Supplementary Methods

Statistical approaches

PS4, PS4_moderate, PS4_supporting

The current ACMG/AMP framework assigns weight to increased prevalence of variants in cases compared with controls. We created thresholds of proband occurrences that qualify for supporting, moderate, and strong weights. This approach represents a, "quasi case-control study," analogous to those made by other ClinGen EPs.[1,2] The true prevalence of FPD/AML families is unknown, but estimated to be at least 5,515 families worldwide based on a population incidence generated from a survey of centers with FPD/AML patients. The estimated ascertainment bias is 3.61 %. The proband cohort size was modeled based on the overall population in gnomAD, which was used as a proxy for healthy controls. The gnomAD population could not be race or ancestry-matched due to the small number of FPD/AML patients and limited clinical information. Using a 2 x 2 contingency table and the chi square test, OR and p-values were calculated for up to fifteen probands carrying a variant that is either absent or present only once in gnomAD's overall control population. OR values were considered confident if the 95% confidence interval did not include 1.

Estimation of CNV frequency in RUNX1

Estimating the frequency of CNVs in *RUNX1* was performed by utilizing Database of Genomic Variants (http://dgv.tcag.ca/dgv/app/home) and UCSC genome browser (https://genome.ucsc.edu/) and searching for reported CNVs, specifically copy loss variants, from published cohorts deposited in these databases. Where reported copy deletion loss overlapped positionally with coding exons of *RUNX1*, the upper bound of frequency of this event was discerned by the publication's reported cohort size. Specifically, DGV Variants **nsv1059864** from Coe *et al* [3] (position hg19 chr21:36404572...3647051) and **nsv587442** from

Cooper *et al*[4] (hg 19, chr21:36261235..36271158) were reportedly observed as one copy loss event in 29,084 and 17,421 observations, respectively with a calculated frequency of 0.003% and 0.0057%, respectively. We assume this 0.003 to 0.006% to be an upper-bound for the frequency of these CNV deletion event, as many other CNV studies deposited into the DGV showed no copy loss at this location.

References

1. Mester JL, Ghosh R, Pesaran T, et al. Gene-specific criteria for PTEN variant curation: Recommendations from the ClinGen PTEN Expert Panel. *Hum Mutat*. 2018;39(11):1581-1592.

2. Kelly MA, Caleshu C, Morales A, et al. Adaptation and validation of the ACMG/AMP variant classification framework for MYH7-associated inherited cardiomyopathies: recommendations by ClinGen's Inherited Cardiomyopathy Expert Panel. *Genet Med*. 2018;20(3):351-359.

3. Coe BP, Witherspoon K, Rosenfeld JA, et al. Refining analyses of copy number variation identifies specific genes associated with developmental delay. *Nat Genet*. 2014;46(10):1063-1071.

4. Cooper GM, Coe BP, Girirajan S, et al. A copy number variation morbidity map of developmental delay. *Nat Genet*. 2011;43(9):838-846.

Supplementary Tables

Supplementary Table S1- Quasi case-control study for modification of PS4.

proband cohort size n=5,515						
analysis	p-values	Odds ratio	p-values	Odds ratio	p-values	Odds ratio
gnomAD population	total (n=138,632)		total (n=138,632)		total (n=138,632)	
Alleles in gnomAD	0	0	1	1	2	2
proband counts						
1	<0.0001	75.4189	0.000663	25.1416	0.007713	12.5707
2	<0.0001	125.721	<0.0001	50.2924	<0.0001	25.146
3	<0.0001	>100	<0.0001	75.4523	<0.0001	37.7259
4	<0.0001	>100	<0.0001	100.6213	<0.0001	50.3103
5	<0.0001	>100	<0.0001	>100	<0.0001	62.8993
6	<0.0001	>100	<0.0001	>100	<0.0001	75.4928
7	<0.0001	>100	<0.0001	>100	<0.0001	88.091
8	<0.0001	>100	<0.0001	>100	<0.0001	100.6937
9	<0.0001	>100	<0.0001	>100	<0.0001	>100
10	<0.0001	>100	<0.0001	>100	<0.0001	>100
11	<0.0001	>100	<0.0001	>100	<0.0001	>100
12	<0.0001	>100	<0.0001	>100	<0.0001	>100
13	<0.0001	>100	<0.0001	>100	<0.0001	>100
14	<0.0001	>100	<0.0001	>100	<0.0001	>100
15	<0.0001	>100	<0.0001	>100	<0.0001	>100
level of significance	Odds Ratio					
0.001 to 0.05	supporting: OR>10					
0.0001 to 0.001	moderate: OR>30					
<0.0001	strong: OR>100					

Supplementary Table S2- Specifications for PS2/PM6.

Points awarded per *de novo* occurrence

	Points per proband	
Phenotypic consistency	**Confirmed *de novo***	**Assumed *de novo***
Phenotype consistent with gene but not highly specific and high genetic heterogeneity*	0.5	0.25
Phenotype not consistent with gene	0	0

* Maximum allowable value of 1 may contribute to overall score (*RUNX1* phenotype is not highly specific and there is substantial genetic heterogeneity).

Determining the level of strength for *de novo* occurrence (PS2/PM6).

Supporting (0.5 points)[#]	**Moderate (1 point)**[#]
1 confirmed *de novo* **(PS2)** or 2 assumed *de novo* **(PM6)**	2 confirmed *de novo* **(PS2)** or 4 assumed *de novo* **(PM6)**

[#] Combining **PS2** and **PM6** criteria (e.g. in the case of the same variant both confirmed and assumed *de novo*) is possible by recognizing that the maximum allowable value is still 1.

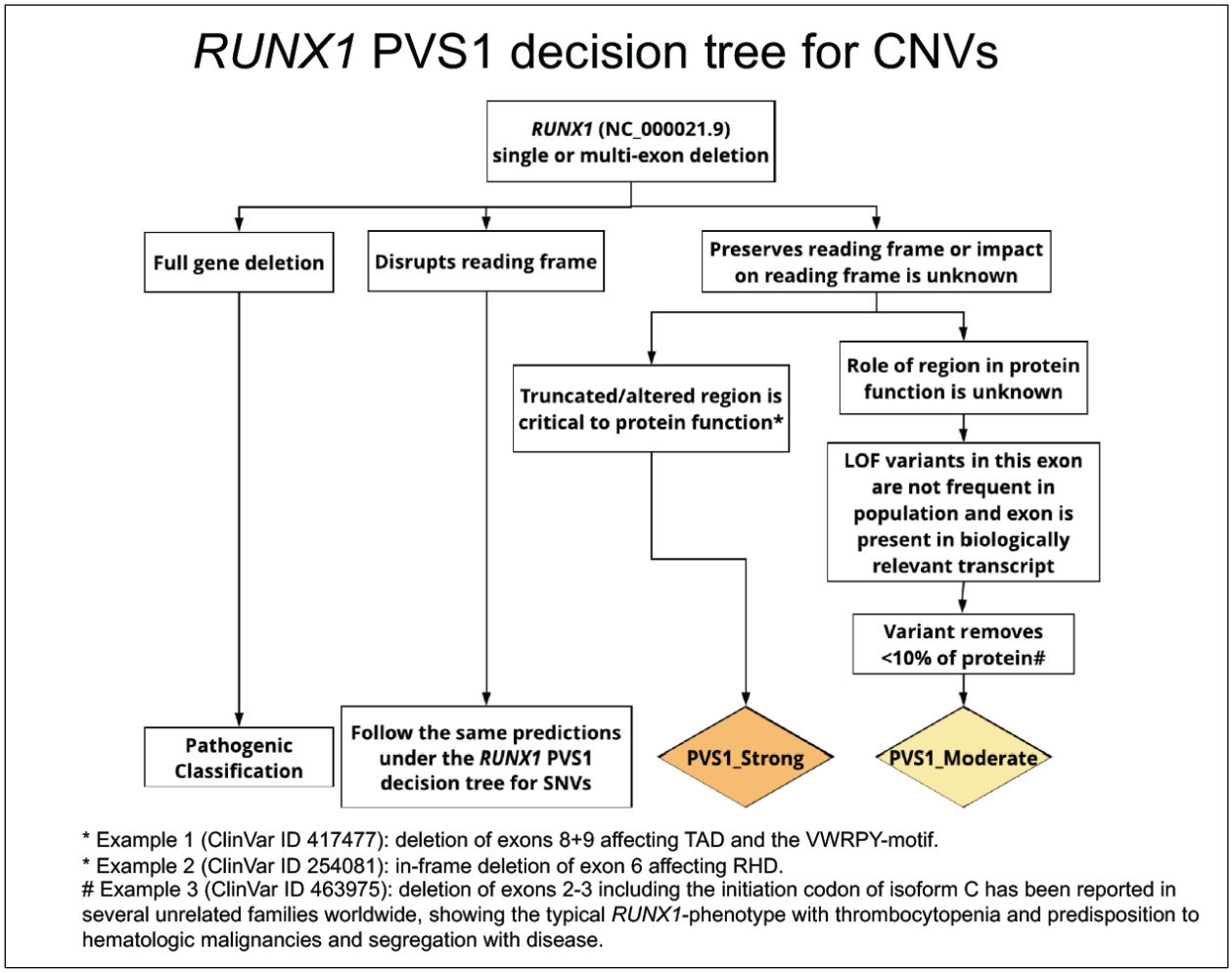
Supplementary Table S3- Summary of splicing effects.

Intron	GT-AG 1,2 Splice site	Location	Predicted or published effects	Classification
Intron 2	Donor	c.58	Only affects isoform C, but not isoform A and B.	**N/A**
	Acceptor	c.59	Only affects 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 it skips exon 4 with frameshift (isoform C) and causes nonsense/frameshift in isoform A/B.	**PVS1**
Intron 4	Donor	c.351	Skips exon 4 with frameshift.	**PVS1**
	Acceptor	c.352	Skips exon 5 with frameshift or uses of cryptic splice acceptor resulting in a frameshift (PMID: 10508512).	**PVS1**
Intron 5	Donor	c.508	Skips exon 5 with frameshift or uses cryptic splice donor resulting in a frameshift (PMID: 11830488).	**PVS1**
	Acceptor	c.509	Skips exon 6 with in-frame Δ171-205 and G170 (GGG->GGA), deletion within RHD.	**PVS1_Strong**
Intron 6	Donor	c.613	Skips exon 6 with in-frame Δ171-205 and G170 (GGG->GGA), deletion within RHD.	**PVS1_Strong**
	Acceptor	c.614	Skips exon 7 with in-frame Δ206-269 and R205N (AGG->AAT), removes 13% of protein.	**PVS1_Strong**
Intron 7	Donor	c.805	Skips exon 7 with in-frame Δ206-269 and R205N (AGG->AAT), removes 13% of protein.	**PVS1_Strong**
	Acceptor	c.806	Skips exon 8 with in-frame Δ270-323 and D269A (GAT->GCG), deletion within TAD.	**PVS1_Strong**
Intron 8	Donor	c.967	Skips exon 8 with in-frame Δ270-323 and D269A (GAT->GCG), deletion within TAD.	**PVS1_Strong**
	Acceptor	c.968	Likely use of cryptic site, the last exon contains 33% of protein.	**PVS1_Strong**

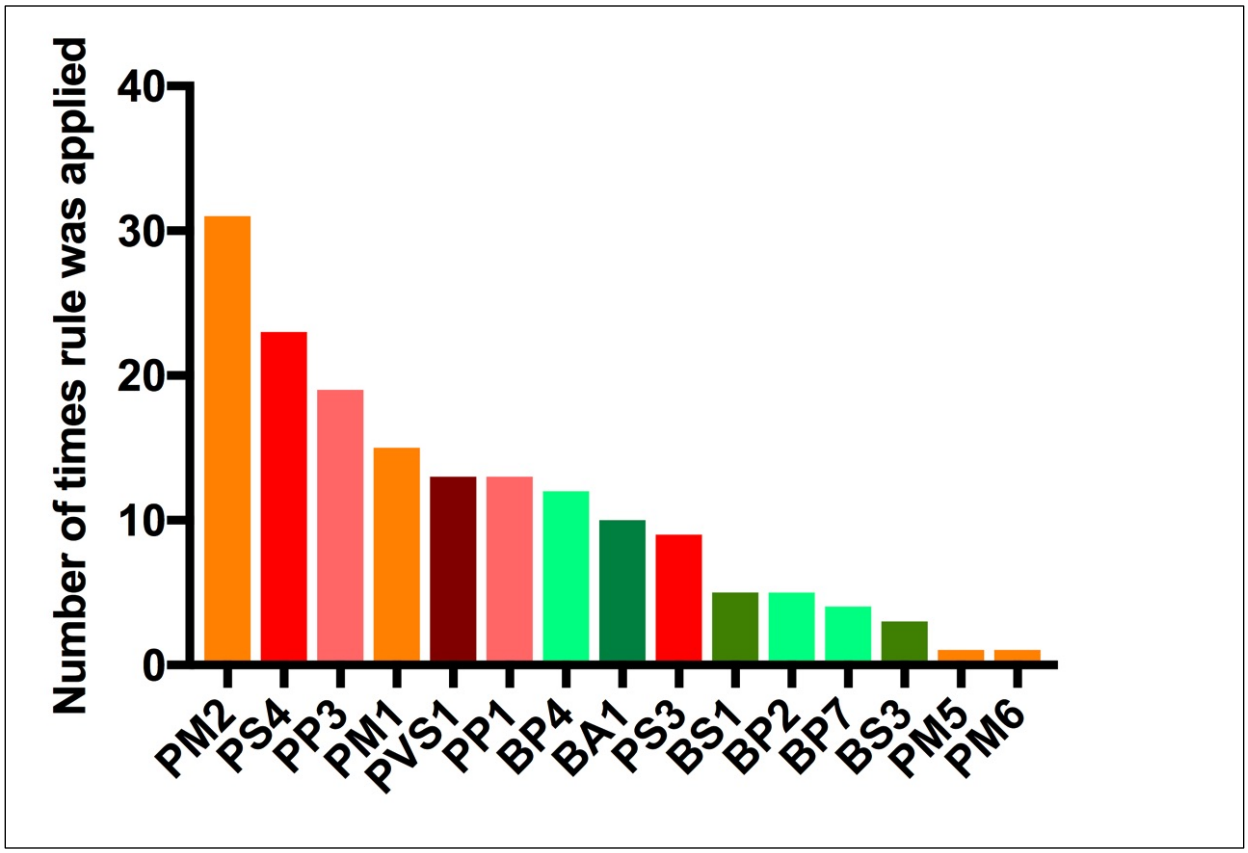
Supplementary Table S4- ClinVar submitters' assertions compared to the final MM-VCEP's classification for *RUNX1* pilot variants.

Supplementary Table S4: List of all *RUNX1* pilot variants, the ClinVar submitters' assertions as well as the MM-VCEP's classifications.			
Single-nucleotide variants/indels			
	ClinVar Assertion	**MM-VCEP Classification**	**Rules applied**
c.601C>T (p.Arg201Ter)	LPATH	PATH	PVS1, PS4, PP1_strong, PM2
c.679G>T (p.Glu227Ter)	LPATH	PATH	PVS1, PM2, PS4_supporting
c.861C>A (p.Tyr287Ter)	PATH	PATH	PVS1, PP1_strong, PM2, PS4_supporting
c.1163C>A (p.Ser388Ter)	LPATH	LPATH	PVS1_strong, PM2, PS4_supporting, PS3_supporting
c.328A>G (p.Lys110Glu)	PATH	PATH	PS3, PP1_strong, PM1, PM2, PP3, PS4_supporting
c.367G>C (p.Asp123His)	PATH	VUS	PP3, PM1_supporting, PS4_supporting
c.400G>C (p.Ala134Pro)	PATH	PATH	PS3, PM1, PM2, PP1, PP3, PS4_supporting
c.467C>A (p.Ala156Glu)	PATH	LPATH	PP1_strong, PM2, PP3, PM1_supporting, PS4_supporting
c.497G>A (p.Arg166Gln)	LPATH	PATH	PS3, PM1, PM2, PS4_moderate, PP1, PP3, PM6_supporting
c.557T>A (p.Val186Asp)	LPATH	VUS	PM2, PP3, PM1_supporting
c.602G>A (p.Arg201Gln)	PATH	PATH	PS3, PS4, PM1, PM2, PP1, PP3
c.215_216dup (p.Ser73Glyfs)	PATH	PATH	PVS1, PM2, PS4_supporting
c.442_449delACCGCAGC (p.Thr148Hisfs)	PATH	PATH	PVS1, PM2, PS4_supporting
c.352-1G>A	PATH	PATH	PVS1, PM2, PP1, PS4_supporting
c.352-1G>T	PATH	PATH	PVS1, PP1_strong, PM2, PS4_supporting
c.351+1G>C	LPATH	LPATH	PVS1, PM2
c.508+3delA	PATH	PATH	PS3, PP1_strong, PM2, PP3, PS4_supporting
c.654C>T (p.Ser218Ser)	BEN/LBEN	LBEN	BS1, BP4
c.1269C>T (p.Arg423Arg)	BEN/LBEN	BEN	BA1, BP4
c.1389C>G (p.Pro463Pro)	BEN	BEN	BA1, BP2, BP4, BP7
c.18A>G (p.Ile6Met)	BEN	LBEN	BS1
c.65T>A (p.Ile22Lys)	LBEN	BEN	BA1
c.824C>T (p.Pro275Leu)	LBEN	BEN	BA1, BP4
c.1005G>T (p.Gln335His)	LBEN	BEN	BA1
c.1355T>G (p.Val452Gly)	LBEN	BEN	BA1, BS3, BP4
c.351+15A>G	BEN/LBEN	BEN	BA1, BP2, BP4, BP7
c.613+8C>T	BEN/LBEN	BEN	BA1, BP2, BP4, BP7
c.614-34C>T	BEN	BEN	BA1, BP2, BP4
c.167T>C (p.Leu56Ser)	BEN/LBEN	BEN	BA1, BP2, BS3
c.1412_1413dup (p.Leu472Alafs)	CONF	PATH	PVS1_strong, PP1_strong, PM2, PS3_moderate, PS4_supporting
c.698G>A (p.Arg233His)	CONF	LBEN	BS1
c.444C>T (p.Thr148Thr)	CONF	LBEN	BP4, BP7
c.253C>A (p.His85Asn)	CONF	LBEN	BS1, BS3, PP3
c.1422G>T (p.Glu474Asp)	VUS	VUS	PM2
c.1257G>A (p.Val419Val)	VUS	VUS	
c.1160G>C (p.Gly387Ala)	VUS	VUS	BP4, PS4_supporting
c.1098_1103dupCGGCAT (p.Gly367_Met368insIleGly)	VUS	VUS	PM2, PS4_moderate
c.259G>T (p.Gly87Cys)	VUS	VUS	PP3
c.155T>A (p.Met52Lys)	VUS	VUS	BS1, PP3
c.97+4T>G	VUS	VUS	BP4
c.563C>G (p.Thr188Ser)	VUS	VUS	PM2, PP3, PM1_supporting
c.532A>C (p.Thr178Pro)	VUS	VUS	PM2, PP3, PM1_supporting, PS4_supporting
c.502G>A (p.Gly168Arg)	VUS	VUS	PM2, PP3, PM1_supporting
c.391A>G (p.Thr131Ala)	VUS	VUS	PM2, PP3, PM1_supporting
c.331A>G (p.Thr111Ala)	VUS	VUS	PM2, PP3, PM1_supporting
c.316T>A (p.Trp106Arg)	VUS	LPATH	PS3, PM2, PP3, PM1_supporting
c.314A>C (p.His105Pro)	VUS	LPATH	PM2, PP3, PS4_supporting, PM1_supporting, PM5_supporting
c.315C>A (p.His105Glu)	NA	LPATH	PS3, PM2, PP3, PM1_supporting
c.1395C>A (p.Asn465Lys)	NA	VUS	PM2, BP4
Copy number variants			
NC_000021.9:g.(?_34787801)_(34799462_?)del	PATH	LPATH	PVS1_strong, PM2, PS4_moderate
NC_000021.9:g.(?_35048836)_(35048905_?)del	PATH	PATH	PS4, PP1_strong, PM2, PVS1_moderate
NC_000021.9:g.(?_34859474)_(34859578_?)del	LPATH	LPATH	PVS1_strong, PM2, PP1, PS4_supporting
RefSeq IDs NM_001754.4 and NC_000021.9 (GRCh38/hg38) were used for all variants.			
Abbreviations: BEN: benign, LBEN: likely benign, LPATH: likely pathogenic, MM-VCEP: Myeloid Malignancy Variant Curation Expert Panel, NA: not applicable, PATH: pathogenic, VUS: variant of uncertain significance.			

Supplementary Figures and Figure Legends



Supplementary Figure S1- PVS1 decision tree for copy-number variants. Application of different levels of strength for PVS1 depending on the impact on the reading frame, the location of the deletion within a known critical protein domain (Runt homology domain (RHD), transactivation domain (TAD) and the VWRPY motif, expression of alternative isoforms and percentage of protein predicted to be removed.



Supplementary Figure S2- Frequency of application of pathogenic/benign evidence codes. The frequency of very strong, strong, moderate, supporting and stand-alone benign/pathogenic codes applied for the 52 pilot variants. The colors indicate the pathogenicity codes as follows: dark-red = pathogenic very strong; red = pathogenic strong; orange = pathogenic moderate; pink = pathogenic supporting; dark-green = benign stand-alone; green = benign strong; light green = benign supporting.