The genetics and clinical manifestations of telomere biology disorders

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Abstract: Telomere biology disorders are a complex set of illnesses defined by the presence of very short telomeres. Individuals with classic dyskeratosis congenita have the most severe phenotype, characterized by the triad of nail dystrophy, abnormal skin pigmentation, and oral leukoplakia. More significantly, these individuals are at very high risk of bone marrow failure, cancer, and pulmonary fibrosis. A mutation in one of six different telomere biology genes can be identified in 50–60% of these individuals. *DKC1*, *TERC*, *TERT*, *NOP10*, and *NHP2* encode components of telomerase or a telomerase-associated factor and *TINF2*, a telomeric protein. Progressively shorter telomeres are inherited from generation to generation in autosomal dominant dyskeratosis congenita, resulting in disease anticipation. Up to 10% of individuals with apparently acquired aplastic anemia or idiopathic pulmonary fibrosis also have short telomeres and mutations in *TERC* or *TERT*. Similar findings have been seen in individuals with liver fibrosis or acute myelogenous leukemia. This report reviews basic aspects of telomere biology and telomere length measurement, and the clinical and genetic features of those disorders that constitute our current understanding of the spectrum of illness caused by defects in telomere biology. We also suggest a grouping schema for the telomere disorders. *Genet Med* 2010:12(12):753–764.

Key Words: *telomere, telomere biology disorder, dyskeratosis congenita, bone marrow failure, idiopathic pulmonary fibrosis*

O ver the past 5–10 years, it has become increasingly clear that alterations in telomere integrity can directly impact human health. Telomeres are the specialized nucleoprotein structures present at the ends of chromosomes and are comprised of long nucleotide repeats $(TTAGGG)_n$ bound by a host of telomere-specific and so-called "accessory" proteins.[1,2] The essential function of telomeres derives from the exquisite sensitivity cells have to double-stranded deoxyribonucleic acid (DNA) ends and the robust mechanisms to detect and repair DNA ends created by DNA double-stranded breaks. Telomeres protect the DNA ends present naturally at the termini of linear chromosomes from being acted on as if they were broken ends, thereby inhibiting telomere fusions, illegitimate recombination, and genomic instability.

Because the semiconservative DNA replication machinery is unable to replicate terminal DNA at chromosome ends, telo-meric sequence is lost with each round of DNA replication.[3] Consequently, telomeres shorten with aging. In peripheral blood leukocytes, the cells most extensively studied, the rate of attrition is greatest during the first year of life.[4] Thereafter, telomeres shorten more gradually. When the extent of telomeric DNA loss exceeds a critical threshold, a robust antiproliferative signal is triggered, leading to cellular senescence or apoptosis. Thus, telomere attrition is thought to contribute to aging phenotypes.[1]

With the 1985 discovery of telomerase,[5] the enzyme that extends telomeric nucleotide repeats, there has been rapid progress both in our understanding of basic telomere biology and the connection of telomere biology to human disease. Although the initial focus had been on the relevance of telomere biology to cancer, we now appreciate that there is a spectrum of human disease that originates from inherent defects in telomere length maintenance, making an understanding of telomere biology disorders of importance to a broader spectrum of clinicians. This review will describe the basis of human telomere length variation and inheritance, various methods available to measure telomere length, how a diagnosis of a telomere biology disorder can be established, and, finally, the medical complications associated with short telomeres.

TELOMERASE AND TELOMERE-ASSOCIATED PROTEINS

The elucidation of factors associated with telomerase through basic biology research has been crucial to the identification of genes mutated in telomere biology disorders. A diagram of these proteins is shown in Figure 1. Telomerase consists of two core components, TERT, a reverse transcriptase, and TERC, a ribonucleic acid (RNA) that contains a template for telomere repeat addition.[3] The telomerase complex catalyzes the synthesis and extension of terminal telomeric DNA. Telomerase is developmentally regulated, with expression in most human tissues only during the first few weeks of embryogenesis.[6] From the neonatal period onward, telomerase activity is largely repressed, except in certain highly proliferative tissues such as skin, intestine, and bone marrow, which are thought to contain stem cell-like subpopulations, and in dividing lymphocytes, ovaries, and testes.[6–9] Telomerase is also upregulated in most cancer cells, reflecting the need for telomere maintenance for proliferative potential.[7,10]

TERT and TERC are sufficient for telomerase reconstitution in vitro, but additional factors are required for telomerase biogenesis, localization, and activity in vivo. Importantly, dyskerin is associated with catalytically active telomerase by TERC and is required for normal TERC levels and telomerase activity in vivo.[11] Dyskerin, which is encoded by *DKC1*, associates with H/ACA box-containing small nucleolar RNAs, including TERC.[12] It is also involved in posttranscriptional pseudouridylation and forms a ribonucleoprotein complex with NOP10, NHP2, and GAR1.[13,14] These factors are also found in a complex with TERC; they do not mediate its pseudouridylation but

Fig. 1. Schematic of genes mutated in telomere biology disorders. The chromosome end consists of (TTAGGG)$_n$ repeats that fold back to form a t loop. This structure is capped by a six-protein complex termed "shelterin." The telomerase enzyme complex is encoded by *TERT*, *TERC*, *DKC1*, *NOP10*, *NHP2*, and *GAR1* (not shown). The inheritance pattern of mutations in these genes is noted. Approximately 50–60% of patients with classic dyskeratosis congenita will have a mutation in one of these genes. *TINF2*, TRF1-interacting nuclear factor 2; *TERT*, telomerase; *TERC,* telomerase RNA component; *DKC1,* dyskerin; *NOP10,* NOLA3, nucleolar protein family A, member 3; *NHP2,* NOLA2, nucleolar protein family A, member 2.

instead are involved in the proper stability, regulation, or trafficking of telomerase.[15] NAF1, which is exchanged for GAR1 in the dyskerin/NOP10/NHP2 complex, and the ATP-ases pontin and reptin are also required for assembly of the catalytically competent telomerase complex but are not associated with the mature enzyme.[16,17] Finally, there is the protein TCAB1, which associates with active telomerase by binding to the CAB box motif of TERC, and is responsible for the localization of telomerase to nuclear Cajal bodies, a critical step for telomere maintenance.[18] Given the importance of these factors, we speculate that a mutation in one or more may be identified in human telomere biology disorders in the future.

In addition to telomerase, proteins that bind to telomeric DNA are important for normal telomere maintenance and function. These include shelterin, a six-protein telomere-specific complex, which serves a number of functions including inhibiting the DNA damage response at telomeres, negatively regulating telomerase, and promoting telomere replication and sister telomere cohesion.[2] The importance of this complex is underscored by the finding that mutations in one of the shelterin components, TIN2, which is encoded by *TINF2*, have been found to cause very short telomeres and dyskeratosis congenita (DC, see later).[19]

TELOMERE LENGTH AND INHERITANCE

Telomere length measurements in a variety of organisms have revealed tremendous variability between species. Individ-ual species, however, maintain their germline telomeres broadly around a defined set point. In humans, telomeres are several kilobases (kb) in length. Telomere length is inversely proportional to age. Although telomere length varies from tissue to tissue, it is correlated within an individual[20–22] (unpublished data). This likely reflects the proliferative history of the constituent cells, the level of telomerase activity, and other factors that may lead to telomere attrition. Thus, highly proliferative tissues may exhibit shorter telomere length.

Genetic factors have been shown to impact telomere length in healthy populations, with heritability estimates ranging from 36 to 82%.[23–26] Telomere length correlated significantly with paternal but not maternal telomere length in a number of studies, suggesting a possible role for genomic imprinting.[27,28] Additionally, inheritance of telomere length follows a polygenic pattern, consistent with a quantitative trait. A number of quantitative trait linkage and genome-wide association studies have been performed to identify genetic factors that modulate telomere length. Several studies have identified single-nucleotide polymorphisms (SNPs) that may contribute to telomere length regulation.[29] For example, SNPs were identified in an intron in *BICD1*, which encodes a protein involved in vacuolar trafficking, and to a region between *BRUNOL4* and *VPS34,* which encode a putative RNA binding protein and a lipid kinase involved in autophagy, respectively.[30] The significance of these associations is unclear, although it is notable that vacuolar protein sorting genes, such as *VPS34,* have been widely implicated in telomere maintenance in a yeast model system.[31] Most

recently, a SNP was identified in a genome-wide association study of mean telomere length in a region 1.5 kb downstream from *TERC*.[32] Whether the SNP affects *TERC* expression levels has yet to be evaluated; however, this is an attractive hypothesis as cells are very sensitive to reductions in TERC (see later).

TELOMERE LENGTH MEASUREMENT

Several methods are available in the research setting to determine telomere length in whole cells and DNA preparations (reviewed in Refs. 33 and 34). The current gold standard of telomere length determination is terminal restriction fragment measurement on Southern blots. This method requires several hundred nanograms of high-quality DNA and uses restriction enzymes to digest telomeric DNA. The resultant fragments are run on a Southern blot and the median length determined. Telomere length can be determined on metaphase chromosomes and on fixed or imbedded tissues through the use of telomere-specific probes and fluorescence in situ hybridization (FISH).[35] The intensity of the telomeric fluorescent signal is measured relative to another DNA-specific probe. Single telomere length analysis, a polymerase chain reaction-based approach, is useful in understanding telomere length differences between chromosomes.[36,37]

Quantitative polymerase chain reaction has emerged as a method that is useful in larger epidemiologic studies of telomere length because it is a high-throughput platform.[38,39] It requires small amounts of high-quality DNA and determines a ratio of telomere signal to single copy gene signal, which serves as a telomere length surrogate.

Telomere length measurement by automated multicolor flow cytometry combined with FISH (flow-FISH) on white blood cell (WBC) subsets is very useful in understanding telomere length differences in the immune system.[35] Unlike other methods, it requires either fresh or cryopreserved blood samples but gives specific measurements on the WBC subset telomere length. Testing of telomere length in WBC subsets using the flow-FISH procedure is currently the only method of determining telomere length that is clinically available. Examples of results from flow-FISH telomere length testing are shown in Figure 2.

The method of telomere length measurement, cell type studied, as well as study design and statistical power, must be considered in the interpretation of data in studies evaluating telomere lengths in individuals with various conditions. For example, there can be considerable interindividual variability in telomere length, even among age-matched subjects. Therefore, a large number of controls are essential to properly interpret data. Many earlier studies reported either the mean telomere length in patients compared with age-matched controls or the difference between the average telomere lengths of patients and controls (deltaTEL). The measurements are useful in understanding the characteristics of a group of individuals but not always applicable to the individual patient. In more recent studies, individual telomere length is plotted against the distribution of age-dependent telomere lengths observed in a large number of "normal" individuals to determine an individual's percentile telomere length (e.g., Fig. 2).[35,40]

THE TELOMERE BIOLOGY DISORDERS

Abnormalities in telomere biology resulting in clinically significant disease were first recognized in patients with DC.[12] Studies of individuals with severe aplastic anemia (SAA), a frequent complication of DC, found that a subset of individuals with SAA had shorter telomeres and mutations in telomere biology genes that overlapped with those that cause DC.[41–47] Short telomeres and mutations in the same genes have also been identified in a small percentage of individuals with pulmonary fibrosis,[48,49] as well as nonalcoholic liver disease[50] and acute myelogenous leukemia (AML).[51,52] These disorders represent a broad spectrum of related diseases caused by defective telomere
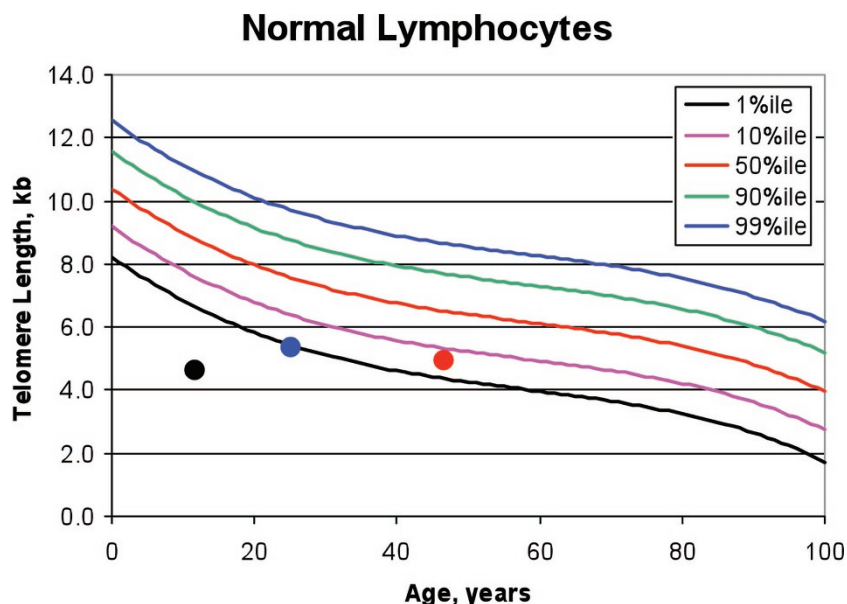
Fig. 2. Example of lymphocyte telomere length measured by flow cytometry with fluorescence in situ hybridization. Black circle, patient with classic dyskeratosis congenita; blue circle, patient with aplastic anemia; and red circle, patient with isolated pulmonary fibrosis. kb, kilobases; %ile, percentile. Graph with courtesy from Drs. Blanche Alter and Peter Lansdorp.

maintenance. This section will describe the clinical presentation, diagnosis, and overlapping features of these related disorders (Box 1).

DYSKERATOSIS CONGENITA

Clinical features

The disorder now known as DC was first described between 1906 and 1910.[53] Case reports initially described the diagnostic triad of nail dystrophy, lacy reticular pigmentation of the neck and upper chest, and oral leukoplakia in males. As additional cases were identified, including in females, the presence of bone marrow failure (BMF), cancer, and numerous other medical problems was noted.[54,55] Figure 3 shows features of the diagnostic triad. The clinical characteristics may also include abnormal pigmentation changes not restricted to the upper chest and neck, eye abnormalities (epiphora, blepharitis, sparse eyelashes, ectropion, entropion, and trichiasis),[56] dental abnormalities (caries and periodontal disease),[57] esophageal stenosis, urethral stenosis, avascular necrosis of the femur and/or humerus, osteopenia, pulmonary fibrosis, and liver disease. Patients with DC are at risk of progressive BMF, myelodysplastic syndrome or AML, and solid tumors (usually squamous cell carcinoma of the head/neck or anogenital cancer).[58]

Two disorders, Hoyeraal-Hreidarsson syndrome and Revesz syndrome, represent severe variants of DC. Hoyeraal-Hreidarsson syndrome is characterized by many of the DC features listed above plus the presence of cerebellar hypoplasia.[59,60] These patients also have significant developmental delay and immunologic abnormalities. Individuals with Revesz syndrome have bilateral exudative retinopathy and intracranial calcifications, in addition to many features of DC.[61,62] The determination of very short telomeres and mutations in telomere biology genes in both Hoyeraal-Hreidarsson (*DKC1* and *TINF2*) and Revesz (*TINF2*) syndromes firmly establishes that these disorders are indeed within the DC spectrum.[19,60,63]

Inheritance

DC is inherited in X-linked recessive, autosomal dominant, and autosomal recessive forms. Approximately one half of patients with DC have a mutation identified in one of six known genes, *DKC1*, *TERC*, *TERT*, *TINF2*, *NHP2,* and *NOP10* (Table 1).[55,64] The mutations in the X-linked recessive gene, *DKC1*, were the first link to the role of telomere biology in DC pathogenesis.[12,65] Fibroblasts from patients with *DKC1* mutations were shown to have very short telomeres.[12] This is thought to be due to a decrease in TERC in *DKC1* mutant cells, which results in a deficiency in cellular telomerase activity.[12]

Mutations in *TERC* or *TERT* were subsequently identified in the autosomal dominant form of DC.[46,47,66] These patients may have classic DC with many complications, whereas others may be less severely affected. Functional analysis of several disease-associated *TERC* and *TERT* mutations revealed that they result in short telomeres due to haploinsufficiency of telomerase rather than a dominant negative effect, indicating that telomerase is limiting in normal cells. A small number of severely affected patients have also been shown to have DC due to autosomal recessive inheritance of *TERT* mutations, which result in significantly decreased levels of telomerase.[47,67] Patients with biallelic *TERT* mutations seem to be more severely affected (i.e., Hoyeraal-Hreidarsson variant) than those with the autosomal dominant *TERT* mutations.

Homozygosity mapping of consanguineous families with DC identified autosomal recessive inheritance of mutations in

Box 1: Telomere biology disorder groupings

Individuals with short telomeres and/or a germline mutation in a telomere biology gene should be considered to have a telomere biology disorder. One schema for how they can be further grouped is presented below. The groupings are based on our current clinical knowledge. It is important to recognize that an individual's placement in a particular group may change over time if additional disease manifestations become apparent.

Classical Dyskeratosis Congenita (DC)—Patients with the mucocutaneous triad of dysplastic nails, skin pigmentation abnormalities, and oral leukoplakia or those who have one component of the triad in combination with a hypoplastic bone marrow and two or more of the other somatic manifestations of DC as suggested by Vulliamy *et al.*[74] These individuals have very short telomeres (<1st percentile for age) in leukocytes measured by flow-FISH.[40]

Hoyeraal Hreidarsson Syndrome—Individuals with documented cerebellar hypoplasia and additional manifestations of classical DC.

Revesz Syndrome—Individuals with bilateral exudative retinopathy and additional manifestations of classical DC.

Mutation Carrier—Asymptomatic or presymptomatic individuals with a mutation in a gene known to cause DC. These individuals are typically identified by screening of family members of affected individuals. In families in which affected individuals do not carry a mutation in one of the known six genes, an asymptomatic individual may be presumed to be a mutation carrier based on the presence of telomeres less than the 1st percentile for age.

DC-like—Individuals who have several features suggestive of DC but do not yet meet diagnostic criteria. Examples could be a patient with bone marrow failure, developmental delay, and a family history of pulmonary fibrosis, in the absence of another diagnosis. Telomeres are typically short but may not be as short as in classical DC. A mutation in a telomere biology gene may or may not be identified. These patients may fulfill the criteria for classical DC over time and should be monitored appropriately.

Isolated Subtypes—Individuals who carry a known mutation in a telomere biology gene and/or have telomeres less than the 1st percentile for age who have the isolated presentation of a condition known to be associated with a telomere biology disorder and dyskeratosis congenita. These conditions include isolated bone marrow failure, pulmonary fibrosis, unexplained liver disease, myelodysplastic syndrome, acute myelogenous leukemia, or early age of onset cancers within the DC spectrum. For example, a patient with isolated bone marrow failure and very short telomeres (<1st percentile for age) or one with isolated pulmonary fibrosis and a *TERT* mutation would be considered to have an isolated subtype. These patients, too, may fulfill the criteria for classical DC or the DC-like grouping over time.

Additional Considerations—The extent to which mutation carriers, DC-like, or isolated subtype individuals need monitoring for DC-related complications is unclear and should be considered on a case-by-case basis. For example, a 3-year-old child with isolated bone marrow failure and a *TINF2* mutation is at very high risk of complications during hematopoietic stem-cell transplantation (HSCT) and should receive a modified HSCT regimen. On the other hand, a 70-year-old individual with isolated pulmonary fibrosis and a *TERT* mutation may not have additional risks but their family members should be counseled for potential disease risk and the existence of genetic anticipation.
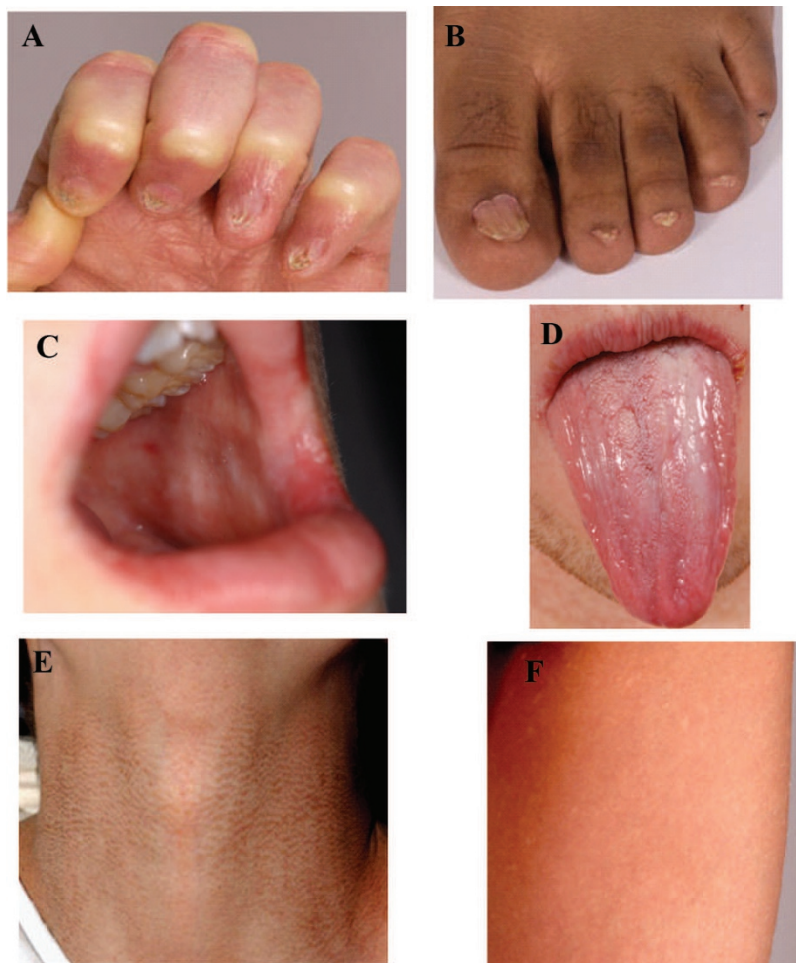
Fig. 3. The diagnostic triad of dyskeratosis congenita. A, Fingernail dysplasia; (B) toenail dysplasia; (C) leukoplakia of the buccal mucosa; (D) leukoplakia of the tongue; (E and F) skin pigmentation abnormalities can be diverse and occur anywhere on the body; (E) hyperpigmentation of the neck; and (F) hypopigmented areas on the upper thigh.

NOP10 (one family) or *NHP2* (two families) as causative of DC.[53,67] The NHP2 and NOP10 proteins associate with dyskerin and GAR1 in the H/ACA small nuclear ribonucleoprotein structures. *NHP2* and *NOP10* mutations seem to result in reduced levels of TERC, similar to the effects of a *DKC1* mutation. Individuals with these mutations have the more severe form of DC, Hoyeraal-Hreidarsson syndrome.

The sixth gene implicated in DC, *TINF2*, encodes not a component of telomerase but rather a component of the telomeric shelterin complex, TIN2.[2,19] In fact, mutations in *TINF2* are responsible for a large proportion of autosomal dominant DC.[19,68] Patients with *TINF2* mutations may have severe forms of DC, including the Revesz syndrome variant but silent carriers (i.e., individuals who have a *TINF2* mutation and very short telomeres but lack the clinical phenotype) have also been identified.[19] In contrast to the molecular mechanism by which mutations in telomerase components lead to telomere shortening, the mechanism by which TIN2 mutations result in telomere shortening has not yet been identified.

Diagnosis

The ability to diagnose DC has improved during the last 3–4 years, in part, through studies of telomere length in individuals with inherited BMF syndromes (IBMFSs) and other disorders. The diagnosis is quite straightforward in patients who present with the diagnostic triad of dysplastic nails, abnormal skin pigmentation, and oral leukoplakia, with or without additional medical complications. However, the diagnosis is often challenging because the clinical manifestations vary widely in age of onset, even in individuals from the same family. The clinical complications of DC, including the diagnostic triad, can evolve over time. Clinical telomere length testing has helped improve our ability to diagnosis DC.

Measurement of telomere length peripheral WBCs by flow-FISH was found to be both sensitive and specific for identifying patients with DC.[40] In that study, telomere length in leukocyte subsets (granulocytes, total lymphocytes, naïve T cells, memory T cells, B cells, and NK cells) was determined by flow-FISH on cells from patients with DC, their relatives, and patients with other IBMFS. Percentiles of telomere length were generated by comparison to a reference group of 400 healthy controls (newborn through 100 years of age).[35,40] Values below the 1st percentile for age were considered "very short." The diagnostic sensitivity and specificity of very short telomeres were >90% in total lymphocytes, naïve T cells, and B cells for the diagnosis of DC in comparison with healthy relatives of patients with DC or

Table 1 Genes that cause dyskeratosis congenita and other telomere biology disorders

Gene, protein name(s)	Chromosomal locus	MIM no.	Inheritance	No. reported mutations	Approximate percent of patients with mutations				
					DC[a]	Isolated SAA	Isolated IPF	Isolated fibrotic liver disease	Isolated AML
DKC1 DKC1, dyskerin	Xq28	300126	XLR	>40	17–36	NR	NR	NR	NR
TINF2 TIN2, TERF1 (TRF1)-interacting nuclear factor 2	14q11.2	604319	AD	~17	11–24	5[b]	NR	NR	NR
TERC TERC, telomerase RNA component	3q26.3	602322	AD	~40	6–10	1–5[c]	1–3[d]	3 families[e]	NR
TERT TERT, telomerase reverse transcriptase	5p15.53	187270	AD, AR	~40	1–7	3–4[f]	7–12[d]	2 families[e]	2[g]
NOP10 NOP10, NOLA3, nucleolar protein family A, member 3	15q14-q15	606471	AR	1	<1	NR	NR	NR	NR
NHP2 NHP2, NOLA2 nucleolar protein family A, member 2	5q35.5	606470	AR	3	<1	NR	NR	NR	NR

The approximate percent of patients with causative mutations in telomere biology genes in the disorders is listed.
[a]Based on reports from Dyskeratosis Congenita Registry[64] and National Cancer Institute's Dyskeratosis Congenita cohort.
[b]Derived from Du et al.,[98] based on 6 of 109 children with SAA who had a *TINF2* mutation.
[c]Derived from Yamaguchi et al.[44] with 3 of 210 patients with SAA and from Vulliamy et al.[45] with 2 of 17 patients with SAA reported to have a *TERC* mutation.
[d]Based on reports of Tsakiri et al.[49] and Armanios et al.[48] who identified five individuals with *TERT* and one with *TERC* mutations from 46 families and five individuals with *TERT* and one with *TERC* mutations from 72 probands with IPF and no features of DC, respectively.
[e]Number of families reported by Calado et al.[50] Incidence in unrelated individuals is not known.
[f]Derived from Yamaguchi et al.[46] with 7 of 200 patients with moderate or SAA reported to have a *TERT* mutation.
[g]Calado et al.[52] reported that 20 of 596 patients with AML (1.9%) had the A1062T variant in *TERT* compared with 13 of 1110 controls (0.6%). In their initial screen of 222 patients with AML, nine had the A1062T variant and five had different *TERT* mutations.
XLR, X-linked recessive; AD, autosomal dominant; AR, autosomal recessive; DC, dyskeratosis congenita; SAA, severe aplastic anemia; IPF, idiopathic pulmonary fibrosis; AML, acute myelogenous leukemia; NR, none reported.

non-DC IBMFS patients.[40] Rare apparently unaffected relatives with very short telomeres (silent carriers) were later shown to have mutations in the same DC genes as the affected probands.[19] Figure 2 shows an example of telomere length results that could be expected from a patient with classic DC compared with patients with SAA and pulmonary fibrosis associated with mutations in *TERC* or *TERT*.

A subsequent study from a different laboratory used a modified flow-FISH method, which involved comparison of telomere signal intensity in peripheral blood mononuclear cells with that of an aneuploid (4N) cell line.[69] Individual telomere length percentiles were determined based on the age-dependent distribution of telomere length found in a population of 234 healthy controls, ranging from 1 day to 94 years of age. That study found that telomere length was sensitive for DC but not specific. The lack of specificity in that study may have been a consequence, in part, of a smaller number of controls, which resulted in a wider extrapolated 1st percentile interval. The differences between the laboratory and analytic methods of the two studies suggest that the results are not directly comparable.

Most studies to date have evaluated telomere length measurement in total WBCs, which has proven to be a useful surrogate for constitutional telomere length, even when a nonhematopoietic tissue (e.g., lung) is primarily affected. Although tissue-specific telomere factor(s), other than telomerase have yet to be described, it is possible that such factor(s) exists, for which testing of telomere length in WBC may lead to an erroneous conclusion that a particular condition is not a telomere biology disorder.
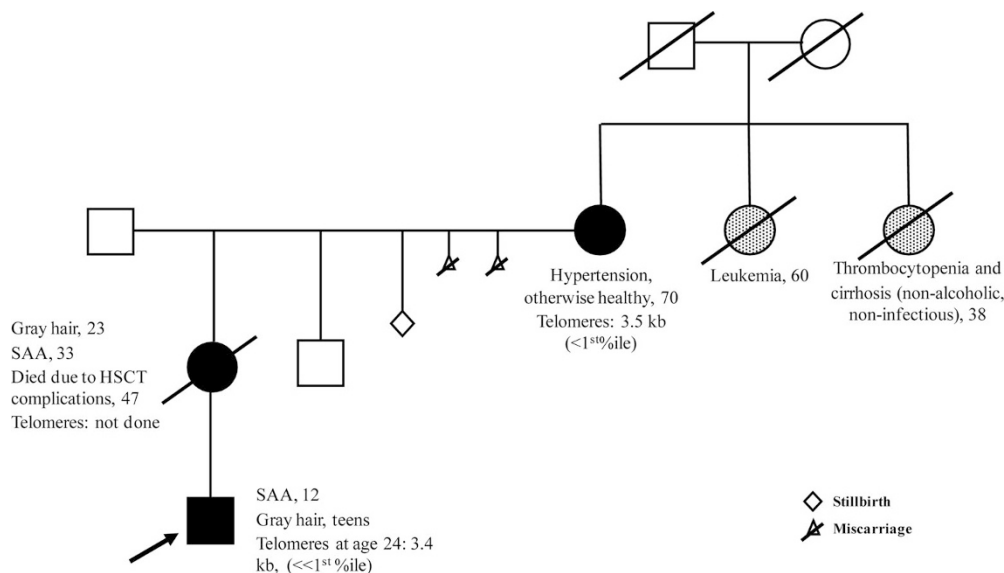
Genetic anticipation

The penetrance, severity, and time of onset of the clinical features of DC are variable even among family members with the same mutation. The telomere length of paternal, more so than maternal, chromosomes in the zygote is thought to contribute to this variability in healthy individuals.[24,27,28,70] Nonetheless, disease anticipation (increasing disease severity and earlier onset with succeeding generations) has been observed in several multigeneration families with autosomal dominant DC.[71–74] Mechanistically, disease anticipation has been shown to be associated with progressive telomere shortening and increasing numbers of chromosomes with very short telomeres.[71,73] Figure 4 shows the pedigree of a family with autosomal dominant inheritance of a *TINF2* mutation. The importance of disease anticipation is that an affected individual may have a phenotypically unaffected but mutation-positive parent, and their siblings may also be at risk. Additionally, carrier offspring may need to be screened for associated illnesses at a younger age than the presentation age of their parents.

Clinical management

The clinical management of DC is complicated due to the highly variable onset and progression of DC manifestations, even between family members.[55,75] Individuals may have few physical findings and normal bone marrow function, and others may present at very young ages with the diagnostic triad, early-onset BMF, and other medical problems. Genetic counseling of the patient and family should be incorporated into the management as soon as the diagnosis of DC is considered.[75]

BMF is often the complication that requires the most intensive management. Hematopoietic stem-cell transplantation (HSCT) is the only curative treatment for BMF and leukemia, but historically, it has had poor long-term efficacy, in part due to treatment-related morbidity and mortality.[76–85] Pulmonary fibrosis and hepatic and gastrointestinal complications are fre-

Fig. 4. Example of a family with genetic anticipation due to a *TINF2* mutation. The arrow indicates the proband. Mutation carriers and the obligate mutation carrier are shaded in black. The history of the proband's paternal family is not available. Possible, but untested, affected relatives are shown in gray. The history of the proband's maternal great grandparents is unknown, but one of them was likely a mutation carrier. Specific medical complications, if present, are noted on the pedigree. The numbers indicate the age in years of the finding. The family is part of our Institutional Review Board-approved study.[132] Lymphocyte telomere lengths measured by flow-FISH are shown. The median lymphocyte telomere length for a healthy 70-year-old individual is 5.8 kb and for a healthy 24-year-old individual is 7.6 kb. SAA, severe aplastic anemia; HSCT, hematopoeitic stem-cell transplantation; %ile, percentile for age.

quently reported.[78] Recent clinical trials that use nonmyeloablative conditioning regimens and exclude agents known to be associated with severe pulmonary, and hepatic toxicity show promising results but have limited follow-up time.[86]

If a suitable HSCT donor is not available, androgen therapy may be considered for BMF.[75,87] Clinical reports indicate that patients with DC may have an improvement in cytopenias while on androgens, although efficacy data are lacking. Androgens have been shown to affect *TERT* gene expression and telomerase activity in hematopoietic cells, including *TERT* mutant cells, providing a potential mechanism of action.[88] As with other patients with an IBMFS, patients with DC on androgen therapy need to have careful monitoring for abnormalities in cholesterol, triglycerides, and liver function, and for the development of liver adenomas. It is recommended that BMF patients taking androgens do not use concurrent growth factors, such as granulocyte-colony stimulating factor or erythropoietin, due to reports of splenic peliosis and rupture.[89]

Individuals with DC are at an 11-fold increased risk of cancer compared with the general population. The risk of certain head and neck cancers, for example, tongue squamous cell cancer, is elevated by about 1000-fold.[58] Annual cancer screening by a dermatologist, gynecologist for females, and an otolaryngologist, and biannual dental examinations are important for early detection.[75,90] Because the risk of tongue squamous cell cancer is so dramatically elevated in DC, DC should be carefully considered in any individual presenting with this cancer, particularly in young, nonsmoking, nondrinking adults.

APLASTIC ANEMIA

Aplastic anemia is diagnosed based on well-established peripheral blood count and bone marrow cellularity criteria.[91] Nonetheless, it is a complex, heterogeneous BMF disorder. It can present at any age and is now often subclassified into inherited and acquired forms, based on underlying etiology. Acquired aplastic anemia may be characterized by the presence of immune-mediated BMF.[91] Exposures to putative environmental risk factors, chemicals such as benzene, idiosyncratic reactions to drugs such as chloramphenicol, and certain infections contribute to the etiology of acquired aplastic anemia,[91] although in most cases an instigating factor cannot be identified. Immune-mediated T-cell destruction of the bone marrow has been shown both in vitro and in vivo, but the reason for T-cell activation in these patients is not clear. A combination of genetic risk factors, including SNPs in tumor necrosis factor-alpha[92,93] and gamma-interferon[94] may be contributory. Despite this knowledge, there is no diagnostic test to establish immune-mediated aplastic anemia.

Treatment approaches, long-term prognosis, disease surveillance, and counseling differ substantially for patients with acquired aplastic anemia versus those with an IBMFS. For example, patients with telomere biology disorders do not respond to immunosuppressive therapy for BMF,[87] may be at increased risk of HSCT complications, require additional molecular or genetic screening of related HSCT donors, and have an increased risk of certain cancers. Thus, it is crucial to carefully consider a telomere biology disorder as an underlying cause of aplastic anemia. Importantly, BMF may be the first sign of an inherited telomere biology disorder, and, historically, these patients were often initially diagnosed as having acquired aplastic anemia. Today, studies of telomere length by flow-FISH in WBC subsets and mutation analyses of telomere biology genes can be instrumental in differentiating inherited aplastic anemia because of a telomere biology disorder from acquired aplastic anemia and other IBMFS.

Telomere length in patients with acquired aplastic anemia may be relatively shorter than normal, but it is not as short as in patients with telomere biology disorders. Early studies of aplastic anemia suggested that telomeres were shorter than average in mononuclear cells or granulocytes in about one third of patients compared with controls.[41–43] Telomerase activity was shown to be upregulated in acquired aplastic anemia, possibly to counteract telomere loss.[95] Patients with acquired aplastic anemia may have short telomeres because of the rapid turnover of hematopoietic progenitor cells to compensate for a failing bone marrow.[43,96] This effect may be more notable in granulocytes, which turnover rapidly and often have shorter telomeres than mononuclear cells in controls.[97]

Individuals with apparently acquired aplastic anemia who did not respond to immunosuppression had the shortest telomeres in several studies.[41–43] This led to the hypothesis that some individuals with apparently acquired aplastic anemia may have an underlying genetic defect, manifested by short telomeres. This was confirmed by the identification of mutations in *TERC*[44,45] or *TERT*[46,47] in patients with what had been previously diagnosed as having acquired aplastic anemia. The *TERC* and *TERT* mutations reported in aplastic anemia do affect enzyme function and, thus, are likely the cause of the short telomeres. Similarly, *TINF2* exon 6 mutations were identified in 6 of 106 pediatric anonymous registry patients who underwent unrelated donor HSCT for SAA,[98] a cohort for whom immunosuppressive therapy presumably failed. Four of the mutations had been previously reported to be associated with early-onset DC.

These mutations are rare but, taken together, do explain the presence of short telomeres in up to 10% of patients with aplastic anemia. Some of these patients may have a family history of cancer, mild hematopoietic abnormalities, pulmonary fibrosis, or liver disease. Telomere length testing by flow-FISH in WBC subsets is recommended for all patients with aplastic anemia or BMF. This will identify patients with a telomere biology disorder and has major implications for their treatment and long-term outcome, as reviewed earlier in the text.

HEMATOPOIETIC MALIGNANCIES

A number of reports have suggested a pathologic role for telomerase mutations in the development of certain hematologic malignancies. Hypomorphic *TERC* or *TERT* mutations were identified in four families in a study of familial myelodysplastic syndrome/AML.[51] The mucocutaneous features of DC were absent in these families, although BMF, gastric cancer, and pulmonary fibrosis were reported in some mutation carriers. Constitutional *TERT* mutations were also reported in cases of sporadic AML,[52] although the most frequently associated gene variant, *TERT* A1062T, was also found in unaffected controls, albeit at a significantly lower frequency (the frequency was increased 3-fold in patients). In a separate study, the hypomorphic A1062T *TERT* allele was also found at a higher frequency in individuals with chronic lymphocytic lymphoma and diffuse large B-cell lymphoma relative to normal controls.[99] Thus, *TERT* A1062T status is emerging, at the very least, as a risk factor for these particular hematologic malignancies.

IDIOPATHIC PULMONARY FIBROSIS

Idiopathic pulmonary fibrosis (IPF) is a complex disorder characterized by progressive and irreversible lung scarring that ultimately leads to respiratory failure. Most cases are sporadic, although 2–20% seem to be familial with autosomal dominant inheritance and variable penetrance.[100] A number of environ-

mental and genetic factors have been implicated, including defective telomere maintenance. In a study of 73 probands from families with IPF, six were found to be heterozygous for mutations in *TERT* or *TERC*.[48] They also had short telomeres compared with related, wild-type family members as determined in lymphocytes by flow-FISH. Although IPF was the dominant phenotype, one of these probands had other relatives with aplastic anemia. Additional *TERT* and/or *TERC* mutations were identified in a separate study of familial and sporadic IPF.[49] Again, some family members in these IPF pedigrees had clinical features similar to DC, including anemia, cancer, and osteoporosis/osteopenia. Consistent with a role of defective telomere maintenance in IPF, defective telomere maintenance, pulmonary fibrosis and other pulmonary diseases have been noted in 20% of individuals with DC.[101] Patients with DC who undergo HCST are at increased risk of pulmonary complications as well.[85] IPF was among the variable clinical phenotypes present in a study of subjects with novel *TERC* mutations.[102] Thus, patients with IPF should be evaluated for germline mutations in *TERC* and *TERT*. Those that harbor such a mutation should be considered to be within the DC spectrum and should be managed and counseled in the same way as patients with classical DC.

LIVER DISEASE

Liver disease, peptic ulceration, or enteropathy has also been noted in approximately 7% of individuals with DC.[103] The clinicopathologic features of these gastrointestinal manifestations of defective telomere maintenance, however, remain poorly defined. Most information is found in single case reports or small case series. Noncirrhotic portal hypertension[104–106] and hepatopulmonary syndrome[107] have been reported in individuals with DC. Similar to IPF, liver disease may be the presenting clinical manifestation of a telomere biology disorder. In a study of family members of five unrelated individuals with BMF and *TERT* or *TERC* mutations, severe liver disease was found to occur in some mutation carriers independently of BMF or other apparent organ involvement.[50] Although the hepatic pathology findings were variable in the seven liver biopsies available for review, combined inflammation and fibrosis, and nodular regeneration were common. Again, as recommended for individuals with IPF, a telomere biology disorder should be considered in individuals with unexplained severe liver disease and family histories with clinical features found in DC.

TELOMERIC REPEAT CONTAINING RNA AND IMMUNODEFICIENCY-CENTROMERIC INSTABILITY-FACIAL ANOMALIES SYNDROME TYPE 1

Once thought to be transcriptionally silent, it is now clear that human telomeres are transcribed to produce telomeric repeat containing RNA (TERRA).[108,109] TERRA, which is noncoding RNA, localizes to telomeric foci and has been implicated in the formation of telomeric heterochromatin and telomerase inhibition. Recently, changes in TERRA levels and other aspects of telomere biology were described in immunodeficiency-centromeric instability-facial anomalies syndrome (ICF syndrome type 1, OMIM #242860). ICF type 1 is caused by biallelic mutations in DNA methyltransferase 3b (*DNMT3B*).[110–112] This defect results in abnormal subtelomeric methylation and abnormally short or undetectable telomeres on some chromosome arms.[113] Transcription of TERRA is significantly increased in

these individuals, possibly due to defects in the telomeric structure caused by aberrant methylation. Thus, ICF type 1 represents the first syndrome in which changes in TERRA have been described. Whether any of the clinical manifestations of ICF type 1 are the result of changes in TERRA remains to be determined.

TELOMERES IN EPIDEMIOLOGY STUDIES

The association of telomere length in surrogate tissues, such as blood or buccal cell DNA, and disease has been evaluated in numerous epidemiologic studies. Several case-control studies have suggested that individuals with cancer may have shorter leukocyte or buccal cell telomere length. These include studies of bladder,[114–116] head and neck,[116] lung,[117] gastric,[118,119] and ovarian[120] cancer. Case-control and cohort studies of individuals with breast cancer have yielded inconsistent results.[121,122] One cohort study of prostate cancer, which evaluated precancer diagnosis specimens, did not find an association between telomere length and prostate cancer.[123] Similar null results were found in skin cancer studies.[124]

Numerous association studies between telomere length and nonmalignant diseases or conditions have been reported as well, including cardiovascular disease,[125,126] diabetes,[127] obesity,[128] and perception of stress.[129] A number of these studies have suggested that affected individuals have shorter telomeres than unaffected individuals. Some of these and other studies have evaluated lifestyle factors, such as diet, exercise, and smoking, and telomere length.[123,130] Telomere length does seem to be shorter in smokers than nonsmokers, but the effect may be small. Studies of healthy (i.e., normal body mass index, more exercise, higher intakes of fruits and vegetables, and nonsmokers) compared with unhealthy lifestyles have suggested that individually these variables do not significantly contribute to telomere length but that in combination, there is an association between longer telomeres and a healthier lifestyle.[123,130]

Telomere epidemiology studies seek to find statistically significant associations between relatively small changes in telomere length between cases and controls. This is in contrast to the very short telomeres found in individuals with telomere biology disorders. These combined approaches will advance understanding of the complex mechanisms of telomere length regulation in inherited disorders and common diseases.

SUMMARY AND FUTURE DIRECTIONS

A broad spectrum of clinical phenotypes is now appreciated in telomere biology disorders (Fig. 5 and Box 1). We propose the grouping schema listed in Box 1 in an effort to better define the overlap of telomere biology disorders. DC is the first disease identified to be caused by abnormalities in genes important in telomere biology. It also has the most severe phenotype. Individuals with isolated SAA or IPF may also have a genetic defect in telomere biology. The clinical manifestations of DC develop at highly variable rates, even within family members with the same mutation. Individuals with apparently isolated SAA, IPF, or liver disease warrant consideration aimed at identifying a mutation in a telomere biology gene. Genetic counseling and a detailed family history should be obtained. Individuals with mutations in a telomere biology gene should be counseled for a potential increased risk of cancer because patients with DC have very high cancer rates. The rates of cancer in individuals with isolated SAA or IPF due to *TERT* or *TERC* mutations are not known; future studies are needed to better understand cancer incidence in these individuals.

Although mutations in six genes have been shown to cause DC and/or related telomere biology disorders, approximately 40–50% of patients with DC lack a detectable mutation in one
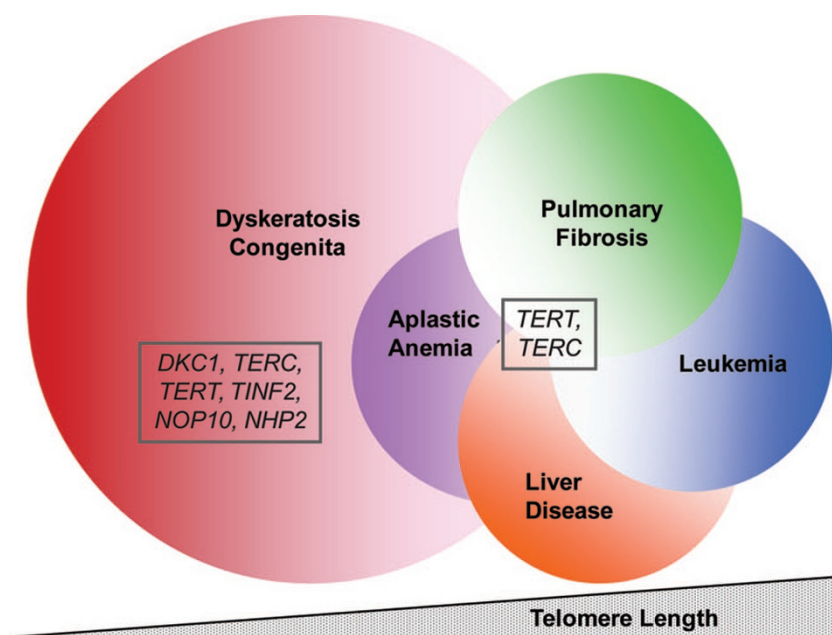
Fig. 5. Telomere biology disorders overlap phenotypically and genetically. Patients with DC are the most severely affected and have extremely short telomeres. Telomeres are shorter than normal, although not as short as in DC, in some individuals with aplastic anemia, pulmonary fibrosis, leukemia, fibrotic liver disorders, and possibly others. Genes that are mutated in these disorders are shown in the gray boxes.

of these genes, which suggests that there are others to be identified. DC inheritance follows essentially all inheritance patterns (X-linked recessive, autosomal dominant, and autosomal recessive), but many cases seem to occur sporadically (i.e., with a negative family history). This could be due to new mutations in a family, incomplete disease penetrance, or variable expressivity of the consequences of the mutation. Thus, the absence of a positive family history should not preclude further evaluation of a suspected case.

The complexities of telomere length regulation suggest that there may be many more genes that contribute to DC and related telomere biology disorders. Numerous genes that regulate telomere length are still being identified and further characterized. In addition, there is an increasing body of research literature that supports nontelomeric functions of TERT such as in the Wnt/β-catenin signaling pathway.[131] Findings such as these lead to the possibility that certain aspects of human disease associated with *TERT* mutations or SNPs may be secondary to loss of one or more of these extracurricular activities.

With the availability of telomere length testing and an increasing awareness of the variable presentations, it is likely we will appreciate a greater frequency of telomere biology disorders than previously thought. As laboratory research leads to a greater understanding of the factors that control telomere length, directed therapies for telomere biology disorders may be at hand in the future.

ACKNOWLEDGMENTS

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