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Genetics of human telomere biology disorders

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Abstract

Telomeres are specialized nucleoprotein structures at the end of linear chromosomes that prevent the activation of DNA damage response and repair pathways. Numerous factors localize at telomeres to regulate their length, structure and function, to avert replicative senescence or genome instability and cell death. In humans, Mendelian defects in several of these factors can result in abnormally short or dysfunctional telomeres, causing a group of rare heterogeneous premature ageing diseases, termed telomeropathies, short-telomere syndromes or telomere biology disorders (TBDs). Here, we review the TBD-causing genes identified so far and describe their main functions associated with telomere biology. We present molecular aspects of TBDs, including genetic anticipation, phenocopy, incomplete penetrance and somatic genetic rescue, which underlie the complexity of these diseases. We also discuss the implications of phenotypic and genetic features of TBDs on fundamental aspects related to human telomere biology, ageing and cancer, as well as on diagnostic, therapeutic and clinical approaches.

[H1] Introduction

Telomeres are nucleoprotein complexes at the end of linear chromosomes. Their essential function is to prevent the chromosome end from being sensed as a free end generated by a DNA double-strand break, which would activate the DNA damage repair machinery inappropriately, resulting in telomere–telomere fusion and

genomic instability. The DNA component comprises tandem double-stranded hexameric repeats, *TTAGGG* in humans, spanning 5–15 kb in length depending on the cell type and replicative history, and terminating with a 3' single-stranded sequence dubbed the 3' G-overhang (**FIG. 1a**). In addition, telomeric DNA is bound by a cadre of proteins that are required for normal telomere length maintenance, structure and function.

Telomeres have evolved robust mechanisms to solve the 'end protection problem'¹, such as adopting a lariat-like structure, called the T-loop, in which the 3' G-overhang invades an upstream telomeric duplex sequence, thereby sequestering chromosome ends from DNA repair factors (**FIG. 1a**). The structure of telomeres poses specific problems for semiconservative DNA replication^{1,2}. Because ends of chromosomes cannot be fully replicated, known as the 'end replication problem', telomeres shorten progressively over repeated cell divisions (**FIG. 1b**). When telomeres become too short, a checkpoint response is activated, leading to senescence or apoptosis. When the checkpoints are dysfunctional, telomeres further shorten and become unprotected, leading to a state called 'crisis', which is characterized by genome instability and the potential for cell death or cellular transformation. In addition to the end replication problem, R-loops [G] formed with telomeric repeat-containing RNA (TERRA), G-quadruplexes [G] and 8-oxoguanine (8-oxo-G) base modifications further hinder DNA replication and promote telomeric instability (**FIG. 1a**). Thus, although telomeres represent only ~0.04% of the human genome, they require specific factors and processes to ensure their proper replication and control their length, structure and stability.

In humans, the progressive telomere shortening that occurs naturally contributes to normal ageing and age-related ailments³. By contrast, germline defects that lead to either accelerated telomere shortening or telomere instability without global telomere shortening cause a set of rare and heterogeneous Mendelian diseases termed telomeropathies, short-telomere syndromes or telomere biology disorders (TBDs). We prefer the term TBD, in part because global telomere shortening is not inevitably observed⁴. TBDs, which include dyskeratosis congenita, pulmonary fibrosis, Høyeraal–Hreidarsson syndrome, Coats plus syndrome and Revesz syndrome (**Box 1**), manifest as a broad array of clinical features involving mainly tissues and organs with high proliferation rates and renewal potential, such as the epithelium and haematopoietic system. In some tissues not associated with high

basal proliferation rates, such as the lung and liver, telomere defects sensitize cells to environmental stressors, for example, cigarette smoke, alcohol or a high-fat diet^{5,6}.

Numerous mouse models have provided a wealth of information on the factors and processes involved in telomere maintenance, structure and function⁷. However, species-specific features can limit the extrapolation of observations obtained in mouse models to humans. For example, mice have short life spans (~2–3 years), residual *Tert* expression in many adult tissues (discussed below)⁸, and, in the common *Mus musculus* laboratory strain, long telomeres (~20–65 kb)⁹. Thus, the identification and analysis of genetic causes of human TBDs remain fundamental to advancing our understanding of the biology of telomeres in human health and disease.

In this Review, we describe the 17 known TBD-causing genes in humans, the roles of the encoded factors at telomeres, mutational spectrum and phenotypic associations. Additionally, we discuss recently proposed new TBD-causing genes. We also highlight the forms of somatic genetic rescue [G] (SGR) that have been detected in patient blood clones [G] and their association with specific germline defects. Together, these observations in individuals with TBDs shed light on fundamental aspects of telomere biology, its relationship to ageing, and open avenues for therapeutic approaches.

[H1] TBD-causing genes

In 1998, deleterious variants in *DKC1* were first reported as the genetic cause of the TBD dyskeratosis congenita¹⁰. Since then, 16 additional TBD-causing genes have been identified (**Table 1**). As predicted, they all affect telomere biology but do so by impinging on various aspects of telomere maintenance, structure and function (**FIG. 2**). Telomere length reduction is observed in most TBDs and underlies characteristics of TBDs such as genetic anticipation [G] and phenocopy [G]. Numerous methods have been developed to measure telomere length in human cells, each with strengths and disadvantages¹¹. Flow cytometry and fluorescence in situ hybridization analysis of blood leukocytes (commonly referred to as telomere flow-FISH) has emerged as a powerful tool to measure median telomere length and identify individuals with TBDs¹². Applying it to 100 individuals with TBDs revealed that paediatric-onset presentations are typically associated with telomeres well below

the first percentile for age, whereas adult-onset disease is more often associated with telomeres just below, at, or even within the first to 10th percentile range for age¹³. However, telomere length measurement, although informative to diagnose most TBDs, should not be used as the sole diagnostic tool since, as discussed below, TBDs are not inevitably associated with global short telomeres.

[H2] Telomerase core components

[H3] TERT

TERT encodes the specialized reverse transcriptase TERT, which uses the telomeric 3' G-overhang as a primer and sequences within human telomerase RNA (hTR) as a template to add telomeric repeats onto the chromosome end. TERT expression is tightly regulated, with expression generally restricted to prenatal development, testes, stem cells and some activated cells^{14,15}. Consequently, the progressive shortening of telomeres limits the replicative capacity of somatic cells (**FIG. 1b**). Importantly, ectopic expression of TERT in human somatic cells, such as primary fibroblasts, is sufficient to maintain telomere length and endow indefinite cell division potential (that is, immortalization)¹⁶. Remarkably, replicative immortality in most cancers is due to the induction of *TERT* expression through mutation of the *TERT* promoter^{17,18}. Telomerase is capable of synthesizing multiple telomeric repeats with a single binding event, termed processivity¹⁹. Numerous monoallelic pathogenic variants are found all along the TERT protein, with many clustering in conserved regions, including the telomerase amino-terminal domain, which is critical for telomerase recruitment to telomeres and processivity; the telomerase RNA binding domain, which binds hTR; the reverse transcriptase domain, which is responsible for catalysis; and the carboxy-terminal element, or thumb domain, which mediates high-affinity DNA binding. These mutations reduce telomerase activity or processivity and, thereby, impair telomere length maintenance in telomerase-positive cells.

TERT was the first TBD gene to be associated with both **monoallelic [G]** and **biallelic [G]** disease, and is associated with a range of clinical phenotypes. The majority of monoallelic *TERT* pathogenic variants are associated with adult-onset presentations, most notably of pulmonary fibrosis, but they have also been reported in association with myelodysplastic syndrome, bone marrow failure, liver disease and dyskeratosis congenita^{20,21}. Biallelic *TERT* variants have been reported in rare

patients presenting in childhood with dyskeratosis congenita and Høyeraal–Hreidarsson syndrome²²⁻²⁸.

[H3] *TERC*

TERC consists of a single exon that encodes hTR. The production and steady-state level of the mature 451 nucleotide hTR molecule depend on multiple steps of RNA processing, including polyadenylation and trimming^{14,29-31}. The template/pseudoknot and conserved regions 4/5 (CR4/5) of mature hTR are bound by TERT to form the catalytic core of telomerase, whereas the H/ACA domain at the hTR 3' end is bound by the ribonucleoproteins (RNPs) dyskerin, GAR1, NHP2, NOP10 and TCAB1 to compose the telomerase holoenzyme³² (**FIG. 2**).

TERC was the first gene associated with autosomal dominant dyskeratosis congenita³³. Since then, numerous monoallelic pathogenic variants have been reported, mostly single nucleotide variants and insertion and deletions (indels) that localize to the critical template/pseudoknot and CR4/5 domains and reduce telomerase activity. Pathogenic variants in *TERC* have been found in individuals and families with pulmonary fibrosis, liver disease, myelodysplastic syndrome, aplastic anaemia and dyskeratosis congenita presenting in adolescence and early adulthood (Table 1). Two adult siblings with pulmonary fibrosis and mucocutaneous features carrying a homozygous r.202U>G variant³⁴ have been the only cases of biallelic disease reported to date. This variant was classified as a variant of uncertain significance owing to the limited information available from family members. The late onset of disease in both the proband and sibling bearing the variant in a homozygous state strongly suggests it is **hypomorphic [G]**.

[H2] **Additional components of the telomerase holoenzyme**

[H3] *DKC1*

The X-linked gene *DKC1* encodes dyskerin which, together with NHP2, NOP10 and the box H/ACA RNP assembly factor NAF1, forms a complex that interacts with box H/ACA RNAs including hTR and participates in their maturation and stability^{31,35,36} (**FIG. 2**). Pathogenic variants in *DKC1* are responsible for the only known X-linked TBDs to date^{10,37}. Most pathogenic variants cluster in two domains that affect the capacity of dyskerin to interact with itself and with hTR^{32,38}. Consequently, the

impaired interaction of *DKC1* mutants with hTR leads to defective hTR maturation and stability, resulting in decreased telomerase activity and accelerated telomere shortening. Most male patients with pathogenic variants in *DKC1* present with dyskeratosis congenita and only very rarely with Høyeraal–Hreidarsson syndrome, pulmonary fibrosis or bone marrow failure. TBD manifestations have also been reported in a small number of female individuals carrying monoallelic *DKC1* pathogenic variants^{39,40}; however, the underlying molecular mechanisms responsible for disease development remain undetermined.

Dyskerin also possesses pseudouridine synthase activity, leading to the isomerization of uridines to pseudouridines in many RNAs, including ribosomal RNAs (rRNA) and spliceosomal RNAs, but not hTR³⁰. Whether disruption of this function could contribute in some cases to the TBD clinical phenotype remains unclear. As discussed below, *DKC1* is one of several TBD genes in which potential effects on rRNA and other non-coding RNAs have been investigated but have yet to be demonstrated as major contributors to the clinical phenotype.

[H3] *NOP10*

NOP10, as part of the H/ACA RNP protein complex, contributes to hTR stability³⁸ (**FIG. 2**). Biallelic (homozygous) variants in *NOP10* have been reported in three related individuals, who had a dyskeratosis congenita phenotype and markedly short telomeres⁴¹. The variant abolishes the ability of *NOP10* to interact with hTR and causes a strong reduction in the hTR level^{41,42}. The patients' first-degree relatives, who were monoallelic carriers of the *NOP10* variant, also showed a reduction in hTR and short telomeres, albeit asymptomatic⁴¹. A monoallelic variant in *NOP10* was recently reported in a family with short telomeres and adult-onset pulmonary fibrosis^{43,44}. While the functional impact of this new *NOP10* missense variant remains to be determined, this finding highlights the potential for autosomal dominant *NOP10*-related disease.

[H3] *NHP2*

Like *NOP10*, the H/ACA RNP component *NHP2* is part of the telomerase holoenzyme^{35,38}. Biallelic *NHP2* pathogenic variants (homozygous and **compound heterozygous [G]**) were identified in three unrelated individuals with dyskeratosis congenita and Høyeraal–Hreidarsson syndrome, reduced hTR level and markedly

short telomeres^{45,46}. One of the patients also exhibited cognitive impairment, intracranial calcification and cirrhosis⁴⁵. Recently, the identification of monoallelic *NHP2* variants co-segregating with pulmonary fibrosis and short telomeres in five patients from three unrelated families demonstrated that heterozygous *NHP2* deficiency can cause TBD in adulthood⁴⁶.

In addition to telomerase biogenesis, the dyskerin/NOP10/NHP2/GAR1 complex, via the pseudouridylation activity of dyskerin, is important for rRNA function, leading to the question of whether some of the clinical features observed in dyskeratosis congenita/Høyeraal–Hreidarsson syndrome are a consequence of impaired ribosome function. Indeed, the above *NHP2* missense variants and dyskeratosis congenita-associated *NOP10* variant have been shown to either impair pre-RNP assembly or rRNA biogenesis^{42,46}. Nonetheless, many TBD genes, such as *TERT*, have no known role in pseudouridylation or ribosome function. Moreover, TBD-associated *DKC1* mutations largely spare the TruB pseudouridine synthase domain. The conundrum is likely resolved by the relative impact of pathogenic variants in *DKC1*, *NOP10* and *NHP2* on telomerase biogenesis and hTR stability and pseudouridylation, with pseudouridylation defects, if present, also contributing to aspects of clinical phenotypes. For example, a recent study reported two families — one with six affected male individuals with a *DKC1* missense variant and the other with two affected female individuals with a homozygous *NOP10* missense variant — with a shared phenotype of infantile-onset nephrotic syndrome, cataracts, hearing impairment and enterocolitis⁴⁷. The missense variants reduced the interaction between dyskerin and NOP10 and impaired pseudouridylation of rRNA. Although the patients exhibited short telomeres, the early death (range 2.5–7.5 years of age; median 3 years) may have precluded dyskeratosis congenita symptom detection⁴⁷.

[H3] *WRAP53*

The H/ACA domain of hTR contains two hairpins, each bound by the H/ACA complex proteins, which as noted above, play crucial roles in telomerase biogenesis and hTR stability. TCAB1, encoded by *WRAP53*, binds the apical loop of the 3' hairpin³² whereby it influences trafficking of telomerase to **Cajal bodies [G]**, recruitment of telomerase to telomeres, and folding of the hTR CR4/5 domain, which stimulates telomerase activity^{48,49}. To date, five unrelated individuals with biallelic *WRAP53* (homozygous and compound heterozygous) variants have been reported,

with telomeres below the first percentile and clinical features of dyskeratosis congenita in four and Høyeraal–Hreidarsson syndrome in one⁵⁰⁻⁵³. Their first-degree carrier-relatives were unaffected and had telomere lengths within the normal range, consistent with autosomal recessive inheritance of *WRAP53*-associated TBD. Eight unique missense variants were identified among the five individuals, all mapping to the central WD40 domain. Analysis of several of these mutant proteins revealed impaired folding by the chaperonin TRiC, reduced protein level and failure to localize to Cajal bodies^{50-52,54}. Some mutant TCAB1 proteins were shown to remain in the cytoplasm^{50,52}. TCAB1 has also been implicated in DNA repair⁵⁵. Whether disruption of this function contributes to the TBD clinical phenotype remains unknown.

[H2] hTR biogenesis and stability factors

[H3] *NAF1*

The more recent identification of *NAF1* as a TBD-associated gene⁵⁶ was unsurprising given *NAF1*'s role in an early stage of the biogenesis of H/ACA RNPs, including telomerase, and stabilization of their RNA components, including hTR⁵⁷. Two heterozygous *NAF1* frameshift mutations were identified: one in a family with pulmonary fibrosis, emphysema, extrapulmonary TBD features, short telomeres and reduced hTR levels in lymphoblastoid cells; the other in an individual with sporadic pulmonary fibrosis, bone marrow failure and short telomeres⁵⁶. *Naf1*^{+/-} mice similarly exhibited decreased hTR levels. Importantly, the mouse model allowed for an assessment of the impact of *NAF1* haploinsufficiency on rRNA pseudouridylation and showed that, despite reductions in H/ACA small nucleolar RNAs, pseudouridylation of rRNA was unaffected, arguing against ribosome defects underlying the clinical features.

[H3] *PARN*

As noted above, hTR undergoes multiple modifications. Most nascent hTR transcripts extend beyond the functional terminus with genomically templated tails up to 51 nucleotides in length, which can be processed to mature hTR either directly or indirectly. The indirect pathway involves the sequential action of non-canonical poly(A) RNA polymerase PAPD5, which oligoadenylates the precursor hTR, and polyA-specific ribonuclease PARN, which deadenylates it^{29,31,58}. Reduction of

PAPD5 eliminates hTR adenylation, stabilizes hTR and increases telomerase activity^{29,59}, whereas reduction of PARN leads to an increase in oligoadenylated hTR and a decrease in total 451 nucleotide-long mature hTR and telomerase activity⁶⁰. The opposing activity of PAPD5 and PARN set the rate of hTR precursor maturation²⁹.

PARN-associated TBDs are characterized by both autosomal dominant and autosomal recessive inheritance, and, as will be discussed, **incomplete penetrance [G]**, **variable expressivity [G]** and SGR. Dyskeratosis congenita-features and, in several reports, prominent neurologic phenotypes (Høyeraal–Hreidarsson syndrome) are associated with biallelic variants (deletions, missense and frameshift)⁶¹⁻⁶⁵. Monoallelic variants (stopgain, frameshift, splice site and missense) are generally associated with pulmonary fibrosis⁶⁶, although large monoallelic deletions without a detected mutation in the other allele have been reported in association with developmental delay and psychiatric illness⁶¹. Cells from patients with biallelic pathogenic variants exhibit an increase in oligoadenylated hTR, a decrease in total hTR and telomerase activity, and shortened telomeres^{60,65}.

Interestingly, two novel loss-of-function (LoF) *PARN* variants (splice site and stopgain) were identified in a cohort of 92 probands with chronic kidney disease of unknown aetiology, a frequency 100-fold greater than in control individuals⁶⁷. The splice variant (c.554+1G>A) was present in an affected parent–child pair (ages 44 and 23 years, respectively) and the stop-gain variant (Gln215*) in a 48-year-old. Leukocyte relative telomere content from the three individuals, as measured by quantitative PCR, was within normal range for age. Normal telomere lengths, as measured by telomere flow FISH, were also observed in an unaffected adult with an inherited LoF frameshift mutation⁶². These results underscore both the variable expressivity and variable penetrance of deleterious *PARN* variants that are not always associated with abnormally short telomeres.

Like members of the H/ACA protein tetramer, *PARN* has been implicated in the processing of non-coding RNAs in addition to hTR, with increased levels of oligoadenylated forms of a subset of small nucleolar RNAs (snoRNAs) and small Cajal body RNAs (scaRNAs) and 18S-E pre-ribosomal RNA in *PARN*-deficient cell models and patient cells^{61,62,65}. In addition, reductions in other telomere biology genes, such as *DKC1* and *TERF1* (encoding the shelterin factor TRF1), have been observed in *PARN*-deficient cells^{63,65}. The impact of *PARN* on these RNAs and

downstream processes independent of telomere length may underlie the variation of clinical features that have been observed.

[H3] *ZCCHC8*

ZCCHC8 is a large scaffold protein that, together with the RNA helicase MTR4 (also known as SKIV2L2 and encoded by *MTREX*) and the RNA binding protein RBM7, forms the nuclear exosome targeting (NEXT) complex. NEXT promotes the degradation of short and non-sequence-specific nuclear RNAs via the exosome machinery⁶⁸. *ZCCHC8* also participates in the transcriptional repression of transposable elements in embryonic stem cells via its interaction with the chromatin modifier HUSH complex⁶⁹. A monoallelic missense variant in *ZCCHC8* was found to co-segregate with low level of mature hTR in several individuals from a unique family with short telomeres, pulmonary fibrosis or bone marrow failure⁷⁰. The missense variant localizes to the cysteine-centered arch-interacting motif (C-AIM) domain, which is required for interaction with both the NEXT and HUSH complexes^{69,71-73}. This variant was shown to cause a ~50% reduction of *ZCCHC8* level and thus, *ZCCHC8* was proposed to be haploinsufficient⁷⁰. *ZCCHC8* knockout in human and murine cells resulted in a ~25% reduction of total hTR RNAs (precursors and mature) and caused the accumulation of hTR precursors at the expense of mature hTR production, supporting the notion that *ZCCHC8* participates in hTR maturation⁷⁰. It is, however, unclear whether the reduced level of mature hTR observed in *ZCCHC8*-deficient cells results from a defective NEXT complex function as, unlike *ZCCHC8* depletion, the depletion of two other NEXT factors, MTR4/SKI2VL2 and RBM7, did not reduce global hTR levels and did not affect the maturation process^{31,70}. Furthermore, both the identification of a biallelic (homozygous) *ZCCHC8* nonsense LoF variant in a patient with a non-syndromic cognitive disorder and the description of *Zcchc8* knockout mice, which exhibit postnatal fatal neurodevelopmental defects, demonstrated an important role of *ZCCHC8* in the development of the central nervous system^{70,74}. In sum, although the identification of a pathogenic *ZCCHC8* monoallelic missense variant in a TBD pedigree underscores a central role of this factor in telomere biology, further studies are needed to precisely determine what governs the phenotypic and functional differences observed with biallelic versus monoallelic, and LoF versus missense *ZCCHC8* variants identified in patients.

[H2] Shelterin factors

[H3] *TINF2*

The TIN2 protein, encoded by *TINF2*, is the central component of the shelterin complex, linking the telomeric DNA binding factors TRF1 and TRF2 to the TPP1/POT1 heterodimer (**FIG. 2**). Heterozygous mutation of *TINF2* can result in either abnormally long telomeres (>99th percentile) and cancer predisposition across generations⁷⁵ or markedly short telomeres (<<1st percentile) and TBD phenotypes in the context of *de novo* events in the vast majority of cases⁷⁶⁻⁷⁸. These differences are reflected by distinct mutation landscapes: those that result in telomere elongation disrupt regions required for the recruitment of TIN2 to the telomere⁷⁵, whereas those associated with TBDs cluster in a narrow within exon 6, downstream from the regions encoding the TRF2, TPP1 and TRF1 binding domain^{76,77}. Mutation of *TINF2*, including missense, stopgain and frameshift mutations, is a frequent cause of dyskeratosis congenita and Høyeraal–Hreidarsson syndrome, seems to be the sole cause of Revesz syndrome, and is an infrequent cause of adult-onset pulmonary fibrosis^{76,77,79,80}. Exon 6 is the last exon of the major *TINF2* transcript, allowing for the expression of truncated proteins in cell lines bearing stopgain and frameshift mutations within it⁸¹. Recently, using a human embryonic stem cell (hESC) model, it was found that hemizygous disruption of either the TBD-mutant allele or the wild-type *TINF2* allele rescued the short telomere induced by the TBD mutation⁸². These results are consistent with *TINF2* TBD mutations imparting a gain of function (GoF). The mechanism underlying this GoF remains undetermined.

[H3] *ACD*

ACD encodes the shelterin protein TPP1 (also previously named TINT1, PTP1 or PIP1), which is critical for telomerase recruitment to telomeres and **repeat addition processivity [G]** (RAP)⁸³. These roles are achieved through contact of the TPP1 glutamate and leucine-rich (TEL) patch and amino-terminal region of TPP1 oligonucleotide/oligosaccharide-binding-fold (NOB) with TERT⁸⁴. Two groups reported the first *ACD* variant associated with TBDs, an in-frame deletion of K170, which results in a conformational change within the TEL patch that impairs TPP1–TERT interaction⁸⁵⁻⁸⁷. When heterozygous, this variant was sufficient to result in

telomere lengths below the first percentile and be associated with TBD phenotypes, such as bone marrow failure, pulmonary fibrosis and premature hair greying, consistent with autosomal dominant transmission^{85,86,88}. Patients bearing biallelic *ACD* variants have also been reported in four families and, in two of them, were associated with Høyeraal–Hreidarsson syndrome^{85,89,90}. In one family, an in-frame deletion of E169, located in the TEL patch, was identified, further highlighting the importance of this domain⁸⁹. The other variant was a frameshift variant. While the transcript from this allele was depleted, presumably via nonsense-mediated decay, and *ACD* mRNA levels reduced in lymphoblasts derived from the patient and the frameshift carrying mother, the TPP1 protein levels were not significantly changed. Consistent with this observation, telomere lengths of the mother were well within the normal range. Similar maintenance of TPP1 levels was observed in cells from a child with 16q22.1 microdeletion syndrome, which includes *ACD* hemizyosity⁸⁹. While further study is needed to date these molecular findings, case studies such as these highlight the importance of telomere length testing in ascribing the impact of even presumed deleterious variants. Additionally, a recent report examined the cellular and organ specific effects of the mutation (Lys82del) in mice, which corresponds to human Lys170del, when homozygous and transmitted across four additional generations⁹¹. Strikingly, spontaneous bone marrow failure was not observed, despite progressive telomere shortening and impact on the male germline (the female germline was not studied, although infertility was noted), thus providing additional caution for the use of mice to model at least certain phenotypic aspects of human TBDs.

Like *TINF2*, *ACD* variants have been reported in familial cancer cohorts, including cutaneous malignant melanoma⁹², chronic lymphocytic leukaemia⁹³ and papillary thyroid cancer⁹⁴. The variants include stopgain, splice-site and missense mutations, three of five of which map to the POT1 binding domain. Although telomere lengths in carriers of these variant have not been reported, the variants are proposed to alleviate negative telomere length regulation, thereby resulting in abnormally long telomeres⁹².

[H3] *POT1*

POT1 interacts with *TPP1* and specifically binds the telomeric 3' G-overhang to prevent the recruitment of a nonspecific single-stranded DNA (ssDNA) binding

complex, RPA, and the activation of the ATR-dependent DNA damage response⁹⁵. The loading of POT1 on the 3' G-overhang also prevents inappropriate degradation of the 5' strand. In addition to these protective functions, the POT1–TPP1 complex recruits telomerase to telomeres and modulates its activity and processivity⁸³. Interestingly, telomeres lengthen in cells depleted of POT1 or expressing mutants unable to bind TPP1 or telomeric DNA, indicating that POT1 also exerts a telomerase inhibitory function^{95,96} (**FIG. 2**). POT1 also interacts with the CTC1–STN1–TEN1 (CST) complex, which recruits the DNA polymerase α -primase complex to telomeres to promote C-strand synthesis⁹⁷ and also participates in C-strand fill-in to control the length of the 3' G-overhang⁹⁸ (**FIG. 1a**). In addition to telomere elongation, loss of POT1 in human cells induces ATR signalling, increases 3' G-overhang signal, telomere fragility and telomere fusions^{96,99}.

One study reported a homozygous missense *POT1* variant in two siblings with early-onset and very severe clinical manifestations of Coats plus syndrome⁹⁹. This variant did not compromise POT1 stability, its interaction with TPP1 or its localization at telomeres. However, patient cells exhibited telomere dysfunction-induced foci, increased frequency of rare sudden telomere loss and premature senescence⁹⁹. A cellular model expressing the *POT1* mutant displayed longer telomeres, suggesting that the variant abolished the telomerase inhibitory function of POT1⁹⁹. Accordingly, the global telomere length was much higher in blood cells from one tested patient than in the healthy sibling (Yanick Crow and P. R., unpublished observation). Moreover, the cellular model expressing the *POT1* mutant exhibited abnormal 3' G-overhang extension attributed to an impaired regulation of the CST complex. Furthermore, patient cells and cell lines reconstituted with the *POT1* mutant exhibited higher frequency of stochastic telomere truncations that were, however, partially healed by telomerase. From these observations, it was hypothesized that *POT1*-induced telomere dysfunction would mostly affect telomerase-negative cells, which could explain the clinical manifestations of Coats plus syndrome in comparison to other TBDs that mainly affect the hematopoietic system and epithelium.

Recently, a monoallelic missense *POT1* variant was identified in four related adult patients with pulmonary fibrosis, extrapulmonary TBD features in some, and variably short leukocyte telomere length¹⁰⁰. Functional analyses revealed the mutant

protein was defective for DNA binding and, in cell models, telomere phenotypes were similar to those caused by the variant leading to Coats plus syndrome, including abnormal 3' G-overhang extension and telomere elongation. Two novel heterozygous missense variants have also been recently identified in several individuals from two unrelated families with pulmonary fibrosis and short telomeres further supporting the notion that some *POT1* variants can cause short telomere-associated TBD (C.K. and P.R., unpublished observations).

Given the association of certain *TINF2* and *ACD* variants with certain familial cancer syndromes, it is unsurprising that certain monoallelic missense *POT1* variants are as well. These variants, which lead to increased telomere length, have been associated with cancer predisposition, particularly to malignant melanoma^{101,102}, but also to chronic lymphocytic leukaemia, glioma and cardiac angiosarcoma^{93,103}. Further investigations are needed to determine how different germline *POT1* variants result in either shortened or elongated telomeres leading to pulmonary fibrosis or Coats plus syndrome clinical features, respectively, or long telomeres associated with specific hereditary cancer syndromes.

[H2] Telomeric accessory factors

[H3] RTEL1

RTEL1 is a DNA helicase that exhibits anti-recombinogenic activity by displacing DNA recombination intermediates called D loops and unwinding telomeric G-quadruplex structures *in vitro*^{104,105}. Via its interaction with the DNA replication and repair factors PCNA, SLX4 and PoldIP3, RTEL1 combats DNA replication hindrances encountered genome-wide such as R-loops, G-quadruplexes and DNA interstrand crosslinks¹⁰⁵⁻¹⁰⁹. Furthermore, via an S phase-dependent interaction with TRF2, RTEL1 mediates T-loop opening to facilitate proper replication fork progression at telomeres^{110,111}. Consequently, impaired T-loop opening during telomere replication in RTEL1-deficient cells results in T-loop excision and liberation of extrachromosomal t-circles [G]¹¹². Other studies have suggested that RTEL1 participates in RNA and RNP trafficking and modulates TERRA abundance and localization at telomeres^{113,114}. Thus, RTEL1 shows pleiotropic functions that are mainly devoted to genome protection and telomere integrity.

A few dozen patients carrying biallelic *RTEL1* variants have been reported so far, most of whom exhibit the severe clinical features of Høyeraal–Hreidarsson syndrome associated with extremely short telomeres. Cells from these patients display dysfunctional telomeres, as inferred by an increase in telomere loss and telomere sister chromatid exchanges and diminished 3' G-overhangs¹¹⁵⁻¹²³. Despite the severe telomere phenotype in *RTEL1*-deficient patient cells, t-circles are not consistently detected, suggesting that the impaired resolution of T-loops leading to their excision may not be the main cause of telomere dysfunction in this condition^{116-118,122,123}. In addition to telomere defects, *RTEL1*-deficient patient cells display impaired repair of DNA interstrand crosslinks and hallmarks of spontaneous genome instability including anaphase bridges and radial and broken chromosomes^{117,122,123}.

RTEL1 is also haploinsufficient, with individuals carrying monoallelic deleterious *RTEL1* variants having short telomeres and developing TBD-related clinical manifestations in adulthood, including pulmonary fibrosis, liver diseases, bone marrow failure and myelodysplasia^{66,124-130}.

Intriguingly, the telomere instability observed in *RTEL1*-deficient murine embryonic fibroblasts (MEFs) is alleviated after *Tert* or *Terc* deletion, suggesting that telomerase could inhibit processes required to cope with stalled replication forks or other telomere damage in an *RTEL1*-deficient setting¹³¹. Whether a similar deleterious effect of telomerase exists in cells of *RTEL1*-deficient patients and, if so, whether it could be clinically significant by exacerbating the phenotype in *TERT*-expressing cells remains to be determined.

[H3] *RPA1*

RPA1 encodes RPA70, which, together with RPA32 and RPA14, forms the ubiquitous RPA complex. RPA binds to ssDNA and contributes to faithful DNA replication by disassembling secondary structures and, thanks to its interaction with several factors, preventing fork collapse when the replisome encounters obstacles¹³². Several studies, mainly carried out in yeast, have demonstrated a role for RPA in telomere maintenance and protection¹³³. The recent identification of *RPA1* and *RPA2* as genetic determinants of telomere length in a study involving 472,174 individuals supports the notion that RPA also plays a role in telomere maintenance in humans¹³⁴. This finding was underscored by the recent identification of monoallelic *RPA1* variants in four unrelated individuals presenting with short

telomeres and TBD manifestations, including bone marrow failure, myelodysplastic syndrome, pulmonary fibrosis and mucocutaneous abnormalities¹³⁵. The three identified variants, located in the DNA binding domain of RPA1, did not affect RPA complex formation but rather increased the affinity of RPA for ssDNA *in vitro*. Moreover, two of these variants enhanced RPA binding to telomeric sequences and its capacity to unwind telomeric G-quadruplex structures¹³⁵. Induced pluripotent stem cells carrying one of the *RPA1* variants in the homozygous state exhibited sharp telomere length reduction and impaired haematopoietic potential¹³⁵. Future studies are needed to determine how RPA1 GoF variants provoke telomere shortening and whether these variants compromise the dynamics of the RPA to POT1 switch at the 3' G-overhang after telomere replication, a process necessary to protect telomeres and regulate telomerase function¹³⁶.

[H3] *DCLRE1B*

DCLRE1B encodes Apollo (also known as SNM1B), a 5'-to-3' DNA exonuclease that interacts with several DNA replication and repair factors and participates in different facets of DNA replication, DNA repair and telomere protection¹³⁷. Apollo localizes at telomeres via its interaction with TRF2^{138,139}, an interaction that seems to influence ageing and organismal lifespan, as inferred from phylogenetic analyses conducted in giant tortoises and rockfish species^{140,141}. Studies conducted in human cells showed that Apollo depletion induced telomere fragility and topological stress during replication^{138,139,142}. The study of *Apollo* knockout MEFs revealed reduced 3' G-overhangs leading to an increase in telomere fusions¹⁴³⁻¹⁴⁵. These observations support a model in which the nuclease activity of Apollo is necessary to generate the protective 3' single-strand at leading telomeres (**FIG. 1a**). Furthermore, the first sequencing-based association study, which was conducted on 109,122 individuals, identified *DCLRE1B* as a genetic determinant of telomere length, suggesting that some Apollo variants can affect global telomere length¹⁴⁶.

Recently, biallelic variants in *DCLRE1B* were identified in three unrelated individuals with dyskeratosis congenita and Høyeraal–Hreidarsson syndrome phenotypes¹⁴⁷. All three patients carried a missense variant, which was either homozygous or in *trans* with a nonsense variant. In one patient, the missense variant was Leu142Phe, whereas, in the other two, Leu at position 142 was substituted with Ser. Structural analysis predicted that these missense variants affected the nuclease

activity, and biochemical experiments indicated that they did not affect Apollo expression or stability but partially reduced its capacity to interact with TRF2. Surprisingly, although the Apollo-mutant patient cells exhibited an increase in rare sudden telomere loss events and telomere fusions and a reduction in 3' G-overhang signal, they did not show a global reduction of telomere length. By contrast, human Apollo knockout cell lines exhibited a drastic telomere length reduction, suggesting that the Apollo Leu142Phe and Leu142Ser variants are hypomorphic and retain some protective activity. This is further supported by the perinatal lethality found in Apollo knockout mice¹⁴⁸. In addition to the telomere defects and consistent with a genome-wide protective role for Apollo, Apollo-deficient patient cells showed spontaneous chromosome instability, impaired DNA repair and increased sensitivity to replicative stress. Long-term follow-up of the patients' parents, who carry monoallelic *DCLRE1B* variants, will inform whether *DCLRE1B* is haploinsufficient and heterozygous pathogenic variants convey a risk of TBD manifestations.

Lastly, given that Apollo efficiently digests DNA substrates containing 8-oxo-G *in vitro* and cells with 8-oxo-G at telomeres exhibit some features that overlap with those found in Apollo-deficient patient cells (for example, telomere loss and fusion, fragile telomeres) raise the possibility that Apollo participates in the removal of these lesions at telomeres^{147,149-151}. However, further studies are needed to determine precisely how Apollo participates in telomere stability and genome-wide integrity and the functional impact of TBD-causing variants.

[H3] CTC1

As noted above, CTC1, together with STN1 and TEN1, forms a complex (CST) that possesses ssDNA binding activity and interacts with POT1 and TPP1^{98,152}. CST, via the recruitment of the Pol α -primase, promotes C-strand fill-in, which controls the length of the 3' G-overhang^{97,153,154} (**Fig. 1a**). Accordingly, depletion of CTC1 or STN1 results in abnormally long G-overhangs. Furthermore, the binding of CST to the 3' G-overhang, which competes with TPP1–POT1 binding, results in telomerase inhibition^{98,155}. Beyond telomeres, CST contributes to DNA replication and repair of DNA damage genome-wide¹⁵⁶⁻¹⁵⁹.

Several studies reported biallelic pathogenic *CTC1* variants as a cause of Coats plus syndrome and, in rare cases, dyskeratosis congenita¹⁶⁰⁻¹⁶⁴. Leukocyte telomere

length was not universally reduced in CTC1-deficient patients^{160,161,163}. Furthermore, while *CTC1* knockout human cell lines exhibit progressive telomere shortening, some TBD-causing *CTC1* mutations induced telomere elongation, illustrating the variable consequences that a dysfunctional CST trimer can exert on telomere length regulation¹⁶⁵⁻¹⁶⁷. Importantly, monoallelic *CTC1* pathogenic variant carriers lacked clinical manifestations, demonstrating autosomal recessive inheritance of disease (**FIG. 3a**). Functional analysis of several of the *CTC1* variants identified in patients revealed they affected several aspects of telomere stability; however, most could be alleviated by telomerase expression¹⁶⁷. Thus, the particular clinical manifestations observed in most patients with *CTC1* mutations could specifically reflect defects in tissues that lack telomerase expression, similar to the *POT1* mutation associated with Coats plus syndrome⁹⁹. Furthermore, the detection of non-telomeric DNA damage and signs of genome instability in CTC1-deficient patient cells and cell models suggest that some Coats plus syndrome features could result from non-telomeric sites of genome dysfunction^{159,160,168}.

[H3] *STN1*

STN1 (also known as *OBFC1*) interacts with *TPP1* and is part of the CST complex. As such, and like *CTC1*, it participates in 3' G-overhang length regulation and modulates telomerase activity. Biallelic *STN1* pathogenic variants have been reported in four cases of Coats plus syndrome, whereas monoallelic carriers were healthy¹⁶⁹⁻¹⁷¹. As with patients with Coats plus syndrome harbouring *CTC1* pathogenic variants, telomere length was not consistently reduced in leukocytes of patients with *STN1* mutations¹⁶⁹⁻¹⁷². However, patient-derived *STN1*-deficient pre-senescent fibroblasts were found to exhibit several hallmarks of telomere dysfunction, including increased 3' G-overhang length, telomere fusions and sister chromatid exchange¹⁶⁹. Hence, although additional studies are needed to better understand the link between the particular clinical features of Coats plus syndrome caused by *STN1*, *POT1* or *CTC1* deficiencies and these telomere defects, impaired 3' G-overhang regulation and partial rescue of telomere anomalies by telomerase might be the common features in these conditions^{99,167}.

[H2] Uncertain TBD-causing genes

Several additional genes have been proposed to be associated with TBDs. However, although one or more may ultimately be classified as a *bona fide* TBD-causing gene, their link to telomere biology or TBDs remains uncertain at present.

[H3] *MDM4*

MDM4 inhibits p53 activity, and p53 is known to contribute to certain manifestations of TBDs. A recent study identified a monoallelic missense variant in *MDM4* in a patient with dyskeratosis congenita and short telomeres, suggesting hyperactivation of p53 can lead to telomere shortening and clinical features of TBDs¹⁷³. However, the patient also carried a missense *TERT* variant of unknown significance that affects a residue adjacent to an amino acid mutated in several individuals with aplastic anaemia and short telomeres^{174,175}. Although MEFs carrying the *MDM4* variant in the homozygous state showed slightly shorter telomeres, the phenotypes in MEFs and mice carrying the monoallelic *Mdm4* variant (mimicking the genotype of the human proband) were almost indistinguishable from the wild-type phenotype¹⁷³. Hence, the identification of TBD patients carrying *MDM4* variants without a confounding variant in other TBD-causing genes is required to validate *MDM4* as a *bona fide* TBD-causing gene.

[H3] *NPM1*

NPM1 encodes nucleophosmin 1 (also known as NPM1 and B23), a nucleolar factor that modifies rRNA and enables proper ribosome biogenesis¹⁷⁶. Germline monoallelic variants were found in two unrelated individuals with some features akin to dyskeratosis congenita. However, the clinical description of the patients was rather limited. Patient cells and cellular models expressing the variants showed some defects in ribosome function, supporting a deleterious impact of the variants on ribosomes. However, analysis of telomere phenotypes in these cells (for example, length, stability or 3' G-overhangs) was lacking. Since TBDs and Mendelian disorders associated with ribosome defects share some clinical features, including progressive bone marrow failure, developmental defects and a predisposition to myelodysplastic syndrome and acute myeloid leukaemia, it is unclear whether the clinical features of the two patients with *NPM1* deficiency were solely a consequence of ribosome defects or were associated with some uncharacterized telomere dysfunction¹⁷⁷.

[H3] SON

A recent study demonstrated that the RNA binding factor SON participates in the regulation of *TERT* transcript alternative splicing required to generate functional *TERT*¹⁷⁸. Germline *de novo* monoallelic *SON* variants cause Zhu–Tokita–Takenouchi–Kim (ZTTK) syndrome, a disorder characterized by a spectrum of cognitive and developmental anomalies¹⁷⁹. The analysis of cells from one ZTTK-deficient patient demonstrated reduced *TERT* expression and activity and short telomeres. However, since *SON* regulates the splicing of a myriad of genes, the majority of which participate in brain development and metabolism, and because most of the ZTTK clinical features are different from those found in *TERT*-deficient patients, *SON* cannot be considered a TBD-causing gene.

[H3] SHQ1

SHQ1 is a protein chaperone that interacts with dyskerin and, as *NAF1*, participates in the biogenesis of many H/ACA RNPs, including the telomerase holoenzyme¹⁸⁰. Compound heterozygous missense *SHQ1* variants were identified in a patient with intrauterine growth retardation and cerebellar hypoplasia but no other TBD-associated clinical features, such as abnormal blood cell counts¹⁸¹. The telomere length in patient cells and the impact of the variants on telomerase holoenzyme biogenesis and activity were not evaluated. Thus, for the time being, lack of information does not allow classification of *SHQ1* as a TBD-causing gene.

[H3] USB1

U6 SnRNA Biogenesis Phosphodiesterase 1 (*USB1*) is a 3'–5'-exoribonuclease and cyclic phosphodiesterase responsible for the 3' end maturation of U6 small nuclear RNA (snRNA)¹⁸². Pre-U6 snRNA is initially extended with uridines by terminal uridylyl transferases 1 (*TUT1*)¹⁸³. These are subsequently removed by *USB1*, yielding 3' end with 5-uridines and a terminal 2',3' cyclic phosphate. Analogous to *PARN*-deficient cells, *USB1*-deficient cells have elevated levels of precursor U6 snRNA with extended polyuridine tails and terminal 1–3 adenosines, which are likely also added by *TUT1* and normally removed by *USB1*. Biallelic (mostly homozygous) LoF variants in *USB1* cause poikiloderma with neutropenia (PN; formerly known as Clericuzio-type poikiloderma with neutropenia and Navajo neutropenia¹⁸⁴). Affected

individuals manifest an inflammatory eczematous rash in the first year of life followed by post-inflammatory poikiloderma. The neutropenia is congenital. Additional features include hyperkeratotic palms, soles and dystrophic nails. Given the ectodermal and haematopoietic features, cases of PN have been misdiagnosed as dyskeratosis congenita¹⁸⁵. Although, absence of the *Schizosaccharomyces pombe* *USB1* orthologue *mpn1* results in increased levels of TERRA and shortened telomeres¹⁸⁶, telomere lengths are normal in PN patient cells¹⁸⁵. Thus, *USB1* is not considered a *bona fide* TBD gene.

[H1] Genotype–phenotype features of TBDs

[H2] Varied modes of genetic inheritance

As described above, all modes of inheritance are represented in the TBDs. *DKC1* is the only TBD gene that is X-linked and causes X-linked recessive disease. At present, *bona fide* autosomal recessive mode of inheritance is observed for only 4 of the 17 TBD-causing genes: *CTC1*, *STN1*, *WRAP53* and *DCLRE1B*. In these cases, monoallelic variant carriers (including the patient's parents and siblings) are asymptomatic (**FIG. 3a**). However, the few cases reported so far do not exclude the possibility that they may be at risk of developing some TBD-related manifestations later in adulthood. By contrast, TBD-causing *TINF2* variants are mostly *de novo* and strictly monoallelic (autosomal dominant), with no biallelic *TINF2* variants reported to date. Lastly, of the 11 remaining TBD-genes (*RTEL1*, *TERT*, *TERC*, *PARN*, *NHP2*, *NOP10*, *NAF1*, *ACD*, *ZCCHC8*, *POT1* and *RPA1*) heterozygous pathogenic variants cause premature-ageing clinical manifestations (for example, pulmonary fibrosis, early hair greying, bone marrow failure), asserting monoallelic inheritance of disease. Remarkably, biallelic variants in some of the same genes (for example, *RTEL1*, *TERT*, *PARN*, *NHP2*, *ACD* and *POT1*) cause early-onset and more severe TBDs such as dyskeratosis congenita, Høyeraal–Hreidarsson syndrome and Coats plus syndrome (**FIG. 3b** and **Table 1**).

[H2] Genetic anticipation

The individuals who carry a dominant monoallelic variant in a TBD-causing gene resulting in a telomere maintenance defect can transmit to their progeny not only the deleterious variant but also short telomeres. This can translate into progressive

telomere shortening and earlier onset and extent of clinical manifestations over successive generations, a phenomenon known as genetic anticipation (**FIG. 3c**). Genetic anticipation has been observed in some families with pulmonary fibrosis carrying monoallelic variants in several TBD-causing genes including *TERC*, *TERT*, *PARN*, *RTEL1* and *POT1*^{100,187-192}. In such cases, the descendants carrying the monoallelic variant exhibit earlier-onset pulmonary disease and other TBD complications, including bone marrow failure (**FIG. 3c**).

[H2] Phenocopy

Seminal studies conducted with heterozygous *Tert* and *Terc* knockout mice demonstrated that short telomeres and the associated premature-ageing phenotypes are transmitted to the progeny even in animals with a wild-type genotype^{193,194}. This phenomenon, where the phenotype (here, short telomeres and/or premature ageing) dissociates from the genotype (here, pathogenic variant in a gene involved in telomere maintenance) is dubbed phenocopy. Phenocopying has been observed in TBD families, in which individuals, despite their wild-type genotype, inherited short telomeres from a parent and, consequently, exhibited TBD-related symptoms^{34,195-197} (**FIG. 3c**). Importantly, in TBD families, telomeres recover a normal length in individuals with wild-type genotypes after two successive generations¹⁸⁹.

[H2] Variable expressivity and incomplete penetrance

For a given variant in a TBD-causing gene, several parameters can modulate disease expressivity, including age, the degree of telomere length reduction or dysfunction, environmental factors (such as, tobacco use), genetic modifiers and the acquisition of rescuing genetic variants (see below). Thus, at a given time point, a carrier of a pathogenic variant in a TBD-causing gene does not inevitably have clinical manifestations¹³. This has been observed particularly in families with pulmonary diseases in which variant-carrier siblings can exhibit strikingly different clinical phenotypes, such as emphysema, liver disease or tongue cancer^{21,62,196,198-200} (**FIG. 3c**).

[H1] Somatic genetic rescue in TBDs

In humans, somatic point mutations accumulate over time in the haematopoietic compartment, with a frequency of ~20 mutations/cell/year, giving rise to age-related

clonal haematopoiesis (ARCH) in healthy older individuals (also known as clonal haematopoiesis of indeterminate potential; CHIP)²⁰¹⁻²⁰³. In the context of Mendelian disease, spontaneous genetic events can induce a 'genetic rescue'. SGR is defined as a somatic genetic event that specifically counteracts the deleterious impact of a germline variant at the cellular level and may confer a selective advantage over non-modified cells and lead to expansion of the SGR positive clones, and somatic mosaicism (reviewed in²⁰⁴). Several recent studies suggest that SGR events in the haematopoietic system from patients with a TBD are particularly frequent^{46,80,135,200,205-210} (**FIG. 4**).

[H2] Direct SGR in TBDs

Direct SGR is a somatic genetic event that modifies the germline-mutated gene. Most of the direct SGR mechanisms reported in TBD patients are similar to those found in patients with other Mendelian haematopoietic diseases²⁰⁴. This includes modifications that correct the germline mutation either by **uniparental isodisomy [G]**, leading to copy neutral loss of heterozygosity (CN-LOH), or point mutations that revert the germline variant to the wild-type sequence (back mutation), as has been observed for LoF variants in *DKC1* and *TERC*^{207,208} (**Table 2**). Conversely, direct SGR can occur through the inactivation of a dominant negative or GoF germline variant either via acquisition of a LoF variant, splice variant, or indel in *cis* with the germline variant²⁰⁴ (**FIG. 4a**). These types of direct SGR have been reported in a few cases of patients with TBDs caused by germline mutations in *TINF2* and *RPA1*, providing evidence for the germline-encoded mutant proteins harbouring a GoF mechanism^{80,135} (**Table 2**). Importantly, the expansion of these blood clones with direct SGR in individuals who had not manifested bone marrow failure or had stabilization of blood counts suggests a selective advantage leading to a rescue, at least partial, of haematopoiesis.

Recently, independent studies highlighted a novel type of direct SGR specific to TBDs caused by germline variants in *TERT*. The SGR consisted of base substitutions in the *TERT* promoter at positions -57, -124 or -146 from the start codon^{200,205,206,209} (**FIG. 4b**). These are the same mutations observed in telomerase-positive cancers that create E-twenty-six (ETS) transcription factor binding motifs and result in increased *TERT* expression and telomerase activity²¹¹⁻²¹³. Thus, this type of SGR counteracts the deleterious effect of the germline monoallelic *TERT*

variant^{205,206}. This adaptive process is rather frequent, detected in blood cells in 6% of TBD patients carrying a germline *TERT* variant. Moreover, distinct clones with different *TERT* promoter-activating mutations (*TERTpam*) can be found in a single individual^{205,206,210}. Strikingly, the somatic *TERTpam* preferentially occur on the wild-type *TERT* allele (in trans of the germline-mutated allele), supporting the notion that the selective advantage is stronger when the *TERTpam* allows the overexpression of the wild-type *TERT*²⁰⁶ (**FIG. 4b**).

[H2] Indirect SGR in TBDs

Indirect SGR corresponds to a somatic genetic event that does not modify the germline-mutated gene but rather a gene that participates in the pathway affected by the germline mutation and provides a selective advantage to the modified cells²⁰⁴. Until now, indirect SGR has been identified in very few Mendelian disorders^{46,205,206,209,214,215}. One example of indirect SGR corresponds to *TERTpam* in TBD patients carrying pathogenic germline variants not in *TERT* but in *TERC*, *NAF1*, *NHP2*, *PARN* or *CTC1*^{46,205,206,209} (**Table 3** and **FIG. 4c**). This observation indicates that *TERTpam* represents a recurrent direct and indirect SGR mechanism in TBDs. It also suggests that upregulation of TERT expression can positively affect telomeres shortened or weakened by diverse mechanisms including reduced hTR (that is, *TERC*, *NAF1*, *NHP2*, *PARN*) and altered telomere stability (that is, *CTC1*).

Another form of recurrent indirect SGR in TBDs was recently identified and corresponds to the acquisition of LoF variants in *POT1*. These somatic variants impair *POT1*'s ability to interact with *TPP1* or bind the 3' G-overhang. Functionally, these *POT1* SGR mutations abrogate the inhibitory function of *POT1* on telomerase^{209,210,216,217} (**Table 3** and **FIG. 4c**). Thus, somatic *TERTpam* and *POT1* LoF mutations functionally converge, increasing telomerase activity at telomeres and likely counterbalancing the telomere shortening caused by germline variants in genes involved in telomere maintenance (**FIG. 4c**).

As mentioned above, the production and regulation of mature hTR involves the RNA decay machinery³¹. A recent study reported in four TBD patients carrying germline variants in *DKC1* or *TERC* blood clones harbouring somatic missense mutations in *MTREX* (encoding MTR4/SKIV2L2) and *DIS3*, genes that contribute to RNA decay²⁰⁹. This observation suggests that the somatic variants in *DIS3* and *MTREX* could increase the level of hTR and, thus, represent indirect SGR by

counterbalancing the reduced level of hTR caused by the germline variants in *DKC1* or *TERC*^{31,70}. This hypothesis is also supported by the observation that the impaired telomerase activity detected in *DKC1*- and *PARN*-deficient cell models is rescued by the depletion of the RNA decay factors DCP2, XRN1 and EXOSC10³⁶. In addition, several genes involved in RNA decay (that is, *DIS3*, *EXOSC6*, *EXOSC9*, *EXOSC10* and *ZCCHC8*) have been implicated recently as genetic determinants of telomere length¹³⁴. Altogether, somatic mutations in genes regulating RNA decay could theoretically represent recurrent indirect SGR mechanisms in TBDs in which the level of mature hTR is affected (for example, *PARN*, *DKC1*, *NHP2*, *NOP10*, *ZCCHC8* or *TERC*).

Lastly, a clonal somatic missense mutation in *TERF2IP*, which encodes the shelterin component RAP1, was detected in a TBD patient carrying a germline variant in *TERT*. Since the functional impact of this somatic *TERF2IP* mutation in the setting of *TERT* deficiency has not been assessed, it is unclear whether it represents a *bona fide* SGR event²⁰⁹.

[H2] Features and implications of SGR in TBDs

Since somatic mutation and chromosomal alteration accumulate over time in haematopoietic cells from normal individuals, it is not surprising that SGR is more frequently detected in patients with TBD manifestations that develop in adulthood (for example, pulmonary fibrosis and liver disease) than in young patients with dyskeratosis congenita and Høyeraal–Hreidarsson syndrome features^{202,203,205,210}. Importantly, the frequency of positive SGR clones in blood can be highly variable and not necessarily correlated with an increase in global telomere length or improvement of haematopoietic parameters^{206,210}. However, recent longitudinal studies demonstrated that, in some cases, the SGR could be associated with telomere length stabilization, blood count improvement, delayed onset of clinical manifestations and reduced frequency of myelodysplastic syndrome and acute myeloid leukaemia development, suggesting a protective potential for SGR events in these patients^{135,209,210}. Remarkably, both the somatic *TERT**pam* and *POT1* LoF mutations that are recurrently identified in blood clones from patients with TBDs and correspond to SGR in this setting are identified as somatic or germline variants in several sporadic and familial cancers^{211,216-218}. It is therefore conceivable that the *TERT**pam* and *POT1* mutations, by promoting telomere maintenance, contribute to

cellular immortalization and oncogenesis in individuals in whom telomere maintenance is not compromised, whereas, by offsetting accelerated telomere shortening due to pathogenic germline variants, act as *bona fide* SGR in patients with TBDs. Long-term analysis of patients with TBDs harbouring SGR-positive clones is required to determine their impact on clinical manifestations and evolution over time. Lastly, since several organs are affected in TBDs (**Box 1**), it is possible that SGR-positive clones could emerge in tissues other than in the blood, especially those with high renewal potential such as the skin, gut and liver.

[H1] Conclusions and future perspectives

For nearly 25 years since the identification of the first genetic and molecular cause of TBD, clinical and genetic analyses in patients with Mendelian premature ageing diseases have contributed substantially to the understanding of the processes involved in the control of telomere length and stability. However, a non-negligible proportion of TBDs remain without defined molecular aetiology (estimated between 15% to 40%, depending on the type of TBD). This can be due, at least in part, to specific aspects of TBDs, including the phenomena of genetic anticipation, phenocopy, incomplete penetrance and SGR, which can complicate interpretation of inheritance patterns and disease diagnosis. Furthermore, although the tools used to detect abnormal global telomere length reduction are reliable and effective to diagnose most TBDs, they can miss specific presentations¹³. Indeed, excessive global telomere shortening is not consistently observed in TBDs, as demonstrated in Coats plus syndrome caused by pathogenic variants in *CTC1*, *POT1* or *STN1*, or in dyskeratosis congenita/Høyeraal–Hreidarsson syndrome associated with variants in *DCLRE1B*^{99,147,160,161,169}. Nonetheless, in these conditions, other signs of telomere dysfunction can be detected, including abnormal 3' G-overhang length and increased frequency of sudden telomere loss events. Unfortunately, the approaches used to assess 3' G-overhang length (in-gel hybridization) and sudden telomere loss (STELA, TeSLA and molecular combing) are technically tricky and time-consuming and, therefore, not suitable as routine clinical diagnostics²¹⁹⁻²²². The development of new techniques, likely based on single-molecule sequencing, should help overcome these limitations and expand the diagnostic toolbox for TBDs in the future.

Moreover, the use of whole-genome sequencing should facilitate the identification of novel TBD-causing variants located in non-coding sequences such

as those affecting regulatory regions, 5' and 3' untranslated regions of transcripts, or causing splicing defects as described in a few examples of TBDs²²³⁻²²⁵. Also, synonymous variants, often neglected in genetic analysis, should be taken into consideration as they can alter splicing and cause TBDs, as recently reported¹⁹⁹. Thus, in the future, it is likely that next-generation sequencing approaches will allow the genetic diagnosis of many other TBDs (the prevalence of which is likely underestimated). Furthermore, while the first descriptions of a TBD date to the early 1900s²²⁶, novel clinical manifestations continue to emerge (for example, hepatopulmonary syndrome and neuropsychiatric)^{227,228}. Thus, some TBD manifestations may continue to be underestimated. We anticipate that the clinical spectrum of TBDs will broaden with the identification of pathogenic variants in TBD genes associated with unexpected disease presentations.

Recent studies suggest that the emergence of SGR-positive clones is particularly frequent in the haematopoietic system of patients with TBDs^{46,80,135,200,205-210}. It is therefore conceivable that in some cases, the presence of a clone carrying a direct SGR that eliminates the germline variant (that is, CN-LOH or back mutation) may hamper the identification of the genetic variant, as observed in some Mendelian haematopoietic diseases^{208,229-231}. Thus, it is preferable to carry out genetic studies of TBDs with DNA extracted from cells or tissues other than blood (for example, skin fibroblasts, hair follicles, buccal epithelial cells) in which a major expansion of SGR-positive clones is less likely.

By contrast, SGR-positive blood clones, especially those carrying somatic *TERT*_{pam} and *POT1* LoF mutations, are recurrent in patients with TBDs, even in patients who do not exhibit TBD-associated blood disease. This suggests that the search for such SGR-positive clones in blood cells can be a useful diagnostic approach in individuals with a suspicion of TBD but without an identified molecular aetiology. This may be particularly important before haematopoietic cell transplantation or lung transplantation because severe complications are frequently observed in TBDs patients undergoing these procedures²³²⁻²³⁶.

Lastly, the identification of SGR and the study of their functional impact in TBD patients could inspire new therapeutic approaches in these diseases. This is substantiated by the observation that, similarly to some SGRs that affect the RNA decay machinery (for example, *DIS3* or *MTREX*; **FIG. 4c**) and likely increase the level of functional hTR, an inhibitor of the hTR Poly(A) polymerase PAPD5 increases

the level of mature hTR and improves the potential of haematopoietic stem and progenitor cells in the context of PARN or dyskerin deficiency^{59,237-239}. In addition, the association of certain types of SGR with germline mutations in certain TBD genes may inform which groups of patients might benefit from specific therapeutic approaches (for example, patients with germline mutations in genes in which *TERT*^{pam} are frequently observed might benefit from therapies that upregulate telomerase activity, as proposed for danazol²⁴⁰).

Thus, the continuous study of human TBDs should provide further insights into our knowledge of telomere biology, which will help to not only develop new strategies to fight these diseases but also limit telomere alterations that accumulate with normal ageing and contribute to age-related diseases, including cancer.

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The authors declare no competing interests.

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Table 1. Telomere biology disorder genes, inheritance and primary disease association

Gene	Gene product	Main function related to telomere biology	Genetic status	Disease associations	Number of patients reported	Global telomere length in blood
<i>DKC1</i> (ENSG00000130826)	Dyskerin	<i>hTR</i> stability	X-linked	Dyskeratosis congenita ^{10,37,40,241-252}	>50	Reduced
				Høyeraal–Hreidarsson syndrome ²⁵³⁻²⁵⁶	8 from 5 families	
				Pulmonary fibrosis ^{199,248,252,257}	8 from 6 families	
				Bone marrow failure or aplastic anaemia ¹⁹⁹	1	
<i>TERC</i> (ENSG00000270141)	hTR (Telomerase RNA component)	Telomerase RNA scaffold, template	Monoallelic	Dyskeratosis congenita ^{33,34,187,208,241,242,258-261}	>50	Reduced
				Høyeraal–Hreidarsson syndrome ²⁵⁵	1	
				Pulmonary fibrosis ^{20,21,190,241,261-266}	>40	
				Liver disease ²⁶⁷⁻²⁶⁹	8 from 8 families	
				Aplastic anaemia or myelodysplastic syndrome ^{241,258,261,268,270-274}	25 from 19 families	
			Biallelic	Pulmonary fibrosis and mucocutaneous features. ³⁴	2 from 1 family	
<i>TERT</i> (ENSG00000164362)	TERT	Catalytic subunit of telomerase, telomere elongation	Mono- & biallelic	Dyskeratosis congenita ^{27,28,34,174,241,258}	>25	Reduced
			Biallelic	Høyeraal–Hreidarsson syndrome ^{24-26,28,174,275}	7 from 6 families	
			Monoallelic	Pulmonary fibrosis ^{20,21,127,190,197,206,241,264,265,276}	>100	
				LD ^{267,269}	14 from 11 families	
<i>NOP10</i> (ENSG00000182117)	NOP10	<i>hTR</i> stability	Biallelic	Dyskeratosis congenita ⁴¹	3 from 1 family	Reduced
			Monoallelic	Pulmonary fibrosis ⁴³	3 from 1 family	
<i>NHP2</i> (ENSG00000145912)	NHP2	<i>hTR</i> stability	Biallelic	Dyskeratosis congenita ^{45,277}	2 from 2 families	Reduced
			Monoallelic	Høyeraal–Hreidarsson syndrome ⁶⁵	1	

				Pulmonary fibrosis ⁴⁶	5 from 3 families	
<i>TINF2</i> (ENSG00000092330)	TIN2	Negative regulator of telomere length	Monoallelic (mostly <i>de novo</i>)	Dyskeratosis congenita ⁷⁶⁷⁷ Sarper, 2010 #8512; Sasa, 2012 #8513; Gleeson, 2012 #8514; Panichareon, 2015 #8516; Norberg, 2018 #8520; Du, 2018 #8521; Roake, 2021 #8523}	45	Reduced
				Høyeraal–Hreidarsson syndrome ^{77,78,278}	5 from 5 families	
				Revesz syndrome ^{76,81,255,279-282}	7 from 7 families	
				Pulmonary fibrosis ⁸⁰	1	
				Aplastic anaemia or myelodysplastic syndrome ²⁸³⁻²⁸⁵	8 from 8 families	
<i>WRAP53</i> (ENSG00000141499)	TCAB1	Telomerase maturation, activation, and trafficking	Biallelic	Dyskeratosis congenita ^{50,51,53,286}	5 from 5 families	Reduced
				Høyeraal–Hreidarsson syndrome ⁵²	1	
				Liver disease ⁵¹	1	
<i>CTC1</i> (ENSG00000178971)	CTC1	3' G-overhang regulation	Biallelic	Dyskeratosis congenita ^{162,163}	2 from 2 families	Not consistently reduced
				Coats plus syndrome ^{160,161,163,164,168,172,287-290}	>20	
<i>RTEL1</i> (ENSG00000258366)	RTEL1	Telomere replication, 3' G-overhang	Monoallelic & biallelic	Dyskeratosis congenita ^{115,119,122,130}	6 from 6 families	Reduced
				Høyeraal–Hreidarsson syndrome ¹¹⁶⁻¹²¹ ; Walne, 2013 #4777; Moriya, 2016 #6508; Ziv, 2020 #8393; Speckmann, 2017 #6254}	>20	
			Monoallelic	Pulmonary fibrosis ^{66,124,125,127,128,190,266,276}	>100	Not consistently Reduced
<i>PARN</i> (ENSG00000140694)	PARN	hTR maturation/stability, stability of telomere-factor transcripts	Biallelic	Dyskeratosis congenita ^{60,291}	4 from 3 families	Reduced in most cases
				Høyeraal–Hreidarsson syndrome ⁶¹⁻⁶⁵	9 in 8 families	
			Monoallelic & biallelic	Pulmonary fibrosis ^{13,61,66,127,190,195,261,266,276,292-294}	>50	
ACD ENSG000001	TPP1	Telomerase recruitment	Biallelic	Kidney disease ⁶⁷	3 from 2 families	Reduced
				Høyeraal–Hreidarsson	2 from 2 families	

02977)		via TEL-patch and NOB domains		syndrome ⁸⁵ ; Hensle e, 2021 #8487}		
			Monoallelic & biallelic	Bone marrow failure or aplastic anaemia ^{86,90}	5 from 2 families	
			Monoallelic	Pulmonary fibrosis ^{88,90}	4 from 2 families	
<i>POT1</i> (ENSG00000128513)	POT1	3' G-overhang regulation, telomerase activity/processivity, telomere stability	Biallelic	Coats plus syndrome ⁹⁹	2 from 1 family	Normal/elongated
			Monoallelic	pulmonary fibrosis ¹⁰⁰ (CK and PR, unpublished observations)	3 from 2 families	Reduced
<i>NAF1</i> (ENSG00000145414)	NAF1	hTR stability	Monoallelic	Pulmonary fibrosis ⁵⁶	5 from 2 families	Reduced
				Myelodysplastic syndrome ⁵⁶		
				LD ⁵⁶		
<i>STN1</i> (ENSG00000107960)	STN1	3' G-overhang fill-in	Biallelic	Coats plus syndrome ¹⁶⁹⁻¹⁷²	5 from 5 families	Not consistently reduced
<i>ZCCHC8</i> (ENSG00000033030)	ZCCHC8	hTR maturation/stability	Monoallelic	Pulmonary fibrosis ⁷⁰	3 from 1 family	Reduced
				Bone marrow failure ⁷⁰	1	
<i>RPA1</i> (ENSG00000132383)	RPA1	Telomere replication?; regulation of telomerase activity?	Monoallelic	Pulmonary fibrosis ¹³⁵	1	Reduced
				Dyskeratosis congenita ¹³⁵	1	
				Myelodysplastic syndrome ¹³⁵	1	
				Immunodeficiency ¹³⁵	1	
<i>DCLRE1B</i> (ENSG00000118655)	Apollo	3' G-overhang regulation, telomere stability	Biallelic	Dyskeratosis congenita ¹⁴⁷	2 from 2 families	Not reduced
				Høyeraal-Hreidarsson syndrome ¹⁴⁷	1	

Only patients for whom molecular sequencing was performed and reported are included.

Table 2. Direct somatic genetic rescue events in telomere biology disorders

Germline mutated TBD-causing gene	Type of somatic genetic event in the germline mutated gene	Functional/genetic consequence of the SGR	Ref.
<i>DKC1</i>	Back mutation and CN-LOH	Replacement of the mutated allele by a WT sequence	²⁰⁷
<i>POT1</i>	Splicing and LoF point mutations	Loss of inhibitory function on telomerase activity or processivity	(C.K. and P.R., unpublished observations)
<i>RPA1</i>	LoF point mutation (in <i>cis</i>), CN-LOH	Inactivation of the dominant effect of the germline variant Replacement of the	¹³⁵

		mutated allele by a WT copy	
<i>TINF2</i>	Small out-of-frame deletion (in <i>cis</i>)	Inactivation of the dominant effect of the germline variant	⁸⁰
<i>TERC</i>	CN-LOH	Replacement of the mutated allele by a WT copy	²⁰⁸
<i>TERT</i>	<i>TERT</i> <i>pam</i> (mostly in <i>trans</i>)	Increased TERT expression/telomerase activity	^{200,205,206,209}

CN-LOH, copy number loss of heterozygosity; LoF, loss of function; TBD, telomere biology disorder; WT, wild type.

Table 3. Indirect somatic genetic rescue events in telomere biology disorders

Gene with somatic variant	Type of somatic genetic event	Functional consequence	Germline-mutated TBD-causing gene
<i>TERT</i>	<i>TERT</i> <i>pam</i>	Increased TERT expression or telomerase activity	<i>CTC1</i> (Fernanda Gutierrez-Rodrigues, Rodrigo T. Calado, Neal S. Young; personal communication)
			<i>NAF1</i> ²⁰⁹
			<i>NHP2</i> ⁴⁶
			<i>PARN</i> ²⁰⁶
			<i>TERC</i> ^{205,209}
<i>POT1</i>	Splicing and LoF point mutations	Loss of inhibitory function on telomerase activity or processivity	<i>RTEL1</i> ²⁰⁹ (Fernanda Gutierrez-Rodrigues, Rodrigo T. Calado, Neal S. Young; personal communication)
			<i>TERC</i> ²⁰⁹
			<i>TINF2</i> ^{209,210}
			<i>TERT</i> ²⁰⁹
<i>DIS3</i>	Missense point mutation	Unknown functional consequence, but possibly increased level of mature hTR	<i>DKC1</i> ²⁰⁹
			<i>TERC</i> ²⁰⁹
<i>MTREX</i>	Missense point mutation	Unknown functional consequence, but possibly increased level of mature hTR	<i>TERC</i> ²⁰⁹
<i>TERF2IP</i>	Missense point mutation	Unknown functional consequence	<i>TERT</i> ²⁰⁹

LoF, loss of function; TBD, telomere biology disorder.

Figure 1. Causes and consequences of telomere dysfunction in normal ageing and TBDs.

A. Several features of telomeres confer potential hindrances that can compromise telomere replication or stability. (Aa) The G-richness of telomeric repeats favours the formation of G-quadruplexes, DNA secondary structures that can impede fork progression, resulting in fork collapse^{295,296}. Reactive oxygen species can induce 8-oxoguanine (8-oxo-G) base lesions, which are particularly deleterious at telomeres¹⁴⁹. Transcription from the subtelomeric region can give rise to a non-coding RNA termed telomeric repeat-containing RNA (TERRA). TERRA can interact with DNA to form telomeric R-loop structures, which may affect telomere replication²⁹⁷. (Ab) Fork progression at the very end of the telomere is inherently unidirectional; therefore, convergent DNA replication cannot compensate for arrested forks. (Ac) After semi-conservative telomere replication, the RNA primer associated with synthesis of the lagging-strand is removed, giving rise to a 3' G-overhang. The terminus of the leading-strand telomere, however, is (nearly) blunt. This inability of DNA polymerases to fully replicate the DNA ends is the major contributor to progressive telomere shortening observed in somatic cells over successive cell divisions^{1,2,97}. (Ad and Ae) Telomeric ends are subject to exonucleolytic and fill-in activities that generate and control the 3' G-overhang length of both leading and lagging telomeres¹. (Af) The single-stranded 3' G-overhang can invade telomeric double-stranded DNA to form the T-loop and sequester the DNA end from the DNA repair machinery. Some of the factors participating in the different steps are listed in brackets. **B.** (left) In somatic cells from normal individuals, telomeres progressively shorten over repeated cell divisions. When telomeres reach too short a length, protective mechanisms become inoperative, resulting in dysfunctional telomeres, DNA damage response induction, senescence and apoptosis. These events contribute to ageing. (middle) Pathogenic variants in genes involved in telomere maintenance cause accelerated telomere shortening and some features of premature ageing. (right) A subset of TBDs is characterized by dysfunctional or fragile telomeres that are not inevitably associated with abnormal global telomere shortening. Examples of genes associated with telomere maintenance defects or telomere instability are listed in brackets.

Figure 2. Factors associated with TBDs. Schematic representation of a telomere, shelterin components, the telomerase holoenzyme, accessory factors participating in telomere replication and stability, and the proteins involved in hTR processing/maturation and telomerase complex biogenesis. To counteract the ‘end replication problem’, the ribonucleoprotein (RNP) telomerase can add telomeric repeats onto the terminus of the 3' G-overhang²⁹⁸. The catalytic core of telomerase contains a reverse transcriptase, TERT, and a H/ACA RNA, hTR, which provides several critical regions for telomerase assembly and function, including the template for reverse transcription of telomeric repeats³⁸. The telomere-dedicated shelterin complex, composed of TRF1, TRF2, RAP1, TIN2, TPP1 and POT1, is essential to inhibit the activation of DNA repair factors at telomeres, promote T-loop formation, and regulate the length of telomere DNA via the modulation of telomerase recruitment and activity¹. TRF1 and TRF2 homodimers bind the double-stranded telomeric repeats, while POT1 binds to the single-stranded 3' G-overhang. RAP1, TIN2 and TPP1 are tethered to telomeres via interactions with TRF1, TRF2 or POT1¹. Multiple factors involved in global genome integrity contribute important roles at telomeres by facilitating telomere replication or participating in 3' G-overhang production (for example, RTEL1, CTC1, STN1, TEN1, RPA1, Apollo and Exo1)¹. The factors in colour are products of TBD-causing genes. Representation of telomerase H/ACA RNP structure adapted with permission from³⁸.

Figure 3. Specific genotype–phenotype features in TBDs. **a.** Schematic representation of an autosomal recessive TBD inheritance as observed in Coats plus syndrome (CTC1 and STN1 deficiencies) and dyskeratosis congenita/Høyeraal–Hreidarsson syndrome (TCAB1 and Apollo deficiencies), where only biallelic variant carriers exhibit clinical symptoms. Note that in these conditions, the patients' cells do not inevitably exhibit global telomere shortening^{99,147,161,169}. **b.** Schematic representation of genotype–phenotype association in a family with autosomal dominant inheritance of pathogenic variants. Monoallelic carriers can exhibit late-onset TBD-related symptoms in adulthood (for example, pulmonary fibrosis, macrocytic anaemia or thrombocytopenia) while biallelic carriers show early-onset and severe TBD manifestations. **c.** Schematic representation of a pedigree with individuals carrying a monoallelic dominant variant in a gene associated with short telomeres. Genetic anticipation (highlighted in blue) is exemplified by telomere length

reduction, the precocity of disease, and more extensive clinical features over successive generations. Phenocopy in TBDs (highlighted in purple) describes an individual that does not carry the pathogenic variant but exhibits one or more TBD manifestations (for example, pulmonary fibrosis) caused by short telomeres inherited from an affected parent. Incomplete penetrance (highlighted in orange) refers to the existence of individuals who carry a pathogenic variant(s) and short telomeres but are asymptomatic at the time of clinical evaluation. The genotype status (Mut: mutated; WT: wild type), the age of onset, and TBD phenotype are noted (y: years). The white to black colour gradient illustrates the severity of the clinical manifestations (white: healthy/asymptomatic; black very severe TBD symptoms). AA, aplastic anaemia.; BMF, bone marrow failure; CP: Coats plus; DC, dyskeratosis congenita; HH, Høyeraal–Hreidarsson syndrome; PF, pulmonary fibrosis.

Figure 4. Mechanisms of somatic genetic rescue in telomere biology disorders.

a. (1) Direct somatic genetic rescue (SGR) refers to the replacement of the germline pathogenic variant with the wild type sequence either by uniparental isodisomy leading to copy neutral-loss of heterozygosity (CN-LOH) or by point mutation (back mutation²⁰⁴)^{135,207,208}. (2) Another type of direct SGR occurs with the introduction of a loss-of-function (LoF) point mutation or indel in the gene carrying the germline variant that leads to the inactivation of the dominant effect of the germline variant^{80,135}. **b.** Direct SGR in TBD patients carrying a germline pathogenic variant in the *TERT* gene can result from the introduction of specific somatic point mutations in the *TERT* promoter located either at position -57, -124, or -146 from the transcriptional start site^{191,206,209,210}. These somatic mutations create a *de novo* binding site for ETS family transcription factors and induce *TERT* expression and activity²¹³. **c.** Schematic representation of the different types of indirect SGR reported in TBDs. (1) Somatic *TERT**pam* mutation counterbalancing accelerated telomere shortening (as depicted in **b.**) in TBDs caused by germline variants in genes other than *TERT* (for example, *PARN*, *TERC*, *NHP2*, *DKC1*, and *NAF1*)^{46,191,206,209,210}. (2) Somatic LoF mutations in *POT1* that alleviate its telomerase inhibitory role represent indirect SGR by limiting telomere shortening^{209,210}. (3) Somatic mutations in genes involved in RNA decay that could increase the amount of hTR have been proposed to represent SGR in TBDs associated with a reduced level of mature hTR (for example, germline variants in *TERC* and *DKC1*)²⁰⁹.

Box 1. Overview of clinical aspects of telomere biology disorders

Clinical aspects of telomere biology disorders (TBDs) have been described in detail in several recent reviews and cohort studies^{4,79,299-301}. In brief, dyskeratosis congenita is classically defined by a mucocutaneous triad of oral leukoplakia, lacy, reticular skin pigmentation and nail dystrophy, which appear most often in the second decade of life. With advances in telomere length measurements and genetic testing, dyskeratosis congenita is frequently diagnosed before these features develop due to progressive bone marrow failure. Underlying bone marrow failure is an accelerated depletion of haematopoietic stem and progenitor cells, leading to impaired production of one or several blood cell lineages. Bone marrow failure may ultimately require haematopoietic stem cell transplantation or evolve to myelodysplastic syndrome or acute myeloid leukaemia^{4,301-303}. Patients with dyskeratosis congenita are also at risk of solid cancers (for example, carcinomas) and interstitial lung diseases^{20,301,304,305}. Cirrhotic liver disease, hepatopulmonary syndrome and gastrointestinal haemorrhage have emerged as additional life-threatening manifestations^{172,227,301}. Numerous other features may develop that affect quality of life (see the figure). Indeed, few organ systems are spared, underscoring the importance of telomere biology in replicative tissues.

Høyeraal–Hreidarsson syndrome, Revesz syndrome and Coats plus syndrome are severe, early-onset TBDs. Patients with Høyeraal–Hreidarsson syndrome most often present at birth or early infancy with intrauterine growth restriction, microcephaly, cerebellar hypoplasia and early-onset severe bone marrow failure²⁹⁹. Immunodeficiency, mainly characterized by a profound reduction of B cells and NK cells, is a frequent cause of death in the first few years of life due to severe infections. Notably, some patients lack profound immunodeficiency but have marked impairments in neurodevelopment, a difference that is likely genotype-dependent.

Revesz syndrome and Coats plus syndrome share features, including bilateral exudative retinopathy (known as Coats disease), intracranial calcification and cysts, bone marrow failure, gastrointestinal haemorrhage, osteopenia, intrauterine growth restriction, postnatal growth failure and sparse hair^{79,306,307}. Revesz syndrome and Coats plus syndrome may be clinically distinguished by the greater severity of bone marrow failure in Revesz syndrome and the leukodystrophy, distinctive pattern of cerebral calcifications and cysts, prominence of gastrointestinal haemorrhage

without prior history of haematopoietic cell transplantation, and propensity of orthopaedic fractures in Coats plus syndrome. They are also distinguishable by genotypes and the resulting impact on global telomere length.

Lastly, the most prevalent type of TBD takes the form of adult-onset lung diseases, including pulmonary fibrosis and emphysema^{20,262,308}. Other clinical manifestations of adult-onset TBDs include bone marrow failure, myelodysplastic syndrome, acute myeloid leukaemia, solid cancers, liver and kidney diseases and gastrointestinal complications.

Glossary

R-loops

Nucleic acid structures composed of an RNA–DNA hybrid and a displaced single-stranded DNA that accumulate at specific regions genome-wide, including telomeres. R-loops can cause replication stress and genome instability.

G-quadruplexes

Four DNA stranded secondary structures formed in G-rich sequences where four guanines form a planar array via Hoogsteen base-pairing. These structure can cause replication stress.

Somatic genetic rescue

In Mendelian disorders, an in vivo somatic genetic event that partially or totally counteracts the deleterious effect of the pathogenic germline mutation and provides a selective advantage over non-somatically modified cells.

Clones

Cells that originate from a common cell ancestor (progenitor) with identical genetic identity.

Genetic anticipation

A phenomenon observed in autosomal dominant diseases in which some clinical manifestation(s) develop earlier and are more severe with successive generations.

Phenocopy

A phenomenon where the phenotype dissociates from the genotype. In the present review, when individuals from familial TBD exhibit short telomeres without carrying the causing germline variant.

Monoallelic variation

One genetic variant located on one allele of a gene.

Biallelic variation

Two (possibly different) variants located on both alleles of the same gene.

Hypomorphic

Refers to a variant that results in reduced but not eliminated function of the gene product.

Compound heterozygous

The existence of distinct mutations on opposite alleles of a single gene located on an autosomal chromosome.

Cajal bodies

Distinct sub-nuclear structures present in eukaryotic cells associated with RNA metabolism and ribonucleoprotein biogenesis.

Incomplete penetrance

Refers to the phenomenon of some individuals carrying a pathogenic variant will not exhibit clinical signs.

Variable expressivity

Refers to the phenomenon of individuals affected by a Mendelian disease exhibit different clinical features.

Repeat addition processivity

The ability of telomerase to synthesize multiple telomeric repeats without dissociating from the telomere.

t-circles

Extrachromosomal circular DNA molecules containing telomeric repeat sequences.

Uniparental isodisomy (UPD)

Refers to both copies of a chromosome originating from one parent (maternal or paternal) and the chromosome from the other parent is absent. Segmental uniparental disomy occurs when only part of a chromosome is affected.

ToC blurb

Mendelian defects in genes that encode factors regulating telomere length, structure and function cause telomeropathies, or telomere biology disorders (TBDs). The authors review confirmed as well as potential TBD-causing genes and their main functions in telomere biology. They also discuss genetic features that underlie the complex nature of these diseases.