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LETTER TO THE EDITOR

Structural modeling of a novel *TERC* variant in a patient with aplastic anemia and short telomeres

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Dear Editor,

Inherited bone marrow failure syndromes (IBMFS) are a heterogeneous group of genetic blood disorders in which there is usually some form of aplastic anemia, associated with a family history of the same disorder. In addition, patients with IBMFS have a high risk of malignancies [1]. Some IBMFS have excessively short telomeres at the peripheral nucleated blood cell level, referred to as telomeropathies. The discovery of loss-of-function mutations in genes of telomerase complex (*TERC* and *TERT*) established a genetic etiology for telomere attrition in these patients [2, 3]. Several *TERC* variants, more than 40 nucleotide changes and small deletions, in patients with bone marrow failure have been described [4]. *TERC* gene, encoding the RNA component of telomerase, spans 451 nucleotides and comprises one non-coding exon that contains several conserved regions essential for its stability. Vertebrate telomerase RNA is composed by four highly conserved structural regions: pseudoknot domain, CR4-CR5 domain, box H/ACA domain, and CR7 domain [5, 6]. The mutations are distributed throughout TERC molecule, indicating that all domains contribute to function and that intricately base-paired structure is critical for its biologic activity [7, 8]. Here, we report on the case of a 33-year-old female affected by a severe anemia and pancytopenia since the age of 3. Family history is positive for blood disorders. Patient's mother was diagnosed with a severe anemia at the age of 20 and died for a lymphoma, whereas maternal grandfather died for an unspecified form of blood cancer at the age of 80. A bone marrow biopsy performed on our patient showed a markedly hypocellular marrow with a reduction of all cell lines, consistent with the diagnosis of aplastic anemia. Molecular analysis of a panel of genes associated with hereditary anemia was performed by next generation sequencing on a sample of genomic DNA from peripheral blood. Genetic testing revealed a novel heterozygous variant in *TERC* gene, n.179 T>A (NG_016363.1). The variant was confirmed and checked in healthy father and brother for segregation by direct Sanger sequencing (Fig. 1a). Mother's DNA sample was not available but we suppose that she harbored the n.179 T>A *TERC* variant based on of her hematological history. Analysis of mean telomere lengths by Q-FISH in metaphase spreads established from patient's lymphocytes showed significantly shorter telomeres when compared with those of seven age-matched controls (Fig. 1b, c) confirming that the identified *TERC* variant has functional implications. Moreover, when compared to normal controls, the patient displayed also a very higher percentage of undetectable and short telomeres (Fig. 1d).

We used structural and molecular modeling to gain insight into the effects of the identified *TERC* variant. The n.179 T>A nucleotide substitution destroys the complementarity with nucleotide A111 in the stem 2 (P3) of the hTER pseudoknot (Fig. 1e), thus breaking the W-C base pairing in this stem. Furthermore, distortions might occur in the arrangements of nearby paired residues to accommodate the larger replacing purine nucleotide. In yeast, base pairing of the central region of stem 2 of the conserved pseudoknot is important for in vivo binding of Est2p (TERT) and telomere maintenance [9]. Of note, both nucleotides flanking 179 T are sites of know pathogenic mutations, 178 G>A and 180 C>T [10]. We propose a pathogenic role for the 179 T>A replacement based on the structural changes envisaged in the central portion of stem 2, which is a region important for the catalytic activity of the

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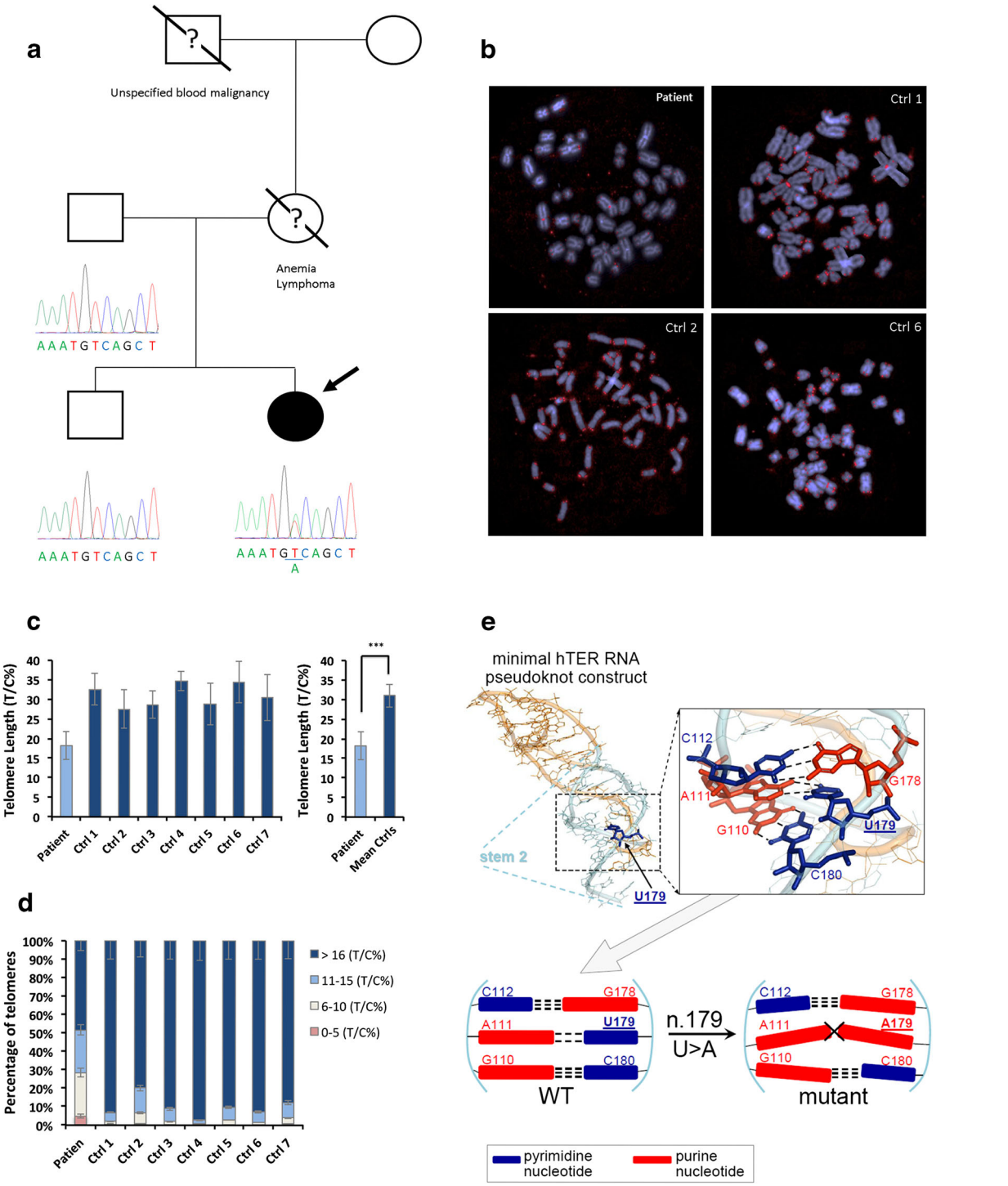

Fig. 1 **a** Family pedigree and Sanger electropherograms. We suppose that patient's mother and grandfather harbor the identified *TERC* variant based on their clinical history. **b** Representative images of metaphase spreads (from patient and controls 1, 2, and 6) stained using centromere-calibrated Q-FISH. For each metaphase, telomere length was calculated as the ratio between the fluorescence of each telomere signal and that of the centromere of the chromosome 2, used as the internal reference. **c** Mean telomere length value assessed in patient lymphocytes was compared with that of seven age-matched controls (left) and with the mean of the control individuals (right) (Student *t* test, ***p value < 0.005). **d** Telomere lengths were arbitrarily categorized in four different classes that are (i) absent or very short telomeres (T/C% lower than 5); (ii) short telomeres (T/C% comprised between 6 and 10); (iii) moderately short telomeres (T/C% comprised between 11 and 15); (iv) normal and long telomeres (T/C% higher than 16). Histograms clearly indicate that patient has a very higher percentage of all the short telomere classes compared to age-matched control individuals. **e** Mapping of the site of the n.179 T>A mutation on the minimal hTER RNA pseudoknot construct (Protein Data Bank structure 2K95, first multimodel conformer). The stem 2 region, which includes the mutation site, is indicated by cyan colored backbone. The inset shows a detailed view highlighting the Watson-Crick (W-C) base pairing involving 179 T and flanking residues (pyrimidine and purine nucleotides are respectively in blue and red color, W-C hydrogen bonding are indicated by black dashed lines). Also shown is a schematic view of W-C base pairing involving 179 T and the immediately flanking residues as well as the perturbation introduced by the 179 T>A mutation

TERT/TERC complex. In conclusion, this novel variant can be included among the pseudoknot mutations reported up to date, expanding the mutational spectrum of TERC related telomeropathies.

Compliance with ethical standards

Clinical investigations and genetic analyses were approved by the institutional scientific board of Bambino Gesù Children's Hospital. Informed consent was obtained from the patient.

Conflict of interest The authors declare that they have no conflict of interest.

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