02	1.03×10^{-6}	1
7	E143K	p.Glu143Lys	546G>A	c.427G>A	5.35×10^{-4}	Easton et al., 2007	0.02	1.09×10^{-5}	1
8	Q155E	p.Gln155Glu	582C>G	c.463C>G	1.91×10^{-3}	Easton et al., 2007	0.02	3.89×10^{-5}	1
8	Y179C	p.Tyr179Cys	655A>G	c.536A>G	8.50×10^{-11}	Spurdle et al., 2008	0.02	1.73×10^{-12}	1
9	S186Y	p.Ser186Tyr	676C>A	c.557C>A	3.83×10^{-10}	Easton et al., 2007	0.02	7.82×10^{-12}	1
9	V191I	p.Val191Ile	690G>A	c.571G>A	7.69×10^{-6}	Easton et al., 2007	0.02	1.57×10^{-7}	1
11	L246V	p.Leu246Val	855T>G	c.736T>G	2.60×10^{-5}	Tavtigian et al., 2006	0.02	5.31×10^{-7}	1
11	A280G	p.Ala280Gly	958C>G	c.839C>G	3.76×10^{-3}	Easton et al., 2007	0.02	7.67×10^{-5}	1
11	M297I	p.Met297Ile	1010G>A	c.891G>A	3.11×10^{-3}	Easton et al., 2007	0.02	6.34×10^{-5}	1
11	P334H	p.Pro334His	1120C>A	c.1001C>A	1.34	Spearman et al., 2008	0.02	0.02	2
11	P334L	p.Pro334Leu	1120C>T	c.1001C>T	1.33×10^{-5}	Easton et al., 2007	0.02	2.71×10^{-7}	1
11	Q356R	p.Gln34Arg	1186A>G	c.1067A>G	$<1.00 \times 10^{-10}$	Tavtigian et al., 2006	0.02	$<2.04 \times 10^{-12}$	1
11	D369del	p.Asp369del	1225del3	c.1105_1107del	3.00×10^{-4}	Easton et al., 2008	0.02	6.13×10^{-6}	1

BRCA1 exon	Codon	HGVS: protein level	BIC: DNA level	HGVS: DNA level	Odds in favor of causality	Reference	Prior Probability of being deleterious	Posterior Probability of being deleterious	IARC Class
11	D369N	p.Asp369Asn	1224G>A	c.1105G>A	2.78×10^{-4}	Easton et al., 2007	0.02	5.68×10^{-6}	1
11	D420Y	p.Asp420Tyr	1377G>T	c.1258G>T	0.01	Easton et al., 2007	0.02	1.66×10^{-4}	1
11	N473S	p.Asn473Ser	1537A>G	c.1418A>G	1.65×10^{-4}	Easton et al., 2007	0.02	3.37×10^{-6}	1
11	F486L	p.Phe486Leu	1575T>C	c.1456T>C	9.13×10^{-11}	Spurdle et al., 2008	0.02	1.86×10^{-12}	1
11	R496H	p.Arg496His	1606G>A	c.1487G>A	9.20×10^{-5}	Tavtigian et al., 2006	0.02	1.88×10^{-6}	1
11	R496C	p.Arg496Cys	1605C>T	c.1486C>T	0.04	Chenevix-Trench et al., 2006	0.02	8.91×10^{-4}	1
11	R504H	p.Arg504His	1630G>A	c.1511G>A	5.21×10^{-5}	Easton et al., 2007	0.02	1.06×10^{-6}	1
11	N550H	p.Asn550His	1767A>C	c.1648A>C	8.00×10^{-11}	Spurdle et al., 2008	0.02	1.63×10^{-12}	1
11	E597K	p.Glu597Lys	1908G>A	c.1789G>A	3.40×10^{-9}	Easton et al., 2007	0.02	6.92×10^{-11}	1
11	A622V	p.Ala622Val	1984C>T	c.1865C>T	1.00×10^{-3}	Easton et al., 2007	0.02	2.12×10^{-5}	1
11	D642H	p.Asp642His	2043G>C	c.1924G>C	6.87×10^{-4}	Easton et al., 2007	0.02	1.40×10^{-5}	1
11	K654Q	p.Lys654Gln	2079A>C	c.1960A>C	0.34	Osorio et al., 2007	0.02	6.86×10^{-3}	2
11	L668F	p.Leu668Phe	2121C>T	c.2002C>T	9.52×10^{-3}	Easton et al., 2007	0.02	1.94×10^{-4}	1
11	D693N	p.Asp693Asn	2196G>A	c.2077G>A	1.00×10^{-10}	Tavtigian et al., 2006	0.02	2.04×10^{-12}	1
11	N723D	p.Asn723Asp	2286A>G	c.2167A>G	1.55×10^{-8}	Easton et al., 2007	0.02	3.17×10^{-10}	1
11	E736A	p.Glu736Ala	2326A>C	c.2207A>C	0.48	Spearman et al., 2008	0.02	9.62×10^{-3}	2
11	V772A	p.Val772Ala	2434T>C	c.2315T>C	9.40×10^{-6}	Tavtigian et al., 2006	0.02	1.92×10^{-7}	1
11	Q804H	p.Gln804His	2531G>C	c.2412G>C	0.02	Chenevix-Trench et al., 2006	0.02	4.46×10^{-4}	1
11	N810Y	p.Asn810Tyr	2547A>T	c.2428A>T	1.14×10^{-11}	Easton et al., 2007	0.02	2.33×10^{-13}	1
11	K820E	p.Lys820Gln	2577A>G	c.2458A>G	$<1.00 \times 10^{-10}$	Tavtigian et al., 2006	0.02	$<2.04 \times 10^{-12}$	1
11	T826K	p.Thr826Lys	2596C>A	c.2477C>A	9.97×10^{-8}	Spurdle et al., 2008	0.02	2.03×10^{-9}	1
11	R841W	p.Arg841Trp	2640C>T	c.2521C>T	1.12×10^{-10}	Goldgar et al., 2004	0.02	2.29×10^{-12}	1
11	E842G	p.Glu842Gly	2644A>G	c.2525A>G	2.11×10^{-4}	Easton et al., 2007	0.02	4.30×10^{-6}	1
11	Y856H	p.Tyr856His	2685T>C	c.2566T>C	8.80×10^{-3}	Tavtigian et al., 2006	0.02	1.80×10^{-4}	1
11	K862E	p.Lys862Glu	2703A>G	c.2584A>G	4.86×10^{-4}	Easton et al., 2007	0.02	9.91×10^{-6}	1
11	R866C	p.Arg866Cys	2715C>T	c.2596C>T	8.00×10^{-13}	Easton et al., 2007	0.02	1.63×10^{-14}	1
11	P871L	p.Pro871Leu	2731C>T	c.2612C>T	$<1.00 \times 10^{-10}$	Tavtigian et al., 2006	0.02	$<2.04 \times 10^{-12}$	1

BRCA1 exon	Codon	HGVS: protein level	BIC: DNA level	HGVS: DNA level	Odds in favor of causality	Reference	Prior Probability of being deleterious	Posterior Probability of being deleterious	IARC Class
11	G890V	p.Gly890Val	2788G>T	c.2669G>T	2.00×10^{-4}	Easton et al., 2007	0.02	4.08×10^{-6}	1
11	V920A	p.Val920Ala	2878T>C	c.2759T>C	1.02	Spurdle et al., 2008	0.02	0.02	2
11	I925L	p.Ile925Leu	2892A>C	c.2773A>C	1.6×10^{-4}	Easton et al., 2007	0.02	3.26×10^{-6}	1
11	M1008I	p.Met1008Ile	3143G>A	c.3024G>A	9.6×10^{-7}	Tavtigian et al., 2006	0.02	1.96×10^{-8}	1
11	M1008V	p.Met1008Val	3141A>G	c.3022A>G	$<1.00\times10^{-10}$	Tavtigian et al., 2006	0.02	$<2.04\times10^{-12}$	1
11	R1028H	p.Arg1028His	3202G>A	c.3083G>A	4.90×10^{-4}	Easton et al., 2007	0.02	9.98×10^{-6}	1
11	E1038G	p.Gln1038Gly	3232A>G	c.3113A>G	$<1.00\times10^{-10}$	Tavtigian et al., 2006	0.02	$<2.04\times10^{-12}$	1
11	S1040N	p.Ser1040Asn	3238G>A	c.3119G>A	$<1.00\times10^{-10}$	Tavtigian et al., 2006	0.02	$<2.04\times10^{-12}$	1
11	I1044V	p.Ile1044Val	3249A>G	c.3130A>G	1.15×10^{-4}	Easton et al., 2007	0.02	2.34×10^{-6}	1
11	P1099L	p.Pro1099Leu	3415C>T	c.3296C>T	1.52×10^{-9}	Spurdle et al., 2008	0.02	3.09×10^{-11}	1
11	S1101N	p.Ser1101Asn	3421G>A	c.3302G>A	3.10×10^{-7}	Easton et al., 2007	0.02	6.32×10^{-9}	1
11	K1109N	p.Lys1109Asn	3446A>C	c.3327A>C	1.51×10^{-3}	Easton et al., 2007	0.02	3.07×10^{-5}	1
11	S1140G	p.Ser1140Gly	3537A>G	c.3418A>G	2.90×10^{-4}	Tavtigian et al., 2006	0.02	5.92×10^{-6}	1
11	D1155H	p.Asp1155His	3582G>C	c.3463G>C	3.50×10^{-4}	Chenevix-Trench et al., 2006	0.02	7.14×10^{-6}	1
11	K1183R	p.Lys1183Arg	3667A>G	c.3548A>G	$<1.00\times10^{-10}$	Tavtigian et al., 2006	0.02	$<2.04\times10^{-12}$	1
11	Q1200H	p.Gln1200His	3719G>C	c.3600G>C	0.35	Osorio et al., 2007	0.02	7.09×10^{-3}	2
11	R1203Q	p.Arg1203Gln	3727G>A	c.3608G>A	1.79×10^{-4}	Easton et al., 2007	0.02	3.65×10^{-6}	1
11	E1214K	p.Glu1214Lys	3759G>A	c.3640G>A	3.38×10^{-8}	Easton et al., 2007	0.02	6.89×10^{-10}	1
11	P1238L	p.Pro1238Leu	3832C>T	c.3713C>T	9.52×10^{-7}	Easton et al., 2007	0.02	1.94×10^{-8}	1
11	V1247I	p.Val1247Ile	3858G>A	c.3739G>A	1.23×10^{-4}	Easton et al., 2007	0.02	2.51×10^{-6}	1
11	E1250K	p.Glu1250Lys	3867G>A	c.3748G>A	1.00×10^{-5}	Tavtigian et al., 2006	0.02	2.04×10^{-7}	1
11	S1266T	p.Ser1266Thr	3916G>C	c.3797G>C	5.56×10^{-5}	Easton et al., 2007	0.02	1.13×10^{-6}	1
11	I1275V	p.Ile1275Val	3942A>G	c.3823A>G	1.62×10^{-5}	Easton et al., 2007	0.02	3.31×10^{-7}	1
11	R1347G	p.Arg1347Gly	4158A>G	c.4039A>G	$<1.00\times10^{-10}$	Tavtigian et al., 2006	0.02	$<2.04\times10^{-12}$	1
11	T1349M	p.Thr1349Met	4165C>T	c.4046C>T	2.16×10^{-3}	Easton et al., 2007	0.02	4.42×10^{-5}	1
11	M1361L	p.Met1361Leu	4200A>C	c.4081A>C	9.62×10^{-3}	Easton et al., 2007	0.02	1.96×10^{-4}	1
13	H1402Y	p.His1402Tyr	4323C>T	c.4204C>T	6.06×10^{-3}	Easton et al., 2007	0.03	1.87×10^{-4}	1

BRCA1 exon	Codon	HGVS: protein level	BIC: DNA level	HGVS: DNA level	Odds in favor of causality	Reference	Prior Probability of being deleterious	Posterior Probability of being deleterious	IARC Class
13	E1419Q	p.Glu1419Gln	4374G>C	c.4255G>C	4.93×10^{-3}	Easton et al., 2007	0.03	1.52×10^{-4}	1
13	R1443G	p.Arg1443Gly	4446C>G	c.4327C>G	<0.02	Carvalho et al., 2007	0.03	$<6.18\times10^{-4}$	1
14	N1468H	p.Asn1468His	4521A>C	c.4402A>C	2.97×10^{-3}	Easton et al., 2007	0.03	9.18×10^{-5}	1
14	#R1495M	p.Arg1495Met	4603G>T	c.4484G>T	1.25×10^{6}	Easton et al., 2007	0.81	1.00	1
15	S1512I	p.Ser1512Ile	4654G>T	c.4535G>T	$<1.00\times10^{-10}$	Tavtigian et al., 2006	0.03	$<3.09\times10^{-12}$	1
15	V1534M	p.Val1534Met	4719G>A	c.4600G>A	2.39×10^{-9}	Easton et al., 2007	0.03	7.40×10^{-11}	1
15	D1546N	p.Asp1546Asn	4755G>A	c.4636G>A	5.60×10^{-3}	Tavtigian et al., 2006	0.03	1.73×10^{-4}	1
15	D1546Y	p.Asp1546Tyr	4755G>T	c.4636G>T	9.19×10^{-4}	Easton et al., 2007	0.03	2.84×10^{-5}	1
16	L1564P	p.Leu1564Pro	4810T>C	c.4691T>C	3.30×10^{-3}	Tavtigian et al., 2006	0.03	1.02×10^{-4}	1
16	S1613G	p.Ser1613Gly	4956A>G	c.4837A>G	$<1.00\times10^{-10}$	Tavtigian et al., 2006	0.03	$<3.09\times10^{-12}$	1
16	P1614S	p.Pro1614Ser	4959C>T	c.4840C>T	2.27×10^{-9}	Easton et al., 2007	0.03	7.03×10^{-11}	1
16	M1628T	p.Met1628Thr	5002T>C	c.4883T>C	1.60×10^{-4}	Tavtigian et al., 2006	0.03	4.95×10^{-6}	1
16	P1637L	p.Pro1637Leu	5029C>T	c.4910C>T	2.03×10^{-3}	Easton et al., 2007	0.03	6.27×10^{-5}	1
16	M1652I	p.Met1652Ile	5075G>A	c.4956G>A	4.80×10^{-4}	Osorio A et al., 2007	0.03	1.48×10^{-5}	1
16	M1652T	p.Met1652Thr	5074T>C	c.4955T>C	2.68×10^{-4}	Easton et al., 2007	0.29	1.10×10^{-4}	1
16	F1662S	p.Phe1662Ser	5104T>C	c.4985T>C	5.21×10^{-3}	Easton et al., 2007	0.03	1.61×10^{-4}	1
17	E1682K	p.Glu1682Lys	5163G>A	c.5044G>A	3.15×10^{-3}	Easton et al., 2007	0.03	9.76×10^{-5}	1
17	T1685A	p.Thr1685Ala	5172A>G	c.5053A>G	107.00	Easton et al., 2007	0.66	1.00	5
17	T1685I	p.Thr1685Ile	5173C>T	c.5054C>T	140.00	Easton et al., 2007	0.81	1.00	5
17	V1688del	p.Val1688del	5181del3	c.5181_5183delGTT	268.00	Easton et al., 2007	0.81	1.00	5
17	M1689R	p.Met1689Arg	5185T>G	c.5066T>G	46.00	Easton et al., 2007	0.66	0.989	4
18	R1699Q	p.Arg1699Gln	5215G>A	c.5096G>A	142.00	Chenevix-Trench et al., 2006	0.66	1.00	5
18	R1699W	p.Arg1699Trp	5214C>T	c.5095C>T	4.00×10^{4}	Easton et al., 2007	0.81	1.00	5
18	G1706A	p.Gly1706Ala	5236G>C	c.5117G>C	2.55×10^{-5}	Lovelock et al., 2006	0.66	4.95×10^{-5}	1
18	G1706E	p.Gly1706Glu	5236G>A	c.5117G>A	589.00	Easton et al., 2007	0.81	1.00	5
18	A1708E	p.Ala1708Glu	5242C>A	c.5123C>A	2.11×10^{12}	Easton et al., 2007	0.81	1.00	5
18	S1715R	p.Ser1715Arg	5262A>C	c.5143A>C	45.00	Easton et al., 2007	0.81	0.99	5
19	T1720A	p.Thr1720Ala	5277A>G	c.5158A>G	1.11×10^{-11}	Easton et al., 2007	0.03	3.44×10^{-13}	1

BRCA1 exon	Codon	HGVS: protein level	BIC: DNA level	HGVS: DNA level	Odds in favor of causality	Reference	Prior Probability of being deleterious	Posterior Probability of being deleterious	IARC Class
20	G1738R	p.Gly1738Arg	5533G>A	c.5212G>A	114.00	Easton et al., 2007	0.81	1.00	5
20	R1751Q	p.Arg1751Gln	5371G>A	c.5252G>A	6.67×10^{-3}	Easton et al., 2007	0.03	2.06×10^{-4}	1
21	L1764P	p.Leu1764Pro	5410T>C	c.5291T>C	350.00	Easton et al., 2007	0.29	0.99	5
21	I1766S	p.Ile1766Ser	5416T>G	c.5297T>G	139.00	Easton et al., 2007	0.66	1.00	5
21	M1775K	p.Met1775 Lys	5443T>A	c.5324T>A	235.00	Tischkowitz et al., 2008	0.66	1.00	5
21	M1775R	p.Met1775Arg	5443T>G	c.5324T>G	>1000	Carvalho et al., 2007	0.66	1.00	5
21	P1776H	p.Pro1776His	5446C>A	c.5327C>A	1.04	Spearman et al., 2008	0.03	0.03	2
22	C1787S	p.Cys1787Ser	5478T>A	c.5359T>A	2032.80	Goldgar et al., 2004	0.81	1.00	5
22	G1788V	p.Gly1788Val	5482G>T	c.5363G>T	7054.00	Easton et al., 2007	0.81	1.00	5
23	V1804D	p.Val1804Asp	5530T>A	c.5411T>A	1.21×10^{-3}	Easton et al., 2007	0.03	3.74×10^{-5}	1
24	V1838E	p.Val1838Glu	5632T>A	c.5513T>A	3973.97	Spurdle et al., 2008	0.66	1.00	5
24	I1858L	p.Ile1858Leu	5691A>C	c.5572A>C	2.25×10^{-3}	Easton et al., 2007	0.03	6.97×10^{-5}	1
24	P1859R	p.Pro1859Arg	5695C>G	c.5576C>G	3.35×10^{-5}	Easton et al., 2007	0.03	1.04×10^{-6}	1

VUS shown to result in aberrant gene splicing

GenBank Reference BRCA1 U14680.1

Nucleotide numbering in "HGVS: DNA level" reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

Nucleotide numbering in "BIC-DNA level" refers to the original nomenclature for BRCA1 and BRCA2 before adoption of HGVS standards where BRCA1 +119 and BRCA2 +228 correspond to the A of the ATG translation initiation codons in the reference sequence.

Table 5

BRCA2 VUS Classified as Class 1, 2 or Class 4, 5

BRCA2 exon	Codon	HGVS: protein level	BIC: DNA level	HGVS: DNA level	Odds in favor of causality	Reference	Prior Probability of being deleterious	Posterior Probability of being deleterious	IARC Class
2	R18H	p.Arg18His	281G>A	c.53G>A	0.01	Spurdle et al., 2008	0.03	3.50×10^{-4}	1
3	Y42C	p.Tyr42Cys	353A>G	c.125A>G	5.96×10^{-17}	Goldgar et al., 2004	0.02	1.22×10^{-18}	1
3	N56T	p.Asn56Thr	395A>C	c.167A>C	1.50×10^{-4}	Easton et al., 2007	0.02	3.06×10^{-6}	1
3	A75P	p.Ala75Pro	451G>C	c.223G>C	4.55×10^{-7}	Easton et al., 2007	0.02	9.28×10^{-9}	1
6	P168T	p.Pro168Thr	730C>A	c.502C>A	1.55×10^{-3}	Easton et al., 2007	0.02	3.16×10^{-5}	1
10	N319T	p.Asn319Thr	1184A>C	c.956A>C	0.02	Farrugia et al., 2008	0.02	4.01×10^{-4}	1
10	S326R	p.Ser326Arg	1206C>A	c.978C>A	0.03	Spearman et al., 2008	0.02	5.83×10^{-4}	1
10	N372H	p.Asn372His	1342C>A	c.1114C>A	1.00×10^{-7}	Farrugia et al., 2008	0.02	2.04×10^{-9}	1
10	P375S	p.Pro375Ser	1351C>T	c.1123C>T	3.17×10^{-3}	Easton et al., 2007	0.02	6.48×10^{-5}	1
10	S384F	p.Ser384Phe	1379C>T	c.1151C>T	2.08×10^{-2}	Chenevix-Trench et al., 2006	0.02	4.24×10^{-4}	1
10	E462G	p.Glu462Gly	1613A>G	c.1385A>G	1.44×10^{-4}	Easton et al., 2007	0.02	2.93×10^{-6}	1
10	A487E	p.Ala487Glu	1688C>A	c.1440C>A	0.17	*This manuscript*	0.02	3.52×10^{-3}	2
10	K513R	p.Lys513Arg	1766A>G	c.1538A>G	9.26×10^{-3}	Easton et al., 2007	0.02	1.89×10^{-4}	1
10	N517S	p.Asn517Ser	1778A>G	c.1550A>G	1.28	Spearman et al., 2008	0.02	2.54×10^{-2}	2
10	C554W	p.Cys554Trp	1890T>G	c.1662T>G	1.94×10^{-5}	Easton et al., 2007	0.02	3.96×10^{-7}	1
10	T582P	p.Thr582Pro	1972A>C	c.1744A>C	1.10×10^{-3}	Easton et al., 2007	0.02	2.24×10^{-5}	1
10	N588D	p.Asn588Asp	1990A>G	c.1762A>G	1.52	Spearman et al., 2008	0.02	3.00×10^{-2}	2
10	G602R	p.Gly602Arg	2032G>A	c.1804G>A	4.95×10^{-5}	Easton et al., 2007	0.02	1.01×10^{-7}	1
10	K607T	p.Lys607Thr	2048A>C	c.1820A>C	0.26	Spurdle et al., 2008	0.02	5.28×10^{-3}	2
10	T630I	p.Thr630Ile	2117C>T	c.1889C>T	5.95×10^{-6}	Easton et al., 2007	0.02	1.21×10^{-7}	1
11	P655R	p.Pro655Arg	2192C>G	c.1964C>G	3.36×10^{-3}	Goldgar et al., 2004	0.02	6.86×10^{-5}	1
11	D806H	p.Asp806His	2644G>C	c.2416G>C	3.92×10^{-3}	Easton et al., 2007	0.02	8.00×10^{-5}	1
11	V894I	p.Val894Ile	2908G>A	c.2680G>A	4.10×10^{-4}	Easton et al., 2007	0.02	8.36×10^{-6}	1
11	L929S	p.Leu929Ser	3014T>C	c.2786T>C	3.63×10^{-3}	Spearman et al., 2008	0.02	7.41×10^{-5}	1
11	D935N	p.Asp935Asn	3031G>A	c.2803G>A	2.59×10^{-5}	Spurdle et al., 2008	0.02	5.29×10^{-7}	1

BRCA2 exon	Codon	HGVS: protein level	BIC: DNA level	HGVS: DNA level	Odds in favor of causality	Reference	Prior Probability of being deleterious	Posterior Probability of being deleterious	IARC Class
11	N987I	p.Asn987Ile	3188A>T	c.2960A>T	3.63×10^{-3}	Spearman et al., 2008	0.02	7.41×10^{-5}	1
11	L1019V	p.Leu1019Val	3283C>G	c.3055C>G	3.50×10^{-7}	Easton et al., 2007	0.02	7.14×10^{-9}	1
11	N1102Y	p.Asn1102Tyr	3532A>T	c.3304A>T	4.00×10^{-3}	Easton et al., 2007	0.02	8.30×10^{-5}	1
11	S1172L	p.Ser1172Leu	3743C>T	c.3515C>T	1.54×10^{-5}	Easton et al., 2007	0.02	3.15×10^{-7}	1
11	R1190W	p.Arg1190Trp	3796C>T	c.3568C>T	5.32×10^{-6}	Easton et al., 2007	0.02	1.09×10^{-8}	1
11	G1194D	p.Gly1194Asp	3809G>A	c.3581G>A	3.16×10^{-3}	Easton et al., 2007	0.02	6.46×10^{-5}	1
11	N1228D	p.Asn1228Asp	3910A>G	c.3682A>G	2.68×10^{-4}	Easton et al., 2007	0.02	5.48×10^{-6}	1
11	C1265S	p.Cys1265Ser	4021T>A	c.3793T>A	0.04	Chenevix-Trench et al., 2006	0.02	8.81×10^{-4}	1
11	D1280V	p.Asp1280Val	4067A>T	c.3839A>T	1.40×10^{-3}	Easton et al., 2007	0.02	2.86×10^{-5}	1
11	V1306I	p.Val1306Ile	4144G>A	c.3916G>A	3.36×10^{-4}	Easton et al., 2007	0.02	6.85×10^{-6}	1
11	I1349T	p.Ile1349Thr	4274T>C	c.4046T>C	4.31×10^{-4}	Easton et al., 2007	0.02	8.80×10^{-6}	1
11	D1352Y	p.Asp1352Tyr	4282G>T	c.4054G>T	2.12	Spearman et al., 2008	0.02	4.14×10^{-2}	2
11	T1354M	p.Thr1354Met	4289C>T	c.4061C>T	3.94×10^{-3}	Easton et al., 2007	0.02	8.03×10^{-5}	1
11	C1365Y	p.Cys1365Tyr	4322G>A	c.4094G>A	6.67×10^{-3}	Easton et al., 2007	0.02	1.36×10^{-4}	1
11	Q1396R	p.Gln1396Arg	4415A>G	c.4187A>G	4.39×10^{-4}	Easton et al., 2007	0.02	8.95×10^{-6}	1
11	D1420Y	p.Asp1420Tyr	4486G>T	c.4258G>T	0.01	Spearman et al., 2008	0.02	1.02×10^{-4}	1
11	K1434I	p.Lys1434Ile	4529A>T	c.4301A>T	2.00	Spearman et al., 2008	0.02	4.05×10^{-2}	2
11	F1524V	p.Phe1524Val	4798T>G	c.4570T>G	1.02×10^{-3}	Easton et al., 2007	0.02	2.08×10^{-5}	1
11	G1529R	p.Gly1529Arg	4813G>A	c.4585G>A	8.13×10^{-9}	Easton et al., 2007	0.02	1.66×10^{-10}	1
11	K1690N	p.Lys1690Asn	5298A>C	c.5070A>C	9.52×10^{-6}	Easton et al., 2007	0.02	1.94×10^{-7}	1
11	S1733F	p.Ser1733Phe	5426C>T	c.5198C>T	7.52×10^{-9}	Easton et al., 2007	0.02	1.53×10^{-10}	1
11	S1760A	p.Ser1760Ala	5506T>G	c.5278T>G	0.60	Spurdle et al., 2008	0.02	1.21×10^{-2}	2
11	G1771D	p.Gly1771Asp	5540G>A	c.5312G>A	7.52×10^{-6}	Easton et al., 2007	0.02	1.53×10^{-7}	1
11	P1819S	p.Pro1819Ser	5683C>T	c.5455C>T	5.88×10^{-6}	Easton et al., 2007	0.02	1.20×10^{-7}	1
11	L1904V	p.Leu1904Val	5938C>G	c.5710C>G	1.02×10^{-3}	Easton et al., 2007	0.02	2.09×10^{-5}	1
11	H1918Y	p.His1918Tyr	5980C>T	c.5752C>T	2.82×10^{-4}	Easton et al., 2007	0.02	5.75×10^{-6}	1
11	I1929V	p.Ile1929Val	6013A>G	c.5785A>G	2.55×10^{-4}	Easton et al., 2007	0.02	5.21×10^{-6}	1
11	R2034C	p.Arg2034Cys	6328C>T	c.6100C>T	2.80×10^{-4}	Spearman et al., 2008	0.02	5.71×10^{-6}	1

BRCA2 exon	Codon	HGVS: protein level	BIC: DNA level	HGVS: DNA level	Odds in favor of causality	Reference	Prior Probability of being deleterious	Posterior Probability of being deleterious	IARC Class
11	N2048I	p.Asn2048Ile	6371A>T	c.6143A>T	1.56×10^{-3}	Easton et al., 2007	0.02	3.19×10^{-5}	1
11	H2074N	p.His2074Asn	6448C>A	c.6220C>A	1.54×10^{-4}	Easton et al., 2007	0.02	3.13×10^{-6}	1
11	R2108H	p.Arg2108His	6551G>A	c.6323G>A	1.82×10^{-11}	Easton et al., 2007	0.02	3.72×10^{-13}	1
11	N2113S	p.Asn2113Ser	6566A>G	c.6338A>G	5.72×10^{-4}	Easton et al., 2007	0.02	1.17×10^{-11}	1
11	T2250A	p.Thr2250Ala	6976A>G	c.6748A>G	9.95×10^{-4}	Easton et al., 2007	0.02	2.03×10^{-5}	1
11	G2274V	p.Gly2274Val	7049G>T	c.6821G>T	0.60	Chenevix-Trench et al., 2006	0.02	1.17×10^{-2}	2
12	I2285V	p.Ile2285Val	7081A>G	c.6853A>G	1.27×10^{-8}	Easton et al., 2007	0.02	2.60×10^{-10}	1
12	D2312V	p.Asp2312Val	7163A>T	c.6935A>T	2.66×10^{-5}	Easton et al., 2007	0.02	5.43×10^{-7}	1
13	R2318Q	p.Arg2318Gln	7181G>A	c.6953G>A	0.13	Chenevix-Trench et al., 2006	0.02	2.65×10^{-3}	2
14	A2351G	p.Ala2351Gly	7280C>G	c.7052C>G	1.38	Spearman et al., 2008	0.02	2.74×10^{-2}	2
14	G2353R	p.Gly2353Arg	7285G>C	c.7513G>C	0.03	*This manuscript*	0.02	6.12×10^{-4}	1
14	Q2384K	p.Gln2384Lys	7378C>A	c.7150C>A	1.64×10^{-5}	Easton et al., 2007	0.02	3.34×10^{-7}	1
14	L2396F	p.Leu2396Phe	7416G>T	c.7150C>A	9.00×10^{-3}	Easton et al., 2007	0.02	1.84×10^{-4}	1
14	K2411T	p.Lys2411Thr	7460A>C	c.7232A>C	8.40×10^{-3}	Easton et al., 2007	0.03	2.60×10^{-4}	1
14	R2418G	p.Arg2418Gly	7480A>G	c.7252A>G	1.13	Spearman et al., 2008	0.03	3.38×10^{-2}	2
14	N2436I	p.Asn2436Ile	7535A>T	c.7307A>T	7.60×10^{-3}	Easton et al., 2007	0.03	2.34×10^{-4}	1
14	K2446E	p.Lys2446Glu	7564A>G	c.7336A>G	1.27	Walker et al., 2010	0.03	3.78×10^{-2}	2
14	K2472T	p.Lys2472Thr	7643A>C	c.7415A>C	5.26×10^{-6}	Spurdle et al., 2008	0.03	1.63×10^{-7}	1
15	T2515I	p.Thr2515Ile	7772C>T	c.7544C>T	2.08×10^{-3}	Spurdle et al., 2008	0.03	6.43×10^{-5}	1
16	P2589H	p.Pro2589His	7994C>A	c.7766C>A	1.48	*This manuscript*	0.03	0.04	2
17	G2609D	p.Gly2609Asp	8054G>A	c.7826G>A	4.12	*This manuscript*	0.81	0.95	4
17	W2626C	p.Trp2626Cys	8106G>C	c.7878G>C	48.14	Easton et al., 2007	0.81	1.00	5
17	I2627F	p.Ile2627Phe	8107A>T	c.7879A>T	1046.43	Easton et al., 2007	0.29	1.00	5
17	L2647P	p.Leu2647Pro	8168T>C	c.7940T>C	4.58	Farrugia et al., 2008	0.81	0.95	4
17	L2653P	p.Leu2653Pro	8186T>C	c.7958T>C	24.06	Easton et al., 2007	0.81	0.99	5
17	#R2659K	p.Arg2659Lys	8204G>A	c.7976G>A	3311.30	Farrugia et al., 2008	0.29	1.00	5
18	#E2663V	p.Glu2663Val	8216A>T	c.7988A>T	233.00	Easton et al., 2007	0.81	1.00	5
18	D2665G	p.Asp2665Gly	8222A>G	c.7994A>G	3.40×10^{-4}	*This manuscript*	0.81	1.45×10^{-3}	2

BRCA2 exon	Codon	HGVS: protein level	BIC: DNA level	HGVS: DNA level	Odds in favor of causality	Reference	Prior Probability of being deleterious	Posterior Probability of being deleterious	IARC Class
18	M2676T	p.Met2676Thr	8255T>C	c.8027T>C	0.96	*This manuscript*	0.03	0.03	2
18	L2688P	p.Leu2686Pro	8285T>C	c.8057T>C	4.00	*This manuscript*	0.81	0.95	4
18	A2717S	p.Ala2717Ser	8377G>T	c.8149G>T	1.60×10^{-4}	Spurdle et al., 2008	0.03	4.95×10^{-6}	1
18	T2722R	p.Thr2722Arg	8393C>G	c.8165C>G	93.50	Farrugia et al., 2008	0.81	1.00	5
18	D2723H	p.Asp2723His	8395G>C	c.8167G>C	5.62×10^{11}	Farrugia et al., 2008	0.81	1.00	5
18	#D2723G	p.Asp2723Gly	8396A>G	c.8168A>G	31.31	Walker et al., 2010	0.81	0.99	5
18	K2729N	p.Lys2729Asn	8415G>T	c.8187G>T	1.64×10^{-3}	Farrugia et al., 2008	0.03	5.04×10^{-5}	1
18	G2748D	p.Gly2748Asp	8471G>A	c.8243G>A	2493.78	Easton et al., 2007	0.81	1.00	5
18	A2770T	p.Ala2770Thr	8536G>A	c.8308G>A	0.49	Chenevix-Trench et al., 2006	0.03	0.01	2
19	R2787H	p.Arg2787His	8588G>A	c.8360G>A	1.31	*This manuscript*	0.03	0.04	2
20	R2842H	p.Arg2842His	8753G>A	c.8525G>A	6.35×10^{-4}	Easton et al., 2007	0.29	2.59×10^{-4}	1
20	E2856A	p.Glu2856Ala	8795A>C	c.8567A>C	3.89×10^{-11}	*This manuscript*	0.03	1.20×10^{-12}	1
20	Q2858R	p.Gln2858Arg	8801A>G	c.8573A>G	0.62	Spurdle et al., 2008	0.03	0.02	2
21	R2888C	p.Arg2888Cys	8890C>T	c.8662C>T	8.03×10^{-4}	Easton et al., 2007	0.03	2.48×10^{-5}	1
21	V2908G	p.Val2908Gly	8951T>G	c.8723T>G	3.91×10^{-3}	Easton et al., 2007	0.66	7.54×10^{-3}	2
22	E2947K	p.Glu2947Lys	9067G>A	c.8839G>A	1.21	Walker et al., 2010	0.03	0.04	2
22	K2950N	p.Lys2950Asn	9078G>T	c.8850G>T	4.23×10^{-3}	Spurdle et al., 2008	0.66	8.14×10^{-3}	2
22	D2965H	p.Asp2965His	9121G>C	c.8893G>C	1.15	*This manuscript*	0.03	0.03	2
22	V2969M	p.Val2969Met	9133G>A	c.8905G>A	3.56×10^{-4}	Easton et al., 2007	0.03	1.10×10^{-5}	1
22	R2973C	p.Arg2973Cys	9145C>T	c.8917C>T	1.76×10^{-4}	Farrugia et al., 2008	0.03	5.44×10^{-6}	1
24	R3052Q	p.Arg3052Gln	9383G>A	c.9155G>A	2.82×10^{-3}	Easton et al., 2007	0.66	5.45×10^{-3}	2
24	R3052W	p.Arg3052Trp	9382C>T	c.9154C>T	9376.00	Farrugia et al., 2008	0.81	1.00	5
24	P3063S	p.Pro3063Ser	9415C>T	c.9187C>T	0.73	*This manuscript*	0.03	0.02	2
24	V3079I	p.Val3079Ile	9463G>A	c.9235G>A	6.47×10^{-4}	Easton et al., 2007	0.03	2.00×10^{-5}	1
25	Y3092C	p.Tyr3092Cys	9503A>G	c.9275A>G	6.03×10^{-3}	Easton et al., 2007	0.81	0.03	2
25	D3095E	p.Asp3095Glu	9513C>G	c.9285C>G	22.59	Easton et al., 2007	0.66	0.98	4
25	Y3098H	p.Tyr3098His	9520T>C	c.9292T>C	3.46×10^{-4}	Easton et al., 2007	0.03	1.07×10^{-5}	1
25	N3124I	p.Asn3124Ile	9599A>T	c.9371A>T	7.23	*This manuscript*	0.81	0.97	4

BRCA2 exon	Codon	HGVS: protein level	BIC: DNA level	HGVS: DNA level	Odds in favor of causality	Reference	Prior Probability of being deleterious	Posterior Probability of being deleterious	IARC Class
26	D3170G	p.Asp3170Gly	9737A>G	c.9509A>G	1.30×10^{-3}	Easton et al., 2007	0.02	2.64×10^{-5}	1
26	C3198R	p.Cys3198Arg	9820T>C	c.9592T>C	1.80×10^{-4}	Easton et al., 2007	0.02	3.68×10^{-6}	1
27	K3326X	p.Lys3326X	10204A>T	c.9976A>T	1.00×10^{-7}	Farrugia et al., 2008	0.02	2.04×10^{-9}	1
27	T3349A	p.Thr3349Ala	10273A>G	c.10045A>G	4.50×10^{-6}	Easton et al., 2007	0.02	9.19×10^{-8}	1

[#] VUS shown to result in aberrant gene splicing

GenBank Reference BRCA2 NM_000059.3

Nucleotide numbering in "HGVS: DNA level" reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

Nucleotide numbering in "BIC-DNA level" refers to the original nomenclature for BRCA1 and BRCA2 before adoption of HGVS standards where BRCA1 +119 and BRCA2 +228 correspond to the A of the ATG translation initiation codons in the reference sequence.

Table 6

Classification of *BRCA1* and *BRCA2* candidate splicing variants as Class 1, 2 or Class 4, 5

Gene	Exon	Codon	HGVS: protein level	HGVS: DNA level	BIC: DNA level	Odds in favor of causality	Reference	Prior Probability of being deleterious	Posterior Probability of being deleterious	IARC Class
BRCA1	3	IVS2-11delT		c.81-11delT	200-11delT	1.32×10^{-4}	Easton et al., 2007	0.26	4.62×10^{-5}	1
BRCA1	3	IVS2-13C>G		c.81-13C>G	200-13C>G	8.58×10^{-4}	Easton et al., 2007	0.26	3.01×10^{-4}	1
BRCA1	5	IVS5+3A>G		c.212+3A>G	331+3A>G	1417.00	Easton et al., 2007	0.26	1.00	5
BRCA1	6	IVS6+7G>A		c.301+7G>A	420+7G>A	1.79×10^{-4}	Easton et al., 2007	0.26	6.27×10^{-5}	1
BRCA1	7	IVS6-1G>C		c.302-1G>C	421-1G>C	72.00	Easton et al., 2007	0.96	1.00	5
BRCA1	7	IVS6-3C>G		c.302-3C>T	421-3C>G	225.00	Easton et al., 2007	0.26	0.99	5
BRCA1	9	IVS8-17G>T		c.548-17G>T	667-17G>T	9.09×10^{-7}	Easton et al., 2007	0.26	3.19×10^{-7}	1
BRCA1	9	IVS9+4A>G		c.593+4A>G	712+4A>G	0.02	Whiley et al., 2011	0.26	0.01	2
BRCA1	12	IVS11-1G>A		c.4097-1G>A	4216-1G>A	24.00	Easton et al., 2007	0.96	1.00	5
BRCA1	12	IVS11-11T>C		c.4097-11T>C	4216-11T>C	1.54×10^{-3}	Easton et al., 2007	0.26	5.42×10^{-4}	1
BRCA1	12	IVS12+10G>C		c.4185+10G>C	4304+10G>C	1.25×10^{-3}	Easton et al., 2008	0.26	4.37×10^{-4}	1
BRCA1	13	IVS13+1G>A		c.4357+1G>A	4476+1G>A	4.82×10^{6}	Easton et al., 2007	0.96	1.00	5
BRCA1	15	IVS15+1G>A		c.4675+1G>A	4794+1G>A	6109.20	Whiley et al., 2011	0.96	1.00	5
BRCA1	16	IVS15-7C>T		c.4676-7C>T	4795-7C>T	0.01	Easton et al., 2007	0.26	2.80×10^{-3}	2
BRCA1	17	IVS16-20A>G		c.4987-20A>G	5106-20A>G	5.62×10^{-7}	Easton et al., 2007	0.26	1.97×10^{-7}	1
BRCA1	17	IVS17+1G>A		c.5074+1G>A	5193+1G>A	196.00	Easton et al., 2007	0.96	1.00	5
BRCA1	18	IVS17-9A>T		c.5075-9A>T	5194-9A>T	4.59×10^{-3}	Easton et al., 2007	0.26	1.61×10^{-3}	2
BRCA1	18	IVS18+1G>T		c5152+1G>T	5271+1G>T	1.12×10^{7}	Spurdle et al., 2008	0.96	1.00	5
BRCA1	19	IVS18-13A>G		c.5153-13A>G	5272-13A>G	1.47×10^{-4}	Easton et al., 2007	0.26	5.17×10^{-5}	1
BRCA1	19	IVS18-1G>C		c.5153-1G>C	5272-1G>C	24.00	Easton et al., 2007	0.96	1.00	5
BRCA1	19	IVS18-6C>A		c.5153-6C>A	5272-6C>A	2.56×10^{-5}	Easton et al., 2007	0.26	9.01×10^{-6}	1
BRCA1	20	IVS19-12G>A		c.5194-12G>A	5313-12G>A	1.85×10^{5}	Whiley et al., 2011	0.26	1.00	5
BRCA1	20	IVS20+1G>A		c.5277+1G>A	5396+1G>A	4.31×10^{11}	Easton et al., 2007	0.96	1.00	5
BRCA1	22	IVS21-8C>T		c.5333-8C>T	5452-8C>T	3.19×10^{-3}	Easton et al., 2007	0.26	1.12×10^{-3}	2
BRCA1	23	IVS23+5G>C		c.5467+5G>C	5586G>C	0.13	Whiley et al., 2011	0.26	0.04	2
BRCA2	6	IVS5-2A>G		c.476-2A>G	704-2A>G	22.00	Easton et al., 2007	0.96	1.00	5

Gene	Exon	Codon	HGVS: protein level	HGVS: DNA level	BIC: DNA level	Odds in favor of causality	Reference	Prior Probability of being deleterious	Posterior Probability of being deleterious	IARC Class
BRCA2	6	IVS6+1G>T		c.516+1G>T	744+1G>T	1.10	Whiley et al., 2011	0.96	0.96	4
BRCA2	9	IVS8-12delTA		c.791delTA	910-12delTA	0.01	Spearman et al., 2008	0.26	2.80×10^{-3}	2
BRCA2	12	IVS11-20T>A		c.6842-20T>A	7070-20T>A	7.76×10^{-4}	Easton et al., 2007	0.26	2.73×10^{-4}	1
BRCA2	13	IVS13+1G>C		c.7007+1G>C	7235+1G>C	2.45	Whiley et al., 2011	0.96	0.98	4
BRCA2	15	IVS14-14T>G		c.7436-14T>G	7664G>C	0.02	Whiley et al., 2011	0.26	6.98×10^{-3}	2
BRCA2	16	IVS15-1G>A		c.7618-1G>A	7846-1G>A	93.20	Whiley et al., 2011	0.96	1.00	5
BRCA2	19	IVS19+1G>A		c.8487+1G>A	8715+1G>A	21.00	Easton et al., 2007	0.96	1.00	5
BRCA2	21	IVS20-16C>G		c.8633-16C>G	8861-16C>G	0.01	Easton et al., 2007	0.26	2.12×10^{-3}	2
BRCA2	21	IVS21+4A>G		c.8754+4A>G	8982+4A>G	36.00	Easton et al., 2007	0.26	0.93	4
BRCA2	22	IVS22+1G>T		c.8953+1G>T	9181+1G>T	14.06	Whiley et al., 2011	0.96	1.00	5
BRCA2	23	P3039P	p.Pro3039Pro	c.9117G>A	9345G>A	146.00	Easton et al., 2007	0.96	1.00	5
BRCA2	25	IVS24-1G>C		c.9257-1G>C	9485-1G>C	55.00	Easton et al., 2007	0.96	1.00	5
BRCA2	25	IVS25+9A>C		c.9501+9A>C	9729+9A>C	6.44×10^{-4}	Easton et al., 2007	0.26	2.26×10^{-4}	1
BRCA2	27	IVS26-20C>T		c.9649-20C>T	9877-20C>T	3.29×10^{-5}	Easton et al., 2007	0.26	1.16×10^{-5}	1

GenBank Reference BRCA1 U14680.1

GenBank Reference BRCA2 NM_000059.3

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