# PALB2 PVS1 decision tree

1. PVS1 decision tree, based on ACMG/AMP rationale (Abou Tayoun et al, 2018), introducing some code strength modifications (upgrades and downgrades, color coded as indicated), and a few instances not considered by Tayoun et al (e.g. de novo functional GC site,. Color coded as indicated)

2. We have considered **NM_024675.3** the clinically relevant reference transcript: 13 exons, 13 coding exons, coding a 1186aa protein (UniProtKB **Q86YC2**)

3. We are not aware of any potential rescue transcripts (i.e. for the sake of simplicity, in the decision tree we will not refer to "exon is absent from biologically-relevant transcripts") (Lopez-Perolio et al, 2019)

4. We have considered two clinically relevant domains:

(i) a Coiled-coil (CC) domain spaning residues 10-40 (Song et al, 2018)

-and-

(ii) a WD40 domain spanning residues 853-1186 (Oliver et al, 2009)

5. Based on clinical, functional and structural data, we have considered in-frame alterations predicted damaging to the CC and WD40 as PVS1

6. As far as we know, p.Tyr1183X is the most C-terminal PTC variant known to be pathogenic (Reid et al 2007)

7. p.Tyr1183X pathogenicity suggests that the last four residues (YHYS) of PALB2, or at least some of them, as critical for PALB2 function. This is supported by structural considerations (Oliver et al, 2009).


-Abou Tayoun AN, Pesaran T, DiStefano MT, Oza A, Rehm HL, Biesecker LG, et al. Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion. Hum Mutat. 2018;39(11):1517-24.

-Lopez-Perolio I, Leman R, Behar R, Lattimore V, Pearson JF, Castéra L, et al. Alternative splicing and ACMG-AMP-2015-based classification of PALB2 genetic variants: an ENIGMA report. J Med Genet. 2019;56(7):453-60.

-Song F, Li M, Liu G, Swapna GVT, Daigham NS, Xia B, et al. Antiparallel Coiled-Coil Interactions Mediate the Homodimerization of the DNA Damage-Repair Protein PALB2. Biochemistry. 2018;57(47):6581-91.

-Oliver AW, Swift S, Lord CJ, Ashworth A, Pearl LH. Structural basis for recruitment of BRCA2 by PALB2. EMBO Rep. 2009;10(9):990-6.

-Boonen RACM, Vreeswijk MPG, van Attikum H. Functional Characterization of PALB2 Variants of Uncertain Significance: Toward Cancer Risk and Therapy Response Prediction. Front Mol Biosci. 2020;7:169.

-Nepomuceno TC, Carvalho MA, Rodrigue A, Simard J, Masson J-Y, Monteiro ANA. PALB2 Variants: Protein Domains and Cancer Susceptibility. Trends Cancer. 2021;7(3):188-97.

-Reid S, Schindler D, Hanenberg H, Barker K, Hanks S, Kalb R, et al. Biallelic mutations in PALB2 cause Fanconi anemia subtype FA-N and predispose to childhood cancer. Nat Genet. 2007;39(2):162-4.

-Byrd PJ, Stewart GS, Smith A, Eaton C, Taylor AJ, Guy C, et al. A Hypomorphic PALB2 Allele Gives Rise to an Unusual Form of FA-N Associated with Lymphoid Tumour Development. PLoS Genet. 2016;12(3):e1005945.

-Li SC, Goto NK, Williams KA, Deber CM. Alpha-helical, but not beta-sheet, propensity of proline is determined by peptide environment. Proc Natl Acad Sci USA. 1996;93(13):6676-81.

-Valenzuela-Palomo A, Bueno-Martínez E, Sanoguera-Miralles L, et al. Splicing predictions, minigene analyses, and ACMG-AMP clinical classification of 42 germline PALB2 splice-site variants. J Pathol. 2022; 256(3):321-334.

-Xia B, Dorsman JC, Ameziane N et al. Fanconi anemia is associated with a defect in the BRCA2 partner PALB2. Nat Genet. 2007 Feb;39(2):159-61.

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|---|---|---|---|---|
| **Initiation Codon** | | | **Alternative star codon discarded**<br>Closest potential in-frame start codon p.Met296 in Exon 4. If used, critical CC domain will be lost (Song et al, 2018).<br>p.Val132fs, p.Leu253fs clinically relevant FA-N (Reid et al, 2007) | **PVS1**<br>**(upgraded from Moderate)** |
| **Nonsense or Frameshift** | **Not predicted to undergo NMD**<br>PTC located in the 3' most exon or within the 3'-most 50 nucleotides of the penultimate exon (i.e. PTC downstream of p.Thr1100, downstream of c.3300 | | **Predicted to undergo NMD**<br><br>PTC upstream of the 3'-most 50 nucleotides of the penultimate exon (i.e. PTC upstream of p.Leu1101, upstream of c.3301)<br>*e.g. NM_024675.4(PALB2):c.3298dup p.(Thr1100Asnfs*23)* | **PVS1** |
| | | **Nonsense**<br>Nonsense SNV<br>-or-<br>Nonsense Indel | **Truncated region is critical to protein function**<br>**Nonsense introduces a PTC upstream of p.His1184**<br><br>*e.g. NM_024675.4(PALB2):c.3549C>A p.(Tyr1183*),* NM_024675.4(PALB2):c.3548dup p.(Tyr1183*) | **PVS1[A]**<br>**(upgraded from Strong)** |
| | | | **Role of truncated region in protein function is unknown**<br>**Nonsense introduces a PTC downstream of p.Tyr1183**<br><br>*nonsense allele codes for p.His1184X, p.Tyr1185X, or p.Ser1186X* | **PVS1_Moderate [B]** |
| | | **Frameshift** | **Truncated region is critical to protein function**<br>**Frameshift introduces a PTC upstream of p.His1184**<br><br>*e.g. NM_024675.4(PALB2):c.3445del p.(Ala1149Profs*14)* | **PVS1[A]**<br>**(upgraded from Strong)** |
| | | | **Altered region is critical to protein function**<br>**Frameshift starting upstream of p.His1184 codes for an alternative C-terminal end**<br>Critical WD40 C-terminal end substituted by an alternative extended sequence<br>Extension will be relatively short. 4 residues (for frame+2/-1) or 2 residues (frame+1/-2)<br><br>*e.g. NM_024675.4(PALB2):c.3542_3543del p.(Phe1181Cysfs*8)* | **PVS1_Strong [C]** |
| | | | **Altered region in protein function is unknown**<br>**Frameshift starting downstream of p.Tyr1183 codes for an alternative C-terminal end**<br>Critical WD40 C-terminal end <u>partially</u> substituted by an alternative extended sequence<br>Extension will be relatively short. 4 residues (for frame+2/-1) or 2 residues (frame+1/-2)<br><br>*e.g. NM_024675.4(PALB2):c.3556del p.(Ser1186Hisfs*5)* | **PVS1_Supporting [D]**<br>**(downgraded from Moderate)** |

A) Two different C-terminal truncating mutations (c.3549C>A, c.3549C>G) introducing the same stop codon [p.(Tyr1183Ter)] were identified in trans with *PALB2* stop-gain variants in three unrelated FA (FA-N) patients (Reid 2007). For that reason, variants introducing stop gains upstream of His1184 are considered PVS1, regardless of NMD status. Pathogenicity of p.(Tyr1183Ter) points to last four residues (YHYS), or at least some of them, as critical for PALB2 function. Probably, this critical role has a structural basis, as WD40 C-terminal residues interacting with WD40 N-terminal residues seal the toroidal structure in a "molecular Velcro" interaction, and lack of residues YHYS (is predicted to) prevent the closure of the WD40 ring (Oliver et al, 2009). Note that, at present, we do not know if all four residues or only a subset are structurally critical.

B) Depending on the location, these variant will preserve one, two, or three residues at the C-terminal YHYS end. The role of deleted residues is not necessarily critical (i.e. the preserved residues might be sufficient to seal the toroidal structure), and <10% protein removed, qualifying for moderate.

C) These variants will encode proteins with alternative C-terminal ends. We expect these alternative C-terminal ends being unable to recapitulate the "molecular Velcro" interaction with WD40 N-terminal residues, but we cannot discard the possibility of an alternative C-terminal end (partially) mimicking the critical role of the native C-terminal end in WD40 folding. A similar mechanism has been proposed at the N-terminal end of the WD40 domain (upstream sequence mimicking 7D β-strand) to explain the apparent hypomorphic nature of exon 6 skipping (Byrd et al, 2016)

D) Depending on the location, these variants will preserve one, two or three residues at the C-terminal YHYS end (as *per* B). Compare with B, these variant will code additional residues. We cannot discard the possibility of an alternative C-terminal end mimicking the critical role of YHYS in WD40 folding

| | | |
|---|---|---|
| **≥ 1 Exon Deletion** (Single exon to full gene) | Full gene deletion | **PVS1_SA (upgraded from PVS1)** |
| | **Disrupts reading frame and is predicted to undergo NMD** | **PVS1** |
| | **Disrupts reading frame and is not predicted to undergo NMD**<br>Necessarily, PTC upstream of p.His1184<br>*e.g. Δ(E12) p.(Leu1069Serfs*6)* | |
| | **Preserves reading frame**<br>**Targeting exon 2 (CC domain)**<br>-and/or-<br>**targeting ≥1 WD40 coding exons (exons 6, 7, 8, 9, 10, 11, 12, 13)** | **PVS1 (upgraded from Strong)** |
| | *Exon 6 codes for WD40 β-strand 7D that is critical for WD-40 toroidal folding (Oliver et al, 2009). Yet, Byrd et al 2016 suggests that exon 6 deletion might be to some extent hypomorphic due to some ability of exon 5 residues to mimic WD40 β-strand 7D.* | |
| | **Not targeting CC domain (exon 2)**<br>-and-<br>**Not targeting WD40 domain (exons 6-13)** | **Exon deletion necessarily removes >10% (i.e.>356nt) of coding sequence**<br>Two only possibilities:<br>**Del (E4)**<br>**Del (E3_E5)** |
| | | **PVS1_Strong** |
| **Duplication** (≥1 exon in size and must be completely contained within gene) | **PTC_NMD predicted**<br>e.g. dup (E8) | **PVS1** (if proven in tandem)<br>**-or-**<br>**PVS1_Strong** (if presumed in tandem) |
| | **Preserves reading frame: completely contained within the WD40 domain**<br>e.g. dup (E11_E12) | **PVS1_Strong** (if proven in tandem)<br>**-or-**<br>**PVS1_Moderate** (if presumed in tandem) |
| | **Preserves reading frame: not contained (or not completely contained) within the WD40 domain**<br>e.g. dup(E4), dup (E6_E7) | **PVS1_Supporting** (if proven in tandem)<br>**-or-**<br>**PVS1_N/A** (if presumed in tandem) |
| | Proven not in tandem | PVS1_N/A |
| | *cDNA analysis proving tandem and reading-frame status strongly recommended* | |

Instance not specifically addressed in:
Abou Tayoun et al, 2018

Code strenght is shown, but be hihglihgt that no specific *PALB2* variant fulfills requeriments

**GT--AG 1,2 splice Sites**

| | | | |
|---|---|---|---|
| **Disrupts reading frame and is predicted to undergo NMD** | | | **PVS1 (variants listed in A)** |
| **Disrupts reading frame and is not predicted to undergo NMD** | **Truncated/Altered region critical to protein function Splicing alteration introduces a PTC upstream of p.His1184** | | **PVS1 (upgraded from Strong) (variants listed in B)** |
| **In-frame splicing alteration predicted** | **Target region critical to protein function** (predicted damaging to CC -or- WD40 domains)[1] | | **PVS1 (upgraded from Strong) (variants listed in C)** |
| | **Role of target region unkown** (damaging to CC -or- WD40 not predicted)[2] | **alteration removes >10% of coding sequence** | **PVS1_Strong (variants listed in D)** |
| | | **alteration removes <10% of coding sequence** | **PVS1_Moderate [2)]**<br>**c.49-2A>** **C, T**<br>**c.49-1G>** **A, C, T** |
| | | **alteration inserts <10% coding sequence** | **PVS1_Supporting [3)]**<br>**c.211+1G>** **A, C, T**<br>**c.211+2T>** **A, C, G** |
| No splicing alteration predicted | | Variant creates a GC site predicted functional | **PVS1_N/A**<br>**c.108+2T>** **C** |
| | | Non-canonical GC improved to GT | **PVS1_N/A**<br>**c.3350+2C>** **T** |

1) Predicted damaging to CC (exon 2) -or- WD40 (exons 6 to 13) domains if ≥1 coding exon skipped
2) For variants targeting the exon 2 acceptor site, the predicted/observed outcome ∆(E2p6) skips two residues located at the CC domain p.(Leu17_Lys18del). p.Lys18Arg has no impact on HR efficiency (Boonen et al, 2020). p.Leu17Pro shows HR efficiency reduced >50%. Yet, we do not consider the latter result a formal proof that Leu 17 is critical for HR activity, as proline is a well establish α-helix breaker (Li et al, 1996)
3) For variants targeting the exon 3 donor site, the predicted/observed outcome ▼(E3q48) introduces 17 novel residues in the protein p.E71delinsGKSRPFTYACFIIHFPE. Supporting strength is based on bioinformatics (PROVEAN score -15.84)

Splicing predictions based on SpliceAI Delta Scores >0.2. GC sites predicted functional only if supported by SpliceAI Donor Loss (DL) Delta Score <0.8 + perfect "canonical AG/GCAAGT" match, or if experimentally validated. c.108+2T>C (DL Delta Score =0.31) is a experimentally validated leaky variant (85% FL, PMID: 34846068) creating a perfect canonical AG/GCAAGT" match.

Individual genetic variants with experimental splicing data (PVS1_O_variable strength) have been underlined and color highlighted

Instance not specifically addressed in:
Abou Tayoun et al, 2018

| PVS1 (list A) | |
|---|---|
| c.48+1G> | A, C, T |
| c.48+2T> | A, C, G |
| c.49-2A> | G |
| c.109-2A> | C, G, T |
| c.109-1G> | A, C, T |
| c.1685-1G> | A |
| c.2514+1G> | A, C, T |
| c.2514+2T> | A, C, G |
| c.2587-2A> | C, G, T |
| c.2587-1G> | A, C, T |
| c.2749-2A> | C, G, T |
| c.2749-1G> | A, C, T |
| c.2834+1G> | A, C, T |
| c.2834+2T> | A, C, G |
| c.2835-1G> | A |
| c.2997-2A> | C |
| c.2997-1G> | A, C, T |
| c.3114-2A> | C, G, T |
| c.3114-1G> | A, C, T |
| c.3201+1G> | A, C, T |
| c.3201+2T> | A, C, G |

| PVS1 (list B) (upgraded from Strong) | |
|---|---|
| c.3202-2A> | C, G, T |
| c.3202-1G> | A, C, T |
| c.3350+1G> | A, C, T |
| c.3350+2C> | A, G |
| c.3351-2A> | C, G, T |
| c.3351-1G> | A, C, T |

| PVS1 (list C) (upgraded from Strong) | |
|---|---|
| c.108+1G> | A, C, T |
| c.108+2T> | A, G |
| c.2515-2A> [4)] | C, G, T |
| c.2515-1G> [4)] | A, C, T |
| c.2586+1G> [4)] | A, C, T |
| c.2586+2T> [4)] | A, C, G |
| c.2748+1G> | A, C, T |
| c.2748+2T> | A, C, G |
| c.2835-2A> | C, G, T |
| c.2835-1G> | C, T |
| c.2996+2T> | A, C, G |
| c.2996+1G> | A, C, T |
| c.2997-2A> | G, T |
| c.3113+1G> | A, C, T |
| c.3113+2T> | A, C, G |

| PVS1_Strong (list D)[5)] | |
|---|---|
| c.212-2A> | C, G, T |
| c.212-1G> | A, C, T |
| c.1684+1G> | A, C, T |
| c.1684+2T> | A, C, G |
| c.1685-2A> | C, G, T |
| c.1685-1G> | C, T |

**4)** Variants targeting exon 6 donor and acceptor sites are predicted to cause ∆(E6) p.(Thr839_Lys862del). Exon 6 codes for WD40 β-strand 7D that is critical for WD-40 toroidal folding (Oliver et al, 2009). Yet, Byrd et al 2016 suggests that exon 6 deletion might be to some extent hypomorphic due to some ability of exon 5 residues to mimic WD40 β-strand 7D.

**5)** Due to its size, splicing analyses involving *PALB2* exons 4 (1437nt) and/or exon 5 (830nt) are challenging. Indeed, we are aware of conflicting data regarding the impact of exon 4 acceptor/donor site variants on splicing. In agreement with ACMG/AMP recommendations (lowest PVS1 strength among the different scenarios, Abou Tayoun et al, 2018), we have assigned a strong evidence strength to exon 4 acceptor/donor site variants based on Δ(E4) (No-FS, not targeting critical domains, >10% of the coding sequence). We have assigned a strong evidence strength to exon 5 acceptor site variants based on ∆(5Ep510) (No-FS, not targeting critical domains, >10% of the coding sequence)

Splicing predictions based on SpliceAI Delta Scores >0.2.
Individual genetic variants with experimental splicing data (PVS1_O_variable strength) have been underlined and color highlighted