Chapter 3

Diagnosing Telomere Biology Disorders

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Introduction

Dyskeratosis congenita (DC) was the first Telomere Biology Disorder (TBD) recognized in the biomedical literature and initially defined by the mucocutaneous triad of lacy reticulated skin pigmentation, nail dystrophy and oral leukoplakia (see Chapter 1, Introduction) [1]. In most cases, DC presents over the first two decades of life. In others, symptoms begin to appear in early adulthood [2, 3]. DC is related to three other syndromes that present in infancy or early childhood, Hoyeraal Hreidarsson syndrome (HH), Revesz syndrome (RS), and Coats plus, which, like DC, arise from defective telomeres (see Chapter 2, Why Telomeres Matter). Further expanding what is now appreciated as a spectrum of TBDs is telomere-mediated pulmonary fibrosis, which typically presents in later adulthood. This chapter will discuss the importance of telomere length testing in rendering a diagnosis of a TBD, describe the evaluation and clinical features of the various TBDs, basic diagnostic criteria, and specific genetic associations. While the specific diagnostic

labels do convey important clinical information, there is clinical overlap and, importantly, clinical features can evolve over time or may be apparent only with systematic evaluation.

Telomere Length Testing

Several methods have been developed to measure telomere length:

- **Automated multicolor flow cytometry combined with fluorescent *in situ* hybridization (flow FISH).** Flow FISH provides a measurement of average telomere length in cells of leukocyte subsets [4, 5]. It is the only test that is clinically available in certified labs and validated for the diagnosis of TBDs.
- **Southern blot analysis of telomere restriction fragments.** This method is often used in biomedical research and aided the initial discovery of DC as a TBD. Given its various limitations, it is not suitable nor available for clinical diagnostic purposes.
- **Telomere quantitative polymerase chain reaction (qPCR)** [6, 7]. While the stalwart of epidemiologic telomere-related research, qPCR is less accurate, reproducible, sensitive and specific for the diagnosis of TBDs than flow FISH [8].
- **High throughput single telomere length analysis (HT-STELA).** This newer approach, which can measure very short telomeres, may emerge as an additional sensitive and specific clinical test for TBDs with further development and research [9].

Flow FISH as a diagnostic test: Clinically certified testing of telomere length by flow FISH is available in the USA, Canada, Switzerland, Germany, and Australia (see

Resources). Importantly, since this testing is performed on fresh peripheral blood cells, it may only be used as a diagnostic tool prior to hematopoietic cell transplantation (HCT); after HCT, donor, rather than native, cells would be assayed. Individuals with DC, HH, and RS have very short telomere lengths across cell types, defined as telomere lengths less than the first percentile for age [10-13]. Specifically, very short telomere length in practically all leukocyte subsets (granulocytes, naïve T cells, memory T cells, B cells, and NK/NKT cells) as determined by flow FISH is both highly sensitive and specific for a diagnosis of one of these TBDs (see Table 1 and Figure 1) [10-12]. The severity of disease correlates with telomere length with the most severely affected, typically those with RS or HH, having the greatest degree of telomere shortening from the age-adjusted mean [12].

Table 1. Telomere lengths in DC patients compared with DC relatives [12].

	DC Patients N abnormal	DC Relatives N abnormal	OR	95% CI	Sens (%)	Spec (%)	PPV (%)	NPV (%)
Granulocytes	60/62	22/123	138	31-1200	97	82	73	98
Lymphocytes	63/65	11/127	332	68-2942	97	91	85	98
$CD45RA^+/CD20^-$ naïve T cells	61/64	9/127	266	64-1468	95	93	87	98
$CD45^-$ memory T cells	61/64	11/127	214	53-1161	95	91	85	98
$CD20^+$ B cells	54/58	12/127	129	37-546	93	91	82	97
$CD57^+$ NK/NKT cells	50/59	12/119	50	18-140	85	90	81	92
≥4/6 lineages	61/64	9/117	244	58-1346	95	92	87	97
≥3/5 lymphocyte lineages	62/64	9/119	379	74-3390	97	92	87	98

4/4 lymphocyte subsets	42/55	7/127	55	19-170	76	94	86	90
≥3/4 lymphocyte subsets	54/55	7/127	926	113-37479	98	94	89	99
3/3 naïve and memory T and B cells	51/58	7/127	125	38-437	88	94	88	94
≥2/3 naïve and memory T and B cells	57/58	9/127	747	97-30241	98	93	86	99
2/2 naïve and memory T cells	59/64	7/127	202	55-806	92	94	89	96
1/2 naïve and memory T cells	63/64	13/127	552	77-22333	98	89	83	99
Granulocytes + lymphocytes	58/65	11/127	87	30-273	89	91	84	94

Denominators vary according to the number of patients in whom each included lineage had sufficient numbers of cells for analysis. The best performance characteristics are in lymphocytes alone, and at least three of the four lymphocyte subsets. Abnormal: below the first percentile for age in normals. OR: odds ratio in favor of being a DC patient compared with an unaffected relative. CI: confidence interval; sens: sensitivity; spec: specificity; PPV: positive predictive value; NPV: negative predictive value. Table reproduced from Alter et al., *Haematologica* 2012 [12]. Note, the DC population (n=65) included those with classical DC (40), HH (14), RS (4) or were silent carriers (7).

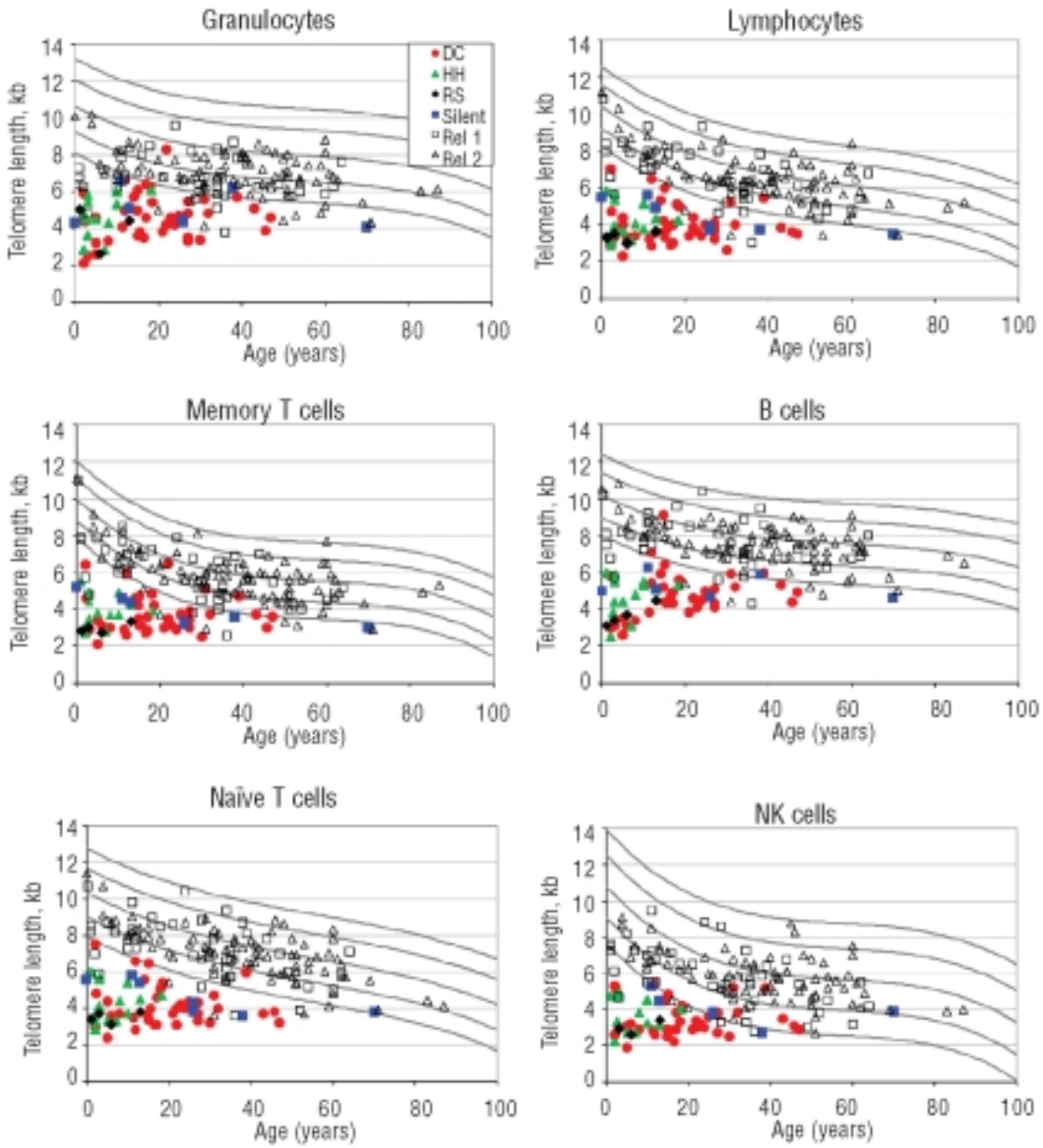


Figure 1. Telomere length according to age in patients with DC and related TBDs and their relatives. The vertical axis represents telomere length in kb. The curved lines in the figures indicate the first, tenth, 50th, 90th, and 99th percentiles of results from 400 normal controls. Colored symbols represent patients with DC and their relatives. Red circles: classical DC patients; green triangles: HH; black diamonds: RS; blue squares: silent carriers; open black squares: DC relatives in families with unknown genes; open black triangles: DC relatives without pathogenic variants in the probands' genes. Top panels show granulocytes, lymphocytes, and CD45RA+/CD20- naïve T cells. Bottom panels show CD45RA- memory T cells, CD20+ B cells, and total NK/NKT cells. Figure adapted and Figure Legend directly from Alter et al., *Haematologica* 2012 [12].

Telomere length slightly below the first percentile in three of the lymphocyte populations is only very rarely observed in patients with other inherited bone marrow failure syndromes, such as Fanconi anemia, Diamond-Blackfan anemia, Shwachman-Diamond syndrome [10-12]. Very short lymphocyte telomere lengths may also be found in association with hepatitis-associated severe aplastic anemia, with lengths increasing into normal range following treatment [14]. Additionally, individual patients with several rare disorders, such as *LIG4*-, *CORO1A*-, and *RUNX1*-associated disease, have been reported to have very short telomere lengths [15-17]. Further study is needed in these rare disorders to better understand the significance of these findings. Thus, while highly sensitive and specific for DC/RS/HH, very short telomeres alone are insufficient to render a TBD diagnosis. Results should be interpreted in the context of the patient's other clinical features and family history.

Bone marrow failure can precede the development of other DC features. Therefore, telomere length in leukocyte subsets by flow FISH testing is highly recommended for all patients with aplastic anemia. This is particularly true for patients being considered for HCT, as the diagnosis of a TBD has a major impact on the conditioning regimen and may influence donor selection. For example, siblings with very short telomeres would be considered suboptimal donors even in the absence of overt disease (see Chapter 13, Hematopoietic Stem Cell Transplantation). Additionally, medical treatment for TBD-related bone marrow failure might include androgens, while immunosuppressive therapy might be indicated in cases of acquired bone marrow failure (Chapter 10). Thus, telomere length testing has the potential to greatly influence treatment strategy.

In contrast to DC, HH, and RS, the extent of telomere shortening observed in individuals with Coats plus when measured by flow FISH varies from well below to just at the first percentile [18]. Analysis by qPCR and telomere restriction fragment analyses have also yielded telomere lengths in normal range [19, 20]. Thus, flow FISH results may not aid in the diagnosis of Coats plus. Laboratory-based research studies have nonetheless uncovered telomere defects associated with Coats plus variants, consistent with the role of genes mutated in Coats plus, *CTC1*, *STN1* and *POT1*, in telomere biology [20-23].

Lastly, adult-onset TBDs, such as telomere-mediated pulmonary fibrosis, have telomeres that may be below or within the first to tenth age-adjusted percentiles by flow FISH [24].

Dyskeratosis Congenita

Classic DC is diagnosed by the presence of the mucocutaneous triad. DC, however, can impact every organ system and lead to a wide range of clinical manifestations, with bone marrow failure being a major clinical feature (see Table 2) [25, 26]. The identification of pathogenic germline genetic variants causative of DC and development of telomere length testing have facilitated diagnosis (see Chapter 4, The Genetics of Dyskeratosis Congenita and Telomere Biology Disorders and Chapter 5, Genetic Counseling for Families). As a result, diagnostic criteria for DC have evolved over the past two decades, although expert opinions vary in the details [26-29]. The term DC is now widely used to also describe patients presenting during the first two decades of life with very short telomeres, with or without a pathogenic variant in a known TBD gene, and bone marrow failure or multiple other clinical features as outlined in Table 2.

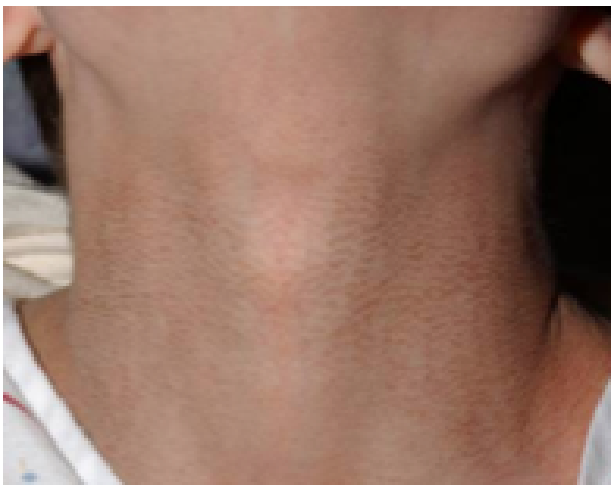
Major Clinical Features of DC

1. **The mucocutaneous triad**

   The mucocutaneous triad of reticulated skin pigmentation, nail dystrophy, and oral leukoplakia typically manifests in mid-to-late childhood. The features need not present simultaneously nor do all need to be present to make a diagnosis of DC (see Chapter 6, Dermatologic Manifestations).

   a. Reticulated skin pigmentation (Figure 2)

   Skin changes most often appear as reticular or lacy hypo- and hyperpigmentation, but may also be more punctate. All areas of skin may be affected, although changes may be restricted to neck, upper chest, and proximal parts of the limbs initially. In some cases, the pigmentation follows Blaschko lines [30]. Skin findings may simulate manifestations of graft versus host disease, a complication of HCT. Some unrelated disorders also manifest reticular skin pigmentation including dermatopathia pigmentosa reticularis, Naegeli syndrome, poikiloderma with neutropenia (also known as poikiloderma Clericuzio type), and Kindler syndrome.



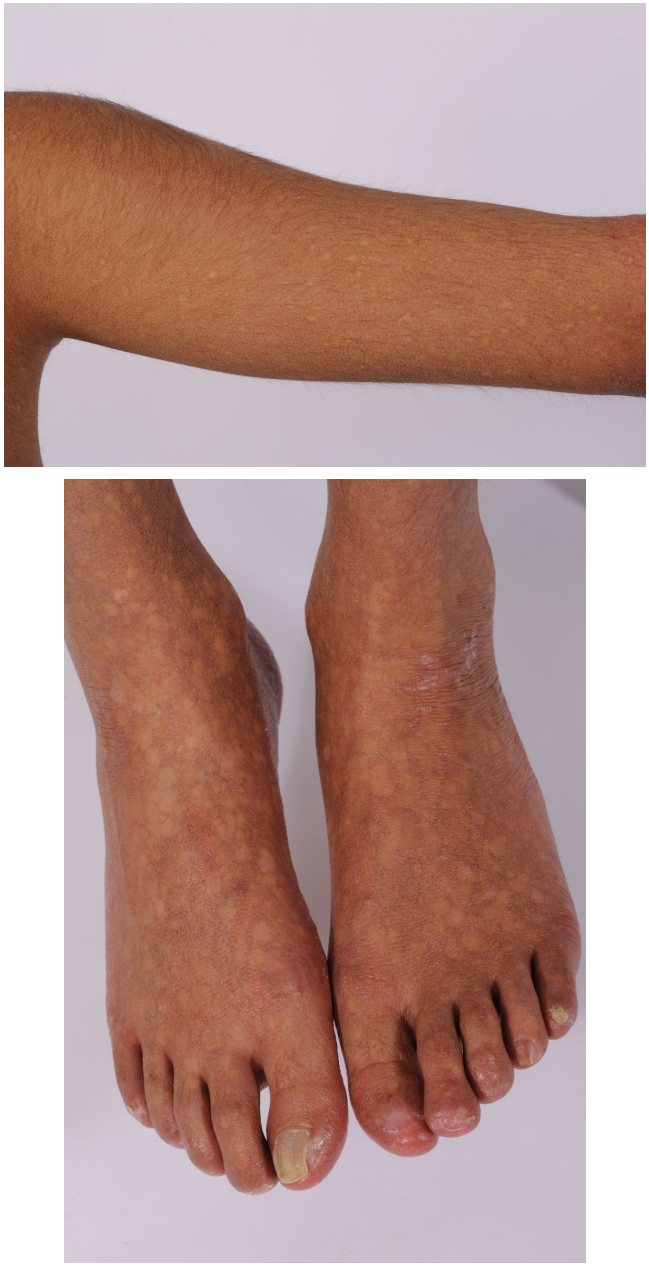


Figure 2. Skin pigmentation changes in TBDs. Images obtained after informed consent from participants in the Cancer in Inherited Bone Marrow Failure Syndromes Study, ClinicalTrials.gov Identifier: NCT00027274. Courtesy of Neelam Giri, MD and Sharon Savage, MD, National Cancer Institute.

b. Nail dystrophy (Figure 3)

Changes to the finger and toe nails may be subtle or severe, with ridging, thinning, peeling, or slow growth. Nail changes in a given patient may be asynchronous, with normal appearing nails adjacent to nails that are clearly affected. With age, nails may even seem to “disappear”.

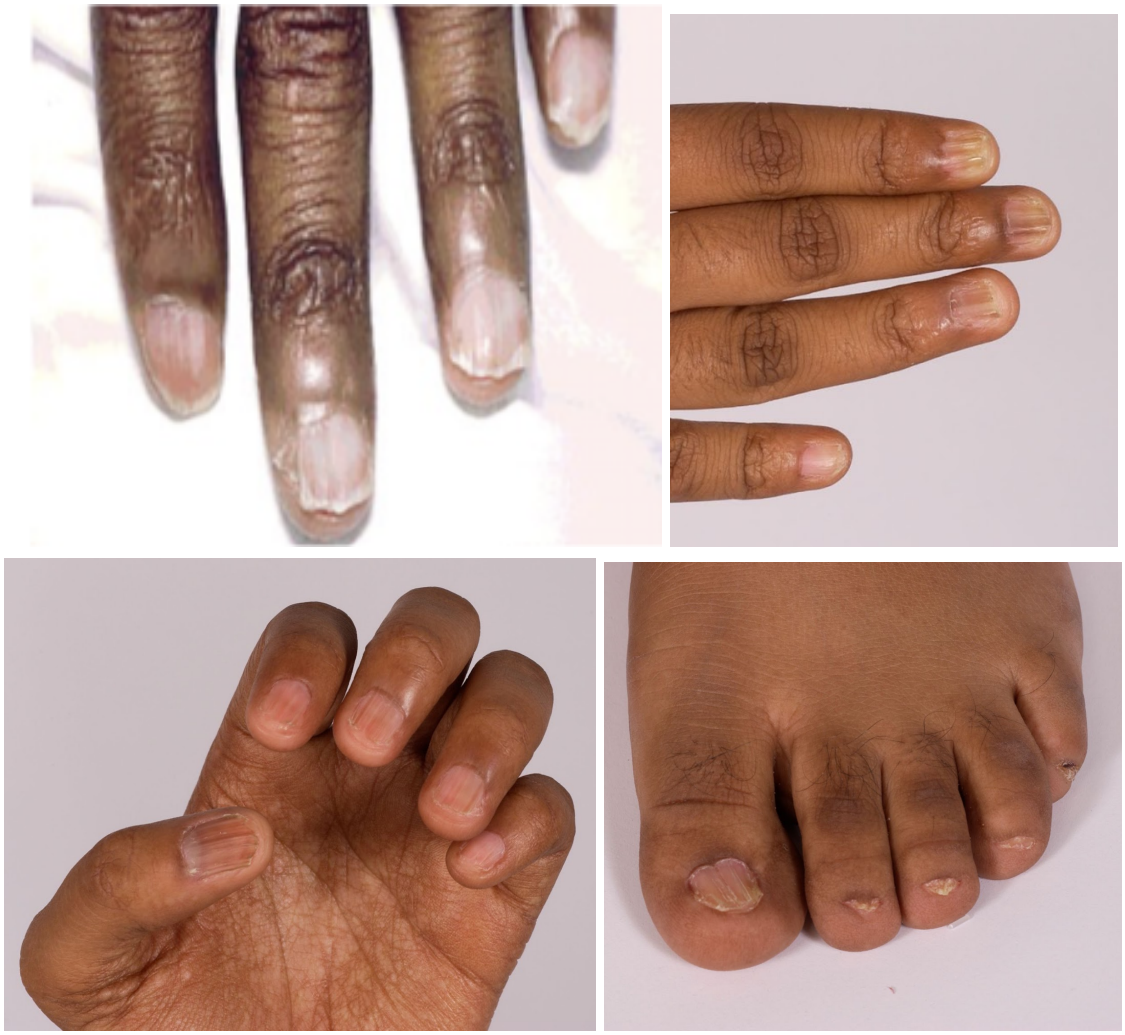


Figure 3. Nail dystrophy in TBDs. Images obtained after informed consent from participants in the Cancer in Inherited Bone Marrow Failure Syndromes Study, ClinicalTrials.gov Identifier: NCT00027274. Courtesy of Neelam Giri, MD and Sharon Savage, MD, National Cancer Institute.

c. Oral leukoplakia (Figure 4)

Oral leukoplakia appears as thickened, white patches that cannot be scraped off the buccal mucosa or along the edges and surface of the tongue. An experienced otolaryngologist (ear, nose, and throat doctor) or oral surgeon best evaluates oral leukoplakia.

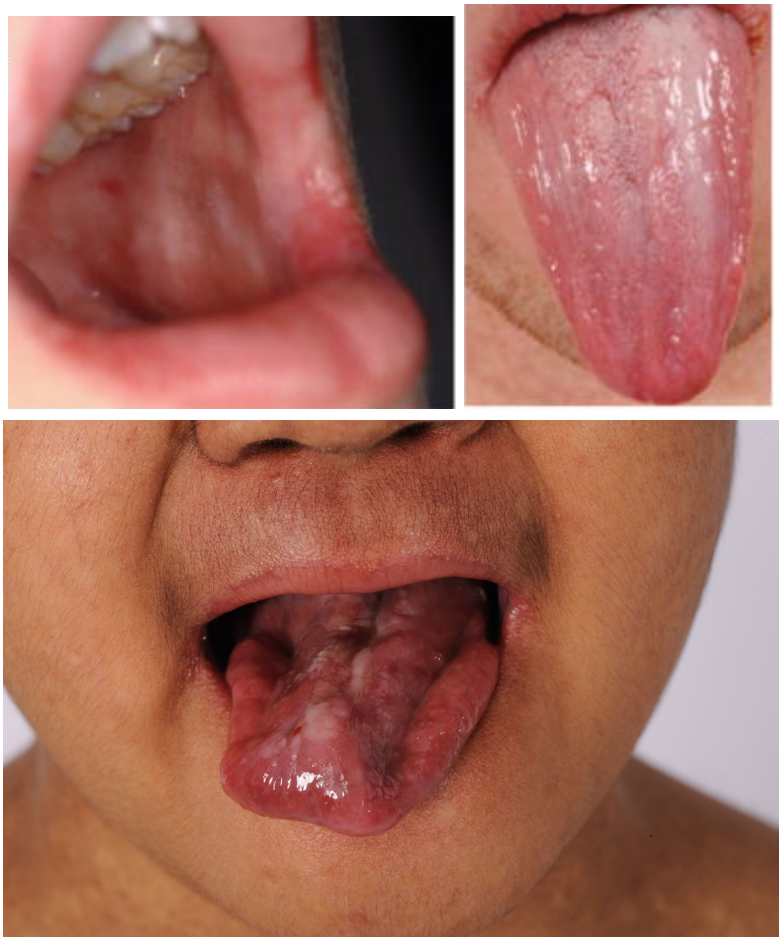


Figure 4. Leukoplakia in TBDs Image 1 and 2 from Savage and Bertuch *Genet Med*. 2010 [28]. Image 3 obtained after informed consent from participant in the Cancer in Inherited Bone Marrow Failure Syndromes Study, ClinicalTrials.gov Identifier: NCT00027274. Courtesy of Neelam Giri, MD and Sharon Savage, MD, National Cancer Institute.

2. **Bone marrow failure**

   Bone marrow failure is generally defined as bone marrow cellularity less than normal for age, and with one or more peripheral blood cytopenias [absolute neutrophil count, hemoglobin (reflecting red blood cell count), or platelet count below the lower limit of normal for age]. It is a common feature of DC, with up to 85% of patients in the London Dyskeratosis Congenita Registry reporting bone marrow failure by the age of 30 years [25]. In a competing risk analysis, the cumulative incidence of bone marrow failure in the National Cancer Institute Inherited Bone Marrow Failure Syndrome Study was 50% by age 50 [31]. The extent of bone marrow failure can be mild to severe, and can precede the mucocutaneous features of DC. Bone marrow failure at any age should prompt consideration of a diagnosis of DC or related TBD.

   Diagnostic evaluations for bone marrow failure include:

   - Complete blood count, including mean corpuscular volume (MCV). An elevated MCV may indicate long standing stress erythropoiesis, as occurs in DC and other inherited bone marrow failure syndromes, rather than more acute bone marrow failure as in most cases of immune aplastic anemia.
   - Absolute reticulocyte count
   - Hemoglobin F measurement. As with MCV, an elevated hemoglobin F measurement may reflect an underlying inherited bone marrow failure syndrome rather than an acute marrow failure process.
   - Bone marrow aspiration and biopsy
   - Bone marrow cytogenetic analysis by G banding
   - Bone marrow fluorescence in situ hybridization to detect 5q-, 7q-/monosomy 7, trisomy 8 and 20q-, if clinically indicated.

   Additional evaluations that may be considered:

- In the absence of nail dystrophy or reticulated skin pigmentation, chromosome breakage analysis should be performed to rule out Fanconi anemia. Because individuals with Fanconi anemia may also develop leukoplakia, this finding cannot be used to distinguish DC from Fanconi anemia.
- RBC folate and vitamin B12 to assess stores if MCV is elevated.

Testing available but of uncertain utility:

- Next-generation sequencing (NGS) panels to detect somatic variants in blood or bone marrow samples in genes associated with sporadic hematologic malignancies and aplastic anemia are now readily available. However, prognostic utility of identifying a variant in such a gene in patients with a TBD is not known.

The hematologic manifestations of DC and related TBDs and their treatment are presented in detail in Chapter 10, Medical Management of Bone Marrow Failure in Telomere Biology Disorders.

Identifying Additional Features of DC and Related TBDs

Table 1 lists the multitude of clinical findings that may be observed in DC. Some of these findings may be apparent on physical examination, whereas others require specific testing. Clinical evaluations that may be done to uncover additional features of DC and related TBDs are listed below. These should be considered on an individual patient basis, as clinically indicated.

Table 2. Diagnostic Findings in DC. These features present with variable severity and may not be present in all individuals

Physical Features	
Mucocutaneous triad	Dystrophic nails
	Lacy reticulated pigmentation, especially neck and thorax
	Leukoplakia (white patches), usually oral
Additional features (in order of frequency) [26]	
Eyes	Epiphora (tearing), lacrimal duct stenosis, blepharitis, exudative retinopathy
Hair	Early graying, loss, sparse eyelashes
Gastrointestinal	Esophageal stricture; liver fibrosis, cirrhosis; hepatopulmonary syndrome; peptic ulceration, enteropathy
Stature	Short
Dental	Caries, missing teeth, periodontitis, decreased crown/root ratio, taurodontism
Skeletal	Osteoporosis, hip avascular necrosis
Head/ Neuro-developmental	Microcephaly, cerebellar hypoplasia (ataxia, spasticity, hypotonia), intracranial calcification
Perinatal	Low birth weight, intrauterine growth restriction
Lung	Fibrosis, restrictive; arterio-venous malformations

Males	Small testes, undescended testes; phimosis, meatal stenosis, urethral stricture, hypospadias, leukoplakia
Skin	Hyperhidrosis
Neurodevelopmental	
Learning disability, developmental delay, intellectual disability, depression, anxiety	
Laboratory Features	
Blood	Anemia, and/or thrombocytopenia, and/or neutropenia
	Pancytopenia
	High MCV for age
	High fetal hemoglobin (Hb F) for age
Bone Marrow	Aplastic: Hypocellular for age
	Myelodysplastic syndrome: significant dyspoieses (per WHO classification) +/- cytogenetic clone
	Leukemia: > 20% blasts in marrow
Telomeres	Below first percentile for age by automated multicolor flow-FISH in three of four lymphocyte subsets ($CD45^+$ naïve T cells, $CD45^-$ memory T cells, $CD20^+$ B cells, $CD57^+$ NK/NKT cells) and granulocytes
Genes	Pathogenic variant in a DC-associated gene

Growth delay

- Birth weight and length measurements and gestational age at birth to assess for intrauterine growth restriction
- Current weight and length to assess for short stature and/or failure to thrive

Developmental delay/intellectual disability (see also Chapter 24, Neuropsychiatric Complications)

- Neuropsychological testing

Ophthalmologic manifestations (see also Chapter 7, Ophthalmic Manifestations) [32]

Examination should be performed by an ophthalmologist and include a retinal exam. Findings may include:

- Epiphora (constant tearing) due to lacrimal duct stenosis or its congenital absence
- Blepharitis
- Retinal neovascularization
- Retinal hemorrhages
- Exudative retinopathy, can be observed in some patients with DC but should prompt consideration of RS or Coats plus

Hearing loss

- Audiogram or auditory brain-stem evoked response testing

Dental involvement (see also Chapter 8, Dental and Oral Complications)

In addition to oral leukoplakia, screening should allow for detection of:

- Extensive caries or tooth loss
- Periodontal disease

- Taurodontism (enlarged tooth pulp chambers) or decreased tooth root/crown ratio

Lung involvement (see also Chapter 14, Pulmonary Fibrosis and Chapter 15, Lung Transplantation)

Initial evaluations to assess involvement of the lungs include:

- Pulse oximetry
- Pulmonary function tests (PFTs)
- Diffusion capacity of the lung for carbon monoxide (DLCO testing)
- Six-minute walk test for young children unable to perform PFTs

In cases in which lung involvement is suspected, additional testing includes:

- Chest radiography
- Non-contrast high resolution chest computed tomography
- Agitated saline echocardiogram or bubble study

Gastrointestinal tract and liver involvement (see also Chapter 17, Gastrointestinal Disease - Luminal, Chapter 18, Hepatic Complications, and Chapter 19, Liver Transplantation)

- A patient may report dysphagia due to the presence of an esophageal web or stricture, which is diagnosed by barium swallow or esophagram.
- Upper and lower gastrointestinal tract bleeding due to ulceration, telangiectasias, or varices may be diagnosed by upper and lower tract endoscopy.
- Liver disease may be revealed by the following testing:
    - Aspartate aminotransferase (AST/SGOT)
    - Alanine aminotransferase (ALT/SGPT)
    - Alkaline phosphatase (Alk phos)
    - Gamma-glutamyltransferase (GGT)
    - Conjugated and unconjugated bilirubin

    - Albumin
    - Prothrombin time (PT)
    - Ammonia
    - Liver ultrasound with Doppler, liver elastrography (fibroscan), or MRI
    - Liver biopsy, which may be indicated if the above studies are abnormal, should include assessment of liver iron stores in addition to histopathology

Genitourinary tract involvement (see also Chapter 20, Genitourinary Complications)

- Physical examination may reveal
    - Urethral stricture
    - Hymenal stricture in females
    - Phimosis in males
    - Hypogonadism (small testes) in males
- Urinalysis may uncover microscopic hematuria due to hemorrhagic cystitis or ureteral bleeding.

Musculoskeletal and endocrine disease (see also Chapter 22, Endocrine and Skeletal Disorders)

Complaints of hip or shoulder pain may be due to avascular necrosis (AVN) of the humeral or femoral head. AVN can be diagnosed by:

- X-ray – most sensitive for late-stage disease
- Bone scan
- MRI – may pick up early changes in bone

Osteoporosis may be present and is diagnosed by:

- Dexascan
- Spine X-ray, which may also reveal compression fractures

Additional mucocutaneous findings (see also Chapter 6, Dermatologic Manifestations)

- Atrophy of the papillae on the dorsum of the tongue
- Complete or patchy alopecia
- Premature graying of the hair
- Sparse eyebrows and lashes
- Telangiectasias
- Hyperpigmentation of the gums, tongue, palms, and soles have been anecdotally reported in individuals of African descent
- Glyphs (fingerprints) may disappear over time

Immunologic abnormalities (see also Chapter 11, Immunologic Complications in Dyskeratosis Congenita and Hoyeraal-Hreidarsson Syndrome)

Patients may present with common variable immunodeficiency [33, 34]. Severe combined immunodeficiency, if present, warrants further consideration of HH as discussed below. Testing for immunodeficiency may include:

- Determination of T, B, and NK cell percentages and absolute numbers
- Quantitative immunoglobulin levels for IgG, IgM, IgA, and IgE
- Lymphocyte proliferation panel for mitogens and antigens

Neurologic manifestations

- Frontal-occipital head circumference measurement to detect microcephaly
- Brain magnetic resonance imaging (MRI) to detect cerebellar hypoplasia (Figure 4), cerebral atrophy, corpus callosum abnormalities, small pons [35]
- Head X-ray or brain computed tomography (CT) to detect calcification
- Brain MRI or CT may also reveal cerebral cysts
- Neurodevelopmental assessment

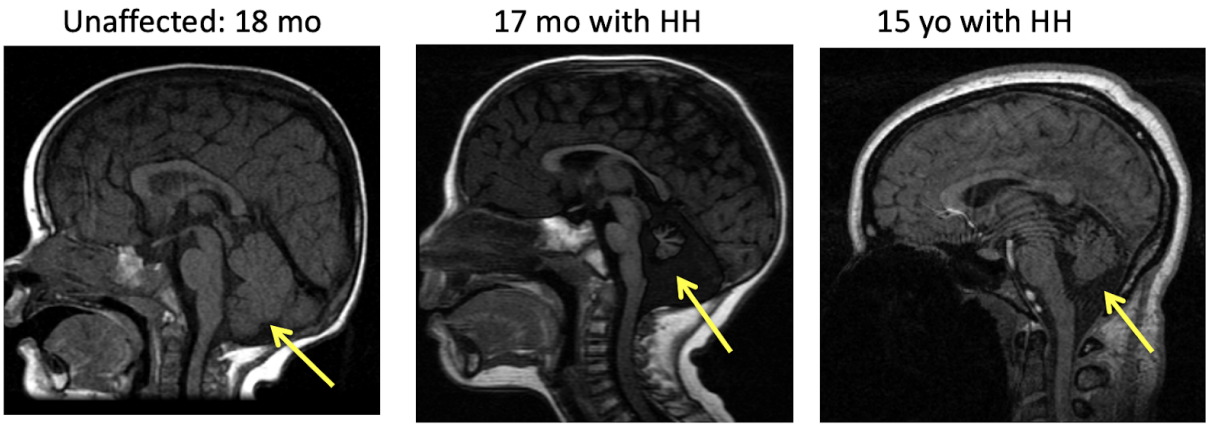


Figure 5. Cerebellar hypoplasia in Hoyeraal-Hreidarsson syndrome [35].
Abbreviations: mo, months old; yo, years old; HH, Hoyeraal Hreidarsson syndrome.

Pertinent History

Cancer history of patient (see also Chapter 9, Solid Tumors)

Cancer may be the presenting feature of DC. The most common neoplasms seen in DC include:

- Myelodysplastic syndrome and acute myeloid leukemia
- Head/neck cancer, especially squamous cell carcinoma of the tongue
- Anogenital squamous cell carcinoma

Family History

Obtaining a thorough family history is crucial. As discussed in Chapter 4, The Genetics of Dyskeratosis Congenita and Telomere Biology Disorders and Chapter 5, Genetic Counseling for Families, disease anticipation, with earlier onset and more severe disease manifestations, may be observed in successive generations. Patient pedigree may include history of:

- DC, HH, RS, Coats plus
- Pulmonary fibrosis
- Liver fibrosis or cirrhosis of nonalcoholic, noninfectious etiology

- Hepatopulmonary syndrome
- Bone marrow failure, myelodysplastic syndrome, leukemia or lymphoma
- Cancer in young relatives (age less than 50 years), especially of the head and neck
- Infant or early childhood death due to immunodeficiency or severe enterocolitis

Molecular Diagnosis

Gene sequencing

To date, pathogenic variants in 15 genes have been found to cause TBDs: *ACD*, *CTC1*, *DKC1*, *NAF1*, *NOP10*, *NHP2*, *PARN*, *POT1*, *RTEL1*, *STN1*, *TERC*, *TERT*, *TINF2*, *WRAP53* (TCAB1), and *ZCCHC8* [36-39]. (See Chapter 4, The Genetics of Dyskeratosis Congenita and Telomere Biology Disorders and Chapter 5, Genetic Counseling for Families for further discussion of the genetics of the TBDs.) *MDM4* has been recently proposed as an additional TBD gene but awaits additional evidence [40]. Clinical laboratories offer NGS panels of many of these genes, and whole exome sequencing is also available as an alternative approach. Copy number analysis via chromosomal microarray or NGS may also uncover pathogenic changes in TBD genes. Sequencing of the known TBD-associated disease in large cohorts of individuals, including both children and adults, with a clinical diagnosis of a TBD will identify causative variants in ~70-80% of cases. It is important to recognize that the absence of a known disease-associated variant does not rule out a TBD. In addition to aiding the diagnosis of an individual patient, obtaining a molecular diagnosis through gene sequencing provides a mechanism to screen family members and to offer genetic counseling, as well as permit pre-implantation genetic diagnosis (see Chapter 5, Genetic Counseling for Families).

Additional Considerations

Female carriers of *DKC1* pathogenic variants

Although *DKC1* variants result in X-linked recessive disease [41], female carriers may occasionally manifest clinical features of a TBD, such as delayed wound healing, abnormal pigmentation, and nail dystrophy [42]. In addition, they may have skewed leukocyte X chromosome inactivation [30] defined as greater than 90 percent of cells inactivating the same X chromosome (for example, the X chromosome inherited from the mother). Clinical X chromosome inactivation analysis utilizing the human androgen receptor assay is offered by numerous testing facilities, and may be useful in determining the carrier status of females when *DKC1* testing is not available or a variant of uncertain significance is identified.

Revertant somatic mosaicism

Similar to that seen in Fanconi anemia, revertant somatic mosaicism has been reported in autosomal dominant DC [43]. This phenomenon refers to the presence (within the same person) of cells bearing a variant originating from the germline, as well as a subpopulation of cells in which the abnormal (mutant) allele has reverted to wildtype. Reversion is thought to occur via mitotic homologous recombination. These hematopoietic stem cells no longer bear a DC-associated gene variant, and so may have stabilized or possibly lengthened the telomeres. They may thereby have the potential to drive effective hematopoiesis. Such growth advantage is not observed in other somatic tissues like lung, liver, and skin. These patients may have clinical features suggestive of DC but with minimal hematopoietic abnormalities. Sequencing of blood cell DNA may fail to detect the presence of the mutant allele due to its relatively smaller proportion in peripheral blood. Therefore, in cases in which revertant somatic mosaicism is suspected, for example in patients with solely extrahematopoietic manifestations of DC, DNA from nonhematopoietic tissue, like skin fibroblasts, should be analyzed.

Hoyeraal-Hreidarsson Syndrome

A diagnosis of Hoyeraal-Hreidarsson syndrome (HH) should be considered in children meeting diagnostic criteria of DC (see above), plus

- Cerebellar hypoplasia

Additional features with high penetrance in HH include

- Intrauterine growth restriction
- Developmental delay and intellectual disability
- Microcephaly
- Immunodeficiency

Classic HH typically presents in early childhood as a progressive, multisystem disorder. Cerebellar hypoplasia is considered by most as a defining feature of HH and may result in signs of cerebellar dysfunction such as ataxia and speech difficulties. Additional central nervous system findings include delayed myelination, hydrocephalus, brain atrophy, and calcification [44]. Microcephaly is frequently present.

Immunodeficiency is an additional major feature and may progress to severe combined immunodeficiency syndrome (SCID) of the T+, B-, NK- cell type, with lethal viral infection in infancy [45-47]. This has raised the possibility that there are young patients with SCID who succumb to infection prior to the recognition of underlying HH. Gastrointestinal problems with chronic bloody diarrhea and feeding difficulties have also been reported [48]. The mucocutaneous triad and additional features of DC may also be present [49].

Historically, the vast majority of individuals with HH reported in the literature have died within the first decade of life due to immunodeficiency or bone marrow failure [50]. However, with improved diagnosis, supportive care, and HCT, longer term survival is possible today [51, 52].

All of the genes associated with HH to date are associated with telomere maintenance (see Chapter 4, The Genetics of Dyskeratosis Congenita and Telomere Biology Disorders). These are:

- *DKC1*, which transmits X-linked recessive HH [53], accounting for the large male preponderance
- *TINF2*, which results in sporadic HH due to de novo heterozygous pathogenic variants
- *ACD*, *TERT*, and *RTEL1*, which result in autosomal recessive HH due to either compound heterozygous or homozygous pathogenic variants

The carrier frequency of the HH-associated *RTEL1* variant c.3791G>A (p.R1264H) is 1% in the orthodox Ashkenazi Jewish and 0.45% in the general Ashkenazi Jewish populations [54]. Therefore, targeted sequencing may be considered initially in these populations.

In addition to variants in the above genes, a heterozygous splice variant of *DCLRE1B* (SMN1B), which encodes the nuclease Apollo, was reported in a child with HH [55]. Apollo is implicated in telomere maintenance, the hallmark abnormality of HH, but it also has a role in certain forms of general DNA repair. In contrast to most individuals with HH who have very short telomeres, the case with the *DCLRE1B* splice variant lacks a defect in telomere length [55], although there is evidence of telomere dysfunction [47]. Thus, telomere length above the first percentile does not necessarily rule out a diagnosis of HH.

Revesz Syndrome

A diagnosis of Revesz syndrome (RS) should be considered in a child who meets diagnostic criteria of DC (see above) plus

- Bilateral exudative retinopathy (bilateral Coats disease)

Additional features may include

- Intrauterine growth restriction
- Sparse hair
- Intracranial calcification

RS is another pediatric onset TBD, with the defining feature of bilateral exudative retinopathy, also known as Coats disease. (See Chapter 7, Ophthalmic Manifestations for further information on ophthalmologic manifestations of TBDs, including RS.) Additional features of RS include intrauterine growth restriction, intracranial calcification, sparse hair, and bone marrow failure [56]. Patients may also have microcephaly, cerebellar hypoplasia, failure to thrive, and additional features of DC, including components of the mucocutaneous triad, most often nail dystrophy and least often oral leukoplakia [57].

The phenotypic overlap of RS and DC has long been appreciated [58]; however, only 18 cases of RS have been well described in the medical literature (clinical features of each tabulated by Karremann, et al) [57]. Of those reported, the vast majority present to medical attention before the age of five years, with the original case described in a six month old infant [56]. Bone marrow failure is most often evident by the second year of life. This early age of presentation, along with the severity and spectrum of disease manifestations, has led to the frequent description of RS as a severe variant of DC. Consistent with this, patients with RS not only have very short telomeres, but telomeres that are shorter than in patients with classic DC and similar to those observed in HH [13]. Lastly, a slight majority of reported cases are males; whether this reflects a true male predilection or simply represents a reporting or recognition bias remains unknown.

The only gene found to date with pathogenic variants in patients with RS is *TINF2*, which encodes TIN2, a member of the telomeric shelterin complex (see Chapter 4, The Genetics of Dyskeratosis Congenita and Telomere Biology Disorders) [59, 60]. Therefore, targeted sequencing of *TINF2* is a reasonable first step toward a molecular diagnosis in a patient with RS. Not all patients with RS, however, will have a *TINF2* pathogenic variant

[12]. Heterozygous *TINF2* pathogenic variants are also associated with classic DC and HH, and are often de novo [60]. While it is probable that most cases of *TINF2*-associated RS are due to *de novo* pathogenic variants, there is one case in the literature of RS in which the *TINF2* variant was inherited, although the carrier parent was a mosaic [61].

A family has been described in which two siblings with exudative retinopathy were found to carry a novel *TERT* variant, c.2603A>G, p.D868G [62]. Although these children had very short telomeres, bone marrow failure, and early pulmonary fibrosis, as seen in DC, they did not have the intracranial calcifications or neurodevelopmental deficits frequently observed in RS. A large number of *TERT* variants have been reported in the literature, including homozygous pathogenic variants in patients with very short telomeres. These are not reported to be associated with exudative retinopathy, so it remains to be determined whether the ocular phenotype in this family is due to the *TERT* variant or is unrelated.

Coats Plus

The diagnosis of Coats plus is made by the presence of the following clinical features:

- Distinctive pattern of intracranial calcification involving the thalamus, basal ganglia, dentate, and deep cortex, with associated leukoencephalopathy and brain cysts
- Retinal telangiectasia and exudates (as seen in Coats disease)
- Osteopenia with tendency to fracture and with poor bone healing
- Recurrent gastrointestinal hemorrhage due to vascular ectasias in the stomach, small intestine, and liver
- Intrauterine growth restriction
- Additional features overlapping with DC may be present: dystrophic nails, sparse hair, and abnormal skin pigmentation

Coats plus is the clinical syndrome most recently placed within the spectrum of TBDs. Similar to RS, patients with Coats plus have bilateral exudative retinopathy or telangiectasias, as well as a characteristic pattern of asymmetric intracranial calcification involving the thalamus, basal ganglia, dentate, and deep cortex, with associated leukoencephalopathy and brain cysts; osteopenia with tendency to fracture and poor bone healing; recurrent gastrointestinal hemorrhage due to vascular ectasias in the stomach, small intestines and liver; and pre- and postnatal growth restriction [63]. Additional features include the mucocutaneous triad of DC and bone marrow involvement, although not typically marrow failure.

Consistent with these overlapping clinical features of DC, the vast majority of patients with Coats plus have biallelic variants in *CTC1*, a gene that encodes a factor important for telomere maintenance (see Chapter 4, The Genetics of Dyskeratosis Congenita and Telomere Biology Disorders) [18, 19] and patients diagnosed with classic DC have also been found to have biallelic *CTC1* pathogenic variants [64, 65]. In addition to *CTC1*, biallelic pathogenic *STN1* variants have been reported in three unrelated patients with Coats plus [20, 66], an expected association given that *CTC1* and *STN1* proteins form a complex. Lastly, a homozygous pathogenic *POT1* variant was identified in two siblings with Coats plus, implicating this shelterin complex gene in Coats plus as well [23].

Whether very short telomeres are a molecular feature of *CTC1*-associated disease remains to be determined. In the initial two reports on patients with Coats plus and *CTC1* variants, one group found affected individuals had age-adjusted lymphocyte telomere length below the first percentile, as determined by flow FISH [18], whereas the other group found no difference in the relative leukocyte telomere length between affected and control individuals, as determined by qPCR [19]. Similarly, a report describing a patient with biallelic *CTC1* variants and classic DC with intracranial calcifications and non-specific vascular retinal changes, found very short lymphocyte telomere length by flow FISH [64]. In contrast, another report describing six individuals with DC or related bone marrow failure disorders and *CTC1* variants found no difference in relative telomere lengths between the affected individuals and controls. However,

these measurements were by qPCR [65]. Simultaneous measurements of telomere length using both methods in individual Coats plus and DC patient samples may ultimately resolve this question.

Distinguishing Revesz Syndrome from Coats Plus

As evident from the above descriptions, RS and Coats plus share several features: intrauterine growth restriction, bilateral exudative retinopathy, intracranial calcifications, sparse hair, nail dystrophy, and cutaneous changes. Recent evidence suggests that telangiectatic gastrointestinal bleeding, which was previously noted to be a feature of Coats plus, may be observed in some patients with RS [67]. However, they are distinct both clinically and genetically. Severe bone marrow failure is a dominant feature of RS, whereas this is not frequently described in patients with Coats plus. Patients with RS frequently have cerebellar hypoplasia, which is rare in Coats plus. Conversely, patients with Coats plus have a very distinctive pattern of intracranial calcification. Further, a skeletal phenotype of osteoporosis and easy fracture are common. Genetically, *TINF2* pathogenic variants are associated with RS [59, 60] whereas *CTC1* and *STN1* pathogenic variants are associated with Coats plus [18, 20]. Thus, the clinical features should lead to direct testing for variants in *TINF2* versus *CTC1* and *STN1*.

Isolated Aplastic Anemia, Myelodysplastic Syndrome, and Acute Leukemia

Aplastic anemia associated with very short lymphocyte telomere lengths or with a pathogenic variant in a telomere biology gene should raise suspicion for a TBD even in the absence of other features of DC. In young children, aplastic anemia may be the first manifestation of DC. As these children age, they may develop additional clinical features, including the mucocutaneous features characteristic of DC. In contrast, there are individuals who are well into adulthood when they develop aplastic anemia as the sole manifestation of a TBD. Notably, variants in *TERC* or *TERT*, and finding of short

telomeres have been reported in isolated adult cases of aplastic anemia, as well as in up to 5 to 10% of individuals in cohorts of seemingly acquired severe aplastic anemia [3, 68-70]. Although the reported individuals lacked physical features of DC, many had relatives who were also pathogenic variant carriers and had histories of macrocytosis, blood count abnormalities including aplastic anemia, myelodysplastic syndrome, or leukemia. Immunosuppressive therapy, which is typically effective in immune-aplastic anemia, was ineffective in these cases. Thus, a thorough family history and telomere length testing is recommended not only for children, but also adults with newly diagnosed aplastic anemia.

Similarly, rare germline variants in TBD genes, such as *TERT* and *TERC*, have been identified in sporadic adult cases of myelodysplastic syndrome and acute myeloid leukemia and in kinships predominantly manifesting these myeloid neoplasms in the absence of other features of DC [71-75]. Thus, as with patients with aplastic anemia, a thorough family history of patients with myeloid malignancy may reveal similarly afflicted relatives or those with varying degrees of bone marrow failure, which would prompt consideration of a familial telomerase or other telomere maintenance gene variant that presents predominantly as an isolated hematologic phenotype.

Telomere-Mediated Pulmonary Fibrosis

Pulmonary fibrosis (PF) is the most common manifestation of disease due to shortened telomeres [76], and up to 25% of familial cases and up to 10% of sporadic, idopathic cases are associated with a rare variant in a TBD gene, *TERT*, *TERC*, *DKC1*, *TINF2*, *RTEL1*, or *PARN* [24, 77-89]. In addition, variants in *NAF1* have been found in association with PF-emphysema [90]. The reader is referred to Chapter 14, Pulmonary Fibrosis, where PF and other TBD-associated lung disease are discussed in more detail. In brief, PF cases due to TBD gene pathogenic variants generally present in mid-adulthood. The majority are familial. The pedigrees of some familial cases are characterized by PF as the predominant phenotype [91], whereas other pedigrees evolve from a PF-predominant to bone marrow failure–predominant phenotype over successive generations [92]. The

presence of an underlying germline TBD gene pathogenic variant is highly suggested when PF is accompanied by cytopenias or other hematologic abnormalities such as macrocytosis or cryptogenic liver disease. Thus, thorough medical histories, examination of peripheral blood counts and liver function, and detailed family history are warranted with PF presentations.

Liver Disease-Predominant Phenotype

Similar to familial PF, pedigrees with a liver disease-predominant phenotype, as well as individuals with sporadic cryptogenic liver disease have been described with germline *TERT*, *TERC*, or *RTEL1* pathogenic variants [93-96]. The reader is referred to Chapter 18, Hepatic Complications, which describes in detail the hepatic manifestations associated with the TBDs. Here, we emphasize the importance of taking a thorough family history focused not only on familial liver disease, but also surveying for bone marrow and lung disease as steps in uncovering these cases [93].

Silent Carriers

Uncovering a pathogenic variant in an individual with clinical features of a TBD has the potential to lead to genetic testing and the discovery of additional family members who carry the variant but are asymptomatic, so-called silent carriers. The ability to anticipate the likelihood of developing disease or having offspring with disease may vary from relatively easy (as for a newborn male sibling with a pathogenic *DKC1* variant, who would be likely to develop disease) to more difficult (as for the highly unpredictable occurrence of myelodysplastic syndrome at 40 years of age in the offspring of a 60-year-old with a pathogenic *TERT* variant). Even more difficult are cases in which a familial variant is not identified, but testing revealed telomere lengths around the first percentile in asymptomatic relatives. As discussed in Chapter 5, Genetic Counseling, knowledge of silent carrier status may impact health-related behaviors (like avoidance of smoking or alcohol use), facilitate decisions on pre-implantation genetic counseling, and lead to disease surveillance (as with periodic CBCs or oral, head, and neck exams).

Lastly, silent carrier status would have significant implications with respect to related hematopoietic cell donation as such carriers would be unsuitable donors.

References

1. Walne AJ, Dokal I. Dyskeratosis Congenita: a historical perspective. *Mech Ageing Dev*. 2008;129(1-2):48-59.
2. Vulliamy TJ, Kirwan MJ, Beswick R, et al. Differences in disease severity but similar telomere lengths in genetic subgroups of patients with telomerase and shelterin mutations. *PloS One*. 2011;6(9):e24383.
3. Fogarty PF, Yamaguchi H, Wiestner A, et al. Late presentation of dyskeratosis congenita as apparently acquired aplastic anaemia due to mutations in telomerase RNA. *Lancet*. 2003;362(9396):1628-1630.
4. Baerlocher GM, Vulto I, de Jong G, Lansdorp PM. Flow cytometry and FISH to measure the average length of telomeres (flow FISH). *Nat Protoc*. 2006;1(5):2365-2376.
5. Baerlocher GM, Mak J, Tien T, Lansdorp PM. Telomere length measurement by fluorescence in situ hybridization and flow cytometry: tips and pitfalls. *Cytometry*. 2002;47(2):89-99.
6. Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. *Nucleic Acids Res*. 2009;37(3):e21.
7. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res*. 2002;30(10):e47.
8. Gutierrez-Rodrigues F, Santana-Lemos BA, Scheucher PS, Alves-Paiva RM, Calado RT. Direct comparison of flow-FISH and qPCR as diagnostic tests for telomere length measurement in humans. *PLoS One*. 2014;9(11):e113747.
9. Norris K, Walne AJ, Ponsford MJ, et al. High-throughput STELA provides a rapid test for the diagnosis of telomere biology disorders. *Hum Genet*. 2021;140(6):945-955.
10. Alter BP, Baerlocher GM, Savage SA, et al. Very short telomere length by flow fluorescence in situ hybridization identifies patients with dyskeratosis congenita. *Blood*. 2007;110(5):1439-1447.
11. Alter BP, Giri N, Savage SA, Rosenberg PS. Telomere length in inherited bone marrow failure syndromes. *Haematologica*. 2015;100(1):49-54.

12. 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;97(3):353-359.

13. 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.

14. Babushok DV, Grignon AL, Li Y, et al. Disrupted lymphocyte homeostasis in hepatitis-associated acquired aplastic anemia is associated with short telomeres. *Am J Hematol*. 2016;91(2):243-247.

15. Stewart DR, Pemov A, Johnston JJ, et al. Dubowitz syndrome is a complex comprised of multiple, genetically distinct and phenotypically overlapping disorders. *PLoS One*. 2014;9(6):e98686.

16. Stray-Pedersen A, Jouanguy E, Crequer A, et al. Compound heterozygous CORO1A mutations in siblings with a mucocutaneous-immunodeficiency syndrome of epidermodysplasia verruciformis-HPV, molluscum contagiosum and granulomatous tuberculoid leprosy. *J Clin Immunol*. 2014;34(7):871-890.

17. Alder JK, Hanumanthu VS, Strong MA, et al. Diagnostic utility of telomere length testing in a hospital-based setting. *Proc Natl Acad Sci U S A*. 2018;115(10):E2358-E2365.

18. Anderson BH, Kasher PR, Mayer J, et al. Mutations in CTC1, encoding conserved telomere maintenance component 1, cause Coats plus. *Nat Genet*. 2012;44(3):338-342.

19. Polvi A, Linnankivi T, Kivela T, 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;90(3):540-549.

20. Simon AJ, Lev A, Zhang Y, et al. Mutations in STN1 cause Coats plus syndrome and are associated with genomic and telomere defects. *J Exp Med*. 2016;213(8):1429-1440.

21. Gu P, Chang S. Functional characterization of human CTC1 mutations reveals novel mechanisms responsible for the pathogenesis of the telomere disease Coats plus. *Aging Cell*. 2013;12(6):1100-1109.

22. Chen LY, Majerska J, Lingner J. Molecular basis of telomere syndrome caused by CTC1 mutations. *Genes Dev*. 2013;27(19):2099-2108.

23. Takai H, Jenkinson E, Kabir S, et al. A POT1 mutation implicates defective telomere end fill-in and telomere truncations in Coats plus. *Genes Dev*. 2016;30(7):812-826.

24. Armanios MY, Chen JJ, Cogan JD, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N Engl J Med*. 2007;356(13):1317-1326.

25. Kirwan M, Dokal I. Dyskeratosis congenita: a genetic disorder of many faces. *Clin Genet*. 2008;73(2):103-112.

26. Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. *Blood Rev*. 2010;24(3):101-122.

27. Dokal I. Dyskeratosis congenita. *Hematology Am Soc Hematol Educ Program*. 2011;2011:480-486.

28. Savage SA, Bertuch AA. The genetics and clinical manifestations of telomere biology disorders. *Genet Med*. 2010;12(12):753-764.

29. Dokal I, Vulliamy T, Mason P, Bessler M. Clinical utility gene card for: Dyskeratosis congenita - update 2015. *Eur J Hum Genet*. 2015;23(4).

30. Devriendt K, Matthijs G, Legius E, et al. Skewed X-chromosome inactivation in female carriers of dyskeratosis congenita. *Am J Hum Genet*. 1997;60(3):581-587.

31. Alter BP, Giri N, Savage SA, et al. Malignancies and survival patterns in the National Cancer Institute inherited bone marrow failure syndromes cohort study. *Br J Haematol*. 2010;150(2):179-188.

32. 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;117(3):615-622.

33. Jyonouchi S, Forbes L, Ruchelli E, Sullivan KE. Dyskeratosis congenita: a combined immunodeficiency with broad clinical spectrum--a single-center pediatric experience. *Pediatr Allergy Immunol*. 2011;22(3):313-319.

34. Allenspach EJ, Bellodi C, Jeong D, et al. Common variable immunodeficiency as the initial presentation of dyskeratosis congenita. *J Allergy Clin Immunol*. 2013;132(1):223-226.

35. Bhala S, Best AF, Giri N, et al. CNS manifestations in patients with telomere biology disorders. *Neurol Genet*. 2019;5(6):370.

36. Ballew BJ, Savage SA. Updates on the biology and management of dyskeratosis congenita and related telomere biology disorders. *Expert Rev Hematol*. 2013;6(3):327-337.

37. Dodson LM, Bertuch AA**.** Dyskeratosis congenita and the telomere biology disorders. In: Bone Marrow Failure (Kupfer GM, Reaman GH and Smith FO, Eds). *Springer International Publishing.* 2018; 111-135.

38. Gable DL, Gaysinskaya V, Atik CC, et al. *ZCCHC8*, the nuclear exosome targeting component, is mutated in familial pulmonary fibrosis and is required for telomerase RNA maturation. *Genes Dev*. 2019;33(19-20):1381-1396.

39. Kocak H, Ballew BJ, Bisht K, et al. Hoyeraal-Hreidarsson syndrome caused by a germline mutation in the TEL patch of the telomere protein TPP1. *Genes Dev*. 2014;28(19):2090-2102.

40. Toufektchan E, Lejour V, Durand R, et al. Germline mutation of MDM4, a major p53 regulator, in a familial syndrome of defective telomere maintenance. *Sci Adv*. 2020;6(15):eaay3511.

41. Heiss NS, Knight SW, Vulliamy TJ, et al. X-linked dyskeratosis congenita is caused by mutations in a highly conserved gene with putative nucleolar functions. *Nat Genet*. 1998;19(1):32-38.

42. Alder JK, Parry EM, Yegnasubramanian S, et al. Telomere phenotypes in females with heterozygous mutations in the dyskeratosis congenita 1 (DKC1) gene. *Hum Mutat*. 2013;34(11):1481-1485.

43. Jongmans MC, Verwiel ET, Heijdra Y, et al. Revertant somatic mosaicism by mitotic recombination in dyskeratosis congenita. *Am J Hum Genet*. 2012;90(3):426-433.

44. Zhang MJ, Cao YX, Wu HY, Li HH. Brain imaging features of children with Hoyeraal-Hreidarsson syndrome. *Brain Behav*. 2021;11(5):e02079.

45. Ballew BJ, Joseph V, De S, 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;9(8):e1003695.

46. Lamm N, Ordan E, Shponkin R, Richler C, Aker M, Tzfati Y. Diminished telomeric 3' overhangs are associated with telomere dysfunction in Hoyeraal-Hreidarsson syndrome. *PLoS One*. 2009;4(5):e5666.

47. Touzot F, Gaillard L, Vasquez N, et al. Heterogeneous telomere defects in patients with severe forms of dyskeratosis congenita. *J Allergy Clin Immunol*. 2012;129(2):473-482, 482 e471-473.

48. Sznajer Y, Baumann C, David A, et al. Further delineation of the congenital form of X-linked dyskeratosis congenita (Hoyeraal-Hreidarsson syndrome). *Eur J Pediatr*. 2003;162(12):863-867.

49. Yaghmai R, Kimyai-Asadi A, Rostamiani K, et al. Overlap of dyskeratosis congenita with the Hoyeraal-Hreidarsson syndrome. *J Pediatr*. 2000;136(3):390-393.

50. Ozdemir MA, Karakukcu M, Kose M, Kumandas S, Gumus H. The longest surviving child with Hoyeraal-Hreidarsson Syndrome. *Haematologica*. 2004;89(9):ECR38.

51. Burris AM, Ballew BJ, Kentosh JB, et al. Hoyeraal-Hreidarsson Syndrome due to PARN Mutations: Fourteen Years of Follow-Up. *Pediatr Neurol*. 2016;56:62-68 e61.

52. Dodson LM, Baldan A, Nissbeck M, et al. From incomplete penetrance with normal telomere length to severe disease and telomere shortening in a family with monoallelic and biallelic PARN pathogenic variants. *Hum Mutat*. 2019;40(12):2414-2429.

53. Knight SW, Heiss NS, Vulliamy TJ, 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;107(2):335-339.

54. Fedick AM, Shi L, Jalas C, et al. Carrier Screening of RTEL1 Mutations in the Ashkenazi Jewish Population. *Clin Genet*. 2014;88(2):177-181.

55. Touzot F, Callebaut I, Soulier J, et al. Function of Apollo (SNM1B) at telomere highlighted by a splice variant identified in a patient with Hoyeraal-Hreidarsson syndrome. *Proc Natl Acad Sci U S A*. 2010;107(22):10097-10102.

56. 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;29(9):673-675.

57. Karremann M, Neumaier-Probst E, Schlichtenbrede F, et al. Revesz syndrome revisited. *Orphanet J Rare Dis*. 2020;15(1):299.

58. Kajtar P, Mehes K. Bilateral coats retinopathy associated with aplastic anaemia and mild dyskeratotic signs. *Am J Med Genet*. 1994;49(4):374-377.

59. Savage SA, Giri N, Baerlocher GM, Orr N, Lansdorp PM, Alter BP. TINF2, a component of the shelterin telomere protection complex, is mutated in dyskeratosis congenita. *Am J Hum Genet*. 2008;82(2):501-509.

60. 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;112(9):3594-3600.

61. Gleeson M, O'Marcaigh A, Cotter M, Brosnahan D, Vulliamy T, Smith OP. Retinal vasculopathy in autosomal dominant dyskeratosis congenita due to TINF2 mutation. *Br J Haematol*. 2012;159(5):498.

62. Sharma A, Myers K, Ye Z, D'Orazio J. Dyskeratosis congenita caused by a novel TERT point mutation in siblings with pancytopenia and exudative retinopathy. *Pediatr Blood Cancer*. 2014;61(12):2302-2304.

63. Crow YJ, McMenamin J, Haenggeli CA, et al. Coats' plus: a progressive familial syndrome of bilateral Coats' disease, characteristic cerebral calcification, leukoencephalopathy, slow pre- and post-natal linear growth and defects of bone marrow and integument. *Neuropediatrics*. 2004;35(1):10-19.

64. Keller RB, Gagne KE, Usmani GN, et al. CTC1 Mutations in a patient with dyskeratosis congenita. *Pediatr Blood Cancer*. 2012;59(2):311-314.

65. Walne A, Bhagat T, Kirwan M, et al. Mutations in the telomere capping complex in bone marrow failure and related syndromes. *Haematologica*. 2013;98(3):334-338.

66. Passi GR, Shamim U, Rathore S, et al. An Indian child with Coats plus syndrome due to mutations in STN1. *Am J Med Genet A*. 2020;182(9):2139-2144.

67. Himes RW, Chiou EH, Queliza K, et al. Gastrointestinal Hemorrhage: A Manifestation of the Telomere Biology Disorders. *J Pediatr*. 2021;230:55-61 e54.

68. Yamaguchi H, Baerlocher GM, Lansdorp PM, et al. Mutations of the human telomerase RNA gene (TERC) in aplastic anemia and myelodysplastic syndrome. *Blood*. 2003;102(3):916-918.

69. Yamaguchi H, Calado RT, Ly H, et al. Mutations in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia. *N Engl J Med*. 2005;352(14):1413-1424.

70. Aalbers AM, Kajigaya S, van den Heuvel-Eibrink MM, van der Velden VH, Calado RT, Young NS. Human telomere disease due to disruption of the CCAAT box of the TERC promoter. *Blood*. 2012;119(13):3060-3063.

71. Reilly CR, Myllymaki M, Redd RA, et al. The clinical and functional effects of TERT variants in myelodysplastic syndrome. *Blood*. 2021;138(10):898-911.

72. Tomlinson CG, Sasa G, Aubert G, et al. Clinical and functional characterization of telomerase variants in patients with pediatric acute myeloid leukemia/myelodysplastic syndrome. *Leukemia*. 2021;35(1):269-273.

73. Feurstein S, Adegunsoye A, Mojsilovic D, et al. Telomere biology disorder prevalence and phenotypes in adults with familial hematologic and/or pulmonary presentations. *Blood Adv*. 2020;4(19):4873-4886.

74. Holme H, Hossain U, Kirwan M, Walne A, Vulliamy T, Dokal I. Marked genetic heterogeneity in familial myelodysplasia/acute myeloid leukaemia. *Br J Haematol*. 2012;158(2):242-248.

75. Kirwan M, Vulliamy T, Marrone A, et al. Defining the pathogenic role of telomerase mutations in myelodysplastic syndrome and acute myeloid leukemia. *Hum Mutat*. 2009;30(11):1567-1573.

76. Armanios M. Telomerase and idiopathic pulmonary fibrosis. *Mutat Res*. 2012;730(1-2):52-58.

77. Cronkhite JT, Xing C, Raghu G, et al. Telomere shortening in familial and sporadic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2008;178(7):729-737.

78. Diaz de Leon A, Cronkhite JT, Katzenstein AL, et al. Telomere lengths, pulmonary fibrosis and telomerase (TERT) mutations. *PLoS One*. 2010;5(5):e10680.

79. Newton CA, Batra K, Torrealba J, et al. Telomere-related lung fibrosis is diagnostically heterogeneous but uniformly progressive. *Eur Respir J*. 2016;48(6):1710-1720.

80. Borie R, Bouvry D, Cottin V, et al. Regulator of telomere length 1 (RTEL1) mutations are associated with heterogeneous pulmonary and extra-pulmonary phenotypes. *Eur Respir J*. 2019;53(2).

81. Stuart BD, Choi J, Zaidi S, et al. Exome sequencing links mutations in PARN and RTEL1 with familial pulmonary fibrosis and telomere shortening. *Nat Genet*. 2015;47(5):512-517.

82. Kannengiesser C, Borie R, Menard C, et al. Heterozygous RTEL1 mutations are associated with familial pulmonary fibrosis. *Eur Respir J*. 2015;46(2):474-485.

83. Cogan JD, Kropski JA, Zhao M, et al. Rare variants in RTEL1 are associated with familial interstitial pneumonia. *Am J Respir Crit Care Med*. 2015;191(6):646-655.

84. Kropski JA, Mitchell DB, Markin C, et al. A novel dyskerin (DKC1) mutation is associated with familial interstitial pneumonia. *Chest*. 2014;146(1):e1-7.

85. Kropski JA, Reiss S, Markin C, et al. Rare Genetic Variants in PARN Are Associated with Pulmonary Fibrosis in Families. *Am J Respir Crit Care Med*. 2017;196(11):1481-1484.

86. Alder JK, Stanley SE, Wagner CL, Hamilton M, Hanumanthu VS, Armanios M. Exome Sequencing Identifies Mutant TINF2 in a Family With Pulmonary Fibrosis. *Chest*. 2015;147(5):1361-1368.

87. Dressen A, Abbas AR, Cabanski C, et al. Analysis of protein-altering variants in telomerase genes and their association with MUC5B common variant status in patients with idiopathic pulmonary fibrosis: a candidate gene sequencing study. *Lancet Respir Med*. 2018;6(8):603-614.

88. Borie R, Tabeze L, Thabut G, et al. Prevalence and characteristics of TERT and TERC mutations in suspected genetic pulmonary fibrosis. *Eur Respir J*. 2016;48(6):1721-1731.

89. Petrovski S, Todd JL, Durheim MT, et al. An Exome Sequencing Study to Assess the Role of Rare Genetic Variation in Pulmonary Fibrosis. *Am J Respir Crit Care Med*. 2017;196(1):82-93.

90. Stanley SE, Gable DL, Wagner CL, et al. Loss-of-function mutations in the RNA biogenesis factor NAF1 predispose to pulmonary fibrosis-emphysema. *Sci Transl Med*. 2016;8(351):351ra107.

91. Alder JK, Cogan JD, Brown AF, et al. Ancestral mutation in telomerase causes defects in repeat addition processivity and manifests as familial pulmonary fibrosis. *PLoS Genet*. 2011;7(3):e1001352.

92. 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;117(21):5607-5611.

93. Calado RT, Regal JA, Kleiner DE, et al. A spectrum of severe familial liver disorders associate with telomerase mutations. *PLoS One*. 2009;4(11):e7926.

94. Calado RT, Brudno J, Mehta P, et al. Constitutional telomerase mutations are genetic risk factors for cirrhosis. *Hepatology*. 2011;53(5):1600-1607.

95. Hartmann D, Srivastava U, Thaler M, et al. Telomerase gene mutations are associated with cirrhosis formation. *Hepatology*. 2011;53(5):1608-1617.

96. Gorgy AI, Jonassaint NL, Stanley SE, et al. Hepatopulmonary syndrome is a frequent cause of dyspnea in the short telomere disorders. *Chest*. 2015;148(4):1019-1026.