Comment on Simon et al, page 1882

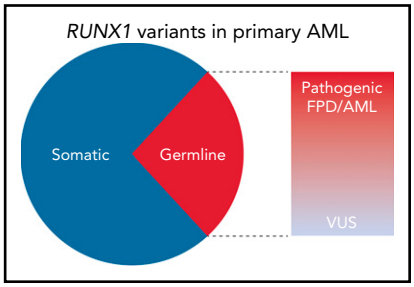
Born to RUNX1

Pamela J. Sung[1] and Daria V. Babushok[1,2] | [1]University of Pennsylvania; [2]Children's Hospital of Philadelphia

In this issue of *Blood*, Simon et al evaluated 430 samples from patients with acute myeloid leukemia (AML) for germline and somatic mutations in RUNX family transcription factor 1 (*RUNX1*). They found that nearly 30% of the identified variants were germline.[1]

The importance and the unique biology of *RUNX1* in AML have been recognized by the World Health Organization (WHO) by adding 2 separate categories of AML with *RUNX1* mutations in the 2016 WHO classification of hematologic neoplasms.[2] AML with mutated *RUNX1* makes up ~10% of newly diagnosed AML and is associated with poor event-free and overall survival.[3] Although the vast majority of *RUNX1* mutations are presumed to be somatic, germline mutations in *RUNX1* cause a rare leukemia predisposition syndrome called familial platelet disorder (FPD) with associated myeloid malignancy (FPDMM, also referred to as FPD/AML), acknowledged in its own WHO category.

Despite the growing awareness of leukemia predisposition syndromes, their prevalence in patients diagnosed with de novo AML or myelodysplastic syndrome (MDS) remains poorly defined. Published reports estimate that 4% to 9% of adults with myeloid neoplasms have germline predisposition.[4,5] More recent analyses of larger cohorts of patients with myeloid malignancies suggest that germline myeloid malignancy predisposition syndromes may be even more common.[6-8] Until now, the prevalence of germline *RUNX1* mutations in AML was largely unexplored.



Among the nonpolymorphic *RUNX1* variants identified in 430 adult AML specimens from the Leucegene AML cohort, 29% were germline. Of the 10 distinct germline variants identified, 6 were classified as pathogenic or likely pathogenic by the MM VCEP criteria; 8 of the 10 were predicted to be deleterious to *RUNX1* function by functional studies or prediction algorithms. VUS, variant of unknown significance.

To determine the frequency of *RUNX1* variants in primary AML, Simon et al sequenced 430 adult leukemia samples from the Quebec Leukemia Cell Bank (Leucegene cohort). After excluding common polymorphisms, 48 patients (11% of the Leucegene cohort) had *RUNX1* variants that were either absent from or exceedingly rare in the general population. To determine which of these were present in the germline, the authors sequenced the mutated regions in paired buccal DNA. An elaborate genotyping scheme was used to exclude false positives resulting from hematopoietic cell contamination, with non-*RUNX1* somatic mutations serving as a marker of contaminating leukemic DNA. By using this approach, 12 of 42 evaluable *RUNX1* variants (~29%) were confirmed to be germline (see figure). Among the 40 *RUNX1* variants present at a high (≥0.3) allelic fraction, 30% were germline.

The strikingly high frequency of germline *RUNX1* variants in the Leucegene cohort deserves attention. Perhaps the closest comparison is a study from the University of Chicago, in which the investigators assessed the germline status of variants in familial leukemia–associated genes identified by a "somatic" next-generation sequencing (NGS) panel in 360 patients with hematologic malignancies.[9] By applying the filtering criteria from Simon et al, the AML patients in the Chicago cohort had 20 nonpolymorphic *RUNX1* variants, of which only 2 (10%) were germline. If restricted to mutations with an allelic fraction ≥0.3, germline variants accounted for 14% (2 of 14) of variants. The 2 cohorts were different enough to preclude direct comparison. Nonetheless, the observed differences in *RUNX1* variant frequencies between the 2 studies are not statistically robust at this relatively small sample size, which underscores the need for future validation with more patients who have *RUNX1*-mutated AML.

Because of the high allelic heterogeneity of germline *RUNX1* variants, many of which have been reported only in single patients or families, the determination of variant pathogenicity requires caution.[3] A personal and family history of life-long thrombocytopenia, platelet dysfunction, and an autosomal dominant pattern of familial AML can go a long way in supporting a diagnosis of FPD/AML; however, this information is not available for members of the Leucegene cohort. This quandary is not uncommon in the clinic when evaluating a patient with *RUNX1*-mutated AML, where a germline mutation could have arisen de novo, or family history could be unknown or incomplete. The recently published ClinGen Myeloid Malignancy Variant Curation Expert Panel (CG MM-VCEP) recommendations for interpreting the pathogenicity of *RUNX1* germline variants put forth a set of rules to systematically evaluate a *RUNX1* variant.[10] Applying the CG MM-VCEP curation rules, 6 of 10 distinct germline variants identified by Simon et al were predicted to be pathogenic or likely pathogenic, and 8 of 10 were predicted to be deleterious to *RUNX1* function by functional studies or prediction algorithms. The nuance in interpreting *RUNX1* variant pathogenicity is illustrated by a family in the Leucegene cohort, in which 2 first-degree relatives developed AML and were found to carry germline variants in both *RUNX1* and *CEBPA* genes, previously reported in their respective familial leukemia syndromes. Both patients also acquired second-hit somatic *CEBPA* mutations, making a case that in this family, the germline *RUNX1* variant may serve as a modifier for primarily *CEBPA*-driven leukemia.

Where does this leave us with respect to germline *RUNX1* mutations or—even more broadly—germline mutations in familial leukemia-associated genes in 2020? With great testing power comes great responsibility. Identifying germline predisposition can significantly impact patient care in positive ways, starting with a more accurate interpretation of bone marrow morphology and prognostic

significance of somatic alterations within the context of a known familial syndrome. Awareness of syndrome-specific hematopoietic and extra-hematopoietic complications, such as platelet dysfunction in *RUNX1* mutation carriers or predisposition to liver cirrhosis in telomere biology disorders, will impact management and disease surveillance. Finally, accurate genetic diagnosis is important for treatment decisions, including donor choice, and, in certain situations, the timing and choice of a conditioning regimen for allogeneic transplantation.

To accomplish all of this, we have to get better at performing and interpreting germline variant testing. The gold standard of cultured skin fibroblasts takes several weeks and requires a facility to culture skin fibroblasts. If not started soon after AML diagnosis, testing may lead to a costly delay at the time of transplant evaluation for an AML patient. As we enter the golden age of AML predisposition genetics, we need improvement in 3 major areas. To expand, simplify, and speed up genetic evaluation, "somatic" NGS panels could be expanded to enhance the capture of genes linked to MDS/AML germline predisposition syndromes. When nonpolymorphic variants in *RUNX1* or other predisposition-associated genes are identified in a blood or marrow specimen, we need to have protocols to ensure appropriate reflexing to confirmatory germline testing. Finally, a centralized, expert-run variant interpretation service could go a long way toward expanding access to evidence-based variant interpretation for rare and emerging MDS/AML predisposition syndromes.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391-2405.
3. Gaidzik VI, Teleanu V, Papaemmanuil E, et al. RUNX1 mutations in acute myeloid leukemia are associated with distinct clinico-pathologic and genetic features. *Leukemia*. 2016;30(11):2282.
4. Lu C, Xie M, Wendl MC, et al. Patterns and functional implications of rare germline variants across 12 cancer types. *Nat Commun*. 2015;6(1):10086.
5. Wartiovaara-Kautto U, Hirvonen EAM, Pitkänen E, et al. Germline alterations in a consecutive series of acute myeloid leukemia. *Leukemia*. 2018;32(10):2282-2285.
6. Lindsley RC, Saber W, Mar BG, et al. Prognostic mutations in myelodysplastic syndrome after stem-cell transplantation. *N Engl J Med*. 2017;376(6):536-547.
7. Sébert M, Passet M, Raimbault A, et al. Germline DDX41 mutations define a significant entity within adult MDS/AML patients. *Blood*. 2019;134(17):1441-1444.
8. Taskesen E, Bullinger L, Corbacioglu A, et al. Prognostic impact, concurrent genetic mutations, and gene expression features of AML with CEBPA mutations in a cohort of 1182 cytogenetically normal AML patients: further evidence for CEBPA double mutant AML as a distinctive disease entity. *Blood*. 2011;117(8):2469-2475.
9. Drazer MW, Kadri S, Sukhanova M, et al. Prognostic tumor sequencing panels frequently identify germ line variants associated with hereditary hematopoietic malignancies. *Blood Adv*. 2018;2(2):146-150.
10. 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.

DOI 10.1182/blood.2020006152

THROMBOSIS AND HEMOSTASIS

Comment on Poothong et al, page 1899

Clogging up the pipeline: factor VIII aggregates

Denise E. Sabatino | The Children's Hospital of Philadelphia; University of Pennsylvania

In this issue of *Blood*, Poothong and colleagues report that increased expression of factor VIII (FVIII) can activate the endoplasmic reticulum (ER) stress response through the formation of amyloid-like fibrils that prevent the further trafficking and secretion of the protein. This study provides insights into the factors that influence the effective secretion of FVIII that may have implications for in vitro protein production systems and in vivo gene therapy.[1]

FVIII deficiency causes the bleeding disorder hemophilia A. It has been known for decades that FVIII is a difficult protein to express both in vitro and in vivo. The first observations were made in vitro in the setting of recombinant protein production. Stable mammalian cell lines (such as Chinese hamster ovary cells, baby hamster kidney cells) were established to synthesize FVIII for the purpose of recombinant protein production used to treat hemophilia A patients. However, the yield of FVIII from these cells was low due to poor expression of the protein. Studies suggested that this low yield may be due to low FVIII messenger RNA levels or poor secretion of FVIII.[2]

FVIII circulates in the plasma at a concentration of 0.1 to 0.2 μg/mL, which is the lowest concentration of all coagulation factors by at least a 10-fold margin. Early work by Kaufman and colleagues compared FVIII to factor V (FV), which has a similar domain structure and protein size. Even in the setting of identical in vitro expression systems, FV was expressed at 5- to 10-fold higher levels than FVIII, suggesting a unique feature of FVIII that prevents effective expression of the protein.[3]

Studies to investigate the cellular processing of FVIII revealed that high-level expression of FVIII can cause ER stress and activate the unfolded protein response (UPR).[4] The UPR is a strategy used by the cells to adapt to cellular stress; however, under conditions of prolonged activation, the cell may undergo apoptosis. In the current study by Poothong et al, several factors that influence FVIII protein misfolding and activation of the UPR pathways are uncovered. When FVIII synthesis is increased or glucose metabolism is inhibited, FVIII aggregates into amyloid-like fibrils that accumulate in the ER. The study also demonstrates that a specific region of the FVIII A1 domain appears to be responsible for the initiation of the amyloid formation. Further analysis comparing FV with FVIII pinpointed specific