COMMENTARY

blood advances

TO THE EDITOR:

Accurate germline *RUNX1* variant interpretation and its clinical significance

Simone Feurstein,[1,*] Liying Zhang,[2,*] and Courtney D. DiNardo[3,*]

[1]Section of Hematology/Oncology, Department of Medicine, The University of Chicago, Chicago, IL; [2]Department of Pathology and Laboratory Medicine, University of California Los Angeles, Los Angeles, CA; and [3]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX

The frequency of germline *RUNX1* variants in an unselected acute myeloid leukemia (AML) population is poorly defined and likely underestimated. The recent study by Simon et al[1] is particularly important as a first attempt to define this underlying frequency. Because *RUNX1* is part of most next-generation sequencing panels performed on leukemic samples, germline variants are invariably found, highlighted by this and other studies.[1-4] Human and medical geneticists, genetic counselors, molecular pathologists, hematopathologists, and hematologists are particularly likely to encounter patients with germline *RUNX1* variants and may benefit from guidance on how to interpret these variants and their clinical implications.

In the Simon et al[1] study, 10.7% (44/430) of AML patients had a somatic or germline *RUNX1* variant. Germline variants represented 27.3% (12/44) of *RUNX1* variants, suggesting a 2.8% frequency of germline *RUNX1* variants in an unselected AML population. However, it was not clearly delineated whether the identified germline variants were all disease causing (ie, pathogenic or likely pathogenic), although the term "mutation" implies pathogenicity. Inconsistent usage of "variant" and "mutation" can lead to miscommunication of scientific findings, as well as clinical testing results: "mutation" refers to pathogenic/likely pathogenic variations that are deleterious and found less frequently in a population or are nongermline changes in a tumor cell (somatic mutations) that are predictive/therapeutic, diagnostic, or prognostic biomarkers (Table 1).[5]

Germline variant classification is performed using 5 ranks of pathogenicity: pathogenic, likely pathogenic, variant of uncertain significance, likely benign, and benign. Variants of uncertain significance, as well as likely benign and benign variants, should not be attributed to disease causality (Table 1). Accurate variant classification is critically important for attribution of pathogenicity of the identified variants and their actionability, because the identification of a deleterious germline variant has clinical implications that extend far beyond the treatment of the diagnosed individual.

In response to interlaboratory curation differences, the Clinical Genome Resource (ClinGen) has launched Variant Curation Expert Panels (VCEPs) to develop gene- or disease-specific American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) criteria.[6] The Myeloid Malignancy (MM)-VCEP was formed in 2018 and published *RUNX1*-specific ACMG/AMP criteria in 2019.[7,8] Given our familiarity with the *RUNX1* variant curation rules, we have reviewed the variants described in the Simon et al[1] study and found that only 7 of the 12 germline variants meet the criteria for pathogenic/likely pathogenic classification (Table 2). Thus, the actual yield of deleterious germline *RUNX1* variants is 16% (7/44) of all *RUNX1* variants and 1.6% (7/430) of all AML patients. Other than early truncating variants leading to non-sense–mediated decay, most causative *RUNX1* variants are dependent on a variety of pathogenic evidence. In the case of *RUNX1*, this is usually a combination of computational and predictive, functional, population, and segregation data in a Bayesian framework.[7,9]

With regard to *RUNX1* variant curation in the Simon et al[1] study as an example, we would like to highlight the following points. (1) Three major *RUNX1* isoforms (A, B, and C) are expressed by the use of 2 promoters and alternative splicing. Isoform function, biological relevance, and expression differ in hematopoietic tissue,[10,11] which makes PVS1 not applicable for N-terminal truncating variants affecting only isoform C.[7,12] (2) Different strength levels of pathogenic functional evidence (PS3) are based on

Submitted 31 August 2020; accepted 2 November 2020; published online 15 December 2020. DOI 10.1182/bloodadvances.2020003304.

*S.F., L.Z., and C.D.D. contributed equally to this work.

Data sharing requests should be sent to Simone Feurstein (feurstein@uchicago.edu).

Table 1. Nomenclature of variants and likelihood of being disease causing

Nomenclature	Definition	Likelihood of being disease causing[9,19-21,*]
Mutation	"Mutation" is used for germline variations that are pathogenic and found less frequently in a population or are nongermline changes in a tumor cell (somatic mutations).[5]	Should only be used when there is clear evidence for pathogenicity
Variant	An alteration in the most common DNA nucleotide sequence. The term "variant" can be used to describe an alteration that may be benign, pathogenic, or of unknown significance. The term "variant" is increasingly being used in place of the term "mutation."[22]	Variant is further classified in a 5-tier system: benign, likely benign, uncertain significance, likely pathogenic, pathogenic
Benign variant	This variant does not cause disease.	<0.1%
Likely benign variant	This variant is not expected to cause disease. Additional evidence may confirm this assertion of benign, but there is a small chance that new evidence may demonstrate that this variant does have clinical significance.	Between 0.1% and 10%
Variant of uncertain significance	There is insufficient evidence to put this variant into a benign or pathogenic category. Further evidence, such as population, segregation, or functional data, may up- or downgrade this variant. This variant is not clinically actionable.	Between 10% and 90%
Likely pathogenic variant	This variant is expected to cause disease. Additional evidence may confirm this assertion of pathogenicity, but there is a small chance that new evidence may demonstrate that this variant does not have clinical significance.	Between 90% and 99%
Pathogenic variant	This variant does cause disease.	>99%

*Most variants do not have data to support a quantitative assignment of variant certainty to any of the 5 categories given the heterogeneous nature of most diseases.

decreased or enhanced transactivation activity, with or without a secondary assay showing decreased DNA binding affinity, diminished heterodimerization ability with CBFb, abnormal cellular localization, reduced colony-forming potential, or abnormal function of mutant RUNX1 in vivo. Of note, other functional assays, such as interaction of RUNX1 with MLL, are not valid secondary assays for RUNX1 function.[13,14] (3) The presence of a *RUNX1* germline variant in a proband with hereditary myelodysplastic syndrome (MDS)/acute leukemia, even with the typical phenotype including lifelong thrombocytopenia and platelet dysfunction, does not justify a pathogenic classification, but it is always dependent on a combination of additional functional, cosegregation, predictive, or population data. More information regarding the application of *RUNX1*-specific ACMG/Association for Molecular Pathology criteria in the classification of variants identified in this study is shown in Table 2.

As of 30 June 2020, 591 *RUNX1* variants have been reported in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/). Many germline disease-causing *RUNX1* variants are unique to individuals or families; thus, detailed annotation is not always available for reference when a new *RUNX1* variant is identified.[15] Only 21% of *RUNX1* variants are clinically significant (pathogenic/likely pathogenic), whereas the majority (79%) are benign/likely benign or variants of uncertain significance, which are not clinically actionable (Figure 1). It is worth mentioning that 50% of *RUNX1* variants are variants of uncertain significance that warrant more collaborative efforts for the scientific community to up- or downgrade them based on new evidence, such as observation in multiple probands, segregation with disease, or functional impact of the variant or absence in affected individuals, nonsegregation with disease, or no effects on protein function.

Phenotypic criteria have been proposed by the ClinGen MM-VCEP, and they can be helpful in the determination of *RUNX1* variant pathogenicity, because a high penetrance, with regard to thrombocytopenia and/or underlying platelet dysfunction, is typically recognized, and patients display ≥1 of the following features[7]: mild to moderate thrombocytopenia with normal platelet size and volume in the absence of other causative factors; platelet ultrastructural and/or functional defects; and diagnosis of a hematologic malignancy, most commonly affecting the myeloid lineage (causing AML or MDS) and less frequently involving the lymphoid lineage and manifesting as T-cell acute lymphoblastic leukemia or others.

The following example highlights the importance of variant annotation for management decisions. A 56-year-old female with a diagnosis of MDS and a family history of hematologic malignancies was identified to have a germline *RUNX1* c.167T>C (p.Leu56Ser) variant and was counseled that this *RUNX1* variant was disease causing. Family members were tested for this variant to determine who lacked the variant and, thus, could be an appropriate stem cell transplant donor for the index patient and who in the family carries the variant and should receive surveillance on research protocols for *RUNX1*-associated familial platelet disorder with myeloid malignancy. Importantly, upon further review at the time of a second opinion, the *RUNX1* c.167T>C (p.Leu56Ser) variant was reclassified to be a benign germline variant and a "red herring" in the evaluation of this family.

Patients with chronic otherwise unexplained thrombocytopenia, platelet ultrastructural and/or functional defects, and/or AML, MDS, or T-cell acute lymphoblastic leukemia should undergo genetic testing whenever there is a positive family history for a *RUNX1* phenotype and when the patient has been diagnosed at a young age or a *RUNX1* variant has been identified upon molecular testing of the leukemic clone. Germline material for testing should represent tissues that are not contaminated with blood/circulating blasts, such as cultured skin fibroblasts, which are the gold standard. Upon confirmation of a germline disease-causing *RUNX1* variant, additional family members can be tested and followed-up long-term, including a baseline bone marrow biopsy with cytogenetic/molecular analysis and additional biopsies at the time of any significant/persistent change in blood counts. Most importantly, a family member with the *RUNX1* variant should not be considered as a related stem cell donor, which makes recognition of the underlying germline syndrome paramount.[16-18]

Our clinical example and the variant interpretation by Simon et al[1] highlight how easily variants can be misclassified when criteria are

Table 2. Comparison of *RUNX1* variant curation between the study by Simon et al and the MM-VCEP

ID*	Variant cDNA/ protein*	Described in MDS/AML*	Described in *RUNX1* FPD*	Functional impact on RUNX1*	MM-VCEP ACMG/AMP criteria code*	MM-VCEP *RUNX1*-specific criteria†	Further explanation of criteria†	MM-VCEP classification†
1	c.44_45delAG/ p.Q15fsX	—	—	Truncating	PVS1_moderate, PS4_supporting, PM2	PS4_supporting, PM2	PVS1 cannot be used for early truncating variants only affecting RUNX1 isoform C.	VUS
2	c.179C>T/p.A60V	Carnicer et al[23]	Lorente[24]	—	BS1	BS1, BS3	This variant meets the calculated BS1 threshold (Latino subpopulation) and BS3 (normal transactivation and normal DNA binding/subcellular localization).[25] The presence of the variant in patients with a *RUNX1* phenotype is not sufficient to call a variant PATH, in particular not if the variant is present in gnomAD at a MAF incompatible with disease prevalence.	BEN
3+4‡	c.421T>G/p.S141A	—	RUNX1db	Normal transactivation[26]	PS4_supporting, PP3, BS3_supporting	PM1_supporting, PP3	Variant not present in RUNX1db. Although there is no effect on heterodimerization ability with CBF,[26] data from an additional secondary assay or transactivation assay are missing; this does not permit application of any BS3 strength level. PS4 cannot be applied (2 alleles in gnomAD).	VUS
5	c.427G>T/p.E143X	—	—	Truncating	PVS1, PS4_supporting, PM2	PVS1, PS4_supporting, PM2		PATH
6	c.454_456insA/ p.K152fsX	Ernst et al[27]	—	Truncating	PVS1, PS4_supporting, PM2	PVS1, PS4_supporting, PM2	Variant nomenclature does not conform with HGVS recommendations for sequence variants. We assume this variant is not present in gnomAD (PM2) and leads to NMD (PVS1).	PATH
7	c.496C>G/p.R166G	Imai et al[28]	—	LOF/dominant negative[28]	PS4_supporting, PM2, PM5, PP3	PS4_supporting, PM1, PM2, PM5, PP3	R166Q has been curated by the MM-VCEP as PATH.	LPATH
8	c.496C>T/p.R166X	Preudhomme et al[29]	Bluteau et al[30]	Truncating	PVS1, PS4, PM2, PP1	PVS1, PS4, PM2, PP1_strong		PATH
9+10	c.610C>T/p.R204X	Osato et al[31]	Song et al[32]	LOF[31]	PVS1, PS4, PM2, PP1	PVS1, PS4, PM2, PP1_strong		PATH
11	c.619C>T/p.R207W	You et al[33]	—	—	PS4_supporting, PM2, PP3	PS4_moderate, PM2, PP3	In silico prediction alone (ie, in this case pathogenic predictions by using SIFT, Polyphen, VEST, CHASM, and REVEL) is only supporting evidence and insufficient to classify a variant as PATH.	VUS
12	c.1243_1244insC/ p.Q415fsX	—	—	Elongated RUNX1 isoform	PVS1_strong, PS4_supporting, PM2	PVS1_strong, PS4_supporting, PM2		LPATH

All variants are annotated using RefSeq ID NM_001754.4. PS4 is applied assuming that the variants in the Simon et al[1] study are germline variants. Germline status should be confirmed in DNA derived from cultured skin fibroblasts, cultured bone marrow mesenchymal stromal cells, or hair roots.

—, no data; BEN, benign; cDNA, complementary DNA; FPD, familial platelet disorder; gnomAD, Genome Aggregation Database; HGVS, Human Genome Variation Society; LOF, loss of function; LPATH, likely pathogenic; MAF, minor allele frequency; NMD, non-sense–mediated decay, PATH, pathogenic, VUS, variant of unknown significance.

*From the Simon et al[1] study.

†MM-VCEP assessment.

‡Patients are related.

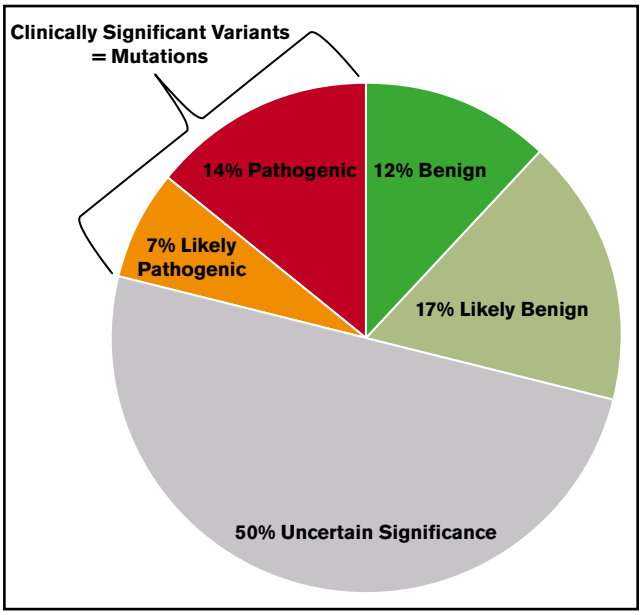

Figure 1. *RUNX1* variants in ClinVar and their clinical significance. All 591 *RUNX1* variants deposited in ClinVar as of 30 June 2020 and their clinical significance based on the 5-tier system for germline variants (benign, likely benign, variant of uncertain significance, likely pathogenic, pathogenic) are shown in the pie chart. Only 21% of the *RUNX1* variants are clinically actionable (ie, likely pathogenic and pathogenic).

not applied correctly, too much weight is put on the observation of the variant in affected probands, or the criteria are not combined correctly to reach the level of clinical significance (ie, disease causing). The accuracy of *RUNX1* variant classification and interpretation is of great importance for treatment and follow-up of affected patients, related donor selection, and counseling of family members. Therefore, we emphasize that MM-VCEP *RUNX1*-specific rules, as the most accurate standards of germline *RUNX1* variant classification, should be applied in clinical and research settings.[7,8]

Acknowledgments: This work was supported by the National Human Genome Research Institute National Institutes of Health (grants U41HG009649 and U41HG009650) and a 2018 National Institutes of Health/National Cancer Institute Leukemia SPORE DRP award (P50CA100632-16, project 00007529) (C.D.D.). The results provided in this publication were generated by the American Society of Hematology in collaboration with Baylor College of Medicine and the University of North Carolina, National Institutes of Health–funded ClinGen grant award recipients.

This commentary was written on behalf of the collaborative group of the American Society of Hematology - Clinical Genome Resource MM-VCEP, which is cochaired by Lucy A. Godley (University of Chicago) and David Wu (University of Washington). A complete list of MM-VCEP members is available at: https://clinicalgenome.org/affiliation/50034/#heading_membership.

Contribution: S.F. reviewed and classified the *RUNX1* variants and explained the use of *RUNX1*-specific criteria; L.Z. worked on the nomenclature of variants; C.D.D. focused on the clinical impact of germline *RUNX1* variants; and all authors wrote and edited the manuscript.

Conflict-of-interest disclosure: L.Z. has received honoraria from Future Technology Research LLC, BGI, and Illumina and honoraria and travel and accommodation expenses from Roche Diagnostics Asia Pacific; family members hold leadership positions and ownership interests in the Shanghai Genome Center. The remaining authors declare no competing financial interests.

ORCID profiles: S.F., 0000-0002-9996-4277; C.D.D., 0000-0001-9003-0390.

Correspondence: Simone Feurstein, The University of Chicago, KCBD 7123A, 900 East 57th St, Chicago, IL 60637; e-mail: feurstein@uchicago.edu.

References

1. Simon L, Spinella J-F, Yao C-Y, et al. High frequency of germline RUNX1 mutations in patients with RUNX1-mutated AML. *Blood*. 2020; 135(21):1882-1886.
2. DiNardo CD, Routbort MJ, Bannon SA, et al. Improving the detection of patients with inherited predispositions to hematologic malignancies using next-generation sequencing-based leukemia prognostication panels. *Cancer*. 2018;124(13):2704-2713.
3. DiNardo CD, Bannon SA, Routbort M, et al. Evaluation of patients and families with concern for predispositions to hematologic malignancies within the Hereditary Hematologic Malignancy Clinic (HHMC). *Clin Lymphoma Myeloma Leuk*. 2016;16(7):417-428.e2.
4. Yannakou CK, Jones K, Ryland GL, et al. Incidental detection of germline variants of potential clinical significance by massively parallel sequencing in haematological malignancies. *J Clin Pathol*. 2018; 71(1):84-87.
5. Leonard DGB, ed. Molecular Pathology in Clinical Practice. 2nd ed. Cham, Switzerland: Springer International Publishing; 2016.
6. Rivera-Muñoz EA, Milko LV, Harrison SM, et al. ClinGen Variant Curation Expert Panel experiences and standardized processes for disease and gene-level specification of the ACMG/AMP guidelines for sequence variant interpretation. *Hum Mutat*. 2018;39(11):1614-1622.
7. Luo X, Feurstein S, Mohan S, et al. ClinGen Myeloid Malignancy Variant Curation Expert Panel recommendations for germline RUNX1 variants. *Blood Adv*. 2019;3(20):2962-2979.
8. Wu D, Luo X, Feurstein S, et al; collaborative group of the American Society of Hematology - Clinical Genome Resource Myeloid Malignancy Variant Curation Expert Panel. How I curate: applying American Society of Hematology-Clinical Genome Resource Myeloid Malignancy Variant Curation Expert Panel rules for *RUNX1* variant curation for germline predisposition to myeloid malignancies. *Haematologica*. 2020;105(4):870-887.
9. Tavtigian SV, Greenblatt MS, Harrison SM, et al; ClinGen Sequence Variant Interpretation Working Group (ClinGen SVI). Modeling the ACMG/AMP variant classification guidelines as a Bayesian classification framework. *Genet Med*. 2018;20(9):1054-1060.
10. Brady G, Elgueta Karstegl C, Farrell PJ. Novel function of the unique N-terminal region of RUNX1c in B cell growth regulation. *Nucleic Acids Res*. 2013;41(3):1555-1568.
11. Tsuzuki S, Seto M. Expansion of functionally defined mouse hematopoietic stem and progenitor cells by a short isoform of RUNX1/AML1. *Blood*. 2012;119(3):727-735.
12. Abou Tayoun AN, Pesaran T, DiStefano MT, et al; ClinGen Sequence Variant Interpretation Working Group (ClinGen SVI).

Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion. *Hum Mutat*. 2018;39(11):1517-1524.

13. Koh CP, Wang CQ, Ng CEL, et al. RUNX1 meets MLL: epigenetic regulation of hematopoiesis by two leukemia genes. *Leukemia*. 2013; 27(9):1793-1802.
14. Huang G, Zhao X, Wang L, et al. The ability of MLL to bind RUNX1 and methylate H3K4 at PU.1 regulatory regions is impaired by MDS/AML-associated RUNX1/AML1 mutations. *Blood*. 2011;118(25): 6544-6552.
15. Sood R, Kamikubo Y, Liu P. Role of RUNX1 in hematological malignancies. *Blood*. 2017;129(15):2070-2082.
16. Feurstein S, Drazer MW, Godley LA. Genetic predisposition to leukemia and other hematologic malignancies. *Semin Oncol*. 2016; 43(5):598-608.
17. Churpek JE, Godley LA; University of Chicago Hematopoietic Malignancies Cancer Risk Team. How I diagnose and manage individuals at risk for inherited myeloid malignancies. *Blood*. 2016; 128(14):1800-1813.
18. Rafei H, DiNardo CD. Hereditary myeloid malignancies. *Best Pract Res Clin Haematol*. 2019;32(2):163-176.
19. Plon SE, Eccles DM, Easton D, et al; IARC Unclassified Genetic VariantsWorking Group. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. *Hum Mutat*. 2008;29(11): 1282-1291.
20. Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424.
21. Harrison SM, Rehm LR. Is "likely pathogenic" really 90% likely? Reclassification data in ClinVar. *Genome Med*. 2019;11(1):72.
22. National Institutes of Health. NCI dictionary of genetics terms. https://www.cancer.gov/publications/dictionaries/genetics-dictionary. Accessed 18 October 2020.
23. Carnicer, MJ, Nomdedéu JF, Lasa A, et al. AML-1 mutations outside the RUNT domain: description of two cases in myeloid malignancies. *Leukemia*. 2002;16(11):2329-2332.
24. Lorente NP. *Molecular Characterization of Patients with Hereditary Myeloid Neoplasms by Next-Generation Sequencing* [thesis]. Valencia, Spain: Universitat Politècnica de València; 2018.
25. Huang G, Zhao X, Wing L, et al. The ability of MLL to bind RUNX1 and methylate H3K4 at PU.1 regulatory regions is impaired by MDS/AML-associated RUNX1/AML1 mutations. *Blood*. 2011;118(25): 6544-6552.
26. Zhang L, Li Z, Yan J, et al. Mutagenesis of the Runt domain defines two energetic hot spots for heterodimerization with the core binding factor beta subunit. *J Biol Chem*. 2003;278(35):33097-33104.
27. Ernst T, Chase A, ZoiK, et al. Transcription factor mutations in myelodysplastic/myeloproliferative neoplasms. *Haematologica*. 2010; 95(9):1473-1480.
28. Imai Y, Kurokawa M, Izutsu K, et al. Mutations of the AML1 gene in myelodysplastic syndrome and their functional implications in leukemogenesis. *Blood*. 2000;96(9):3154-3160.
29. Preudhomme C, Warot-Loze D, Roumier C, et al. High incidence of biallelic point mutations in the Runt domain of the AML1/PEBP2 alpha B gene in Mo acute myeloid leukemia and in myeloid malignancies with acquired trisomy 21. *Blood*. 2000;96(8):2862-2869.
30. Bluteau O, Sebert M, Leblanc T, et al. A landscape of germ line mutations in a cohort of inherited bone marrow failure patients. *Blood*. 2018;96(8):2862-2869.
31. Osato M, Asou N, Abdalla E, et al. Biallelic and heterozygous point mutations in the runt domain of the AML1/PEBP2alphaB gene associated with myeloblastic leukemias. *Blood*. 1999;93(6): 1817-1824.
32. Song WJ, Sullivan MG, Legare RD, et al. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. *Nat Genet*. 1999;23(2):166-175.
33. You E, Cho Y-U, Jang S, et al. Frequency and clinicopathologic features of RUNX1 mutations in patients with acute myeloid leukemia not otherwise specified. *Am J Clin Pathol*. 2017;148(1):64-72.