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Short Telomere Syndromes in Clinical Practice: Bridging Bench and Bedside

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Abstract

Short telomere syndromes (STSs) are accelerated aging syndromes often caused by inheritable gene mutations resulting in decreased telomere lengths. Consequently, organ systems with increased cell turnover, such as the skin, bone marrow, lungs, and gastrointestinal tract, are commonly affected. Owing to diverse clinical presentations, STSs pose a diagnostic challenge, with bone marrow failure and idiopathic pulmonary fibrosis being frequent manifestations, occurring in association with gene mutations involving *DKC1* (for expansion of gene symbols, use search tool at www.genenames.org), *TERT*, *TERC*, and others. Inherited STSs demonstrate genetic anticipation, occurring at an earlier age with more severe manifestations in the affected progeny. Telomere lengths can be assessed in peripheral blood granulocytes and lymphocytes using a sensitive technique called flow cytometry—fluorescence in situ hybridization, and mutational analysis can be performed using next-generation sequencing assays. In approximately 40% of patients with shortened telomere lengths, gene mutations cannot be identified due to the fact that all STS-associated genes have not yet been defined or due to alternative mechanisms of telomere shortening. Danazol, an anabolic steroid, has been associated with hematologic responses in patients with STSs and associated bone marrow failure; however, its reported ability to increase telomerase activity and reduce telomere attrition needs further elucidation. Organ transplant is reserved for patients with end-organ failure and is associated with substantial morbidity and mortality. Herein, we summarize the clinical and laboratory characteristics of STSs and offer a stepwise approach to diagnose and manage complications in affected patients.

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For editorial comment, see page 815; for related article, see page 834

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Telomeres are hexanucleotide tandem repeats (TTAGGG) present at the ends of chromosomes, protecting them from gradual degradation in the process of aging.[1,2] Physiologic telomere losses occur over time, contributing to the process of aging. Once telomeres become critically short, the ends of chromosomes are exposed as double-stranded DNA breaks, resulting in DNA damage response, p53 activation, and apoptosis/senescence. This forms the basis for telomere lengths representing the "molecular clock" in human beings. Telomeres are distinguished by a 30- to 400-nucleotide long overhang of a guanosine-rich strand, also known as the G-strand overhang, that folds back to a double-stranded area, thereby forming a T-loop and a displacement or D-loop. Telomeres are flanked by special regions, called the shelterin complex, that regulate telomere lengths and protect them from DNA damage response.[1] The shelterin complex comprises telomeric repeat binding factors 1 and 2 (TRF1 and TRF2), protection of telomeres protein 1 (POT1), TRF1-interacting protein 2 (TIN2), repressor activator protein 1 (RAP1), and POT1-interacting protein (TPP1).[3]

Telomerase is a ribonucleoprotein enzyme complex (DNA polymerase) that builds new telomere sequences onto the ends of chromosomes. It is composed of the telomerase reverse transcriptase (TERT) and its RNA template (TERC). In addition, there are associated proteins, such as dyskerin, NHP2 (nuclear protein family A, member 2), NOP10 (nuclear protein family A, member 3), NAF1 (nuclear assembly factor 1), and GAR1 (nuclear protein family A, member 1), that help in telomere assembly, trafficking, recruitment, and stability (Figure 1).[2,4]

The aforementioned telomere repair process is not foolproof, and physiologic telomere attrition occurs at an estimated rate of 50 to 150 base pairs per cell division, resulting in

a gradual decrease in median telomere lengths with increasing age.[5] Germline mutations that impact the telomere complex can lead to telomere loss during cell replication, thus shortening the stem cell pool. Regenerative replicative stresses that occur in patients with bone marrow failure, postchemotherapy, or hematopoietic stem cell transplant (HCT) can enhance telomere shortening secondary to the increased mitotic activity. Finally, reactive oxygen species generated secondary to inflammation, toxins, or radiation exposure can cause DNA damage and telomere loss.[2]

Secondary to advances in genomics, shortened telomere lengths have now been identified to play a critical role in the pathogenesis of a variety of multisystemic disorders, resulting in clinical manifestations such as bone marrow failure, primary immunodeficiency, enterocolitis, idiopathic pulmonary fibrosis (IPF), premature-onset emphysema, cryptogenic cirrhosis of the liver, nodular regenerative hyperplasia of the liver (NRH), premature graying of hair, fibrous cartilage dysplasia, osteoporosis, and cancer predisposition syndromes (epithelial and hematologic malignancies).[6-8] In this critical review, we discuss the implications and management strategies for patients with short telomere syndromes (STSs).

ARTICLE HIGHLIGHTS

- Short telomere syndromes are multisystem disorders with widespread clinical manifestations.
- Organs with high cell turnover, such as the bone marrow, liver, lungs, and immune system, are commonly affected.
- Key clinical cues to suspect short telomeres in a patient are a personal or family history of premature graying of hair (at age <30 years), unexplained cytopenias, idiopathic pulmonary fibrosis, and cryptogenic cirrhosis.
- Flow cytometry–fluorescence in situ hybridization is the initial screening test, followed by genetic sequencing.
- Treatment requires a multidisciplinary approach.

CLINICAL FEATURES OF STSs

The STSs can involve multiple organ systems and are clinically defined by premature loss of progenitor stem cells, thereby affecting the regenerative capacity of involved cells/organs. An important characteristic of telomere diseases is the phenomenon of genetic anticipation wherein the disease manifestations tend to occur at an earlier age in subsequent generations, with severe manifestations. This is largely due to the fact that offspring inherit not only the telomere-related mutation but also shortened germline telomere lengths.[8]

Shortened Telomeres and Bone Marrow Failure

Shortened telomeres are commonly associated with bone marrow failure syndromes, including aplastic anemia. Among these, the classical telomere biology disorder that commonly presents in early childhood is dyskeratosis congenita (DKC), which is characterized by the presence of a clinical triad of nail dysplasia, oral leukoplakia, and abnormal skin pigmentation, associated with bone marrow failure.[2] Associated features can include pulmonary fibrosis; emphysema; cryptogenic liver cirrhosis; lacrimal ductal, esophageal, and urethral stenosis; premature graying of hair; avascular necrosis of hips and shoulders; periodontal disease; and an increased predisposition to epithelial and hematologic malignancies. Types of malignancies encountered in DKC include head and neck cancers (~70-fold), anogenital squamous cell carcinomas (~50-fold), myelodysplastic syndromes (~500-fold), and acute myeloid leukemia (~70-fold).[9] Although DKC is characterized by extremely short telomere lengths, the genetic basis and mode of inheritance is variable. Thus far, several mutant genes have been identified affecting the telomerase or the telomere protein complex, resulting in a DKC phenotype. These genes include *DKC1*, *TINF2*, *TERT*, *TERC*, *NHP2*, *NOP10*, *TCAB1*, and *RTEL1*.[8] Hoyeraal-Hreidarsson syndrome is a severe manifestation of DKC, presenting with concomitant cerebellar hypoplasia, usually occurring secondary to mutations involving *DKC1* or *RTEL1*.[10] Revesz syndrome consists of a severe DKC phenotype with associated retinal pathology, usually secondary to dominant mutations in *TINF2*.[11] Mutations involving *TERT* and *TERC* can be associated with bone marrow failure in adolescents and adults (autosomal dominant), with manifestations being more severe with *TERC* mutations, potentially due to a greater effect on telomerase activity.[12,13] Telomere-related adult-onset

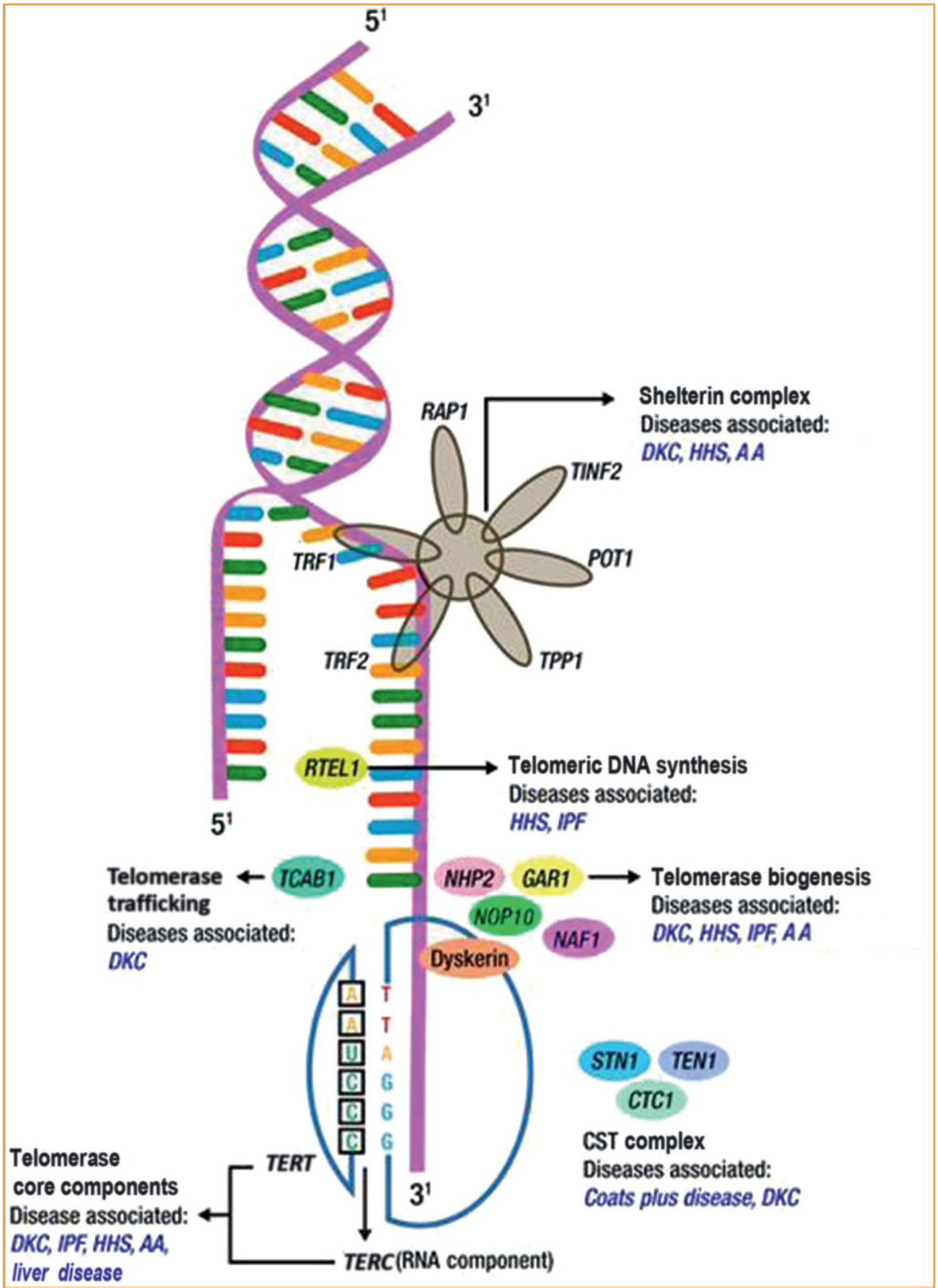

FIGURE 1. Constituents of the human telomere complex and associated genetic anomalies. AA = aplastic anemia; DKC = dyskeratosis congenital; HHS = Hoyeraal-Hreidarsson syndrome; IPF = idiopathic pulmonary fibrosis. For expansion of gene symbols, use search tool at www.genenames.org.

marrow failure is hard to distinguish from idiopathic aplastic anemia, with many patients presenting with macrocytosis and having similar initial responses to immunosuppressive therapies; however, very often these responses are not durable.

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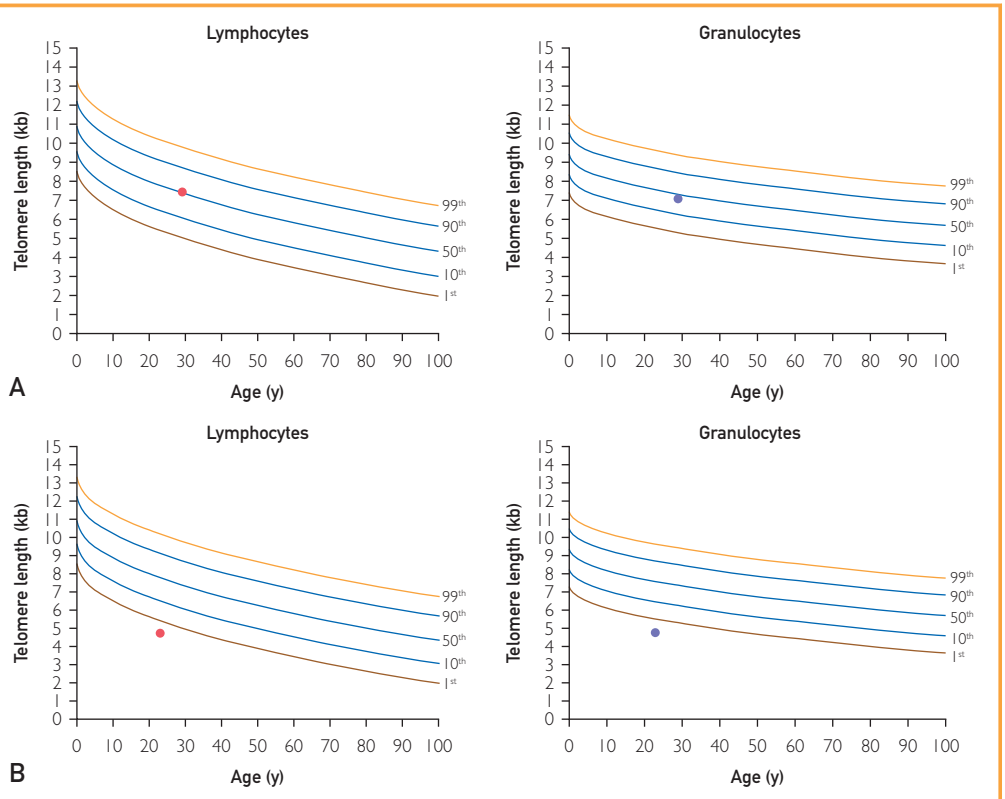

FIGURE 2. Flow cytometry—fluorescence in situ hybridization reports for a control subject and a patient with a suspected short telomere syndrome (STS). The color variations represent telomere length values that are >2 standard deviations away from the population mean. A, Control patient with telomere lengths in lymphocytes and granulocytes at the 50th percentile. B, A patient with telomere lengths less than the 1st percentile, highly suggestive for an STS.

IPF, Emphysema, and Interstitial Pneumonitis

Idiopathic pulmonary fibrosis is the most frequent pulmonary manifestation seen in patients with STSs (70%) and can occur in the setting of familial IPF (25%) or sporadic IPF (1%-3%).[6,14,15] In addition to IPF, additional pulmonary issues that can be encountered include bronchiolitis obliterans with organizing pneumonia, chronic hypersensitivity pneumonitis, interstitial pneumonitis, and emphysema.[16] Familial interstitial pneumonia, a disease entity clinically defined by the diagnosis of an idiopathic interstitial pneumonia or IPF in 2 or more relatives of common ancestry[17], is characterized by deleterious mutations not only in surfactant production genes such as *SFTPA2*, *SFTPC*, and *ABCA3*, among others, but also in telomere biology-associated genes such as *TERT*, *TERC*, *RTEL1*, *TINF2*, and *PARN*.[17,18] Pulmonary fibrosis secondary to telomere dysfunction usually presents in adulthood and is accelerated in patients who smoke. In fact, smokers can present with a mixed restrictive/obstructive pulmonary syndrome with overlapping features of emphysema and IPF.[19-21] A diagnosis of IPF is usually made in the clinical context with the help of pulmonary function testing and high-resolution computed tomography of the chest (inspiratory and expiratory views). In approximately 10% of patients who present with sporadic IPF, a paternal or maternal relative is likely to be diagnosed as having idiopathic interstitial pneumonia during subsequent years, emphasizing the importance of taking a detailed family history.

Hepatobiliary Manifestation of STSs

Hepatic manifestations were first noted in approximately 7% of patients with DKC and

since then have been identified as critical disease manifestations under the spectrum of STS. These manifestations include hepatic parenchymal inflammation, hepatic fibrosis, cryptogenic cirrhosis of the liver, NRH, and portal hypertension.[14,22] In a comprehensive study assessing liver pathology findings in patients with STSs, recurrent manifestations included inflammatory and fibrotic components (hepatocyte necrosis and bridging fibrosis) with several cases of noncirrhotic portal fibrosis, cirrhosis, NRH, and iron accumulation in the absence of a history of blood transfusions or hemochromatosis gene mutations.[22]

Gastrointestinal Manifestations

Gastrointestinal manifestations occur in approximately 16% of patients with STSs and can be the initial, often fatal presentation in young children.[23] Common manifestations include esophageal stenosis, B-cell immunodeficiency with enterocolitis, and a celiac-like enteropathy (telomere-mediated stem cell failure).[23] The intestinal mucosa in affected patients often displays villous atrophy, apoptosis, and anaphase bridging pointing to defects in regeneration in the gastrointestinal epithelium. Gastrointestinal manifestations tend to be most severe in patients with the Hoyeraal-Hreidarsson syndrome.

Primary Immunodeficiency Syndromes

Telomere shortening causes early senescence of B and T lymphocytes and a spectrum of immune defects. Immunodeficiency is most severe in children with the Hoyeraal-Hreidarsson syndrome, whereas milder defects can be seen in other genetic variants. A unique entity associated with short telomeres is the immunodeficiency, centromeric region stability and facial anomalies type 1 syndrome, caused by mutations in the *DNMT3B* gene, thus leading to severe hypomethylation of subtelomeric regions, critically short telomere lengths, and increased levels of telomeric repeat—containing RNA.[24,25] Furthermore, telomere-dependent replicative senescence contributes to immune dysregulation in certain patients with common variable immunodeficiency.[26] The association between altered immunity and telomere shortening is further highlighted by the fact that telomere attrition is thought to be responsible for diminished natural killer cell function in the elderly.[27]

CLINICAL MEASUREMENT OF TELOMERE LENGTH

Several methods have been described to measure telomere length: fluorescence in situ hybridization (FISH) (flow cytometry—FISH [flow-FISH] and quantitative FISH[28-30]), quantitative polymerase chain reaction (qPCR)—based techniques[31] (including single-cell telomere length measurement[32]), monochrome multiplex qPCR, optical techniques (surface-enhanced Raman scattering[33]), hybridization protection assay,[34] and telomere restriction fragment length analysis.[35] Although no single technique is ideal for use in all clinical and research scenarios, the telomere restriction fragment length analysis is considered the gold standard largely due to the simplicity of its protocol and accuracy; however, its widespread use is limited because it requires large amounts of DNA for analysis.[32,36] Despite the low cost, qPCR-based approaches are limited by their high interlaboratory variability and technical challenges.[36] For qPCR methods, telomeres are amplified using primers that attach to both telomeric C- and G-rich areas with mismatches, which thereby reduce primer-dimer formation, and amplification at telomeric templates happens only at low annealing temperatures. Discrepancy in pipetting volume among telomere and control reactions, and a possible consequent inaccuracy in estimating telomere lengths is a major limitation of the qPCR technique, thereby limiting its use in routine clinical practice.[36]

On the other hand, flow-FISH is an extremely accurate technique and can be used for assessing large numbers of samples, making it our preferred testing modality to assess telomere lengths.[36,37] Flow cytometry—FISH involves the use of a fluorescent probe that tags on to telomeres and thereby allows flow cytometry—based assessment of telomere fluorescence in distinct cell populations, such as granulocytes and lymphocytes. The ability to assess telomere lengths in different cell types is particularly advantageous in distinguishing malignancy-associated telomere attrition from a true STS. For example, a patient with myelodysplastic syndrome will have shorter age-adjusted telomere lengths in granulocytes,

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TABLE 1. Short Telomere Syndromes (STSs), Associated Genes, and Their Respective Modes of Inheritance

Disease	Inheritance	Associated genes	Reference, year
Bone marrow failure associated with STSs			
Dyskeratosis congenita[a]	X-linked recessive (more common)	Telomerase biogenesis:	
		DKC1	Heiss et al,[39] 1998
	Autosomal dominant	Telomerase core components:	
		TERT	Vulliamy et al,[45] 2001
		TERC	Armanios et al,[40] 2005
			Vulliamy et al,[41] 2006
		Shelterin component:	
		TIN2	Savage et al,[11] 2008
	Autosomal recessive	Telomerase biogenesis:	
		NOP10 (*NOLA3*)	Walne et al,[42] 2007
		NHP2	Vulliamy et al,[43] 2008
		Telomerase trafficking:	
		TCAB1	Zhong et al,[44] 2011
Hoyeraal-Hreidarsson syndrome[b]	X-linked recessive	Telomerase biogenesis:	
		DKC1	Glousker et al,[46] 2015
			Kocak et al,[47] 2014
	Autosomal dominant	Shelterin components:	
		TIN2	
	Autosomal recessive	Telomerase core components:	
		TERT	
		Shelterin components:	
		ACD (encoding TPP1)	
		Telomeric DNA synthesis:	
		RTEL1	
Revesz syndrome	Autosomal dominant	Shelterin component:	
		TIN2	Savage et al,[11] 2008
Cerebroretinal microangiopathy with calcifications and cysts (Coats plus disease)	Autosomal recessive	Telomeric DNA synthesis:	Anderson et al,[48] 2012
		CTC1, *STN1* (part of the CTC complex)	Simon et al,[49] 2016
Aplastic anemia	Autosomal dominant	Telomerase core components:	
		TERT	Guo et al,[50] 2014
		TERC	Martinez et al,[1] 2017
			Joksic et al,[51] 2012
Fanconi anemia	X-linked recessive	Telomerase biogenesis:	
	Autosomal recessive	*DKC1*	
		NOP10	
		NHP2	
	Autosomal dominant	Shelterin components:	
		ACD	
	Autosomal recessive	*FANCD2*	
Pulmonary STSs			
Idiopathic pulmonary fibrosis; or familial lung fibrosis or fibrotic idiopathic interstitial pneumonia	Autosomal dominant	Telomerase core components:	
		TERC	Armanios et al,[52] 2007
		TERT	Fingerlin et al,[18] 2013
		Telomerase biogenesis:	
		NAF1	Stanley et al,[4] 2016
Gastrointestinal STSs			
Cryptogenic cirrhosis or nodular regenerative hyperplasia	Autosomal dominant	Telomerase core components:	
		TERT	Calado et al,[22] 2009
		TERC	

Continued on next page

TABLE 1. Continued

Disease	Inheritance	Associated genes	Reference, year
Others			
Rothmund-Thomson syndrome	Autosomal recessive	*RECQL4*[c]	Ghosh et al,[53] 2012
Immunodeficiency, centromeric region instability and facial anomalies type 1	Autosomal recessive	*DNMT3B*[d]	Xu et al,[24] 1999 Yehezkel et al,[25] 2013
Facioscapulohumeral muscular dystrophy	Autosomal dominant	*DUX4*	Stadler et al,[54] 2013

[a]Approximately 40% of patients with dyskeratosis congenita still have unidentified mutations.
[b]Considered a severe variant of dyskeratosis congenita.
[c]Plays a role in telomere maintenance.
[d]Causes abnormally short telomeres, hypomethylation of subtelomeric regions, and elevated levels of abnormal telomeric transcripts known as telomeric repeat–containing RNA.

and, therefore, telomere lengths need to be assessed in lymphocytes to diagnose a true underlying STS. A flow-FISH report provides information on telomere lengths relative to age, which is important because telomere attrition is part of the normal aging process (Figure 2). Figure 2A describes a control patient with telomere lengths in granulocytes and lymphocytes at the 50th percentile, which is interpreted as a telomere length value similar to approximately 50% of the healthy population for the same age range. Figure 2B describes a patient with telomere lengths less than the 1st percentile in granulocytes and lymphocytes, highly suggestive of an STS. The main disadvantage of the flow-FISH technique is the requirement of large viable cell populations and expertise for transporting, processing, and analyzing clinical samples.[32] Flow cytometry–FISH testing has had a significant effect on the management of bone marrow failure syndromes, with a recent study reporting that approximately 24% of tested patients had shortened telomere lengths, influencing treatment and transplant decision making.[38]

TELOMERE MUTATION ANALYSIS

Several genes have been associated with STSs and can be subcategorized based on their normal physiologic role in telomere biology. Inheritance modes are usually X-linked recessive, autosomal recessive, and autosomal dominant. Genes associated with telomerase biogenesis include *DKC1*.[39] Telomerase core component genes include *hTERT* and *TERC*, whereas *TCAB1* is involved in telomerase trafficking. *TINF2* and *ACD* (encoding *TPP1*) are part of the shelterin complex.[2,6,7] In DKC, an X-linked recessive gene called dyskerin pseudouridine synthase 1 (DKC1) involved in telomerase biogenesis is the most frequent causative genomic aberration.[39] Other DKC-associated mutations include *hTERT*,[40] *TERC*,[41] and *TINF2*,[11] which are inherited in an autosomal dominant pattern, and the autosomal recessive mutations include *NOP10*,[42] *NHP2*,[43] and *TCAB1*.[44] A comprehensive list of STS-associated mutations, along with modes of inheritance, is detailed in Table 1.

Several targeted exome or next-generation sequencing (NGS) panels are currently available in commercial and research settings to detect causative mutations in patients with clinical suspicion for STSs. In our experience, mutations are identified in approximately 40% of clinically suspected cases, suggesting that there are several yet-to-be characterized genetic and epigenetic mechanisms of telomere length regulation.

THERAPEUTIC OPTIONS FOR PATIENTS WITH STSs

Organ transplant remains the mainstay for treatment of organ failure associated with STSs. Allogeneic HCT for DKC and STS-related bone marrow failure syndromes, lung transplant for IPF or emphysema, and liver transplant for end-stage cryptogenic cirrhosis of the liver have been performed with significant morbidity and mortality.[55-58] For patients with STS-associated bone marrow failure syndromes, we use reduced-intensity conditioning regimens for allogeneic HCT to minimize pulmonary toxicity associated with exposure to ionizing radiation and high doses of cytotoxic

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chemotherapy.[59] Details on modalities and outcomes of organ transplant for STSs are outside the scope of this review.

For several years, androgens have been used with success in patients with aplastic anemia with reported hematologic response rates of approximately 50%.[60-63] In vitro and animal model studies have found that androgens upregulate telomerase gene expression, thus slowing the rate of telomere attrition and enhancing cell regeneration.[50,64,65] In 2016, Townsley et al[66] reported findings from a phase 1/2 clinical trial that included 27 patients with age-adjusted telomere lengths less than the 1st percentile or a known STS mutation with clinical manifestations such as cytopenias, pulmonary fibrosis, or both, treated with danazol at an oral dose of 800 mg, administered twice daily. Telomere length attrition was reduced in 12 of 27 patients (44%) after 12 months of use, with adverse effects including hepatic transaminitis (41%), muscle cramps (33%), edema (26%), and lipid abnormalities (26%).[66] At 3 months, 19 of 24 patients (79%) had a hematologic response, and by 24 months, 10 of 12 (83%) had an improvement in their blood cell counts, and red blood cell transfusion independence was documented in 12 of 13 transfusion-dependent patients (92%). A mean increase in neutrophil counts by 300/mm^3 and in platelet counts by 14,250/mm^3 was documented in responders. At last follow-up (~2 years), 10 of 12 evaluable patients (83%) had an ongoing hematologic response.[66] Danazol therapy also resulted in stability in pulmonary functions in patients with pulmonary fibrosis. Liver fibrosis was objectively measured using ultrasound-mediated transient elastometry (FibroScan; Echosens) at baseline and at 2 years in 4 of 6 patients (66.7%) with an established diagnosis of cirrhosis, with 3 demonstrating a substantial reduction in the degree and extent of liver fibrosis.[66] In this study, the authors used a qPCR technique to measure telomere lengths and found that danazol had the ability to reduce telomere length attrition, presumably by increasing telomerase activity. This finding remains unsubstantiated and is further fraught with technical inaccuracies associated with the qPCR technique. In a previous study, Khincha et al[67] found that telomere lengths (measured at baseline and

TABLE 2. Investigational Treatment Strategies for Short Telomere Syndromes

Type of study	Inclusion criteria	Drug	Dose	No. of patients	Response rate	Adverse effects	Reference, year
Phase 1/2 clinical trial	Age-adjusted telomere length ≤1st percentile, known telomere-associated mutation, or both PLUS cytopenia, pulmonary fibrosis, or both	Danazol	800 mg/d	27	Primary end point: reduction in telomere attrition length; 12 of 27 (44%) at 12 mo	41% Transaminitis, 33% muscle cramps, 26% edema, 26% lipid abnormalities	Townsley et al,[66] 2016
Retrospective	DKC	Oxymetholone (n=14), fluoxymesterone (n=1), nandrolone (n=1)	For oxymetholone: 0.5-1 mg/kg for starting dose; 0.5-2.7 mg/kg per day for maintenance dose	16	70% of patients achieved a hematologic response	Hyperlipidemia (data not available on all patients)	Khincha et al,[67] 2014
Preclinical	Terc$^{+/-}$ mice, Terc$^{-/-}$ mice as controls	TA-65	Not established	NA	NA	NA	Bernardes de Jesus et al,[68] 2011
Preclinical	Mouse models of aplastic anemia	*TERT* gene therapy (AAV9-Tert delivery)	Not established	NA	NA	NA	Bär et al,[69] 2016

AAV9 = adeno-associated virus 9; DKC = dyskeratosis congenital; NA = not applicable.

at a median of 2.6 years on therapy), when assessed by the more sensitive flow-FISH technique in 4 androgen-responsive patients with DKC (oxymetholone-3, fluoxymesterone-1), were consistently the same or declined after androgen exposure. Hence, more evidence is needed before the routine use of danazol can be recommended to reduce telomere attrition rates in clinically affected and asymptomatic patients with STSs. Danazol should be used with caution in older men due to the risk of potentially worsening prostatic hypertrophy and stimulating the growth of preexisting prostatic adenocarcinoma. In addition, longer-term follow-up with danazol is much needed to mitigate concerns about potentially stimulating malignant clonal hematopoiesis in patients with bone marrow failure.

Retrospective studies in DKC have also reported clinical benefit with other anabolic steroids, such as oxymetholone, fluoxymesterone, and nandrolone.[67] Exciting new therapeutic options, such as gene therapy, are currently being studied in animal models and are outlined in Table 2.[68,69] TA-65 is a small molecule that has been reported to activate telomerase activity in mice, thereby effectively increasing telomere lengths.[68] Adeno-associated virus 9 gene therapy vector-induced telomerase activation has also shown efficacy in 2 independent mouse models of aplastic anemia.[70] These studies need additional safety and efficacy measures before they can transition to human trials but are exciting prospects for affected patients.

OUR APPROACH FOR MANAGEMENT OF TELOMERE DISORDERS

At Mayo Clinic, with the help of the Center for Individualized Medicine and the Division of Hematology, we established an Inherited Bone Marrow Failure Precision Genomics Clinic (ClinicalTrials.gov Identifier: NCT02958462) to aid with the diagnosis and management of inherited bone marrow failure syndromes, including STSs.[71] We suspect STSs when patients present with, or have a strong family history of, premature graying of hair (defined by consensus as onset of graying of hair before age 30 years), IPF, premature emphysema or idiopathic interstitial pneumonia, unexplained cytopenias or bone marrow failure, NRH or cryptogenic cirrhosis of the liver, along with other stigmata of STSs, such as esophageal, lacrimal ductal, and urethral stenosis. We obtain a detailed personal and family history and perform a thorough physical examination, followed by genetic counselor—assisted pedigree charting and counseling. We then assess telomere lengths in these individuals by using the peripheral blood flow-FISH assay. If telomere lengths are less than the 1st percentile in granulocytes and lymphocytes, then the probability of an STS is high and we proceed with mutational analysis by NGS (*DKC1*, *NOP10 [NOLA3]*, *NHP2 [NOLA2]*, *RTEL1*, *TERC [hTR]*, *TERT*, *WRAP53 [WDR79]*, *TINF2*, *TCAB1*, and *NAF1*). Patients with telomere lengths greater than the 10th percentile are unlikely to have an STS, and those with telomere lengths in the 1st to 10th percentile range could possibly have an STS. For these patients with indeterminate telomere lengths, we proceed with mutational analysis based on the strength of clinical suspicion (pretest probability). For patients with negative NGS test results, we do have a research protocol (Mayo Clinic) that allows us to proceed with advanced genomic techniques such as whole exome sequencing and RNA sequencing after obtaining written informed consent. In patients with cytopenia or bone marrow failure, we recommend a bone marrow aspiration and biopsy with cytogenetic studies and myeloid-relevant NGS assays to assess for clonal evolution, if indicated. We work closely with our colleagues in pulmonary medicine and gastroenterology to assess and screen for additional organ involvement. We obtain a baseline set of pulmonary function test results and a baseline high-resolution computed tomography scan of the chest with inspiratory and expiratory views. We assess the hepatobiliary tree with a baseline ultrasound and Doppler study of the abdomen and the portal venous system. If there is suspicion of liver involvement, additional studies, such as magnetic resonance elastography and liver biopsies, are undertaken. Annual head/neck and anogenital screening for cancers is recommended, and in age-eligible patients without substantial immunodeficiency, we strongly recommend the human papilloma virus vaccine.[72] Special focus is placed on bone health and risk of avascular necrosis of the hips and shoulder joints. Appropriate subspecialty and organ transplant referrals are made in a

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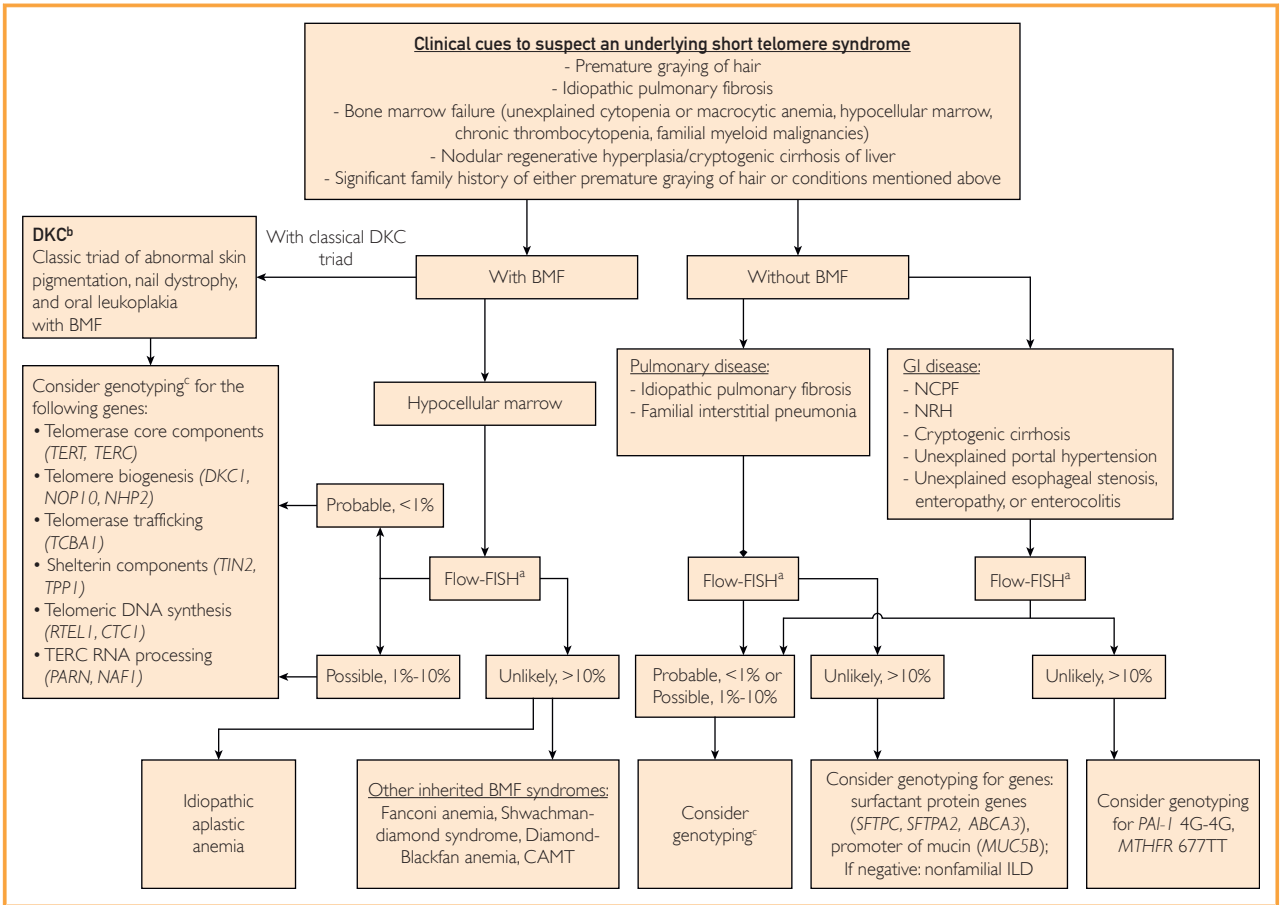

FIGURE 3. Summary of the various disorders associated with telomere shortening and steps in management. BMF = bone marrow failure; CAMT = congenital amegakaryocytic thrombocytopenia; DKC = dyskeratosis congenita; flow-FISH = flow cytometry–fluorescence in situ hybridization; GI = gastrointestinal; ILD = interstitial lung disease; NCPF = noncirrhotic portal fibrosis; NRH = nodular regenerative hyperplasia. For expansion of gene symbols, use search tool at www.genenames.org. [a]Flow cytometry–FISH estimates telomere length and compares it with that of age-matched controls (telomere length is usually measured in leukocytes; however, it may be falsely low in patients after stem cell transplant or myeloid malignancy, in which case lymphocytes are preferred). [b]DKC is a multisystem disorder, and several less common clinical features, such as developmental delay, cerebellar defects, short stature, enteropathy, liver disease, osteoporosis, etc, are identified. [c]Consider genotyping for known telomere biology disorder genes.

timely manner, and family members are screened after extensive counseling. Our schematic approach to manage a suspected STS is summarized in Figure 3.

CONCLUSION

The STSs occur secondary to premature shortening of telomere lengths, resulting in multisystemic disease, often associated with substantial morbidity and mortality. Telomere shortening results in accelerated aging of the stem cell pool, with predominant manifestations seen in organs with high cell turnover. The classical STS often seen in pediatric patients is DKC, characterized by a lacy skin rash, nail and hair dystrophy, oral leukoplakia, and bone marrow failure; milder versions secondary to different gene mutations can occur at all ages. Prominent manifestations of STSs include bone marrow failure, IPF, premature emphysema, cryptogenic cirrhosis of the liver, NRH with portal hypertension, premature graying of hair, luminal stenosis (esophageal, lacrimal ductal, and urethral), and immune deregulation. Telomere lengths can be assessed using the flow-FISH method, whereas gene mutations, seen in

approximately 40% of affected patients, can be identified using NGS techniques. Organ transplant is reserved for patients with end-organ dysfunction, and androgen therapy with drugs such as danazol has been associated with clinical benefit. Advances in understanding telomere biology and the use of gene therapy to augment impaired telomere functioning are exciting future prospects.

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Abbreviations and Acronyms: **AA** = aplastic anemia; **AAV9** = adeno-associated virus 9; **BMF** = bone marrow failure; **CAMT** = congenital amegakaryocytic thrombocytopenia; **DKC** = dyskeratosis congenita; **flow-FISH** = flow cytometry–fluorescence in situ hybridization; **GI** = gastrointestinal; **HCT** = hematopoietic stem cell transplant; **HHS** = Hoyeraal-Hreidarsson syndrome; **ILD** = interstitial lung disease; **IPF** = idiopathic pulmonary fibrosis; **NCPF** = noncirrhotic portal fibrosis; **NGS** = next-generation sequencing; **NRH** = nodular regenerative hyperplasia; **qPCR** = quantitative polymerase chain reaction; **STS** = short telomere syndrome

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