Genetic Drivers of Clonal Hematopoiesis in Patients with Telomere Biology Disorders

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Telomere biology disorders (TBD) are a heterogeneous group of inherited bone marrow failure diseases caused by genetic defects in telomere maintenance. The resultant telomere shortening in TBD patients leads to a multi-system disease, manifesting as cytopenias, mucocutaneous abnormalities, pulmonary fibrosis, cirrhosis, and cancer predisposition. Notably, TBD patients have an increased risk of myeloid malignancies, with up to a 24-fold higher risk of leukemia and a 578-fold higher risk of myelodysplastic syndrome (MDS) than the general population. In previous studies, our group and others have identified a high proportion of TBD patients with clonal hematopoiesis, yet somatic mutations associated with normal aging and sporadic MDS were infrequent. Early clonal events underlying MDS and leukemia predisposition in TBD remain poorly understood.

To determine drivers of pre-malignant, clonal evolution in TBD, we performed a comprehensive analysis of clonal hematopoiesis in 67 TBD patients recruited from 2010 through 2022, a part of 2 IRB-approved patient registries at the University of Pennsylvania and Children's Hospital of Philadelphia. The diagnosis of TBD was established using a combination of lymphocyte telomere lengths (TL), genetic testing, and clinical criteria. Patient TLs were <1st percentile for age in 81.8% and <10th percentile for age in 12.7%. Germline mutations in TBD-associated genes were identified in 53 patients (79%) (ACD n=2, DKC1 n=12, RTEL1 n=9, TERC n=18, TERT n=13). Clonal hematopoiesis was analyzed, as available, using a combination of X chromosome inactivation (n=11), metaphase cytogenetics (n=48), cytogenetic arrays (n=24), whole-exome sequencing (n=14), and massively parallel sequencing via targeted hematologic neoplasm panel (n=30) and/or custom 305-gene panel with telomere maintenance, DNA damage response (DDR), and cell senescence pathway genes (n=44).

Clonal hematopoiesis, as measured by any modality, was identified in 29/67 (43.3%) of patients. The most commonly mutated genes were ATM (10 mutations in 5 of 54 (9.3%) analyzed patients) and TP53 (5 mutations in 4 of 56 (7.1%) analyzed patients). ATM mutations were seen in patients with severe telomere shortening and severe extra-hematopoietic manifestations: 2 had TERC-associated TBD (n=2 patients, with 2 and 4 ATM mutations each) and 3 had no established genetic diagnosis. Three patients with ATM variants were followed for over 2 years with stable blood counts; two subsequently developed worsening cytopenias, which was associated with development of additional mutations (subclonal acquisition of an NPM1 variant in one patient, and clonal replacement with a TP53-mutant clone in another patient). An additional 11 somatic mutations were identified in genes involved in cell senescence, DDR, and telomere maintenance, including KAT6A, KAT6B, PALB2, TEL02, TOP1, and RTEL1. Somatic reversion of TBD-associated germline lesions was identified in 2 patients, one with reversion of a DKC1 variant and the other with 3q loss of heterozygosity and reversion of a TERC variant. Other hematologic malignancy-associated genes were mutated in 7 patients (12.5%), most frequently involving spliceosome genes (U2AF1 n=2, SF3B1 n=1), and 4 of 7 patients with MDS-associated mutations transformed to MDS, with mutations in NPM1, RUNX1, SF3B1, WT1, and FLT3.
cytogenetic changes occurred in 7/48 of evaluable patients (14.6%), the most common being a gain of 1q, identified in 4 patients (8.3%). Two patients (4.2%), both with mutations in TP53, developed complex karyotype.

In conclusion, we identified somatic disruption of key DDR genes, ATM and TP53, as main drivers of pre-malignant clonal evolution, particularly in TERC-mutated patients. Our findings point to telomere dysfunction-induced DDR and cellular senescence mechanisms as the main drivers of bone marrow failure and clonal selection in this patient population. Ongoing studies aim to validate our results and explore therapeutic targeting of DDR in TERC-mutated TBD.