A novel TERT variant associated with a telomere biology disorder and challenges in variant classification

ABSTRACT

Telomere biology disorders (TBDs) are inherited conditions characterised by dysfunctional telomere maintenance and potentially severe multi-organ clinical manifestations. We report a novel TERT variant (K710R) identified in a patient with classic TBD features, including pancytopenia and very short telomeres. His father had a milder phenotype consistent with anticipation. Functional assessment of the variant showed significantly reduced telomerase specific activity and processivity. Although clinical and functional evidence links the variant to TBD, it was classified as a variant of uncertain significance according to current variant curation guidelines highlighting the need for further refinement of variant classification for TBDs.

KEYWORDS

Telomere biology disorders, genetic anticipation, variant curation, Telomerase activity, TERT variant

Telomere biology disorders (TBDs) are multisystem inherited disorders related to dysfunction in telomere maintenance mechanisms, which are crucial for genomic stability and cellular proliferation. Disruption of telomere homoeostasis promotes premature aging and may manifest as bone marrow failure, idiopathic pulmonary fibrosis, liver cirrhosis, premature greying, and increased susceptibility to cancer [1]. Telomerase reverse transcriptase (TERT) is one of the core components of the telomerase ribonucleoprotein enzyme complex, which utilises a template within the

telomerase RNA subunit (TERC) to add telomere repeats to chromosome ends [2]. Germline loss of function variants in *TERT* are one of the most common causes of TBDs [3]. Herein we describe the clinical and functional characterisation of a novel *TERT* variant in an adult with a TBD which also highlights potential issues for variant curation of causative genes in TBDs.

A previously healthy 27-year-old male presented with bruising following minor trauma. The patient had no past medical history but had noted early greying (from age 18) and fingernail dystrophy. There was no significant medical history in the family; however, his father was noted to also have had early greying and mild nail ridging (Figure 1A, B).

Full blood examination of the patient showed pancytopenia with moderate thrombocytopenia (platelet count 48 x 10^9/L) and a mild macrocytic anaemia (Hb 126 g/L, MCV 108fL) (Figure 1C). The patient had a bone marrow biopsy which showed a markedly hypocellular aspirate and trephine but no morphological dysplasia or blast excess. Conventional karyotype performed on the bone marrow aspirate sample was normal. No variants were detected in a next generation sequencing (NGS) targeted panel covering 80 genes (not including TERT) recurrently mutated in haematological malignancy. Telomere length was tested by both flow-FISH and High Throughput Single Telomere Length Analysis (HT-STELA) [4], which both revealed telomere lengths less than 1st centile for age (Figure 1D).

Given the clinical diagnosis of a TBD, clinically accredited germline whole genome sequencing (WGS) was performed on DNA extracted from hair follicles which revealed

a heterozygous *TERT* variant (c.2129A>G; p.(K710R)), subsequently confirmed by the PanHaem NGS panel covering the TERT gene [5]. No other potentially causative variants were detected including in *TERC*, *DKC1*, *TINF2*, *RTEL1*, *NOP10*, *NHP2* and *RPA1*.

The lysine at position 710 is located within the reverse transcriptase domain of TERT and is a conserved amino acid occurring very close to an essential catalytic aspartate residue (D712) [6]. A catalytic triad of aspartates (D712, D868, and D869 in human TERT) coordinates positively charged magnesium ions critical for telomerase-catalysed nucleotide addition by the telomerase complex [2]. K710 forms part of a beta sheet that positions this catalytic triad (Figure 2A) and substitution of an arginine at K710 may disrupt the conformation of the enzyme active site.

The patient's bone marrow aspirate sample was also tested for the presence of a *TERT* promoter (*TERTp*) variant by Sanger sequencing which revealed a hotspot c.−124C>T *TERTp* variant. *TERTp* variants may be observed in patients with TBDs as a compensatory mechanism in conjunction with germline loss of function variants in genes including *TERT*, *TERC*, *RTEL1*, *CTC1*, and *PARN* [7, 8]. *TERTp* variants appear to functionally compensate for the adverse effects of disease-associated germline TBD variants by recruiting the GA-binding protein alpha transcription factor (GABPA) to the mutated *TERT* promoter and boosting telomerase activity [9].

Parental segregation testing confirmed paternal inheritance of the *TERT* K710R variant. Interestingly, the father had a normal full blood count with only mild physical manifestations of a TBD, including early greying and mild nail rigidity (Figure 1B). Telomere length assessment of the father by HT-STELA showed telomere lengths

between the 1st and 10th centile for age (Figure 1D). No *TERTp* variant was detected in the father's peripheral blood.

We then went on to functionally assess the effect of the K710R variant. Telomerase activity was assessed using an *in vitro* telomerase extension assay performed using K710R telomerase that was reconstituted in HEK293T cells and immunopurified as previously described [10]. The K710R variant significantly reduced telomerase specific activity (Figure 2B, C) and processivity (Figure 2B, D) relative to the wild-type enzyme ($p < 0.0001$). When co-expressed with wild-type telomerase to mimic the heterozygous state, K710R reduced telomerase activity to approximately 40% of wild-type levels ($p < 0.0001$) (Figure 2B, C). Other heterozygously-inherited likely pathogenic TERT variants have telomerase activities of approximately 50–70% of wild-type levels [11, 12].

Despite the compelling clinical and functional data in this patient, this variant was classified as a variant of uncertain significance (VUS) [PM2; Absent from population databases (Moderate), PP3; Computational evidence predicting deleterious effect (Supporting)] according to current variant curation guidelines (ACMG) [13]. The categorisation of this variant as a VUS is in part due to the stringent criteria used to define functional studies able to be used to inform curation of variants. Recently, a systematic review of functional assays in TBDs [14] has proposed that evidence may be applied for the 'direct' (i.e. non-PCR based) telomerase activity assay (PS3). However, the current recommendation is to only apply this evidence at a "supporting" level of strength, which would not change the categorisation of this variant from a VUS.

Another potentially important avenue of evidence in these types of variants is co-segregation of the variant with disease phenotype. However, in the current kindred

this is potentially confounded due to genetic anticipation. The absence of significant end-organ dysfunction in the proband's father, along with his short telomeres (in contrast to the very short telomeres in the proband), is consistent with the phenomenon of genetic anticipation, due to a defect in the ability of telomerase to fully reset telomere lengths in successive generations, which is commonly observed in TBDs [15].

One potential modification to the curation of germline *TERT* variants would be to integrate the presence of *TERTp* variants as supportive evidence for the phenotype given the relative specificity of the presence of *TERTp* variants to TBDs [8]. A similar principle has been used to incorporate somatic *DDX41* variants as a criterion for pathogenicity in germline *DDX41* variant curation [16]. However, in order to integrate *TERTp* using the same statistical method, the specificity of *TERTp* for germline *TERT*/*TERC* variants would need to be more comprehensively understood in a larger cohort.

In summary, we have described the clinical and functional characteristics of a novel *TERT* variant (K710R) associated with a TBD. This description highlights several challenges for the field, including integration of functional studies into variant curation, the presence of genetic anticipation affecting phenotype-genotype segregation, and the potential use of acquired mutations to inform variant curation. To enhance the accuracy of variant classification in TBDs, future efforts should focus on refining curation guidelines to better incorporate functional and familial data, ultimately leading to more precise and actionable genetic insights.

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Figure legends

Figure 1. Family pedigree, clinicopathological findings, and telomere length analysis associated with the TERT c.2129A>G variant. **(A)** Pedigree of the family indicating the proband and his father both carrying the TERT c.2129A>G allele. Amino acid changes resulting from the TERT variant are highlighted in blue. The arrow indicates the proband. **(B-C)** Clinicopathological findings, including mild nail ridging in the father (B) and pancytopenia in the proband (C). **(D)** Relative telomere length plotted against age measured by High Throughput Single Telomere Length Analysis (HT-STELA) in blood samples from the proband (red dot) and the proband's father (blue dot). Lines represent the 1st, 10th, 50th, 90th, and 99th percentiles of 17p telomere length collected from a pool of 227 healthy controls. Germline and somatic TERT variants identified in the proband and his father are denoted in red and blue font, respectively.

Figure 2. The effects of TERT K710R variant on telomerase activity and processivity. **(A)** Location of the K710 residue relative to the telomerase active site within a TERT structural model. Red: K710; blue: catalytic triad (D712, D868, D869); green: Mg^{2+} ion in active site; magenta: DNA; orange: RNA. **(B)** Direct telomerase activity assay demonstrating the extension of a telomeric DNA primer *in vitro* in the presence of radiolabeled ^{32}P-dGTP, for WT and K710R telomerase variants, expressed individually or together. LC: ^{32}P-labeled 30-mer oligonucleotide included as a control for recovery and loading. Number of nucleotides added to primer indicated on left. **(C)** Telomerase specific activity relative to wild-type. **(D)** Telomerase repeat addition processivity values relative to wild-type. Both graphs depict mean ± SEM of three independent experiments; significance determined by one-way ANOVA followed by Dunnett's multiple comparison testing.