Accurate germline \textit{RUNX1} variant interpretation and its clinical significance

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

In the Simon et al\textsuperscript{1} study, 10.7% (44/430) of AML patients had a somatic or germline \textit{RUNX1} variant. Germline variants represented 27.3% (12/44) of \textit{RUNX1} variants, suggesting a 2.8% frequency of germline \textit{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).\textsuperscript{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.\textsuperscript{6} The Myeloid Malignancy (MM)-VCEP was formed in 2018 and published \textit{RUNX1}-specific ACMG/AMP criteria in 2019.\textsuperscript{7,8} Given our familiarity with the \textit{RUNX1} variant curation rules, we have reviewed the variants described in the Simon et al\textsuperscript{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 \textit{RUNX1} variants is 16% (7/44) of all \textit{RUNX1} variants and 1.6% (7/430) of all AML patients. Other than early truncating variants leading to non-sense–mediated decay, most causative \textit{RUNX1} variants are dependent on a variety of pathogenic evidence. In the case of \textit{RUNX1}, this is usually a combination of computational and predictive, functional, population, and segregation data in a Bayesian framework.\textsuperscript{7,9}

With regard to \textit{RUNX1} variant curation in the Simon et al\textsuperscript{1} study as an example, we would like to highlight the following points. (1) Three major \textit{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,\textsuperscript{10,11} which makes PVS1 not applicable for N-terminal truncating variants affecting only isoform C.\textsuperscript{7,12} (2) Different strength levels of pathogenic functional evidence (PS3) are based on
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. 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 $\geq 1$ of the following features': 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>;/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.

Our clinical example and the variant interpretation by Simon et al highlight how easily variants can be misclassified when criteria are
| 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            | PVSI cannot be used for early truncating variants only affecting RUNX1 isoform C. | VUS                      |
| 2   | c.179C>T/p.A60V       | Carniero et al23       | Lorentz24              | —                           | BS1                           | BS1, BS3                        | This variant meets the calculated BS1 threshold (Latino subpopulation) and BS3 (normal transactivation and normal DNA binding/subcellular localization).26 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 transactivation26     | 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                  | PVSI, PS4_supporting, PM2     | PVSI, PS4_supporting, PM2      |                           | PATH                     |
| 6   | c.454_456insA/p.K152fsX | Ernst et al27        | —                      | Truncating                  | PVSI, PS4_supporting, PM2     | PVSI, 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 al28          | —                      | LOF/dominant negative28     | PS4_supporting, PM2, PM5, PP3 | PS4_supporting, PM1, PM2, PM5, PP3 | R166G has been curated by the MM-VCEP as PATH. | LPATH                    |
| 8   | c.496C>T/p.R166X      | Preudhomme et al29    | Bluteau et al30        | Truncating                  | PVSI, PS4, PM2, PP1           | PVSI, PS4, PM2, PP1            |                           | PATH                     |
| 9+10| c.610C>T/p.R204X      | Osato et al31         | Song et al32           | LOF31                       | PVSI, PS4, PM2, PP1_strong    | PVSI, PS4, PM2, PP1_strong    |                           | PATH                     |
| 11  | c.619C>T/p.R207W      | You et al33           | —                      | PS4_supporting, PM2, PP3    | PS4 Moderate, PM2, PM3         | —                              | 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     | PVSI_strong, PS4_supporting, PM2 | PVSI_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 al1 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 al1 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).

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