DICER1 CanVIG-UK Gene-Specific Guidance

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CanVIG-UK review of *DICER1* 12/01/2024: Consensus to use relevant recommendations from the **ClinGen DICER1 and miRNA-Processing Gene Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for DICER1, v1.3.0** (available at: https://cspec.genome.network/cspec/ui/svi/affiliation/50050**,** PDF attached below). Additional points of specification are given below where applicable.

Evidence Combinations: Please combine evidence using the corresponding evidence points as per the CanVIG-UK Consensus Specification.

Evidence element	Evidence strengths allowed				Thresholds/data-sources/applications specifically relevant to *DICER1*
PS4		_STR	_MOD	_SUP	As per VCEP specification
PP4				_SUP	As per VCEP specification
PM2				_SUP	As per VCEP specification
PVS1	_VSTR	_STR	_MOD		As per VCEP specification
PS1		_STR			As per VCEP specification
PM4			_MOD	_SUP	As per VCEP specification
PM5			_MOD		As per VCEP specification
PP3				_SUP	As per VCEP specification
PM1			_MOD	_SUP	As per VCEP specification
PS3		_STR	_MOD	_SUP	As per VCEP specification
PP1		_STR	_MOD	_SUP	As per VCEP specification
PP2					Not applicable, as per VCEP
PS2	_VSTR	_STR	_MOD	_SUP	As per VCEP specification
PM6					Not applicable, as per VCEP
PM3					Not applicable, as per VCEP
PP5					Not applicable (code discontinued)

BA1/BS1	_SA	_STR		As per VCEP specification
BS2		_STR		As per VCEP specification
BP4			_SUP	As per VCEP specification
BP1				Not applicable, as per VCEP
BP7			_SUP	As per VCEP specification
BP3				Not applicable, as per VCEP
BS3		_STR	_SUP	As per VCEP specification
BS4		_STR		As per VCEP specification
BP2			_SUP	As per VCEP specification
BP6				Not applicable (code discontinued)
BP5				Not applicable, as per VCEP

Version History/Amendments

Version	Date	Section	Update	Amended by	Approved by
1.0	26/03/2024	--	Initial Version	--	

Criteria Specification

ClinGen DICER1 and miRNA-Processing Gene Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for DICER1 Version 1.3.0

Affiliation: DICER1 and miRNA-Processing Gene VCEP
Description : 1. BA1/BS1/PM2 Clarification: In light of the recent release of gnomAD v4.0.0 without a (non-cancer) filter, removed the (non-cancer) text and added the following clarifying instruction: "In general, the most recent/most comprehensive gnomAD version should be used." 2. Criteria Combination Clarification: Added a general comment to the C Spec asking users to disregard the "Rules for Combining Criteria" section and instead use the "Evidence Criteria Combinations" table.
Version : 1.3.0
Released : 1/30/2024
Release Notes :
1. BA1/BS1/PM2 Clarification: In light of the recent release of gnomAD v4.0.0 without a (non-cancer) filter, removed the (non-cancer) text and added the following clarifying instruction: "In general, the most recent/most comprehensive gnomAD version should be used."
2. Criteria Combination Clarification: Added a general comment to the C Spec asking users to disregard the "Rules for Combining Criteria" section and instead use the "Evidence Criteria Combinations" table.

Rules for DICER1

General Comments: Please note that the DICER1 VCEP utilizes a Bayesian points system for final classification. Please disregard the "Rules for Combining Criteria" section of the C Spec and instead use the "Evidence Criteria Combinations" table at the bottom of the page. For more information about the Bayesian points combination, please see our open access publication: https://www.hindawi.com/journals/humu/2023/9537832/

Gene: DICER1 (HGNC:17098)
Transcripts:
NM_177438.2

HGNC Name: dicer 1, ribonuclease III
Disease:
DICER1-related tumor predisposition (MONDO:0100216) **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

Follow SVI guidance, using DICER1-specific information. Per the PVS1 workflow guidance provided in Tayoun et al. 2018[1], the following will apply:

- Nonsense or frameshift variants:
- PVS1 applies to variants predicted to result in nonsense-mediated decay (NMD); the predicted NMD cutoff for DICER1 occurs at p.Pro1850.
- PVS1_Moderate applies to variants resulting in protein truncation 3’ of this cutoff
- Canonical splice variants (+/- 1,2 intronic positions): PVS1 applies with the following exceptions:
- Exon 10 SDS/SAS: PVS1_Strong (in-frame but exon includes >10% protein)
- Exons 5, 15, 18, 22 SDS/SAS: PVS1_Moderate (in-frame and each <10% of protein)
- Exon 27 SAS: PVS1_Moderate (final exon)
- Exon 1: no criteria (non-coding)
- Variants that disrupt the translation start site (p.M1?): no criteria applied given p.M1 is not highly conserved, there are three in-frame possible alternate start codons (p.Met11, p.Met17, p.Met24), and multiple lab cases of p.Met1? without DICER1 phenotype. SDS = splice donor site; SAS = splice acceptor site. Refer to PS3 weight guidelines when a variant meets criterion for application of both PVS1 and PS3. A disease-specific PVS1 decision tree incorporating the above bullets is also included at the end of this document as an additional curation tool.

Modification Type: Disease-specific,General recommendation

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

For same AA change, must confirm there is no difference in splicing using RNA data or in-silico modeling data (concordance of MaxEntScan and SpliceAI). For non-canonical intronic

splicing variants at same nucleotide should have equal or worse splicing impact. This rule code can only be used to compare variants asserted as pathogenic by the ClinGen DICER1 VCEP. Likely pathogenic changes do not apply.

Modification Type: General recommendation

Instructions: All variants should be assessed by MaxEntScan (MES) and SpliceAI for predicting de novo and cryptic splice sites. However, for predicting impact to consensus splice sites, SpliceAI scores alone should be considered for variants outside the MES validation threshold, as MES is not capable of predicting native splice site impact for such variants. (MES validation threshold = last 3 nucleotides of exon through intronic position +6 (donor sites); intronic position -20 through first 3 nucleotides of exon (acceptor sites))

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 points

Modification Type: Strength

Strong

≥2 but less than 4 de novo points

Modification Type: Strength

Moderate

≥1 but less than 2 de novo points

Modification Type: General recommendation

Supporting

≥0.5 but less than 1 de novo points

Modification Strength

Type:

Instructions: De novo points should be tallied using the simplified table for tallying proband points and used to determine the applied strength of PS2, consistent with SVI guidance. To avoid redundancy and increase consistency, the EP has opted to drop PM6 and exclusively use PS2 for de novo evidence.

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.

Strong

RNA assay shows splicing impact that is out-of-frame, in-frame ≥193 residues, or in-frame with RNase IIIb disruption. (PS3_Moderate if PVS1_Strong is applied).

Modification Type: Disease-specific

Moderate

RNA assay shows in-frame splicing impact with change in protein length <193 residues AND RNase IIIb domain not disrupted.

Modification Type: Disease-specific,General recommendation

Supporting

In vitro cleavage assay shows failure or severely reduced capacity to produce either 5p or 3p microRNAs from a premiRNA (positive and negative controls also performed).

Modification Type: Disease-specific,Strength

Instructions: This rule should be used and weighted appropriately for variants with functional evidence of a splicing impact and/or reduced DICER1 ability to cleave pre-miRNA. Follow SVI guidance regarding control numbers for functional studies. Do not apply PS3 at any strength if PVS1 is applied at full 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.

Strong

≥4 phenotype points

Modification Type: General recommendation

Moderate

2 – 3.5 phenotype points

Modification Type: Strength

Supporting

1 – 1.5 phenotype points

Modification Type: Strength

Instructions: Unrelated probands may contribute up to 1 point each based on phenotype (see Tables 2 & 3 in ruleset) Caveats:

- Do not apply PS4 if variant meets BA1/BS1 criteria.
- Do not apply points for a phenotype in an individual with a likely pathogenic germline variant in a second gene that could have reasonably contributed to the phenotype (e.g. Wilms tumor in an individual with a P/LP WT1 variant).
- Do not apply points for a proband whose tumor sequencing is consistent with a likely sporadic event (i.e. sequencing reveals a somatic, VCEPcurated, non-hotspot, likely pathogenic DICER1 variant in addition to a somatic hotspot variant and the germline variant under assessment). Of note, DICER1 tumors that consistently or occasionally follow a classical 2- hit hypothesis (i.e. LOF of both alleles) are exempt from this caveat. For example, identification of a somatic pathogenic non-hotspot DICER1 variant in pineoblastoma[2],

pituitary blastoma[3], and lung cysts or cystic nephroma lacking mesenchymal cells[4,5] should not exclude the proband from PS4.

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.

Moderate

Putative missense variants at residues affecting metal ion-binding: codons p.S1344, p.E1705, p.D1709, p.D1713, p.G1809, p.D1810, p.E1813

Modification Type: Disease-specific

Supporting

Putative missense variants at residues in the RNase IIIb domain (p.Y1682 – p.S1846), besides the metal ion-binding residues (see PM1).

Modification Type: Strength

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

Allele frequency <0.000005 across gnomAD with no more than one allele in any subpopulation and at least 20x coverage.

Modification Type: Disease-specific,Strength

Instructions: In general, the most recent/most comprehensive gnomAD version should be used.

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

Comments: Autosomal dominant.

PM4

Original ACMG
Summary

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

Moderate

In-frame indels with a residue within the RNase IIIb domain (p.Y1682 – p.S1846).

Modification Type: Disease-specific

Supporting

In-frame indels outside of the RNase IIIb domain (p.Y1682 – p.S1846) and repeat regions (p.D606-p.D609; p.E1418-p.E1420; p.E1422-p.E1425).

Modification Type: Strength

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

Missense variant under evaluation should have equal or worse Grantham score. Splicing should be ruled out with either RNA data or agreement in splicing predictors (MaxEntScan and SpliceAI) that show no splicing effects. The other variant must be interpreted as pathogenic by the ClinGen DICER1 VCEP. Likely pathogenic changes do not apply. This rule cannot be applied in combination with PM1 or PS1.

Modification Type: General recommendation

Instructions: All variants should be assessed by MaxEntScan (MES) and SpliceAI for predicting de novo and cryptic splice sites. However, for predicting impact to consensus splice sites, SpliceAI scores alone should be considered for variants outside the MES validation threshold, as MES is not capable of predicting native splice site impact for such variants. (MES validation threshold = last 3 nucleotides of exon through intronic position +6 (donor sites); intronic position -20 through first 3 nucleotides of exon (acceptor sites))

PM6

Original ACMG Summary

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

Not Applicable

Comments: Combined with PS2. Use PS2 instead of PM6.

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

≥7 meioses across ≥2 families

Modification Type: Strength

Moderate

5 – 6 meioses across ≥1 family

Modification Type: Strength

Supporting

3 – 4 meioses across ≥1 family

Modification Type: General recommendation

Instructions: Phenotype-positive individuals should have high, moderate, or low-

specificity phenotypes (see phenotype table). (Caveat: segregation with a single low-specificity phenotype across multiple individuals (e.g. familial Wilms tumor) does not fulfill PP1.) Do not apply PP1 if variant meets BA1/BS1 criteria.

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: While DICER1 does meet recommended cutoff for missense constraint z score of ≥3.09 established by the SVI (4.23 on gnomAD) the VCEP recommends this rule not be used for DICER1 due to the presence of various missense variants throughout the gene that are clinically interpreted as benign (9) or likely benign (30) in ClinVar.

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

For missense variants, REVEL score ≥ 0.75 OR agreement in splicing predictors predict splicing effects. For splicing variants, concordance of MaxEntScan and SpliceAI.

Modification Type: Disease-specific

Instructions: All variants should be assessed by MaxEntScan (MES) and SpliceAI for predicting de novo and cryptic splice sites. However, for predicting impact to consensus splice sites, SpliceAI scores alone should be considered for variants outside the MES validation threshold, as MES is not capable of predicting native splice site impact for such variants. (MES validation threshold = last 3 nucleotides of exon through intronic position +6 (donor sites); intronic position -20 through first 3 nucleotides of exon (acceptor sites))

PP4

Original ACMG Summary

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

Supporting

Somatic tumor testing identifies somatic hotspot second hit and no additional somatic LOF variants. Tumor testing[6] of a neoplasm with known DICER1 association in a proband who carries the germline variant under evaluation reveals the following:

- A previously reported somatic second hit of DICER1 in an RNase IIIb-disrupting “hotspot” codon (p.S1344, p.E1705, p.D1709, p.D1713, p.G1809, p.D1810, or p.E1813) AND
- Retention of the germline DICER1 variant under evaluation. PP4 is NOT applicable if:
- The germline variant is a missense variant in one of the seven RNase IIIb “hotspot” codons (see PM1), OR
- Somatic sequencing reveals additional DICER1 non-hotspot variants (could be consistent with sporadic tumorigenesis).

Modification Type: Disease-specific

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

Frequency >0.003 (0.3%) in gnomAD subpopulations. Subpopulations must have >2,000 alleles tested and a minimum of 5 alleles present.

Modification Disease-specific

Type:

Instructions: In general, the most recent/most comprehensive gnomAD version should be used.

BS1

Original ACMG Summary

Allele frequency is greater than expected for disorder.

Strong

Frequency >0.0003 (0.03%) in gnomAD subpopulations. Subpopulations must have >2,000 alleles tested and a minimum of 5 alleles present.

Modification Type: Disease-specific

Instructions: In general, the most recent/most comprehensive gnomAD version should be used.

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

40+ unrelated females from a single source are tumor-free through age 50 (caveat: ratio of BS2-eligible females to PS4-eligible probands must be ≥ 40:1) OR 2+ observations of homozygosity in healthy individuals OR 1+ observation(s) of homozygosity in a healthy individual with status confirmed by parental testing.

Modification Type: Disease-specific

Supporting

10+ unrelated females from a single source are tumor-free through age 50 (caveat: ratio of BS2-eligible females to PS4-eligible probands must be ≥ 10:1) OR 2+ observations of homozygosity in individuals lacking clinical information

Modification Type: Disease-specific

BS3

Original ACMG Summary

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

Strong

For intronic or synonymous variants, no splicing impact observed via RNA assay. (Should be observed more than once.)

Modification Type: Disease-specific

Supporting

An in vitro cleavage assay must demonstrate the variant produces both 5p and 3p microRNAs from a pre-miRNA (positive and negative controls also performed). An example of an appropriate assay to which criteria could be applied is Wu et al. 2018[7].

Modification Type: Disease-specific

Instructions: This rule should be used and weighted appropriately for variants with functional evidence of no splicing impact and/or no reduced DICER1 ability to cleave pre-miRNA. Follow SVI guidance regarding control numbers for functional studies.

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

Family members should be phenotype-positive (must be high- or moderatespecificity phenotype; see phenotype table), genotype-negative 1st, 2nd, or 3rd degree relatives of the proband.

Modification Type: General recommendation

BP1

Original ACMG Summary

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

Not Applicable

Comments: This rule code does not apply to this gene, as truncating variants account for only a portion of disease-causing variants.

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

≥1 observation in trans with P/LP DICER1 variant or ≥3 observations in cis or phase unknown with 2+ different P/LP DICER1 variants.

Modification Type: Disease-specific

BP3

Original ACMG Summary

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

Not Applicable

Comments: Not applicable at this time.

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

For missense variants, REVEL score < 0.50 and agreement in splicing predictors that no

splicing effects are predicted. For synonymous/intronic/non-coding variants concordance of MaxEntScan and SpliceAI.

Modification Type: Disease-specific

Instructions: All variants should be assessed by MaxEntScan (MES) and SpliceAI for predicting de novo and cryptic splice sites. However, for predicting impact to consensus splice sites, SpliceAI scores alone should be considered for variants outside the MES validation threshold, as MES is not capable of predicting native splice site impact for such variants. (MES validation threshold = last 3 nucleotides of exon through intronic position +6 (donor sites); intronic position -20 through first 3 nucleotides of exon (acceptor sites))

BP5

Original ACMG Summary

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

Not Applicable

Comments: Given the broad spectrum of DICER1-related neoplasms and the General recommendation lack of evidence of other high-penetrance germline variants that could account for such neoplasms (except perhaps for some already low-specificity phenotypes such as Wilms tumor), this rule should not be used at this time.

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

Silent variant OR Intronic variant at or beyond +7 to -21 positions OR Other intronic or non-coding variant if the variant is the reference nucleotide in ≥1 primate and/or ≥4 mammalian species. Caveat: Variant must meet BP4 to apply BP7

Modification Type: General recommendation

Rules for Combining Criteria

Pathogenic

1 Very Strong *(PVS1, PS2_Very Strong)* **AND ≥ 1 Strong** *(PS1, PS2, PS3, PS4, PP1_Strong)*

1 Very Strong *(PVS1, PS2_Very Strong)* **AND 1 Moderate** *(PS2_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate)* **AND 1 Supporting** *(PS2_Supporting, PS3_Supporting, PS4_Supporting, PM1_Supporting, PM2_Supporting, PM4_Supporting, PP1, PP3, PP4)*

1 Very Strong *(PVS1, PS2_Very Strong)* **AND ≥ 2 Supporting** *(PS2_Supporting, PS3_Supporting, PS4_Supporting, PM1_Supporting, PM2_Supporting, PM4_Supporting, PP1, PP3, PP4)*

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

1 Strong *(PS1, PS2, PS3, PS4, PP1_Strong)* **AND ≥ 3 Moderate** *(PS2_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate)*

1 Strong *(PS1, PS2, PS3, PS4, PP1_Strong)* **AND 2 Moderate** *(PS2_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate)* **AND ≥ 2 Supporting** *(PS2_Supporting, PS3_Supporting, PS4_Supporting, PM1_Supporting, PM2_Supporting, PM4_Supporting, PP1, PP3, PP4)*

1 Very Strong *(PVS1, PS2_Very Strong)* **AND ≥ 1 Moderate** *(PS2_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate)*

Likely Pathogenic

1 Strong *(PS1, PS2, PS3, PS4, PP1_Strong)* **AND 1 Moderate** *(PS2_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate)*

1 Strong *(PS1, PS2, PS3, PS4, PP1_Strong)* **AND ≥ 2 Supporting** *(PS2_Supporting, PS3_Supporting, PS4_Supporting, PM1_Supporting, PM2_Supporting, PM4_Supporting, PP1, PP3, PP4)*

≥ 3 Moderate *(PS2_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate)*

2 Moderate *(PS2_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate)* **AND ≥ 2 Supporting** *(PS2_Supporting, PS3_Supporting, PS4_Supporting, PM1_Supporting, PM2_Supporting, PM4_Supporting, PP1, PP3, PP4)*

1 Moderate *(PS2_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate)* **AND ≥ 4 Supporting** *(PS2_Supporting, PS3_Supporting, PS4_Supporting, PM1_Supporting, PM2_Supporting, PM4_Supporting, PP1, PP3, PP4)*

1 Strong *(PS1, PS2, PS3, PS4, PP1_Strong)* **AND 2 Moderate** *(PS2_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PP1_Moderate)*

Benign

1 Stand Alone *(BA1)*

≥ 2 Strong *(BS1, BS2, BS3, BS4)*

Likely Benign

2 Supporting *(BS2_Supporting, BS3_Supporting, BS4_Supporting, BP4_Supporting, BP5, BP7)*

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

≥ **1 Strong** *(BS1, BS2, BS3, BS4)*

Files & Images

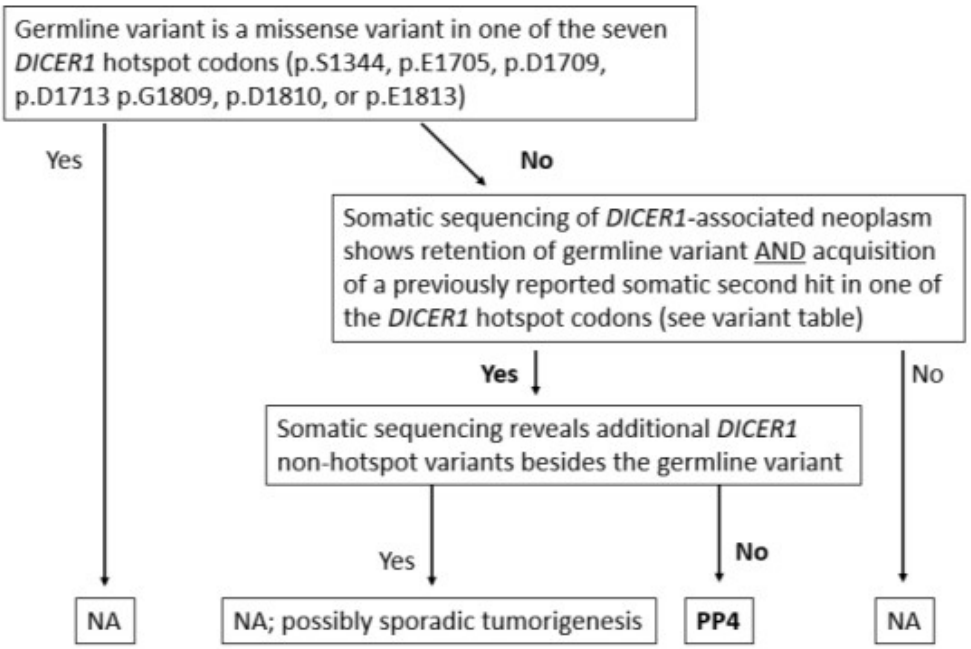
PVS1: Decision Tree Guide for PVS1

Phenotype Table: Phenotypes specificity table for use with PS4, PS2, PP1, PP4, BS4

2. ***DICER1* Phenotype table** for use with PS4 PS2, PP1, PP4, BS4.

Specificity	Phenotypes
High-specificity *(much more likely than not to have germline P/LP DICER1)*	PPB (Including Type 1r) Pituitary Blastoma Anaplastic renal sarcoma Ciliary body medulloepithelioma Cystic nephroma (<18 yrs) Embryonal rhabdomyosarcoma (Ovarian) Embryonal rhabdomyosarcoma (Cervix)
Moderate-specificity *(more likely than not to have germline P/LP DICER1)*	Differentiated thyroid cancer and/or Multinodular goiter (<18 years) Nasal chondromesenchymal hamartoma Ovarian Sertoli-Leydig cell tumors Ovarian sex-cord stromal tumor of mixed type (specifically, gynandroblastoma)
Low-specificity *(less likely to have DICER1)*	Non-parasitic liver cysts (childhood) Wilms tumor Pineoblastoma Cerebral sarcoma Lung cysts (<18 yrs)
****For PP4 use ONLY** Additional neoplasms of very low or undetermined specificity**	Thyroid neoplasms (any age) Sarcomas Juvenile hamartomatous polyps Primitive neuroectodermal/neuroepithelial neoplasms Infantile cerebellar embryonal tumors Fetal lung adenocarcinoma

PP4 Flowchart and Second Hits: Flowchart for application of PP4 and table of qualifying, previously reported somatic second hits.



Previously reported somatic second hits (PMIDs: 31342592; 23620094; 28825729):

	WT	Alternate
1344	**Ser (S)**	Leu (L)
1705	**Glu (E)**	Asp (D), Gln (Q), Lys (K), Val (V)
1709	**Asp (D)**	Asn (N), Glu (E), Gly (G), Tyr (Y), Val (V)
1713	**Asp (D)**	Val (V)
1809	**Gly (G)**	Arg (R), Glu (E), Trp (W)
1810	**Asp (D)**	Asn (N), Gly (G), His (H), Tyr (Y), Val (V)
1813	**Glu (E)**	Ala (A), Asp (D), Gln (Q), Gly (G), Lys (K), Val (V)

Table for Tallying Proband Points: Table for tallying points for PS4 and PS2. Use in conjunction with Phenotype Table.

3. **Simplified table for tallying proband points** for PS2 and PS4. Modified from "SVI Recommendation for *De Novo* Criteria (PS2 & PM6)" – Version 1.0

Phenotypic Consistency	Points per Proband			Proband Phenotype (use Phenotype Table)
	PS2		PS4	
	Confirmed	Assumed		
Phenotype highly specific for gene	2	1	1	I. ≥1 High OR II. ≥2 Moderate OR III. 1 Moderate **AND** A. 1-2 Low OR B. High or Moderate in 1st or 2nd-degree relative (unless known not to carry variant).*
Phenotype consistent with gene but not highly specific	1	0.5	0.5	IV. 1 Moderate
Phenotype consistent with gene but not highly specific and high genetic heterogeneity**	0.5	0.25	0	V. ≥1 Low

* If PP1 is applied and the proband's family contributed to the PP1 meiosis count, use IV (1 Moderate) instead of III.B to avoid double counting family history.

** Maximum allowable value of 1 may contribute to overall PS2 score to avoid counting multiple probands with only low-specificity phenotypes.

Code Strength		Total Points
PS2	PS4	
Very Strong	Strong	≥4
Strong	Moderate	2 to <4
Moderate	Supporting	1 to <2
Supporting	NA	0.5 to <1

Evidence Criteria Combinations: Modified Bayesian point system for variants with conflicting evidence codes. Adapted from Tables 2 and 3 of Tavtigian et al. 2020 (PMID: 32720330)

	Supporting	Moderate	Strong	Very Strong
Pathogenic	+1	+2	+4	+8
Benign	-1	-2	-4	-8

Category	Point ranges
Pathogenic	≥ 10
Likely Pathogenic	6 to 9
Uncertain	0 to 5
Uncertain with caveat*	-1
Likely Benign	-2 to -6
Benign	≤ -7

*A final point value of -1 may be overridden to Likely Benign in cases where at least 2 benign evidence codes are applied AND PM2_Supporting is the only pathogenic code applied.

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