APC CanVIG-UK Gene-Specific Guidance

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A. Garrett[1,2], S. Allen[1], L. Loong[1], M Durkie[3], G.J. Burghel[4], R. Robinson[5], A. Callaway[6], J. Field[7], B. Frugtniet[2], S. Palmer-Smith[8], J. Grant[9], J. Pagan[10], T. McDevitt[11], T. McVeigh[12], H. Hanson[1,13,14], C. Turnbull[1,11]

1) Division of Genetics and Epidemiology, The Institute of Cancer Research, London, UK.
2) St George's University Hospitals NHS Foundation Trust, Tooting, London, UK
3) Sheffield Diagnostic Genetics Service, NEY Genomic Laboratory Hub, Sheffield Children's NHS Foundation Trust, Sheffield, UK
4) Manchester Centre for Genomic Medicine and NW Laboratory Genetics Hub, Manchester University Hospitals NHS Foundation Trust, Manchester, UK
5) Yorkshire Regional Genetics Service, Leeds Teaching Hospitals NHS Trust, Leeds, UK
6) Wessex Regional Genetics Laboratory, Salisbury NHS Foundation Trust, Salisbury, UK
7) Genomics and Molecular Medicine Service, Nottingham University Hospitals NHS Trust, Nottingham, UK
8) Institute of Medical Genetics, University Hospital of Wales, Cardiff and Vale University Health Board, Cardiff, UK
9) Laboratory Genetics, Queen Elizabeth University Hospital, NHS Greater Glasgow and Clyde, Glasgow, UK
10) South East Scotland Clinical Genetics, Western General Hospital, Edinburgh, UK.
11) Department of Clinical Genetics, CHI at Crumlin, Dublin, Ireland
12) The Royal Marsden NHS Foundation Trust, Fulham Road, London
13) Peninsula Regional Genetics Service, Royal Devon University Healthcare NHS Foundation Trust, Exeter, UK
14) Faculty of Health and Life Sciences, University of Exeter, Exeter, UK

CanVIG-UK review of *APC* January 2024: Consensus to use relevant recommendations from the **ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis VCEP guidance for *APC* v2.1.0** (available at: https://clinicalgenome.org/affiliation/50099/, PDF attached below). Additional points of specification are given below where applicable.

Summary: Evidence towards Pathogenicity

Evidence element	Evidence strengths allowed				Thresholds/data-sources/applications specifically relevant to *APC*
PS4	_VSTR	_STR	_MOD	_SUP	As per VCEP
PP4					*Not applicable as per VCEP*
PM2				_SUP	As per VCEP
PVS1	_VSTR	_STR	_MOD	_SUP	As per VCEP
PS1		_STR	_MOD		As per VCEP
PM4					*Not applicable as per VCEP*
PM5			_MOD	_SUP	As per VCEP
PP3				_SUP	As per VCEP
PM1 and PP2					*Not applicable as per VCEP*
PS3	_VSTR	_STR	_MOD	_SUP	As per VCEP
PP1		_STR	_MOD	_SUP	As per VCEP
PS2 and PM6	_VSTR	_STR	_MOD	_SUP	As per VCEP
PM3					*Not applicable as per VCEP*
PP5					*Not applicable as per VCEP*

Summary: Evidence towards Benignity

BA1/BS1	_SA	_STR		As per VCEP
BS2		_STR	_SUP	As per VCEP
BP4			_SUP	As per VCEP
BP1			_SUP	As per VCEP
BP7			_SUP	As per VCEP
BP3				*Not applicable as per VCEP*
BS3		_STR	_SUP	As per VCEP
BS4		_STR	_SUP	As per VCEP
BP2			_SUP	As per VCEP
BP6				*Not applicable as per VCEP*
BP5			_SUP	As per VCEP

Version History/Amendments

Revised version	Date	Section	Update	Amended by	Approved by

1.0	14/11/2023	--	Initial Version	--	CStAG
1.1	25/01/2024	Statement	Update to confirm alignment with latest APC VCEP version (v2.1.0)	Allen	CStAG

Criteria Specification

ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for APC Version 2.1.0

Affiliation: InSiGHT Hereditary Colorectal Cancer/Polyposis VCEP

Description : The following criteria are for classic or attenuated familial adenomatous polyposis only and does not apply to Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS, MONDO:0017790). The preferred transcript for coding, intronic and promoter 1A variants is NM_000038.6 (MANE transcript). The NM_001127510.2 transcript differs from NM_000038.6 in the number of "non-coding" exons in the 5' region, which results in different exon numbering (in NM_000038.6 there is only one non-coding exon, in NM_001127510.2 there is one additional non-coding exon and one non-coding exon overlapping with NM_000038.6; the 15 coding exons are the same). For the promoter 1B deletion the preferred transcript is NM_001127511.3, which has an alternative coding exon 1. The LRG_130 summarizes all three “additional” exons of the previously mentioned transcripts, resulting in 18 exons). To standardize, variants in this document are described in HGVS nomenclature according to their positions in the NM_000038.6 transcript unless otherwise specified. Numbered exons in this document refers to exons 1-16 in the NM_000038.6transcript. Refer to Supplementary Table 1 for exon number conversions. It is important to note that these criteria are not developed for low/moderate penetrant variants (e. g. c.3920T>A p.(Ile1307Lys) and c.3949G>C p.(Glu1317Gln)).

Version : 2.1.0

Released : 11/24/2023

Release Notes :

- Correction of some inaccuracies in the Rules for Combining Criteria.
- Addition of some relevant instructions out of the supplementary material
- Change in Fig. 1A: Update of the possible pathways for “G to non-G changes”
- Change in Fig. 1B: Transfer of splice variants c.136-1G>A,C,T; c.136-2A>C,G,T; c.220+1G>A,C,T and c.220+2T>A,C,G from List E to List A and transfer of c.220G>A,C,T from List E to List B based on RNA and phenotype data
- Update of the supplementary material file.

Rules for APC

Gene: APC (HGNC:583)

Transcripts:
NM_000038.6

HGNC Name: APC regulator of WNT signaling pathway

Disease:
familial adenomatous polyposis 1 (MONDO:0021056)

Mode of Inheritance:
Autosomal dominant inheritance

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

Null variant in a gene where LOF is a known mechanism of disease. As per modified decision tree (**Figure 1**) [Reference 1].

Modification Type: Gene-specific,Strength

Strong

Null variant in a gene where LOF is a known mechanism of disease. As per modified decision tree (**Figure 1**) [Reference 1].

Modification Type: Gene-specific,Strength

Moderate

Null variant in a gene where LOF is a known mechanism of disease. As per modified decision tree (**Figure 1**) [Reference 1].

Modification Type: Gene-specific,Strength

Supporting

Null variant in a gene where LOF is a known mechanism of disease. As per modified decision tree (**Figure 1**) [Reference 1].

Modification Type: Gene-specific,Strength

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

The previously established variant was classified as Pathogenic according to the APC-specific modifications.

This criterion can be applied to both missense and splice variants in *APC*.

Missense variants: when the variant under assessment results in the same amino acid change as previously established Pathogenic variant(s).

There are currently only two Likely Pathogenic missense variants: c.3077A>G p.(Asn1026Ser) and c.3084T>A p.(Ser1028Arg). Other variants leading to the same missense change at these positions meet PS1_Moderate. No missense variant has been classified as Pathogenic based on current evidence.

Splice variants: when the variant under assessment affects splicing at the same nucleotide as a previously established Pathogenic variant. The splice prediction must be above defined thresholds (see instructions) or similar to the previously established variant by multiple *in silico* predictors.

Modification Type: Gene-specific,Strength

Moderate

The previously established variant was classified as Likely Pathogenic according to the APC-specific modifications.

This criterion can be applied to both missense and splice variants in *APC*.

Missense variants: when the variant under assessment results in the same amino acid change as previously established Likely Pathogenic variant(s).

There are currently only two Likely Pathogenic missense variants: c.3077A>G p.(Asn1026Ser) and c.3084T>A p.(Ser1028Arg). Other variants leading to the same missense change at these positions meet PS1_Moderate. No missense variant has been classified as Pathogenic based on current evidence.

Splice variants: when the variant under assessment affects splicing at the same nucleotide as a previously established Likely Pathogenic variant. The splice prediction must be above defined thresholds (see instructions) or similar to the previously established variant by multiple *in silico* predictors.

Modification Type: Gene-specific,Strength

Instructions: Recommended splice prediction programs:

- SpliceAI: https://spliceailookup.broadinstitute.org/,
- MaxEntScan : http://hollywood.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html for 5’splice sites and

http://hollywood.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq_acc.html for 3'splice sites
- VarSeak: https://varseak.bio/

For SpliceAI a loss of the native splice site is considered for scores between 0.8 and 1. A gain of a cryptic splice site is considered strong for scores between 0.8 and 1 and as moderate for a score between 0.2 and 0.8.

For MaxEntScan predictions a score of >3 is required for credibility of a native site prediction and a threshold of -15% is considered for native splice site loss (Houdayer et al. 2012, PMID 22505045). A score >3 is used as a conservative measure for cryptic site use in the context of native site loss.

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.

Very Strong

≥ 4 *de novo* scores. For curation of *de novo* score see **Tables 1** and **2**.

Modification Type: Gene-specific,Strength

Strong

2-3.5 *de novo* scores. For curation of *de novo* score see **Tables 1** and **2**.

Modification Type: Gene-specific,Strength

Moderate

1-1.5 *de novo* score. For curation of *de novo* score see **Tables 1** and **2**.

Modification Type: Gene-specific,Strength

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.

Very Strong

RNA assays show

1. a premature stop codon
   OR
2. inframe skipping of exon 13 or 14

AND the absence of full-length transcript.

Modification Type: Gene-specific,Strength

Strong

RNA assays show

1. a premature stop codon
   OR
2. inframe skipping of exon 13 or 14

AND < 10% of full-length transcript.

Modification Type: Gene-specific,Strength

Moderate

RNA assays show

1. a premature stop codon AND reports of exon deletion/skipping/loss, insertion of intronic nucleotides
   OR
2. inframe skipping of exon 13 or 14 AND reports of exon deletion/skipping/loss, insertion of intronic nucleotides
   OR
3. other inframe skipping AND absent or < 10% full-length transcript.

Modification Type: Gene-specific,Strength

Supporting

RNA assays show

1. inframe skipping of exons other than exon 13 or 14 AND reports of exon deletion/skipping/loss, insertion of intronic nucleotides
   OR
2. over-expression of an alternative transcript (exons 10, 11 or 15)

Protein assays show

Increased β-catenin regulated transcription activity and/or decreased binding to β-catenin by surface plasmon resonance (only for variants within the β-catenin binding domain, which refers to codons 959-2129 of *APC*) [Reference 2].

Modification Type: Gene-specific,Strength

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.

Very Strong

≥ 16 phenotype points. For phenotype points curation see **Table 1**.

Modification Type: Gene-specific,Strength

Strong

4-15.5 phenotype points. For phenotype points curation see **Table 1**.

Modification Type: Gene-specific,Strength

Moderate

2-3.5 phenotype points. For phenotype points curation see **Table 1**.

Modification Type: Gene-specific,Strength

Supporting

1-1.5 phenotype point. For phenotype points curation see **Table 1**.

Modification Type: Gene-specific,Strength

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

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

Rare in controls, defined by an allele frequency ≤ 0.0003% (0.000003) if the allele count is > 1 OR by an allele frequency < 0.001% (0.00001) if the allele count is ≤ 1.

Modification Type: Gene-specific,Strength

Instructions: General recommendation: Use the total population from the non-cancer dataset from gnomAD (v2.1.1)

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.

Not Applicable

PM4

Original ACMG Summary

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

Not Applicable

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.

Moderate

The reported missense variant was determined to be Pathogenic according to the APC-specific modifications.

There are currently only two Likely Pathogenic missense variants: c.3077A>G p.(Asn1026Ser) and c.3084T>A p.(Ser1028Arg). Other different missense variants at these positions meet PM5_supporting. No missense variant has been classified as Pathogenic based on current evidence.

Grantham´s distance of the variant under assessment must have an equal or higher score than the reported variant [Reference 3].

Modification Type: Gene-specific,Strength

Supporting

The reported missense variant was determined to be Likely Pathogenic according to the APC-specific modifications.

There are currently only two Likely Pathogenic missense variants: c.3077A>G p.(Asn1026Ser) and c.3084T>A p.(Ser1028Arg). Other different missense variants at these positions meet PM5_supporting. No missense variant has been classified as Pathogenic based on current evidence.

Grantham´s distance of the variant under assessment must have an equal or higher score than the reported variant [Reference 3].

Modification Type: Gene-specific,Strength

PM6

Original ACMG
Summary

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

Strong

2-3.5 *de novo* scores. For curation of *de novo* score see **Tables 1** and **2**.

Modification Gene-specific,Strength

Type:

Moderate

1-1.5 *de novo* scores. For curation of *de novo* score see **Tables 1** and **2**.

Modification Type: Gene-specific,Strength

Supporting

0.5 *de novo* scores. For curation of *de novo* score see **Tables 1** and **2**.

Modification Type: Gene-specific,Strength

Instructions: PM6_VeryStrong: ≥ 4 de novo scores. For curation of de novo score see Tables 1 and 2.

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

Variant segregates in ≥ 7 meioses in ≥ 2 families.

Modification Type: Strength

Moderate

Variant segregates in 5-6 meioses in ≥ 1 family.

Modification Type: Strength

Supporting

Variant segregates in 3-4 meioses in ≥ 1 family.

Modification Type: Strength

Instructions: Affected individuals exhibit at least 0.5 point of the phenotype point system (see Table 1), for relatives also ≥ 10 or “multiple” colorectal adenomas are considered as 0.5 point.

Only genotype and phenotype positive individuals and obligate carriers with phenotype are counted (note: carriers who have received chemoprevention and may have a milder phenotype can also be counted).

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

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

Missense variants: Do not use computational prediction models for conservation, evolution, etc. *In silico* splicing predictors should be used for presumed missense variants to reveal possible splicing effects.

Non-canonical splicing variants: Multiple *in silico* splicing predictors support a deleterious effect.

Modification Type: Gene-specific,Strength

Instructions: Recommended splice prediction programs: see PS1

PP4

Original ACMG Summary

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

Not Applicable

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 Popmax Filtering Allele Frequency (AF) **≥ 0.1%** (0.001).

Modification Type: Gene-specific

Instructions: General recommendation: Use the non-cancer dataset from gnomAD (v2.1.1)

BS1

Original ACMG Summary

Allele frequency is greater than expected for disorder.

Strong

GnomAD Popmax Filtering Allele Frequency (AF) **≥ 0.001%** (0.00001).

Modification Type: Gene-specific

Instructions: General recommendation: Use the non-cancer dataset from gnomAD (v2.1.1)

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

≥ 10 points for healthy individuals **OR** ≥ 2 times in homozygous state.

A **healthy individual** worth 1 point is defined by:

Age ≥ 50 years
+ Less than 5 adenomatous polyps in a colonoscopy
+ Absence of features in Table 1

OR

Age ≥ 50 years
+ Colorectal cancer/polyposis was not the indication for testing

A **healthy individual** worth 0.5 points is defined by keywords including control, non-cancer, normal, unaffected population.

Modification Type: Gene-specific,Strength

Supporting

≥ 3 points for healthy individuals.

A **healthy individual** worth 1 point is defined by:

Age ≥ 50 years
+ Less than 5 adenomatous polyps in a colonoscopy
+ Absence of features in Table 1

OR

Age ≥ 50 years
+ Colorectal cancer/polyposis was not the indication for testing

A **healthy individual** worth 0.5 points is defined by keywords including control, non-cancer, normal, unaffected population.

Modification Type: Gene-specific,Strength

Instructions: The non-cancer dataset from gnomAD (v2.1.1) cannot be used for ”heterozygous healthy individuals”, because of the limited phenotype information and since it is usually already used for BA1/BS1. However, the non-cancer dataset from gnomAD (v2.1.1) can be used to search for homozygous individuals.

BS3

Original ACMG

Summary

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

Strong

RNA assay of a synonymous or intronic variant in constitutional patient sample demonstrates no mRNA aberration

AND

biallelic expression is shown and/or nonsense-mediated decay inhibition was used.

Modification Type: Gene-specific,Strength

Supporting

RNA assay of a synonymous or intronic variant in constitutional patient sample demonstrates no mRNA aberration, without demonstration of biallelic expression or use of nonsense-mediated decay inhibition

OR

Protein assay show retention of β-catenin regulated transcription activity comparable to wild-type (only for variants within the β-catenin binding domain, which refers to codons 959-2129 of *APC*, see PMID: 33348689)

Modification Type: Gene-specific,Strength

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

Affected member without the variant must score at least 1 phenotype point or at least two affected members without the variant must each score at least 0.5 phenotype points (see **Table 1**).

Modification Type: Gene-specific,Strength

Supporting

Affected member without the variant must score at least 0.5 phenotype points (see **Table 1**).

Modification Type: Gene-specific,Strength

BP1

Original ACMG Summary

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

Supporting

BP1 is applicable to APC with the exception of missense variants located in the first 15-amino acid repeat of the β-catenin binding domain (codon 1021-1035).

Modification Type: No change

Instructions: A number of assumed “missense” variants are in fact splice variants. At least several splice prediction tools should be used.

Recommended splice prediction programs: see PS1

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.

Supporting

Observed *in trans* with a (Likely) Pathogenic *APC* variant **OR** ≥ 3 times in an unknown phase with different (Likely) Pathogenic *APC* variants.

Modification Type: Gene-specific

BP3

Original ACMG Summary

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

Not Applicable

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

Missense variants: BP4 is not applicable.

Synonymous (silent) or intronic variants: Multiple in silico splicing predictors suggest no impact on gene or gene product.

Modification Type: Gene-specific

Instructions: Recommended splice prediction programs: see PS1

BP5

Original ACMG Summary

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

Supporting

Only applicable for an alternate genetic basis of the colorectal polyposis phenotype.

Modification Type: No change

Instructions: (Likely) Pathogenic variant in another adenomatous polyposis gene (heterozygous variants in *POLD1* or *POLE*; biallelic variants in *MUTYH, NTHL1* or *MSH3*; in patients with onset in childhood / adolescence: biallelic variants in *MLH1, MSH2, MSH6* or *PMS2*). This rule is only applicable when a colorectal polyposis phenotype is present.

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.

Supporting

A synonymous (silent) or intronic variant at or beyond +7/–21 for which multiple splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site.

Modification Type: General recommendation

Instructions: The use of BP7 with BP4 is allowed.

Recommended splice prediction programs: see PS1

Rules for Combining Criteria

Pathogenic

≥ 2 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)* **AND ≥ 3 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)* **AND 2 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)* **AND ≥ 2 Supporting** *(PVS1_Supporting, PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PM6_Supporting, PP1, PP3)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)* **AND ≥ 4 Supporting** *(PVS1_Supporting, PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PM6_Supporting, PP1, PP3)*

1 Very Strong *(PVS1)* **AND ≥ 1 Strong** *(PS1, PS2, PS4, PM6_Strong, PP1_Strong)*

1 Very Strong *(PS2_Very Strong, PS3_Very Strong, PS4_Very Strong)* **AND ≥ 1 Strong** *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)*

1 Very Strong *(PVS1)* **AND ≥ 2 Moderate** *(PS1_Moderate, PS2_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)*

1 Very Strong *(PS2_Very Strong, PS3_Very Strong, PS4_Very Strong)* **AND ≥ 2 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)*

1 Very Strong *(PVS1)* **AND 1 Moderate** *(PS1_Moderate, PS2_Moderate, PS4_Moderate, PM5, PM6,*

PP1_Moderate) **AND 1 Supporting** *(PS4_Supporting, PM2_Supporting, PM5_Supporting, PM6_Supporting, PP1)*

1 Very Strong *(PS2_Very Strong, PS3_Very Strong, PS4_Very Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)* **AND 1 Supporting** *(PVS1_Supporting, PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PM6_Supporting, PP1, PP3)*

1 Very Strong *(PVS1)* **AND ≥ 2 Supporting** *(PS4_Supporting, PM2_Supporting, PM5_Supporting, PM6_Supporting, PP1)*

1 Very Strong *(PS2_Very Strong, PS3_Very Strong, PS4_Very Strong)* **AND ≥ 2 Supporting** *(PVS1_Supporting, PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PM6_Supporting, PP1, PP3)*

Likely Pathogenic

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)* **AND ≥ 2 Supporting** *(PVS1_Supporting, PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PM6_Supporting, PP1, PP3)*

≥ 3 Moderate *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)*

2 Moderate *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)* **AND ≥ 2 Supporting** *(PVS1_Supporting, PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PM6_Supporting, PP1, PP3)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)* **AND 2 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)*

1 Very Strong *(PVS1)* **AND 1 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)*

1 Very Strong *(PS2_Very Strong, PS3_Very Strong, PS4_Very Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)*

1 Very Strong *(PVS1)* **AND 1 Supporting** *(PVS1_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PM6_Supporting, PP1)*

1 Very Strong *(PS2_Very Strong, PS3_Very Strong, PS4_Very Strong)* **AND 1 Supporting** *(PVS1_Supporting, PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PM6_Supporting, PP1, PP3)*

1 Moderate *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PS4_Moderate, PM5, PM6, PP1_Moderate)* **AND ≥ 4 Supporting** *(PVS1_Supporting, PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PM6_Supporting, PP1, PP3)*

Benign

1 Stand Alone *(BA1)*

≥ 2 Strong *(BS1, BS2, BS3, BS4)*

Likely Benign

≥ 2 Supporting *(BS2_Supporting, BS3_Supporting, BS4_Supporting, BP1, BP2, BP4, BP5, BP7)*

1 Strong *(BS1, BS2, BS3, BS4)*

Files & Images

Fig. 1: Modified decision tree for PVS1_Variable [Reference 1]

Table 1&2: Table 1. Point system for phenotypic description relevant to criteria PS2, PS4, PM6, PP1 and BS4 & Table 2. Curation of de novo score for PS2 / PM6 based on the phenotype point system

APC-specifi c rules for combining criteria:

Supplementary material_V2:

References

1. Abou Tayoun AN Pesaran T et al. *Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion.* **Hum Mutat** (2018) 39 (11) p. 1517-1524. 10.1002/humu.23626 30192042
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3. Grantham R *Amino acid difference formula to help explain protein evolution.* **Science** (1974) 185 (4154) p. 862-4. 10.1126/science.185.4154.862 4843792