

## Telomeropathies - A Genetic Conglomerate

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**Received:** February 16, 2021

**Published:** March 20, 2021

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### Abstract

Telomeres are repeated TTAGGG hexanucleotides and proteins associated with them. Telomeres function to form a stable end of the chromosome and protect it from being recognized as a DNA fragment that needs repair. Telomeropathies or 'telomere biology disorders' are rare and heterogenous diseases that are caused by mutations in the maintenance of telomerase or the damage response system (DDR). Telomeropathies or telomere biology disorders (TBDs) may be classified as primary and secondary. Primary telomeropathies are due to defects in the telomere maintenance machinery whereas secondary telomeropathies are caused by failures in the DDR.

Dyskeratosis congenita (DKC) which is an inherited bone marrow failure syndrome is the classical example of the telomeropathy syndromes. Progressive telomere loss or attrition is associated with and is likely to be an etiology of malignant transformation. Over 85% of malignant tumors show positivity for telomerase, in contrast most normal tissues are telomerase-negative, and owing to this telomerase enzyme has become an excellent target in research for newer therapies that inhibit telomerase and shorten telomeres in malignant cells. In aplastic anemia, patients with the shortest telomeres are four- to five times more likely to have clonal evolution of disease and undergo progression to myelodysplastic syndrome and leukemia. The telomeropathies hence resemble a spectrum of disorders rather than being distinct diseases.

Diagnosis of a telomeropathies needs awareness with regards to the clinical features the laboratory results and the molecular testing. Due to the rarity of telomeropathies and a low degree of suspicion the diagnosis may often be missed. For some telomeropathies early diagnosis will translate into overall survival benefits and improved quality of life. In this review, we give an overview of the pathophysiology, signs and symptoms of the disorders caused by defects in telomere biology and touch upon the detection of telomeropathies in the laboratory.

**Keywords:** Telomeres; Telomerase Complex; Dyskeratosis Congenita; Telomere Length; Flow-FISH

### Introduction

Telomeropathies or 'telomere biology disorders' (TBD) are a group of rare genetic disorders caused by defects in the maintenance of the telomerase complex along with the DNA damage

response sensing mechanisms. As the cell ages there is progressive loss of telomeres with every cycle of replication. This loss is however harmless as these are non coding regions. Besides ageing environmental factors such as oxidative damage and regenerative

stress can also lead to shortening. Critical shortening leads to senescence, apoptosis, or, rarely malignant transformation. The progressive shortening of telomeres is counteracted by the telomerase enzyme that extends telomeres. When cell division continues despite critically short telomeres this protective function is gradually lost.

The association of Telomeres and bone marrow failure was postulated after identifying shortening of telomeres in the leukocytes of acquired AA patients [1,2].

Thereafter followed the correlation shortened telomeres were the genetic caused of inherited aplastic anemia or Dyskeratosis congenita [3]. Progressive telomeres loss is an explanation for the 'Hayflick limit' a concept that explains the mechanisms underlying cellular aging. This concept states that a normal human cell after forty to sixty divisions break down by programmed cell death or apoptosis as it cannot divide beyond this limit Somatic cells such as lymphocytes have telomeric DNA ranging from 8 to 14 kilobases (kb) at birth, 50 to 100 base pairs of this DNA is removed per cell division. The normal rate of telomere shortening in normal individuals is approx 60 bp/yr whereas in telomere disorders it increases to approximately 120 bp/ yr [4-6].

**TELOMERES - The normal biology**

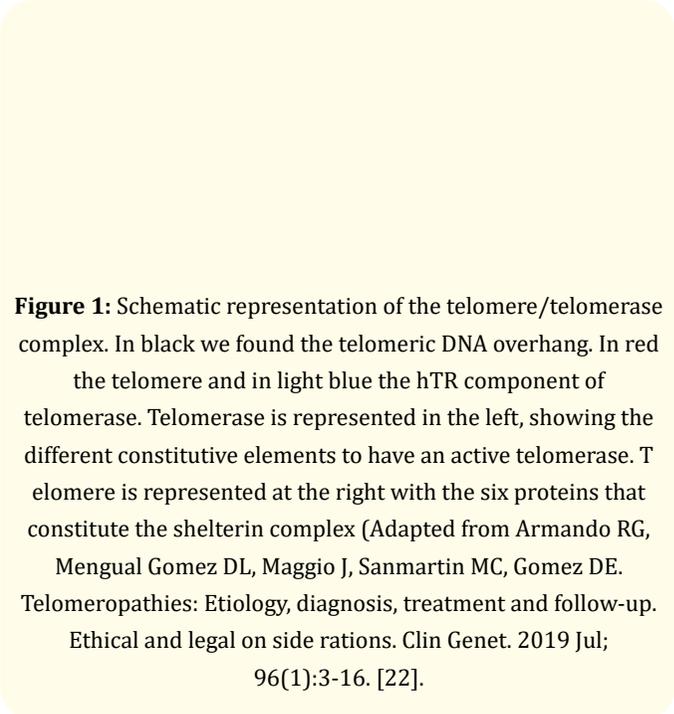
Telomeres are nucleoproteins, and their DNA consists of repetitive sequences, TTAGGG in humans. Telomeres are formed by a 30- to 400-nucleotide long overhang of a guanosine-rich strand, also known as the G-strand overhang which folds back to a double-stranded area forming a T-loop and a displacement or D-loop. Telomerase is a ribonucleoprotein enzyme complex that synthesizes telomeres.

The Telomerase complex includes-

- Telomerase: a reverse transcriptase (encoded by the gene TERT).
- A RNA template (encoded by TERC), and
- Associated proteins that affect assembly, trafficking, recruitment of telomerase to telomeres, and stability of telomerase, a number of accessory proteins including dyskerin, NHP2,NOP10, and GAR1.

Telomeres on are flanked by special regions, called the shelterin complex that regulate telomere lengths and protect them

from DNA damage response. The shelterin complex is a 6-protein complex - telomeric repeat binding factors 1 and 2 (TRF1, TRF2) TRF1-interacting protein 2(TIN2) protection of telomeres Protein1 (POT1) POT1-interacting protein (TPP1) and repressor activator protein 1(RAP1) as shown in figure 1 [8].



The gene coding for human TERC maps to chromosome 3q and comprises of 451 nucleotides, which includes a 11 bp long RNA template (50-CUAACCCUAAC-30) complementary to human telomere repeats (TTAGGG).

While TERC can be found in abundance in tumor and non-tumor cells, the catalytic subunit TERT is expressed increasingly in cells with self-renewal capacity such as hematopoietic stem and progenitor cells (HSPCs), proliferating lymphocytes and the regenerating cells in the basal layer of the epidermis [9].

The other associated proteins include dyskerin, NHP2 (nuclear protein family A, member 2), NOP10 (nuclear protein family A, member3), NAF1 (nuclear assembly factor 1), and GAR1 (nuclear protein family A, member 1). These proteins help to recruiting, trafficking and stability of the telomerase complex. If telomeres lack shelterin, the extreme end of telomeres are recognized as double

strand breaks (DSB) p53 is activated and this leading to apoptosis senescence or both. This forms an integral part of the DNA damage response pathway.

Part of the mechanism by which telomere ends are hidden is via a structure known as a T-loop; During DNA replication owing to the action of RTEL1 or other helicases the T loop dissociates and further excision of The T-loop occurs by the SLX4 nuclease leading to rapid telomere loss.

**Telomeropathies and their pathogenesis**

The pathogenesis of telomere biology disorders includes decreased telomerase activity the most common mechanism of which is TERT, TERC, DKC1, NHP2, NOP10, and NAF1 mutations leading to decreased telomerase levels or activity. There can be impaired telomerase recruitment to telomeres due to defective TCAB1. Impaired telomere duplication and impaired regulation of the telomere biology related gene transcripts involving the CST complex are other causes for impaired function [10]. These candidate genes associated process and the diseases resulting thereof are depicted in table 1.

There are a few additional factors and these include TRF1 and TRF2-associated factors, such as Apollo, MRN complex, PNUTS, MCPH1, WRN/FEN1, TNKS and the ORC complex TERRA. These have a variety of functions including telomere maintenance, response to certain types of DNA damage; regulation of telomere length and protecting telomeres from DNA repair [11].

**Dyskeratosis congenita- The prototype Telomeropathy**

DC is the second most common type of inherited bone marrow failure syndromes (IBMFS) and is named after its clinical manifestation of ectodermal dysplasia, a triad of oral leukoplakia dystrophic nails, skin hyperpigmentation. DC was initially described by Zinsser and was recognized as a clinical entity by Engman and Cole [12,13].

Three different inheritance patterns have been described in DC: X-linked, autosomal dominant (AD), and autosomal recessive. In large registries, the most common inheritance pattern reported is X-linked, affecting the DKC1 gene, which encodes dyskerin, a component of the telomerase complex. The clinical features in DC present early in childhood. Skin pigmentation and nail dystrophy appear first, usually by the first decade of life. Hyper- or hypopig-

Candidate gene	Process/complex	Diseases
TIN2	Forms Shelterin complex inhibits TRF1 PARsylation	DKC, Revesz syndrome, HHS
RTEL1	Associated with T-loop dissociation, target of cytosolic iron-sulfur protein assembly (CIA) complex	DKC/HHS
CTC1	Associated with CST complex	Coats plus syndrome
Apollo	Overhang processing	HHS
TERT, TERC, Dyskerin, NHP2, NOP10	Form the Telomerase complex	DKC, aplastic anemia IPF
TCAB1	Cajal body, telomerase assembly	DKC

**Table 1:** Genes and their respective complexes in telomere biology disorders.

mentation of tan-to-gray colour can be seen which on Histopathology presents as Poikilodermatous changes with an atrophic and telangiectatic epidermis. Ectodermal abnormalities such as alopecia of the scalp, eyebrows, and eyelashes; premature graying hair; hyperhidrosis; hyperkeratosis of the palms and soles; and adermatoglyphia (loss of dermal ridges on fingers and toes) are commonly encountered. BM failure usually develops below the age of 20 years. Macrocytosis with or without anemia is common in the telomeropathies. Over patients 90% have peripheral cytopenias by the third decade of life. The bone marrow in such patients may be indistinguishable from acquired aplastic anaemia [14].

80% cases have underlying pulmonary complications, including fibrosis and abnormalities of pulmonary vasculature. On high resolution computerized tomography patchy, basal, and peripheral reticular opacities, ground glass opacities, and honey combing is seen. Bronchiolitis obliterans organizing pneumonia, chronic hypersensitivity pneumonitis, emphysema alone, or combined pulmonary fibrosis and emphysema can also be seen [15,16]. Hepatic involvement in telomere diseases may show changes related to cir-

rphosis, portal hypertension, or steatosis. Presence of both pulmonary fibrosis and bone marrow failure is highly specific for an underlying telomere biology disorder [17]. Esophageal and lacrimal duct stenosis, enteropathy, enterocolitis, osteoporosis, avascular necrosis, and immunodeficiency have been described [18].

The main causes for mortality include bone marrow failure and immunodeficiency (60–70%), pulmonary complications (~10–15%) and cancers (~10%) [14].

Owing to the association of DKC and its more severe variants with underlying malignant transformation these patients should undergo annual health checkups and avoid alcohol, sun exposure, smoking etc.

**Severe forms of DC- These include the following variants**

**Hoyeraal-Hreidarsson syndrome**

Hoyeraal-Hreidarsson syndrome (HHS) with a path gnomic cerebellar hypoplasia is a serious and extremely rare presentation of DC. It has an X linked recessive inheritance. Clinical features include intrauterine growth retardation bone marrow failure, immunodeficiency and microcephaly. So far only about 50 cases have been described in literature. HHS-causing mutations have been found in TRF1, TIN2 and RTEL1 genes. The RTEL gene encodes the helicases that unwinds the T loop [19].

**Revesz syndrome**

Revesz syndrome (RS) is defined by symptoms of HHS along with prominent exudative retinopathy which characterizes this syndrome. The mode of inheritance is autosomal dominant and the underlying mutation is in the TINF2 gene.

**Coats plus syndrome- Cerebroretinal microangiopathy with calcifications and cysts**

This resembles Revesz but cerebellar and hematologic manifestations are less prominent. It is due to compound heterozygous mutations in CTC1 [20].

**Secondary telomeropathies**

The secondary telomeropathies are disorders which have an underlying telomeropathy but whether this is a primary driver of disease or acts just as a catalyst is yet to be known [21]. These are briefly described in the table below in table 2.

Disease	Inheritance	Genes	Salient features
Aplastic anemia	AD	Telomerase core components: TERT TERC	Symptoms of marrow failure
Fanconi anemia	X-LR AR AD AR	Telomerase biogenesis: DKC1 NOP10 NHP2 Shelterin c omponents ACD FANCD2	
Idiopathic pulmonary fibrosis	AD	Telomerase core components: TERT TERC Telomerase biogenesis: NAF1	Bronchiolitis obliterans with organizing pneumonia, chronic hypersensitivity pneumonitis, interstitial pneumonitis, and emphysema  familial interstitial pneumonia
Cryptogenic cirrhosis or nodular regenerative hyperplasia	AD	Telomerase core components: TERT TERC	Hepatic parenchymal inflammation, hepatic fibrosis, cryptogenic cirrhosis of the liver, nodular regenerative hyperplasia, and portal hypertension

Rothmund-Thomson syndrome	AR	RECQL4	
Immunodeficiency, centromeric region instability and facial anomalies type 1	AR	DNMT3B	Dimorphism in the middle portion of the face, growth retardation and a psychomotor retardation senescence of B and T lymphocytes and a spectrum of immune defects
Baller-Gerold syndrome		RECQL4, FGFR2, TWIST	Coronal craniosynostosis with radial ray anomalies, facial dimorphism, delayed growth
Bloom syndrome	Autosomal recessive	BLM	Dolichocephalism, a narrow face, a prominent nose and ears and mandibular hypoplasia, facial telangiectasia, low white blood cell count and progressive lung disease
Xeroderma pigmentosum	AR	XPC, DDB2, ERCC2, EERCC3, ERCC4, ERCC5, POLH and XPA	Premature ageing, cutaneous cancer, neurological manifestations propensity to cancer

Trichothiodystrophy		ERCC2( most frequent) TTD2, ERCC3/XPB, GTF2H5, MPLKI, RNF113A	Brittle fragile hair, often combined growth retardation intellectual deficit, congenital ichthyosis, ocular and nail abnormalities, short stature, frequent infections, infertility
Cockayne syndrome	AR	ERCC6(most common) (CSA, CSB, XPB, XPD and XPG	Growth failure, premature aging, photosensitivity, progressive neurological dysfunction
Seckel syndrome	AR	ATR	microcephalic primordial dwarfism, dysmorphic face, clinodactyly
Ataxia telangiectasia		ATM kinase	Staggering gait, muscular incoordination, immunodeficiency, neurodegeneration and premature aging, increased occurrence of malignancy
Facioscapulothumeral muscular dystrophy	Autosomal dominant	DUX4	

**Table 2:** The secondary telomeropathies their inheritance pattern and salient features.

### Diagnosis of telomere biology disorders

Detection of a telomere disease requires awareness with regards to the clinical features the laboratory testing and subsequent genetic testing. Due to the rarity of telomeropathies and a low degree of suspicion the diagnosis may often be missed. Several methods are available for measuring the length of telomere repeats of these available methods analysis of terminal restriction fragment (TRF) length by Southern blot analysis is the gold standard. However, this method overestimates the telomere length by several kb because the distance between terminal restriction sites in the genomic DNA and the actual telomeres varies between chromosomes. The TRF method is advantageous in being reproducible however it needs large amounts of DNA and takes a longer time. The PCR based methods that are available measure either average or chromosome specific length. Measuring the average chromosome length provides a measure of telomere DNA in relation to the germline DNA (usually a single-copy gene) as a single ratio value whereas measuring chromosome specific length gives information on the actual length of telomere repeats at a specified chromosome arm [23,24].

Fluorescence in situ hybridization of telomere repeats measured by flow cytometry (flow-FISH) is a quick, relatively inexpensive technique to measure telomere length. It was first described as a modification of Q FISH (quantitative) by Rufer, *et al.* in 1998 [25]. The underlying principle of Q FISH is that at low ionic strength the peptide nucleic acids (PNA) can anneal to complementary single stranded DNA sequences while single stranded DNA sequences cannot. Quantitative hybridization to telomere repeats is achieved using conditions that only allow labeled (CCCTAA) PNA to hybridize to (TTAGGG) target sequences. Flow FISH is essentially the combination of flow cytometry with cytogenetics. The mean telomere length in lymphocytes by flow-FISH is less than the first percentile when compared with age-matched controls accurately identifies patients with telomerase gene mutations. Flow FISH involves six basic steps of cell separation, DNA denaturation, hybridization with PNA probe, a washing step to remove excess probe, DNA counterstaining and finally acquisition and analysis. Sample is collected in sodium heparin or EDTA or citrate vacutainer tubes and is processed within 24–48 hrs. Single-cell suspensions of cow thymocytes are used as internal control. White blood cells are purified or separated by osmotic lysis of RBCs with NH<sub>4</sub>Cl. A high con-

centration of RBCs or hemoglobin (corresponding to a haematocrit of more than 2%) interferes with telomere fluorescence measurements by quenching of the relatively weak FITC fluorescence of the telomere probe. Bovine thymocytes are used as internal control, and the telomere length in bovine thymocytes is about 2–3 times longer than that in human cells. The advantage of Flow FISH is that it can determine mean length for specific cell populations, can provide cell type specific information and can be automated. Samples with relatively low cell counts ( $10^5$ ) can be processed. Minimum detectable difference in telomere length is in range of 0.2 to 0.5 kb. The disadvantages are that it is labour intensive, requires highly skilled labour and telomere length expressed as relative fluorescence unit [2,26].

### Conclusion

The telomeropathies, especially when they are mild or occur in a chronic form may be misdiagnosed or may go unrecognized. Therefore, a high degree of suspicion is essential on the part of the treating clinicians. The association of telomerase mutations with disease in three separate organ systems - the lungs, the liver and the bone marrow has important consequences for the patients and the treating physician. In the family history, attention should be paid to even mild blood count abnormalities in relatives, as well as more severe hematologic disease, especially acute myeloid leukemia. In other organ systems, usually neglected in a standard history, pulmonary fibrosis and hepatic cirrhosis are important clues to the diagnosis of a telomeropathy. If treatment protocols for the TBD are available then these schemes and updated recommendations should be followed if already available and any new therapies or clinical studies should be kept in mind. If the disease does not have a cure preventive care strategy, symptomatic treatment, screening for potential complications and supportive treatment should be given [22].

The importance of telomeres in repair and regeneration with regards to fibrogenesis and adipogenesis may be of particular interest. Telomere attrition linking chronic inflammation and carcinogenesis may be a pathway that could be modulated by drugs or hormones that maintain or elongate telomeres. Explanations for variations in the genotype and phenotype, high degree of variable penetrance, organ specific involvement, and clinical course are still missing and are areas of potential research.

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