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DOI 10.1182/blood.2020005756

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TO THE EDITOR:

RUNX1 germline variants in *RUNX1*-mutant AML: how frequent?

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RUNX1 mutations are recurrent aberrations in acute myeloid leukemia (AML) that are either somatically acquired or originate in the germline. Monoallelic pathogenic germline *RUNX1* variants cause familial platelet disorder with propensity to AML (FPD/AML), featuring predisposition to develop myeloid malignancies. Recently, Simon et al[1] found that 30% of *RUNX1*-mutated AML patients carried a (nonpolymorphic) *RUNX1* germline variant. This high frequency contrasted previously reported frequencies in AML cohort studies.[2-5]

To obtain further insight into the frequency of germline variants within *RUNX1*-mutated AML patients, we retrieved data from a large independent cohort of AML patients that was previously interrogated by paired genetic sequencing at diagnosis and in complete remission (CR).[6] Targeted next-generation sequencing (NGS) at diagnosis was performed in 763 AML patients, enrolled in the Dutch-Belgian Hemato-Oncology Cooperative Group (HOVON)102 clinical trial (2010-2013),[7] using the TruSight Myeloid Sequencing Panel (Illumina). The HOVON102 trial was designed to investigate the added value of clofarabine in combination with standard remission-induction chemotherapy in adult (18-65 years) AML or myelodysplastic syndrome patients. NGS data were analyzed as previously described,[6] and nonpolymorphic *RUNX1* variants were annotated to RefSeq ID NM_001754.5.

At diagnosis, at least 1 *RUNX1* mutation was detected in 115 of 763 AML patients (15.1%) for a total of 142 *RUNX1* mutations (data not shown), corresponding to frequencies of 10% to 15% reported in literature.[3,4,8,9] We detected 2 or more mutations in 23 of 115 patients.

To distinguish germline variants from somatic mutations, we retrieved data from paired diagnostic and remission HOVON102 AML samples to identify persisting *RUNX1* mutations. Remission samples of 287 CR patients were available for NGS analysis.[6] Among these 287 AML patients, 48 *RUNX1* mutations were detected in 37 patients (12.9%), of whom 9 carried 2 or more mutations (supplemental Tables 1 and 2, available on the *Blood* Web site). The distribution of the variant allele frequencies (VAF) at diagnosis of the *RUNX1* mutations of the selected samples (n = 48 in 37/287 patients) was similar to the initial HOVON102 cohort (n = 142 in 115/763 patients) (Figure 1A). In the majority of cases (34/37), *RUNX1* mutations were acquired because the VAF of these mutations (n = 45) was ≤10% in CR (Figure 1A). However, in 3 of the 37 AML cases (8.1%), a *RUNX1* mutation was present at a VAF of 50% in both diagnostic and remission samples (Figure 1A), highly indicative for germline origin. Other cooccurring mutations present at diagnosis in these 3 AML patients were either cleared or persisted at much lower VAF in CR (Figure 1B-D), supporting the germline status of these *RUNX1* variants. Thus, in the current cohort of 287 AML patients that attained CR, 3 (1.0%) harbored a germline *RUNX1* variant.

We identified 2 different *RUNX1* germline variants in these 3 AML patients. A single patient (no. 748) carried *RUNX1* p.(Arg232Trp) (exon 7), a missense variant located between the runt homology domain and the transactivation domain (Figure 1E). The 2 other patients (no. 124 and no. 740) shared variant *RUNX1* c.97+1G>A, affecting the splice donor site of exon 3 (Figure 1E). A familial bond between these 2 patients could not be confirmed.

In accordance to recently published guidelines by the ClinGen Myeloid Malignancy Variant Curation Expert Panel,[10] we classified both identified *RUNX1* germline variants as variants of unknown significance (supplemental Table 3). Neither of these have been reported as *RUNX1* germline variants before in the context of FPD/AML traits,[11] although *RUNX1* p.(Arg232Trp) has been recurrently reported as somatically acquired mutation in AML.[1,3,4] Both variants occur outside of the runt homology domain, in which most missense *RUNX1* germline variants cluster.[11] *RUNX1* c.97+1G>A is presumed to specifically affect isoform RUNX1C, 1 of 3 major *RUNX1* isoforms, complicating its interpretation.[10] However, deletions of exon 2 and 3, also putatively affecting RUNX1C specifically, have been reported in the context of FPD/AML before.[12]

Additional somatic mutations were found in all 3 patients, most of which have been reported in the context of germline *RUNX1*-mutant AML before, including additional *RUNX1* mutations and concomitant mutations in *DNMT3A, FLT3*, and *GATA2*.[1,11,13-15] Secondary mutations in *RUNX1* are a frequent recurrent event in

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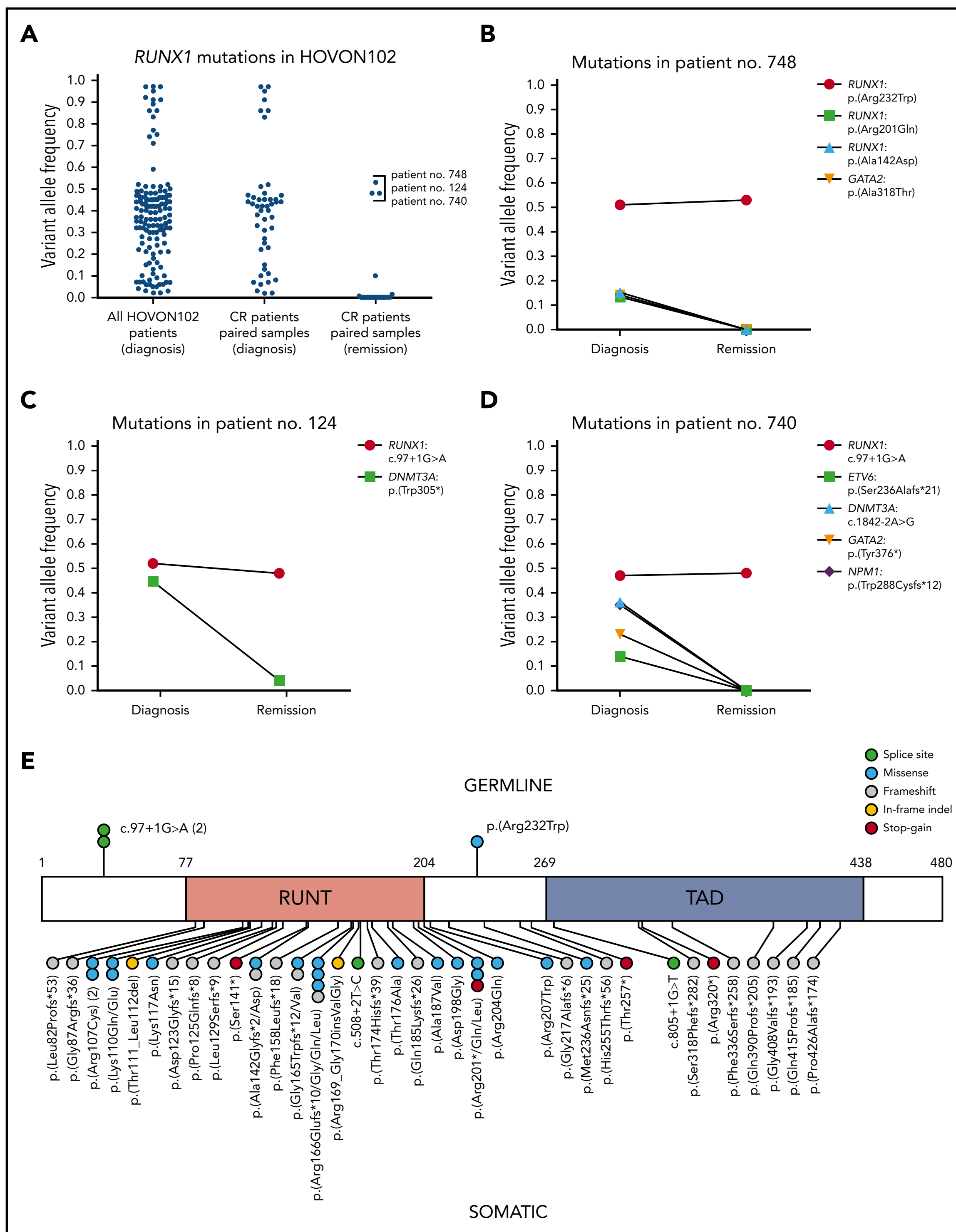

Figure 1. *RUNX1* mutations detected in the HOVON102 cohort of AML patients. (A) Variant allele frequencies (VAFs) of all *RUNX1* mutations in the entire HOVON102 cohort at diagnosis (n = 142 in 115/763 patients), VAFs of all *RUNX1* mutations in the subcohort of patients who attained complete remission (CR), and for whom paired samples were available, at diagnosis and at remission (n = 48 in 37/287 patients). (B-D) VAFs of all mutations of patients no. 748, no. 124, and no. 740, at diagnosis and during remission. (E) Visualization of the 48 *RUNX1* mutations identified in the 37 paired samples from CR patients, annotated to NM_001754.5 (supplemental Table 2). *, stop codon; (2), identical variant is identified in 2 different patients; Gln/Glu, the nucleotide at this location is mutated in 2 patients, changing to Gln and Glu, respectively; indel, insertion or deletion; RUNT, runt homology domain; TAD, transactivation domain.

FPD/AML-related AML,[11] and cooccurred in patient no. 748 carrying the germline variant *RUNX1* p.(Arg232Trp). Besides somatic mutations in *GATA2*, which were also reported to be overrepresented in germline *RUNX1* AML,[11] none of the mutated genes were specific to the germline *RUNX1* patients (supplemental Table 4). We revealed a somatic mutation in *ETV6*, a gene that has not been previously reported in AML with *RUNX1* germline variants, in one of our cases (no. 740).

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Table 1. Clinical characteristics of AML patients with a germline *RUNX1* variant

	Patient no. 748	Patient no. 124	Patient no. 740
Sex	Female	Female	Male
Age, y	26	38	63
Relevant medical history	Familial platelet disorder	Breast cancer	None
FAB	M6	M5	M0
Cytogenetics	46,XX	46,XX,t(9;11)(p22;q23)	46,XY
Consolidation	Allogeneic HSCT, MUD	Allogeneic HSCT, sib	Allogeneic HSCT, CB
Survival	Alive (67.1 mo)	Deceased (19.9 mo)	Alive (66.3 mo)
Relapse (DFS)	No (65.7 mo)	Yes (14.4 mo)	No (65.2 mo)
RUNX1 mutation	*RUNX1* p.(Arg232Trp)	*RUNX1* c.97+1G>A	*RUNX1* c.97+1G>A

RUNX1 variants were annotated to RefSeq ID NM_001754.5.

CB, cord blood; DFS, disease-free survival from complete remission; FAB, French-American-British classification of AML; HSCT, hematopoietic stem cell transplantation; MUD, matched unrelated donor; OS, overall survival from diagnosis; sib, sibling.

The clinical characteristics and course of the 3 patients harboring germline *RUNX1* variants is summarized in Table 1. All 3 patients were transplanted. Patient no. 124 had a therapy-related AML with a t(9;11) cytogenetic abnormality after breast cancer therapy. She was transplanted with hematopoietic cells from a sibling donor, relapsed 14 months after achieving CR, and died 4 months later. The origin of the relapse (donor/host) is not known. The other 2 patients achieved long-term survival without relapse after HSCT (of an unrelated donor [no. 748] and cord blood [no. 740]). Interestingly, at the time of study, a familial platelet disorder and a presumptive diagnosis of FPD/AML had been recognized in only 1 of 3 patients (no. 748).

Taken together, data from this independent cohort reveal an 8.1% frequency of germline variants in *RUNX1*-mutated AML. This frequency is lower than that reported by Simon et al (30%),[1] and consistent with previously reported frequencies in *RUNX1*-mutated AML in smaller series as assessed by DNA sequencing in buccal DNA at diagnosis or peripheral blood DNA in CR (8.6%, 10%, and 9.5%).[3-5] Schnittger et al[2] did not detect any germline *RUNX1* variants in remission samples of 60 noncomplex karyotype AMLs.

The apparent discrepancies in germline *RUNX1* mutation frequencies in *RUNX1*-mutant AML between the previously reported (0%-10%) as well as ours (8.1%) and reported by Simon et al (30%) may reflect population differences, selection biases, technical differences in detecting genetic variants, as well as the inherent small group sizes.

Differences in techniques used to detect *RUNX1* mutations may have contributed to the differences between our results and those reported by Simon et al.[1] RNA sequencing, as applied by Simon et al, is dependent on gene expression and detection of *RUNX1* mutations with lower VAFs may therefore be more challenging by NGS-based RNA sequencing than DNA sequencing. This may possibly explain the higher proportion of patients with *RUNX1* mutations at VAF <30% in our cohorts (26/115, 22.6% in the entire HOVON102 cohort; 8/37, 21.6% in the CR subcohort) vs 4.3% in Simon et al.[1] Differences in sensitivity for detection of these low VAF cases could potentially lead to an overestimation of the frequencies of *RUNX1* germline variants in *RUNX1*-mutant AMLs. RNA sequencing may further fail to detect mutations introducing premature stop codons leading to nonsense mediated decay of transcripts, which would result in underestimation of the total number of *RUNX1* mutations. Because this could potentially affect the detection of both germline and somatic variants, the overall effect on germline frequency is uncertain.

We cannot formally exclude the possibility that the frequency of germline variants in our study may be biased by a potentially differential response to induction therapy of patients harboring germline *RUNX1* mutations. In the complete NGS-analyzed HOVON102 cohort of 763 AML patients, 668 (88%) achieved CR after 2 cycles of high-intensity induction chemotherapy (data not shown). *RUNX1*-mutated patients achieved CR less frequently (91/115, 79.1%) compared with *RUNX1* wild-type patients (577/648, 89%), with a relative risk of refractory disease of 1.90 (95% confidence interval, 1.25-2.89), which is in line with the proposed relative chemoresistance of *RUNX1*-mutated AML.[3,4,8,9,16] No difference, however, was found in CR rates between patients carrying a *RUNX1* mutation at a VAF ≥30% (70/89, 78.7%) and patients carrying *RUNX1* mutations at a VAF <30% (21/26, 80.8%) at diagnosis, with a relative risk of refractory disease of 1.11 (95% confidence interval, 0.46-2.68), perhaps arguing against a potential bias introduced by limiting analyses to patients in CR.

On a final note, it is worth considering that not all reported variants are necessarily disease-causing mutations. Therefore, the frequency of *RUNX1* germline variants reported in literature may not directly translate to the frequency of FPD/AML. Moreover, even if the latest classifying algorithms are applied, as exemplified in Simon et al and in the current analysis, pathogenicity of certain variants remains uncertain. Efforts to refine classifying algorithms by initiatives such as ClinGen, as well as continued reporting and curating of variants in databases like ClinVar and RUNX1db, are vital to improve the collective knowledge of variants and thus the ability to more accurately diagnose FPD/AML.

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We conclude that the frequency of germline *RUNX1* variants in *RUNX1*-mutated AML may be lower than recently reported, depending on multiple variables including cohort variation and *RUNX1* detection methods. Future studies in larger cohorts are required to definitively establish this frequency. This does not negate the absolute necessity of germline testing in *RUNX1*-mutated AML (with high VAFs), as stressed by the clinical cases described herein.

Acknowledgments

The authors thank the HOVON/SAKK leukemia working group for conducting the HOVON102 clinical trial, and T. Grob, B. Van der Holt, and Y. Van Norden for providing additional clinical data.

This work was supported by Alex's Lemonade Stand Foundation for Childhood Cancer-Familial RUNX1 Research Grant to M.H.G.P.R.

Authorship

Contribution: M.P.T.E. wrote the article and provided literature background; M.P.T.E. and F.G.K. provided illustrations; F.G.K. and B.L. provided data and scientific input; and P.J.M.V. and M.H.G.P.R. wrote the article and supervised M.P.T.E.

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

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Footnotes

Submitted 31 July 2020; accepted 5 October 2020; prepublished online on *Blood* First Edition 19 October 2020.

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Contact the corresponding author for original data.

The online version of this article contains a data supplement.

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DOI 10.1182/blood.2020008478

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