Telomere attrition is common in patients with germline *RUNX1* pathogenic variants

Familial platelet disorder with associated myeloid malignancy (FPDMM) is a hematologic malignancy (HM) predisposition syndrome caused by germline *RUNX1* pathogenic variants,[1] characterized by a high lifetime incidence of HM. Telomeres are important for maintaining genomic stability, and shortened telomeres have been identified in several inherited blood diseases.[2] FPDMM patients exhibit thrombocytopenia, marrow dysplasia, and increased malignancy risk, but telomere dynamics in FPDMM have not been studied. In this study, we used flow fluorescence *in situ* hybridization (FISH) to determine leukocyte telomere length (TL) in FPDMM patients. Leukocyte TL was significantly below the age-adjusted 50th percentile in all patients for both lymphocytes (P=1.8x10^{-30}) and granulocytes (P=6.5x10^{-42}) across all age groups. More patients had very short telomeres (<1st percentile) in granulocytes than in lymphocytes (57.5% *vs*. 30%). Moreover, the severity of TL shortening in FPDMM is similar to that in telomere biology disorder (TBD) and other inherited blood diseases linked to leukemia. Our data indicate that premature TL shortening is a common genomic alteration in germline *RUNX1* variants and FPDMM, pointing to a new and potentially critical disease attribute.

The natural history study on patients with FPDMM was approved by the NIH Institutional Review Board (protocol number 19HG0059) and was registered at clinicaltrials.gov (identifier NCT03854318). We prospectively assessed 40 participants from the largest, single-center natural history study of FPDMM (clinicaltrials.gov identifier NCT03854318)[1] between May 2024 and March 2025. All 40 participants carry germline *RUNX1* pathogenic (P) or likely pathogenic (LP) variants, encompassing 23 families with 19 unique *RUNX1* variants (*Online Supplementary Table S1*). Informed consent was obtained for all participants in accordance with the Declaration of Helsinki. We did not include participants with hematologic malignancy, *RUNX1* benign / likely benign variants, or variants of uncertain significance. The median age of the cohort was 30 years (range 2-66). Lymphocyte TL in 87.5% (35/40) of the patients was shortened: 57.5% measured short (<10th age-adjusted percentile), and 30% very short (<1st age-adjusted percentile) (Figure 1A, *Online Supplementary Table S2A*). In granulocytes, 95% (38/40) of the patients had shortened TL, including 37.5% short and 57.5% very short (Figure 1B, *Online Supplementary Table S2A*). Overall, TL was significantly below the age-adjusted 50th percentile in all patients (lymphocytes: P=1.8x10^{-30}; granulocytes: P=6.5x10^{-42}) (Figure 1C), demonstrating a 100% negative predictive value for identifying a germline P/LP variant in *RUNX1*. Furthermore, granulocytes had significantly greater TL shortening than lymphocytes (P=8.59x10^{-7}) (Figure 1D). For both lymphocytes and granulocytes, TL shortening occurred in all age groups (Figure 1A, B, *Online Supplementary Table S2A*), with no statistically significant differences in severity (Figure 1E, F).

Thirteen of 40 (32.5%) patients in this study had somatic mutations in myeloid cancer genes with variant allele frequency >2% (*Online Supplementary Figure S1*). However, we found no significant TL difference in the presence or absence of the somatic variants (P≥0.9) (*Online Supplementary Figure S1B-D*). We also did not see any significant difference in expression of telomere-related genes between FPDMM patients and healthy controls, nor did we identify any mutations in such genes in the FPDMM patients. Moreover, no correlations were identified between the severity of TL shortening and the *RUNX1* variant types. Finally, age-adjusted FPDMM TL did not correlate with peripheral blood counts, bone marrow cellularity or International Society on Thrombosis and Hemostasis (ISTH) bleeding criteria. These findings suggest that the TL shortenings observed in these FPDMM patients correlate mainly with the presence of *RUNX1* P/LP variants, rather than any secondary contributing factors.

For 2 siblings with *RUNX1* frameshift Arg346Profs*249 variant, we analyzed chronological changes of TL data at three timepoints each over nine years. For both patients, TL continued to shorten with age and accelerated between the second and third timepoints (Figure 2A, B). We also calculated the relative rates of TL attrition in these 2 siblings. The telomere attrition rate accelerated with age for both patients and both cell types (Figure 2C, D), relative to the normal attrition rates (green curves in Figure 2C, D, *Online Supplementary Table S2B*), suggesting accelerated telomere attrition with increasing age for both patients.

Next, we considered the possibility of genetic anticipation for the TL shortening phenotype. Of the 23 families, 9 had 2 generations with measured TL. There were no families with TL measurements of 3 generations. TL data in those 9 families do not demonstrate a consistent trend pointing to anticipation (Figure 2E, F). The data would suggest that the TL erosion is acquired due to *RUNX1* loss with telomere reset in each generation.

Very short leukocyte TL, typical of TBD, results from telomere dysfunction in hematopoietic stem cells (HSC) harboring germline mutations in telomere-related genes. However, telomere shortening can also arise from the impaired hematopoiesis observed in other inherited bone marrow failure syndromes (IBMFS), such as Fanconi anemia, Diamond-Blackfan anemia, and Shwachman-Diamond Syndrome.[3] Additionally, TL shortening may also be a consequence of increased HSC proliferation, as seen in severe

aplastic anemia (SAA).[4] Comparing the TL profile of our FPDMM patients to those with TBD, other IBMFS, and SAA,[5] we found that the percentage of abnormal TL in FPDMM significantly exceeds that of all inherited IBMFS except dyskeratosis congenita (DC), a TBD with telomeres that are almost exclusively very short (< 1st percentile) (Figure 3A). We next compared age-adjusted ΔT between our FPDMM cohort and previously published patients consisting of 62 SAA

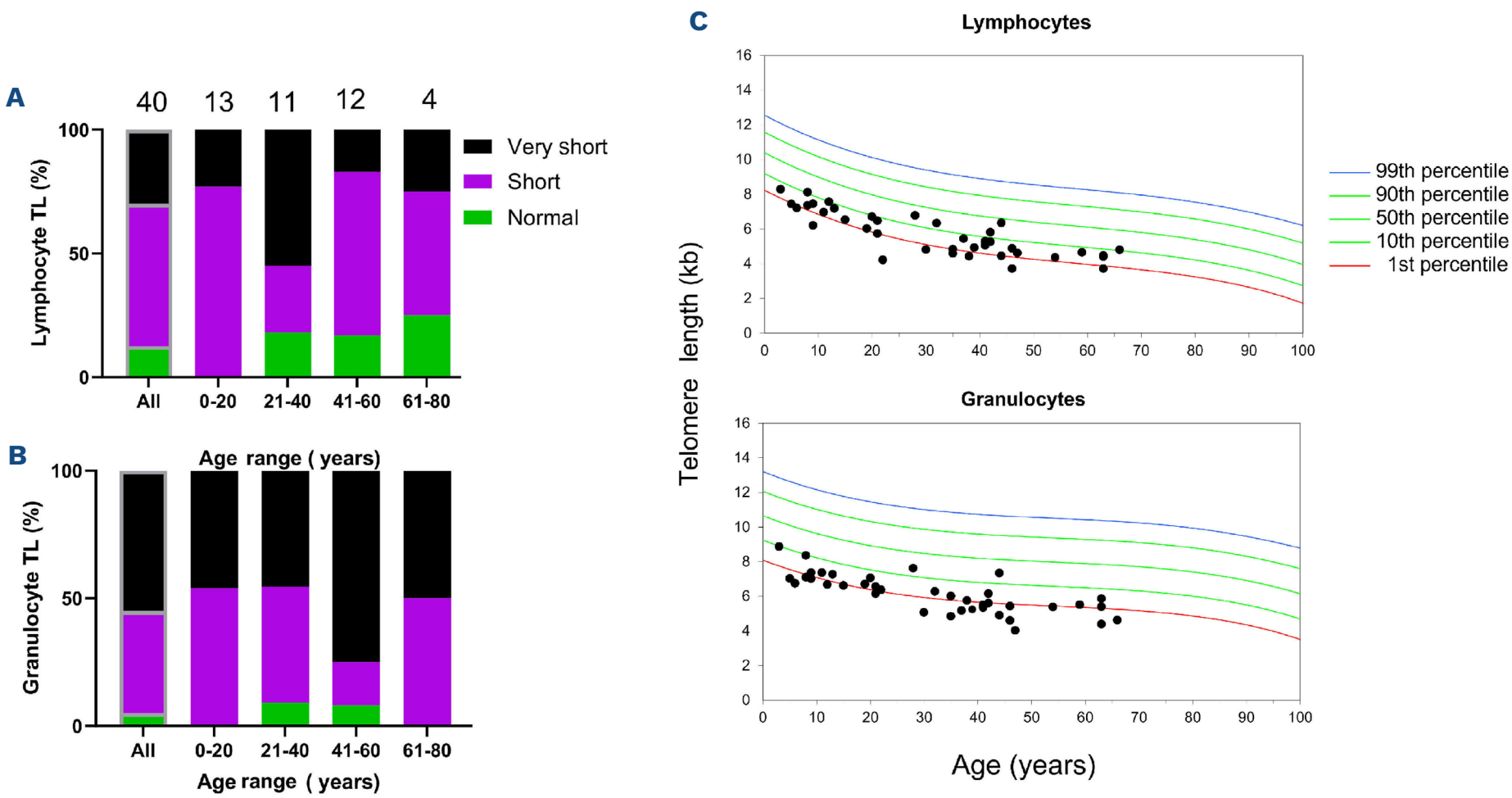

Figure 1. Distribution and changes of age-adjusted leukocyte telomere lengths in patients with germline *RUNX1* familial platelet disorder with associated myeloid malignancy. Stacked bar graphs of normal, short, and very short telomere lengths (TL) percentage in lymphocytes (A) and in granulocytes (B) from patients with familial platelet disorder with associated myeloid malignancy (FPDMM) in 2-decade intervals. The numbers of patients in each age group are presented at the top of panel (A). (C) Nomograms of lymphocyte and granulocyte TL from FPDMM patients with normal control percentile lines as indicated. (D) Statistical comparison between lymphocyte and granulocyte averages of ΔT for each age range. (E and F) The TL mean deviation from age-adjusted median (ΔT) in lymphocytes (E) and granulocytes (F) over 20-year age groups. Average ΔT for each age range are listed under each graph (in kb). ΔT: deviation of TL from the age-adjusted median; kb: kilobase. TL measurements were conducted with lymphocytes and granulocytes from fresh blood samples using CLIA-certified fluorescence *in situ* hybridization and flow cytometry (flow - FISH) by the Repeat Diagnostics Inc. (Vancouver, British Columbia, Canada).

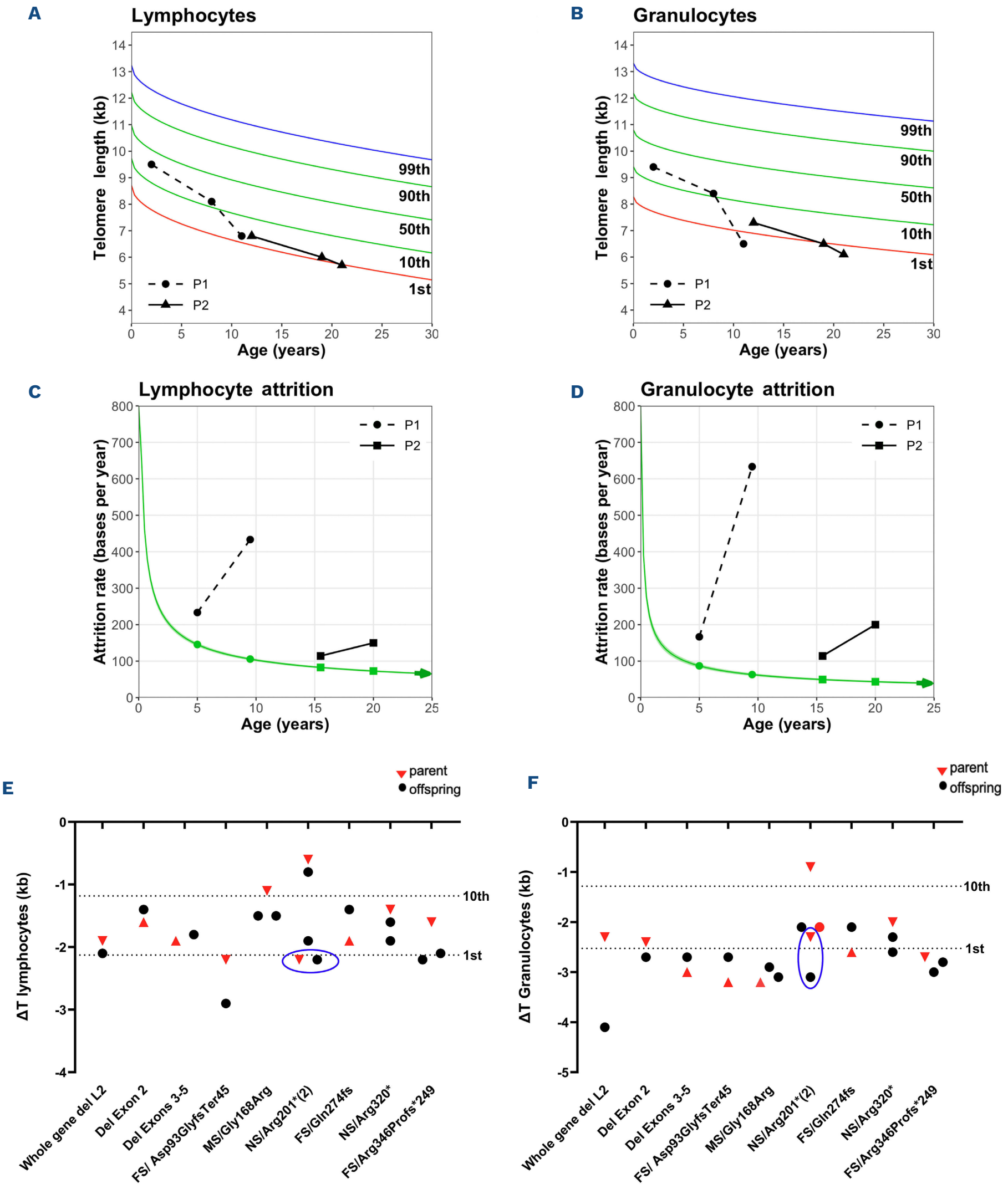

Figure 2. Telomere length in families. Telomere length (TL) longitudinal assessments of 2 siblings with germline *RUNX1* familial platelet disorder with associated myeloid malignancy (FPDMM) are plotted on nomograms for patient 1 (P1) and patient 2 (P2) of lymphocytes (A) and granulocytes (B) over a 9-year interval with three timepoints each. Plots of the attrition rates for lympho-

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cytes (C) and granulocytes (D) in each patient against the published control data.[16] The rate of attrition ([ending TL – starting TL]/time elapsed) is plotted as a function of time, with the indicated point being the mid-point between observations. Green curved lines depict normal, cell type-specific rate of attrition with a 95% Confidence Interval (CI). Black symbols are for the 2 patients (dots for P1 and triangles for P2). Green symbols are expected observations if the patients were to follow the population-based rate of attrition. (E and F) TL erosion in families over 2 generations, showing distribution of age-adjusted deviation in TL according to an individual family and their *RUNX1* variant in lymphocytes (E) and granulocytes (F). Parent: triangles; offspring: circles. ΔT: deviation of TL from the age-adjusted median; kb: kilobase. There are 2 families with the same Arg201* variant, hence the blue circles represent an individual family separated from another family sharing the same variant.

A

Diagnosis	Lymphocytes		Granulocytes	
	Short (%)	Very short (%)	Short (%)	Very short (%)
RUNX1 FPD	57.5	30	37.5	57.5
IBMFS				
DC	8	92	4	96
FA	20	13	33	40
DBA	36	7	21	21
SDS	20	20	40	20

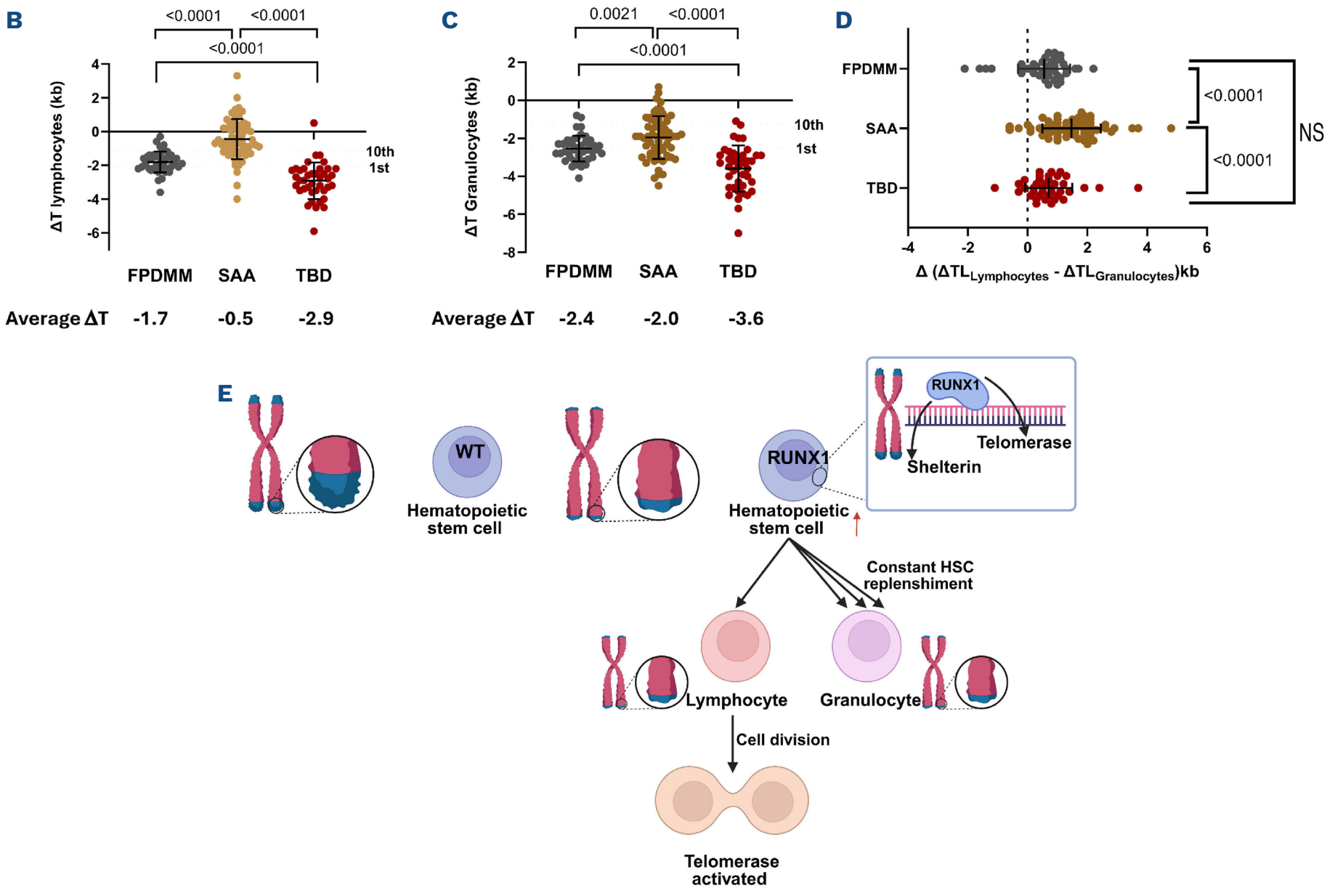

Figure 3. Comparing telomere length abnormalities in familial platelet disorder with associated myeloid malignancy to other telomere biology disorders. (A) Percentage of patients with short (1-10%) and very short (<1%) telomeres in lymphocytes and granulocytes in familial platelet disorder with associated myeloid malignancy (FPDMM) compared to inherited bone marrow failure syndromes (IBMFS). Percentages for IBMFS were obtained from Alter *et al.*[3] Changes in age-adjusted telomere length in FPDMM, severe aplastic anemia (SAA), and telomere biology disorder (TBD) in lymphocytes (B) and granulocytes (C). (D) Comparisons among FPDMM, SAA, and TBD for the differences between lymphocyte and granulocyte for their changes in age-adjusted

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telomere length. $\Delta(\Delta TL_{lymphocytes} - \Delta TL_{granulocytes})$. Only pathogenic (P) or likely pathogenic (LP) variants are included. Each dot represents one patient. ΔT: deviation of TL from the age-adjusted median. (E) Schematic representation of telomere biology in FPDMM. As a defining feature, telomeres in hematopoietic cells in FPDMM patients are shorter than those in healthy controls. The exact mechanism is unclear, but RUNX1 may regulate genes involved in the telomerase complex and/or the shelterin complex. DBA: Diamond-Blackfan anemia; DC: dyskeratosis congenita; FA: Fanconi anemia; HSC: hematopoietic stem cell; kb: kilobase; NS: not significant; SDS: Shwachman-Diamond syndrome.

and 40 TBD, where a majority had germline variants in *TERT*, *TERC*, *RTEL1*, *TINF2*, or *DKC1*.[6,7] Measured FPDMM lymphocyte ΔTL was -1.7kb (Figure 1D), which was greater than -0.5kb ΔTL in SAA patients (P<0.0001), but less than TBD at -2.9kb (P<0.0001) (Figure 3B). The same pattern of differences was observed in granulocytes (Figure 3C). The Δ (ΔTL) between leukocyte subsets $\Delta(\Delta TL_{lymphocytes} - \Delta TL_{granulocytes})$ showed a significant increase in SAA compared to both FPDMM and TBD (P<0.0001), while there was no significant difference between FPDMM and TBD (Figure 3D).

Two observations emerge from our findings: the leukocyte subtype discordance seen in FPDMM resembles the pattern typically detected in SAA,[4,6] yet the frequency and early onset of abnormal telomere lengths is similar to that seen in IBMFS. The significant decrease in granulocytes may result from the fact that lymphocytes (T and B cells) can activate telomerase during cell division to counteract telomere shortening,[8,9] whereas granulocytes do not express telomerase to reduce the loss of TL.[10] In addition, the greater TL attrition in granulocytes could be due to hematopoietic stress placed on the FPDMM HSC.[11] Since lymphocytes are long-lived cells while granulocytes require constant HSC replenishment, HSC dysfunction would manifest immediately in granulocytes but take from years to decades in lymphocytes. In the case of acquired aplastic anemia, the pathophysiology is thought to be due to immune-mediated damage to hematopoietic stem and progenitor cells. Recent studies identified increased CD74 signaling driving inflammation in FPDMM patients,[12] suggesting a likely contributor to TL erosion in this population. However, TL attrition in FPDMM exceeds that in SAA, suggesting immune mechanisms alone are insufficient and additional factors likely contribute. It is plausible that there is a direct role for *RUNX1* regulation of TL through the telomerase complex.[4,5,13-15] However, we do not currently have data to demonstrate this.

The TBD cohort compared in Figure 3B-D[7] included pathogenic germline variants in *TERT*, *TERC*, *RTEL1*, *TINF2*, and *DKC1*. Although *RUNX1* has been shown to modulate the expression of *TERT*, no significant change in the expression of telomere-related genes was detected in FPDMM patients compared to healthy donors. In addition, the percentage of patients with very short or short telomeres in each leukocyte subset in FPDMM is similar to but more severe than IBMFS we analyzed here, except DC. Yet, unlike SAA, FPDMM attrition occurs before bone marrow failure, often in the absence of cytopenia apart from the characteristic thrombocytopenia. Thus, our findings suggest a new disease phenotype for FPDMM, with possible pathogenic implications for cancer, and point to a new direction for understanding the biology of the disease. Additionally, our data show for the first time that germline pathogenic variants in a transcription factor gene are associated with telomere shortening. The exact mechanism is unclear, but given the similarity with IBMFS, RUNX1 may regulate genes involved in the telomerase complex and/or the shelterin complex (Figure 3E). To uncover connections between RUNX1 and telomere length, further studies are required to expand our understanding of FPDMM biology.

Clinically, high frequencies of FPDMM patients with short or very short telomeres have direct implications for the differential diagnosis of IBMFS. TL testing turnaround is much shorter than genetic testing, and reports of short or very short telomeres increase the suspicion of underlying TBD. Patients with thrombocytopenia, short telomeres, and increased risk of malignancy can have different etiologies, and distinguishing these patients by clinical presentation may not be enough, implying a need for genetic testing.

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Disclosures

No conflicts of interest to disclose.

Contributions

RAL drafted the manuscript, and collected and analyzed the data; FG, EMG, BAP and NSY provided guidance and data on SAA and TBD patients; MM contributed data on patients; NTD, KC and SC collected the data and cared for patients; EB, MS and DW contributed and analyzed data on patients; NW, DJY and PL supervised the work and drafted the manuscript. All authors critically reviewed the manuscript for submission.

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Data-sharing statement

We follow the NIH policy to share scientific data to the maximum extent possible, considering ethical, legal, and technical factors. All data are included in the manuscript including associated supplementary files. Any specific request maybe directed to Paul Liu at pliu@mail.nih.gov.

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