ClinGen Myeloid Malignancy Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

This version specified for the following gene: *RUNX1*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50034

Gene	Disease (MONDO ID)	Clinically significant transcript
RUNX1	**Familial platelet disorder with predisposition to acute myeloid leukemia (FPD/AML), MONDO:0011071**	**NM_001754.4 (RUNX1c)**

PATHOGENIC CRITERIA		
Criteria	**Criteria Description**	**Specification**
VERY STRONG CRITERIA		
PVS1	Null variant in a gene where loss of function is a known mechanism of disease. Per modified *RUNX1* **PVS1** decision tree for SNVs and CNVs and table of splicing effects.	Gene-Specific
STRONG CRITERIA		
PVS1_Strong	Null variant in a gene where loss of function is a known mechanism of disease. Per modified *RUNX1* **PVS1** decision tree for SNVs and CNVs and table of splicing effects.	Gene-Specific, Strength
PS1	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change.	None
PS2_PM6_Strong	*De novo* (maternity and paternity confirmed) in a patient with the disease and no family history.	N/A
PS3	Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect. 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**.	Gene-Specific
PS4	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls. ≥ 4 probands meeting at least one of the *RUNX1*-phenotypic criteria.	Disease-Specific

Related publication(s): **Date Approved:** July 10, 2019

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PM5_Strong	Missense change at an amino acid residue where a different missense change which has been determined to be pathogenic before. 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.	Strength
PP1_Strong	Co-segregation with disease in multiple affected family members. ≥ 7 meioses observed within one or across multiple families.	Disease-Specific, Strength
MODERATE CRITERIA		
PVS1_Moderate	Null variant in a gene where loss of function is a known mechanism of disease. Per modified *RUNX1* **PVS1** decision tree for SNVs and CNVs and table of splicing effects.	Gene-Specific, Strength
PS1_Moderate	Same amino acid change as a previously established likely pathogenic variant regardless of nucleotide change.	Strength
PS2_PM6_Moderate	*De novo*, proven or assumed (using SVI recommendation). 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	Disease-Specific, Strength
PS3_Moderate	Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect. 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.	Gene-Specific, Strength
PS4_Moderate	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls. 2-3 probands meeting at least one of the *RUNX1*-phenotypic criteria.	Disease-Specific, Strength

PM1	Located in a mutational hot spot and/or critical and well-established functional domain without benign variation. Variant affecting one of the following amino acid residues within the RHD: R107, K110, A134, R162, R166, S167, R169, G170, K194, T196, D198, R201, R204.	Gene-Specific
PM2	Variant is absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes or ExAC. Variant must be completely absent from all population databases.	General recommendation
PM3	For recessive disorders, detected *in trans* with a pathogenic variant.	N/A
PM4	Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants. 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.	Gene-Specific
PM5	Missense change at an amino acid residue where a different missense change which has been determined to be pathogenic before.	None
PP1_Moderate	Co-segregation with disease in multiple affected family members. 5 or 6 meioses observed within one or across multiple families.	Disease-Specific, Strength
SUPPORTING CRITERIA		
PS2_PM6_Supporting	*De novo*, proven or assumed (using SVI recommendation). 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	Disease-Specific, Strength
PS3_Supporting	Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect. Transactivation assays demonstrating enhanced transactivation (>115% of wt).	Gene-Specific, Strength
PS4_Supporting	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls.	Disease-Specific, Strength

	1 proband meeting at least one of the *RUNX1*-phenotypic criteria.	
PM1_Supporting	Located in a mutational hot spot and/or critical and well-established functional domain without benign variation. Variant affecting one of the other amino acid residues 105-204 within the RHD.	Gene-Specific, Strength
PM4_Supporting	Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants. In-frame deletion/insertion impacting at least one of the other amino acid residues 105-204 within the RHD.	Gene-Specific, Strength
PM5_Supporting	Missense change at an amino acid residue where a different missense change which has been determined to be pathogenic before. Missense change at an amino acid residue where a different missense change which has been determined to be likely pathogenic before.	Strength
PP1	Co-segregation with disease in multiple affected family members. 3 or 4 meioses observed within one or across multiple families.	Disease-Specific
PP2	Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease.	N/A
PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product. For missense variants: REVEL score > 0.75 OR agreement in splicing predictors predict splicing effects (See the detailed description in the PP3 section bellow) For synonymous variants or intronic variants (intron 4-8): agreement in splicing predictors predict splicing effects	General recommendation
PP4	Patient's phenotype or family history is highly specific for a disease with a single genetic etiology.	N/A
PP5	Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation.	N/A

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BENIGN CRITERIA		
Criteria	**Criteria Description**	**Specification**
STAND ALONE CRITERIA		
BA1	Allele frequency is > 5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium. Minor allele frequency ε 0.0015 (0.15%) in any general continental population dataset with ≥ 2,000 alleles tested and variant present in ≥ 5 alleles.	Disease-Specific
STRONG CRITERIA		
BS1	Allele frequency is greater than expected for disorder. 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.	Disease-Specific
BS2	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.	N/A
BS3	Well-established *in vitro* or *in vivo* functional studies shows no damaging effect on protein function or splicing. Transactivation assays demonstrating normal transactivation (80-115% of wt) AND data from a secondary assay demonstrating normal function.	Gene-Specific
BS4	Lack of segregation in affected members of a family. Applied when seen in ≥ 2 informative meioses.	General recommendation
SUPPORTING CRITERIA		
BS3_Supporting	Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing. Transactivation assays demonstrating normal transactivation (80-115% of wt).	Gene-Specific, Strength
BP1	Missense variant in gene for which primarily truncating variants are known to cause disease.	N/A
BP2	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.	None

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BP3	In-frame deletions/insertions in a repetitive region without a known function.	N/A
BP4	Multiple lines of computational evidence suggest no impact on gene or gene product. For missense variants: REVEL score < 0.15 AND agreement in splicing predictors predict no splicing effects (See the detailed description in the BP4 section bellow) For synonymous/Intronic/Non-coding variants: Agreement in splicing predictors predict no splicing effects.	General recommendation
BP5	Variant found in a case with an alternate molecular basis for disease.	N/A
BP6	Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation.	N/A
BP7	A synonymous (silent) 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. BP7 is also applicable for intronic/non-coding variants at or beyond positions +7/-21 for which (1) SSF and MES predict either an increase in the canonical splice site score or a decrease of the canonical splice site score by no more than 10% AND no putative cryptic splice sites are created. (2) evolutionary conservation prediction algorithms predict the site as not conserved (variant is the reference nucleotide in one primate and/or 3 mammal species or PhyloP score < 0.1).	General recommendation

Key: **Gene-Specific:** Gene-specific modifications are based on data specific to *RUNX1*; **Disease-Specific:** Disease-specific modifications are based on what is known about FPD/AML; **Strength:** Increasing or decreasing strength of criteria are based on the level of evidence; **N/A:** not applicable for *RUNX1*; **General recommendation:** Criterion is applicable per the original ACMG/AMP guidelines with general notes from the MM-EP; **None:** No changes were made to existing criteria.

This version specified for the following gene: *RUNX1*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50034

VERY STRONG EVIDENCE OF PATHOGENICITY

PVS1 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:

- Use caution interpreting loss-of-function (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

ClinGen Myeloid Malignancy Expert Panel (MM-EP) notes:

(1) We recommend using RUNX1 isoform c as the default transcript (NM_001754.4), since this is the isoform used for annotation by most clinical laboratories.
(2) Three major isoforms (a, b, c) are expressed by use of two promotors and alternative splicing. Expression of the short human RUNX1a isoform has been shown to favor expansion of the hematopoietic stem cell (HSC) pool, whereas expression of the full length RUNX1b and RUNX1c isoforms function to promote hematopoietic differentiation. *RUNX1* LOF variants are a common mechanism of disease in familial platelet disorder with predisposition to acute myeloid leukemia (FPD/AML). C-terminal truncating variants not predicted to undergo nonsense-mediated mRNA decay (NMD) are classified as **PVS1_strong**, deletions of exon 1-3, presumably only affecting RUNX1 isoform c, meet **PVS1_moderate**.
(3) Most splicing effects are based on predictions. The rules can be modified in the future if new functional evidence becomes available.
(4) The ClinGen copy number variant (CNV) interpretation working group is currently developing a systematic framework for the clinical interpretation of CNVs, which may change the curation of *RUNX1* CNVs in the future.

RUNX1 Specification:

Per modified *RUNX1* **PVS1** decision tree for single-nucleotide variants (SNVs) and CNVs and table of splicing effects. Strength-modified: **PVS1, PVS1_Strong, PVS1_Moderate**

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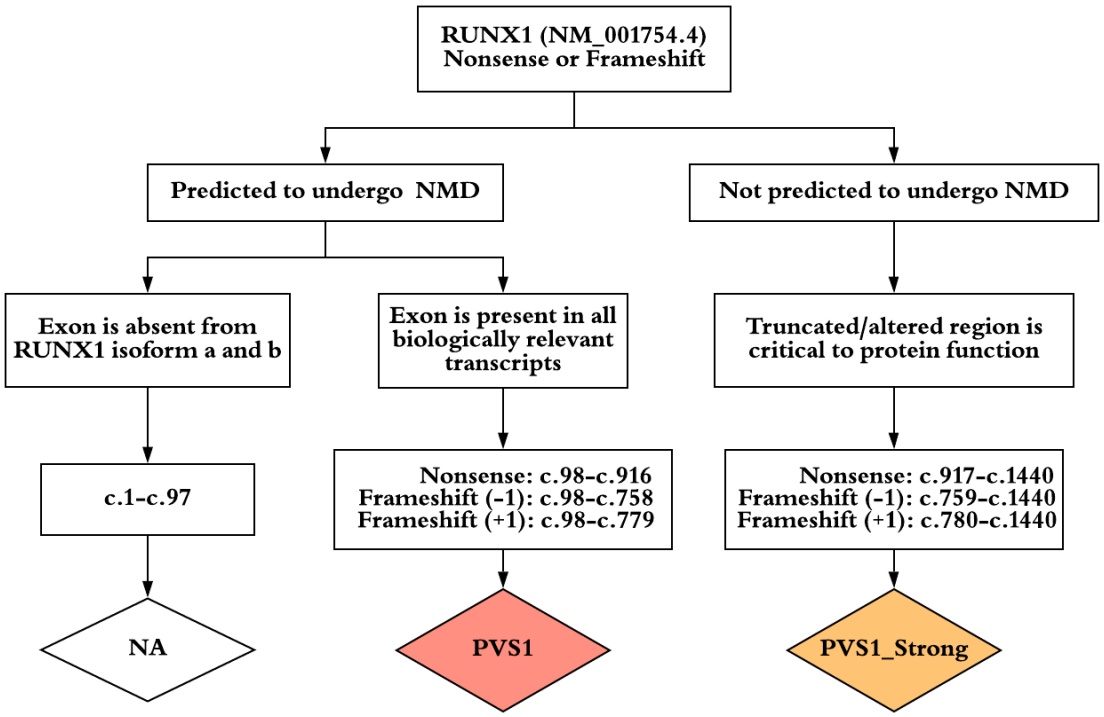
RUNX1 PVS1 decision tree for SNVs



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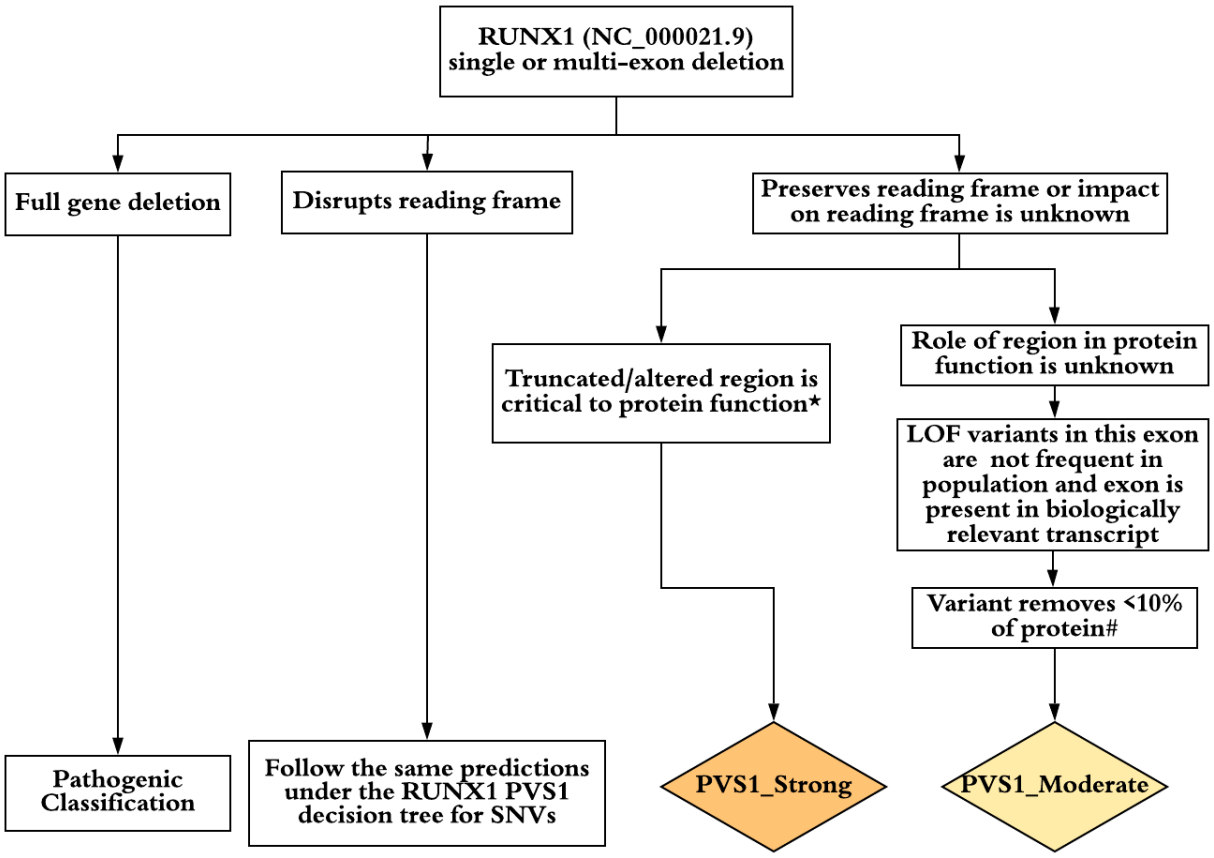
RUNX1 PVS1 decision tree for CNVs



Summary of splicing effects

Intron	GT-AG 1,2 Splice site	Location	Predicted or published effects	Classification
Intron 2	Donor	c.58	Only affect isoform c, but not isoform a and b	N/A
	Acceptor	c.59	Only affect isoform c, but not isoform a and b	N/A
Intron 3	Donor	c.97	Only affect isoform c, but not isoform a and b	N/A
	Acceptor	c.98	Only affect isoform c, but not isoform a and b	N/A
			If Skip Exon 4 with frameshift on isoform c AND cause nonsense/frameshift on isoform a/b	PVS1
Intron 4	Donor	c.351	Skip Exon 4 with frameshift	PVS1
	Acceptor	c.352	Skip Exon 5 with frameshift OR Use of Cryptic splice acceptor with a frameshift, PMID: 10508512.	PVS1
Intron 5	Donor	c.508	Skip Exon 5 with frameshift OR Use of Cryptic splice donor with a frameshift, PMID: 11830488.	PVS1
	Acceptor	c.509	Skip Exon 6 with In frame Δ171-205 and G170 (GGG->GGA), deletion in RHD.	PVS1_Strong
Intron 6	Donor	c.613	Skip Exon 6 with In frame Δ171-205 and G170 (GGG->GGA), deletion in RHD.	PVS1_Strong
	Acceptor	c.614	Skip Exon 7 with In frame Δ206-269 and R205N (AGG->AAT), remove 13% of protein.	PVS1_Strong
Intron 7	Donor	c.805	Skip Exon 7 with In frame Δ206-269 and R205N (AGG->AAT), remove 13% of protein.	PVS1_Strong
	Acceptor	c.806	Skip Exon 8 with In frame Δ270-323 and D269A (GAT->GCG), deletion in TAD.	PVS1_Strong

Related publication(s):

Date Approved: July 10, 2019

STRONG EVIDENCE OF PATHOGENICITY

PS1 Same amino acid change as a previously established pathogenic variant regardless of the nucleotide change.

MM-EP notes:

(1) RNA data or agreement in splicing predictors show no splicing effects (Splice Site Finder (SSF) and MaxEntScan (MES) predict either an increase in the canonical splice site* score or a decrease of the canonical splice site* score by no more than 10% AND no putative cryptic splice sites are created.

* Canonical splice sites are defined as the GT or AG nucleotides, at positions +/- 1 and 2 in reference to exons, for splice donor sites and acceptor sites, respectively.

(2) The previously established pathogenic variant must be reviewed by the MM-EP and asserted pathogenic/likely pathogenic before this rule can be applied.

***RUNX1* Specification:**

PS1: Same amino acid change as a previously established pathogenic variant regardless of nucleotide change.

PS1_Moderate: Same amino acid change as a previously established likely pathogenic variant regardless of the nucleotide change.

PS2 *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.

MM-EP notes:

FPD/AML phenotype is not highly specific and there is substantial genetic heterogeneity. We thus concluded that due to the lack of a highly specific phenotype and genetic heterogeneity, the maximum allowable value is 1 point contributing to the overall score.

(2) The phenotype of a deleterious *RUNX1* mutation encompasses at least one of the following three criteria:

a) **Mild to moderate thrombocytopenia with normal platelet size and volume in the absence of other causative factors** such as autoimmune (e.g. antibodies against platelet surface antigens) or drug-related thrombocytopenia.
b) **Platelet ultrastructural and/or functional defects** including platelet alpha or dense granule secretion defects or impaired platelet aggregation - particularly in response to collagen and epinephrine.
c) Diagnosis of a **hematologic malignancy, most commonly affecting the myeloid lineage causing acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS)**, less frequently involving the lymphoid lineage manifesting as T-acute lymphoblastic leukemia (T-ALL). There are rare case-reports of patients with germline *RUNX1* mutations and mixed myeloproliferative syndromes/MDS such as chronic myelomonocytic leukemia, as well as case-reports of patients with B-ALL, and hairy-cell leukemia.

(3) No family history is defined as the absence of the variant and any of the *RUNX1*-phenotypic criteria in first and second-degree relatives.
(4) The maximum allowable strength by combining **PS2** and **PM6** is to apply one moderate or two supporting rules (the maximum allowable value is still 1 point).

***RUNX1* Specification:**

Following the ClinGen Sequence Variant Interpretation (SVI) Working Group guidance, *de novo RUNX1* variants will be scored at the third tier of the point-based system (" Phenotype consistent with gene but not highly

specific and high genetic heterogeneity") with maximum allowable value of 1 point contributing to overall score:

PS2_Moderate: ≥ 2 proven *de novo* occurrences (both maternity and paternity confirmed) in patients with FPD/AML phenotype.
PS2_Supporting: 1 proven *de novo* occurrence (both maternity and paternity confirmed) in a patient with FPD/AML phenotype.

PS3 Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect on the gene or gene product.

MM-EP notes:

(1) **Transactivation assays** demonstrating altered transactivation compared to wildtype (wt) are often performed as functional studies to evaluate the pathogenicity of a *RUNX1* variant. Promoter sequences of *M-CSFR*, *PF4*, *C-FMS* and *GZMB*, containing consensus *RUNX1* binding sites TGTGGT, have been used for this purpose. The transactivation assay must include wt and known pathogenic controls, as well as co-expression with CBF®.

(2) Data from **secondary assays** are frequently used to evaluate an altered function of mutant RUNX1. Electrophoretic mobility shift assays and yeast hybrid assays are performed to demonstrate decreased DNA binding affinity, and co-immunoprecipitation assays, fluorescence resonance energy transfer assays and affinity assays can demonstrate diminished heterodimerization ability of mutant RUNX1 with CBF®. Abnormal cellular localization of mutant RUNX1 can be shown by immunofluorescence and cell-fractionation with Western Blot. Sorted primary hematopoietic stem and progenitor cells can be used for demonstration of reduced colony-forming potential and xenotransplantation experiments may reveal abnormal function of mutant RUNX1 *in vivo.*

(3) PS3 can also apply for evidence of very low or abnormal mRNA/protein expression of the variant allele as the functional consequence of a null variant or incorrect mRNA/protein products.

***RUNX1* Specification:**

PS3: 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**.

PS3_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.

PS3_supporting: Transactivation assays demonstrating enhanced transactivation (>115% of wt).

PS4 The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls.

MM-EP notes:

(1) There is currently no published *RUNX1* case control study. The criteria of a case control study can be added into the rules, if such a study will be published in the future. The original ACMG/AMP criterion states that in the absence of a published case-control study, the observation of the variant in multiple unrelated patients with the same phenotype and its absence in controls, may be used. The MM-EP created a "quasi-case-control study" with the estimated number of probands worldwide and the overall gnomAD population as control cohort. In order to apply this code, the proband has to meet the *RUNX1*-phenotypic criteria (see **PS2**) and the variant has to be either absent from gnomAD or only present once.

***RUNX1* Specification:**

PS4: ≥ 4 probands meeting at least one of the *RUNX1*-phenotypic criteria (OR 127.1).

PS4_Moderate: 2-3 probands meeting at least one of the *RUNX1*-phenotypic criteria (OR 63.5-95.3).

PS4_Supporting: 1 proband meeting at least one of the *RUNX1*-phenotypic criteria (OR 31.8).

MODERATE EVIDENCE OF PATHOGENICITY

PM1 Located in a mutational hot spot and/or critical and well-established functional domain (*e.g.* active site of an enzyme) without benign variation.

MM-EP notes:

(1) The Runt homology domain (RHD) has been established as highly conserved DNA binding domain without any benign variation in ClinVar. Thirteen somatic and/or germline mutational hotspots within the RHD have been identified: R107, K110, A134, R162, R166, S167, R169, G170, K194, T196, D198, R210, R204.

(2) Variants in other parts of the RHD (amino acid (AA) 105-204) have been described as likely pathogenic/pathogenic before, thus prompting to establish PM1_supporting with reduced strength-level for these variants.

(3) No reported germline *RUNX1* mutations in AA residues 77-104 of the RHD to date. If there is more evidence available, this region may be expanded in the future to other parts of the RHD or the protein.

***RUNX1* Specification:**

PM1: Variant affecting one of the following 13 AA residues within the RHD: R107, K110, A134, R162, R166, S167, R169, G170, K194, T196, D198, R201, R204.

PM1_Supporting: Variant affecting one of the other AA residues 105-204 within the RHD.

PM2 Variant is absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes or ExAC.

MM-EP notes:

(1) The variant must be completely absent from all population databases.
(2) The mean coverage of *RUNX1* in the population database used should be at least 20x.

PM3 For recessive disorders, detected in *trans* with a pathogenic variant.

MM-EP notes:

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

PM4 Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants.

MM-EP notes:

(1) The RHD has been established as highly conserved DNA binding domain without any benign variation in ClinVar. Thirteen somatic and/or germline mutational hotspots within the RHD have been identified: R107, K110, A134, R162, R166, S167, R169, G170, K194, T196, D198, R210, R204.

(2) Variants in other parts of the RHD (AA 105-204) have been described as likely pathogenic/pathogenic before, thus prompting to establish PM4_supporting with reduced strength-level for these variants.

(3) No reported germline *RUNX1* mutations in AA residues 77-104 of the RHD to date. If there is more evidence available, this region may be expanded in the future to other parts of the RHD or the protein.

***RUNX1* Specification:**

PM4: In-frame deletion/insertion impacting at least one of the following AA residues within the RHD: R107, K110, A134, R162, R166, S167, R169, G170, K194, T196, D198, R201, R204.

PM4_Supporting:
In-frame deletion/insertion impacting at least one of the other AA residues 105-204 within the RHD.

PM5 Missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before.

MM-EP notes:

(1) RNA data or agreement in splicing predictors show no splicing effects (Splice Site Finder (SSF) and MaxEntScan (MES) predict either an increase in the canonical splice site score or a decrease of the canonical splice site score by no more than 10% AND no putative cryptic splice sites are created.

(2) The previously established pathogenic variant must be reviewed by the MM-EP and asserted pathogenic/likely pathogenic before this rule can be applied.

***RUNX1* Specification:**

PM5_strong: Missense change at an AA residue where ≥ 2 different missense changes which have been determined to be pathogenic before.

PM5: Missense change at an AA residue where a different missense change which has been determined to be pathogenic before.

PM5_Supporting: Missense change at an AA residue where a different missense change which has been determined to be likely pathogenic before.

PM6 Assumed *de novo*, but without confirmation of paternity and maternity.

MM-EP notes:

FPD/AML phenotype is not highly specific and there is substantial genetic heterogeneity. We thus concluded that due to the lack of a highly specific phenotype and genetic heterogeneity, the maximum allowable value is 1 point contributing to the overall score.

(2) The phenotype of a deleterious *RUNX1* mutation encompasses at least one of the three phenotypic criteria (see **PS2**).

amily history is defined as the absence of the variant and any of the *RUNX1*-phenotypic criteria in first and second-degree relatives.
maximum allowable strength by combining **PS2** and **PM6** is to apply one moderate or two supporting rules (the maximum allowable value is still 1 point).

***RUNX1* Specification:**

Following the SVI guidance, assumed *de novo RUNX1* variants will be scored at the third tier of the point-based system with maximum allowable value of 1 point contributing to overall score:

PM6: ≥ 4 assumed *de novo* occurrences (without confirmation of maternity and paternity) in patients with FPD/AML phenotype.

PM6_Supporting: 2 or 3 assumed *de novo* occurrences (without confirmation of maternity and paternity) in patients with FPD/AML phenotype.

SUPPORTING EVIDENCE OF PATHOGENICITY

PP1 Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease.

MM-EP notes:

(1) The MM-EP adopted the approach being taken by other ClinGen-EPs and supported by the SVI and other work with additional meioses supporting higher evidence levels based on calculated LOD scores of 0.9, 1.5 and 2.1, respectively, with three or four meioses for **PP1**, five or six meioses for **PP1_moderate** and seven or more meioses for **PP1_strong**.

(2) Affected individuals show at least one of the *RUNX1*-phenotypic criteria (see **PS2**).

(3) Only genotype and phenotype positive individuals and obligate carriers are counted.

(4) The MM-EP waived the ACMG/AMP recommendations for demonstrating co-segregation in more than one family given that many *RUNX1* variants are unique to families and do not occur in other unrelated families.

***RUNX1* Specification:**

PP1_Strong: ≥ 7 meioses observed within one or across multiple families.

PP1_Moderate: 5 or 6 meioses observed within one or across multiple families.

PP1: 3 or 4 meioses observed within one or across multiple families.

PP2 Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease.

MM-EP notes:

The recommended cutoff for **PP2** by the SVI is a missense constraint z score of ε 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 Multiple lines of computational evidence support a deleterious effect on the gene or gene product.

MM EP notes:

(1) For *in-silico* evaluation of missense variants, the MM-EP recommends using REVEL, a meta-predictor combining 13 individual tools with high sensitivity and specificity and that has recently demonstrated highest performance compared to any individual tool or other ensemble methods.

(2) **PP3** should be applied for missense variants (1) if REVEL score > 0.75 OR (2) if the variant alters the last three bases of an exon preceding a splice donor site or following an acceptor splice site (PMID: 22505045) and the predicted decrease in the score of the canonical splice site (measured by both MES and SSF) is at least 75% regardless of the predicted creation/presence of a putative cryptic splice site.

(3) **PP3** should be applied for intronic variants (in introns 4-8) located in reference to exons at positions +3 to +5 for donor splice sites or -3 to -5 for acceptor splice sites (PMID: 27313609) and have a predicted decrease in the score of the canonical splice site by at least 75% (measured by both MES and SSF) regardless of the predicted creation/presence of a putative cryptic splice site.

(4) **PP3** should be applied for synonymous variants that alter the last three bases of an exon preceding a splice donor site or following an acceptor splice site (PMID: 22505045) and have a predicted decrease in the score of the canonical splice site by at least 75% (measured by both MES and SSF) regardless of the predicted creation/presence of a putative cryptic splice site
(5) **PP3** cannot be applied for canonical splice site variants.

PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology.

MM-EP notes:

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

MM-EP notes:

PP5 is not applicable following recommendations from the ClinGen SVI Working Group.

STAND ALONE EVIDENCE OF BENIGN IMPACT

BA1 Allele frequency is > 5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium.

Calculation of BA1:

FPD/AML with germline *RUNX1* mutation is a rare disorder. The phenotype of carriers of a germline *RUNX1* mutation includes three criteria (mild to moderate thrombocytopenia, platelet ultrastructural and/or functional defects and diagnosis of a hematologic malignancy). Of these three criteria, thrombocytopenia is the most common feature. Most clinical laboratories establish their platelet count reference values by measuring samples from at

least 120 healthy individuals and identifying the most outlying 5% of observed values. Most often, these outlying observations are split evenly between the ends of the test result distribution in the reference population, 2.5% at each end of the distribution, resulting in a two-sided reference interval. Using this approach, the prevalence of thrombocytopenia can be defined as 1 in 40 (lower 2.5%). The penetrance in families with *RUNX1* germline mutation is high to near-complete. We identified a family with a penetrance of 85% among known carriers of the mutation as the pedigree with the lowest penetrance to date. So far, no founder mutations in *RUNX1* have been reported, *de novo* variants are rare but have been described. The MM-EP modified **BA1** using extremely conservative values to account for the unknown prevalence and disease attribution to *RUNX1*. In order to obtain a *RUNX1*-specific population allele frequency for **BA1**, we utilized the Whiffin/Ware calculator (http://cardiodb.org/allelefrequencyapp/) with a prevalence of 1 in 40, a conservative unascertained penetrance estimate of 85%, an allelic heterogeneity of 100% and a maximum genetic heterogeneity of 10%. A 95% confidence interval was used to develop the threshold. The threshold developed for application of **BA1** as a stand-alone criterion is a minor allele frequency of equal to or higher than 0.0015 (0.15%).

MM-EP notes:

The MM-EP also adopted the SVI recommendation that the variant be present in any general continental population dataset with a minimum number of 2,000 alleles and variant present in ≥ 5 alleles.

***RUNX1* Specification:**

BA1: Minor allele frequency ε 0.0015 (0.15%) in any general continental population dataset with ≥ 2,000 alleles tested and variant present in ≥ 5 alleles.

STRONG EVIDENCE OF BENIGN IMPACT

BS1 Allele frequency is greater than expected for disorder.

Calculation of BS1:

Similarly, for the **BS1** calculation, we utilized the Whiffin/Ware calculator (http://cardiodb.org/allelefrequencyapp/) with a prevalence of 1 in 40, a conservative unascertained penetrance estimate of 85%, an allelic heterogeneity of 100% and a maximum genetic heterogeneity of 1% (one magnitude lower than for **BA1**). A 95% confidence interval was used to develop

the threshold. We developed a range for application of **BS1** for variants with a minor allele frequency between 0.00015 (0.015%) and 0.0015 (0.15%).

MM-EP notes:
(1) The MM-EP also adopted the SVI recommendation that the variant be present in any general continental population dataset with a minimum number of 2,000 alleles and variant present in ≥ 5 alleles.
(2) The variant can be classified as likely benign based on **BS1** alone if there is no contradictory evidence supporting pathogenicity.

***RUNX1* Specification:**

BS1: 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.

BS2 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.

MM-EP notes:

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

BS3 Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing.

MM-EP notes:

(1) **Transactivation assays** demonstrating altered transactivation compared to wt are often performed as functional studies to evaluate the pathogenicity of a *RUNX1* variant. Promoter sequences of *M-CSFR*, *PF4*, *C-FMS* and *GZMB*, containing consensus *RUNX1* binding sites TGTGGT, have been used for this purpose. The transactivation assay must include wt and known pathogenic controls, as well as co-expression with CBF®.

(2) Data from **secondary assays** are frequently used to evaluate an altered function of mutant RUNX1. Electrophoretic mobility shift assays and yeast hybrid assays are performed to demonstrate decreased DNA binding affinity,

and co-immunoprecipitation assays, fluorescence resonance energy transfer assays and affinity assays can demonstrate diminished heterodimerization ability of mutant RUNX1 with CBF®. Abnormal cellular localization of mutant RUNX1 can be shown by immunofluorescence and cell-fractionation with Western Blot. Sorted primary hematopoietic stem and progenitor cells can be used for demonstration of reduced colony-forming potential and xenotransplantation experiments may reveal abnormal function of mutant RUNX1 *in vivo.*

***RUNX1* Specification:**

BS3: Transactivation assays demonstrating normal transactivation (80-115% of wt) AND data from a secondary assay demonstrating normal function.

BS3_supporting: Transactivation assays demonstrating normal transactivation (80-115% of wt).

BS4 Lack of segregation in affected members of a family.

MM-EP notes:

This code should only be applied for genotype-positive, phenotype-negative family members.

***RUNX1* Specification:**

BS4 is applicable when seen in ≥ 2 informative meioses.

SUPPORTING EVIDENCE FOR BENIGN IMPACT

BP1 Missense variant in gene for which primarily truncating variants are known to cause disease.

MM-EP notes:

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

BP2 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.

MM-EP notes:

BP2 is applicable per the original ACMG/AMP guidelines. *In vivo*, mice lacking Runx1 die during mid-embryonic development. Biallelic pathogenic variants in *RUNX1* have never been reported in FPD/AML patients. A variant *in trans* with a known pathogenic variant or observation of the variant in the homozygous state in individuals without FPD/AML phenotype can be considered supporting benign evidence.

BP3 In-frame deletions/insertions in a repetitive region without a known function

MM-EP notes:

RUNX1 does not contain a repetitive region without known function. **BP3** is therefore deemed not applicable.

BP4 Multiple lines of computational evidence suggest no impact on gene or gene product.

MM-EP notes:

(1) For *in-silico* evaluation of missense variants, the MM-EP recommends using REVEL, a meta-predictor combining 13 individual tools with high sensitivity and specificity and that has recently demonstrated highest performance compared to any individual tool or other ensemble methods.

(2) **BP4** should be applied for missense variants (a) if REVEL score < 0.15 AND (b) SSF and MES predict either an increase in the canonical splice site score or a decrease of the canonical splice site score by no more than 10% AND no putative cryptic splice sites are created.

(3) **BP4** should be applied for synonymous, intronic and non-coding variants for which SSF and MES predict either an increase in the canonical splice site score or a

decrease of the canonical splice site score by no more than 10% AND no putative cryptic splice sites are created.

BP5 Variant found in a case with an alternate molecular basis for disease.

MM-EP notes:

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

BP6 Reputable source recently reports variant as benign but the evidence is not available to the laboratory to perform an independent evaluation.

MM-EP notes:

BP6 is not applicable following recommendations from the ClinGen SVI Working Group.

BP7 A synonymous (silent) 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.

MM-EP notes:

BP7 is also applicable for intronic/non-coding variants at or beyond positions +7/-21 for which (1) SSF and MES predict either an increase in the canonical splice site score or a decrease of the canonical splice site score by no more than 10% AND no putative cryptic splice sites are created (2) evolutionary conservation prediction algorithms predict the site as not conserved (variant is the reference nucleotide in one primate and/or 3 mammal species or PhyloP score < 0.1).

ClinGen Myeloid Malignancy Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

This version specified for the following gene: *RUNX1*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50034

RULES FOR COMBINING PATHOGENIC CRITERIA

Pathogenic

1. 1 Very Strong AND
    a. ≥1 Strong OR
    b. ≥2 Moderate OR
    c. 1 Moderate and 1 Supporting OR
    d. ≥2 Supporting
2. ≥2 Strong
3. 1 Strong AND
    a. ≥3 Moderate OR
    b. 2 Moderate AND ≥2 Supporting OR
    c. 1 Moderate AND ≥4 Supporting

Likely Pathogenic

1. 1 Very Strong AND 1 Moderate
2. 1 Strong AND 1-2 Moderate
3. 1 Strong AND ≥2 Supporting
4. ≥3 Moderate
5. 2 Moderate AND ≥2 Supporting
6. 1 Moderate AND ≥4 Supporting

RULES FOR COMBINING BENIGN CRITERIA

Benign

1. 1 Stand-Alone (BA1)
2. ≥2 Strong (BS1-BS4)

Likely Benign

1. 1 Strong and 1 Supporting
2. ≥2 Supporting
3. BS1 alone