<table>
<tr><th></th><th><u>PRESENCE</u> OF ACTIVE EVIDENCE OF EFFECT SIZE</th><th><u>ABSENCE</u> OF EVIDENCE OF EFFECT SIZE</th></tr>
<tr><td>Summary</td><td>• Quantitative evidence of effect size (OR) available</td><td>• No quantitative evidence of effect size (OR) available
• Most evidence suggests pathogenicity but some evidence items (under standard penetrance classification model) are either weakly-pathogenic and/or potentially-contradictory.</td></tr>
<tr><td>Evidence directly quantifying effect size</td><td>• PS4: odds ratio from case-control analysis 2-4[a] (for high penetrance gene)
o lower 95th CI >1 (PS4_mod, 2 EPs)
o lower 95th CI >1.5 (PS4_str, 4 EPs)
o EPs from multiple studies may be summed

AND/OR

• PP1: significant Bayes factor/likelihood ratio from COOL segregation tool or similar with target OR of 2-4[b]
o apply PP1 at full strength</td><td>N/A</td></tr>
<tr><td>Standard evidence towards pathogenicity</td><td colspan="2">• Any of PS1, PS3, PM1, PM2, PM3, PM5, PP2, PP3, PP4 can be used (as per full penetrance guidance)</td></tr>
<tr><td>Weakly pathogenic evidence (can be counted towards assignation as <<Likely pathogenic-reduced penetrance>>)</td><td colspan="2">• PS3: Functional score on a quantitative assay between the mid-point of the intermediate range and the threshold for loss of function[c]
o PS3 can be awarded, but downgraded by one pathogenicity evidence strength level
• PM3: Observation in homozygous state/in trans with a pathogenic variant in an individual with <u>mild</u> phenotype
o PM3 can be awarded, but downgraded by one pathogenicity evidence strength level</td></tr>
<tr><td rowspan="3">Potentially contradictory evidence that may be <u>revised</u>, <u>discounted</u> or used at <u>reduced in strength</u> (in the context of reduced penetrance)</td><td>• Multifactorial analysis from segregation/co-occurrence/family history data or segregation analysis using COOL tool or similar under full-penetrance model (usually target OR of >4):
o BS4/BP5 evidence can be discounted[d]</td><td>• Multifactorial analysis from segregation/co-occurrence/family history data or segregation analysis using COOL tool or similar under full-penetrance model (usually target OR of >4):
o BS4/BP5 evidence can be downgraded by one benignity evidence strength level[e]</td></tr>
<tr><td colspan="2">• Functional assay result indicating functionality (BS3):
o BS3 can be downgraded by one benignity evidence strength level
• Observation in homozygous state/in trans with a pathogenic variant in an individual with normal phenotype (BP2/BS2)
o BP2 can be downgraded by one benignity evidence strength level</td></tr>
<tr><td colspan="2">• Frequency > BS1 threshold:
o Use at standard strength following recalculation of MTAF with reduced penetrance metrics (where available)[f], otherwise downgrade by one benignity evidence strength level</td></tr>
<tr><td>Recommendations on final classification</td><td>Variant may be classified as <<Likely pathogenic-reduced penetrance>>[g] if net EP ≥ 6</td><td>Variant may be classified as <<Likely pathogenic-reduced penetrance>>[g] if net EP ≥ 6 and ≤1 piece of evidence requiring discounting/evidence strength level modification using reduced penetrance framework</td></tr>
</table>

CI: confidence interval; COOL: COsegregation OnLine; EP: Evidence points; OR: odds ratio; MTAF: Maximum tolerated allele frequency
Evidence towards both pathogenicity and benignity may be applied at the following strengths: Very Strong, Strong, Moderate, Supporting.

[a]OR >half of OR associated with full penetrance variant but <OR associated with full penetrance variant in gene of interest. If using enriched dataset, adjust target OR accordingly. OR 2-4 is established for breast cancer as consistent with moderate penetrance; for other genes this OR must be established[1]
[b]When using COOL tool, use custom input files for reduced penetrance variants where available, or select the *BRCA1*:p.R1699Q option where appropriate[2]
[c]Intermediate score should represent an intermediate functional effect, not an indeterminate effect or technical fail. Consider application of higher evidence strength if multiple functional studies indicate intermediate effect. Splice assays with evidence of leakiness may also be appropriate to apply under PS3 in reduced penetrance context. Consider applying PS3 reduced by one evidence strength level if multiple assays give conflicting results but the majority of assays indicate loss of function, with more weighting given to assays assigned higher evidence strength weighting as per Brnich et al guidance. If assays give conflicting results but the majority of assays indicate functionality, consider applying BS3 reduced by one benignity evidence strength level, with more weighting given to assays assigned higher evidence strength weighting as per Brnich et al guidance[3]
[d]Multifactorial analysis of pathology data should still be applied as evidence e.g. tumour pathology likelihood ratio from Parsons et. al, 2019[4]
[e]For example, multifactorial data scoring within the strong range (4-7.9 evidence points) would now be downgraded to moderate (2 evidence points) and multifactorial data scoring within the moderate range (2-3.9 evidence points) would be downgraded to supporting (1 evidence point)
[f]On revision of lifetime breast cancer penetrance for *BRCA1/BRCA2* from 0.71/0.69 to 0.25 (compared to population penetrance of 0.125), the BA1/BS1 thresholds are revised to ~0.003/0.0003
[g]Variants may be classified as <<<u>pathogenic</u> with reduced penetrance>> only where there is international validation of reduced penetrance effect e.g. *BRCA1* 5096G>A p.Arg1699Gln

Version History/Amendments

Revised version	Date	Section	Update	Amended by	Approved by
v1.0	01/10/2024	--	Initial version	--	--

References

1. Easton DF, Pharoah PD, Antoniou AC, et al. Gene-panel sequencing and the prediction of breast-cancer risk. *The New England journal of medicine* 2015;372(23):2243-57. doi: 10.1056/NEJMsr1501341 [published Online First: 2015/05/28]
2. Belman S, Parsons MT, Spurdle AB, et al. Considerations in assessing germline variant pathogenicity using cosegregation analysis. *Genetics in medicine : official journal of the American College of Medical Genetics* 2020;22(12):2052-59. doi: 10.1038/s41436-020-0920-4 [published Online First: 2020/08/11]
3. Brnich SE, Abou Tayoun AN, Couch FJ, et al. Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework. *Genome medicine* 2019;12(1):3. doi: 10.1186/s13073-019-0690-2 [published Online First: 2020/01/02]
4. Parsons MT, Tudini E, Li H, et al. Large scale multifactorial likelihood quantitative analysis of BRCA1 and BRCA2 variants: An ENIGMA resource to support clinical variant classification. *Human mutation* 2019;40(9):1557-78. doi: 10.1002/humu.23818 [published Online First: 2019/05/28]