

PALB2: CanVIG-UK Gene-Specific Guidance

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CanVIG-UK review of PALB2 January 2024: Consensus to use relevant recommendations from the **ClinGen PALB2 VCEP guidance, v1.1.0** (available at: <https://clinicalgenome.org/affiliation/50039/>, PDF attached below) for *PALB2* variants reported under indication R208 of the UK Genomic Test Directory. Evidence items in grey are not relevant as per the ClinGen VCEP guidance. Additional points of specification are given below where applicable.

Evidence towards Pathogenicity

Evidence element and evidence strengths allowed		Thresholds/data-sources/applications specifically relevant to PALB2
PS4: Case-control: The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls	_STR	There should be a minimum of 2 case observations before PS4 can be applied. Female-only population controls are recommended for use. See main CanVIG-UK consensus specification for database recommendations. Otherwise follow VCEP recommendations.
PP4: Phenotypic specificity/case counting (Patient's phenotype or family history is highly specific for a disease with a single genetic aetiology)		Not applicable as per VCEP
PM2: Absent from controls (or at extremely low frequency if recessive) in ESP, 1000GP, or ExAC	_SUP	Female-only population controls are recommended for use. See main CanVIG-UK consensus specification for database recommendations.
PVS1: Predicted null variant (in a gene where LOF is a known mechanism of disease)	_VSTR _STR _MOD _SUP	As per VCEP
PS1: Same amino acid change as an established variant	_STR MOD SUP	As per VCEP
PM4: Protein-length-changing variant		Not applicable as per VCEP
PM5: Novel missense change at an amino acid residue where a different missense change determined to be pathogenic seen before	_SUP	As per VCEP

PP3: In silico: Multiple lines of computational evidence support a deleterious effect on the gene or gene product	_SUP	As per VCEP
PM1, PP2: Enrichment/constraint: PP2: Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease PM1: Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation		Not applicable as per VCEP
PS3: Functional: Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product		Not applicable as per VCEP
PP1: Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease	_STR _MOD _SUP	As per VCEP
PS2/PM6: De novo (maternity and paternity confirmed/unconfirmed) in a patient with the disease and no family history		Not applicable as per VCEP
PM3: in trans with a pathogenic variant (recessive disorders)	_STR _MOD _SUP	As per VCEP
PP5: Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation		Not applicable as per VCEP

Evidence towards Benignity

BA1/BS1: Allele frequency is “too high” in ExAC or gnomAD for disorder	_SA _STR	Female-only population controls are recommended for use. See main CanVIG-UK consensus specification for database recommendations. Otherwise, as per VCEP
BS2: Observation in controls inconsistent with disease penetrance. Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full	_STR _MOD _SUP	As per VCEP

penetrance expected at an early age		
BP4: In silico: Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)	_SUP	As per VCEP
BP1: Missense variant in a gene for which primarily truncating variants are known to cause disease	_SUP	As per VCEP
BP7: Synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence	_STR _MOD _SUP	As per VCEP
BP3: In-frame deletions/insertions in a repetitive region		Not applicable as per VCEP
BS3: Well-established <i>in vitro</i> or <i>in vivo</i> functional studies show no damaging effect on protein function or splicing		Not applicable as per VCEP
BS4: Non segregation with disease	_STR _MOD _SUP	As per VCEP
BP2: Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis		Not applicable as per VCEP
BP6: Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation		Not applicable as per VCEP
BP5: Alternate molecular basis for disease		Not applicable as per VCEP

Version History/Amendments

Revised version	Date	Section	Update	Amended by	Approved by
1.0	02/06/2023	--	Initial Version	--	
1.1	04/08/2023	PM2	Specified to use female-only sex matched controls.	Allen	CStAG
1.1	30/10/2023	PS4	Requirement for a minimum of 2 case observations and female-only sex matched controls.	Garrett	CStAG
1.2	25/01/2024	Top of page	Statement updated to confirm this guidance should follow the VCEP PALB2 guidance v1.1.0	Allen	CStAG
1.2	25/01/2024	BP4	Evidence criteria allowed as per VCEP	Allen	CStAG
1.2	25/01/2024	BA1/BS1	Specified to use female-only sex matched controls.	Allen	CStAG

Criteria Specification

ClinGen Hereditary Breast, Ovarian and Pancreatic Cancer Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for PALB2 Version 1.1.0

Affiliation: [Hereditary Breast, Ovarian and Pancreatic Cancer VCEP](#)

Description : Summary of ACMG-AMP Criteria for PALB2 associated with hereditary breast and pancreatic cancer

Version : 1.1.0

Released : 11/28/2023

Release Notes :

Version 1.1 updates

Updated PS1 table to match Splicing SVI recommendations (PMID: 37352859)

Corrected errors in verbiage/typos for BP4

Changed BP4 from not applicable to applicable to make application of BP4 for splicing more visible in CSPEC

Verbiage update: RNA: At least one well-established in silico predictor (e.g. SpliceAI) shows no impact on splicing [updated from error in typing that said shows impact]

Verbiage update: *NOTE: BP4 splice predictions may not be used in conjunction with BP7_variable (RNA) [updated from just BP7 for clarification].

Added BP4 under the combinations for Benign and LB classification [this was omitted in error]

Rules for PALB2

General Comments: Version 1.1 updates Updated PS1 table to match Splicing SVI recommendations (PMID: 37352859) Corrected errors in verbiage/typos for BP4 Changed BP4 from not applicable to applicable to make application of BP4 for splicing more visible in CSPEC Verbiage update: RNA: At least one well-established in silico predictor (e.g. SpliceAI) shows no impact on splicing [updated from error in typing that said shows impact] Verbiage update: *NOTE: BP4 splice predictions may not be used in conjunction with BP7_variable (RNA) [updated from just BP7 for clarification]. Added BP4 under the combinations for Benign and LB classification [this was omitted in error]

Gene: PALB2 (HGNC:26144) [↗](#)

Transcripts:

NM_024675.3

HGNC Name: partner and localizer of BRCA2

Disease:

hereditary breast carcinoma (MONDO:0016419) [↗](#) **Mode**

of Inheritance: Autosomal dominant inheritance
familial pancreatic carcinoma (MONDO:0015278) [↗](#) **Mode**

of Inheritance: Autosomal dominant inheritance
Fanconi anemia
complementation group N (MONDO:0012565) [↗](#) **Mode**

Criteria & Strength Specifications

PVS1

Original ACMG

Summary

Null variant (nonsense, frameshift, canonical ± 1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease.

Caveats:

- Beware of genes where LOF is not a known disease mechanism (e.g. GFAP, MYH7).
- Use caution interpreting LOF variants at the extreme 3' end of a gene.
- Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact.
- Use caution in the presence of multiple transcripts.

Very Strong

Use PALB2 PVS1 Decision Tree

Modification Gene-specific, Strength

Type:

Strong

Use PALB2 PVS1 Decision Tree.

Modification Gene-specific, Strength

Type:

Moderate

Use PALB2 PVS1 Decision Tree.

Modification Gene-specific, Strength

Type:

Supporting

Use PALB2 PVS1 Decision Tree

Modification Gene-specific, Strength

Type:

-
- Instructions:**
- Use PALB2 PVS1 Decision Tree Per PALB2 Exon Map and PALB2 PVS1 Guide
 - PVS1: Predicted splice defect

- PVS1_Strength(RNA): Observed splice defect
- The default RefSeq transcript for nucleotide (c.) annotation is NM_024675.3/ENST00000261584.8. Several naturally occurring alternate splicing isoforms have been described¹. Yet, after careful examination, none of them is considered a candidate rescue transcript (very low contribution to overall expression, not coding proteins predicted functional, or both). In keeping with that, we have considered that all presumed LoF events (PVS1 decision tree specifications) occur in biologically relevant transcript(s).
- WD40 beta propeller and the Coiled-coil domain (CC) are considered indispensable for PALB2 protein function.
 - PVS1 alterations that are predicted to escape NMD, but that adversely affect the WD40 domain can be granted PVS1 (as opposed to PVS1_Strong as the recommended baseline². The following evidence supports this strength change:
 - The WD40 domain interacts with many different protein partners that are involved in the double strand break repair pathway³
 - Two different C-terminal truncating mutations (c.3549C>A and c.3549C>G) resulting in loss of the last 3 amino acids [p. (Tyr1183Ter)], were identified in trans with PALB2 stop-gain variants in three unrelated FA (FA-N) patients⁴
 - The PALB2 WD40 toroidal structure is “sealed” in the seventh blade by interaction of the C-terminal strand with the incomplete N-terminal blade. The last four residues of PALB2 (Y1183, H1184, Y1185, and S1186) are directly involved in this interaction (molecular Velcro hydrogen bonding)⁰⁰. This is the rationale for the clinical relevance of the last 4 amino acids of the protein.
 - Alterations predicted to lead to in frame losses adversely affecting the WD40 structure/function are found in trans with LoF PALB2 alterations in Fanconi Anemia patients⁴
 - Exon10 donor: c.3113+5G>C (biallelic with c.395delT)
 - Exon 12 donor: c.3350+4A>G (biallelic with c.2393_2394insCT)
 - LoF alterations are rare in GnomAD in all exons
 - GnomAD v2.1 accessed 5/30/2019
 - Total Variants (includes splice acceptor/donor-conservative)
 - 1418 variants
 - 336,349 carriers
 - LoF Flag (excludes splice acceptor/donor-conservative)
 - 95 variants (6.7%)
 - 239 carriers (.07%)
- PVS1 can be applied as per the PVS1 decision tree.
 - PVS1_Variable(RNA) shall be used for observed splice defects, whether from canonical +/-1,2 positions or other spliceogenic

regions (including mid-exonic missense/synonymous variants that cause splice defects) with baseline weight as per the below decision tree. Weight can be further modified based on the quality of the RNA study including consideration of concepts such as:

- Starting material (where patient material is preferable to in vitro minigene)
- Use of NMD inhibitors where translation does occur such as cell lines⁵⁶
- Primer design (to make sure it's comprehensive to capture possible multicassette events)
- Method of quantification
 - where e.g. capillary electrophoresis is preferable to estimation by gel band density
 - where SNP analysis is most preferred (where analysis of exonic SNPs and their relative presence in aberrant and WT transcripts is informative)
- Quantification (where complete effects should have increased weight over incomplete effects)
- Specific guidance on the use of RNA evidence in variant assessment is not a gene-specific consideration for PALB2 at this time, therefore discretion is left to assessors until further guidance is provided for this general concept from the Sequence Variant Interpretation group.

PS1

Original ACMG Summary

Same amino acid change as a previously established pathogenic variant regardless of nucleotide change.

Example: Val->Leu caused by either G>C or G>T in the same codon.

Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

Strong

Use PALB2 PS1 Splicing table

Modification General recommendation

Type:

Moderate

Use PALB2 PS1 Splicing table

Modification General recommendation

Type:

Supporting

Use PALB2 PS1 Splicing table

Modification General recommendation

Type:

- Instructions:**
- **Protein:** Do not use. Missense changes are not yet confirmed as a mechanism of disease for PALB2
 - **Splicing:** See PALB2 PS1 Table (PMID: **37352859**)

PS2

Original ACMG

Summary

De novo (both maternity and paternity confirmed) in a patient with the disease and no family history.

Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, etc. can contribute to non-maternity.

Not Applicable

Comments:

- Do not use for AD or AR disease: Informative de novo occurrences have not yet been observed and de novo AR conditions are unlikely to be informed by phase
- Autosomal Dominant Disease: Do not use - Informative de novo occurrences have not yet been observed for autosomal dominant disease. As breast cancer is relatively common and occurs frequently as an apparently sporadic event, de novo is unlikely to ever be informative unless specific features of PALB2-related cancer predisposition are identified.
- Autosomal Recessive Disease: Do not use - de novo occurrences are too rare to be informative at this time. In addition, in a biallelic state, de novo occurrences have an exceedingly low probability of being able to be confirmed as in trans because parental testing (and identification of one variant in each parent) is typically required without the use of long-range technologies.

PS3

Original ACMG

Summary

Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product.

Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well-established.

Not Applicable

Comments:

- Protein: Do not use: Lack of known positive controls
- RNA: Do not use:

PS4**Original ACMG
Summary**

The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls.

Note 1: Relative risk (RR) or odds ratio (OR), as obtained from case-control studies, is >5.0 and the confidence interval around the estimate of RR or OR does not include 1.0. See manuscript for detailed guidance.

Note 2: In instances of very rare variants where case-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.

Strong

Case-control studies; $p\text{-value} \leq .05$ AND (Odds ratio, hazard ratio, or relative risk ≥ 3 OR lower 95% CI ≥ 1.5).

Modification Disease-specific

Type:

Instructions: PS4_Moderate: Do not use. Proband counting for genes causing a common disorder need to be calibrated in a population-specific way before use.

PM1**Original ACMG
Summary**

Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation.

Not Applicable

Comments: Do not use: Missense pathogenic variation in PALB2 is not yet confirmed as a mechanism of disease.

PM2**Original ACMG
Summary**

Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes or Exome Aggregation Consortium.

Caveat: Population data for indels may be poorly called by next generation sequencing.

Supporting

Variant absent in gnomAD or present in $\leq 1/300,000$ alleles

Modification Gene-specific, Strength

Type:

- Instructions:**
- PM2_supporting is not considered a conflicting piece of evidence for variants that otherwise are likely benign/benign
 - Use as PM2_Supporting (not moderate)

PM3

Original ACMG

Summary

For recessive disorders, detected in trans with a pathogenic variant

Note: This requires testing of parents (or offspring) to determine phase.

Strong

Use Fanconi Anemia PM3 tables

Modification Disease-specific, Strength

Type:

Moderate

Use Fanconi Anemia PM3 tables

Modification Disease-specific, Strength

Type:

Supporting

Use Fanconi Anemia PM3 tables

Modification Disease-specific, Strength

Type:

Instructions: Fanconi Anemia (FA) of any subtype is generally considered an exceedingly rare, severe, early-onset disease with variable features. In the case of *BRCA2*, hypomorphic FA patients have been described who are diagnosed at older ages with less severe phenotypes. The criteria set forth in the tables below are designed to accommodate such hypomorphs and are recommended to be applied to all FA-associated genes which may not be as well described due to the extreme infrequency of their identification, and due to ascertainment bias (for severe phenotype) in the literature.

Variant may not exceed general population frequency $>0.01\%$. Consider other gene panel test results as potential explanation for phenotype.

Multiple unrelated cases are additive.

- Ex. one individual homozygous for a variant with Fanconi gets 2.0 points. Another individual affected with Fanconi Anemia has the same variant and another truncating PALB2 variant with phase unknown gets 2.0 points. In total, there are 4.0 points towards PM3.

Phenotype consistent: Chromosomal breakage with 1 clinical feature OR at least 2 of 3 clinical features from separate categories without chromosomal breakage studies

- Ex. Chromosomal breakage testing + microcephaly / triangular face
- Ex. (Without chromosomal breakage): Myelodysplastic Syndrome and microcephaly / triangular face. Individuals must have features from at least 2 of 3 distinct Clinical Feature categories below.
- Positive for chromosome breakage test:
 - Increased chromosome breakage and/or radial forms on cytogenetic testing of lymphocytes with diepoxybutane (DEB) or mitomycin C (MMC)
- Clinical features indicative of FA, including
 - Physical features (in ~75% of affected persons), include:
 - prenatal and/or postnatal short stature
 - abnormal skin pigmentation (e.g., café au lait macules, hypo-pigmentation)
 - Skeletal malformations (e.g., hypoplastic thumb, hypoplastic radius)
 - Microcephaly, triangular face
 - Ophthalmic anomalies
 - Genitourinary tract anomalies.
 - See Orphanet ¹³ for full list of >100 HPO terms (and their reported frequency).
 - Pathology findings and laboratory findings (non-cancer related) include
 - progressive bone marrow failure (unrelated to cancer treatment)
 - aplastic anemia
 - Myelodysplastic syndrome
 - Inordinate toxicities from chemotherapy or radiation
 - macrocytosis
 - cytopenia (especially thrombocytopenia, leukopenia, and neutropenia)
 - increased fetal hemoglobin (often precedes anemia).
 - Note: FA patients with very early onset cancer (≤ 5 yr) may not present with hematologic disease, which is reported to have median age at onset of 7 years in FA patients in general¹⁴
 - Cancer diagnosis ≤ 5 yr, particularly

- Blood cancers (AML)
- Brain cancers (medulloblastoma, neuroblastoma)
- Wilms Tumor

Specifications are adapted from definitions from GeneReviews (last revision June 3, 2021)

PM4

Original ACMG Summary

Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants.

Not Applicable

Comments:

- Do not use for in-frame deletions/insertions that are not already PVS1-eligible as no information is available to justify the application of this rule.
- In addition, missense and small in-frame indels are not yet confirmed as a mechanism of disease for PALB2.
- Do not use for stop-loss due to lack of data on stop-loss variants.

PM5

Original ACMG Summary

Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before.

Example: Arg156His is pathogenic; now you observe Arg156Cys.

Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

Supporting

Apply to frameshifting or truncating variants with premature termination codons upstream of p.Tyr1183, based on location of the most C-terminal known pathogenic variant, p.Tyr1183*

Modification Gene-specific, Strength
Type:

Instructions:

- For protein: Do not use. Missense changes are not yet confirmed as a mechanism of disease for PALB2.
- Apply as PM5_Supporting to frameshifting or truncating variants with premature termination codons upstream of p.Tyr1183, based on location of the most C-terminal known pathogenic variant, p.Tyr1183*
- Apply to splice variants as PM5_supporting for splice variants can only be applied for variants premature termination codons upstream

of p.Tyr1183 where PVS1_VS(RNA) is applied based on high quality observed splicing impact and must be NMD prone

PM6

Original ACMG Summary

Assumed de novo, but without confirmation of paternity and maternity.

Not Applicable

Comments: Do not use for AD or AR disease: Informative de novo occurrences have not yet been observed and de novo AR conditions are unlikely to be informed by phase

PP1

Original ACMG Summary

Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease.

Note: May be used as stronger evidence with increasing segregation data.

Strong

LOD ≥ 1.26 or Bayes Factor (LR) $\geq 18:1$

Modification Gene-specific

Type:

Moderate

LOD $\geq .60$ or Bayes Factor (LR) $\geq 4:1$

Modification Gene-specific

Type:

Supporting

LOD ≥ 0.3 or Bayes Factor (LR) $\geq 2:1$

Modification Gene-specific

Type:

Instructions:

- AD Condition: use as per Co-Segregation Guidelines
- AR Condition: informative instances of co-segregation in FANCN families are too rare to be considered for weight at this time.
- Co-Segregation Guidelines
 - Quantitative co-segregation analysis is mandated for more accurate assessment of causality for PALB2 alterations. It is

strongly preferred that biocurators use a quantitative method that accommodates both pathologies of AD PALB2: breast cancer, ovarian cancer and pancreatic cancer. These methods may be conducted by biostatisticians, particularly if they are able to compute LR scores⁹ using multiple phenotypes.

- PP1_Strong: LOD ≥ 1.26 or Bayes Factor (LR) $\geq 18:1$
 - PP1_Moderate: LOD $\geq .60$ or Bayes Factor (LR) $\geq 4:1$
 - PP1: LOD ≥ 0.3 or Bayes Factor (LR) $\geq 2:18$
 - BS4_Supporting: LOD $\leq -.32$ or Bayes Factor (LR) $\leq .48$
 - BS4_Moderate: LOD $\leq -.64$ or Bayes Factor (LR) $\leq .23$
 - BS4: LOD ≤ -1.28 or Bayes Factor (LR) $\leq .053:1$
- A freely available tool, COOL (COsegregation OnLine) from Bing-Jian Feng's laboratory can be used to calculate LoD scores for co-segregation analysis
 1. Navigate to COOL (COsegregation OnLine)⁷
 2. Input 'PALB2' into the 'Input a Gene Symbol' field (the PALB2 defaults are approved by this VCEP)
 3. Upload your Pedigree File (see COOL (COsegregation OnLine) manual⁸ for formatting)
 4. Leave all defaults as is. Select 'Submit' to obtain LR based on Full Likelihood Bayes (FLB)

PP2

Original ACMG Summary

Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease.

Not Applicable

Comments: Do not use. Missense is not yet confirmed or refuted as a mechanism of disease for PALB2

PP3

Original ACMG Summary

Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.).

Caveat: As many in silico algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. PP3 can be used only once in any evaluation of a variant.

Supporting

- Protein: Do not use.

- RNA: At least one well-established in silico predictor (e.g. SpliceAI) shows impact on splicing

Modification General recommendation

Type:

-
- Instructions:**
- Protein: Do not use. So far, published predictors have yet to achieve functional outcome for PALB2 missense variants
 - RNA: At least one well-established in silico predictor (e.g. SpliceAI) shows impact on splicing
 - NOTE: Splice analysis needs to be considered for all variant types (including missense, frameshift, nonsense, etc. as any variant has the potential to impact splicing which may preclude any expected protein effects)
 - NOTE: PP3 for splice predictions may not be applied in addition to PVS1 or PVS1_Variable(RNA) codes.
 - Use caution in applying the wrong type of computational evidence (protein vs. RNA) towards the cumulative body of evidence for the opposite mechanism.

PP4

Original ACMG Summary

Patient's phenotype or family history is highly specific for a disease with a single genetic etiology.

Not Applicable

Comments: Do not use for AD disorder as breast cancer is a disease with multiple genetic etiology (genetic heterogeneity) and there are no features that can readily distinguish hereditary from sporadic causes. For AR disorder, use PM3 for specific phenotype considerations

PP5

Original ACMG Summary

Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation.

Not Applicable

This criterion is not for use as recommended by the ClinGen Sequence Variant Interpretation VCEP Review Committee. [PubMed : 29543229](#)

BA1

Original ACMG

Summary

Allele frequency is above 5% in Exome Sequencing Project, 1000 Genomes or Exome Aggregation Consortium.

Stand Alone

GnomAD **Filtering Allele Frequency** Allele frequency

>0.1%

Modification Disease-specific, Gene-specific

Type:

Instructions: Rounded from .118% established using Whiffin calculator¹⁰:

- Prevalence (breast cancer): 1:8
- Allelic Heterogeneity: 1 (differs from BS1)
- Genetic Heterogeneity: .01
- Penetrance: .53

BS1

Original ACMG

Summary

Allele frequency is greater than expected for disorder.

Strong

GnomAD **Filtering Allele Frequency** greater than expected for disease

>.01%

Modification Disease-specific, Gene-specific

Type:

Instructions:

- Rounded from .0118% established using Whiffin calculator¹⁰:

- Prevalence (breast cancer): 8
- Allelic Heterogeneity: 0.1 (differs from BA1)
- Genetic Heterogeneity: .01
- Penetrance: .53

BS2

Original ACMG

Summary

Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an

early age.

Strong

Per Fanconi Anemia BS2 tables

Modification Disease-specific
Type:

Moderate

Per Fanconi Anemia BS2 tables

Modification Disease-specific
Type:

Supporting

Per Fanconi Anemia BS2 tables

Modification Disease-specific
Type:

Instructions:

- See Fanconi Anemia BS2 tables
- Do not use for individuals in population based cohorts, such as gnomAD
- Consider multiple instances of co-occurrence with the same variant are more likely to be in cis in unrelated individuals when assessing BS2 application

BS3

Original ACMG Summary

Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing.

Not Applicable

Comments: ● Do not use: Protein functional studies (BS3) See PS3 for details ● RNA functional studies (Use BP7_Variable(RNA))

BS4

Original ACMG Summary

Lack of segregation in affected members of a family.

Caveat: The presence of phenocopies for common phenotypes (i.e. cancer, epilepsy) can mimic lack of segregation among affected individuals. Also, families may have more than

one pathogenic variant contributing to an autosomal dominant disorder, further confounding an apparent lack of segregation.

Strong

LOD \leq -1.28 or Bayes Factor (LR) $\text{LR} \leq .053:1$

Modification Gene-specific

Type:

Moderate

LOD \leq -.64 or Bayes Factor (LR) $\leq .23$

Modification Gene-specific

Type:

Supporting

LOD \leq -.32 or Bayes Factor (LR) $\leq .48$

Modification Gene-specific

Type:

Instructions:

- Quantitative co-segregation analysis is mandated for more accurate assessment of causality for PALB2 alterations. It is strongly preferred that biocurators use a quantitative method that accommodates both pathologies of AD PALB2: breast cancer, ovarian cancer and pancreatic cancer. These methods may be conducted by biostatisticians, particularly if they are able to compute LR scores⁹ using multiple phenotypes.
 - PP1_Strong: LOD \geq 1.26 or Bayes Factor (LR) \geq 18:1
 - PP1_Moderate: LOD \geq .60 or Bayes Factor (LR) \geq 4:1
 - PP1: LOD \geq 0.3 or Bayes Factor (LR) \geq 2:1
 - BS4_Supporting: LOD \leq -.32 or Bayes Factor (LR) \leq .48
 - BS4_Moderate: LOD \leq -.64 or Bayes Factor (LR) \leq .23
 - BS4: LOD \leq -1.28 or Bayes Factor (LR) $\text{LR} \leq .053:1$
- A freely available tool, COOL (COsegregation OnLine) from Bing-Jian Feng's laboratory can be used to calculate LoD scores for co-segregation analysis
 1. Navigate to COOL (COsegregation OnLine)¹¹
 2. Input 'PALB2' into the 'Input a Gene Symbol' field (the PALB2 defaults are approved by this VCEP)
 3. Upload your Pedigree File (see COOL (COsegregation OnLine) manual¹² for formatting)
 4. Leave all defaults as is. Select 'Submit' to obtain LR based on Full Likelihood Bayes (FLB)

Original ACMG

Summary

Missense variant in a gene for which primarily truncating variants are known to cause disease.

Supporting

Apply to all missense variants.

Modification Gene-specific

Type:

Instructions: Based on published and unpublished functional studies, PALB2 has a low rate of missense variants that are non-functional in relevant assays. True missense pathogenic variants are not yet confirmed or refuted but are thought to be exceedingly rare. Given the very low likelihood that missense variants are pathogenic, this rule applies to all missense variants in PALB2.

BP2

Original ACMG

Summary

Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern.

Not Applicable

Comments: Do not use: See Fanconi Anemia BS2 table

BP3

Original ACMG

Summary

In frame-deletions/insertions in a repetitive region without a known function.

Not Applicable

Comments: Do not use: small in-frame losses are neither confirmed nor refuted as a mechanism of pathogenicity for PALB2. In addition, PALB2 is not considered to have repetitive regions without known function

BP4

Original ACMG

Summary

Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc)

Caveat: As many in silico algorithms use the same or very similar input for their predictions, each algorithm cannot be counted as an independent criterion. BP4 can be used only once in any evaluation of a variant.

Supporting

- Protein: Do not use. So far, published predictors have yet to achieve functional outcome for PALB2 missense variants
- RNA: At least one well-established in silico predictor (e.g. SpliceAI) shows no impact on splicing
 - o NOTE: Splice analysis needs to be considered for all variant types (including missense, frameshift, nonsense, etc. as any variant has the potential to impact splicing which may preclude any expected protein effects)
 - o NOTE: BP4 for splice predictions may not be applied in conjunction with BP7_Variable(RNA) (a lack of observed RNA defect)
 - o Use caution in applying the wrong type of computational evidence (protein vs. RNA) towards the cumulative body of evidence for the opposite mechanism.

Modification General recommendation

Type:

- Instructions:**
- Protein: Do not use. So far, published predictors have yet to achieve functional outcome for PALB2 missense variants
 - RNA: At least one well-established in silico predictor (e.g. SpliceAI) shows no impact on splicing
 - o NOTE: Splice analysis needs to be considered for all variant types (including missense, frameshift, nonsense, etc. as any variant has the potential to impact splicing which may preclude any expected protein effects)
 - o NOTE: BP4 for splice predictions may not be applied in conjunction with BP7_Variable(RNA) (a lack of observed RNA defect)
 - o Use caution in applying the wrong type of computational evidence (protein vs. RNA) towards the cumulative body of evidence for the opposite mechanism.

BP5

Original ACMG Summary

Variant found in a case with an alternate molecular basis for disease.

Not Applicable

Comments: Do not use: Cases with multiple pathogenic variants have been observed

with no noticeable difference in phenotype (e.g. BRCA1 and BRCA2). In addition, PALB2 has moderate penetrance and will naturally occur with other pathogenic variants more frequently due to higher tolerance/presence in the general population.

BP6

Original ACMG Summary

Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation.

Not Applicable

This criterion is not for use as recommended by the ClinGen Sequence Variant Interpretation VCEP Review Committee. [PubMed : 29543229](#) 

BP7

Original ACMG Summary

A synonymous variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.

Strong

BP7_Strong(RNA): Observed lack of aberrant RNA defect for silent substitutions and intronic variants. May reduce weight applied depending on assay quality.

Modification General recommendation
Type:

Moderate

- BP7_Variable(RNA): Observed Lack of aberrant RNA defect with variable weight applied depending on assay quality

Modification General recommendation
Type:

Supporting

- BP7: Synonymous and deep intronic
- BP7_Variable(RNA): Observed Lack of aberrant RNA defect with variable weight applied depending on assay quality

Modification General recommendation
Type:

Instructions: • BP7: Synonymous and deep intronic

- Can be used for deep intronic variants beyond (but not including) +7 (donor) and -21 (acceptor)
- May also apply BP4 to achieve Likely Benign
- Is not considered a conflicting piece of evidence against a body of evidence supporting a pathogenic splice defect
- BP7_Variable(RNA): RNA functional studies
 - Lack of aberrant splice defect: Please see PVS1_Variable(RNA) section (above) for guidance on baseline weights and modifications of weight based on quality for RNA assays
 - NOTE: BP4 splice predictions may not be used in conjunction with BP7_variable(RNA)

Rules for Combining Criteria

Pathogenic

≥ 2 Strong (*PVS1_Strong, PS1, PS4, PM3_Strong, PP1_Strong*)

1 Strong (*PVS1_Strong, PS1, PS4, PM3_Strong, PP1_Strong*) **AND** **≥ 3 Moderate** (*PVS1_Moderate, PS1_Moderate, PM3, PP1_Moderate*)

1 Strong (*PVS1_Strong, PS1, PS4, PM3_Strong, PP1_Strong*) **AND** **2 Moderate** (*PVS1_Moderate, PS1_Moderate, PM3, PP1_Moderate*) **AND** **≥ 2 Supporting** (*PVS1_Supporting, PS1_Supporting, PM2_Supporting, PM3_Supporting, PM5_Supporting, PP1, PP3*)

1 Strong (*PVS1_Strong, PS1, PS4, PM3_Strong, PP1_Strong*) **AND** **1 Moderate** (*PVS1_Moderate, PS1_Moderate, PM3, PP1_Moderate*) **AND** **≥ 4 Supporting** (*PVS1_Supporting, PS1_Supporting, PM2_Supporting, PM3_Supporting, PM5_Supporting, PP1, PP3*)

1 Very Strong (*PVS1*) **AND** **1 Strong** (*PS1, PS4, PM3_Strong, PP1_Strong*)

1 Very Strong (*PVS1*) **AND** **≥ 2 Moderate** (*PS1_Moderate, PM3, PP1_Moderate*)

1 Very Strong (*PVS1*) **AND** **1 Moderate** (*PS1_Moderate, PM3, PP1_Moderate*) **AND** **1 Supporting** (*PS1_Supporting, PM2_Supporting, PM3_Supporting, PM5_Supporting, PP1*)

1 Very Strong (*PVS1*) **AND** **≥ 2 Supporting** (*PS1_Supporting, PM2_Supporting, PM3_Supporting, PM5_Supporting, PP1*)

Likely Pathogenic

1 Strong (*PVS1_Strong, PS1, PS4, PM3_Strong, PP1_Strong*) **AND** **1 Moderate** (*PVS1_Moderate, PS1_Moderate, PM3, PP1_Moderate*)

1 Strong (*PVS1_Strong, PS1, PS4, PM3_Strong, PP1_Strong*) **AND** **≥ 2 Supporting** (*PVS1_Supporting, PS1_Supporting, PM2_Supporting, PM3_Supporting, PM5_Supporting, PP1, PP3*)

≥ 3 Moderate (*PVS1_Moderate, PS1_Moderate, PM3, PP1_Moderate*)

2 Moderate (*PVS1_Moderate, PS1_Moderate, PM3, PP1_Moderate*) **AND** **≥ 2 Supporting** (*PVS1_Supporting, PS1_Supporting, PM2_Supporting, PM3_Supporting, PM5_Supporting, PP1, PP3*)

1 Moderate (*PVS1_Moderate, PS1_Moderate, PM3, PP1_Moderate*) **AND** **≥ 4 Supporting** (*PVS1_Supporting, PS1_Supporting, PM2_Supporting, PM3_Supporting, PM5_Supporting, PP1, PP3*)

1 Strong (*PVS1_Strong, PS1, PS4, PM3_Strong, PP1_Strong*) **AND** **2 Moderate** (*PVS1_Moderate, PS1_Moderate, PM3, PP1_Moderate*)

1 Very Strong (*PVS1*) **AND** **1 Moderate** (*PS1_Moderate, PM3, PP1_Moderate*)

1 Very Strong (*PVS1*) **AND** **1 Supporting** (*PS1_Supporting, PM2_Supporting, PM3_Supporting, PM5_Supporting, PP1*)

Benign

≥ 2 **Strong** (BS1, BS2, BS4, BP7_Strong)

1 **Stand Alone** (BA1)

Likely Benign

1 **Strong** (BS1, BS2, BS4, BP7_Strong)

1 **Strong** (BS1, BS2, BS4, BP7_Strong) **AND** 1 **Supporting** (BS2_Supporting, BS4_Supporting, BP1, BP4, BP7)

≥ 2 **Supporting** (BS2_Supporting, BS4_Supporting, BP1, BP4, BP7)

Files & Images

PALB2 Exon and Domain Maps: [↓](#)



PALB2 PVS1 Decision Tree: [↓](#)

PALB2 PM3 and BS2 Tables: [↓](#)

PALB2 PS1 table : [↓](#)

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