In most eukaryotes, telomeres become critically short when they become inactivated and fail to maintain their length through the life span of a cell. Telomerase activity counteracts the continuous telomere shortening caused by cell replication. However, in humans telomerase activity is not observed after birth in most somatic cells, or occurs only at very low levels. In contrast, germ cells, stem cells and their immediate progeny, activated T cells, monocytes, and notably most cancer cells express telomerase activity, but only in germline cells and cancer cells is telomerase activity sufficient to prevent telomere shortening.4-7

Telomere length is highly variable among different species, but within an individual species the number of telomeric repeats is usually maintained within a well-defined range. In humans telomeres shorten with age.4,8 In peripheral blood cells rapid telomere shortening occurs within the first year of life, followed by a more gradual decline over time.9 Optimal telomere length setting during stages when telomerase is expressed is crucial for long-term survival of the somatic cells that lack telomerase expression, as telomeres must be sufficiently long to prevent premature cell senescence, but short enough to induce cell cycle arrest in cells that have lost normal growth control before they become cancer cells.

**Dyskeratosis congenita**

Dyskeratosis congenita (DC) is the first human disease whose pathogenesis has been directly linked to an impairment of telomere maintenance.10-12 DC is clinically and genetically heterogeneous. Patients with DC typically present with progressive bone marrow failure and the classical triad of mucocutaneous features including abnormal pigmentation, dystrophic nail changes, and leukoplakia of the buccal mucosa.13 However, other somatic abnormalities may occur, including epiphora caused by the blockage of the tear duct, early graying of the hair, premature tooth loss, enteropathy and diarrhea, pulmonary fibrosis, liver cirrhosis, osteoporosis and avascular necrosis of the bone, testicular atrophy, learning difficulties, mental retardation, and cerebellar ataxia caused by cerebellar atrophy.14 Mutations in three different genes have been identified in patients with DC: DKC1, TERC, and TERT. The products of these genes, dyskerin encoded by DKC1, the RNA component of telomerase, TERC, and the catalytic component of telomerase, TERT, form the catalytically active telomerase (Figure 1).15

It is thought that telomere length rather than impaired telomerase activity is responsible for disease in patients with DC. Indeed, all patients with DC and clinically relevant disease have very short telomeres.15 Interestingly, however, the severity of disease, the age of onset, and the spectrum of clinical manifestations vary with the gene mutated and the nature of the mutation responsible for the disease.

**X-linked DC and dyskerin**

DKC1 maps to the X chromosome. Pathogenic mutations in DKC1 cause disease in all male members of the affected family with the mutation, whereas females who carry the mutation show no or only mild disease. Female carriers of DKC1 mutations nearly always show 100% skewing of X-chromosome inactivation such that the chromosome carrying the mutated DKC1 allele is inactivated in all cells.16 Although the age of onset and the severity of disease may vary, by the age of 30 years more than 90% of males with DKC1 mutations show signs of bone marrow failure and at least one of the cutaneous features typical of the disease. Dyskerin is essential for the nuclear accumulation of telomerase RNA TERC.17 Dyskerin mutant cells have greatly reduced levels of TERC RNA and it is thought that the reduction in TERC levels leads to the inability of telomerase to maintain telomere length.18

Hoyeraal-Hreidarrson syndrome (HH - MIM 300240) is a rare variant of DC that presents in early childhood and is characterized by intrauterine growth retardation, microcephaly, cerebellar hypoplasia, mental retardation, progressive combined immune deficiency, and aplastic anemia. Mutations in the DKC1 gene have been identified in some, but not all, patients with HH. HH patients have very short telomeres at the time of diagnosis and it is thought that HH is a very severe variant of DC.19 Dyskerin, in addition to its role in telomere maintenance also has a function in ribosome biogenesis in which it is the catalytic component in ribonucleoprotein
Whether individuals without mucocutaneous manifestations should still be diagnosed as having dyskeratosis congenita, a name that refers to the presence of the classic cutaneous features, is currently a matter of controversy. However, because premature telomere shortening is the common pathogenetic pathway of disease for individuals with DKC1, TERC or TERT gene mutations, clinical disease in the absence of mucocutaneous features is usually referred to as atypical DC. Interestingly, myelodysplastic syndrome (MDS) and acute myeloid leukemia, which are rare in the X-linked form, appear to be more frequent in individuals with DC due to TERT or TERC gene mutations, suggesting that a lag period might be needed to allow malignant transformation. Thus, malignant transformation is more likely to occur in individuals with mild disease and a longer survival than in individuals with severe disease and death in childhood or early adolescence.

**Anticipation**

A unique feature of DC due to TERT and TERC gene mutation is genetic anticipation,\(^{26,27}\) which means an inherited disease manifests at increasingly younger ages and/or with increased severity with each succeeding generation. Thus, if the offspring of patients develop the disease, they will tend to do so at an earlier age and display more severe clinical manifestations than their parents. The inheritance of increasingly shorter telomeres with subsequent generations is thought to be the molecular basis of disease anticipation in TERC and TERT gene mutation carriers. According to this model the disease is caused by the inheritance of a mutated TERC or TERT gene and the inheritance of short telomeres. Anticipation has been elegantly demonstrated in mice lacking telomerase activity.\(^{26,27}\) Several generations of inbreeding is necessary to shorten telomeres to the extent that they cause disease. Disease anticipation has also been demonstrated in several families with DC due to TERC gene mutations and in one family with DC due to a TERT gene mutation.\(^{28-31}\) In all patients the TERC or TERT gene mutations are always inherited, but the number of generations necessary before the mutations lead to telomeres sufficiently short to cause disease is unknown.

In this issue of the Journal Marrone and colleagues describe two patients with two different TERC gene mutations and demonstrate that these have most likely occurred de novo.\(^{33}\) Both mutations are likely to be responsible for disease as both significantly impair in vitro telomerase activity and in vivo are associated with short telomeres. Why a TERC mutation leads to telomeres short enough to cause disease in these two patients is unclear. In both cases the unaffected parent has telomeres length within the normal age-dependent distribution, which excludes the inheritance of preshortened telomeres. It is possible that both individuals experienced some sort of injury either during embryonic

---

**Figure 1.** Components of the catalytically active telomerase ribonucleoprotein complex include telomerase TERT, the telomerase RNA TERC, and dyskerin. Mutations in one of the three components of active telomerase lead to the clinical disease of dyskeratosis congenita.

---

particles responsible for pseudouridylation of uridine residues in ribosomal RNA (rRNA).\(^{26}\) Pseudouridylation of rRNA is an essential step in the early stages of ribosome biogenesis. Indeed, DKC1 null mutations are lethal in early embryonic mice.\(^{21}\) Moreover, defects in ribosome biogenesis have been demonstrated in mouse cells with Dkc1 mutations that are pathogenic in humans.\(^{27}\) However, whether and to what extent defects in ribosome biogenesis or ribosome function contribute to the pathogenesis of X-linked DC is controversial.\(^{26,28}\)

**Autosomal dominant DC**

In families with DC due to TERC or TERT gene mutations the disease usually follows an autosomal dominant pattern of inheritance.\(^{24,25}\) Haploinsufficiency is thought to be the mechanism of disease. Typically, disease in individuals with TERC or with TERT gene mutations is often much milder than in individuals with DKC1 gene mutations, and the onset of disease manifestations is often later in life. Furthermore, in some families not all family members carrying the mutation have clinical disease, indicating a variable penetrance of disease in TERC and TERT gene mutation carriers. Variable disease penetrance in some families results in sporadic disease or mimics the inheritance pattern of autosomal recessive inheritance.\(^{29}\) In addition to disease penetrance, the expressivity of disease is also highly variable in TERC and TERT gene mutation carriers. Mucocutaneous manifestations, characteristic of the X-linked disease, are often absent or very mild, whereas bone marrow failure, pulmonary fibrosis, and liver cirrhosis are more prominent and in some families may be the only clinical manifestations of disease.\(^{26,27}\)
development or during the development of definitive hematopoiesis necessitating an increased replication of the surviving cells, which, due to an impaired telomerase activity, would result in a shorter telomere length setting in cells that become telomerase negative and a more rapid telomere exhaustion in these cells compared to that in individuals with only the TERT gene mutation but no additional injury. Alternatively, despite the lack of a dominant negative effect of the TERC mutation on telomerase activity in vitro, the mutations that occur within two base pairs of each other (nt 178 and nt 180, see Marrone et al.)23 may be associated with accelerated telomere shortening by a mechanism that remains to be determined. Indeed there may be a dominant negative effect on telomere maintenance that is not detected in the in vitro assay. Rapid disease progression would suggest accelerated telomere shortening, whereas a more protracted progression would support the hypothesis of prenatal injury. Longitudinal monitoring of telomere length would provide further valuable insights into the dynamics of telomere length and the impact of the environmental and genetic factors that modulate the disease manifestation and disease severity in individuals with DC.

With the availability of molecular diagnostics our perception of DC has changed dramatically. Previously, the diagnosis was dictated by the clinical appearance and restricted to individuals with severe disease and the classical clinical manifestations. Today, we know that these individuals only represent a small proportion of patients with DC, the tip of an iceberg whose size is now beginning to be appreciated. Patients with atypical DC are probably much more frequent than currently realised due to the atypical presentation causing misdiagnosis. We propose that the time point when telomeres become critically short strongly affects the clinical picture and severity of disease (Figure 2). Most of what we know about DC comes from previous studies that are biased towards patients with more severe disease and the classic clinical presentation. To what extent the course of disease, prognosis, and response to treatment differs between patients with classic DC and patients with atypical DC will be the focus of future studies.

Funding: our studies on bone marrow failure are supported by the National Institutes of Health (NIH) grant RO1 CA105342 (to M.B.), and RO1 CA066995 (to P.J.M).

Acknowledgments: we are grateful to all patients and their physicians for participating in our studies (http://bmfm.im.wustl.edu).

References