RUNX1 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) The Leeds Genetics Laboratory, NEY Genomic Laboratory Hub, Leeds Teaching Hospitals NHS Trust, Leeds, UK
6) Central and South Genomics Laboratory Hub, Wessex Genomics Laboratory Service, University Hospital Southampton 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 *RUNX1* 08/03/2024: Consensus to use relevant recommendations from the **ClinGen Myeloid Malignancy VCEP, Specification for RUNX1 v2.0.0** (available at: https://clinicalgenome.org/affiliation/50034/, PDF attached below). Additional points of specification are given below where applicable.

Summary: Evidence towards Pathogenicity

Evidence element	Evidence strengths allowed				Additional points of specification relevant to *RUNX1*
PVS1	_VSTR	_STR	_MOD		As per VCEP specification. **Point of additional clarification:** For truncating variants which meet the criteria for both PVS1_str and PS3_str, PVS1 should be upgraded to PVS1_vstr, and PS3 should not be used (per communication with VCEP).
PS1		_STR	_MOD		As per VCEP specification
PS2			_MOD	_SUP	As per VCEP specification
PS3		_STR	_MOD	_SUP	As per VCEP specification. **For variants which meet both PVS1_str and PS3, please see clarification under PVS1.**
PS4		_STR	_MOD	_SUP	As per VCEP specification
PM1			_MOD	_SUP	As per VCEP specification
PM2				_SUP	As per VCEP specification
PM3					Not applicable as per VCEP specification
PM4			_MOD	_SUP	As per VCEP specification
PM5		_STR	_MOD	_SUP	As per VCEP specification
PM6			_MOD		As per VCEP specification
PP1		_STR	_MOD	_SUP	As per VCEP specification
PP2					Not applicable as per VCEP specification
PP3				_SUP	As per VCEP specification
PP4					Not applicable as per VCEP specification
PP5					Not applicable as per VCEP specification

Summary: Evidence towards Benignity

BA1/BS1	_SA	_STR		As per VCEP specification
BS2				Not applicable as per VCEP specification
BS3		_STR	_SUP	As per VCEP specification
BS4		_STR		As per VCEP specification
BP1				Not applicable as per VCEP specification

BP2			_SUP	As per VCEP specification
BP3				Not applicable as per VCEP specification
BP4			_SUP	As per VCEP specification
BP5				Not applicable as per VCEP specification
BP6				Not applicable as per VCEP specification
BP7			_SUP	As per VCEP specification

Version History/Amendments

Revised version	Date	Section	Update	Amended by	Approved by
1.0	28/03/2024	--	Initial Version	--	CStAG

Criteria Specification

ClinGen Myeloid Malignancy Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

Affiliation: Myeloid Malignancy VCEP
Description : MM-VCEP ACMG/AMP specifications for RUNX1
Version : 2.0.0
Released : 9/15/2021
Release Notes :
(1) New REVEL thresholds for PP3 ≥ 0.88 and for BP4 ≤ 0.50 (originally PP3 ≥ 0.75 and BP4 ≤ 0.15). (2) Use SpliceAI as the primary in-silico splicing predictor replacing MES and SSF. The thresholds for SpliceAI ≥ 0.38 for PP3 and ≤ 0.20 for BP4. (3) PP3 can be applied for missense, synonymous, intronic and non-coding variants if the variant impacts splicing, including the creation of cryptic novel splice sites. (4) New conservation threshold for phyloP100 way (GRCh38/hg38) ≤2.0 for BP7 (originally PhyloP score < 0.1). (5) New amino acid range for PM1_supporting and PM4_supporting to AA 89-204 (originally AA105-204). (6) Apply PM5_supporting to nonsense/frameshift variants that are downstream of c.98 (in transcript NM_001754.4). (7) PM2 is downgraded to PM2_supporting. (8) Use of the Bayesian point system in curations with conflicting evidence. (9) Remove the previous location requirements (+7/-21 in BP7 and ±3/±5 in PP3) for intronic variants in BP7 and PP3. And remove the usage for UTR variants in PP3/BP4/BP7.

PDF

Rules for RUNX1

Gene: RUNX1 (HGNC:10471)

HGNC Name: RUNX family transcription factor 1

Transcripts:
NM_001754.4 (RUNX1c)

Disease:
hereditary thrombocytopenia and hematologic cancer predisposition syndrome (MONDO:0011071)

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

Per modified RUNX1 PVS1 decision tree for SNVs and CNVs and table of splicing effects.

Modification Type: Gene-specific

Strong

Per modified RUNX1 PVS1 decision tree for SNVs and CNVs and table of splicing effects.

Modification Type: Gene-specific,Strength

Moderate

Per modified RUNX1 PVS1 decision tree for SNVs and CNVs and table of splicing effects.

Modification Type: Disease-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

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

Modification Type: None

Moderate

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

Modification Type: Strength

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.

Moderate

Phenotypic specificity category: “Phenotype consistent with gene but not highly specific and high genetic heterogeneity”. For each proven de novo case give 0.5 points, for each assumed de novo case give 0.25 point. Moderate = 1.0 points total.

Modification Type: Disease-specific,Strength

Supporting

Phenotypic specificity category: “Phenotype consistent with gene but not highly specific and high genetic heterogeneity”. For each proven de novo case give 0.5 points, for each assumed de novo case give 0.25 point. Supporting = 0.5 points total.

Modification Type: Disease-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.

Strong

Transactivation assays demonstrating altered transactivation (<20% of wt, and/or reduced to levels similar to well established pathogenic variants such as R201Q or R166Q) AND data from a secondary assay demonstrating altered function. Not applicable if variant meets PVS1. If variant meets PVS1_strong, either apply PS3_moderate or upgrade to PVS1.

Modification Type: Gene-specific

Moderate

Transactivation assays demonstrating altered transactivation (<20% of wt and/or reduced to levels similar to well established pathogenic variants such as R201Q or R166Q) OR ≥ 2 secondary assays demonstrating altered function.

Modification Type: Gene-specific,Strength

Supporting

Transactivation assays demonstrating enhanced transactivation (>115% of wt).

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.

Strong

≥ 4 probands meeting at least one of the RUNX1-phenotypic criteria.

Modification Type: Disease-specific

Moderate

2-3 probands meeting at least one of the RUNX1-phenotypic criteria.

Modification Type: Disease-specific,Strength

Supporting

1 proband meeting at least one of the RUNX1-phenotypic criteria.

Modification Type: Disease-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.

Moderate

Variant affecting one of the following amino acid residues within he RHD: R107, K110, A134, R162, R166, S167, R169, G170, K194, T196, D198, R201, R204.

Modification Type: Gene-Specific

Supporting

Variant affecting one of the other amino acid residues 89-204 within the RHD.

Modification Type: Gene-specific,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

Variant must be completely absent from all population databases.

Modification Type: Strength

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: FPD/AML is inherited in an autosomal dominant manner, thus PM3 is not applicable.

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 deletion/insertion impacting at least one of the following amino acid residues within the RHD: R107, K110, A134, R162, R166, S167, R169, G170, K194, T196, D198, R201, R204.

Modification Type: Gene-specific

Supporting

In-frame deletion/insertion impacting at least one of the other amino acid residues 89-204 within the RHD.

Modification Type: Gene-specific,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.

Strong

Missense change at an AA residue where ≥ 2 different missense changes which have been determined to be pathogenic before. Not applicable in combination with PM1.

Modification Type: Strength

Moderate

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

Modification Type: None

Supporting

Missense change at an amino acid residue where a different missense change which has been determined to be likely pathogenic before. PM5_supporting is also applied to nonsense/frameshift variants that are downstream of c.98 (in transcript NM_001754.4).

Modification Type: Strength

PM6

Original ACMG Summary

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

Moderate

Phenotypic specificity category: “Phenotype consistent with gene but not highly specific and high genetic heterogeneity” For each proven de novo case give 0.5 points, for each assumed de novo case give 0.25 point. Moderate = 1.0 points total.

Modification Type: Disease-specific,Strength

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 observed within one or across multiple families.

Modification Type: Disease-specific,Strength

Moderate

Co-segregation with disease in multiple affected family members. 5 or 6 meioses observed within one or across multiple families.

Modification Type: Disease-specific,Strength

Supporting

3 or 4 meioses observed within one or across multiple families.

Modification Type: Disease-specific

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: The recommended cutoff for PP2 by the SVI is a missense constraint z score of 3.09 which was not met by RUNX1 (2.48 on ExAC and 2.08 on gnomAD). In addition, there are 9 benign/likely benign missense RUNX1 variants 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 ≥ 0.88. For missense, synonymous and intronic (intron 4-8) variants: SpliceAI ≥ 0.38, including the creation of cryptic novel splice sites.

Modification Type: Gene-specific,Disease-specific

PP4

Original ACMG Summary

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

Not Applicable

Comments: The FPD/AML phenotype is rather unspecific and can be caused by a number of other inherited predisposition syndromes, somatic mutations or environmental factors that are insufficient to meet the original ACMG/AMP rule PP4.

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

Minor allele frequency between 0.0015 (0.15%) in any general continental population dataset with ≥ 2,000 alleles tested and variant present in ≥ 5 alleles.

Modification Type: Disease-specific

BS1

Original ACMG Summary

Allele frequency is greater than expected for disorder.

Strong

Minor allele frequency between 0.00015 (0.015%) and 0.0015 (0.15%) in any general continental population dataset with ≥ 2,000 alleles tested and variant present in ≥ 5 alleles.

Modification Type: Disease-specific

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.

Not Applicable

Comments: BS2 is not applicable since FPD/AML patients display incomplete penetrance and the average age of onset of hematologic malignancies is 33.

BS3

Original ACMG Summary

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

Strong

Transactivation assays demonstrating normal transactivation (80- 115% of wt) AND data from a secondary assay demonstrating normal function.

Modification Type: Gene-specific

Supporting

Transactivation assays demonstrating normal transactivation (80- 115% of wt).

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

Applied when seen in ≥ 2 informative meioses.

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: BP1 is not applicable for RUNX1, because both truncating and missense variants cause FPD/AML.

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

Modification Type: None

BP3

Original ACMG Summary

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

Not Applicable

Comments: RUNX1 does not contain a repetitive region without known function. BP3 is therefore deemed 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

For missense variants: REVEL score <0.50 AND SpliceAI ≤ 0.20. For synonymous and Intronic variants: SpliceAI ≤ 0.20.

Modification Type: Gene-specific,Disease-specific

BP5

Original ACMG Summary

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

Not Applicable

Comments: BP5 is not applicable. In rare circumstances, a patient can carry two pathogenic variants in genes predisposing to hematologic malignancies.

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

BP7 is applicable for synonymous and intronic which SpliceAI ≤ 0.20 AND evolutionary conservation prediction algorithms predict the site as not conserved phyloP100 way (GRCh38/hg38) ≤2.0).

Modification Type: Gene-specific,Disease-specific