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Criteria of the German Consortium for Hereditary Breast and Ovarian Cancer for the Classification of Germline Sequence Variants in Risk Genes for Hereditary Breast and Ovarian Cancer

Kriterien des Deutschen Konsortiums Familiärer Brust- und Eierstockkrebs zur Klassifizierung von Keimbahn-Sequenzvarianten in Risikogenen für familiären Brust- und Eierstockkrebs

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ABSTRACT

More than ten years ago, the German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC) set up a panel of experts (VUS Task Force) which was tasked with reviewing the classifications of genetic variants reported by individual centres of the GC-HBOC to the central database in Leipzig and reclassifying them, where necessary, based on the most recent data. When it evaluates variants, the VUS Task Force must arrive at a consensus. The resulting classifications are recorded in a central database where they serve as a basis for ensuring the consistent evaluation of previously known and newly identified variants in the different centres of the GC-HBOC. The standardised VUS evaluation by the VUS Task Force is a key element of the recall system which has also been set up by the GC-HBOC. The system will be used to pass on information to families monitored and managed by GC-HBOC centres in the event that previously classified variants are reclassified based on new information. The evaluation algorithm of the VUS Task Force was compiled using internationally established assessment methods (IARC, ACMG, ENIGMA) and is presented here together with the underlying evaluation criteria used to arrive at the classification decision using a flow chart. In addition, the characteristics and special features of specific individual risk genes associated with breast and/or ovarian cancer are discussed in separate subsections. The URLs of relevant databases have also been included together with extensive literature references to provide additional information and cover the scope and dynamism of the current state of knowledge on the evaluation of genetic variants. In future, if criteria are updated based on new information, the update will be published on the website of the GC-HBOC (https://www.konsortium-familiaerer-brustkrebs.de/).

ZUSAMMENFASSUNG

Das Deutsche Konsortium für Familiären Brust- und Eierstockkrebs (GC-HBOC) etablierte vor über 10 Jahren eine Expertengruppe (VUS Task Force), um die von den einzelnen Zentren des GC-HBOC an die zentrale Datenbank in Leipzig gemeldeten Varianten hinsichtlich ihrer Klassifizierung zu überprüfen und ggf. nach aktueller Datenlage neu einzustufen. Die innerhalb der VUS-Task Force konsentierten Variantenbewertungen und resultierenden -klassifizierungen werden in einer zentralen Datenbank hinterlegt und sind als Grundlage zu berücksichtigen, um eine einheitliche Bewertung bereits bekannter wie auch neu identifizierter Varianten innerhalb der verschiedenen Zentren des GC-HBOC zu gewährleisten. Die standardisierte VUS-Bewertung durch die VUS Task Force ist ein zentrales Element des vom GC-HBOC ebenfalls etablierten Recall-Systems. Dieses dient der Weitergabe der Informationen an die in den Zentren betreuten Familien im Falle einer aufgrund neuer Erkenntnisse aktualisierten Neubewertung von bereits klassifizierten Varianten. Die in Anlehnung an international etablierte Bewertungsverfahren (IARC, ACMG, ENIGMA) angepassten Bewertungsalgorithmen der VUS Task Force werden in diesem Artikel anhand der zugrundeliegenden Entscheidungskriterien präsentiert, die gemäß eines priorisierenden Fließschemas zum Klassifizierungsergebnis führen. Weiterhin werden genspezifische Regelungen und Besonderheiten, die für einzelne mit Brust- und/oder Eierstockkrebs assoziierte Risikogene zu berücksichtigen sind, in einzelnen Unterkapiteln dargelegt. Um dem Umfang und der Dynamik des aktuellen Wissens zur Variantenbewertung gerecht zu werden, sind neben umfangreichen Literaturverweisen insbesondere auch die URLs von relevanten Datenbanken angegeben. In Zukunft sollen an neue Erkenntnisse angepasste Kriterien auf der Webseite von GC-HBOC (https://www.konsortium-familiaerer-brustkrebs.de/) veröffentlicht werden.

General Principles

The criteria of the German Consortium for Hereditary Breast and Ovarian Cancer (http://www.konsortium-familiaerer-brustkrebs.de/) for the classification of germline sequence variants in risk genes for hereditary breast and ovarian cancer were developed and compiled by the members of the panel of experts on variant evaluation (VUS Task Force) from the German Consortium for Hereditary Breast and Ovarian Cancer listed above. The task of this panel of experts is to specify binding criteria for the German Consortium for Hereditary Breast and Ovarian Cancer to evaluate variants and verify the classification of variants to ensure that variants are uniformly evaluated by the Consortium. The present criteria are based on the IARC[1] 5-class system for high-risk genes[2] which is based on the guidelines issued by the ENIGMA[3] Consortium (ENIGMA *BRCA1/2* Classification Criteria, Version 2.5.1, June 2017), as well as the ACMG[4] and ACGS[5] guidelines. Using this 5-class system, germline sequence variants are evaluated in terms of their relevance for a loss of function of the coded protein (Class1: neutral, Class2: likely neutral, Class3: uncertain evidence/no reliable evaluation, Class4: likely relevant loss of function, Class5: relevant loss of function; level of significance, see [4]). For high-penetrance genes (such as *BRCA1, BRCA2*) for which a clinical correlation (pathogenicity) with loss of function has been described, the functional classification yields a pathogenicity evaluation based on the IARC 5-class system (ranging from Class1: not pathogenic to Class5: pathogenic), as outlined in Appendix A 2, ▸ **Table 1**. The advantage of such a structured approach is that it starts by checking for defined criteria which can be used for a quick and unambiguous classification, and the extensive data and literature search is only carried out afterwards (see Appendix A 2, ▸ **Table 2**: Relevant literature and databases, and A 3, ▸ **Fig. 1**: Evaluation criteria flow chart). As regards the classification of sequence variants of the genes *ATM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, PALB2, RAD51C, RAD51D* and *TP53*, all of them so-called “core genes” according to the TruRisk panel (version 1/2018, see the homepage of the German Consortium: http://www.konsortium-familiaerer-brustkrebs.de/), the specific

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[1] International Agency for Research on Cancer.

[2] High-risk genes: at least one sequence variant with an odds ratio for breast and/or ovarian cancer of > 5 (e.g. *BRCA1, BRCA2, RAD51C, PALB2, TP53, ATM*), see [1, 2].

[3] Evidence-based network for the interpretation of germline mutant alleles: http://enigmaconsortium.org/

[4] American College of Medical Genetics and Genomics (ACMG [3]).

[5] Association for Clinical Genomic Science (ACGS, http://www.acgs.uk.com/).

features of the individual genes listed in Appendix A 5 must be taken into account. Moderate/low-penetrance genes are only evaluated in terms of their functionality (in these cases: loss of function should not be equated with "pathogenicity"). Even variants of high-risk genes may only be associated with an intermediate risk [5, 6].

Criteria for the Interpretation of mRNA Analyses

The criteria of the German Consortium for Hereditary Breast and Ovarian Cancer for the evaluation of sequence variants with subsequent mRNA analysis are based on the guidelines of the ENIGMA Consortium (see also [7]). The respective threshold values of potential splice variants for an empirical predictive prognosis based on three commonly used predictive programmes are given in Appendix A 1. Appendix A 4 (▶ **Fig. 2**) gives a schematic representation of the areas considered by the VUS Task Force when evaluating the splice variants. mRNA analysis is carried out using fresh blood samples, cultured lymphocytes, cultured lymphoblastoid cell lines, etc. and compared in parallel with at least 5 controls of the same type of material. A sequence variant is described as pathogenic if it has the following effect on mRNA transcription: one or more aberrant transcripts of the variant allele are detected, which lead to a stop codon or an in-frame deletion and result in the destruction of known functional domains. Sequencing of the full-length transcript of the variant allele or the presence of an intronic variant of a cis-acting polymorphism is considered sufficient (evidence of monoallelic expression) to determine the transcript amount (using semi-quantitative or quantitative methods). Variants which show a transcript pattern comparable to the mean value of controls are rated as neutral/not pathogenic due to the lack of aberrant mRNA. As regards the cDNA primer design, the physiological splice variants/naturally occurring isoforms must also be taken into account (see [8, 9]).

Caution: Certain *BRCA1* and *BRCA2* variants which are ± 1, 2 bp from the exon border and which are predicted or proven to lead to at least 20–30% naturally occurring in-frame RNA isoforms per allele could presumably result in some residual protein activity (see [9–12]) and are therefore classified as VUS Class3, unless there is evidence to the contrary (see Overview, Appendix 5, ▶ **Table 5**)

Approach of the VUS Task Force

The Consortium recommends routinely testing for 10 genes which are known (as per 8/19) to be associated with breast and/or ovarian cancer: *ATM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, PALB2, RAD51C, RAD51D* and *TP53* (http://www.konsortium-familiaerer-brustkrebs.de/).

The Consortium's panel of experts (VUS Task Force) holds monthly telephone conferences and, if necessary, meetings to reach a consensus on the classification of newly reported sequence variants, discuss any new evidence available for the re-evaluation of already known variants, and evaluate variants of unclear significance. In the event of a re-evaluation, the central database of the Consortium will inform all centres about the reclassification (recall system).

It should be expressly noted that new information can lead to changes in the classification of variants and that these classifications are regularly reviewed by the panel of experts. Similarly, new findings can lead to changes in the list of core genes for which the German Consortium for Hereditary Breast and Ovarian Cancer recommends that patients are tested. All such changes along with the inclusion of new findings into the classification are published on the homepage of the German Consortium for Hereditary Breast and Ovarian Cancer (http://www.konsortium-familiaerer-brustkrebs.de/).

Classification of Sequence Variants According to Their Functional Relevance

1. **Class1 (functionally irrelevant/no loss of function) if one of the following criteria is met:**
   - 1.1 Allele frequency of variants in large population groups (e.g. Caucasians, Africans, or Asians) is ≥ 1% (minor allele frequency [MAF] ≥ 0.01). **Caution:** An allele frequency of ≥ 1% in subpopulations with a low-diversity gene pool (examples: Finnish population, founder mutations!) is not sufficient.
   - 1.2 Variants with a calculated multifactorial probability of < 0.001 of being pathogenic.
     Caution: This currently only applies to the high-risk genes *BRCA1/2* (for an exemplary calculation, see [13]).
   - 1.3 Variants in high-risk genes which occur in at least 10 individuals in appropriate cohorts of persons without disease (▶ **Table 1**).
2. **Class2 (probably no loss of function/functionally irrelevant) if one of the following criteria are met:**
   - 2.1 Allele frequency of variants in large population groups (e.g. Caucasians, Africans, or Asians) is 0.5–1% (MAF 0.005–0.01) **Caution:** An allele frequency of 0.5–1% in subpopulations with a low-diversity gene pool (examples: Finnish population, founder mutations!) is not sufficient.
   - 2.2 Exonic variants (A) which result in substitution of an amino acid (missense variants) or small in-frame insertions/deletions (insertions/deletions of one or fewer amino acid [s]) and whose a priori probability of pathogenicity is ≤ 2% (A-GVGD analysis, http://priors.hci.utah.edu/PRIORS/); intronic variants (B) which are **more than − 20 bp, + 10 bp from the exon border**; and synonymous variants (C) if these variants (A–C) will, according to bioinformatic prediction programmes (see Appendix A 1), in all probability not change the splicing mechanism. In non-*BRCA1/2* genes, the above-mentioned variants must be present in large population groups with an allele frequency of 0.001 ≤ MAF < 0.01.
   - 2.3 Synonymous substitutions or intronic variants which show no mRNA aberrations in the form of exon deletions/duplications or monoallelic expression of the wildtype (wt) transcript in "in-vitro" laboratory tests even if, according to bioinformatic prediction programmes (see Appendix A 1 for programmes and threshold values), in all probability they change the splicing mechanism.

2.4 Variants which occur in the same gene with a clearly pathogenic variant in trans (co-occurrence), if it has been verified that a homozygous or compound heterozygous genotype is associated with a known, clinically unambiguous phenotype.

2.5 Variants with a calculated multifactorial probability of pathogenicity of 0.001–0.049.
Caution: This currently only applies to the high-risk genes *BRCA1/2* (for an exemplary calculation, see: [13]).

2.6 Exon variants which code for the same amino acid exchange as a sequence variant which has already been classified as Class1 but are based on a different nucleotide exchange if no aberrant splicing is predicted.

2.7 Missense variants for which information from functional analyses, etc., is available but not sufficient for multifactorial classification and which have been classified as Class2 by a panel of experts (e.g. ENIGMA).

3. Class3 (unclear functional relevance) if one of the following criteria is met: variants which cannot be unambiguously assigned to Class1, Class2, Class4, or Class5, e.g.:

3.1 Special cases which could be assigned to one of the other classes based on the evaluation criteria but are listed in Appendix A 5 among the characteristics of individual core genes or in Table 5, Appendix of *BRCA1/2* classification criteria, Version 2.5.1, July 2017 (ENIGMA) (▶ **Table 5**).

3.2 Variants where the data used for their evaluation is contradictory and for which further studies are still required.

3.3 Variants which are **− 20 bp, + 10 bp from the exon border** and which, based on bioinformatic predictive programmes (see Appendix A 1), probably change the splicing mechanism as long as no in-vitro mRNA analysis has been done yet (▶ **Fig. 2**, Schematic representation of variants in the vicinity of splice sites).

3.4 Exon duplications which have not been analysed further (e.g. break point analysis, cDNA analysis, etc.).

3.5 Variants with a calculated multifactorial probability of pathogenicity of 0.05–0.949.
Caution: This currently only applies to the high-risk genes *BRCA1/2* (for an exemplary calculation, see [13]).

4. Class4 (probable loss of function/functionally relevant) if one of the following criteria is met:

4.1 Variants with a calculated multifactorial probability of pathogenicity of 0.95–0.99.
Caution: This currently only applies to the high-risk genes *BRCA1/2* (for an exemplary calculation, see Goldgar et al., 2004 [13]).

4.2 Variants which code for a premature termination of protein biosynthesis (nonsense or frameshift variants) and do not necessitate the loss of known clinically relevant functional protein domains as long as the location of the stop codon is not downstream from the Nonsense-mediated decay-(NMD-)relevant site, 50 base pairs before the end of the penultimate exon.

4.3 Intronic variants in position ± 1,2 or G > non-G in the last position of the exon: if there is a positive splicing prediction (see Appendix A 1) and the first 6 bases in the intron are not GTRRGT and an aberrant in-vitro mRNA analysis is **not yet** available (i.e. has not [yet] been confirmed by a panel of experts or the pathomechanism of loss of function has been confirmed to be exon skipping or allele-specific transcript expression).

Exceptions:

- A cryptic splice site (AG/GT) in the vicinity is activated and the (predicted) new exon is spliced in-frame **(→ Class3)**
- The (predicted) skipped exon (or exons) is alternatively spliced in significant quantities **(→ Class3)**.
- The (predicted) skipped exon (or exons) is spliced in-frame and contains no known functional domain **(→ Class3)**

4.4 Variants which code for the same amino acid exchange as pathogenic missense variants which have already been categorised as Class5 but are caused by another nucleotide exchange and for which there is no positive splicing prediction (see Appendix A 1).

4.5 In-frame deletions (even for just one amino acid) which lead to the loss of a missense variant already categorised as Class5 and which result in the interruption of known, functionally important domains.

4.6 Extensive in-frame deletions which lead to the interruption/loss of known, functionally important domains.

4.7 In-frame insertions verified by in-vitro mRNA analysis which result in the interruption of functionally important domains.

4.8 Variants which lead to mutations of the translation initiation codon (AUG, methionine) and for which there is no evidence (e.g. an alternative start codon in the immediate vicinity) which would support an alternative classification.

4.9 Variants for which information from functional analyses, clinical data, etc., is available but insufficient for a multifactorial classification and which are categorised as Class4 by a panel of experts (e.g. ENIGMA).

5. Class5 (loss of function/functionally relevant) if one of the following criteria are met:

5.1 Variants which code for a premature termination of protein biosynthesis (nonsense or frameshift variants) which prevents the expression of known, clinically relevant, functional protein domains.

5.2 Variants with a calculated multifactorial probability of pathogenicity of > 0.99.
Caution: This currently only applies to the high-risk genes *BRCA1/2* (for an exemplary calculation, see Goldgar et al., 2004 [13]).

5.3 Splice variants for which a frameshift effect was established by in-vitro mRNA analysis, which leads to a premature termination of protein biosynthesis and prevents the expression of known, clinically relevant, functional protein domains and for which a wild-type transcript of the mutated allele has not been confirmed (monoallelic expression).

5.4 Splice variants for which in-vitro mRNA analysis detected an in-frame deletion/insertion which leads to the interruption or loss of a known, clinically relevant domain or functional inactivation through changes of the protein structure and for which a wild-type transcript of the mutated allele has not been verified (monoallelic expression).

5.5 Copy-number deletion variants which result in the interruption or loss of one or more exons with known, clinically relevant functional domains or lead to a reading frameshift, which results, according to the prediction, in the inactivation of known, clinically relevant, functional domains.

5.6 Copy-number duplication variants of any size, confirmed by laboratory analysis, which duplicate one or more exons and lead to a reading frameshift, which results, according to the prediction, in the inactivation of known, clinically relevant, functional domains.

Appendix

A 1. Splicing prediction programmes and their threshold values

The splicing prediction programmes MaxEntScan (MES), Splice Site Finder (SSF), and Human Splicing Finder (HSF) are considered relatively reliable and should therefore be used to evaluate possible effects on the splicing process. MaxEntScan results are considered non-normal for a deviation of delta of ≥ 15% [14], Human Splicing Finder for a delta of ≥ 4.1% [10] and Splice Site Finder for a deviation of delta of ≥ 5% [14]. An mRNA analysis should be done for evaluation in cases with non-normal prediction (at least two of the three programmes mentioned below). The precondition is that the physiological splice site is recognised by the respective prediction software based on the following threshold values.

The threshold values (calculated for *BRCA1/2* [14]) are:

1. MES > 3
2. SSF > 60
3. HSF > 80

An approximation of these threshold values can also be for the other genes. Once specific threshold values have been defined, then the defined threshold values must be used.

A 2.

▶ **Tables 1** and **2**.

▶ **Table 1** IARC 5-tiered classification system with accompanying recommendations for family management[a] (excerpt from https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf).

Class	Quantitative measure: probability of pathogenicity	Predictive testing of at-risk relatives	Surveillance of at-risk relatives	Research testing of relatives
5: Pathogenic	> 0.99	Yes	Full high-risk guidelines for variant carriers	Not indicated
4: Likely pathogenic	0.95–0.99	Yes[b]	Full high-risk guidelines for variant carriers	Yes
3: Uncertain	0.05–0.949	No[b]	Based on family history & other risk factors	Yes
2: Likely not pathogenic or of little clinical significance	0.001–0.049	No[b]	Based on family history & other risk factors – treat as “no *BRCA1/2* pathogenic variant detected” for this disorder	Yes
1: Not pathogenic or of no clinical significance	< 0.001	No[b]	Based on family history & other risk factors – treat as “no *BRCA1/2* pathogenic variant detected” for this disorder	Not indicated

[a] Adapted for clarity from the original published tabular presentation (Plon et al., 2008 [4])

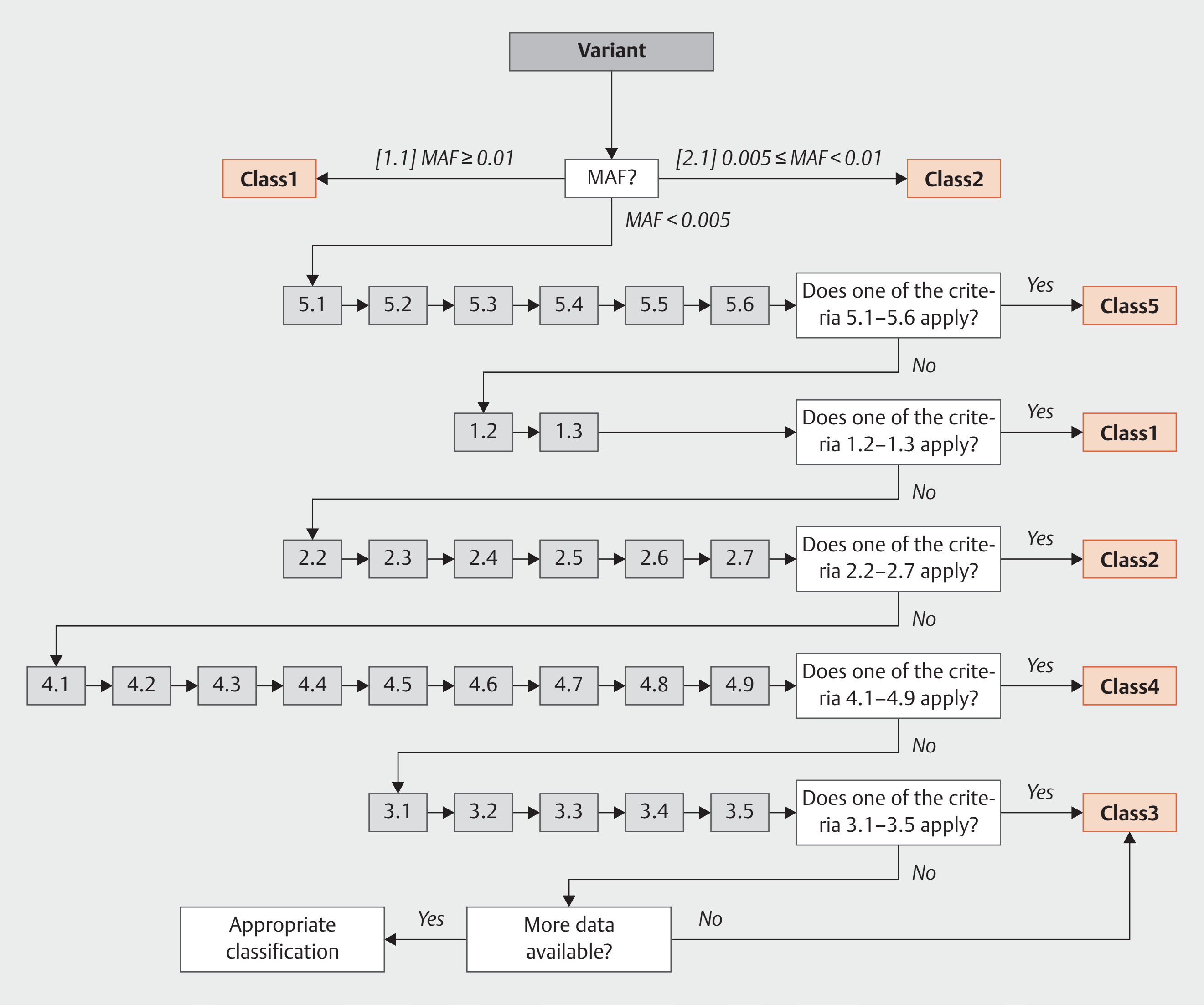
[b] Continued testing of proband for any additional available testing modalities available for BRCA1/2, e.g. rearrangements, is recommended.

▶ **Table 2** Relevant literature and databases available for evaluation.

Database	URL
1000Genomes	http://www.1000genomes.org/
ARUP *(BRCA1)*	http://arup.utah.edu/database/BRCA/Variants/BRCA1
ARUP *(BRCA2)*	http://arup.utah.edu/database/BRCA/Variants/BRCA2
BIC (**B**reast Cancer **I**nformation **C**ore)	http://research.nhgri.nih.gov/bic/
BRCA1 CIRCOS (via BIC, or **Caution:** specify BIC nomenclature!)	https://research.nhgri.nih.gov/projects/bic/circos/search.shtml
BRCA Exchange	http://brcaexchange.org
ClinVar (ClinGen)	https://www.ncbi.nlm.nih.gov/clinvar/
Database of Functional Classifications of *BRCA1* Variants based on Saturation Genome Editing [15, 16]	https://sge.gs.washington.edu/BRCA1/
dbSNP	https://www.ncbi.nlm.nih.gov/snp
EVS/ESP	http://evs.gs.washington.edu/EVS/
Exome Aggregation Consortium ExAC Browser	http://exac.broadinstitute.org/
exUV-LOVD (multifactorial analysis of reclassified *BRCA1/2* variants)	http://hci-exlovd.hci.utah.edu/home.php
FLOSSIES (**F**abulous **L**adies **O**ver **S**eventy: germline variants for 27 breast cancer-relevant genes in approx. 10 000 women aged > 70 years, who have not developed cancer to date)	https://whi.color.com/
Genome Aggregation Database GnomAD	http://gnomad.broadinstitute.org/
Google Scholar	http://scholar.google.de/
HGMD Professional (registration required)	https://portal.biobase-international.com/
IARC *TP53* database	http://p53.iarc.fr/
InSiGHT Consortium (classification database)	http://www.insight-database.org/classifications/
KAVIAR (Known VARiants, genomic)	http://db.systemsbiology.net/kaviar/cgi-pub/Kaviar.pl
LOVD (Leiden Open Variation Database, general)	http://www.lovd.nl/3.0/home
LOVD database, *ATM*	https://databases.lovd.nl/shared/genes/ATM
LOVD database, *BRCA1*	https://databases.lovd.nl/shared/genes/BRCA1
LOVD database, *BRCA2*	https://databases.lovd.nl/shared/genes/BRCA2
LOVD database, *BRIP1*	http://databases.lovd.nl/shared/genes/BRIP1
LOVD database, *CDH1*	http://databases.lovd.nl/shared/genes/CDH1
LOVD database, *CHEK2*	https://databases.lovd.nl/shared/genes/CHEK2
LOVD database, *PALB2*	http://databases.lovd.nl/shared/genes/PALB2
LOVD database, *RAD51C*	https://databases.lovd.nl/shared/genes/RAD51C
LOVD database, *RAD51D*	https://databases.lovd.nl/shared/genes/RAD51D
LOVD database, *TP53*	https://databases.lovd.nl/shared/genes/TP53
PubMed	http://www.ncbi.nlm.nih.gov/pubmed/
TP53, PHANTM (genotype/phenotype correlation)	http://mutantp53.broadinstitute.org/heatMap/login
UMD (**U**niv. **M**utation **D**atabase, LSDB: Locus Specific Databases)	http://www.umd.be/
UMD/BRCAshare-*BRCA1* (registration required)	http://www.umd.be/BRCA1/
UMD/BRCAshare-*BRCA2* (registration required)	http://www.umd.be/BRCA2/
UMD *TP53*	http://p53.fr/tp53-database
VarSeak	https://varseak.bio/
VarSome (automated ACMG classification)	https://varsome.com/

A 3. Evaluation Criteria Flow Chart

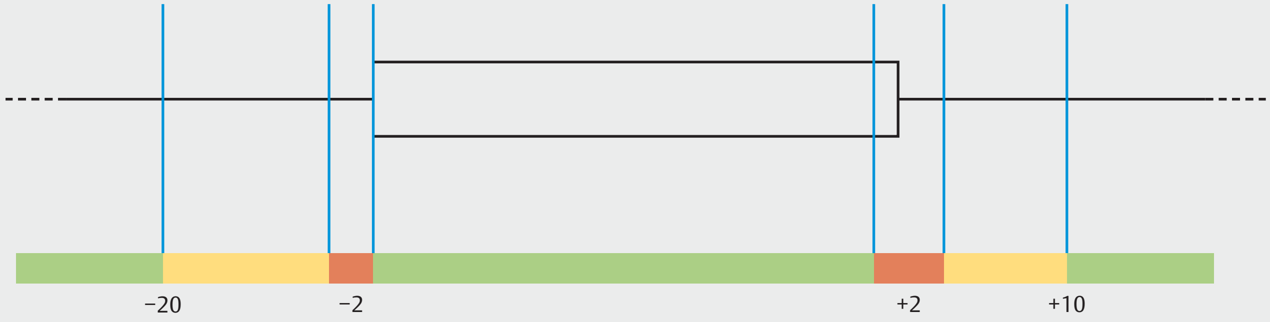
▶ **Fig. 1**.



▶ **Fig. 1** VUS evaluation criteria 1.1.

A 4. Schematic Representation of Variants in the Vicinity of Splice Sites

▸ **Fig. 2**.



🟩 1. Intronic variants at a distance of more than −20/+10 from the exon if the **splicing prediction is normal: Class2**.
2. Exonic (synonymous) variants if the **splicing prediction is normal** and MAF ≥ 0.001 but < 0.01: **Class2**.
3. In both cases: if the splicing prediction is abnormal: **Class3** and RNA analysis is required.

🟨 Variants in this region should be investigated using RNA analysis, particularly if the splicing prediction is abnormal: **Class3**.

🟧 Intronic variants at position –1, –2/+1, +2 or G > ≠ G at the last position of the exon: if the splicing prediction is positive and the first 6 bases in the intron are not GTRRGT even without an RNA analysis: **Class4**.
All other variants at these positions: initially **Class3**, RNA analysis (according to the Consortium) necessary/obligatory!

In addition, synonymous substitutions or intronic variants with an abnormal splicing prediction but which have no mRNA aberrations in the form of exon deletions/duplications or monoallelic expression of the wt transcript in "in-vitro" laboratory tests: **Class1**.

▸ **Fig. 2** Classification of variants which can affect splicing.

The use of predictive programmes to assess possible splicing effects is obligatory for all new mutations including stop mutations as "rescue" effects can occur through alternative transcripts.

A 5. Characteristics of Individual Genes

The above-mentioned general evaluation criteria should apply to all genes. However, some exceptions, variations and special features are present in specific variants and regions of individual genes, which, for the sake of clarity, are listed below.

A 5.1 *BRCA1/2*

The following should be categorised as Class3: truncating *BRCA1* mutations after the amino acid position 1854 and truncating *BRCA2* variants after amino acid position 3308 (they are not categorised as Class1, as structural mutations cannot be excluded). Exception: truncating variants after the polymorphic stop codon p.(Lys3326*) are classified as dispensable/neutral (Class1) [17] and ENIGMA: p.(Lys3326*) is a frequently detected polymorphism which is not associated with a higher risk, OR 1.3–1.5 depending on breast or ovarian cancer. This means that variants which lead to a stop downstream from p.(Lys3326*) will also not be associated with an increased risk of developing disease.

The following should be categorised as Class5 in *BRCA1/2*: all truncating *BRCA1* variants up to the last mutation unequivocally identified as pathogenic at amino acid position 1853 [18] and all truncating *BRCA2* variants up to amino acid position 3308, c.9924C>G [19]. See ENIGMA *BRCA1,2* functional domains, ▸ **Tables 3** and **4**. **Caution:** Be aware of the potential impact of NMD; the last 50 bp in the penultimate exon and variants in the last exon usually are usually not subject to NMD. The predictive value of RNA analysis of blood may be limited as it does not involve the target tissue.

Other special features are listed in the Appendix of the evaluation guidelines of the ENIGMA Consortium, which can be accessed via the following link: https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf, Tables 3, 4 and 6 (▸ **Tables 3** to **5**).

▶ **Table 3** Excerpt from https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf.

Table 3: Catalogue of BRCA1 conserved domains/motifs and currently known, clinically important amino acid residues, and relevance for classification of *BRCA1* in-frame and terminal exon sequence variants.

Domain/Motif	AA start	AA end	AA alterations with demonstrated clinical importance[a]	Classification of in-frame deletions targeting domain/motifs	References and summary interpretation[a]
RING	1	101	L22S (c.65T>C [p.Leu22Ser]) T37K (c.110C>A [p.Thr37Lys]) C39R (c.115T>C [p.Cys39Arg]) H41R (c.122A>G [p.His41Arg]) C44S (c.130T>A [p.Cys44Ser]) C44Y (c.131G>A [p.Cys44Tyr]) C61G (c.181T>G [p.Cys61Gly])	Class5 if at least one clinically relevant residue is removed. Otherwise Class3.	http://www.ncbi.nlm.nih.gov/protein/15988069; http://hci-exlovd.hci.utah.edu; multifactorial analysis for H41R (c.122A>G [p.His41Arg]) (Whiley et al., 2014).
NES	81	99	None reported	Class3	Domain location description (Rodriguez and Henderson, 2000).
NLS1	503	508	None reported	Class3	Domain location description (Chen et al., 1996, Thakur et al., 1997).
NLS2	607	614	None reported	Class3	Domain location description (Chen et al., 1996, Thakur et al., 1997).
NLS3	651	656	None reported	Class3	Domain location description (Chen et al., 1996).
COILED-COIL	1391	1424	None reported	Class3	Domain location description (Hu et al., 2000).
BRCT DOMAINS	1650	1863	T1685A (c.5053A>G [p.Thr1685Ala]) T1685I (c.5054C>T [p.Thr1685Ile]) V1688del (c.5062_5064del [p.Val1688del]) R1699W (c.5095C>T [p.Arg1699Trp]) G1706E (c.5117G>A [p.Gly1706Glu]) A1708E (c.5123C>A [p.Ala1708Glu]) S1715R (c.5143A>C [p.Ser1715Arg]) G1738R (c.5212G>A [p.Gly1738Arg]) L1764P (c.5291T>C [p.Leu1764Pro]) I1766S (c.5297T>G [p.Ile1766Ser]) M1775K (c.5324T>A [p.Met1775Lys]) M1775R (c.5324T>G [p.Met1775Arg]) C1787S (c.5359T>A [p.Cys1787Ser]) G1788V (c.5363G>T [p.Gly1788Val]) V1838E (c.5513T>A [p.Val1838Glu])	Class5 if at least one clinically relevant residue is removed. Otherwise Class3.	Domain boundaries derived from X-ray crystallography data are aa1646-1863 (1T15, http://www.ncbi.nlm.nih.gov/Structure/mmdb/mmdbsrv.cgi?uid=27907), and ENIGMA functional assay data (Monteiro, unpublished). Digestion data indicate aa1860-1863 are dispensable based on susceptibility to digestion (Lee et al., 2010), while pathogenic variant data indicate that 1855-1862 are dispensable (Hayes et al., 2000). Position 1854 is implicated as clinically important by the observation that Y1853X (c.5559C>G [p.Tyr1853Ter]) is a recognised high-risk pathogenic variant. These combined data indicate that position 1854 or 1855 is the C-terminal border of the BRCT/BRCA1 relevant for the clinical interpretation of sequence variants in exon 24 of BRCA1. That is, a variant predicted to disrupt expression of protein sequence only downstream* of position 1855 would not be considered clinically important.

[a] Missense substitutions in specific functional domains that are designated as Class5 pathogenic based on multifactorial likelihood of the posterior probability of pathogenicity > 0.99 (listed in http://hci-exlovd.hci.utah.edu or individual references), and which have no/little effect on the mRNA transcript profile, *unless* the variant results in an aberrant transcript that encodes a discrete in-frame deletion considered informative for the definition of clinically important domains.

* Typo was corrected in version 2.5.1.

Note: The following pathogenic exonic variants known to alter mRNA splicing have been excluded from Table 3 above, as justified below:

Variant	mRNA Change	Predicted protein change	Reason for exclusion
BRCA1 R1495M (c.4484G>T [p.Arg1495Met])	r.[4358_4484del, 4358_4675del]	p.(Ala1453Glyfs Ter10) – predominant transcript	Predominant alternate transcript is out of frame. Loss of function is assumed due to loss of full-length transcript from variant allele (Houdayer et al., 2012, Colombo et al., 2013, Santos et al., 2014).
BRCA1 E1559K (c.4675G>A [p.Glu1559Lys])	r.[4665_4675del]	p.(Gln1366Alafs Ter13)	Alternate transcript is out-of-frame. Level of full-length transcript not assessed (Wappenschmidt et al., 2012).
BRCA1 A1623G (c.4868C>G [p.Ala1623Gly])	r.[4868_4986del]	p.(Ala1623Aspfs Ter16)	Alternate transcript is out of frame. Variant allele produces some full-length transcripts (Walker et al., 2010).
BRCA1 D1692N (c.5074G>A [p.Asp1692Asn])	r.[4987_5074del, 5074_5075ins5074 +1_5074+153]	p.(Val1665Serfs Ter8) – predominant transcript	Predominant alternate transcript, based on minigene assay (Ahlborn et al., 2015), is out of frame.

► **Table 4** Excerpt from https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf.

Table 4: Catalogue of BRCA2 conserved domains/motifs and currently known clinically important amino acid residues, and relevance for classification of *BRCA2* in-frame and terminal exon sequence variants.

Domain/ Motif	AA start	AA end	AA alterations with demonstrated clinical importance[a]	Classification of in-frame deletions targeting domain/motifs	References and summary interpretation[a]
PALB2 Binding	10	40	None reported	Class3	Domain location description (Oliver et al., 2009, Xia et al., 2006)
BRC-1	1002	1036	None reported	Class3	http://www.ncbi.nlm.nih.gov/protein/NP_000050.2
BRC-2	1212	1246	None reported	Class3	http://www.ncbi.nlm.nih.gov/protein/NP_000050.2
BRC-3	1422	1453	None reported	Class3	http://www.ncbi.nlm.nih.gov/protein/NP_000050.2
BRC-4	1518	1549	None reported	Class3	http://www.ncbi.nlm.nih.gov/protein/NP_000050.2
BRC-5	1665	1696	None reported	Class3	http://www.ncbi.nlm.nih.gov/protein/NP_000050.2
BRC-6	1837	1871	None reported	Class3	http://www.ncbi.nlm.nih.gov/protein/NP_000050.2
BRC-7	1971	2005	None reported	Class3	http://www.ncbi.nlm.nih.gov/protein/NP_000050.2
BRC-8	2051	2085	None reported	Class3	http://www.ncbi.nlm.nih.gov/protein/NP_000050.2
DBD (DNA/DSS1 binding domain – helical, OB1, OB2, OB3)	2481	3186	W2626C (c.7878G>C [p.Trp2626Cys]) I2627F (c.7879A>T [p.Ile2627Phe]) E2663V (c.7988A>T [p.Glu2663Val]) T2722R (c.8165C>G [p.Thr2722Arg]) D2723G (c.8168A>G [p.Asp2723Gly]) D2723H (c.8167G>C [p.Asp2723His]) G2748D (c.8243G>A [p.Gly2748Asp]) I2778_Q2829del (c.8332_8487del [p.Ile2778_Gln2829del]) R3052W (c.9154C>T [p.Arg3052Trp])	Class5 if at least one clinically relevant residue (or all of AA2778-2829) is removed. Otherwise Class3.	http://www.ncbi.nlm.nih.gov/protein/NP_000050.2; http://hci-exlovd.hci.utah.edu. Pathogenic variant c.8486G>A (also recorded as Gln2829Arg) results in a transcript encoding an in-frame exon 19 deletion only (Houdayer et al., 2012), indicating that genetic variation encompassing loss of this entire exon (AA2778-2829) should be considered clinically important. The clinical impact of alteration/deletion of individual amino acids in exon 19 is not yet established.
NLS1	3263	3269	None reported	Class3	Domain local description (Guidugli et al., 2014)
BRC-9 or TR2	3265	3330	None reported	Class3	Note: although amino acids 3270-3305 within this fragment are reported to bind RAD51-DNA filaments (Davies and Pellegrini, 2007), there is no sequence conservation with the BRC repeats located between aa1002 and aa2014. Domain boundaries are derived from x-ray crystallography data are aa3265-3330 (Esashi et al., 2005, Esashi et al., 2007). Case-control and frequency data indicate that BRCA2 c.9976A>T (p.Lys3326Ter) does not confer a high risk of cancer (OR 1.3–1.5, dependent on breast or ovarian cancer subtype (Meeks et al., 2016), demonstrating that residues at and downstream of 3327 are likely dispensable. Position 3308 is implicated as clinically important by the observation that a nonsense variant c.9924C>G (p.Tyr3308Ter) is recognized as a high-risk pathogenic variant with known functional relevance ([Vallee et al., 2016]; Bayes score 1122 : 1 from a single large kConFab family, Spurdle unpublished data). There is currently no publicly available clinical information to support pathogenicity of nonsense or frameshift variants located between positions 3309 and 3325. These data combined suggest that the C-terminal border of the BRC-9 relevant to the clinical interpretation of sequence variants in exon 27 of BRCA2 lies between 3309 and 3325. That is, a variant predicted to disrupt expression only of protein sequence downstream of position 3325 would be considered unlikely to be clinically important. Further functional and clinical studies are underway to refine risk, if any, for predicted nonsense or frameshift variants downstream of position 3326.
NLS2	3381	3385	No	Class3	Domain location description (Guidugli et al., 2014). This domain is considered unlikely clinically relevant since it lies downstream of position 3326.

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► **Table 4** Excerpt from https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf. *(Continued)*

[a] Missense substitutions in denoted functional domains that are designated as Class5 pathogenic based on multifactorial likelihood posterior probability of pathogenicity > 0.99, and for which there is no/little effect on mRNA transcript profile – ***unless*** the variant results in an aberrant transcript that encodes a discrete in-frame deletion considered informative to definition of clinically important domains. (Splicing aberrations are reported for *BRCA2* c.7988A>T [p.Glu2663Val] and c.8168A>G [p.Asp2723Gly] (Walker et al., 2010), but these did not lead to complete loss of function of the full length transcript), and missense alterations showed abrogation of functional activity using multiple assays (Walker et al., 2010). An additional conserved region not commonly recognized as a BRCA2 domain/motif is located AA 1110-1183, but no pathogenic missense substitutions have been recorded for this region.

Note – The following pathogenic exonic variants known to alter mRNA splicing have been excluded from Table 4 above, as justified below:

Variant	mRNA Change	Predicted protein change	Reason for exclusion
BRCA2 R2659K (c.7976G>A [p.Arg2659Lys])	r.[7806_7976del]	p.(Ala2603_ Arg2659del)	Alternate transcript is in-frame but level of full length transcript not assessed (Farrugia et al., 2008)
BRCA2 R2659T (c.7976G>C [p.Arg2659Thr])	r.[7806_7976del]	p.(Ala2603_ Arg2659del)	Alternate transcript is in-frame but level of full length transcript not assessed (Farrugia et al., 2008)
BRCA2 P3039P (c.9117G>A [p.Pro3039Pro])	r.[8954_9117del]	p.(Val2985 Glyfs*4)	Allele-specific assay shows out-of-frame transcript (Houdayer et al., 2012)

► **Table 5** Excerpt from https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf.

Table 6: *BRCA1* and *BRCA2* exon boundary variants predicted or known to lead to naturally occurring in-frame RNA isoforms that may rescue gene functionality. ***Variants at these positions should be considered Class3 Uncertain unless proven otherwise.****

Gene	Alternative splicing event	Variants implicated	Rationale
BRCA1	Δ8p	c.442-1 (IVS7-1) c.442-2 (IVS7-2)	*BRCA1* exon 8 acceptor site is an experimentally validated tandem acceptor site (NAGNAG) subject to alternative splicing (Colombo et al., 2014). c.442-1,-2 variants are predicted to inactivate the 5′ acceptor site, but not the 3′ acceptor site, thus producing Δ8p transcripts.
	Δ9,10	c.548-1 (IVS8-1) c.548-2 (IVS8-2) c.593 to non-G c.593+1 (IVS9+1) c.593+2 (IVS9+2) c.594-1 (IVS9-1) c.594-2 (IVS9-2) c.670 to non-G c.670+1 (IVS10+1) c.670+2 (IVS10+2)	Carriers of variants at these positions are predicted to produce normal (or increased) levels of *BRCA1* Δ(9,10), a major in-frame alternative splicing event (Colombo et al., 2014). The *BRCA1* variant c.594-2A>C (shown from ENIGMA research to co-occur in cis with c.641A>G), has been reported to demonstrate clinical characteristics inconsistent with a high risk of cancer expected for a pathogenic *BRCA1* variant (Rosenthal et al., 2015). The haplotype of c.[594-2A>C; 641A>G] has been shown from mRNA analysis in human samples to produce high levels of Δ10 transcripts (70% of the overall expression, and **has been designated as Class1 Not Pathogenic** by the ENIGMA Consortium using multifactorial likelihood analysis that includes genetic (segregation, case-control analysis) and pathology data (de la Hoya et al., 2016).
	Δ11q, Δ11	c.4096 to non-G c.4096+1 (IVS11+1) c.4097+2 (IVS11+2)	Data collected by the ENIGMA consortium demonstrates that the *BRCA1* c.4096+1G>A variant, proven to result in the production of naturally occurring in-frame transcripts Δ11q (Bonatti et al., 2006) and also Δ11 (Radice, unpublished data), may not exhibit the clinical characteristics of a standard high-risk pathogenic *BRCA1* variant (Spurdle, unpublished data).
	Δ13p	c.4186-1 (IVS12-1) c.4186-2 (IVS12-2)	*BRCA1* exon 13 acceptor site is an experimentally validated tandem acceptor site (NAGNAG) subject to alternative splicing (Colombo et al., 2014). c.4186-1,-2 variants are predicted to inactivate the 5′ acceptor site, but not the 3′ acceptor site, thus producing Δ13p transcripts.
	Δ14p	c.4358-1 (IVS13-1) c.4358-2 (IVS13-2)	*BRCA1* exon 14 acceptor site is an experimentally validated tandem acceptor site (NAGNAG) subject to alternative splicing (Colombo et al., 2014). c.4358-1,-2 variants are predicted to inactivate the 5′ acceptor site, but not the 3′ acceptor site, thus producing Δ14p transcripts.
BRCA2	Δ12	c.6842-1 (IVS11-1) c.6842-2 (IVS11-2) c.6937 to non-G c.6937+1 (IVS12+1) c.6937+2 (IVS12+2)	Carriers of these variants are predicted to produce exon 12 skipping. BRCA2 Δ12 is a naturally occurring in-frame splicing event (Fackenthal et al., 2016). BRCA2 exon 12 is functionally redundant (Li et al., 2009).

* This summary table does not yet capture the possibility of acceptor site changes leading to small in-frame deletions > 3 bp, e.g. due to NAG $(NNN)_n$ NAG sites. It is recommended that bioinformatic prediction analysis is carried out for variation in/near *all* donor and acceptor sites to assess the likelihood that a variant will or will not cause alternative splicing.

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▶ **Table 5** Excerpt from https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf. *(Continued)*

Note: It could be argued that nonsense or frameshift variants in *BRCA1* exon 9, *BRCA1* exon 10, or *BRCA2* exon 12 may not be associated with high risk of cancer due to rescue by the expression of in-frame naturally occurring isoforms that bypass the premature termination codon and thus encode a functional protein. A review of multiple clinical and control datasets for the frequency of unique nonsense or frameshift variants – **adjusted for exon size** – does not provide strong support for this hypothesis at present (Spurdle, de la Hoya, unpublished data). Additional research is underway to further investigate the functional/clinical importance of germline nonsense or frameshift variants in these exons.

Moreover, further work is planned within ENIGMA (led by Paolo Radice) to document variants that have undergone splicing assays and are proven to be "leaky" variants, to provide a record of all spliceogenic variants for which additional research is necessary. This resource will identify variants that have already been classified using clinical data, as positive and negative controls for future quantitative mRNA studies.

A 5.2 *ATM*

The evaluation criteria for *ATM* are based on a combination of the following criteria:

- The 5-class IARC system for the assessment of the pathogenicity of *BRCA1* and *BRCA2* variants.
- The 3-class system to evaluate the pathogenicity of *ATM* variants [20] which includes in silico analyses such as Align-GVGD.
- The ACMG guidelines on the classification of variants [3, 21].
- Additional literature: [22 – 28].

Class1:

- If the allele frequency is ≥ 1 % (MAF ≥ 0.01) in large population groups (e.g. Caucasians, Africans, or Asians) or there is evidence of homozygous variant carriers in control populations. If this is the case, then the variant is always categorised as Class1. An allele frequency of ≥ 1 % in subpopulations with a low-diversity gene pool (examples: Finnish population, founder mutations!) is not sufficient.

Class2:

- If the allele frequency is ≥ 0.5–< 1 % (MAF ≥ 0.005–0.099) in large population groups (e.g. Caucasians, Africans, or Asians), the variant is always categorised as Class2.
- Missense variants which, according to in silico analysis (Align-GVGD, SIFT), are very probably neutral and/or outside the functionally critical domain (FATKIN).

Class3:

- All variants which cannot be categorised as Class1, 2, 4 or 5.

Class4:

- Variants with an in-frame deletion which are within the functionally critical domain (FATKIN).
- Missense variants which are within the functionally critical domain (FATKIN) and are, according to in silico analysis (Align-GVGD, SIFT), very probably harmful and described as functionally inactive.

Class5:

- Truncating *ATM* variants up to the FATKIN domain.
- Missense variants, in-frame deletion or splice mutations which reduce ATM protein expression to < 20 % for the mutated allele [28, 29].
- Variants associated with classic AT.

Splice variants: see *BRCA1* and *BRCA2*.

Functional domains: FATKIN with FAT; PI3K-related kinase; FATC ▶ **Table 6**.

Additional literature: [22 – 26, 28, 33, 35, 38].

▶ **Table 6** *ATM*, functional domains and relevance for the interpretation of the clinical importance of sequence variants – catalogue of clinically relevant functional domains and amino acids.

Region	AA start	AA end	AA alterations with demonstrated clinical importance (AT) characterising known functional domains	References and summary interpretation
Substrate binding	91	97	None reported	Domain location description [30] Also contains p53- and BRCA1-binding domain
NLS	385	388	None reported	Domain location description [28, 31]
Leucine zipper	1218	1238	None reported	Domain location description [25, 28]
Proline rich	1373	1382	None reported	Domain location description [25, 28]
FATKIN	1893	3056	Yes, e.g. p.(Val2424Gly) p.(2546_2548del), in frame p.(Asp2625Glu) p.(Ala2626Pro) p.(Val2716Ala) p.(Ser2855_Val2856delinsArgIle)	AA alterations and in-frame deletions [26, 28, 29, 32 – 35] Domain location description [25, 27, 28, 36, 37] **Domains:** FAT: 1893-2612 KIN: 2612-3056 with ATP-binding: 2716-2730, substrate (nibrin and p53) binding: 2682-3012, FATC with TIP60 binding: 3034-3056 Domain location description

A 5.3 *PALB2*

- The p.(Leu939Trp) mutation should be categorised as Class2 [39].

Additional literature: [35, 38, 40 – 46].

▶ **Table 7**.

▶ **Table 7** *PALB2*, functional domains and relevance for the interpretation of the clinical importance of sequence variants – catalogue of clinically relevant functional domains and amino acids.

Region	AA start	AA end	AA alterations with potential clinical importance	References and summary interpretation
BRCA1 interaction domain	9	43	Yes, e.g. p.(Leu35Pro)	Also covers oligomerisation domain/covers coiled-coiled motif Domain location description [47 – 49]; Amino acid alteration in VUS and functional analysis [50].
DNA-binding site	1	200	None reported	Domain location description [51]
RAD51 binding site	101	184	None reported	Domain location description [51, 52]
DNA-binding site	372	561	None reported	Covers also chromatin association motif (ChAM, 395-446) Domain location description [51, 53]
MRG15 (MORF4L1) interaction domain	611	764	None reported	Domain location description [48]
WD40 repeat	853	1186	Yes, e.g. p.(Thr1030Ile) p.(Leu1143Pro)	BRCA2 (1019-1098), RAD51C, XRCC3 and/or RAD51 complex formation Domain location description [51, 52, 54 – 57]. Amino acid alterations [48, 54, 58]

A 5.4 *CHEK2*

In exons 11–15, highly homologous, functionally inactive sequences (pseudogenes) on various other chromosomes (2, 7, 10, 13, 15, 16, X, and Y) [59, 60] which can superimpose the relevant sequences > long-range PCR of exons 11–15 and bioinformatic filtering of pseudogene reads, where possible.

Functional domains: SQ/TQ-rich domain*, forkhead-associated (FHA)** domain, kinase domain***, nuclear localisation signal (NLS) [61 – 63].

- To date, only truncating variants in the SQ/TQ-rich domain have been classified as pathogenic (▶ **Table 8**).
- Numerous known missense variants in the FHA domain. **Caution:** Consult FLOSSIES database during evaluation! (e.g. c.470C>T; p.Ile157Thr: Class2 [see § footnote ▶ **Table 8**]) or with unclear clinical relevance (e.g. c.434G>A, p.Arg145Gln; c.422A>C, p.Lys141Thr).
- Missense variants in the kinase domain with unclear clinical relevance: e.g. c.1216C>T, p.Arg406Cys.
- Similarly, in the NLS domain, only truncating variants have been classified as pathogenic to date (▶ **Table 8**).

The investigation by Ow et al. [63] gives an overview of the identified mutations in the *CHEK2* gene region. The publication by Roeb et al. (2012) includes a schematic overview of the functional domains as well as the results of functional analyses of the missense mutations located in these different *CHEK2* domains [62].

A 5.5 *TP53*

- IARC *TP53* database; the functional analyses by Kato et al. (2003) and Monti et al. (2007, 2011) are reliable [72 – 77].
- Functional domains: oligomerisation domain, core domain (DNA-binding).
- Possible dominant negative effect of missense variants and stop variants which affect the oligomerisation domain.
- Mosaic mutations and clonal haematopoiesis are possible, therefore watch out for the variant allele fraction when carrying out NGS analysis; if necessary, carry out further analysis to confirm germline mutations.

▶ **Table 9**.

▶ **Table 8** *CHEK2*, functional domains and relevance for the interpretation of the clinical importance of sequence variants – catalogue of clinically relevant functional domains and amino acids.

Region	AA start	AA end	AA alterations with Potential Clinical Importance	References and summary interpretation
SQ/TQ-rich	19	69	e.g. c.85C>T,p.Gln29*	[62, 65]
FHA	92 [115]	205 [175]	p.Arg117Gly, p.Arg145Trp, p.Gly167Arg	[61 – 63]
Kinase	212	501	c.1040A>C, p.Asp347Ala #; c.1100del; c.1164dup; p.Thr476Met, c.1169A>C, p.Tyr390Ser; c.1183G>T, p.Val395Phe#; c.1283C>T, p.Ser428Phe; c.1427C>T, p.Thr476Met	[61, 62], #ClinVar
NLS	515	538	e.g. c.1547delC, p.Ser516Leufs#; c.1555C>T, p.Arg519Ter#;	[63], #ClinVar

* SQ/TQ consensus sites are sites phosphorylated by ATM/ATR [116]. e.g. phosphorylation of Thr-68 is important for CHEK2 activation and oligomerisation.

** The phosphorylated Thr-68 site of CHEK2 interacts with the FHA domain of another CHEK2 molecule and thus leads to the formation of CHEK2 oligomers [66].

*** [67]

§ The *CHEK2* c.470T>C p.Ile157Thr variant, although still present with various classifications (VUS/likely pathogenic/pathogenic) in ClinVar and other databases has been reclassified (date: 28.08.2018) as Class2/likely benign by the German Consortium on HBOC on the following basis:
It is frequently listed in large unaffected control cohorts (0.5 %, gnomAD V. 2.1.1, non-cancer). The population frequency in Finnish Europeans is 2.5 % (10 homozygous carriers). In addition, it is present in 47/7325 individuals (0.64 %) in the FLOSSIES database (non-cancer female controls of European descent aged > 70 years). Although showing functionally impaired dimerisation and autophosphorylation [61, 62, 68], numerous large case-control studies show results indicating low or no increased breast cancer risk [64, 69]: breast cancer OR = 1.58 (1.42–1.75), colon cancer OR = 1.67 (1.24–2.26) [70, 71]. Additionally, it has been observed with a frequency of 2 % in controls [2]. However, it may act as a polygenic risk allele.

▶ **Table 9** *p53*, functional domains and relevance for the interpretation of the clinical importance of sequence variants – catalogue of clinically relevant functional domains and amino acids.

Region	AA start	AA end	AA alterations with demonstrated clinical importance (including conflicting interpretations of pathogenicity but criteria provided)	References and summary interpretation
Transcription activation	1	55	p.(Val10Ile) p.(Val31Ile) p.(Pro47Ser)	Amino acid alterations (ClinVar) Domain location description [78] Also binding site for numerous proteins including HDM2 (amino acids 15-29; IARC)
Proline-rich domain	61	94	p.(Pro82Leu) p.(Ala83Val)	Amino acid alterations (ClinVar) Domain location description [78]
DNA-binding region	102	292	p.(Gly105Asp) p.(Lys120Glu) p.(Thr125Met) p.(Ser127Phe) p.(Asn131Tyr) p.(Cys141Tyr) p.(Pro151Ser) p.(Pro151Thr) p.(Pro152Leu) p.(Arg156His) p.(Arg158Cys) p.(Arg158His) p.(Tyr163Asp) p.(Tyr163Cys) p.(Arg175Leu) p.(Arg175His)	Amino acid alterations (ClinVar) Domain location description (IARC) Also binding site for numerous proteins including 53BP1 (IARC) and RAD51 amino acids 94-160 and 264-315 [79]

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▸ **Table 9** *p53*, functional domains and relevance for the interpretation of the clinical importance of sequence variants – catalogue of clinically relevant functional domains and amino acids. *(Continued)*

Region	AA start	AA end	AA alterations with demonstrated clinical importance (including conflicting interpretations of pathogenicity but criteria provided)	References and summary interpretation
			p.(Cys176Tyr) p.(His179Tyr) p.(Arg181Cys) p.(Arg181His) p.(Ala189Val) p.(His193Arg) p.(His193Leu) p.(Leu194Phe) p.(Ile195Thr) p.(Arg213Gln) p.(Val.216Met) p.(Tyr220Cys) p.(Tyr220Ser) p.(Ile232Thr) p.(Tyr234Cys) p.(Asn235Ser) p.(Tyr236Asp) p.(Met237Val) p.(Met237Ile) p.(Cys238Tyr) p.(Ser241Phe) p.(Cys242Tyr) p.(Gly245Asp) p.(Gly245Ser) p.(Gly245Cys) p.(Met246Val) p.(Met246Leu) p.(Met246Arg) p.(Arg248Gln) p.(Arg248Trp) p.(Ile251Leu) p.(Ile251Ser) p.(Thr256Ala) p.(Leu257Arg) p.(Glu258Lys) p.(Arg267Trp) p.(Arg267Gln) p.(Val272Leu) p.(Arg273Hisv p.(Arg273Cys) p.(Cys275Tyr) p.(Cys277Tyr) p.(Arg280Thr) p.(Asp281Val) p.(Asp281Gly) p.(Arg282Gly) p.(Arg282Leu) p.(Arg282Trp) p.(Arg283His) p.(Arg283Lys) p.(Glu286Lys)	
Oligomerisation region	325	356	p.(Gly325Val) p.(Arg337Leu) p.(Arg337Cys) p.(Glu339Lys) p.(Arg342Pro)	Amino acid alterations (ClinVar) Domain location description (IARC) Also binding site for numerous proteins Covering main nuclear localisation signal (amino acids 316-322) [80, 81]
Basic (repression of DNA-binding region)	369	388	None reported	Domain location description (IARC) Also binding site for numerous proteins including RAD54 [82]

A 5.6 *RAD51D*

Functional domains: N-terminal domains and ATP-binding domain with the highly conserved Walker A and B motifs [83 – 85].

▸ **Table 10**.

Additional literature: [91 – 94]

A 5.7 *RAD51C*

References: [91, 95 – 101]

Functional domains: DNA repair/recombination protein RecA-like, ATP-binding domain

▸ **Table 11**.

A 5.8 *BRIP1*

▸ **Table 12**.

Additional literature: [1, 102, 105 – 110]

▶ **Table 10** *RAD51D*, functional domains and relevance for the interpretation of the clinical importance of sequence variants – catalogue of clinically relevant functional domains and amino acids.

Region	AA start	AA end	AA alterations with demonstrated clinical importance (including conflicting interpretations of pathogenicity)	References and summary interpretation
N-terminal region	1	83	None reported	N-terminal domain required for ssDNA-specific binding function [86]
Linker	60	78	None reported	Proper interaction with RAD51C and XRCC2 [85]
ATPase domain and RAD51B, RAD51C, and XRCC2 binding	99	274	p.(G112A) (disrupts binding of RAD51D to RAD51C [87]) p.(S207L) (disrupts RAD51D-XRCC2 interaction [85]) p.(A210V) (predicted to be potentially pathogenic [88, 89]) p.(R266C) [90], Meindl et al. (unpublished)	ATPase, AAA+ type Walker A and B motifs crucial for HR. These motifs are also implicated in binding to RAD51C and XRCC2.

▶ **Table 11** *RAD51C*, functional domains and relevance for the interpretation of the clinical importance of sequence variants – catalogue of clinically relevant functional domains and amino acids.

Region	AA start	AA end	AA alterations with demonstrated clinical importance (including conflicting interpretations of pathogenicity)	References and summary interpretation
N-terminal region	1	66	None reported	Homology-derived putative DNA-binding domain [91]
ATPase domain and RAD51B, XRCC3, and RAD51D binding	79	376	p.(Gly125Val) p.(Cys135Tyr) p.(Leu138Phe) p.(Gly153Asp) p.(Asp159Asn) p.(Val169Ala) p.(Leu219Ser) p.(Arg258His) p.(Gly264Ser)	Amino acid alterations and functional consequences [95 – 98] Domain location description [91] ATPase domain includes Walker A nucleotide binding motif (amino acids 125-132) and Walker B nucleotide binding motif (amino acids 238-242) [91, 99]
Nuclear localisation signal	366	370	None reported	Domain location description [99]

▶ **Table 12** *BRIP1*, functional domains and relevance for the interpretation of the clinical importance of sequence variants – catalogue of clinically relevant functional domains and amino acids.

Region	AA start	AA end	AA alterations with demonstrated clinical importance (including conflicting interpretations of pathogenicity)	References and summary interpretation
DEAD/DEAH box helicase domain	17	441		Domain location description [102]
Helicase superfamily c-terminal domain	697	851		Domain location description [102]
The BRCA1 interacting region of BRIP1	976	1006	Phosphorylation of FANCJ at Ser-990 is important for its interaction with BRCA1	[103]
MLH1 interaction			Lysines 141 and 142 are required for direct interaction of FANCJ with MLH1	[104]
Nuclear localisation signal	158	175	None reported	Domain location description [102]

A 5.9 CDH1

References:
Focuses predominantly on molecular genetics: [111]
Review of functional analyses: [112]
Review of the HDGC Consortium: [113]
Review of lobular breast cancer: [114]
Distribution of pathogenic variants at the *CDH1* locus [111]

Known pathogenic CDH1 variants are distributed across the entire locus; it is therefore not possible to define a clinically relevant functional protein domain. The last known truncating pathogenic variant in the last exon is c.2506G>T (p.Glu836*) [115]. All truncating variants upstream must therefore be categorised at least as Class4.

The proposal put forwards by the ClinGen Consortium to categorise variants with a MAF > 0.2% as ACMG Class1, contrary to IARC guidelines, is currently being debated.

CDH1 Rule Specifications for the ACMG/AMP Variant Curation Guidelines ClinGen (https://www.clinicalgenome.org/site/assets/files/8816/clingen_cdh1_acmg_specifications_v1.pdf).

Conflict of Interest

The authors declare that they have no conflict of interest.

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Kriterien des Deutschen Konsortiums Familiärer Brust- und Eierstockkrebs zur Klassifizierung von Keimbahn-Sequenzvarianten in Risikogenen für familiären Brust- und Eierstockkrebs

Criteria of the German Consortium for Hereditary Breast and Ovarian Cancer for the Classification of Germline Sequence Variants in Risk Genes for Hereditary Breast and Ovarian Cancer

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ZUSAMMENFASSUNG

Das Deutsche Konsortium für Familiären Brust- und Eierstockkrebs (GC-HBOC) etablierte vor über 10 Jahren eine Expertengruppe (VUS Task Force), um die von den einzelnen Zentren des GC-HBOC an die zentrale Datenbank in Leipzig gemeldeten Varianten hinsichtlich ihrer Klassifizierung zu überprüfen und ggf. nach aktueller Datenlage neu einzustufen. Die innerhalb der VUS-Task Force konsentierten Variantenbewertungen und resultierenden -klassifizierungen werden in einer zentralen Datenbank hinterlegt und sind als Grundlage zu berücksichtigen, um eine einheitliche Bewertung bereits bekannter wie auch neu identifizierter Varianten innerhalb der verschiedenen Zentren des GC-HBOC zu gewährleisten. Die standardisierte VUS-Bewertung durch die VUS Task Force ist ein zentrales Element des vom GC-HBOC ebenfalls etablierten Recall-Systems. Dieses dient der Weitergabe der Informationen an die in den Zentren betreuten Familien im Falle einer aufgrund neuer Erkenntnisse aktualisierten Neubewertung von bereits klassifizierten Varianten. Die in Anlehnung an international etablierte Bewertungsverfahren (IARC, ACMG, ENIGMA) angepassten Bewertungsalgorithmen der VUS Task Force werden in diesem Artikel anhand der zugrundeliegenden Entscheidungskriterien präsentiert, die gemäß eines priorisierenden Fließschemas zum Klassifizierungsergebnis führen. Weiterhin werden genspezifische Regelungen und Besonderheiten, die für einzelne mit Brust- und/oder Eierstockkrebs assoziierte Risikogene zu berücksichtigen sind, in einzelnen Unterkapiteln dargelegt. Um dem Umfang und der Dynamik des aktuellen Wissens zur Variantenbewertung gerecht zu werden, sind neben umfangreichen Literaturverweisen insbesondere auch die URLs von relevanten Datenbanken angegeben. In Zukunft sollen an neue Erkenntnisse angepasste Kriterien auf der Webseite von GC-HBOC (https://www.konsortium-familiaerer-brustkrebs.de/) veröffentlicht werden.

ABSTRACT

More than ten years ago, the German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC) set up a panel of experts (VUS Task Force) which was tasked with reviewing the classifications of genetic variants reported by individual centres of the GC-HBOC to the central database in Leipzig and reclassifying them, where necessary, based on the most recent data. When it evaluates variants, the VUS Task Force must arrive at a consensus. The resulting classifications are recorded in a central database where they serve as a basis for ensuring the consistent evaluation of previously known and newly identified variants in the different centres of the GC-HBOC. The standardised VUS evaluation by the VUS Task Force is a key element of the recall system which has also been set up by the GC-HBOC. The system will be used to pass on information to families monitored and managed by GC-HBOC centres in the event that previously classified variants are reclassified based on new information. The evaluation algorithm of the VUS Task Force was compiled using internationally established assessment methods (IARC, ACMG, ENIGMA) and is presented here together with the underlying evaluation criteria used to arrive at the classification decision using a flow chart. In addition, the characteristics and special features of specific individual risk genes associated with breast and/or ovarian cancer are discussed in separate subsections. The URLs of relevant databases have also been included together with extensive literature references to provide additional information and cover the scope and dynamism of the current state of knowledge on the evaluation of genetic variants. In future, if criteria are updated based on new information, the update will be published on the website of the GC-HBOC (https://www.konsortium-familiaerer-brustkrebs.de/).

Allgemeine Grundlagen

Die Kriterien des Deutschen Konsortiums Familiärer Brust- und Eierstockkrebs (http://www.konsortium-familiaerer-brustkrebs.de/) zur Klassifizierung von Keimbahn-Sequenzvarianten in Risikogenen für Brust-und Eierstockkrebs wurden durch die oben genannten Mitglieder des Expertengremiums zur Variantenbewertung (VUS Task-Force) des Deutschen Konsortiums Familiärer Brust- und Eierstockkrebs erarbeitet. Aufgabe dieser Expertengruppe ist es, innerhalb des Deutschen Konsortiums Familiärer Brust- und Eierstockkrebs verbindliche Kriterien für die Bewertung von Varianten vorzugeben und die Klassifizierung von Varianten zu überprüfen um eine einheitliche Bewertungen von Varianten innerhalb des Konsortiums sicherzustellen. Die vorliegenden Kriterien beruhen auf einem IARC[1] 5-Klassen-System für Hochrisikogene[2] basierend auf den Richtlinien des ENIGMA[3]-Konsortiums (ENIGMA *BRCA1/2* classification criteria Version 2.5.1, June 2017) sowie den ACMG[4]- und ACGS[5]-Richtlinien. Innerhalb dieses 5-Klassen-Systems erfolgt eine Bewertung von Sequenzvarianten der Keimbahn hinsichtlich ihrer Relevanz für einen Funktionsverlust des codierten Proteins (Class1: neutral, Class2: wahrscheinlich neutral, Class3: unklare Datenlage/keine sichere Bewertung, Class4: wahrscheinlicher relevanter Funktionsverlust, Class5: relevanter Funktionsverlust; Signifikanzlevel, siehe [4]). Für Gene mit hoher Penetranz (wie z. B. *BRCA1, BRCA2*), für die eine klinische Korrelation (Pathogenität) mit einem Funktionsverlust beschrieben ist, ergibt die funktionelle Klassifizierung eine Pathogenitätsbeurteilung entsprechend dem IARC-5-Klassensystem (Class1: not pathogenic bis Class5: pathogenic), siehe Anhang A 2, ▶ **Tab. 1**. Vorteil dieses strukturierten Vorgehens ist, dass zunächst Kriterien abgefragt werden, die eine schnelle und eindeutige Klassifizierung erlauben und erst im Anschluss eine umfangreiche Daten- und Literaturrecherche erfolgt (siehe Anhang A 2, ▶ **Tab. 2**: Übersicht relevanter Datenbanken und A 3, ▶ **Abb. 1**: Fließschema zu den Bewertungskriterien). Hinsichtlich der Klassifizierung von Sequenzvarianten der Gene *ATM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, PALB2, RAD51C, RAD51D* und *TP53*, den sogenannten „Core“-Genen des TruRisk-Panels (Version 1/2018, siehe unter: Homepage des Deutschen Konsortiums, http://www.konsortium-familiaerer-brustkrebs.de/) sind die in Anhang A 5 aufgeführten Besonderheiten zu berück-

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[1] International Agency for Research on Cancer.

[2] Hochrisikogene: mind. eine Sequenzvariante mit Odds Ratio für Brust- und/oder Eierstockkrebs OR > 5 (z. B. *BRCA1, BRCA2, RAD51C, PALB2, TP53, ATM*), siehe auch [1, 2].

[3] Evidence based Network for the Interpretation of Germline Mutant Allels, http://enigmaconsortium.org/

[4] American College of Medical Genetics and Genomics (ACMG [3]).

[5] Association for Clinical Genomic Science (ACGS, http://www.acgs.uk.com/).

sichtigen. Bei moderat/niedrig-penetranten Genen erfolgt dagegen lediglich eine Bewertung bezüglich ihrer Funktionalität (hier: Funktionsverlust nicht gleichzusetzen mit „Pathogenität“). Auch bei Hochrisikogenen können Varianten nachweislich nur mit einem intermediären Risiko einhergehen [5, 6].

Kriterien zur Interpretation von mRNA-Analysen

Die Kriterien des Deutschen Konsortiums familiärer Brust- und Eierstockkrebs zur Bewertung von Sequenzvarianten mit nachfolgender mRNA-Analyse beruhen ebenfalls auf den Richtlinien des ENIGMA-Konsortiums (siehe dazu auch [7]). Für die empirische, prädiktive Vorhersage von potenziellen Spleißvarianten mittels der 3 häufig verwandten Vorhersageprogramme sind in A 1 die entsprechenden Grenzwerte angegeben. Eine schematische Darstellung der von der VUS-Task-Force betrachteten Bereiche zur Bewertung von Spleißvarianten zeigt A 4 (▶ **Abb. 2**). Die mRNA-Analysen werden an Frischblut, kultivierten Lymphozyten, kultivierten lymphoblastoiden Zelllinien etc. durchgeführt und parallel mit mindestens 5 Kontrollen gleichen Materialtyps verglichen. Eine Sequenzvariante wird dann als pathogen bezeichnet, wenn diese wie folgt einen Effekt auf die mRNA-Transkription hat: Es werden ausschließlich ein oder mehrere aberrante Transkripte des varianten Allels nachgewiesen, welche zu einem Stopp-Codon oder einer In-Frame-Deletion führen und in der Zerstörung bekannter funktioneller Domänen resultieren. Zur Bestimmung der Transkriptmenge (semiquantitative oder quantitative Methoden) wird die Sequenzierung des Full-Length-Transkripts des varianten Allels oder bei Vorliegen einer intronischen Variante eines in *cis* liegenden Polymorphismus als ausreichend angesehen (Nachweis monoallelischer Expression). Varianten, die dagegen ein Transkriptmuster vergleichbar mit dem Mittelwert der Kontrollen zeigen, werden aufgrund einer fehlenden aberranten mRNA als neutral/nicht pathogen bewertet. Bezüglich des cDNA-Primer-Designs sollten die physiologischen Spleißvarianten/natürlich vorkommenden Isoformen berücksichtigt werden (siehe auch [8, 9]).

Cave: Bestimmte *BRCA1*- und *BRCA2*-Varianten ± 1, 2 bp von der Exongrenze entfernt, welche vorhergesagt oder nachgewiesen per Allel zu mindestens 20–30 % natürlich auftretender In-Frame-RNA-Isoformen führen, können vermutlich zu einer noch vorhandenen Restaktivität des Proteins führen (siehe auch [9–12]) und werden, wenn nicht anders nachgewiesen, als VUS Class3 bewertet (siehe auch Übersicht, Anhang 5, ▶ **Tab. 5**)

Vorgehensweise der VUS Task Force

Das Konsortium empfiehlt routinemäßig die Erstellung eines Genbefundes über 10 Gene (Stand 8/19), die evidenzbasiert mit Brust- und/oder Eierstockkrebs assoziiert werden konnten: *ATM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, PALB2, RAD51C, RAD51D* und *TP53* (http://www.konsortium-familiaerer-brustkrebs.de/).

Zur konsentierten Klassifizierung von neu gemeldeten Sequenzvarianten sowie bei Evidenzen für eine Neubewertung bereits bekannter Varianten finden monatliche Telefonkonferenzen sowie ggf. Arbeitstreffen des „Expertengremiums des Konsortiums zur Bewertung von Varianten mit unklarer Signifikanz (VUS Task-Force) statt. Im Falle einer Neubewertung werden durch die zentrale Datenbank des Konsortiums alle Zentren über diese Neuklassifizierung in Kenntnis gesetzt (Recall-System).

Es wird weiterhin ausdrücklich darauf hingewiesen, dass sich die Bewertung von Varianten aufgrund neuer Erkenntnisse ändern kann und diese regelmäßig einer Überprüfung durch das Expertengremium unterzogen werden. Ebenso kann sich durch neuere Erkenntnisse die Liste der Kerngene ändern, für die, innerhalb des Deutschen Konsortiums für Brust- und Eierstockkrebs, eine Erstellung eines Befundes empfohlen wird. Dieses, wie auch die Einarbeitung neuerer Erkenntnisse in die hier vorgestellten Klassifizierungsregelungen, werden auf der Internetseite des Deutschen Konsortiums für Familiären Brust- und Eierstockkrebs (http://www.konsortium-familiaerer-brustkrebs.de/) veröffentlicht werden.

Klassifizierung von Sequenzvarianten hinsichtlich funktioneller Relevanz

1. **Class1 (funktionell nicht relevant/ohne Funktionsverlust) wenn eines der folgenden Kriterien erfüllt ist:**
   - 1.1 Allelfrequenz der Varianten ≥ 1 % (Minor Allele Frequency [MAF] ≥ 0,01) in den Großpopulationen z. B. Kaukasier, Afrikaner oder Asiaten. **Cave:** Eine Allelfrequenz ≥ 1 % in Subpopulationen mit wenig durchmischtem Genpool (Bsp.: finnische Population, Gründermutationen!) ist nicht ausreichend.
   - 1.2 Varianten mit einer multifaktoriell berechneten Wahrscheinlichkeit von < 0,001, pathogen zu sein.
     Cave: Gilt derzeit nur für die Hochrisikogene *BRCA1/2* (exemplarische Berechnung siehe [13]).
   - 1.3 Varianten in Hochrisikogenen welche in geeigneten Kollektiven von nicht erkrankten Personen (▶ **Tab. 1**) bei mindestens 10 Individuen auftreten.
2. **Class2 (wahrscheinlich ohne Funktionsverlust/funktionell nicht relevant) wenn eines der folgenden Kriterien erfüllt ist:**
   - 2.1 Allelfrequenz der Varianten 0,5–1 % (MAF 0,005–0,01) in den Großpopulationen z. B. Kaukasier, Afrikaner oder Asiaten. **Cave:** Eine Allelfrequenz von 0,5–1 % in Subpopulationen mit wenig durchmischtem Genpool (Bsp.: finnische Population, Gründermutationen!) ist nicht ausreichend.
   - 2.2 Exonische Varianten (A), die zur Substitution einer Aminosäure (Missense-Varianten) oder kleine In-Frame-Insertionen/Deletionen (Insertionen/Deletionen einer oder weniger Aminosäure[n]) führen und deren A-priori-Wahrscheinlichkeit für Pathogenität ≤ 2 % ist (A-GVGD Analyse, http://priors.hci.utah.edu/PRIORS/), intronische Varianten (B), die **mehr als − 20 bp, + 10 bp von der Exongrenze entfernt sind**, und synonyme Varianten (C), wenn diese Varianten (A–C) laut bioinformatischer Vorhersageprogrammen (siehe Anhang A 1) den Spleißmechanismus mit großer Wahrscheinlichkeit nicht verändern. Für die Nicht-*BRCA1/2*-Gene müssen die genannten Varianten in Großpopulationen mit einer Allelfrequenz von 0,001 ≤ MAF < 0,01 auftreten.

2.3 synonyme Substitutionen oder intronische Varianten, die keine mRNA-Aberrationen in Form von Exon-Deletionen/Duplikationen oder monoallelischer Expression des Wildtyp-Transkripts (wt) in „In-vitro“-Labortests zeigen auch wenn sie laut bioinformatischer Vorhersageprogramme (Programme und Schwellenwerte siehe Anhang A 1) den Spleißmechanismus mit großer Wahrscheinlichkeit verändern.

2.4 Varianten, die im gleichen Gen mit einer eindeutig pathogenen Variante in trans auftreten (Co-Occurrence), wenn gesichert ist, dass ein homozygoter oder compound heterozygoter Genotyp mit einem bekannten klinisch eindeutigen Phänotyp assoziiert ist.

2.5 Varianten mit einer multifaktoriell berechneten Wahrscheinlichkeit, pathogen zu sein, von 0,001–0,049.
Cave: Gilt derzeit nur für die Hochrisikogene *BRCA1/2* (exemplarische Berechnung siehe: [13]).

2.6 Exonische Varianten, die für denselben Aminosäureaustausch wie eine bereits als Class1 bewertete Sequenzvariante codieren, aber auf einen abweichenden Nukleotidaustausch beruhen, wenn sie keine auffällige Spleißvorhersage aufweisen.

2.7 Missense-Varianten, für welche Informationen aus funktionellen Analysen etc. vorliegen, die jedoch für eine multifaktorielle Klassifizierung nicht ausreichen, und durch Expertengremien (z. B. ENIGMA) als Class2 eingestuft werden.

3. Class3 (unklare funktionelle Relevanz), wenn eines der folgenden Kriterien erfüllt ist: Varianten, die nicht eindeutig Class1, Class2, Class4, oder Class5 zugeordnet werden können, z. B.:

3.1 Sonderfälle, die nach den Bewertungskriterien in einer der anderen Klassen eingeordnet werden könnten, aber im Anhang A 5 zu den Besonderheiten der einzelnen Kerngene oder in Tabelle 5, Appendix der *BRCA1/2* classification criteria Version 2.5.1, July 2017 (ENIGMA) aufgeführt sind (▶ **Tab. 5**).

3.2 Varianten mit widersprüchlicher Datenlage bezüglich ihrer Bewertung und noch ausstehenden weiterführenden Untersuchungen.

3.3 Varianten, die bis **−20 bp, +10 bp von der Exongrenze entfernt** sind, welche laut bioinformatischer Vorhersageprogrammen (siehe Anhang A 1) den Spleißmechanismus mit großer Wahrscheinlichkeit verändern, sofern eine In-vitro-mRNA-Analyse noch nicht vorliegt (siehe auch ▶ **Abb. 2**, Schematische Darstellung der Spleißstellen).

3.4 Exon-Duplikationen ohne weiterführende Analysen (z. B. Bruchpunktanalysen, cDNA-Analyse etc.).

3.5 Varianten mit einer multifaktoriell berechneten Wahrscheinlich, pathogen zu sein, von 0,05–0,949.
Cave: Gilt derzeit nur für die Hochrisikogene *BRCA1/2* (exemplarische Berechnung siehe [13]).

4. Class4 (wahrscheinlich mit Funktionsverlust/funktionell relevant), wenn eines der folgenden Kriterien erfüllt ist:

4.1 Varianten mit einer multifaktoriell berechneten Wahrscheinlich von 0,95–0,99, pathogen zu sein.
Cave: Gilt derzeit nur für die Hochrisikogene *BRCA1/2* (exemplarische Berechnung siehe Goldgar et al., 2004 [13]).

4.2 Varianten, welche einen frühzeitigen Stopp der Proteinbiosynthese codieren (Nonsense- oder Frame-Shift-Varianten) und welche nicht den Verlust bekannter klinisch relevanter funktioneller Proteindomänen bedingen, sofern die Stopp-Kodons nicht Downstream der Nonsense-mediated-Decay-(NMD-)relevanten Position, 50 Basenpaare vor dem Ende des vorletzten Exons, liegen.

4.3 Intronische Varianten an Position ± 1,2 oder G > Nicht-G an letzter Position des Exons: Wenn positive Spleißvorhersage (siehe Anhang A 1) vorliegt und die ersten 6 Basen im Intron nicht GTRRGT lauten, und eine aberrante In-vitro-mRNA-Analyse **noch nicht** vorliegt (d. h. durch Experten-Review [noch] nicht bestätigt oder z. B. Exonskipping oder allelspezifische Transkript-Expression, Loss-of-Function als Pathomechanismus nachgewiesen wurde).

Ausnahmen:

- Eine kryptische Spleißstelle (AG/GT) in der Nähe wird aktiviert und das (vorhergesagte) neue Exon wird In Frame gespleißt (**→ Class3**)
- Das (vorhergesagte) geskippte Exon (bzw. Exons) wird in relevantem Umfang alternativ gespleißt (**→ Class3**).
- Das (vorhergesagte) geskippte Exon (bzw. Exons) wird In Frame gespleißt und enthält keine bekannte funktionelle Domäne (**→ Class3**)

4.4 Varianten, welche den gleichen Aminosäureaustausch wie eine bereits als Class5 bewertete, pathogene Missense-Variante codieren, aber durch einen anderen Nukleotid-Austausch bedingt sind, und wenn keine positive Spleißvorhersage (siehe Anhang A 1) vorliegt.

4.5 In-Frame-Deletionen (auch schon bei nur einer Aminosäure), welche zum Verlust einer bereits als Class5 bewerteten Missense-Variante und die zur Unterbrechung bekannter, funktionell wichtiger Domänen führen.

4.6 Große In-Frame-Deletionen, die zur Unterbrechung/Verlust bekannter, funktionell wichtiger Domänen führen.

4.7 Über In-vitro-mRNA-Analysen verifizierte In-Frame-Insertionen, die zur Unterbrechung funktionell wichtiger Domänen führen.

4.8 Varianten, welche zu einer Veränderung des Translations-initiations-Codon (AUG, Methionin) führen und für die keine Evidenz (z. B. alternatives Start-Codon in unmittelbarer Nähe) für eine alternative Klassifikation vorliegt.

4.9 Varianten, für die Informationen aus funktionellen Analysen, klinische Daten etc. vorliegen, die jedoch für eine multifaktorielle Klassifizierung nicht ausreichen, und die durch Expertengremien (z. B. ENIGMA) als Class4 eingestuft werden.

5. Class5 (Funktionsverlust/funktionell relevant) wenn eines der folgenden Kriterien erfüllt ist:

5.1 Varianten, welche einen frühzeitigen Stopp der Proteinbiosynthese codieren (Nonsense- oder Frame-Shift-Varianten), welche die Expression bekannter klinisch relevanter funktioneller Proteindomänen unterbindet.

5.2 Varianten mit einer multifaktoriell berechneten Wahrscheinlichkeit, pathogen zu sein, von > 0,99.
Cave: Gilt derzeit nur für die Hochrisikogene *BRCA1/2* (exemplarische Berechnung siehe Goldgar et al., 2004 [13]).

5.3 Spleiß-Varianten, bei denen im Rahmen einer In-vitro-mRNA-Analyse ein Frame-Shift-Effekt nachgewiesen wurde, der zu einem frühzeitigen Stopp der Proteinbiosynthese führt und die Expression bekannter klinisch relevanter funktioneller Proteindomänen unterbindet und für die ein Wildtyp-Transkript des mutierten Allels nicht nachweisbar ist (monoallelische Expression).

5.4 Spleiß-Varianten, bei denen im Rahmen einer In-vitro-mRNA-Analyse eine In-Frame-Deletion/Insertion nachgewiesen wurde, die zur Unterbrechung oder zum Verlust einer bekannten klinisch relevanten funktionellen Domäne oder zu einer funktionell inaktivierenden Änderung der Proteinstruktur führt und für die ein Wildtyp-Transkript des mutierten Allels nicht nachweisbar ist (monoallelische Expression).

5.5 Copy-Number Deletions-Varianten, die in der Unterbrechung oder dem Verlust einer oder mehrerer, bekannter klinisch relevanter funktioneller Domänen beinhaltender Exons resultieren oder in einer Leserasterverschiebung, welche laut Vorhersage zur Inaktivierung bekannter klinisch relevanter funktioneller Domänen führt.

5.6 Copy-Number Duplikations-Varianten jeglicher Größe, die durch Laboranalysen bestätigt wurden, welche ein oder mehrere Exons duplizieren und zu einer Leserasterverschiebung führen, welche laut Vorhersage in einer Inaktivierung bekannter klinisch relevanter funktioneller Domänen resultiert.

Anhang

A 1. Spleißvorhersageprogramme und deren Schwellenwerte

Als recht zuverlässig können die Spleißvorhersageprogramme MaxEntScan (MES), Splice Site Finder (SSF) Human Splicing Finder (HSF) betrachtet werden, deren Verwendung daher zur Bewertung von möglichen Effekten auf den Spleißprozess erfolgen sollte. MaxEnt Scan gilt als auffällig ab einer Abweichung von Delta ≥ 15 % [14], Human Splicing Finder ab einem Delta von ≥ 4,1 % [10] sowie Splice Site Finder ab einem Delta von ≥ 5 % [14]. Bei auffälliger Prädiktion (mindestens 2 der 3 unten genannten Programme) ist zur Abklärung eine mRNA-Analyse notwendig. Voraussetzung ist, dass die physiologische Spleißstelle von der jeweiligen Prädiktionssoftware mit folgenden Schwellenwerten erkannt wird.

Die Schwellenwerte (berechnet für *BRCA1/2* [14]) liegen bei:

1. MES > 3
2. SSF > 60
3. HSF > 80

Diese Schwellenwerte können in Annäherung auch für die weiteren Gene verwendet werden. Sollten spezifische Schwellenwerte definiert werden, sind diese zu verwenden.

A 2.

▶ **Tab. 1** und **2**.

▶ **Tab. 1** IARC 5-tiered classification system with accompanying recommendations for family management[a] (Auszug aus https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf).

Class	Quantitative measure: probability of pathogenicity	Predictive testing of at-risk relatives	Surveillance of at-risk relatives	Research testing of relatives
5: Pathogenic	> 0.99	Yes	Full high-risk guidelines for variant carriers	Not indicated
4: Likely pathogenic	0.95–0.99	Yes[b]	Full high-risk guidelines for variant carriers	Yes
3: Uncertain	0.05–0.949	No[b]	Based on family history & other risk factors	Yes
2: Likely not pathogenic or of little clinical significance	0.001–0.049	No[b]	Based on family history & other risk factors – treat as „no *BRCA1/2* pathogenic variant detected“ for this disorder	Yes
1: Not pathogenic or of no clinical significance	< 0.001	No[b]	Based on family history & other risk factors – treat as „no *BRCA1/2* pathogenic variant detected“ for this disorder	Not indicated

[a] Adapted for clarity from the original published tabular presentation (Plon et al., 2008 [4])

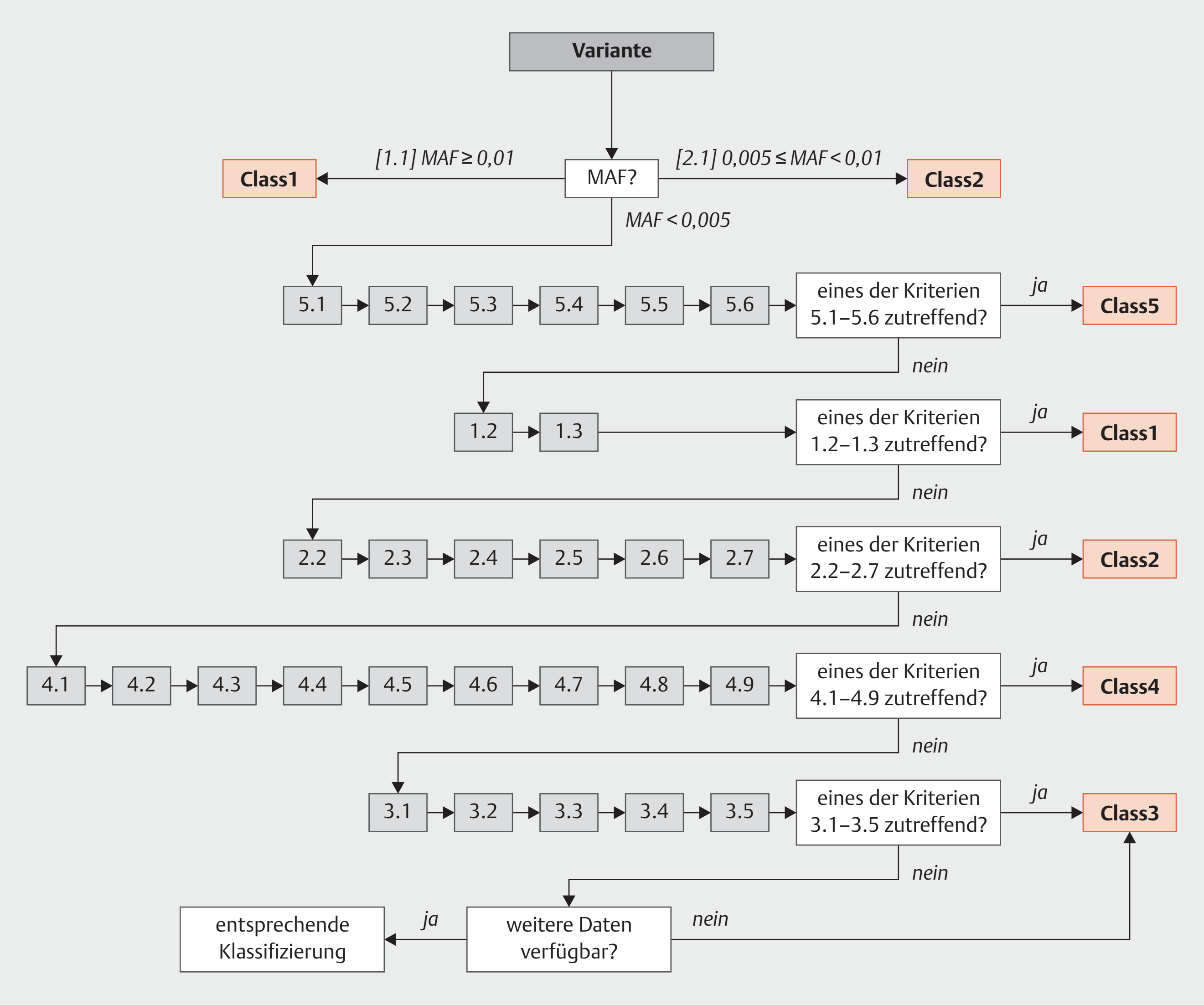
[b] Continued testing of proband for any additional available testing modalities available for BRCA1/2, e. g. rearrangements, is recommended.

▸ **Tab. 2** Für die Bewertung relevante Literatur- und Datenbanken.

Datenbank	URL
1000Genomes	http://www.1000genomes.org/
ARUP *(BRCA1)*	http://arup.utah.edu/database/BRCA/Variants/BRCA1
ARUP *(BRCA2)*	http://arup.utah.edu/database/BRCA/Variants/BRCA2
BIC (**B**reast Cancer **I**nformation **C**ore)	http://research.nhgri.nih.gov/bic/
BRCA1 CIRCOS (via BIC oder, **Cave:** BIC-Nomenklatur angeben!)	https://research.nhgri.nih.gov/projects/bic/circos/search.shtml
BRCA Exchange	http://brcaexchange.org
ClinVar (ClinGen)	https://www.ncbi.nlm.nih.gov/clinvar/
Database of Functional Classifications of *BRCA1* Variants based on Saturation Genome Editing [15, 16]	https://sge.gs.washington.edu/BRCA1/
dbSNP	https://www.ncbi.nlm.nih.gov/snp
EVS/ESP	http://evs.gs.washington.edu/EVS/
Exome Aggregation Consortium ExAC Browser	http://exac.broadinstitute.org/
exUV-LOVD (multifaktoriell reklassifizierte *BRCA1/2*-Varianten)	http://hci-exlovd.hci.utah.edu/home.php
FLOSSIES (**F**abulous **L**adies **O**ver **S**eventy: Keimbahnvarianten in 27 brustkrebsrelevanten Genen bei ca. 10 000 Frauen > 70 J., bisher nicht an Krebs erkrankt)	https://whi.color.com/
Genome Aggregation Database GnomAD	http://gnomad.broadinstitute.org/
Google Scholar	http://scholar.google.de/
HGMD Professional (Registrierung erforderlich)	https://portal.biobase-international.com/
IARC *TP53* Datenbank	http://p53.iarc.fr/
InSiGHT Consortium (Classification Database)	http://www.insight-database.org/classifications/
KAVIAR (Known VARiants, genomic)	http://db.systemsbiology.net/kaviar/cgi-pub/Kaviar.pl
LOVD (Leiden Open Variation Database, allgemein)	http://www.lovd.nl/3.0/home
LOVD-Datenbank, *ATM*	https://databases.lovd.nl/shared/genes/ATM
LOVD-Datenbank, *BRCA1*	https://databases.lovd.nl/shared/genes/BRCA1
LOVD-Datenbank, *BRCA2*	https://databases.lovd.nl/shared/genes/BRCA2
LOVD-Datenbank, *BRIP1*	http://databases.lovd.nl/shared/genes/BRIP1
LOVD-Datenbank, *CDH1*	http://databases.lovd.nl/shared/genes/CDH1
LOVD-Datenbank, *CHEK2*	https://databases.lovd.nl/shared/genes/CHEK2
LOVD-Datenbank, *PALB2*	http://databases.lovd.nl/shared/genes/PALB2
LOVD-Datenbank, *RAD51C*	https://databases.lovd.nl/shared/genes/RAD51C
LOVD-Datenbank, *RAD51D*	https://databases.lovd.nl/shared/genes/RAD51D
LOVD-Datenbank, *TP53*	https://databases.lovd.nl/shared/genes/TP53
PubMed	http://www.ncbi.nlm.nih.gov/pubmed/
TP53, PHANTM (Genotyp-Phenotyp-Korrelation)	http://mutantp53.broadinstitute.org/heatMap/login
UMD (**U**niv. **M**utation **D**atabase, LSDB: Locus Specific Databases)	http://www.umd.be/
UMD/BRCAshare-*BRCA1* (Registrierung erforderlich)	http://www.umd.be/BRCA1/
UMD/BRCAshare-*BRCA2* (Registrierung erforderlich)	http://www.umd.be/BRCA2/
UMD *TP53*	http://p53.fr/tp53-database
VarSeak	https://varseak.bio/
VarSome (automatisierte ACMG-Klassifizierung)	https://varsome.com/

A 3. Fließschema zu den Bewertungskriterien

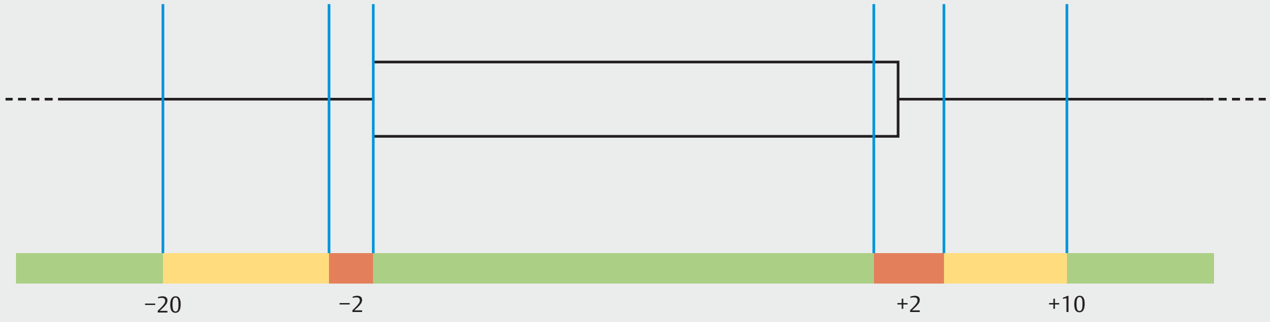
▶ **Abb. 1**.



▶ **Abb. 1** VUS-Bewertungsschema 1.1.

A 4. Schematische Darstellung zu Varianten in der Nähe der Spleißstellen

▶ **Abb. 2**.



🟩 1. intronische Varianten weiter als −20/+10 vom Exon entfernt, wenn **Spleißvorhersage unauffällig: Class2**
2. exonische (synonyme) Varianten, wenn **Spleißvorhersage unauffällig** und MAF ≥ 0,001, aber < 0,01: **Class2**
3. für beide Fälle gilt: wenn die Spleißvorhersage auffällig ist: **Class3** und RNA-Analysen nötig

🟨 Varianten in diesem Bereich sollten insbesondere bei auffälliger Spleißvorhersage mittels RNA-Analyse untersucht werden: **Class3**

🟧 intronische Varianten an Position –1, –2/+1, +2 oder G > ≠ G an letzter Position des Exons: Wenn eine positive Spleißvorhersage vorliegt und die ersten 6 Basen im Intron nicht GTRRGT lauten auch ohne RNA-Analyse: **Class4**
Alle übrigen Varianten an diesen Positionen zunächst **Class3**, RNA-Analyse (im Konsortium) notwendig/verpflichtend!

Zusätzlich gilt: synonyme Substitutionen oder intronische Varianten mit auffälliger Spleißvorhersage, die aber keine mRNA-Aberrationen in Form von Exon-Deletionen/-Duplikationen oder monoallelischer Expression des wt-Transkripts in „in-vitro“-Labortests zeigen: **Class1**

▶ **Abb. 2** Klassifizierung von Varianten, die das Spleißen beeinflussen könnten.

Die Verwendung von Vorhersageprogrammen für mögliche Spleißeffekte ist für alle neuen Veränderungen auch bei Stopp-Mutationen obligat, da beispielsweise „Rescue“-Effekte durch alternative Transkripte auftreten können.

A 5. Besonderheiten der einzelnen Gene

Die oben genannten allgemeinen Bewertungskriterien sollen für alle Gene anwendbar sein. Jedoch bestehen Ausnahmen, Abweichungen und Besonderheiten für bestimmte Varianten und Regionen bei den einzelnen Genen, welche aus Gründen der Übersichtlichkeit erst im Folgenden aufgeführt sind.

A 5.1 *BRCA1/2*

Als Class3 sind zu bewerten: trunkierende *BRCA1*-Mutationen nach der Aminosäureposition 1854 sowie trunkierende *BRCA2*-Varianten nach Aminosäureposition 3308 (hier erfolgt jeweils keine Class1-Bewertung, da strukturelle Veränderungen nicht ausgeschlossen werden können). Ausnahme: Trunkierende Varianten nach dem polymorphen Stopp-Codon p.(Lys3326*) werden dagegen als verzichtbar/neutral gewertet (Class1) [17] und ENIGMA: p.(Lys3326*) ist ein häufig nachweisbarer Polymorphismus, welcher nicht mit einem höheren Risiko assoziiert ist, OR 1.3–1.5 abhängig von Brust- oder Ovarialkrebs. Somit werden Varianten, die stromabwärts von p.(Lys3326*) zu einem Stopp führen, ebenfalls nicht mit einem erhöhten Erkrankungsrisiko assoziiert sein.

Als Class5 in *BRCA1/2* sind zu bewerten: Alle trunkierenden *BRCA1*-Varianten bis zur letzten eindeutig als pathogen beschriebenen Mutation an Aminosäureposition 1853 [18] sowie alle trunkierenden *BRCA2*-Varianten bis Aminosäureposition 3308, c.9924C>G [19]. Siehe ENIGMA *BRCA1,2* funktionelle Domänen, ▶ **Tab. 3** und **4**. **Cave:** NMD beachten; die letzten 50 bp im vorletzten Exon sowie Varianten im letzten Exon unterliegen in der Regel nicht dem NMD. Die Aussagen von RNA-Analysen im Blut sind möglicherweise beschränkt, da es sich nicht um das Zielgewebe handelt.

Weitere Besonderheiten sind im Anhang der Bewertungsrichtlinien des ENIGMA-Konsortiums aufgeführt, die unter folgendem Link abgerufen werden können: https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf, Tabellen 3, 4 und 6 (▶ **Tab. 3** bis **5**).

▶ **Tab. 3** Auszug aus https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf.

Table 3: Catalogue of BRCA1 conserved domains/motifs and currently known, clinically important amino acid residues, and relevance for classification of *BRCA1* in-frame and terminal exon sequence variants.

Domain/ Motif	AA start	AA end	AA alterations with demonstrated clinical importance[a]	Classification of in-frame deletions targeting domain/motifs	References and summary interpretation[a]
RING	1	101	L22S (c.65T>C [p.Leu22Ser]) T37K (c.110C>A [p.Thr37Lys]) C39R (c.115T>C [p.Cys39Arg]) H41R (c.122A>G [p.His41Arg]) C44S (c.130T>A [p.Cys44Ser]) C44Y (c.131G>A [p.Cys44Tyr]) C61G (c.181T>G [p.Cys61Gly])	Class5 if at least one clinically relevant residue is removed. Otherwise Class3.	http://www.ncbi.nlm.nih.gov/protein/15988069; http://hci-exlovd.hci.utah.edu; multifactorial analysis for H41R (c.122A>G [p.His41Arg]) (Whiley et al., 2014).
NES	81	99	None reported	Class3	Domain location description (Rodriguez and Henderson, 2000).
NLS1	503	508	None reported	Class3	Domain location description (Chen et al., 1996, Thakur et al., 1997).
NLS2	607	614	None reported	Class3	Domain location description (Chen et al., 1996, Thakur et al., 1997).
NLS3	651	656	None reported	Class3	Domain location description (Chen et al., 1996).
COILED-COIL	1391	1424	None reported	Class3	Domain location description (Hu et al., 2000).
BRCT DOMAINS	1650	1863	T1685A (c.5053A>G [p.Thr1685Ala]) T1685I (c.5054C>T [p.Thr1685Ile]) V1688del (c.5062_5064del [p.Val1688del]) R1699W (c.5095C>T [p.Arg1699Trp]) G1706E (c.5117G>A [p.Gly1706Glu]) A1708E (c.5123C>A [p.Ala1708Glu]) S1715R (c.5143A>C [p.Ser1715Arg]) G1738R (c.5212G>A [p.Gly1738Arg]) L1764P (c.5291T>C [p.Leu1764Pro]) I1766S (c.5297T>G [p.Ile1766Ser]) M1775K (c.5324T>A [p.Met1775Lys]) M1775R (c.5324T>G [p.Met1775Arg]) C1787S (c.5359T>A [p.Cys1787Ser]) G1788V (c.5363G>T [p.Gly1788Val]) V1838E (c.5513T>A [p.Val1838Glu])	Class5 if at least one clinically relevant residue is removed. Otherwise Class3.	Domain boundaries derived from X-ray crystallography data are aa1646-1863 (1T15, http://www.ncbi.nlm.nih.gov/Structure/mmdb/mmdbsrv.cgi?uid=27907), and ENIGMA functional assay data (Monteiro, unpublished). Digestion data indicate aa1860-1863 are dispensable based on susceptibility to digestion (Lee et al., 2010), while pathogenic variant data indicate that 1855-1862 are dispensable (Hayes et al., 2000). Position 1854 is implicated as clinically important by the observation that Y1853X (c.5559C>G [p.Tyr1853Ter]) is a recognised high-risk pathogenic variant. These combined data indicate that position 1854 or 1855 is the C-terminal border of the BRCT/BRCA1 relevant for the clinical interpretation of sequence variants in exon 24 of BRCA1. That is, a variant predicted to disrupt expression of protein sequence only downstream* of position 1855 would not be considered clinically important.

[a] Missense substitutions in specific functional domains that are designated as Class5 pathogenic based on multifactorial likelihood of the posterior probability of pathogenicity > 0.99 (listed in http://hci-exlovd.hci.utah.edu or individual references), and which have no/little effect on the mRNA transcript profile, *unless* the variant results in an aberrant transcript that encodes a discrete in-frame deletion considered informative for the definition of clinically important domains.

* Typo was corrected in version 2.5.1.

Note: The following pathogenic exonic variants known to alter mRNA splicing have been excluded from Table 3 above, as justified below:

Variant	mRNA Change	Predicted protein change	Reason for exclusion
BRCA1 R1495M (c.4484G>T [p.Arg1495Met])	r.[4358_4484del, 4358_4675del]	p.(Ala1453Glyfs Ter10) – predominant transcript	Predominant alternate transcript is out of frame. Loss of function is assumed due to loss of full-length transcript from variant allele (Houdayer et al., 2012, Colombo et al., 2013, Santos et al., 2014).
BRCA1 E1559K (c.4675G>A [p.Glu1559Lys])	r.[4665_4675del]	p.(Gln1366Alafs Ter13)	Alternate transcript is out-of-frame. Level of full-length transcript not assessed (Wappenschmidt et al., 2012).
BRCA1 A1623G (c.4868C>G [p.Ala1623Gly])	r.[4868_4986del]	p.(Ala1623Aspfs Ter16)	Alternate transcript is out of frame. Variant allele produces some full-length transcripts (Walker et al., 2010).
BRCA1 D1692N (c.5074G>A [p.Asp1692Asn])	r.[4987_5074del, 5074_5075ins5074 +1_5074+153]	p.(Val1665Serfs Ter8) – predominant transcript	Predominant alternate transcript, based on minigene assay (Ahlborn et al., 2015), is out of frame.

▶ **Tab. 4** Auszug aus https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf.

Table 4: Catalogue of BRCA2 conserved domains/motifs and currently known clinically important amino acid residues, and relevance for classification of *BRCA2* in-frame and terminal exon sequence variants.

Domain/ Motif	AA start	AA end	AA alterations with demonstrated clinical importance[a]	Classification of in-frame deletions targeting domain/motifs	References and summary interpretation[a]
PALB2 Binding	10	40	None reported	Class3	Domain location description (Oliver et al., 2009, Xia et al., 2006)
BRC-1	1002	1036	None reported	Class3	http://www.ncbi.nlm.nih.gov/protein/NP_000050.2
BRC-2	1212	1246	None reported	Class3	http://www.ncbi.nlm.nih.gov/protein/NP_000050.2
BRC-3	1422	1453	None reported	Class3	http://www.ncbi.nlm.nih.gov/protein/NP_000050.2
BRC-4	1518	1549	None reported	Class3	http://www.ncbi.nlm.nih.gov/protein/NP_000050.2
BRC-5	1665	1696	None reported	Class3	http://www.ncbi.nlm.nih.gov/protein/NP_000050.2
BRC-6	1837	1871	None reported	Class3	http://www.ncbi.nlm.nih.gov/protein/NP_000050.2
BRC-7	1971	2005	None reported	Class3	http://www.ncbi.nlm.nih.gov/protein/NP_000050.2
BRC-8	2051	2085	None reported	Class3	http://www.ncbi.nlm.nih.gov/protein/NP_000050.2
DBD (DNA/DSS1 binding domain – helical, OB1, OB2, OB3)	2481	3186	W2626C (c.7878G>C [p.Trp2626Cys]) I2627F (c.7879A>T [p.Ile2627Phe]) E2663V (c.7988A>T [p.Glu2663Val]) T2722R (c.8165C>G [p.Thr2722Arg]) D2723G (c.8168A>G [p.Asp2723Gly]) D2723H (c.8167G>C [p.Asp2723His]) G2748D (c.8243G>A [p.Gly2748Asp]) I2778_Q2829del (c.8332_8487del [p.Ile2778_Gln2829del]) R3052W (c.9154C>T [p.Arg3052Trp])	Class5 if at least one clinically relevant residue (or all of AA2778-2829) is removed. Otherwise Class3.	http://www.ncbi.nlm.nih.gov/protein/NP_000050.2; http://hci-exlovd.hci.utah.edu. Pathogenic variant c.8486G>A (also recorded as Gln2829Arg) results in a transcript encoding an in-frame exon 19 deletion only (Houdayer et al., 2012), indicating that genetic variation encompassing loss of this entire exon (AA2778-2829) should be considered clinically important. The clinical impact of alteration/deletion of individual amino acids in exon 19 is not yet established.
NLS1	3263	3269	None reported	Class3	Domain local description (Guidugli et al., 2014)
BRC-9 or TR2	3265	3330	None reported	Class3	Note: although amino acids 3270-3305 within this fragment are reported to bind RAD51-DNA filaments (Davies and Pellegrini, 2007), there is no sequence conservation with the BRC repeats located between aa1002 and aa2014. Domain boundaries are derived from x-ray crystallography data are aa3265-3330 (Esashi et al., 2005, Esashi et al., 2007). Case-control and frequency data indicate that BRCA2 c.9976A>T (p.Lys3326Ter) does not confer a high risk of cancer (OR 1.3–1.5, dependent on breast or ovarian cancer subtype (Meeks et al., 2016), demonstrating that residues at and downstream of 3327 are likely dispensable. Position 3308 is implicated as clinically important by the observation that a nonsense variant c.9924C>G (p.Tyr3308Ter) is recognized as a high-risk pathogenic variant with known functional relevance ([Vallee et al., 2016]; Bayes score 1122 : 1 from a single large kConFab family, Spurdle unpublished data). There is currently no publicly available clinical information to support pathogenicity of nonsense or frameshift variants located between positions 3309 and 3325. These data combined suggest that the C-terminal border of the BRC-9 relevant to the clinical interpretation of sequence variants in exon 27 of BRCA2 lies between 3309 and 3325. That is, a variant predicted to disrupt expression only of protein sequence downstream of position 3325 would be considered unlikely to be clinically important. Further functional and clinical studies are underway to refine risk, if any, for predicted nonsense or frameshift variants downstream of position 3326.
NLS2	3381	3385	No	Class3	Domain location description (Guidugli et al., 2014). This domain is considered unlikely clinically relevant since it lies downstream of position 3326.

Fortsetzung nächste Seite

▶ **Tab. 4** Auszug aus https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf. *(Fortsetzung)*

[a] Missense substitutions in denoted functional domains that are designated as Class5 pathogenic based on multifactorial likelihood posterior probability of pathogenicity > 0.99, and for which there is no/little effect on mRNA transcript profile – ***unless*** the variant results in an aberrant transcript that encodes a discrete in-frame deletion considered informative to definition of clinically important domains. (Splicing aberrations are reported for *BRCA2* c.7988A>T [p.Glu2663Val] and c.8168A>G [p.Asp2723Gly] (Walker et al., 2010), but these did not lead to complete loss of function of the full length transcript), and missense alterations showed abrogation of functional activity using multiple assays (Walker et al., 2010). An additional conserved region not commonly recognized as a BRCA2 domain/motif is located AA 1110-1183, but no pathogenic missense substitutions have been recorded for this region.

Note – The following pathogenic exonic variants known to alter mRNA splicing have been excluded from Table 4 above, as justified below:

Variant	mRNA Change	Predicted protein change	Reason for exclusion
BRCA2 R2659K (c.7976G>A [p.Arg2659Lys])	r.[7806_7976del]	p.(Ala2603_ Arg2659del)	Alternate transcript is in-frame but level of full length transcript not assessed (Farrugia et al., 2008)
BRCA2 R2659T (c.7976G>C [p.Arg2659Thr])	r.[7806_7976del]	p.(Ala2603_ Arg2659del)	Alternate transcript is in-frame but level of full length transcript not assessed (Farrugia et al., 2008)
BRCA2 P3039P (c.9117G>A [p.Pro3039Pro])	r.[8954_9117del]	p.(Val2985 Glyfs*4)	Allele-specific assay shows out-of-frame transcript (Houdayer et al., 2012)

▶ **Tab. 5** Auszug aus https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf.

Table 6: *BRCA1* and *BRCA2* exon boundary variants predicted or known to lead to naturally occurring in-frame RNA isoforms that may rescue gene functionality. *Variants at these positions should be considered Class3 Uncertain unless proven otherwise.* *

Gene	Alternative splicing event	Variants implicated	Rationale
BRCA1	Δ8p	c.442-1 (IVS7-1) c.442-2 (IVS7-2)	*BRCA1* exon 8 acceptor site is an experimentally validated tandem acceptor site (NAGNAG) subject to alternative splicing (Colombo et al., 2014). c.442-1,-2 variants are predicted to inactivate the 5′ acceptor site, but not the 3′ acceptor site, thus producing Δ8p transcripts.
	Δ9,10	c.548-1 (IVS8-1) c.548-2 (IVS8-2) c.593 to non-G c.593+1 (IVS9+1) c.593+2 (IVS9+2) c.594+1 (IVS9-1) c.594-2 (IVS9-2) c.670 to non-G c.670+1 (IVS10+1) c.670+2 (IVS10+2)	Carriers of variants at these positions are predicted to produce normal (or increased) levels of *BRCA1* Δ(9,10), a major in-frame alternative splicing event (Colombo et al., 2014). The *BRCA1* variant c.594-2A>C (shown from ENIGMA research to co-occur in cis with c.641A>G), has been reported to demonstrate clinical characteristics inconsistent with a high risk of cancer expected for a pathogenic *BRCA1* variant (Rosenthal et al., 2015). The haplotype of c.[594-2A>C; 641A>G] has been shown from mRNA analysis in human samples to produce high levels of Δ10 transcripts (70% of the overall expression, and **has been designated as Class1 Not Pathogenic** by the ENIGMA Consortium using multifactorial likelihood analysis that includes genetic (segregation, case-control analysis) and pathology data (de la Hoya et al., 2016).
	Δ11q, Δ11	c.4096 to non-G c.4096+1 (IVS11+1) c.4097+2 (IVS11+2)	Data collected by the ENIGMA consortium demonstrates that the *BRCA1* c.4096+1G>A variant, proven to result in the production of naturally occurring in-frame transcripts Δ11q (Bonatti et al., 2006) and also Δ11 (Radice, unpublished data), may not exhibit the clinical characteristics of a standard high-risk pathogenic *BRCA1* variant (Spurdle, unpublished data).
	Δ13p	c.4186-1 (IVS12-1) c.4186-2 (IVS12-2)	*BRCA1* exon 13 acceptor site is an experimentally validated tandem acceptor site (NAGNAG) subject to alternative splicing (Colombo et al., 2014). c.4186-1,-2 variants are predicted to inactivate the 5′ acceptor site, but not the 3′ acceptor site, thus producing Δ13p transcripts.
	Δ14p	c.4358-1 (IVS13-1) c.4358-2 (IVS13-2)	*BRCA1* exon 14 acceptor site is an experimentally validated tandem acceptor site (NAGNAG) subject to alternative splicing (Colombo et al., 2014). c.4358-1,-2 variants are predicted to inactivate the 5′ acceptor site, but not the 3′acceptor site, thus producing Δ14p transcripts.
BRCA2	Δ12	c.6842-1 (IVS11-1) c.6842-2 (IVS11-2) c.6937 to non-G c.6937+1 (IVS12+1) c.6937+2 (IVS12+2)	Carriers of these variants are predicted to produce exon 12 skipping. BRCA2 Δ12 is a naturally occurring in-frame splicing event (Fackenthal et al., 2016). BRCA2 exon 12 is functionally redundant (Li et al., 2009).

* This summary table does not yet capture the possibility of acceptor site changes leading to small in-frame deletions > 3 bp, e. g. due to NAG $(NNN)_n$ NAG sites. It is recommended that bioinformatic prediction analysis is carried out for variation in/near *all* donor and acceptor sites to assess the likelihood that a variant will or will not cause alternative splicing.

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▶ Tab. 5 Auszug aus https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf. *(Fortsetzung)*

Note: It could be argued that nonsense or frameshift variants in *BRCA1* exon 9, *BRCA1* exon 10, or *BRCA2* exon 12 may not be associated with high risk of cancer due to rescue by the expression of in-frame naturally occurring isoforms that bypass the premature termination codon and thus encode a functional protein. A review of multiple clinical and control datasets for the frequency of unique nonsense or frameshift variants – **adjusted for exon size** – does not provide strong support for this hypothesis at present (Spurdle, de la Hoya, unpublished data). Additional research is underway to further investigate the functional/clinical importance of germline nonsense or frameshift variants in these exons.

Moreover, further work is planned within ENIGMA (led by Paolo Radice) to document variants that have undergone splicing assays and are proven to be “leaky” variants, to provide a record of all spliceogenic variants for which additional research is necessary. This resource will identify variants that have already been classified using clinical data, as positive and negative controls for future quantitative mRNA studies.

A 5.2 *ATM*

Die Bewertungskriterien für *ATM* beruhen auf einer Kombination aus folgenden Kriterien:

- dem 5-Klassen-IARC-System für die Einschätzung der Pathogenität von *BRCA1*- und *BRCA2*-Varianten,
- dem 3-Klassen-System zur Einschätzung der Pathogenität von *ATM*-Varianten [20], welches In-silico-Analysen wie Align-GVGD mitberücksichtigt,
- den ACMG-Guidelines zur Variantenklassifizierung [3, 21],
- weiteren Literaturstellen: [22 – 28].

Class1:

- Bei Allelfrequenz ≥ 1 % (MAF ≥ 0,01) in den Großpopulationen Kaukasier, Afrikaner oder Asiaten oder Nachweis von homozygoten Variantenträgern in Kontrollpopulationen. Ist dies der Fall, wird die Variante immer als Class1 bewertet. Eine Allelfrequenz ≥ 1 % in Subpopulationen mit wenig durchmischtem Genpool (Bsp.: finnische Population, Gründermutationen!) ist nicht ausreichend.

Class2:

- Bei Allelfrequenz ≥ 0,5–< 1 % (MAF ≥ 0,005–0,099) in den Großpopulationen Kaukasier, Afrikaner oder Asiaten wird die Variante immer als Class2 bewertet.
- Missense-Variante, die gemäß In-silico-Analyse (Align-GVGD, SIFT) mit hoher Wahrscheinlichkeit neutral ist und/oder außerhalb der funktionell kritischen Domäne (FATKIN) liegt.

Class3:

- Alle Varianten, die nicht in Class1, 2, 4 oder 5 eingeordnet werden können.

Class4:

- Varianten mit einer In-Frame-Deletion, die innerhalb der funktionell kritischen Domäne (FATKIN) liegt.
- Missense-Varianten, die innerhalb der funktionell kritischen Domäne (FATKIN) liegen, und gemäß In-silico-Analyse (Align-GVGD, SIFT) mit hoher Wahrscheinlichkeit schädigend und als funktionell inaktiv beschrieben wurden.

Class5:

- Trunkierende *ATM*-Varianten bis zur FATKIN-Domäne.
- Missense-Varianten, In-Frame-Deletion oder Spleißveränderungen, die eine Reduktion der ATM-Proteinexpression auf < 20 % für das mutierte Allel verursachen [28, 29].
- Varianten, die mit klassischer AT assoziiert sind.

Spleißvarianten: siehe *BRCA1* und *BRCA2*.
Funktionelle Domänen: FATKIN mit FAT; PI3K related Kinase; FATC **▶ Tab. 6**.
Weitere Literaturstellen: [22 – 26, 28, 33, 35, 38].

▶ Tab. 6 *ATM*, funktionelle Domänen und Relevanz für die Interpretation der klinischen Bedeutung von Sequenzvarianten – Katalog klinisch relevanter funktioneller Domänen und Aminosäuren.

Region	AA start	AA end	AA alterations with demonstrated clinical importance (AT) characterising known functional domains	References and summary interpretation
Substrate binding	91	97	None reported	Domain location description [30] Also contains p53- and BRCA1-binding domain
NLS	385	388	None reported	Domain location description [28, 31]
Leucine zipper	1218	1238	None reported	Domain location description [25, 28]
Proline rich	1373	1382	None reported	Domain location description [25, 28]
FATKIN	1893	3056	Yes, e.g. p.(Val2424Gly) p.(2546_2548del), in frame p.(Asp2625Glu) p.(Ala2626Pro) p.(Val2716Ala) p.(Ser2855_Val2856delinsArgIle)	AA alterations and in-frame deletions [26, 28, 29, 32 – 35] Domain location description [25, 27, 28, 36, 37] **Domains:** FAT: 1893-2612 KIN: 2612-3056 with ATP-binding: 2716-2730, substrate (nibrin and p53) binding: 2682-3012, FATC with TIP60 binding: 3034-3056 Domain location description

A 5.3 *PALB2*

- Die Veränderung p.(Leu939Trp) ist als Class2 zu werten [39].

Weitere Literaturstellen: [35, 38, 40 – 46].

▶ **Tab. 7**.

▶ **Tab. 7** *PALB2*, funktionelle Domänen und Relevanz für die Interpretation der klinischen Bedeutung von Sequenzvarianten – Katalog klinisch relevanter funktioneller Domänen und Aminosäuren.

Region	AA start	AA end	AA alterations with potential clinical importance	References and summary interpretation
BRCA1 interaction domain	9	43	Yes, e.g. p.(Leu35Pro)	Also covers oligomerisation domain/covers coiled-coiled motif Domain location description [47 – 49]; Amino acid alteration in VUS and functional analysis [50].
DNA-binding site	1	200	None reported	Domain location description [51]
RAD51 binding site	101	184	None reported	Domain location description [51, 52]
DNA-binding site	372	561	None reported	Covers also chromatin association motif (ChAM, 395-446) Domain location description [51, 53]
MRG15 (MORF4L1) interaction domain	611	764	None reported	Domain location description [48]
WD40 repeat	853	1186	Yes, e.g. p.(Thr1030Ile) p.(Leu1143Pro)	BRCA2 (1019-1098), RAD51C, XRCC3 and/or RAD51 complex formation Domain location description [51, 52, 54 – 57]. Amino acid alterations [48, 54, 58]

A 5.4 *CHEK2*

Im Bereich der Exons 11–15 hochhomologe, funktionsinaktive Sequenzbereiche (Pseudogene) auf verschiedenen anderen Chromosomen (2, 7, 10, 13, 15, 16, X, and Y) [59, 60], die relevante Sequenzbereiche überlagern können > Long Range PCR der Exons 11–15 und bioinformatische Herausfilterung der Pseudogen-reads falls möglich.

Funktionelle Domänen: SQ/TQ-rich-Domäne*, Forkhead associated (FHA)**-Domäne, Kinase-Domäne***, nukleäres Lokalisationssignal (NLS) [61 – 63].

- In der SQ/TQ-rich-Domäne sind bisher nur trunkierende Varianten als pathogen eingestuft (▶ **Tab. 8**).
- Zahlreiche Missense-Varianten in der FHA-Domäne bekannt. **Cave:** Bei Bewertung Flossies-Datenbank berücksichtigen! (z. B. c.470C>T; p.Ile157Thr: Class2 [siehe § Fußnote ▶ **Tab. 8**]) oder mit unklarer klinische Relevanz (z. B. c.434G>A, p.Arg145Gln; c.422A>C, p.Lys141Thr).
- Missense-Varianten in der Kinase-Domäne mit unklarer klinischer Relevanz: z. B. c.1216C>T, p.Arg406Cys.
- In der NLS-Domäne sind bisher ebenfalls nur trunkierende Varianten als pathogen eingestuft (▶ **Tab. 8**).

Einen Überblick über identifizierte Mutationen innerhalb des *CHEK2*-Genbereichs findet sich in der Arbeit von Ow et al. [63]. Die Veröffentlichung von Roeb et al., 2012 beinhaltet einen schematischen Überblick der funktionellen Domänen sowie Ergebnisse der funktionellen Analyse von in diesen verschiedenen *CHEK2*-Domänen lokalisierten missense Mutationen [62].

A 5.5 *TP53*

- IARC *TP53*-Datenbank, funktionelle Analysen von Kato et al., 2003 und Monti et al., 2007, 2011 sind verlässlich [72 – 77].
- Funktionelle Domänen: Oligomerisierungs-Domäne, Core-Domäne (DNA-Bindung).
- Möglicher dominant negativer Effekt von Missense-Varianten und Stopp-Varianten, welche die Oligomerisierungsdomäne betreffen.
- Mosaikmutationen sowie klonale Hämatopoese sind möglich, daher bei NGS-Analysen besonders auf die Variant-Allel-Fraction achten und ggf. weitere Analysen zur Bestätigung von Keimbahnmutationen durchführen.

▶ **Tab. 9**.

▶ **Tab. 8** *CHEK2*, funktionelle Domänen und Relevanz für die Interpretation der klinischen Bedeutung von Sequenzvarianten – Katalog klinisch relevanter funktioneller Domänen und Aminosäuren.

Region	AA start	AA end	AA alterations with Potential Clinical Importance	References and summary interpretation
SQ/TQ-rich	19	69	e.g. c.85C>T,p.Gln29*	[62, 65]
FHA	92 [115]	205 [175]	p.Arg117Gly, p.Arg145Trp, p.Gly167Arg	[61 – 63]
Kinase	212	501	c.1040A>C, p.Asp347Ala #; c.1100del; c.1164dup; p.Thr476Met, c.1169A>C, p.Tyr390Ser; c.1183G>T, p.Val395Phe#; c.1283C>T, p.Ser428Phe; c.1427C>T, p.Thr476Met	[61, 62], #ClinVar
NLS	515	538	e.g. c.1547delC, p.Ser516Leufs#; c.1555C>T, p.Arg519Ter#;	[63], #ClinVar

* SQ/TQ consensus sites are sites phosphorylated by ATM/ATR [116]. e. g. phosphorylation of Thr-68 is important for CHEK2 activation and oligomerisation.

** The phosphorylated Thr-68 site of CHEK2 interacts with the FHA domain of another CHEK2 molecule and thus leads to the formation of CHEK2 oligomers [66].

*** [67]

§ The *CHEK2* c.470T>C p.Ile157Thr variant, although still present with various classifications (VUS/likely pathogenic/pathogenic) in ClinVar and other databases has been reclassified (date: 28.08.2018) as Class2/likely benign by the German Consortium on HBOC on the following basis:
It is frequently listed in large unaffected control cohorts (0.5 %, gnomAD V. 2.1.1, non-cancer). The population frequency in Finnish Europeans is 2.5 % (10 homozygous carriers). In addition, it is present in 47/7325 individuals (0.64 %) in the FLOSSIES database (non-cancer female controls of European descent aged > 70 years). Although showing functionally impaired dimerisation and autophosphorylation [61, 62, 68], numerous large case-control studies show results indicating low or no increased breast cancer risk [64, 69]: breast cancer OR = 1.58 (1.42–1.75), colon cancer OR = 1.67 (1.24–2.26) [70, 71]. Additionally, it has been observed with a frequency of 2 % in controls [2]. However, it may act as a polygenic risk allele.

▶ **Tab. 9** *p53*, funktionelle Domänen und Relevanz für die Interpretation der klinischen Bedeutung von Sequenzvarianten – Katalog klinisch relevanter funktioneller Domänen und Aminosäuren.

▶ **Tab. 9** *p53*, funktionelle Domänen und Relevanz für die Interpretation der klinischen Bedeutung von Sequenzvarianten – Katalog klinisch relevanter funktioneller Domänen und Aminosäuren. *(Fortsetzung)*

Region	AA start	AA end	AA alterations with demonstrated clinical importance (including conflicting interpretations of pathogenicity but criteria provided)	References and summary interpretation
Transcription activation	1	55	p.(Val10Ile) p.(Val31Ile) p.(Pro47Ser)	Amino acid alterations (ClinVar) Domain location description [78] Also binding site for numerous proteins including HDM2 (amino acids 15–29; IARC)
Proline-rich domain	61	94	p.(Pro82Leu) p.(Ala83Val)	Amino acid alterations (ClinVar) Domain location description [78]
DNA-binding region	102	292	p.(Gly105Asp) p.(Lys120Glu) p.(Thr125Met) p.(Ser127Phe) p.(Asn131Tyr) p.(Cys141Tyr) p.(Pro151Ser) p.(Pro151Thr) p.(Pro152Leu) p.(Arg156His) p.(Arg158Cys) p.(Arg158His) p.(Tyr163Asp) p.(Tyr163Cys) p.(Arg175Leu) p.(Arg175His)	Amino acid alterations (ClinVar) Domain location description (IARC) Also binding site for numerous proteins including 53BP1 (IARC) and RAD51 amino acids 94–160 and 264–315 [79]

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▶ **Tab. 9** *p53*, funktionelle Domänen und Relevanz für die Interpretation der klinischen Bedeutung von Sequenzvarianten – Katalog klinisch relevanter funktioneller Domänen und Aminosäuren. *(Fortsetzung)*

Region	AA start	AA end	AA alterations with demonstrated clinical importance (including conflicting interpretations of pathogenicity but criteria provided)	References and summary interpretation
			p.(Cys176Tyr) p.(His179Tyr) p.(Arg181Cys) p.(Arg181His) p.(Ala189Val) p.(His193Arg) p.(His193Leu) p.(Leu194Phe) p.(Ile195Thr) p.(Arg213Gln) p.(Val.216Met) p.(Tyr220Cys) p.(Tyr220Ser) p.(Ile232Thr) p.(Tyr234Cys) p.(Asn235Ser) p.(Tyr236Asp) p.(Met237Val) p.(Met237Ile) p.(Cys238Tyr) p.(Ser241Phe) p.(Cys242Tyr) p.(Gly245Asp) p.(Gly245Ser) p.(Gly245Cys) p.(Met246Val) p.(Met246Leu) p.(Met246Arg) p.(Arg248Gln) p.(Arg248Trp) p.(Ile251Leu) p.(Ile251Ser) p.(Thr256Ala) p.(Leu257Arg) p.(Glu258Lys) p.(Arg267Trp) p.(Arg267Gln) p.(Val272Leu) p.(Arg273Hisv) p.(Arg273Cys) p.(Cys275Tyr) p.(Cys277Tyr) p.(Arg280Thr) p.(Asp281Val) p.(Asp281Gly) p.(Arg282Gly) p.(Arg282Leu) p.(Arg282Trp) p.(Arg283His) p.(Arg283Lys) p.(Glu286Lys)	
Oligomerisation region	325	356	p.(Gly325Val) p.(Arg337Leu) p.(Arg337Cys) p.(Glu339Lys) p.(Arg342Pro)	Amino acid alterations (ClinVar) Domain location description (IARC) Also binding site for numerous proteins Covering main nuclear localisation signal (amino acids 316-322) [80, 81]
Basic (repression of DNA-binding region)	369	388	None reported	Domain location description (IARC) Also binding site for numerous proteins including RAD54 [82]

▶ **Tab. 9** *p53*, funktionelle Domänen und Relevanz für die Interpretation der klinischen Bedeutung von Sequenzvarianten – Katalog klinisch relevanter funktioneller Domänen und Aminosäuren. *(Fortsetzung)*

A 5.6 *RAD51D*

Funktionelle Domänen: N-terminale Domäne und „ATP binding domain“ mit den hochkonservierten Walker A und B Motiven [83 – 85].

▶ **Tab. 10**.

Weitere Literaturstellen: [91 – 94]

A 5.7 *RAD51C*

Literatur: [91, 95 – 101]

Funktionelle Domänen: „DNA repair/recombination protein RecA-like ATP binding Domain“

▶ **Tab. 11**.

A 5.8 *BRIP1*

▶ **Tab. 12**.

Weitere Literaturstellen: [1, 102, 105 – 110]

▶ **Tab. 10** *RAD51D*, funktionelle Domänen und Relevanz für die Interpretation der klinischen Bedeutung von Sequenzvarianten – Katalog klinisch relevanter funktioneller Domänen und Aminosäuren.

Region	AA start	AA end	AA alterations with demonstrated clinical importance (including conflicting interpretations of pathogenicity)	References and summary interpretation
N-terminal region	1	83	None reported	N-terminal domain required for ssDNA-specific binding function [86]
Linker	60	78	None reported	Proper interaction with RAD51C and XRCC2 [85]
ATPase domain and RAD51B, RAD51C, and XRCC2 binding	99	274	p.(G112A) (disrupts binding of RAD51D to RAD51C [87]) p.(S207L) (disrupts RAD51D-XRCC2 interaction [85]) p.(A210V) (predicted to be potentially pathogenic [88, 89]) p.(R266C) [90], Meindl et al. (unpublished)	ATPase, AAA+ type Walker A and B motifs crucial for HR. These motifs are also implicated in binding to RAD51C and XRCC2.

▶ **Tab. 11** *RAD51C*, funktionelle Domänen und Relevanz für die Interpretation der klinischen Bedeutung von Sequenzvarianten – Katalog klinisch relevanter funktioneller Domänen und Aminosäuren.

Region	AA start	AA end	AA alterations with demonstrated clinical importance (including conflicting interpretations of pathogenicity)	References and summary interpretation
N-terminal region	1	66	None reported	Homology-derived putative DNA-binding domain [91]
ATPase domain and RAD51B, XRCC3, and RAD51D binding	79	376	p.(Gly125Val) p.(Cys135Tyr) p.(Leu138Phe) p.(Gly153Asp) p.(Asp159Asn) p.(Val169Ala) p.(Leu219Ser) p.(Arg258His) p.(Gly264Ser)	Amino acid alterations and functional consequences [95–98] Domain location description [91] ATPase domain includes Walker A nucleotide binding motif (amino acids 125–132) and Walker B nucleotide binding motif (amino acids 238–242) [91, 99]
Nuclear localisation signal	366	370	None reported	Domain location description [99]

▶ **Tab. 12** *BRIP1*, funktionelle Domänen und Relevanz für die Interpretation der klinischen Bedeutung von Sequenzvarianten – Katalog klinisch relevanter funktioneller Domänen und Aminosäuren.

Region	AA start	AA end	AA alterations with demonstrated clinical importance (including conflicting interpretations of pathogenicity)	References and summary interpretation
DEAD/DEAH box helicase domain	17	441		Domain location description [102]
Helicase superfamily c-terminal domain	697	851		Domain location description [102]
The BRCA1 interacting region of BRIP1	976	1006	Phosphorylation of FANCJ at Ser-990 is important for its interaction with BRCA1	[103]
MLH1 interaction			Lysines 141 and 142 are required for direct interaction of FANCJ with MLH1	[104]
Nuclear localisation signal	158	175	None reported	Domain location description [102]

A 5.9 CDH1

Literatur:
Vorwiegend auf die Molekulargenetik eingehend: [111]
Review funktionelle Analysen: [112]
Review des HDGC-Konsortiums: [113]
Review zu lobulärem Brustkrebs: [114]
Verteilung von pathogen Varianten über den *CDH1*-Locus [111]

Bisher bekannte pathogene CDH1-Varianten sind über den gesamten Locus verteilt und es kann daher keine klinisch relevante funktionelle Protein-Domäne definiert werden. Letzte bekannte trunkierende pathogene Variante im letzten Exon ist c.2506G>T (p.Glu836*) [115]. Alle trunkierenden Varianten Upstream müssten daher mindestens als Klasse 4 eingestuft werden.

Der Vorschlag des ClinGen-Konsortiums, abweichend von IARC Guidelines, Varianten mit einer MAF > 0,2% als Klasse 1 nach ACMG zu klassifizieren, ist derzeit Gegenstand der Diskussion.

CDH1 Rule Specifications for the ACMG/AMP Variant Curation Guidelines ClinGen (https://www.clinicalgenome.org/site/assets/files/8816/clingen_cdh1_acmg_specifications_v1.pdf).

Interessenkonflikt

Die Autorinnen/Autoren geben an, dass kein Interessenkonflikt besteht.

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