Reviewer's report

Title: A novel mutation in H/ACA box of telomerase RNA component gene (TERC) in a young patient with myelodysplastic syndrome

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Reviewer: Judy Wong

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The telomere syndromes include a spectrum of tissue-renewal disorders that originate from deficiencies in the synthesis (from genetic mutations associated with telomerase components), or structural protection (from genetic mutations in the telomere binding proteins), of telomeres. Clinical presentation of these disorders can vary, but often includes hematopoietic dysfunctions. Manifestations range from bone marrow failure to several forms of blood cancer.

The submitted case study of a myelodysplastic syndrome patient revealed a novel mutation in the telomerase RNA (TERC) component. Notably, this mutation was found in the functionally significant Hinge box (H-box) of the TERC sca/sno RNA domain. Previous biochemical studies reported that H-box mutations were associated with dysfunctional assembly of ribonucleoprotein, and a loss of steady-state TERC accumulation. Thus, it is not surprising that PBMC from this patient exhibited very short telomere lengths, as assayed by QPCR, or with the STELA assay that measures chromosome-specific telomere repeat lengths. A small-scale pedigree analysis suggested paternal inheritance of this mutation.

The genetic and telomere measurements were well executed. The last experiment raised a few questions and did not provide information beyond what is already reported in the literature. I suggest that it be removed. Specific recommendations are discussed below:

MAJOR COMPULSORY REVISION

1) Given the importance of the H-box in binding with H/ACA proteins, and the steady-state accumulation of TERC, it is surprising that the patient’s father, who inherited the same TERC-377G mutation, did not exhibit any notable clinical symptoms. The authors should comment on this, and its implications for the patient’s siblings.

2) Figure 1 depicts the secondary structure of TERC, and the H-box location. The authors’ rendition (using sticks and balls) is not entirely accurate. This can easily be corrected using available RNA-drawing software. While TERC structure can be obtained from the literature, it would be helpful if an up-to-date accurate version be included in the manuscript.

3) There are a number of inconsistencies in the description of the methods, and missing controls for the transient transfection assay in VA13 cells:
a) In the methods section, the authors describe generating T7-promoter-driven WT and mutant TERC, subcloned into the pUC57 plasmids. T7 is a bacteriophage-derived promoter, and pUC57 lacks any mammalian promoter sequence. The method, as it is written, is not predicted to mediate recombinant TERC expression in VA13 cells. To clarify, the authors should state whether the TERC gene (with its endogenous promoter) was cloned, or if VA13 cells were cotransfected with a T7 polymerase expression vector.

b) Cells extracts were assayed for telomerase activity two days after the transient transfection of two recombinant telomerase subunits (TERT; WT versus mutant TERC) in VA13 cells. Relative telomerase activities, measured in WT-TERC-transfected cells versus mutant TERC-transfected cells, were used as a functional indicator of the mutation’s effect on telomerase activity. Transient transfection efficiency could be highly variable between different treatments. How would the authors control for this discrepancy?

c) How sure are the authors that the mutant TERC affects normal telomerase activity by 50%? The over-expression of the mutant TERC through transient transfection in VA13 cells may alleviate some of the pre-RNP assembly defects, leading to measurement of higher telomerase activity, compared to constitutively low TERC expression levels. The authors should include this caveat in their comments.

MINOR ESSENTIAL REVISIONS:
1) page 3, “recruit TERC into a unique structure called cajal body in the nucleolus”. This statement is not accurate. Cajal bodies are unique nuclear bodies that are structurally distinct from the nucleolus, which is an organelle.

2) page 7, TERC accumulation and RNA stability refer to the same observation in this context; the use of both terms in the same sentence is redundant.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: 
I declare that I have no competing interests