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Serine Avagyan, Anna L. Brown

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Commentary

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To T or not to B: germline *RUNX1* mutation preferences in pediatric ALL predisposition

Serine Avagyan¹ and Anna L. Brown^{2,3,4}

¹Dana-Farber/Boston Children's Hospital Cancer and Blood Disorders Center, Boston, Massachusetts, USA. ²Department of Genetics and Molecular Pathology, SA Pathology, and ³Centre for Cancer Biology, SA Pathology and University of South Australia, Adelaide, South Australia, Australia. ⁴School of Medicine, University of Adelaide, Adelaide, South Australia, Australia.

Germline *RUNX1* variants have been identified in relation to myeloid malignancy predisposition, with lymphoid hematological malignancies present at a lower frequency in families. In this issue of the *JCI*, Li and Yang et al. examined the frequency and type of germline *RUNX1* variants in pediatric patients with acute lymphoblastic leukemia (ALL). Patients with T cell ALL (T-ALL) harbored rare, damaging *RUNX1* mutations that were not seen in patients with B cell ALL (B-ALL). Further, several of the T-ALL-associated *RUNX1* variants had potential dominant-negative activity. *RUNX1*-mutated T-ALL cases were also associated with somatic *JAK3* mutations and enriched for the early T cell precursor (ETP) leukemia subtype, a finding that was validated when *RUNX1* and *JAK3* mutations were combined in mice. This study confirms germline *RUNX1* predisposition beyond myeloid malignancy, demonstrates the importance of examining both germline and somatic mutations in malignancy cohorts, and demarcates the ETP ALL subtype as a flag for germline predisposition in patients.

Germline predisposition to hematological malignancy

Acute lymphoblastic leukemia (ALL) is the most common cancer in children and increasingly recognized as having an inherited component in some cases. Initially, ALL was annotated as part of more general cancer predisposition syndromes, such as germline *TP53* mutations in patients manifesting as low-hypodiploid ALL in Li-Fraumeni syndrome (1). In recent years, syndromes with a predominant predisposition to hematological malignancies and in some cases exclusive predisposition to ALL (e.g., *PAX5*, *IKZF1*) have emerged (Figure 1A) (1). It has been more than 20 years since the first report of germline *RUNX1* variants as causative for familial platelet disorder (*RUNX1*-FPD), most commonly observed with myeloid malignancy (2). Germline *RUNX1* variants are present in approximately 12%

of families with inherited myelodysplastic syndrome(s) and acute myeloid leukemia and 3% of patients with inherited bone marrow failure syndromes, excluding Fanconi anemia (3, 4). Ongoing clinical and research studies have identified several hundred families (5) and broadened the range of hematological malignancies in the germline *RUNX1* spectrum to also include lymphoid subtype malignancies, most commonly childhood T cell ALL (T-ALL), but also including lymphoma and, in some rare cases, B cell ALL (B-ALL) (Figure 1B) (6–8). Guideline recommendations for germline *RUNX1* mutations in hematological malignancy predisposition by the WHO and others suggest mainstream germline testing is approaching (9). However, due to the range of phenotype and age of presentation, testing remains clinically challenging (10). Therefore, a complementary approach to the traditional clinical family

history that may miss predisposition due to many factors, such as mild symptoms (e.g., low-normal platelets in germline *RUNX1*) and de novo mutations, includes the ability to identify germline predisposition variants in relevant tumor cohorts through unbiased genomic analysis. The increased use of clinical tumor next-generation sequencing (NGS) profiling panels provides both an opportunity and a challenge in this area. There is much discussion about how to identify and appropriately deal with germline findings from tumor sequencing, with published guideline suggestions for assessing myeloid predisposition, including potential germline *RUNX1* variants in this scenario as well as a recent position paper from the American College of Medical Genetics and Genomics (ACMG) encompassing guidelines across all tumor types (11, 12).

Potential germline *RUNX1* variants

In this issue of the *JCI*, Li and Yang et al. (13) take an unbiased approach to annotating and testing potential germline *RUNX1* variants. The researchers sequenced *RUNX1* in remission samples from over 4800 cases of B-ALL and over 1300 cases of T-ALL from a large cohort of pediatric patients. Selecting for rare variants in remission samples, the authors identified putative germline variants for further assessment. Variants in both B-ALL and T-ALL cohorts differed in their characteristics. B-ALL variants were predominantly missense mutations concentrated toward the C terminus of the protein and outside of the RUNT homology domain (RHD), which is involved with DNA binding and where most known pathogenic *RUNX1* missense variants are found (5). In contrast, the variants found in T-ALL were almost all stop-gain and frameshift variants and spread across the protein, consistent with potential loss-of-function variants. Functional assessment of variants is important

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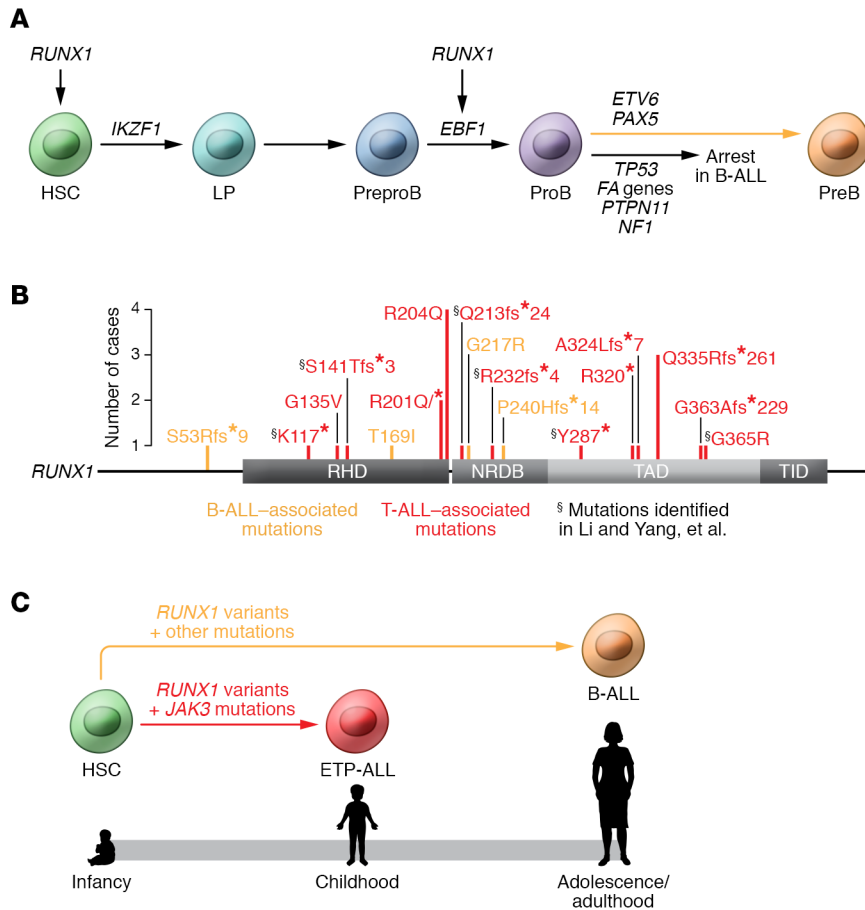


Figure 1. Model for B- and T-ALL predisposition. (A) Schematic of B cell development and normal function of known transcription factors involved in B-ALL predisposition. *RUNX1* is required for the emergence of developmental hematopoietic stem cells (HSCs), and it also regulates the induction of *EBF1* essential for transition to pro-B cell. Germline variants of *IKZF1*, *ETV6*, and *PAX5* predominantly predispose patients to ALLs, while germline variants in *PTPN11*, *NF1*, *TP53*, and other genes involved in MAPK signaling and DNA damage are associated with both ALL and other malignancies. (B) *RUNX1* germline variants reported in Li and Yang et al. (13) (§) and previously published work (*) associate with lymphoid malignancies B-ALL and T-ALL. (C) Damaging germline *RUNX1* variants are observed in pediatric T-ALL patients and collaborate with somatic *JAK3* mutations, whereas they are absent from pediatric B-ALL patients, with rare examples from other studies in which they are associated with older age of B-ALL onset, but do not have recurrent shared somatic variants. LP, lymphoid progenitor; PreproB, prepro B cell; proB, pro-B cell; preB, pre-B cell; FA, Fanconi anemia; NRDB, negative regulatory domain for DNA binding; TID, transcriptional inhibitory domain.

for the determination of pathogenicity of alleles and assists clinical classification. The ClinGen Myeloid Malignancy Variant Curation Expert Panel (MM-VCEP) recommendations for *RUNX1* include a luciferase-based transactivation assay with a known *RUNX1* target gene (14). Employing such an assay, the authors found that, consistent with the predicted effect on function, within the 20 B-ALL variants assessed, none had a reduction in transactivation activity, whereas in the 11 T-ALL-associated variants, six had reduced transactivation capacity. The combination of this functional data with other properties of these variants allowed four variants to be provisionally classified as pathogenic or likely pathogenic according to modified ACMG criteria (14). Given the rarity of germline *RUNX1* mutations, unsurprisingly, these mutations are all novel in the germline *RUNX1*-FPD context, with two variants seen previously in a somatic context in the Catalogue of Somatic Mutations in Cancer (COSMIC) database. The commonality of the same

variant occurring in both a somatic and germline context in tumors highlights a substantial challenge in identifying when *RUNX1* variants are germline. Therefore, it is essential to confirm variants as germline using a nonhematological source of genetic material (e.g., hair follicle, cultured skin fibroblasts).

The role of *RUNX1* variants in lymphoid leukemogenesis

Out of the T-ALL-associated *RUNX1* variants of interest identified by Li and Yang et al., two variants resulted in a loss of function due to nonsense and frameshift mutations in the RHD (13). Interestingly, four T-ALL-associated *RUNX1* variants, in addition to the loss of transcriptional activity, appeared to have dominant-negative activity in assays of cellular context, adding support to previous data suggesting some germline *RUNX1* variants have dominant-negative function (10). Further, functional assays in CD34⁺ cells performed on three variants with intact RHD showed phenotypes consistent with known myeloid

skewing that has also been observed in *Runx1* deficiency models and *RUNX1*-FPD preclinical models (10, 15). Importantly, all variants resulted in decreased production of CD41a⁺CD42b⁺ megakaryocytes, a feature of *RUNX1*-FPD associated with mild thrombocytopenia in patients.

Li, Yang, and coauthors next tested the specific role of the *RUNX1* variants in leukemogenesis. First, gene-expression analysis of patient T-ALLs showed that leukemias with either germline or somatic *RUNX1* mutations clustered closer to T-ALL with early T cell precursor (ETP) or near-ETP phenotype when compared with those with WT *RUNX1*. These results suggest that mutant *RUNX1* can contribute to leukemia maintenance regardless of whether the mutation was inherited or acquired. Whole-genome sequencing analysis revealed an enrichment of missense *JAK3* mutations in the T-ALL samples evaluated (83%) (Figure 1C). Similarly, *JAK3* mutations were also enriched in T-ALL cases with somatic *RUNX1* (27%) compared with *RUNX1* WT T-ALL cases

(less than 8%). The authors used a murine transplantation model to test the effect of these two mutations *in vivo*. The recipients of *JAK3*-transfected hematopoietic stem and progenitor cells (HSPCs) had higher total peripheral WBC counts compared with controls. The *RUNX1* variant recipients had lower WBC counts than controls, consistent with previous observations of mildly reduced reconstitution capacity of *RUNX1* mutant HSPCs (15). Leukemia was observed only in recipients of *JAK3*- or *JAK3/RUNX1*-transfected HSPCs, arguing for the role of acquired *JAK3* mutations in the context of *RUNX1* variants for leukemogenesis. Interestingly, the leukemia in *JAK3/RUNX1* recipients compared with *JAK3*-only recipients was more consistent with an ETP phenotype, further suggesting their collaboration is essential for driving this phenotypic expression of *RUNX1* leukemia predisposition (13).

Conclusions and future directions

Where does the striking difference between *RUNX1* variants in B-ALL and T-ALL stem from? Among lymphoid malignancies described in *RUNX1*-FPD to date, the predominance of those of T cell origin is clear. The role of *RUNX1* in T cell biology has been described in T cell maturation and tolerance (16). *Runx1*-deficient mice have decreased common lymphoid progenitors (CLPs) and CD3⁺ T cells, which persist upon transplantation, suggesting a cell-autonomous effect (15). *RUNX1* is commonly mutated in adult and pediatric T-ALL and promotes T-ALL growth (17); its loss leads to dysregulated oncogenes critical for maintenance of T-ALL (18), and it participates in a core transcriptional regulatory circuit regulated by *TAL1* in T-ALL (19). Li and Yang et al. demonstrated that the *RUNX1* variant negatively affected T cells and, when combined with the *JAK3* mutation, led to leukemia. It is not yet known how its role in T cell development relates to T-ALL leukemogenesis.

RUNX1 is involved in various stages in normal B cell development (Figure 1A and ref. 20). In childhood B-ALL, *RUNX1* most commonly associates with the proto-oncogene *ETV6* as a fusion protein (*ETV6-RUNX1* fusion protein) (21). In the fusion complex, *RUNX1* remains largely intact,

possessing the RHD and the transcriptional activation domain (TAD). However, when *ETV6-RUNX1* fusion protein is expressed in developing HSPCs, it blocks B cell development at the transition to CLPs (22). Studies in preclinical models suggest that *ETV6-RUNX1* fusion has a specific role in childhood B-ALL, acting through its effect on developmental HSPCs, and it is largely absent in adult B-ALL (22). Given the effect on later developmental stages of B cells in *RUNX1* deficiency, is it possible that B cell leukemogenesis requires a longer latency to acquire transforming mutations and thus would be unlikely to associate with childhood B-ALL? The study cohort in Li and Yang et al. (13) was relatively young (10.5 years plus or minus 5.7 years in AALLO232) (23). *RUNX1* loss affects B lineage priming at the stages of lymphoid-primed multipotential progenitor and CLPs, and a transforming secondary mutation in these short-lived, self-limiting populations is unlikely to occur in the first decade of life. Consequently, *RUNX1* deficiency-related B-ALL may more likely be observed in adolescence or adulthood, when the transforming somatic mutation would occur in an earlier progenitor (Figure 1C). Asymptomatic occurrence of clonal hematopoiesis due to mutations in HSPCs has been described in *RUNX1*-FPD and is usually observed after the second decade of life (10). In fact, the four cases of B-ALL in *RUNX1*-FPD known to us were diagnosed in two adolescents and two adults in their 40s (6–8). The pathophysiologic link between *RUNX1* loss and B-ALL leukemogenesis is yet to be established.

The work by Li and Yang et al. (13) highlights the importance of annotating and characterizing gene variants identified on widely used sequencing platforms in pediatric oncology today and builds on previous such efforts that have added *TP53*, *IKZF1*, and others to our repertoire of genes known to predispose to particular subtypes of ALL (1). Ongoing research efforts to build detailed clinical annotations of these rare disease cohorts with specific *RUNX1* variants will contribute to our understanding of age of disease onset, treatment-related toxicities, and outcome of patients harboring these variants. Comprehensive clinical annotations will provide further opportunities to identify, monitor, and treat individuals at risk for ALL.

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Address correspondence to: Anna L. Brown, SA Pathology, Frome Road, Adelaide, South Australia 5070, Australia. Phone: 61.8.82223885; Email: Anna.Brown@sa.gov.au.

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