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Chapter 5

Molecular Diagnosis and Precision Therapeutic Approaches for Telomere Biology Disorders

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Abstract

Telomeres are nucleo-protein structures located at the end of chromosomes that protect them from degradation. Telomeres length is maintained by the activity of the telomerase complex. These structures are protected by a specialized protein complex named shelterin. In the absence of telomerase activity and/or protection telomeres are shortened after each round of DNA replication. When a critical size is reached, telomeres are recognized as damaged DNA by the cell p53-dependent DNA-repair system. Persistent activation of this pathway finally results in cell apoptosis or senescence.

There are a number of rare hereditary diseases caused by the presence of shortened telomeres, collectively named telomeropathies or telomere biology disorders. In these diseases, cell proliferation is impaired, which results in premature aging and dysfunction of highly proliferative tissues (bone morrow, skin and other epithelia). Among them are Dyskeratosis congenita, the Hoyeraal-Hreidarsson, Revesz and Coats plus syndromes, Aplastic anemia, Idiopathic pulmonary fibrosis and nonalcoholic, noninfectious liver disease. Mutations present in the genes coding for component of the telomerase and shelterin complexes and other proteins involved in telomere replication are the cause of these diseases. Clinical manifestations, causative mutations, diagnosis and possible therapeutic approaches to these diseases will be discussed in this chapter.

Keywords: telomere, telomere biology disorders, telomeropathies, pulmonary fibrosis, bone marrow failure

1. Introduction

Eukaryotic chromosomes are capped at their ends by specialized nucleo-protein structures, named telomeres that protect them from degradation. Human telomeres have a specific nucleotide sequence composed by thousand of repetitions of the TTAGGG hexanucleotide [1]. A protein complex, named shelterin associates to this DNA region to form the telomere-specific chromatin structure. Telomeres protects the chromosomal ends from degradation and are, therefore, essential for chromosomal and genome stability [2]. In their absence chromosomal ends are recognized as damaged DNA by the cell and can be degraded or recombined with other chromosomal ends resulting in the fusion and reorganization of chromosomes [3]. The maintenance of telomeres is, therefore, of critical importance for the genetic stability of cells and organisms.

Replication of telomeric DNA requires the contribution of a specific enzymatic machinery. DNA polymerases responsible for replication of the rest of the chromosomal DNA cannot completely synthesize telomeric DNA. DNA polymerases always require a primer molecule that cover the 5′ end of the DNA and are not able to complete the synthesis of the lagging strand of lineal DNA molecules, such as chromosomes. This end-replication problem results in the shortening of each telomere by 50-100 nucleotides at each DNA replication cycle and, therefore, at each cell division [4]. In most eukaryotic organisms, including humans, telomere length is maintained by the activity of the telomerase complex that elongates the telomeres by a replication-independent mechanism [5]. The complex is formed by a protein with reverse-transcriptase activity (TERT) and one RNA with a region of homology to the telomere DNA that is used as template for elongation [6]. Telomerase activity is, therefore, required for unlimited cell proliferation. Telomerase components and, in particular the TERT gene, are expressed to high relative levels during embryonic development allowing high cell proliferation rates. Expression of the TERT gene is, however, repressed in most human adult cells [7]. TERT expression is found only in germinal cells, in stem cells, specially in those of highly proliferative tissues such as bone marrow and epithelia and in lymphocytes [8]. The rest of the cells express very low TERT levels and their telomeres get progressively shorter after each cell division. When telomeres reach a critical size get unprotected and are recognized as damaged DNA. The ATM and ATR kinases, that regulate cellular responses to DNA damage are recruited to critically-short telomeres and activate the p53-dependent pathway that results in cell cycle arrest [3]. Prolonged arrest would finally induce apoptotic cell death or cellular senescence. Actually, most tissue-specific stem cells do not express enough TERT protein to completely replicate their telomeres at each cell division and their proliferative capacity decreases with the age of the organism [9]. With time, stem cell exhaustion impairs tissue renewal. Because of this reason, telomere shortening has been recognized as one of the hallmarks of human aging [10].

Telomere replication is also involved in the acquisition of the unlimited proliferative capacity that characterizes tumor cells [7]. Telomerase expression and activity is induced in about 85% of tumors, which allows tumor cells to completely elongate their telomeres at each cell division. In the other about 15% of tumors, telomeres length is maintained by a telomerase-independent

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mechanism, know as Alternative Lengthening of the Telomeres (ALT) that elongates telomeres through DNA recombination mechanisms [11].

The importance of telomere homeostasis is further enforced by the existence of a number of rare hereditary diseases that are caused by the presence of shortened telomeres, collectively named telomeropathies, short telomere syndromes or telomere biology disorders [12]. These diseases are caused by mutations in genes coding for proteins involved in telomere lengthening (telomerase complex and related proteins) or in the maintenance of telomere structure (shelterin complex). These diseases are commonly characterized by the presence of very short telomeres in the cells of the affected patients. The molecular pathology of these diseases, their diagnosis and emerging therapies will be summarized in this chapter. It is necessary to enforce the importance of telomere homeostaxis for healthy life since excessively long telomeres are also causative of disease. Recent reports have associated the presence of long telomeres to increased frequency of cancers such as melanoma or glioma [13]. Mutations in the promoter region of the TERT gene that increase gene expression are frequently found in these and other tumors [14, 15]. In addition, mutations in the coding region of genes coding for protein of the shelterin complex have been found in human tumors [16, 17].

2. Main body

2.1. Telomere structure

Telomeres have a very specialized chromatin structure that is required to protect chromosome ends from degradation and to avoid telomere-telomere fusions [2]. The general structure will be briefly summarized in this section of the chapter and is schematically shown in the upper panel of Figure 1 where the genes mutated in telomere biology disorders are indicated by asterisks. Telomere structure has been the subject of several recent excellent reviews [2, 12, 18, 19]. The nucleotide sequence of telomeres is composed for multiple repetitions of the TTAGGG hexanucleotide in humans and several other animals. The length of these regions is variable in different organisms. In humans, telomeres have and average size of 8-14 kb in peripheral blood cells in new born children [20]. The size decreases with age so that the average size in a 90-years old person is of 3-7 kb [12]. In contrast, most mice strains used in research have an average telomere length of 50-100 kb which has made more difficult the development of mouse models of telomere biology disorders [21, 22].

Telomere ends are not formed by blunt-ended double-stranded DNA, as might be expected. Instead, the 3′ strand is about 75-300 bases longer than the 5′ end strand forming an over-hanging single-stranded DNA fragment (Fig 1, upper panel) [1]. The overhanging strand contains the TTAGGG sequence and is, therefore, known as the G-rich strand. The complementary strand contains the complementary CCCTAA repeats and is named the C-rich strand. The overhanging strand is not unstructured. Instead, it turns over the telomere DNA and intercalates in the neighbouring double-stranded DNA forming a loop, named the T-loop, as schematically shown in the upper panel of Figure 1 [23]. Looping results in the formation of a

triple stranded DNA region known as D-loop that is required for the stability of the terminal telomeric DNA region [24].

Telomeres are further stabilized by the presence of a specific protein complex, the shelterin complex. It is composed by six different proteins: TRF1, TRF2, RAP1, TIN2, TPP1 and POT1 (upper panel of Figure 1] [2]. TRF1 (telomeric repeat binding factor 1, encoded by the TERF1 gene) binds to telomeric double-stranded DNA as a dimmer [25]. TRF2 (telomeric repeat binding factor 2, encoded by the TERF2 gene) also binds double-stranded DNA as a dimmer and associates with TRF1 [26]. The TIN2 protein (TRF1-interacting protein 2] interacts with TRF1 and TRF2 [27] and recruits the POT1(Protection of telomeres protein 1]/TPP1(POT1-interacting protein 1] heterodimer [28]. POT1 binds with high affinity to the G-rich strand overhang [29]. RAP1 (repressor-activator protein 1] incorporates to the shelterin complex via TRF2 interaction [28]. In addition, telomeres and subtelomeric regions are enriched in heterochromatin components including the association of HP1 proteins and histone 3 Lysine 9 and histone 4 lysine 20 trimethylation which further contributes to their stability [30].

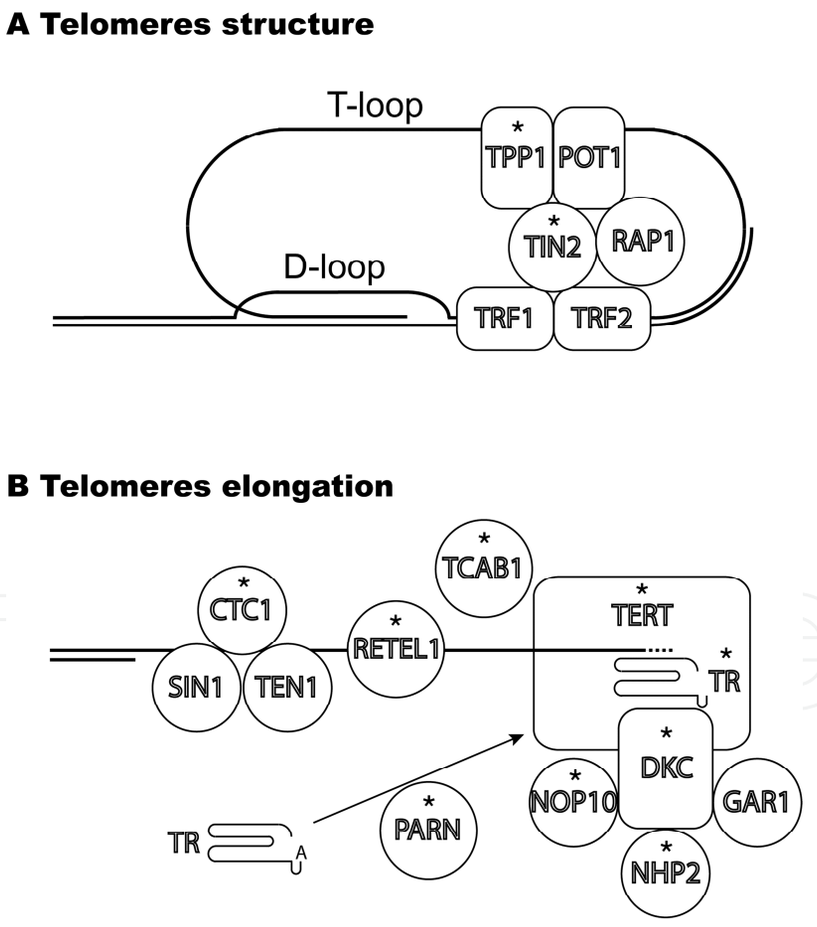

Figure 1. Telomere structure and elongation mechanism.

The shelterin complex is required for telomere maintenance and function and prevents the recognition of telomeres as damaged DNA. TRF2 inhibits the ATM kinase that induces the

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canonical non-homologous-end-joining DNA repair pathway that would result in telomere-telomere fusions [31]. In addition, POT1 inhibits signalling by the ATR kinase in response to DNA damage by double-strand and single-strand breaks and alkylating agents [32]. POT1 also inhibits sister-telomere associations [33].

Telomere structure is schematically shown in upper panel A of the figure. Telomeric DNA is formed by repetitions of the TTAGGG hexanucleotide and is represented as two lines in the figure. The upper line represents the leading, G-rich strand. The 3′ end of this strand is single-stranded and forms a loop (T-loop) to hybridize to a region of the upstream double-stranded DNA forming a smaller loop (D-loop). The lower line represents the lagging DNA strand. Proteins of the shelterin complex, which binds to telomeric DNA, are represented as boxes. Asterisks indicate the proteins whose encoding gene have been found mutated in patients with telomere biology disorders. Panel B represent the components involved in telomere elongation. DNA is represented as two lines and proteins as boxes, as indicated on panel A. The DNA leading strand is the upper one and the DNA being synthesized is represented as a broken line. The components of the telomerase complex that catalyzes telomere elongation are represented on the right of the panel. Telomerase complex is composed by the TERT, DKC, NOP10, NHP2 and GAR1 proteins and the RNA molecule TR. The protein TCAB1 is required for telomerase recruitment to telomeres. PARN is required for TR RNA processing. The RETEL1 helicase and the CTC1/SIN1/TEN1 complex facilitate telomere elongation by disrupting DNA secondary structures. Asterisks indicate the proteins whose encoding genes have been found mutated in patients with telomere biology disorders.

2.2. Telomere elongation

In this section a brief summary of the telomere elongation process and its regulation will be presented because many of the proteins involved are related to telomere biology disorders. A schematic representation of the process is shown at the lower panel of Figure 1. Genes mutated in telomere biology disorders, as described in next sections, are indicated by asterisks. Telomere replication has been reviewed recently by several authors [18, 19, 34-36]. Cellular DNA polymerases cannot complete the replication of DNA double-strand ends which is know as the end-replication problem [37], as mentioned in the Introduction section. In mammals, telomere DNA 3′ ends are elongated by the telomerase complex through reverse transcription. The catalytic activity of the complex resides in the TERT (telomerase reverse transcriptase) protein [6] while the 454 nucleotides long TR (telomerase RNA, encoded by the TERC gene) is used as template [38] (Figure 1, lower panel) [36]. This is because TR has an internal region complementary to the TTAGGG repeats that allows hybridization to the 3′ end of the telomere DNA [36]. The TR RNA has similarity to other small nucleolar RNAs and contains a H/ACA motif [39]. It is transcribed by RNA polymerase II and contains a Cap structure and its 5′end and a 3′ oligo-Adenosine tail [40]. It has been recently shown that the 3′ end of TR has to be processed through exonuclease cleavage and that the Poly(A)-specific ribonuclease (PARN) is required for this maturation process [41].

Additional proteins form part of the telomerase complex and are required for its assembly and stability. Among them, dyskerin (encoded by the DKC1 gene) binds to TR through its inter-

action with the dyskerin PUA RNA binding domain [42]. Dyskerin binding is required for TR stability and for its recruitment to the telomerase complex. The proteins NHP2 and NOP10 associate to dyskerin and are also required for telomerase assembly [43].

Telomerase activity is dependent on TERT gene expression that is tissue-dependent and developmentally regulated, as mentioned in the Introduction section. In addition, it is dependent on protein-protein interactions that regulate telomerase-complex assembly and its recruitment to telomeres. One of the proteins involved is TCAB1 (encoded by the WRAP53 gene) that is an essential component of the subnuclear Cajal body structures. TACB1associates to TR, contributing to telomerase assembly in these structures [44]. Depletion of TCAB1 results in a relocation of the telomerase complex to the nucleolus and reduced recruitment to telomeres [44]. The shelterin-complex component TPP1 is also required for the recruitment of telomerase to telomeres. The TEL patch of TPP1, rich in Glutamate and Leucine residues, interacts with the TEN domain of TERT to mediate telomerase recruitment [45]. This interaction also promotes the telomerase ability to catalyze repeated cycles of DNA synthesis at the telomeres [46].

After elongation of the G-rich overhanging strand by the telomerase complex the C-rich strand is synthesized by the activity of the primase/DNA polymerase complex [35]. Proteins with helicase activity are required to facilitate telomere elongation through disruption of DNA structures that impair telomerase and DNA polymerase activity. The G-rich nature of the overhanging strand favours the formation of secondary structures such as G-quartets [47]. In addition, the D-loop structure of telomeres can also impair DNA synthesis. One of the proteins involved in solving DNA structures at the telomeres is RETEL1 (regulator of telomere length 1], a DNA helicase with D-loop-disrupting activity that has been proposed to facilitate T loop unwinding and to counteract the formation of G-quartets [48, 49]. In addition, RETEL1 has additional functions at other DNA loci [50]. The CST complex, formed by the CTC1, STN1 and TEN1 proteins promotes the initiation of the lagging C-rich strand synthesis [51]. CTC1 binds to the single-stranded G-rich DNA strand and recruits the initiator Polα primase complex to accomplish C-rich strand synthesis [52].

Once DNA synthesis is completed at telomeres, DNA is further processed to generate the G-strand overhangs [35]. This is a highly regulated process so that G-strands overhangs are between 30 and 400 nucleotides long and the C-rich strand ends at the 3′-CCAATC-5′ sequence in most telomeres [53]. Processing requires the activity of several factors: the Apollo/SNMB1 nuclease, Exonuclease I, the CST complex and two shelterin proteins, POT1 and TRF2 [54].

It is important to enforce that although telomere structure and replication have been presented separately for simplicity they are highly interconnected processes. For example, proteins of the shelterin complex play essential roles in telomere elongation through recruitment of the telomerase complex and proteins involved in the regulation of telomere length.

2.3. Telomere biology disorders

Several diseases related to telomere biology will be described in this section. The clinical manifestations of each disease will be described together with their molecular bases. These

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diseases are multisystem genetic disorders that share many of the affected genes (see [12, 13, 18, 19, 55-58] for recent reviews). They also have in common the presence of very short telomeres in the affected patients. However, time of onset, phenotype and clinical severity of these diseases are very heterogeneous. Some of the disorders manifest in young children as it is the case of Dyskeratosis congenita, Hoyeraal-Hreidarsson syndrome, Revesz syndrome and Coats plus syndrome that are rare diseases presented with very low frequency in the population. Other diseases that manifest at older ages, generally in adults, are less severe and more frequent in the population. Among these are Aplastic anemia, Lung disease and Non-alcoholic, non-infectious liver disease. A summary of these diseases, their clinical manifestations, time of presentation and the genes that have been found mutated is shown in Table 1.

Disease	**Symptoms**	**Presentation time**	**Mutated genes (inheritance)***
Dyskeratosis congenita	Nail dysplasia	Childhood	DKC1 (XL)
	Abnormal skin pigmentation		TERT (AD,AR)
	Oral leukoplakia		TERC (AD)
	Bone marrow failure		TINF2 (AD)
	Pulmonary fibrosis		WRAP53 (AR)
	Liver abnormalities		NOP10 (AR)
	Avascular necrosis of the hips		NHP2 (AR)
	Stenosis of the exophagus, lacrimal ducts and/or uretra		CTC1 (AR)
	Increased cancer risk		RTEL1 (AD,AR)
	Osteopenia, risk of bone fractures		PARN (AR)
	Psychiatric disorders		
Hoyeraal-Hreidarsson syndrome	Intrauterine growth retardation	Early childhood	DKC1 (XL)
	Microcephaly		TINF2 (AD)
	Cerebellar hypoplasia		TERT (AR)
	Thrombocytopenia		RTEL1 (AR)
	Immunodeficiency		TPP1 (AR)
	Nonspecific enteropathies		PARN (AR)
	Bone marrow failure		
Revesz syndrome	Bilateral exudative retinopathy	Early childhood	TINF2 (AD)
	Bone marrow failure		
	Intrauterine growth retardation		
	Intracraneal calcifications		

Disease	Symptoms	Presentation time	Mutated genes (inheritance)*
	Developmental delay		
	Fine, sparse hair		
	Nail dystrophy		
Coats plus syndrome	Bilateral exudative retinopathy	Early childhood	CTC1 (AR)
	Retinal telangiectasias		
	Intrauterine growth retardation		
	Bone abnormalities with poor healing		
	Gastrointestinal vascular ectasias		
Aplastic anemia	Bone marrow failure	Middle age	TERC (AD) TERT (AD)
Idiopathic pulmonary fibrosis	Pulmonary fibrosis	Middle age	TERC (AD)
	Emphysema		TERT (AD)
	Interstitial pneumonitis		PARN (AD)
	Honeycombing in high resolution computarized tomography		RTEL1 (AD)
Nonalcoholic, noninfectious liver disease	Hepatic fibrosis	Middle age	TERC (AD)
	Noncirrhotic portal hypertension		TERT (AD)
	Hepatopulmonary syndrome		

*XL: X-linked; AD: autosomal dominant; AR: Autosomal recesive

Table 1. Clinical characteristics of telomere biology disorders.

2.3.1. Dyskeratosis congenita

Dyskeratosis congenita (DC) was the first telomere biology disease described in early 1900s [59]. It is an inherited disorder that is usually diagnosed in early childhood. The most characteristic clinical feature is a triad of mucocutaneous features: leukoplakia, reticulated skin pigmentation and nail dystrophy, as shown in Table 1. Lacy reticular pigmentation use to be observed at neck and upper chest. However, DC symptoms have variable expressivity and/or incomplete penetrance and this triad is not always present. Some patients worsen with age and the triad might not be evident in the firsts examinations. The median age of appearance of the triad is approximately 8 years [59]. The variability in the nail phenotype can go from ridging to complete nail loss and can involve both the finger and toenails. The skin may be hyper or hypo pigmented. Leukoplakia may affect other mucosa surfaces. However, additional

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reports expanded the phenotype and it is now recognized as a multisystem disease. One of the most common haematological manifestations is bone marrow failure that is the most significant cause of mortality in DC patients, up to 60-70% [60]. Patients present hypocellular bone marrow and severe cytopenias.

DC patients frequently show other skin manifestations, some of them are atrophy of the dorsal surface of hands and feet,hyperhydrosis and hyperkeratosis of palms and soles. Other mucosal surfaces can also be affected leading to stenosis of the oesophagus, urethra or lacrimal duct. Oesophageal strictures and non-specific enteropathies are common. Dental abnormalities can be also observed including extensive caries in 13-17% of the patients [61]. Early greying and loss of hair also occur. Skeletal abnormalities are also observed in up to 5% of the patients [62] including osteoporosis and avascular necrosis. Osteoporosis resembles that seen in natural aging and can led to fractures [63].

Respiratory abnormalities are also a significant cause of morbidity and mortality and cause the death of 10-15% of DC patients [64]. The main clinical manifestation is pulmonary fibrosis that usually is posterior to the mucocutaneous or bone marrow features. Hepatic disease can be also observed including cirrhosis, fibrosis, portal hypertension and portal vein thrombosis and several cases of non-alcoholic, non-infectious liver diseases have been reported [65].

Neuropsychiatric disorders were recently described in 55% of children and 75% of adult DC patients [66]. These disorders include mood, anxiety, psychotic and adjustment disorders, attention deficit/hyperactivity, intellectual disability, learning disabilities and pervasive developmental disorders.

DC patients also present increased risk of cancer development. Co-occurrence of Myelodysplastic syndrome (MDS), Acute myelogenous leukemia (AML) and head and neck squamous cell cancer has been described. A literature review reported a 11-fold increased risk of cancer in DC patients including AML, MDS, tongue cancer, cervical squamous cell carcinoma and non-Hodgkin's lymphoma, with a risk of 40% of developing any cancer by the age of 50 [67].

This diversity in clinical manifestations makes the diagnosis of DC challenging. When the mucocutaneous triad is observed the diagnosis is relatively clear but this is not always the case. Vulliamy et al proposed in 2006 clinical criteria for the diagnosis of DC [68]. These criteria require the presence of the three components of the mucocutaneous triad or one feature of the triad, bone marrow failure and two other clinical manifestations usually found in DC patients, as described above. However, the diagnosis of some patients can still be difficult because the triad might evolve late in time and other clinical manifestations might not be associated to DC because of their diversity.

2.3.1.1. Dyskeratosis congenita as a telomere biology disease, implications in diagnosis

Dyskeratosis congenita may have X-linked inheritance which allowed the identification of one X-chromosome gene that presented missense mutations in several unrelated DC patient. The encoded protein was named dyskerin and the gene DKC1 [60, 69]. Dyskerin was characterized as a highly conserved protein with possible nucleolar functions [60]. Soon afterwards, fibroblasts derived from DC patients were shown to have very short telomeres [70]. These cells

also presented reduced telomerase activity and decreased levels of TERC expression. The correlation between Dyskeratosis and telomere length was strengthened when a large family was found carrying an autosomal dominant mutation in the TERC gene [71].

The recognition of DC as a telomere biology disease helped to understand the biology of this disease. As mentioned in the Introduction, critically short telomeres are recognized as damaged DNA by DNA-damage response pathways that involve the ATM and ATR protein kinases and the p53 protein. Activation of these pathways induces apoptotic cell death and cellular senescence. Telomere shortening is associated to DNA replication, which is specially relevant in cells that have impaired systems of telomere elongation and protection, as is the case of DC patients cells. Therefore, highly proliferative cells are expected to be the firstly affected by telomere shortening. Some of these highly proliferative cells are the stem cells of tissues with high capacity of renewal such epithelia, bone marrow cells and lymphocytes. These cells types are characterized by the expression of high telomerase activity in healthy individuals. Depletion of these stem cell populations can explain the main clinical manifestations of DC patients. Among them are the deficit observed in epithelial tissues such as different mucosa and skin that could be due to insufficient cell renewal because of exhaustion of stem cell populations. Impaired proliferation of bone marrow stem cells also could explain the existence of hypocellular bone marrow and severe cytopenia. Pulmonary alveolar stem cell failure has also been recently described in patients with telomere dysfunction [72]. The reduction in the number and proliferative capacity of stem cells can also explain the premature aging of DC patients as manifested by hair loss and early greying. These data would recognize DC as a stem cell disease.

A second clinical characteristic associated to progressive telomere shortening is the existence of genetic anticipation. It is defined by the occurrence of increasing disease severity and early onset with successive generations, as observed in multigenerational families with autosomal dominant DC [73-75]. Genetic anticipation is due, in these diseases, to non-complete telomere replication in germinal cells due to impaired telomerase activity. Successive generations inherit progressively short telomeres and, therefore, critically short telomeres appear at an early age in highly proliferative tissues of the affected patients.

The identification of telemere shortening also provides one important diagnostic criteria. DC patients are characterized by the presence of very short telomeres in peripheral blood cells. Usually bellow the 1% of the telomere size of control populations of the same age as the patient. Measuring telomere length provides one differential diagnostic criteria for telomere biology diseases. The length of telomeres can be determined by different methods in patient samples [76] and compared with controls of the same age. Variation of telomere length with ethnicity has also been described [77]. One of the methods estimates telomere length by Southern blot. Purified DNA is digested with a restriction enzyme that has recognition sites close to the telomeres (sub-telomeric region) but not at the telomere. Digestion products are separated in agarose gels and blotted to membranes that are hybridized to telomere-specific probes. The distribution of telomeres size and average length can be determined by comparison to the migration of DNA molecular weight markers. The use of this technique requires relatively high amounts of pure DNA.

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Telomere length can also be determined by quantitative PCR methods from clinical samples. Some of these methods use telomere-specific oligonucleotides to determine the amount of telomeric DNA in comparison to non-telomeric DNA in each sample. This method has the advantage that a large number of samples can be easily analyzed but it gives and average length of all the cellular telomeres. However, variation in the length of individual chromosomes can be also important in disease progression [78]. Telomeres rearrangements can also have a large effect on the cell [79] and would not be detected by measuring average telomeric DNA content. A PCR-based method that determines single telomere length (Single telomere length assay, STELA) has also been developed [80]. Telomere length can also be determined by flow fluorescence in situ hybridization (flow-FISH) using peripheral blood lymphocytes [81]. This technique can be used in clinical settings and has been shown to be highly sensitive and specific in identifying patients with DC from their unaffected relatives and healthy controls [81, 82]. Flow-FISH is presently the only clinically certified test for DC.

2.3.1.2. Molecular genetics of Dyskeratosis congenita

DC is a rare inherited disease caused by mutations in genes coding for proteins involved in telomere synthesis and protection. Mutations in ten genes have been identified to date in DC patients [55]. Mutations in these genes explain about 60% of the cases of DC so that there are many cases where the causative gene has not been identified. Until few years ago, molecular diagnosis was made through PCR amplification and DNA sequencing of each exon of the candidate genes, pre-analyzed by High Resolution Melting [83]. Discovery of new genes involved in DC and related telomeropathies required positional cloning and were challenging projects. The development of techniques of massive parallel DNA sequencing makes now possible to sequence either all the genes of a patient (genome sequencing) or all the gene exons (exome sequencing) [84]. Analyses of massive sequencing data greatly facilitates molecular diagnosis as well as the discovery of genes whose causative relationship with the disease was previously unknown. One example is the recent identification of mutations in the PARN gene, coding for a Poly(A)-specific ribonuclease in DC patients [85]. The genetic mutations found in DC patients will be reviewed in this section of the chapter and have been described in recent reviews [18, 86]. A summary is also presented in Table 1. Detailed updated information on the nucleotide variants found in DC-related genes can also be found at the Telomerase Database (http://telomerase.asu.edu/diseases.html).

2.3.1.2.1 Dyskerin (DKC1]

Dyskerin is a 524 amino acids long protein that is highly conserved during evolution. It is an essential nucleolar protein that is expressed in all tissues [42]. Dyskerin participates in two very relevant cellular activities, telomere maintenance and RNA pseudouridylation. The first activity has been described in the Telomere elongation section of this chapter (section 2.2]. For the second activity dyskerin binds to small nucleolar RNAs containing the H/ACA box to form small nucleolar RNP (snoRNP) complexes. The proteins NHP2, NOP10 and GAR1, involved in telomerase assembly are also part of these complexes [42]. Small nucleolar RNAs guide snoRNPs to specific uridine residues that are converted to pseudouridines by dyskerin. Pseudouridylation takes place in many cellular RNAs including ribosomal RNAs but also

small nuclear and nucleolar RNAs and mRNAs, as recently described [87]. This modification is important for folding and processing of these RNAs [88]. One subset of snoRNPs (Cajal body RNPs, scaRNPs) is directed to Cajal bodies by the TCAB1 protein [89]. The telomerase RNA, TR, assembles as a typical scaRNP, which is important for TR stability and telomerase recruitment [39, 70] as previously indicated (section 2.1). Because of this pseudouridylation activity of rRNAs, dyskerin is important for ribosome biogenesis and function, and dyskerin mutations could impair protein synthesis. However, human cells obtained from X-linked DC patients showed intact or only slightly affected ribosome biogenesis and function and very reduced TR levels [90, 91]. These data support the hypothesis that impaired TR stability and telomerase activity are the main cause of DC. However, Bellodi et al. have reported that impaired protein synthesis could contribute to the cancer predisposition of DC patients [92].

Sequence analyses in the Pfam databank identified three functional domains in dyskerin: the dyskerin-like domain (amino acids 48-106] with a yet unknown function; the TruB pseudouridine synthase catalytic domain (aa 110-126], and the PUA RNA binding domain (aa 297-370]. To date, over 50 different DKC1 mutations have been found in association with DC. Many of them were inherited but there were some that were generated de novo in the patients. Not all these mutations show the same severe phenotype and 13 of them cause the Hoyeraal-Hreidarsson syndrome, a more severe manifestation that will be described in the section 2.3.2 of this chapter. Two of the DKC1 mutations are only found in this syndrome. Most DKC1 mutations cluster in two regions of the gene: the region coding amino acids 2-72, at the N-terminus of the protein, and the region coding amino acids 314-420, at the PUA domain [68, 86]. These two domains are contiguous in the three-dimensional structure of the protein and might form a binding site for other proteins [93]. Some disease-causing mutations have been show to alter dyskerin-TR binding because they affect binding of the RNP assembly factor SHQ1 [94]. N-terminal DKC1 mutations overlap a SUMOylation motif and Brault et al have shown that impaired SUMOylation leads to reduced dyskerin, and TR, levels [95]. A mutation in the promoter region of DKC1 that affected dyskerin expression was also identified in a DC patient [96] suggesting that protein levels could have an important role in DC pathogenesis. DKC1 is encoded in the X-chromosome and dyskerin mutations have X-linked transmission with affected males and carrier mothers. In most of the cases carriers do not show any clinical manifestation but carriers of some mutations can manifest late onset diseases such as pulmonary fibrosis that will be described in section 2.3.6.

2.3.1.2.2 Telomerase Reverse Transcriptase (TERT)

The catalytic protein component of the telomerase complex is one 1132 amino acid long protein that contains three major functional domains conserved trough evolution [97]. The telomerase essential N-terminal (TEN) domain is highly conserved among vertebrate proteins and has been implicated in telomere DNA binding upstream of the primer-template interaction [98]. The TEN domain contains a DAT motif involved in telomerase recruitment to the telomeres through interaction with the TEL patch of TPP1 shelterin component. The TERT RNA-binding domain (TRBD) is located next to the TEN domain and precedes the reverse transcriptase domain, which contains the active site for reverse

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transcription. In addition, the reverse transcriptase motif also participates in TR RNA binding ensuring the maintenance of a stable telomerase complex [99]. Finally, TERT contains a less-conserved C-terminal extension region.

Comparative analysis of the TERT gene in healthy individuals and telomere biology disorder patients has shown a high degree of nucleotide variation. More than 200 distinct missense nucleotide variants are described in the TERT gene at the Exome Aggregation Consortium (ExAC) database (http://exac.broadinstitute.org/). This database compiles all the nucleotides variants found in the different Exome sequencing projects and presently accumulates information from about 60.700 individuals [121.400 alleles for each gene). Data from both healthy individuals and patients from different diseases are incorporated to this database. Over 75 TERT mutations, most of them novel, have been reported in telomere biology disorders diseases [100], including missense, stop gain, frameshift and splice site mutations. However, the existence of a given mutation in a patient does not imply that it is causative of the disease. It might be a mutation that does not affect protein functionality. The existence of a familiar history showing a strong correlation between the presence of the mutation and disease manifestation would support the causative role of this mutation. However, if it is a novel mutation, or the family history is short, experimental assay of the activity of the mutated protein is required to ascertain the possible causal role. For this purpose, mutated TERT proteins are expressed in cells that have very low telomerase activity, if any. The activity of the mutated protein can be consequently tested on this background using the telomere repeat amplification protocol (TRAP) or primer extension assays (see Collopy et al [101] for a recent example).

TERT mutations associated with DC and other telomere biology disorders are found all over the protein although their frequency is higher at the reverse transcriptase domain. Most reported patients with TERT mutations are monoallelic heterozygous. The telomerase activity found in cells from these patients is an average of homozygous wild type and mutant cells and might indicate that haploinsufficiency is the cause of the clinical phenotype [74, 102, 103].

2.3.1.2.3 Telomerase RNA, TR (TERC)

The RNA component of the telomerase complex is 454 nucleotides in length and is encoded by the TERC gene. This RNA provides the template sequence for reverse transcription and is involved in assemblage of the RNP complex [104]. The interaction between TR and TERT regulates the catalytic activity, processivity and telomere-binding activity of the telomerase complex [105]. TR presents domains conserved through evolution that are involved in RNA stabilization, accumulation, subcellular localization and telomerase assembly. They are the template/pseudoknot domain and the CR4/5 motif [106]. These two domains are sufficient to restore telomerase activity when combined with TERT [107, 108]. An additional H/ACA domain at the 3′ end of TR allows binding of the proteins required for telomerase biogenesis dyskerin, NOP10, NHP2 and GAR1 [43].

Mutations in TERC are less frequent than in TERT in DC patients and approximately 60 different mutations have been reported [100]. Nucleotide variants are also less frequent in the general population and only 62 are reported in ExAC exomes database. Among the mutations

found in DC patients some deleted large segments that affect functional domains while others are nucleotide substitutions. Mutations are particularly frequent at the pseudoknot domains and present an autosomal dominant inheritance (http://telomerase.asu.edu/diseases.html). As indicated for TERT, the functional significance of TERC mutations, specially nucleotide substitutions, has to be determined experimentally. A relevant example determined functional properties such as TR stability, TERT interaction, telomerase activity and processivity of 13 TR mutations [109].

2.3.1.2.4 TERF1- interacting nuclear factor 2 (TINF2]

The gene TINF2 codes for the protein TIN2, component of the shelterin complex that protect the telomere and regulates telomerase recruitment and activity. TIN2 links the double-stranded DNA binding proteins TRF1and TRF2 to the single-stranded DNA binding proteins TPP-1 and POT1 within the shelterin complex. TIN2 also interacts with heterochromatin protein 1 gamma (HP1γ) [110] through the canonical PTVML binding site [111] which is crucial for sister telomere cohesion.

Over 20 mutations in TINF2 have been described in DC patients. Many of them are novel mutations and results in early-onset disease. Familiar mutations are usually inherited in autosomal-dominant manner [111, 112]. All TINF2 mutations reported to date cluster in a segment coding for 34 amino acids centrally located in the gene. The function of this short protein fragment is not clear at the present time. One of the functions affected in some TINF2 mutants is HP1γ binding [110] which could be explained because the PTVML binding site is located within the mutation cluster [111]. Impaired HP1γ binding resulted in reduced sister telomere cohesion. On the contrary, the interaction of TIN2 with TERF1 is not affected in most of the mutated proteins [112, 113]. Impairment of telomerase recruitment to telomeres in a TIN2 mutant has been reported [114]. However, the telomere shortening observed in a mouse model of TINF2 mutation was recently reported to be telomerase independent [115].

2.3.1.2.5 TCAB1: Driving telomerase to Cajal bodies (WRAP53]

The protein TCAB1 (encoded by the WRAP53 gene) [116] binds to the telomerase RNA, TR, through the 4 nucleotides CAB box, present on small Cajal body-associated RNAs. Telomerase recruitment to Cajal Bodies is required for consequent assembly on the telomeres [44, 117].

Compound heterozygous mutations in WRAP53 have been identified in DC patients [118]. Telomerase localization to Cajal Bodies was disrupted in this patients leading to TR accumulation in the nucleoli. Mutations map to a region that mediates interaction between TCAB1 and the TCP-1 Ring Complex (TRiC) that is required for TCAB1 folding.

2.3.1.2.6 Nucleolar protein 10 (NOP10, Nola3]

Nucleolar protein 10 (NOP10] is encoded by the Nola3 gene (nucleolar protein family A, member 3]. This protein is a H/ACA snoRNA-binding protein that binds the TR RNA in association with dyskerin and NHP2 [43]. One homozygous mutation has been found in a DC patient that impaired TR binding and RNP assembly [119].

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2.3.1.2.7 NHP2 ribonucleoprotein (Nola 2]

Similarly to NOP10, the NHP2 protein, encoded by the NHP2 gene, also named Nola2, is a H/ACA snoRNA-binding protein that associates to TR together with dyskerin [43]. One homozygous missense mutation in the NHP2 gene was described in a patient with severe DC while compound heterozygous mutations were described in a second DC patient [120]. These patients had decreased TR levels and short telomeres because of impaired telomerase assembly and stability [119].

2.3.1.2.8 Conserved telomere maintenance component 1 (CTC1]

The protein CTC1 is one of the components of the CST complex (CTC1, STN1, TEN1] that promotes re-start of the telomere lagging strand synthesis and fill-in C-rich strand synthesis at the telomeres [51, 52]. CTC1 binds to single-stranded DNA at telomeres and associates with the replication initiator polα primase complex.

Biallelic, compound heterozygous, CTC1 mutations were identified in a group of DC patients [121, 122]. These mutations impaired the association of CSC1 with STN1, TEN1 and polα primase, telomeric DNA binding and cellular localization [123]. To date, 10 CTC1 mutations have been associated with DC patients [122].

2.3.1.2.9 Regulator of telomere elongation helicase 1 (RTEL1]

Regulator of telomere elongation helicase 1 is an essential DNA helicase that belongs to a small family of these proteins involved in different genomic instability diseases [124]. At telomeres, RTEL1 disrupts the D-loops resolving the T-loop structure [48]. RTEL1 is recruited to telomeres by TRF2 in late S phase and is essential to prevent nuclease-dependent excision of telomere T-circles [49]. RTEL1 also show G-quartet unwinding activity required for telomeric DNA replication although this activity is independent of TRF2 [50]. In addition, RTEL1 has important non-telomeric functions in processes such as DNA-replication, DNA repair and homologous recombination [125].

Whole-exome sequencing in DC patients and their families identified RTEL1 mutations that could cause DC and related telomere biology disease such as the Hoyeraal–Hreidarsson syndrome and Pulmonary Fibrosis, that will be described later (sections 2.3.2 and 2.3.6] [126-128]. To date almost 30 RTEL1 variants have been reported in telomere biology diseases patients. Most RTEL1 mutations are transmitted in autosomal recessive manner but autosomal dominance has been also reported [126]. Some mutations map to functional protein domains such as the helicase, harmonin homology or the C4C4 metal-binding motifs [129]. The RTEL1 R1264H mutation, that impairs RTEL1 interaction with TRF2, has been found in 1% of the Ashkenazi Jewish population [130].

2.3.1.2.10 Poly(A)-specific 3′ exoribonuclease (PARN)

Poly(A)-specific 3′ exoribonuclease is a widely expressed protein with Poly(A) deadenylase activity that participates in the regulation of global mRNA levels during development [131]. In addition, PARN also deadenylates small nucleolar RNAs [40]. A recent exome sequencing study linked PARN mutations with pulmonary fibrosis and telomere shortening [128].

A subsequent study, also based on exome sequencing, identified bialelic mutations in the PARN gene in three families with individuals exhibiting severe DC [85]. Two of the families were homozygous for one missense variant and one Splicing-altering variant, respectively. The third affected patient was a compound heterozygous. These patients exhibited reduced TERC, DKC1, RTEL1 and TERF1 mRNA levels. Cells from these patients showed activated DNA-damage response associated to nuclear p53 regulation, cell-cycle arrest and reduced cell viability upon UV treatment [85]. These results supported a potential link between PARN, the p53-dependent pathway and telomere shortening [132]. A subsequent study using cells derived from these patients has shown that PARN is required for the 3'-maturation of the telomerase RNA component [41]. Specifically, PARN is required for removal of the oligo(A) tails post-transcriptionally added to the TR 3' end and that target nuclear RNAs for degradation.

2.3.2. Hoyeraal-Hreidarsson syndrome

The Hoyeraal-Hreidarsson syndrome (HH) is frequently considered a severe variant of DC, typically presented in infancy [133]. The first patients with this syndrome were described by Hoyeraal et al [134] and Hreidarsson et al [135]. However, the eponym was first proposed in 1995 in a case report of a child with clinical features very similar to those described by Hoyeraal and Hreidarsson [136]. About 50 cases of HH have been reported since the first description [86]. HH is a multisystem genetic disorder and represents the extreme phenotype of the telomere biology disorders. Peripheral blood cells from these patients present very short telomeres, below the first percentile for their age. Clinical manifestations typically present early in childhood. These patients present developmental problems such as cerebellar hypoplasia, microcephaly, developmental delay and intrauterine growth retardation (IUGR). In addition, typically present immunodeficiency and progressive bone marrow failure. In addition to these specific symptoms, HH patients can also present clinical manifestations found in DC patients. For example, the typical triad of mucocutaneous alterations shown in DC patients can also be present at diagnosis or develop with time in HH patients. Other DC-associated symptoms that are also present in some HH patients include immunodeficiency, prematurity, dysmorphology, gastrointestinal features and neurological symptoms. Among these symptoms, cerebellar hypoplasia is considered a requirement for the diagnosis of HH [75, 137]. Other neurological complications include impaired myelination, seizures, hypoplastic corpus callosum and intracranial calcifications (reviewed in Glousker et al. [86]). Immunodeficiency is observed in a large proportion of HH patients with increased susceptibility to life-threatening infections. Over half of the patients present with lymphopenia [86]. The T cell compartment is less frequently affected although abnormalities of T cell proliferation have been observed [138]. There are also some reports of severe combined immunodeficiency [139]. Therefore, any child presenting with humoral deficiency or combined immunodeficiency and neurological features (microcephaly, cerebellar hypoplasia) should be considered a possible HH patient. Digestive tract anomalies are frequent in HH patients and include oesophageal strictures, severe enteropathy and colitis [140]. Other clinical complications include skeletal malformations [141], urinary tract abnormalities [136, 142], and ophthalmological signs [136, 142].

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2.3.2.1. Molecular genetics of Hoyeraal-Hreidarsson syndrome

As mentioned above, HH can be considered as severe form of DC and, in agreement with this consideration, some of the genes mutated in DC are also found mutated in HH patients [86]. The specific mutations present in HH patients can be different to those found in DC so that mutations that affect more importantly protein function are associated to HH. In other cases the difference is found in allele composition so that some mutations are found in homozygosis or compound heterozygosis in HH and in heterozygosis in DC. The mutations presently associated to HH will be briefly described in the following paragraphs and the genes affected are indicated in Table 1.

2.3.2.1.1 Dyskerin (DKC1]

DKC1 mutations cause DC and also HH so that 13 out of the over 50 different mutations presently known cause DC and HH and two of them are only found in HH (T49M and S304N) [68, 143]. No clear correlation has been found between the location of the mutation on dyskerin functional domains and the severity of the disease. Indeed, some mutations are associated with variable severity from mild DC to HH, like the A353V mutation [144]. The two HH-associated mutations are located to the catalytic TruB domain suggesting that pseudouridylation activity is important for telomerase function [143].

2.3.2.1.2 Telomerase Reverse Transcriptase (TERT)

Mutations in the TERT gene have been found in HH patients but not as frequently as in DC patients. From the more than 50 TERT mutations related to telomere biology disorders only five are implicated in HH. Four of them cause HH in homozygosis (T567M, R901W) [145, 146] or compound heterozygosis (P530L, A880T) [147]. Carriers of these mutations have short telomeres without reported clinical manifestations. The A880T and R901W mutations fall into the TERT catalytic reverse transcriptase domain and the T576M mutation in the RNA-binding domain. These mutations greatly impair telomerase activity and processivity, respectively [146]. Only one autosomal dominant TERT mutation has been associated with HH (F1127L) but it was also found in the healthy mother with could indicate the presence of a second, paternal, mutation or disease anticipation [148].

2.3.2.1.3 TERF1- interacting nuclear factor 2 (TINF2]

The shelterin component TIN2 is encoded by the gene TINF2 and has an important role by interacting with the double-stranded DNA binding proteins TRF1/TRF2 and the single-stranded DNA binding heterodimer TPP1/POT1, as mentioned above. Three of the over 20 DC-associated TINF2 mutations have been found in HH patients. These mutations were de novo or inherited in an autosomal-dominant manner [100, 111].

2.3.2.1.4 Regulator of telomere elongation helicase 1 (RTEL1]

The RTEL1 protein is a DNA helicase required for telomere replication, as mentioned above. Presently, 18 RTEL1 mutations have been described in 17 HH patients. Most RTEL1 mutations were biallelic, with either homozygous or compound heterozygous recessive inheritance. The mutations were located in domains involved in protein-protein interaction or ubiquitin transfer [127, 142].

2.3.2.1.5 TPP1 (ACD)

The protein TPP1 (TINT1, PTOP, PIP1] is encoded by the Adrenocortical Dysplasia Homolog (ACD) gene and is a component of the shelterin complex, as previously described (Section 2.1]. Three functional domains have been identified in this protein. The N-terminal OB domain is involved in the interaction of TPP1 with TERT that participates in the recruitment of the telomerase complex to telomeres through the TEL patch and increases telomerase processivity [45, 46]. The central domain is required for heterodimer formation with POT1 [28, 149]. The C-terminal domain binds TIN2 to form the shelterin complex, as mentioned above [150]. Whole exome sequencing discovered a mutation at the TEL patch of TPP1 together with a missense mutation in this same gene in a compound heterozygous HH patient [151]. The TEL patch mutation was a single amino acid deletion and resulted in a reduction of telomerase processivity and recruitment to telomeres. This same mutation has been identified in a family with aplastic anemia and other DC symptoms and was transmitted in a dominant inheritance manner [152].

2.3.2.1.6 Poly(A)-specific 3′ exoribonuclease PARN

The Poly(A)-specific 3′ exoribonuclease is involved in processing of the telomerase RNA, TR, as mentioned above. A recent work identified PARN mutations in three families with individuals exhibiting severe DC [85]. Actually, some of these patients had a disease classified as HH syndrome associating PARN mutations to this disease [132]. These patients presented biallelic mutations in the PARN gene indicating a recessive manner of inheritance.

2.3.3. Revesz syndrome

The syndrome of Revesz (RS) is a telomere biology disorder that affects young children. This disease was first reported by Revesz et al as a case of a 6-month-old children with bilateral exudative retinopathy that developed a severe bone marrow failure [153]. This and subsequent reports indicated the following symptoms, summarized in Table 1: intrauterine growth retardation, intracranial calcifications, developmental delay, fine spare hair and nail dystrophy. The clinical presentations have several symptoms in common with DC and the specific diagnosis of RS requires identification of bilateral exudative retinopathy [154]. Besides common manifestations, the relation of RS with DC and other telomere biology disorders was confirmed because RS patients have very short telomeres and present mutations in the TINF2 gene, that encodes the TIN2 shelterin component [111].

2.3.4. Coats plus syndrome/CRMCC

The Coats plus syndrome (CPS) is also known as cerebroretinal microangiopathy with calcifications and cysts (CRMCC). Coats plus patients have bilateral exudative retinopathy, retinal telangiectasias, intrauterine growth retardation, intracranial calcifications, bone abnormalities with poor healing, and gastrointestinal vascular ectasias (Table 1). Some patients also present DC-related features such as dystrophic nails, sparse or greying hair and anemia. Intracranial calcifications and bilateral exudative retinopathy are also present in RS patients but Coats plus patients also present cerebellar and hematologic manifestations [12, 56].

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Autosomal recessive compound heterozygous mutations in CTC1 have been described in CPS identifying this syndrome as a telomere biology disorder [155-157]. As mentioned above, the protein Conserved telomere maintenance component 1 (CTC1] is required for telomere elongation. Actually, mutations in CTC1 probably account for most of the CTS cases. In addition, telomeres that are bellow the first percentile for age have been found in CTS patients and telomeres from heterozygous carriers have a length bellow average [155].

2.3.5. Aplastic anemia

Aplastic anemia is one of the clinical manifestations of telomere biology disorders in adults usually associated to mutations in TERT and TERC. Symptoms in these patients are milder than in children and mucocutaneous features are infrequent [12, 56]. Aplastic anemia can have very different causes and there are inherited and acquired forms of the disease. Acquired forms can be related to environmental exposures and infectious, among other factors, and is immune-mediated. Inherited aplastic anemia has been reported to occur in patients with Fanconi anemia, Shwachman-Diamond syndrome and other inherited bone marrow failures, including DC. It has been described that approximately 10% of patients with isolated aplastic anemia have mutations in TERC and TERT genes [158]. These mutations usually present an autosomal dominant manner of inheritance. Telomere length in these patients is usually bellow the 10% percentile for age [159]. The existence of symptoms related to telomere biology disorders in relatives of these patients, such as pulmonary fibrosis, mild cytopenias, leukemia and squamous cell cancer, can be of great help for their diagnosis [12].

2.3.6. Pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a lung disease characterized by progressive interstitial fibrosis that has a poor prognosis (median survival time of 2-3 years) [160]. Diagnosis of pulmonary fibrosis, also known as interstitial pneumonia, is made by the presence of honeycombing on high-resolution computarized tomography (HRCT). In addition to pulmonary fibrosis, these patients can present a range of pulmonary pathologies, including bronchiolitis, obliterans organizing pneumonia, chronic hypersensitive pneumonia and emphysema alone or combined with pulmonary fibrosis (Table 1) [56]. Familial forms of pulmonary fibrosis have been also described and might represent up to 20% of the cases [161]. The study of these familial forms identified mutations in TERT and TERC in 8-15 % of the cases [162, 163], establishing IPF as a telomere biology disorder. IPF is inherited in these families as an autosomal dominant trail. This observation is supported by animal models since TERT null mice have decreased number or alveolar epithelial cells [164]. TRF1 deletion in type II alveolar cells also causes pulmonary fibrosis in mice [165].

Heterozygous mutations in genes coding for telomere-related proteins have been found in 15-20% of IPF families without a history of DC [162, 163, 166] and 1-3% of sporadic cases of IPF [167]. In addition, 20% of patients with DC develop pulmonary fibrosis [57, 58]. In agreement with these observations, IPF patients have significantly shorter telomeres than age-matched controls. Actually, IPF is the most common manifestation of telomere biology disorders since DC and AA have much lower prevalence [168]. IPF due to telomere disfunction

presents in adulthood, into middle age [164]. The gene most frequently mutated in IPF patients is TERT [8-15% of familial cases) but mutations have been also found in TERC (<1%), DKC1 (<1%)[169], TINF2 (<1%)[170], RTEL1 [5%)[128, 166] and PARN [4%) [128]. TERT mutations have been also found in smokers with severe emphysema at a frequency of 1% [171]. Telomere dysfunction due to these genetic mutations can originate irreversible alveolar stem cell failure that would be at the origin of pulmonary fibrosis and emphysema [72, 162]. IPF patients that carry mutations in telomere-related genes can also present extra-pulmonary manifestations related to telomere biology disorders such as bone marrow failure including red blood cells, single lineage cytopenias or aplastic anemia [164]. Actually, the complex syndrome of IPF and bone marrow failure predicted the presence of TERT or TERC mutations in 10 families that presented these diseases in consecutive generations [172].

Short telomere length is a common finding in IPF patients, even in those without mutations in telomere-related genes [167]. These results could indicate that IPF may be more likely to develop in those individuals that naturally present shorter telomeres in the general population. These individuals might also have increased incidence of other telomere-related disorders such as cryptogenic liver cirrhosis and diabetes [58].

2.3.7. Liver disease

The study of five families with liver disease in combination with hematologic and autoimmune disorders identified mutations in TERT and TERC [65]. A subsequent study of patients with idiopathic live cirrhosis also found an increased frequency of TERT and TERC mutations [3.7% vs 0,85% in the control population) [173]. Affected patients presented reduced telomerase activity and short telomere length in peripheral blood cells. They also have increased probability to progress to end-stage liver disease. In addition, liver disease, including hepatic fibrosis, noncirrhotic portal hypertension, and hepatopulmonary syndrome has been reported in DC patients (Table 1) [62, 64].

2.4. Treatment of telomere biology disorders

Treatment of diseases with several organs potentially compromised has many practical complications. Presently, there are no curative therapies for many of the clinical manifestations of telomere biology disorders. The major causes of decease in these patients are bone marrow failure and pulmonary fibrosis and in this section we will summarize the present treatment of these pathologies. In the second part we will describe some of the experimental strategies that are being used to generate new therapies for telomere biology disorders.

2.4.1. Present treatment of telomere biology diseases

Hematopoietic stem cells transplantation (HSCT) is the only treatment than can cure bone marrow failure in these patients. Donor's selection requires special attention in telomere biology disorders since relatives might be silent carriers of the mutations, given the clinical heterogeneity of these diseases. This circumstance has been reported in two cases and there was a failure either to engraft or to mobilize stem cells from the graft [174]. Analysis of the

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outcome of 34 DC patients transplanted with bone marrow indicated higher rates of mortality and morbidity due to respiratory complications and graft failure [175]. Best results were obtained transplanting grafts from HLA-matched siblings but the 10-year probability of survival was 30% in this study. Conditioning of transplanted DC patients may also contribute to long-term development of pulmonary fibrosis and liver disease. Therefore, the use of reduced intensity conditioning, avoiding radiotherapy, busulfan and high dose of cyclophosphamide might benefit to these patients [176].

Androgen therapy has been also used for telomere biology disorder patients with bone marrow failure. These patients seem to be responsive to male hormones [177]. The mechanism involved seems to be that male hormones modulate TERT gene expression and increase telomerase activity [178]. In a retrospective analysis of 16 DC patients treated with androgens, 11 achieved clinically significant hematologic response [179]. Telomere elongation after androgen treatment has been reported in one case [180]. However, androgens can have side effects such as masculinisation, liver function abnormalities, hyperlipidemia and splenic peliosis (when androgens are used in conjunction with GCSF) and telomere biology disorders patients can be specially sensitive to these effects [181]. The androgen-stimulating hormone Danazol has less masculinising side effects and has been also used for treatment of DC patients [182].

Treatment of idiopathic pulmonary fibrosis is presently mainly supportive with pulmonary rehabilitation therapy and the administration of supplemental oxygen. Recently, two pharmacological agents, pirfenidone [183] and nintedanib [184] were shown to reduce lung function decline in IPF patients. Danazol administration has also been described to slow down the progression of pulmonary fibrosis in DC patients [182]. However, lung transplantation is the only curative strategy available. Lung transplantation was successfully used in a patient after HSCT [185]. The study of a small series of IPF patients with TERC or TERT mutations showed a favourable short term output with 7 of 8 patients alive after a median follow-up of 1,9 years. However, frequent haematological, renal and infectious complications were observed [186].

Because of the lack of curative therapies, telomere biology disorder's management are presently based on supportive measures and close follow-up for medium and long term complications [55]. Regular clinical review to monitor organ-specific disease progression, such as haematological analysis and pulmonary function testing must be performed. Surveillance for the appearance of dermatological and digestive tumours is important for early detection and complete surgical resection. Preventive measures such as avoidance of potential carcinogens (tobacco smoke, sun exposure) and adequate dental hygiene are also very important.

2.4.2. Experimental strategies for treatment of telomere biology disorders

Important efforts for the development of mice models of telomere biology disorders aimed to the development of novel therapies have been made in the last years [187]. However, the existence of very long telomeres in the mice strains used for experimentation [50-100 kb) has made of this a difficult task. Mice strains with defective telomerase activity have been generated [187, 188] but they have to be crossed for 4-5 generations before their telomeres are sufficiently short to manifest telomere-associated defects [189]. The use of mice with short

telomeres to generate telomerase-deficient strains provided a better experimental model [190]. Mouse models carrying mutations in DKC1 have been also generated [191]. More recently, tissue-specific inactivation of genes related to telomere biology has been used to generate mice models of these diseases. For example, mice models of bone marrow failure and pulmonary fibrosis were generated by deleting the TRF1 gene in the hematopoietic compartment and type II alveolar cells, respectively [165, 192]. However, these mouse models did not completely reproduce the human disease and telomere size was not reduced [190, 191]. Mice lacking the p53 C-terminal domain had short telomeres and suffer from aplastic anemia and pulmonary fibrosis and could be a useful model for the study of telomere biology disorders [193].

Telomere biology disorders are caused by mutations in a single gene in most patients and could be, therefore, amenable for gene therapy strategies. An important caveat is that telomere length is narrowly controlled and excessively long telomeres increase the probability of developing some cancers such as melanoma [13]. Mice over-expressing TERT also develop a large number of tumors unless the tumor-suppressor protein p53 is also overexpressed [194]. Raval et al recently showed that inducible reactivation of telomerase activity could reverse defective hematopoiesis caused by telomere shortening in TERT-deleted mice [189] opening new perspectives to gene therapy approaches. Transient expression of TERT also extends telomeres in human cells [195]. Recent reports indicate that TERT plays roles beyond telomeres and contributes to stem cells maintenance and cell reprogramming which might offer new therapeutics targets for telomere biology disorders [196].

Telomere shortening results in the accumulation of DNA damage at telomeres and the activation of the p53 pathway, as mention above. A DC mouse model in which mice carries a DKC1 exon 15 deletion demonstrated that mutant cells had a growth retardation compared to wild-type cells [191]. Mutant cells accumulated increased levels of DNA damage. In addition, these cells are hypersensitive to oxygen and accumulate reactive oxygen species. Treatment of these cells with the antioxidant N-acetyl cysteine increased cell growth, both in vitro and in vivo. Competitive bone marrow repopulation studies showed that the DCK1 mutation is associated with a functional stem cell defect consistent with accelerated senescence. This stem cell defect was partially reverted by N-acetyl cystein treatment of the animals [197]. These results suggest that antioxidant treatment may prevent or delay some DC manifestations.

A new therapeutic opportunity came from the observation that a dyskerin motif, corresponding to the TruB domain of the protein (GSE24.2], reactivated telomerase activity in DC-patients and human telomerase-deficient cells [198]. This peptide activated human TERT promoter in a c-myc expression-dependent manner. GSE24.2 rescued DC-fibroblasts from premature senescence. The peptide also increased the telomerase RNA, TR, expression trough stabilization of the molecule [199]. DC-cells presented increased DNA damage at the telomeres and increased levels of oxidative stress. Expression of GSE24.2 decreased both DNA damage and oxidative stress of the cells that expressed a DKC1 mutant protein [200]. Subsequent studies demonstrated that a shorter fragment of GSE24.2, named GSE4, maintained the same biological activity and induced telomerase activity and cell proliferation of DKC-mutant cells. In addition, DNA damage, oxidative stress and cell senescence were reduced upon expression of GSE4 [201]. GSE24.2 could be delivered to cells using surface modified biodegradable

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polymeric nanoparticles, which might facilitate their administration to patients [202]. These results open a new therapeutic opportunity for the treatment of telomere biology disorders and GSE24.2 was recently approved by the EMA as an orphan drug for DC treatment (EU/3/12/1070-EMA/OD/136/11].

2.5. Conclusion

Telomere biology disorders, also named telomeropathies, compose a group of diseases with diverse clinical presentations, affecting several systems, but a common genetic etiology. Telomere maintenance or protection is defective in the patients affected by these diseases and, as a consequence, they present short telomeres. Critically short telomeres induce cell death or senescence impairing cell proliferation. Cell renewal in adult tissues depends on the proliferation of stem cells. In patients with defects in telomere biology, telomeres of stem cells are shortened after each cell cycle and get exhausted much earlier than in healthy individuals which impairs tissue renewal. This effect is specially important in highly proliferative tissues such as bone marrow, lymphocytes and epithelial tissues, including lung alveolar epithelia, that are the tissues mainly affected in patients with telomere biology disorders.

The severity of the disease seems to be dependent on the functional alterations that each mutation causes in the biological activity of the corresponding protein. The genetic doses is also very important since patients that are homozygous for the mutation or compound heterozygous for more than one mutation usually present more severe symptoms of the disease that manifest at an early age. These forms of the disease are inherited in a recessive or X-linked manner. Heterozygous patients might be healthy carriers but they can also present milder forms of the disease that present at an older age, generally in adults. These disease manifestations are inherited in an autosomal dominant manner. There is also an association between the severity of the disease and the tissues affected. High-turnover tissues are affected in younger patients and as a more severe disease. For example, the main manifestation in infancy is severe immunodeficiency affecting B cells, T cells and NK cells that have high replication rates. Bone marrow defects manifests later in children and young adults as isolated cytopenias and aplastic anemia. The gastrointestinal epithelium is also affected in children and young adults. In contrast, telomere phenotypes predominantly manifest in slow-turnover tissues such as lung and liver in adults [9]. There is also a strong correlation between the size of the telomeres, as determined in peripheral blood cells, the severity of the disease and the time of presentation so that patients younger and with more severe presentation have shorter telomeres [12].

Genetic anticipation can also have a relevant influence in the presentation and evolution of these diseases. As previously described, patients and healthy carriers of telomere biology disease can transmit shortened telomeres to their descendants. If descendants are affected by the disease, their stem cells will present critically short telomeres at an early age that the previous generation, anticipating the time of onset and increasing the severity of the disease [74]. For example, a large family has been described with several patients manifesting IPF, bone marrow failure or a combined phenotype. There seemed to be a difference of several

decades in the onset of the disease between generations with IPF manifesting in older individuals (mean 51 years) and bone marrow failure in younger ones [14 years) [172].

The heterogeneous presentation of telomere biology diseases make difficult their diagnosis as already mentioned in the description of DC (section 2.3.1]. In addition, some of the symptoms also occur in patients with other diseases. For example, inherited bone marrow failure is also observed in patients with Fanconi anemia, Shwachmann-Diammond syndrome and other inherited BMFs. However, differentiating DC-associated BMF is important for patient management since, for example, these patients often do not respond to immunosuppressive therapy. One important criterion for diagnosis of telomere biology disorders is the presence of short telomeres in comparison to the aged healthy population, usually lower than the 1% percentile. However, some IPF patients present short telomeres even in the absence of mutations in telomere biology related genes [167]. Therefore, an accurate diagnosis requires establishing the molecular basis of the disease, identifying the causative mutation. Molecular diagnosis can be done sequencing the exons contained in the genes presently known to be mutated in telomere biology disorders. However, the high number of candidate genes and the large number of exons present in some of them makes this approach time-consuming and expensive. Alternatively, sequencing by exome sequencing techniques all exons of patients and close relatives is becoming a more attractive, faster and cheaper method [84]. In addition, exome sequencing allows the identification of mutations in genes that have not been previously related to telomere biology (see [128] for a recent example).

These diseases can be considered and example of the importance of precision medicine for diagnosis but also for patient managing and genetic counselling. The importance in diagnosis is enforced by the elevated heterogeneity of the clinical presentations in several systems that can create some uncertainty until a genetic analysis is performed. The importance for patient management derives from the possible progressive alteration of different organs, as mentioned above. This progression with time is very characteristic of telomerase biology disorders and can only be predicted by a precise molecular diagnosis. Disease progression also determines the therapeutic treatment. As mentioned above, the only curative alternatives are organ transplantation, either hematopoietic stem cell transplantation (HSCT) or lung transplantation. However, in telomere biology disorders both transplants have specific complications. Reduced intensity conditioning is advised and patients frequently present graft failure. In the case of HSCT respiratory complications have been described. In contrast, haematological, renal and infectious complications were observed after lung transplantation. Both complications could be expected for the multi-systemic nature of these diseases.

Precision medicine is also important for genetic counselling in telomere biology disorders [12]. Depending on the mutation, these diseases can have different manners of inheritance. The phenotypic penetrance of each mutation can be different. Heterozygous clinically silent carriers can be found whose long-term evolution is not yet understood. Patients treated for a symptom can develop a different one later on. As mentioned above, supportive measures and close follow-up of patients and carrier relatives is also very important. All these characteristics, specific to these diseases, might be difficult to transmit to the patients and their relatives what

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makes even more important to provide appropriate genetic education and counselling to the families.

As mentioned above, presently there are no really curative alternatives for these diseases. Lung transplantation and HSCT are important therapeutic interventions but, unfortunately, with a short time of survival. Some experimental therapies are promising but new curative therapies are urgently needed and should be the focus of intensive research in the near future.

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References

[1] McElligott R, Wellinger RJ. The terminal DNA structure of mammalian chromosomes. EMBO J. 1997 Jun 16;16(12):3705-14.

[2] de Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. Genes Dev. 2005 Sep 15;19(18):2100-10.

[3] Karlseder J, Broccoli D, Dai Y, Hardy S, de Lange T. p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2. Science. 1999 Feb 26;283(5406):1321-5.

[4] Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature. 1990 May 31;345(6274):458-60.

[5] Blackburn EH, Greider CW, Szostak JW. Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. Nat Med. 2006 Oct;12(10): 1133-8.

[6] Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell. 1985 Dec;43(2 Pt 1):405-13.

[7] Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, et al. Specific association of human telomerase activity with immortal cells and cancer. Science. 1994 Dec 23;266(5193):2011-5.

[8] Wright WE, Piatyszek MA, Rainey WE, Byrd W, Shay JW. Telomerase activity in human germline and embryonic tissues and cells. Dev Genet. 1996;18(2):173-9.

[9] Armanios M. Telomeres and age-related disease: how telomere biology informs clinical paradigms. J Clin Invest. 2013 Mar;123(3):996-1002.

[10] Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013 Jun 6;153(6):1194-217.

[11] Nabetani A, Ishikawa F. Alternative lengthening of telomeres pathway: recombination-mediated telomere maintenance mechanism in human cells. J Biochem. 2010 Jan; 149(1):5-14.

[12] Savage SA. Human telomeres and telomere biology disorders. Prog Mol Biol Transl Sci. 2014;125:41-66.

[13] Stanley SE, Armanios M. The short and long telomere syndromes: paired paradigms for molecular medicine. Curr Opin Genet Dev. 2015 Aug;33:1-9.

[14] Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, et al. TERT promoter mutations in familial and sporadic melanoma. Science. 2013 Feb 22;339(6122):959-61.

[15] Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. Science. 2013 Feb 22;339(6122):957-9.

[16] Aoude LG, Pritchard AL, Robles-Espinoza CD, Wadt K, Harland M, Choi J, et al. Nonsense mutations in the shelterin complex genes ACD and TERF2IP in familial melanoma. J Natl Cancer Inst. 2015 Feb;107(2):pli:dju408.

[17] Bainbridge MN, Armstrong GN, Gramatges MM, Bertuch AA, Jhangiani SN, Doddapaneni H, et al. Germline mutations in shelterin complex genes are associated with familial glioma. J Natl Cancer Inst. 2015 Jan;107(1):384.

[18] Bertuch AA. The Molecular Genetics of the Telomere Biology Disorders. RNA Biol. 2016; 13(8):696-706.

[19] Giardini MA, Segatto M, da Silva MS, Nunes VS, Cano MI. Telomere and telomerase biology. Prog Mol Biol Transl Sci. 2014;125:1-40.

[20] Aubert G, Baerlocher GM, Vulto I, Poon SS, Lansdorp PM. Collapse of telomere homeostasis in hematopoietic cells caused by heterozygous mutations in telomerase genes. PLoS Genet. 2012;8(5):e1002696.

[21] Kipling D, Cooke HJ. Hypervariable ultra-long telomeres in mice. Nature. 1990 Sep 27;347(6291):400-2.

[22] Blasco MA. Telomeres and human disease: ageing, cancer and beyond. Nat Rev Genet. 2005 Aug;6(8):611-22.

[23] de Lange T, Shiue L, Myers RM, Cox DR, Naylor SL, Killery AM, et al. Structure and variability of human chromosome ends. Mol Cell Biol. 1990 Feb;10(2):518-27.

[24] de Lange T. T-loops and the origin of telomeres. Nat Rev Mol Cell Biol. 2004 Apr; 5(4):323-9.

[25] Cooper JP, Nimmo ER, Allshire RC, Cech TR. Regulation of telomere length and function by a Myb-domain protein in fission yeast. Nature. 1997 Feb 20;385(6618): 744-7.

[26] Broccoli D, Smogorzewska A, Chong L, de Lange T. Human telomeres contain two distinct Myb-related proteins, TRF1 and TRF2. Nat Genet. 1997 Oct;17(2):231-5.

[27] Ye JZ, Donigian JR, van Overbeek M, Loayza D, Luo Y, Krutchinsky AN, et al. TIN2 binds TRF1 and TRF2 simultaneously and stabilizes the TRF2 complex on telomeres. J Biol Chem. 2004 Nov 5;279(45):47264-71.

[28] Liu D, O'Connor MS, Qin J, Songyang Z. Telosome, a mammalian telomere-associated complex formed by multiple telomeric proteins. J Biol Chem. 2004 Dec 3;279(49): 51338-42.

[29] Chen LY, Redon S, Lingner J. The human CST complex is a terminator of telomerase activity. Nature. 2012 Aug 23;488(7412):540-4.

[30] Garcia-Cao M, O'Sullivan R, Peters AH, Jenuwein T, Blasco MA. Epigenetic regulation of telomere length in mammalian cells by the Suv39h1 and Suv39h2 histone methyltransferases. Nat Genet. 2004 Jan;36(1):94-9.

[31] Karlseder J, Hoke K, Mirzoeva OK, Bakkenist C, Kastan MB, Petrini JH, et al. The telomeric protein TRF2 binds the ATM kinase and can inhibit the ATM-dependent DNA damage response. PLoS Biol. 2004 Aug;2(8):E240.

[32] Wu L, Multani AS, He H, Cosme-Blanco W, Deng Y, Deng JM, et al. Pot1 deficiency initiates DNA damage checkpoint activation and aberrant homologous recombination at telomeres. Cell. 2006 Jul 14;126(1):49-62.

[33] Diotti R, Loayza D. Shelterin complex and associated factors at human telomeres. Nucleus. 2011 Mar-Apr;2(2):119-35.

[34] Nandakumar J, Cech TR. Finding the end: recruitment of telomerase to telomeres. Nat Rev Mol Cell Biol. 2013 Feb;14(2):69-82.

[35] Martinez P, Blasco MA. Replicating through telomeres: a means to an end. Trends Biochem Sci. 2015 Sep;40(9):504-15.

[36] Schmidt JC, Cech TR. Human telomerase: biogenesis, trafficking, recruitment, and activation. Genes Dev. 2015 Jun 1;29(11):1095-105.

[37] Watson JD. Origin of concatemeric T7 DNA. Nat New Biol. 1972 Oct 18;239(94): 197-201.

[38] Greider CW, Blackburn EH. A telomeric sequence in the RNA of Tetrahymena telomerase required for telomere repeat synthesis. Nature. 1989 Jan 26;337(6205):331-7.

[39] Mitchell JR, Cheng J, Collins K. A box H/ACA small nucleolar RNA-like domain at the human telomerase RNA 3' end. Mol Cell Biol. 1999 Jan;19(1):567-76.

[40] Berndt H, Harnisch C, Rammelt C, Stohr N, Zirkel A, Dohm JC, et al. Maturation of mammalian H/ACA box snoRNAs: PAPD5-dependent adenylation and PARN-dependent trimming. RNA. 2012 May;18(5):958-72.

[41] Moon DH, Segal M, Boyraz B, Guinan E, Hofmann I, Cahan P, et al. Poly(A)-specific ribonuclease (PARN) mediates 3'-end maturation of the telomerase RNA component. Nat Genet. 2015 Dec;47(12):1482-8.

[42] Angrisani A, Vicidomini R, Turano M, Furia M. Human dyskerin: beyond telomeres. Biol Chem. 2014 Jun;395(6):593-610.

[43] Egan ED, Collins K. Specificity and stoichiometry of subunit interactions in the human telomerase holoenzyme assembled in vivo. Mol Cell Biol. 2010 Jun;30(11): 2775-86.

[44] Venteicher AS, Abreu EB, Meng Z, McCann KE, Terns RM, Veenstra TD, et al. A human telomerase holoenzyme protein required for Cajal body localization and telomere synthesis. Science. 2009 Jan 30;323(5914):644-8.

[45] Zhong FL, Batista LF, Freund A, Pech MF, Venteicher AS, Artandi SE. TPP1 OB-fold domain controls telomere maintenance by recruiting telomerase to chromosome ends. Cell. 2012 Aug 3;150(3):481-94.

[46] Nandakumar J, Bell CF, Weidenfeld I, Zaug AJ, Leinwand LA, Cech TR. The TEL patch of telomere protein TPP1 mediates telomerase recruitment and processivity. Nature. 2012 Dec 13;492(7428):285-9.

[47] Williamson JR, Raghuraman MK, Cech TR. Monovalent cation-induced structure of telomeric DNA: the G-quartet model. Cell. 1989 Dec 1;59(5):871-80.

[48] Vannier JB, Pavicic-Kaltenbrunner V, Petalcorin MI, Ding H, Boulton SJ. RTEL1 dismantles T loops and counteracts telomeric G4-DNA to maintain telomere integrity. Cell. 2012 May 11;149(4):795-806.

[49] Sarek G, Vannier JB, Panier S, Petrini JH, Boulton SJ. TRF2 recruits RTEL1 to telomeres in S phase to promote t-loop unwinding. Mol Cell. 2015 Feb 19;57(4):622-35.

[50] Vannier JB, Sandhu S, Petalcorin MI, Wu X, Nabi Z, Ding H, et al. RTEL1 is a replisome-associated helicase that promotes telomere and genome-wide replication. Science. 2013 Oct 11;342(6155):239-42.

[51] Stewart JA, Wang F, Chaiken MF, Kasbek C, Chastain PD, 2nd, Wright WE, et al. Human CST promotes telomere duplex replication and general replication restart after fork stalling. EMBO J. 2012 Aug 29;31(17):3537-49.

[52] Wang F, Stewart JA, Kasbek C, Zhao Y, Wright WE, Price CM. Human CST has independent functions during telomere duplex replication and C-strand fill-in. Cell Rep. 2012 Nov 29;2(5):1096-103.

[53] Sfeir AJ, Chai W, Shay JW, Wright WE. Telomere-end processing the terminal nucleotides of human chromosomes. Mol Cell. 2005 Apr 1;18(1):131-8.

[54] Wu P, Takai H, de Lange T. Telomeric 3' overhangs derive from resection by Exo1 and Apollo and fill-in by POT1b-associated CST. Cell. 2012 Jul 6;150(1):39-52.

[55] Barbaro P, Ziegler DS, Reddel RR. The wide-ranging clinical implications of the Short Telomere Syndromes. Intern Med J. 2016; 46(4):393-403.

[56] Townsley DM, Dumitriu B, Young NS. Bone marrow failure and the telomeropathies. Blood. 2014 Oct 30;124(18):2775-83.

[57] Kropski JA, Blackwell TS, Loyd JE. The genetic basis of idiopathic pulmonary fibrosis. Eur Respir J. 2015 Jun;45(6):1717-27.

[58] Armanios M. Telomerase and idiopathic pulmonary fibrosis. Mutat Res. 2012 Feb 1;730(1-2):52-8.

[59] Dokal I. Dyskeratosis congenita in all its forms. Br J Haematol. 2000 Sep;110(4): 768-79.

[60] Heiss NS, Knight SW, Vulliamy TJ, Klauck SM, Wiemann S, Mason PJ, et al. X-linked dyskeratosis congenita is caused by mutations in a highly conserved gene with putative nucleolar functions. Nat Genet. 1998 May;19(1):32-8.

[61] Atkinson JC, Harvey KE, Domingo DL, Trujillo MI, Guadagnini JP, Gollins S, et al. Oral and dental phenotype of dyskeratosis congenita. Oral Dis. 2008 Jul;14(5):419-27.

[62] Ballew BJ, Savage SA. Updates on the biology and management of dyskeratosis congenita and related telomere biology disorders. Expert Rev Hematol. 2013 Jun;6(3): 327-37.

[63] Pignolo RJ, Suda RK, McMillan EA, Shen J, Lee SH, Choi Y, et al. Defects in telomere maintenance molecules impair osteoblast differentiation and promote osteoporosis. Aging Cell. 2008 Jan;7(1):23-31.

[64] Dokal I. Dyskeratosis congenita. Hematology Am Soc Hematol Educ Program. 2011;2011:480-6.

[65] Calado RT, Regal JA, Kleiner DE, Schrump DS, Peterson NR, Pons V, et al. A spectrum of severe familial liver disorders associate with telomerase mutations. PLoS One. 2009;4(11):e7926.

[66] Rackley S, Pao M, Seratti GF, Giri N, Rasimas JJ, Alter BP, et al. Neuropsychiatric conditions among patients with dyskeratosis congenita: a link with telomere biology? Psychosomatics. 2012 May-Jun;53(3):230-5.

[67] Alter BP, Giri N, Savage SA, Rosenberg PS. Cancer in dyskeratosis congenita. Blood. 2009 Jun 25;113(26):6549-57.

[68] Vulliamy TJ, Marrone A, Knight SW, Walne A, Mason PJ, Dokal I. Mutations in dyskeratosis congenita: their impact on telomere length and the diversity of clinical presentation. Blood. 2006 Apr 1;107(7):2680-5.

[69] Knight SW, Heiss NS, Vulliamy TJ, Greschner S, Stavrides G, Pai GS, et al. X-linked dyskeratosis congenita is predominantly caused by missense mutations in the DKC1 gene. Am J Hum Genet. 1999 Jul;65(1):50-8.

[70] Mitchell JR, Wood E, Collins K. A telomerase component is defective in the human disease dyskeratosis congenita. Nature. 1999 Dec 2;402(6761):551-5.

[71] Vulliamy T, Marrone A, Goldman F, Dearlove A, Bessler M, Mason PJ, et al. The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita. Nature. 2001 Sep 27;413(6854):432-5.

[72] Alder JK, Barkauskas CE, Limjunyawong N, Stanley SE, Kembou F, Tuder RM, et al. Telomere dysfunction causes alveolar stem cell failure. Proc Natl Acad Sci U S A. 2015 Apr 21;112(16):5099-104.

[73] Vulliamy T, Marrone A, Szydlo R, Walne A, Mason PJ, Dokal I. Disease anticipation is associated with progressive telomere shortening in families with dyskeratosis congenita due to mutations in TERC. Nat Genet. 2004 May;36(5):447-9.

[74] Armanios M, Chen JL, Chang YP, Brodsky RA, Hawkins A, Griffin CA, et al. Haploinsufficiency of telomerase reverse transcriptase leads to anticipation in autosomal dominant dyskeratosis congenita. Proc Natl Acad Sci U S A. 2005 Nov 1;102(44): 15960-4.

[75] Savage SA, Bertuch AA. The genetics and clinical manifestations of telomere biology disorders. Genet Med. 2010 Dec;12(12):753-64.

[76] Aubert G, Hills M, Lansdorp PM. Telomere length measurement-caveats and a critical assessment of the available technologies and tools. Mutat Res. 2012 Feb 1;730(1-2): 59-67.

[77] Diez Roux AV, Ranjit N, Jenny NS, Shea S, Cushman M, Fitzpatrick A, et al. Race/ethnicity and telomere length in the Multi-Ethnic Study of Atherosclerosis. Aging Cell. 2009 Jun;8(3):251-7.

[78] Hemann MT, Strong MA, Hao LY, Greider CW. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. Cell. 2001 Oct 5;107(1):67-77.

[79] Letsolo BT, Rowson J, Baird DM. Fusion of short telomeres in human cells is characterized by extensive deletion and microhomology, and can result in complex rearrangements. Nucleic Acids Res. 2010 Apr;38(6):1841-52.

[80] Baird DM, Rowson J, Wynford-Thomas D, Kipling D. Extensive allelic variation and ultrashort telomeres in senescent human cells. Nat Genet. 2003 Feb;33(2):203-7.

[81] Alter BP, Baerlocher GM, Savage SA, Chanock SJ, Weksler BB, Willner JP, et al. Very short telomere length by flow fluorescence in situ hybridization identifies patients with dyskeratosis congenita. Blood. 2007 Sep 1;110(5):1439-47.

[82] Alter BP, Rosenberg PS, Giri N, Baerlocher GM, Lansdorp PM, Savage SA. Telomere length is associated with disease severity and declines with age in dyskeratosis congenita. Haematologica. 2012 Mar;97(3):353-9.

[83] Carrillo J, Martinez P, Solera J, Moratilla C, Gonzalez A, Manguan-Garcia C, et al. High resolution melting analysis for the identification of novel mutations in DKC1 and TERT genes in patients with dyskeratosis congenita. Blood Cells Mol Dis. 2012 Oct 15-Dec 15;49(3-4):140-6.

[84] Sastre L. Exome sequencing:what clinicians need to know. Advances in Genomics and Genetics. 2014;4:15-27.

[85] Tummala H, Walne A, Collopy L, Cardoso S, de la Fuente J, Lawson S, et al. Poly(A)-specific ribonuclease deficiency impacts telomere biology and causes dyskeratosis congenita. J Clin Invest. 2015 May;125(5):2151-60.

[86] Glousker G, Touzot F, Revy P, Tzfati Y, Savage SA. Unraveling the pathogenesis of Hoyeraal-Hreidarsson syndrome, a complex telomere biology disorder. Br J Haematol. 2015 Aug;170(4):457-71.

[87] Schwartz S, Bernstein DA, Mumbach MR, Jovanovic M, Herbst RH, Leon-Ricardo BX, et al. Transcriptome-wide mapping reveals widespread dynamic-regulated pseudouridylation of ncRNA and mRNA. Cell. 2014 Sep 25;159(1):148-62.

[88] Kiss T, Fayet-Lebaron E, Jady BE. Box H/ACA small ribonucleoproteins. Mol Cell. 2010 Mar 12;37(5):597-606.

[89] Tycowski KT, Shu MD, Kukoyi A, Steitz JA. A conserved WD40 protein binds the Cajal body localization signal of scaRNP particles. Mol Cell. 2009 Apr 10;34(1):47-57.

[90] Wong JM, Collins K. Telomerase RNA level limits telomere maintenance in X-linked dyskeratosis congenita. Genes Dev. 2006 Oct 15;20(20):2848-58.

[91] Carrillo J, Gonzalez A, Manguan-Garcia C, Pintado-Berninches L, Perona R. p53 pathway activation by telomere attrition in X-DC primary fibroblasts occurs in the absence of ribosome biogenesis failure and as a consequence of DNA damage. Clin Transl Oncol. 2013 Jun;16(6):529-38.

[92] Bellodi C, Kopmar N, Ruggero D. Deregulation of oncogene-induced senescence and p53 translational control in X-linked dyskeratosis congenita. EMBO J. 2010 Jun 2;29(11):1865-76.

[93] Walbott H, Machado-Pinilla R, Liger D, Blaud M, Rety S, Grozdanov PN, et al. The H/ACA RNP assembly factor SHQ1 functions as an RNA mimic. Genes Dev. 2011 Nov 15;25(22):2398-408.

[94] Grozdanov PN, Fernandez-Fuentes N, Fiser A, Meier UT. Pathogenic NAP57 mutations decrease ribonucleoprotein assembly in dyskeratosis congenita. Hum Mol Genet. 2009 Dec 1;18(23):4546-51.

[95] Brault ME, Lauzon C, Autexier C. Dyskeratosis congenita mutations in dyskerin SUMOylation consensus sites lead to impaired telomerase RNA accumulation and telomere defects. Hum Mol Genet. 2013 Sep 1;22(17):3498-507.

[96] Salowsky R, Heiss NS, Benner A, Wittig R, Poustka A. Basal transcription activity of the dyskeratosis congenita gene is mediated by Sp1 and Sp3 and a patient mutation in a Sp1 binding site is associated with decreased promoter activity. Gene. 2002 Jun 26;293(1-2):9-19.

[97] Lingner J, Hughes TR, Shevchenko A, Mann M, Lundblad V, Cech TR. Reverse transcriptase motifs in the catalytic subunit of telomerase. Science. 1997 Apr 25;276(5312): 561-7.

[98] Moriarty TJ, Ward RJ, Taboski MA, Autexier C. An anchor site-type defect in human telomerase that disrupts telomere length maintenance and cellular immortalization. Mol Biol Cell. 2005 Jul;16(7):3152-61.

[99] Bryan TM, Goodrich KJ, Cech TR. Telomerase RNA bound by protein motifs specific to telomerase reverse transcriptase. Mol Cell. 2000 Aug;6(2):493-9.

[100] Podlevsky JD, Bley CJ, Omana RV, Qi X, Chen JJ. The telomerase database. Nucleic Acids Res. 2008 Jan;36(Database issue):D339-43.

[101] Collopy LC, Walne AJ, Cardoso S, de la Fuente J, Mohamed M, Toriello H, et al. Triallelic and epigenetic-like inheritance in human disorders of telomerase. Blood. 2015 Jul 9;126(2):176-84.

[102] Xin ZT, Beauchamp AD, Calado RT, Bradford JW, Regal JA, Shenoy A, et al. Functional characterization of natural telomerase mutations found in patients with hematologic disorders. Blood. 2007 Jan 15;109(2):524-32.

[103] Zaug AJ, Crary SM, Jesse Fioravanti M, Campbell K, Cech TR. Many disease-associated variants of hTERT retain high telomerase enzymatic activity. Nucleic Acids Res. 2013 Oct;41(19):8969-78.

[104] Stone MD, Mihalusova M, O'Connor C M, Prathapam R, Collins K, Zhuang X. Stepwise protein-mediated RNA folding directs assembly of telomerase ribonucleoprotein. Nature. 2007 Mar 22;446(7134):458-61.

[105] Lai CK, Miller MC, Collins K. Roles for RNA in telomerase nucleotide and repeat addition processivity. Mol Cell. 2003 Jun;11(6):1673-83.

[106] Chen JL, Opperman KK, Greider CW. A critical stem-loop structure in the CR4-CR5 domain of mammalian telomerase RNA. Nucleic Acids Res. 2002 Jan 15;30(2):592-7.

[107] Tesmer VM, Ford LP, Holt SE, Frank BC, Yi X, Aisner DL, et al. Two inactive fragments of the integral RNA cooperate to assemble active telomerase with the human protein catalytic subunit (hTERT) in vitro. Mol Cell Biol. 1999 Sep;19(9):6207-16.

[108] Mitchell JR, Collins K. Human telomerase activation requires two independent interactions between telomerase RNA and telomerase reverse transcriptase. Mol Cell. 2000 Aug;6(2):361-71.

[109] Robart AR, Collins K. Investigation of human telomerase holoenzyme assembly, activity, and processivity using disease-linked subunit variants. J Biol Chem. 2009 Feb 12;285(7):4375-86.

[110] Canudas S, Houghtaling BR, Bhanot M, Sasa G, Savage SA, Bertuch AA, et al. A role for heterochromatin protein 1gamma at human telomeres. Genes Dev. 2011 Sep 1;25(17):1807-19.

[111] Walne AJ, Vulliamy T, Beswick R, Kirwan M, Dokal I. TINF2 mutations result in very short telomeres: analysis of a large cohort of patients with dyskeratosis congenita and related bone marrow failure syndromes. Blood. 2008 Nov 1;112(9):3594-600.

[112] Sasa GS, Ribes-Zamora A, Nelson ND, Bertuch AA. Three novel truncating TINF2 mutations causing severe dyskeratosis congenita in early childhood. Clin Genet. 2012 May;81(5):470-8.

[113] Xin ZT, Ly H. Characterization of interactions between naturally mutated forms of the TIN2 protein and its known protein partners of the shelterin complex. Clin Genet. 2012 Mar;81(3):301-2.

[114] Yang D, He Q, Kim H, Ma W, Songyang Z. TIN2 protein dyskeratosis congenita missense mutants are defective in association with telomerase. J Biol Chem. 2011 Jul 1;286(26):23022-30.

[115] Frescas D, de Lange T. A TIN2 dyskeratosis congenita mutation causes telomerase-independent telomere shortening in mice. Genes Dev. 2014 Jan 15;28(2):153-66.

[116] Henriksson S, Farnebo M. On the road with WRAP53beta: guardian of Cajal bodies and genome integrity. Front Genet. 2015;6:91.

[117] Stern JL, Zyner KG, Pickett HA, Cohen SB, Bryan TM. Telomerase recruitment requires both TCAB1 and Cajal bodies independently. Mol Cell Biol. 2012 Jul;32(13): 2384-95.

[118] Zhong F, Savage SA, Shkreli M, Giri N, Jessop L, Myers T, et al. Disruption of telomerase trafficking by TCAB1 mutation causes dyskeratosis congenita. Genes Dev. 2011 Jan 1;25(1):11-6.

[119] Trahan C, Martel C, Dragon F. Effects of dyskeratosis congenita mutations in dyskerin, NHP2 and NOP10 on assembly of H/ACA pre-RNPs. Hum Mol Genet. 2009 Mar 1;19(5):825-36.

[120] Vulliamy T, Beswick R, Kirwan M, Marrone A, Digweed M, Walne A, et al. Mutations in the telomerase component NHP2 cause the premature ageing syndrome dyskeratosis congenita. Proc Natl Acad Sci U S A. 2008 Jun 10;105(23):8073-8.

[121] Keller RB, Gagne KE, Usmani GN, Asdourian GK, Williams DA, Hofmann I, et al. CTC1 Mutations in a patient with dyskeratosis congenita. Pediatr Blood Cancer. 2012 Aug;59(2):311-4.

[122] Walne AJ, Bhagat T, Kirwan M, Gitiaux C, Desguerre I, Leonard N, et al. Mutations in the telomere capping complex in bone marrow failure and related syndromes. Haematologica. 2013 Mar;98(3):334-8.

[123] Chen LY, Majerska J, Lingner J. Molecular basis of telomere syndrome caused by CTC1 mutations. Genes Dev. 2013 Oct 1;27(19):2099-108.

[124] White MF. Structure, function and evolution of the XPD family of iron-sulfur-containing 5'-->3' DNA helicases. Biochem Soc Trans. 2009 Jun;37(Pt 3):547-51.

[125] Uringa EJ, Lisaingo K, Pickett HA, Brind'Amour J, Rohde JH, Zelensky A, et al. RTEL1 contributes to DNA replication and repair and telomere maintenance. Mol Biol Cell. 2012 Jul;23(14):2782-92.

[126] Ballew BJ, Yeager M, Jacobs K, Giri N, Boland J, Burdett L, et al. Germline mutations of regulator of telomere elongation helicase 1, RTEL1, in Dyskeratosis congenita. Hum Genet. 2013 Apr;132(4):473-80.

[127] Le Guen T, Jullien L, Touzot F, Schertzer M, Gaillard L, Perderiset M, et al. Human RTEL1 deficiency causes Hoyeraal-Hreidarsson syndrome with short telomeres and genome instability. Hum Mol Genet. 2013 Aug 15;22(16):3239-49.

[128] Stuart BD, Choi J, Zaidi S, Xing C, Holohan B, Chen R, et al. Exome sequencing links mutations in PARN and RTEL1 with familial pulmonary fibrosis and telomere shortening. Nat Genet. 2015 May;47(5):512-7.

[129] Stanley SE, Noth I, Armanios M. What the genetics "RTEL"ing us about telomeres and pulmonary fibrosis. Am J Respir Crit Care Med. 2015 Mar 15;191(6):608-10.

[130] Fedick AM, Shi L, Jalas C, Treff NR, Ekstein J, Kornreich R, et al. Carrier screening of RTEL1 mutations in the Ashkenazi Jewish population. Clin Genet. 2014 Aug;88(2): 177-81.

[131] Korner CG, Wahle E. Poly(A) tail shortening by a mammalian poly(A)-specific 3'-exoribonuclease. J Biol Chem. 1997 Apr 18;272(16):10448-56.

[132] Mason PJ, Bessler M. mRNA deadenylation and telomere disease. J Clin Invest. 2015 May;125(5):1796-8.

[133] Ohga S, Kai T, Honda K, Nakayama H, Inamitsu T, Ueda K. What are the essential symptoms in the Hoyeraal-Hreidarsson syndrome? Eur J Pediatr. 1997 Jan;156(1): 80-1.

[134] Hoyeraal HM, Lamvik J, Moe PJ. Congenital hypoplastic thrombocytopenia and cerebral malformations in two brothers. Acta Paediatr Scand. 1970 Mar;59(2):185-91.

[135] Hreidarsson S, Kristjansson K, Johannesson G, Johannsson JH. A syndrome of progressive pancytopenia with microcephaly, cerebellar hypoplasia and growth failure. Acta Paediatr Scand. 1988 Sep;77(5):773-5.

[136] Aalfs CM, van den Berg H, Barth PG, Hennekam RC. The Hoyeraal-Hreidarsson syndrome: the fourth case of a separate entity with prenatal growth retardation, progressive pancytopenia and cerebellar hypoplasia. Eur J Pediatr. 1995 Apr;154(4):304-8.

[137] Savage SA, Alter BP. Dyskeratosis congenita. Hematol Oncol Clin North Am. 2009 Apr;23(2):215-31.

[138] Sznajer Y, Baumann C, David A, Journel H, Lacombe D, Perel Y, et al. Further delineation of the congenital form of X-linked dyskeratosis congenita (Hoyeraal-Hreidarsson syndrome). Eur J Pediatr. 2003 Dec;162(12):863-7.

[139] Cossu F, Vulliamy TJ, Marrone A, Badiali M, Cao A, Dokal I. A novel DKC1 mutation, severe combined immunodeficiency (T+B-NK- SCID) and bone marrow transplantation in an infant with Hoyeraal-Hreidarsson syndrome. Br J Haematol. 2002 Dec;119(3):765-8.

[140] Borggraefe I, Koletzko S, Arenz T, Fuehrer M, Hoffmann F, Dokal I, et al. Severe variant of x-linked dyskeratosis congenita (Hoyeraal-Hreidarsson Syndrome) causes significant enterocolitis in early infancy. J Pediatr Gastroenterol Nutr. 2009 Sep;49(3): 359-63.

[141] Malbora B, Avci Z, Ozbek N. Aplastic anemia and Hoyeraal-Hreidarsson syndrome. Skinmed. 2014 Mar-Apr;12(2):117-8.

[142] Ballew BJ, Joseph V, De S, Sarek G, Vannier JB, Stracker T, et al. A recessive founder mutation in regulator of telomere elongation helicase 1, RTEL1, underlies severe immunodeficiency and features of Hoyeraal Hreidarsson syndrome. PLoS Genet. 2013 Aug;9(8):e1003695.

[143] Knight SW, Heiss NS, Vulliamy TJ, Aalfs CM, McMahon C, Richmond P, et al. Unexplained aplastic anaemia, immunodeficiency, and cerebellar hypoplasia (Hoyeraal-Hreidarsson syndrome) due to mutations in the dyskeratosis congenita gene, DKC1. Br J Haematol. 1999 Nov;107(2):335-9.

[144] Lai W, Deng WP, Liu X, Chen HM, Dai Sh X. A recurrent p. A353V mutation in DKC1 responsible for different phenotypes of dyskeratosis congenita in a Chinese family. J Dermatol Sci. 2011 Aug;63(2):122-4.

[145] Marrone A, Sokhal P, Walne A, Beswick R, Kirwan M, Killick S, et al. Functional characterization of novel telomerase RNA (TERC) mutations in patients with diverse clinical and pathological presentations. Haematologica. 2007 Aug;92(8):1013-20.

[146] Gramatges MM, Qi X, Sasa GS, Chen JJ, Bertuch AA. A homozygous telomerase T-motif variant resulting in markedly reduced repeat addition processivity in siblings with Hoyeraal Hreidarsson syndrome. Blood. 2013 May 2;121(18):3586-93.

[147] Vogiatzi P, Perdigones N, Mason PJ, Wilson DB, Bessler M. A family with Hoyeraal-Hreidarsson syndrome and four variants in two genes of the telomerase core complex. Pediatr Blood Cancer. 2013 Jun;60(6):E4-6.

[148] Vulliamy TJ, Walne A, Baskaradas A, Mason PJ, Marrone A, Dokal I. Mutations in the reverse transcriptase component of telomerase (TERT) in patients with bone marrow failure. Blood Cells Mol Dis. 2005 May-Jun;34(3):257-63.

[149] Ye JZ, Hockemeyer D, Krutchinsky AN, Loayza D, Hooper SM, Chait BT, et al. POT1-interacting protein PIP1: a telomere length regulator that recruits POT1 to the TIN2/TRF1 complex. Genes Dev. 2004 Jul 15;18(14):1649-54.

[150] Takai KK, Kibe T, Donigian JR, Frescas D, de Lange T. Telomere protection by TPP1/POT1 requires tethering to TIN2. Mol Cell. 2011 Nov 18;44(4):647-59.

[151] Kocak H, Ballew BJ, Bisht K, Eggebeen R, Hicks BD, Suman S, et al. Hoyeraal-Hreidarsson syndrome caused by a germline mutation in the TEL patch of the telomere protein TPP1. Genes Dev. 2014 Oct 1;28(19):2090-102.

[152] Guo Y, Kartawinata M, Li J, Pickett HA, Teo J, Kilo T, et al. Inherited bone marrow failure associated with germline mutation of ACD, the gene encoding telomere protein TPP1. Blood. 2014 Oct 30;124(18):2767-74.

[153] Revesz T, Fletcher S, al-Gazali LI, DeBuse P. Bilateral retinopathy, aplastic anaemia, and central nervous system abnormalities: a new syndrome? J Med Genet. 1992 Sep; 29(9):673-5.

[154] Tsilou ET, Giri N, Weinstein S, Mueller C, Savage SA, Alter BP. Ocular and orbital manifestations of the inherited bone marrow failure syndromes: Fanconi anemia and dyskeratosis congenita. Ophthalmology. 2010 Mar;117(3):615-22.

[155] Anderson BH, Kasher PR, Mayer J, Szynkiewicz M, Jenkinson EM, Bhaskar SS, et al. Mutations in CTC1, encoding conserved telomere maintenance component 1, cause Coats plus. Nat Genet. 2012 Mar;44(3):338-42.

[156] Polvi A, Linnankivi T, Kivela T, Herva R, Keating JP, Makitie O, et al. Mutations in CTC1, encoding the CTS telomere maintenance complex component 1, cause cerebroretinal microangiopathy with calcifications and cysts. Am J Hum Genet. 2012 Mar 9;90(3):540-9.

[157] Savage SA. Connecting complex disorders through biology. Nat Genet. 2012 Mar; 44(3):238-40.

[158] Yamaguchi H, Baerlocher GM, Lansdorp PM, Chanock SJ, Nunez O, Sloand E, et al. Mutations of the human telomerase RNA gene (TERC) in aplastic anemia and myelodysplastic syndrome. Blood. 2003 Aug 1;102(3):916-8.

[159] Scheinberg P, Cooper JN, Sloand EM, Wu CO, Calado RT, Young NS. Association of telomere length of peripheral blood leukocytes with hematopoietic relapse, malignant transformation, and survival in severe aplastic anemia. JAMA. 2010 Sep 22;304(12):1358-64.

[160] Kim HJ, Perlman D, Tomic R. Natural history of idiopathic pulmonary fibrosis. Respir Med. 2015 Jun;109(6):661-70.

[161] Loyd JE. Pulmonary fibrosis in families. Am J Respir Cell Mol Biol. 2003 Sep;29(3 Suppl):S47-50.

[162] Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, Markin C, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. N Engl J Med. 2007 Mar 29;356(13):1317-26.

[163] Tsakiri KD, Cronkhite JT, Kuan PJ, Xing C, Raghu G, Weissler JC, et al. Adult-onset pulmonary fibrosis caused by mutations in telomerase. Proc Natl Acad Sci U S A. 2007 May 1;104(18):7552-7.

[164] Diaz de Leon A, Cronkhite JT, Katzenstein AL, Godwin JD, Raghu G, Glazer CS, et al. Telomere lengths, pulmonary fibrosis and telomerase (TERT) mutations. PLoS One. 2010;5(5):e10680.

[165] Povedano JM, Martinez P, Flores JM, Mulero F, Blasco MA. Mice with Pulmonary Fibrosis Driven by Telomere Dysfunction. Cell Rep. 2015 Jul 14;12(2):286-99.

[166] Cogan JD, Kropski JA, Zhao M, Mitchell DB, Rives L, Markin C, et al. Rare variants in RTEL1 are associated with familial interstitial pneumonia. Am J Respir Crit Care Med. 2015 Mar 15;191(6):646-55.

[167] Alder JK, Chen JJ, Lancaster L, Danoff S, Su SC, Cogan JD, et al. Short telomeres are a risk factor for idiopathic pulmonary fibrosis. Proc Natl Acad Sci U S A. 2008 Sep 2;105(35):13051-6.

[168] Armanios M. Syndromes of telomere shortening. Annu Rev Genomics Hum Genet. 2009;10:45-61.

[169] Kropski JA, Mitchell DB, Markin C, Polosukhin VV, Choi L, Johnson JE, et al. A novel dyskerin (DKC1) mutation is associated with familial interstitial pneumonia. Chest. 2014 Jul;146(1):e1-7.

[170] Fukuhara A, Tanino Y, Ishii T, Inokoshi Y, Saito K, Fukuhara N, et al. Pulmonary fibrosis in dyskeratosis congenita with TINF2 gene mutation. Eur Respir J. 2013 Dec; 42(6):1757-9.

[171] Stanley SE, Chen JJ, Podlevsky JD, Alder JK, Hansel NN, Mathias RA, et al. Telomerase mutations in smokers with severe emphysema. J Clin Invest. 2015 Feb;125(2): 563-70.

[172] Parry EM, Alder JK, Qi X, Chen JJ, Armanios M. Syndrome complex of bone marrow failure and pulmonary fibrosis predicts germline defects in telomerase. Blood. 2011 May 26;117(21):5607-11.

[173] Calado RT, Brudno J, Mehta P, Kovacs JJ, Wu C, Zago MA, et al. Constitutional telomerase mutations are genetic risk factors for cirrhosis. Hepatology. 2011 May;53(5): 1600-7.

[174] Fogarty PF, Yamaguchi H, Wiestner A, Baerlocher GM, Sloand E, Zeng WS, et al. Late presentation of dyskeratosis congenita as apparently acquired aplastic anaemia due to mutations in telomerase RNA. Lancet. 2003 Nov 15;362(9396):1628-30.

[175] Gadalla SM, Sales-Bonfim C, Carreras J, Alter BP, Antin JH, Ayas M, et al. Outcomes of allogeneic hematopoietic cell transplantation in patients with dyskeratosis congenita. Biol Blood Marrow Transplant. 2013 Aug;19(8):1238-43.

[176] Ayas M, Nassar A, Hamidieh AA, Kharfan-Dabaja M, Othman TB, Elhaddad A, et al. Reduced intensity conditioning is effective for hematopoietic SCT in dyskeratosis congenita-related BM failure. Bone Marrow Transplant. 2013 Sep;48(9):1168-72.

[177] Islam A, Rafiq S, Kirwan M, Walne A, Cavenagh J, Vulliamy T, et al. Haematological recovery in dyskeratosis congenita patients treated with danazol. Br J Haematol. 2013 Sep;162(6):854-6.

[178] Calado RT, Yewdell WT, Wilkerson KL, Regal JA, Kajigaya S, Stratakis CA, et al. Sex hormones, acting on the TERT gene, increase telomerase activity in human primary hematopoietic cells. Blood. 2009 Sep 10;114(11):2236-43.

[179] Khincha PP, Wentzensen IM, Giri N, Alter BP, Savage SA. Response to androgen therapy in patients with dyskeratosis congenita. Br J Haematol. 2014 May;165(3): 349-57.

[180] Ziegler P, Schrezenmeier H, Akkad J, Brassat U, Vankann L, Panse J, et al. Telomere elongation and clinical response to androgen treatment in a patient with aplastic anemia and a heterozygous hTERT gene mutation. Ann Hematol. 2012 Jul;91(7):1115-20.

[181] Giri N, Pitel PA, Green D, Alter BP. Splenic peliosis and rupture in patients with dyskeratosis congenita on androgens and granulocyte colony-stimulating factor. Br J Haematol. 2007 Sep;138(6):815-7.

[182] Zlateska B, Ciccolini A, Dror Y. Treatment of dyskeratosis congenita-associated pulmonary fibrosis with danazol. Pediatr Pulmonol. 2015 50(12):48-51.

[183] Noble PW, Albera C, Bradford WZ, Costabel U, du Bois RM, Fagan EA, et al. Pirfenidone for idiopathic pulmonary fibrosis: analysis of pooled data from three multinational phase 3 trials. Eur Respir J. 2015 Jan;47(1):243-53.

[184] Richeldi L, du Bois RM, Raghu G, Azuma A, Brown KK, Costabel U, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. N Engl J Med. 2014 May 29;370(22):2071-82.

[185] Giri N, Lee R, Faro A, Huddleston CB, White FV, Alter BP, et al. Lung transplantation for pulmonary fibrosis in dyskeratosis congenita: Case Report and systematic literature review. BMC Blood Disord. 2011;11:3.

[186] Silhan LL, Shah PD, Chambers DC, Snyder LD, Riise GC, Wagner CL, et al. Lung transplantation in telomerase mutation carriers with pulmonary fibrosis. Eur Respir J. 2014 Jul;44(1):178-87.

[187] Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, et al. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell. 1997 Oct 3;91(1):25-34.

[188] Lee HW, Blasco MA, Gottlieb GJ, Horner JW, 2nd, Greider CW, DePinho RA. Essential role of mouse telomerase in highly proliferative organs. Nature. 1998 Apr 9;392(6676):569-74.

[189] Raval A, Behbehani GK, Nguyen le XT, Thomas D, Kusler B, Garbuzov A, et al. Reversibility of Defective Hematopoiesis Caused by Telomere Shortening in Telomerase Knockout Mice. PLoS One. 2015;10(7):e0131722.

[190] Herrera E, Samper E, Martin-Caballero J, Flores JM, Lee HW, Blasco MA. Disease states associated with telomerase deficiency appear earlier in mice with short telomeres. EMBO J. 1999 Jun 1;18(11):2950-60.

[191] Gu BW, Bessler M, Mason PJ. A pathogenic dyskerin mutation impairs proliferation and activates a DNA damage response independent of telomere length in mice. Proc Natl Acad Sci U S A. 2008 Jul 22;105(29):10173-8.

[192] Beier F, Foronda M, Martinez P, Blasco MA. Conditional TRF1 knockout in the hematopoietic compartment leads to bone marrow failure and recapitulates clinical features of dyskeratosis congenita. Blood. 2012 Oct 11;120(15):2990-3000.

[193] Simeonova I, Jaber S, Draskovic I, Bardot B, Fang M, Bouarich-Bourimi R, et al. Mutant mice lacking the p53 C-terminal domain model telomere syndromes. Cell Rep. 2013 Jun 27;3(6):2046-58.

[194] Garcia-Cao I, Garcia-Cao M, Martin-Caballero J, Criado LM, Klatt P, Flores JM, et al. "Super p53" mice exhibit enhanced DNA damage response, are tumor resistant and age normally. EMBO J. 2002 Nov 15;21(22):6225-35.

[195] Ramunas J, Yakubov E, Brady JJ, Corbel SY, Holbrook C, Brandt M, et al. Transient delivery of modified mRNA encoding TERT rapidly extends telomeres in human cells. FASEB J. 2015 May;29(5):1930-9.

[196] Maida Y, Masutomi K. Telomerase reverse transcriptase moonlights: Therapeutic targets beyond telomerase. Cancer Sci. 2015 106(11):1486-92.

[197] Gu BW, Fan JM, Bessler M, Mason PJ. Accelerated hematopoietic stem cell aging in a mouse model of dyskeratosis congenita responds to antioxidant treatment. Aging Cell. 2011 Apr;10(2):338-48.

[198] Machado-Pinilla R, Sanchez-Perez I, Murguia JR, Sastre L, Perona R. A dyskerin motif reactivates telomerase activity in X-linked dyskeratosis congenita and in telomerase-deficient human cells. Blood. 2008 Mar 1;111(5):2606-14.

[199] Machado-Pinilla R, Carrillo J, Manguan-Garcia C, Sastre L, Mentzer A, Gu BW, et al. Defects in mTR stability and telomerase activity produced by the Dkc1 A353V mutation in dyskeratosis congenita are rescued by a peptide from the dyskerin TruB domain. Clin Transl Oncol. 2012 Oct;14(10):755-63.

[200] Manguan-Garcia C, Pintado-Berninches L, Carrillo J, Machado-Pinilla R, Sastre L, Perez-Quilis C, et al. Expression of the genetic suppressor element 24.2 (GSE24.2) decreases DNA damage and oxidative stress in X-linked dyskeratosis congenita cells. PLoS One. 2014;9(7):e101424.

[201] Iarriccio L, Manguan-Garcia C, Pintado-Berninches L, Mancheno JM, Molina A, Perona R, et al. GSE4, a Small Dyskerin- and GSE24.2-Related Peptide, Induces Telomerase Activity, Cell Proliferation and Reduces DNA Damage, Oxidative Stress and Cell Senescence in Dyskerin Mutant Cells. PLoS One. 2015;10(11):e0142980.

http://dx.doi.org/10.5772/65353

[202] Egusquiaguirre SP, Manguan-Garcia C, Pintado-Berninches L, Iarriccio L, Carbajo D, Albericio F, et al. Development of surface modified biodegradable polymeric nanoparticles to deliver GSE24.2 peptide to cells: a promising approach for the treatment of defective telomerase disorders. Eur J Pharm Biopharm. 2015 Apr;91:91-102.

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