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Review

# The role of telomere biology in bone marrow failure and other disorders

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**Abstract**

Telomeres, consisting of nucleotide repeats and a protein complex at chromosome ends, are essential in maintaining chromosomal integrity. Dyskeratosis congenita (DC) is the inherited bone marrow failure syndrome (IBMFS) that epitomizes the effects of abnormal telomere biology. Patients with DC have extremely short telomere lengths (<1st percentile) and many have mutations in telomere biology genes. Interpretation of telomere length in other IBMFSs is less straightforward. Abnormal telomere shortening has been reported in patients with apparently acquired hematologic disorders, including aplastic anemia, myeolodysplasia, paroxysmal nocturnal hemoglobinuria, and leukemia. In these disorders, the shortest-lived cells have the shortest telomeres, suggestive of increased hematopoietic stress. Telomeres are also markers of replicative and/or oxidative stress in other complex disease pathways, such as inflammation, stress, and carcinogenesis.

The spectrum of related disorders caused by mutations in telomere biology genes extends beyond classical DC to include marrow failure that does not respond to immunosuppression, idiopathic pulmonary fibrosis, and possibly other syndromes. We suggest that such patients be categorized as having an inherited disorder of telomere biology. Longitudinal studies of patients with very short telomeres but without classical DC are necessary to further understand the long-term sequelae, such as malignancy, osteonecrosis/osteoporosis, and pulmonary and liver disease. Published by Elsevier Ireland Ltd.

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## 1. Bone marrow failure

Bone marrow failure (BMF), also referred to as aplastic anemia (AA), is the result of the inability of the bone marrow to produce an adequate number of circulating blood cells (white blood cells [WBCs], red blood cells and/or platelets). Varying degrees of peripheral blood leukopenia, anemia, and thrombocytopenia are often present. The severity of BMF ranges from incidental findings on routine blood counts to life-threatening cytopenias. BMF is typically classified as inherited or acquired. Inherited bone marrow failure syndromes (IBMFS), such as Fanconi anemia (FA) and dyskeratosis congenita (DC), usually come to clinical attention in childhood, while acquired AAs are more common in adulthood. However, it is important to recognize that inherited syndromes, especially those with variable phenotypes, can present at any age, as can acquired AA.

Telomere length and telomere dynamics have been shown to be abnormal in almost all patients with DC, and in some patients with an IBMFS other than DC, as well as in some cases of acquired AA or other bone marrow disorders. Making the distinction between an IBMFS and acquired AA is critical in order to provide proper counseling and treatment for individual patients. In addition, since IBMFS are also cancer predisposition syndromes proper surveillance for this increased risk of cancer is a necessary component of clinical care.

## 2. Telomeres and telomere length

Telomeres consist of long TTAGGG nucleotide repeats and associated proteins at the ends of chromosomes that are essential for the maintenance of chromosomal integrity. In order to preserve the chromosome end, telomerase reverse transcriptase (gene name *TERT*), its RNA component (*TERC*) and an ordered protein complex, termed shelterin, protect the

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telomere from end-to-end fusion (Collins and Mitchell, 2002; de Lange, 2005; Nakamura and Cech, 1998). Shelterin consists of six proteins (gene name, protein name abbreviations), telomeric repeat-binding factor 1 (*TERF1*, TRF1), telomeric-repeat binding factor 2 (*TERF2*, TRF2), TRF1-interacting nuclear factor 2 (*TINF2*, TIN2), TERF2-interacting protein (*TERF2IP*, Rap1), TIN2-interacting protein 1 (*ACD*, protein names include TPP1, TINT1, PIP1, and PTOP) and protection of telomeres (*POT1*, POT1). Telomeric repeats are lost with each cell division, in part due to incomplete replication of the 3′-end of the chromosome. Telomeric attrition can result in critically short telomeres, prompting cellular senescence or cellular crisis, including apoptosis, genomic instability or a reduction in cellular proliferative potential (Maser and DePinho, 2002; Shay et al., 2001).

Telomere shortening with age is very well described. It occurs most rapidly during infancy and subsequently slows but continues at approximately 30–40 bp per year (Frenck et al., 1998; Rufer et al., 1999; Slagboom et al., 1994; Zeichner et al., 1999). Intra-individual telomere lengths appear to be consistent between tissues including cerebral cortex, myocardium, liver, renal cortex, and liver (Takubo et al., 2002) as well as between whole blood WBCs, umbilical artery and foreskin specimens (Okuda et al., 2002). There does appear to be more intra-individual variation in telomere length within bone marrow-derived cells: specifically intra-individual granulocyte telomere lengths in subjects with BMF are often shorter than those of other WBC subsets (Alter et al., 2007a; Ball et al., 1998; Brummendorf et al., 2001c; Lee et al., 2001; Polychronopoulou and Koutroumba, 2004; Rufer et al., 1998; Weng et al., 1998).

Numerous methods are available to determine telomere length in blood and other cell types, and have been reviewed elsewhere (Baird, 2005; Lin and Yan, 2005). Telomere length in genomic DNA is often determined by terminal restriction fragment (TRF) measurement on Southern blots, or by quantitative polymerase chain reaction (QPCR). Fluorescence *in situ* hybridization (FISH) and immunostaining (telomere/immunostaining-FISH [TELI-FISH] and single telomere length analysis [STELA]) are used on tissue specimens and for individual chromosome measurements. FISH combined with flow-cytometry (flow-FISH) uses fresh blood samples and has the advantage of providing telomere length data on specific white blood cell subsets (Baerlocher and Lansdorp, 2003). The method of telomere length measurement, cell type studied, as well as study design, must be considered in data interpretation and clinical application.

Until recently, most studies of BMF, inherited or acquired, used TRF to determine telomere lengths in whole blood WBCs or mononuclear cells and reported either mean telomere length in patients compared with age-matched controls, or the difference between the average telomere lengths of patients and controls (deltaTEL). The focus of those reports was the average telomere length in groups of patients compared with groups of controls, rather than in specific individuals whose telomeres were abnormally short. Refinements in telomere length measurements and advances in understanding of telomere biology and clinical phenotype are progressing rapidly. Large case-control studies of telomere length and disease association are being conducted to evaluate telomere length in groups of cases with telomere length in groups of age-matched control subjects. In order to apply this growing literature to the individual patient, interpretation of telomere length data must consider the presence of age-appropriate controls, the method of telomere length measurement, the cell type studied, as well as the definition of short telomeres used in the study.

## 3. Telomere length inheritance and genetics

Numerous studies suggest that telomere length is a heritable trait, despite the high degree of inter-individual variation. A study of monozygotic and dyzygotic twin pairs which measured telomere length in DNA isolated from whole blood WBCs showed high levels of concordance between twin pairs and telomere length, with an estimated heritability of 78% (Slagboom et al., 1994); this was subsequently confirmed (Jeanclos et al., 2000). Others have evaluated telomere length in female sibling pairs and parent–child trios and found varying degrees of telomere length heritability (Andrew et al., 2006; Bischoff et al., 2005; Graakjaer et al., 2006).

Identification of the genetic region and/or genes responsible for telomere length has proven difficult. X-linked inheritance of telomere length was suggested by analysis of multi-generational pedigrees and comparison of parent/child pairs (Nawrot et al., 2004). A quantitative-trait linkage analysis of 400 microsatellite markers in 258 sibling pairs showed significant linkage on chromosome 12, and identified a candidate gene in that region, *DDX11*, a DNA helicase (Vasa-Nicotera et al., 2005). Significant linkage at 14q23.2 and 3p26.1 was found in another quantitative-trait linkage analysis study of 1025 female dizygotic twin pairs (Andrew et al., 2006). Single nucleotide polymorphisms (SNPs), the most common germline genetic variants in the human genome, may also play a role in telomere length regulation. Individuals with one or two copies of the −1381 promoter T allele in the telomerase gene (*TERT*, T > C, rs2735940) had significantly longer telomeres and increased telomerase activity than in individuals with the CC genotype (Matsubara et al., 2006). These data suggest that polygenic inheritance of telomere length may occur.

## 4. Disorders with mutations in telomere biology genes

### 4.1. Dyskeratosis congenita

DC is a clinically heterogeneous IBMFS that epitomizes the clinical consequences of very short telomeres and mutations in telomere biology (Walne et al., 2005; Yamaguchi, 2007). The classical diagnosis of DC requires the triad of dysplastic nails, lacey reticular pigmentation of the upper chest and neck, and oral leukoplakia (Drachtman and Alter, 1992). This diagnostic triad is still important in defining clinically significant disease, but as more patients with DC and their family members have been studied, an extremely broad clinical phenotype is now appreciated. Additional features include epiphora, blepharitis, premature gray hair, alopecia, developmental delay, short