TELOMERI

La cromatina telomerica

Telomeres also bind to nucleosomes, which are rich in modified histones.

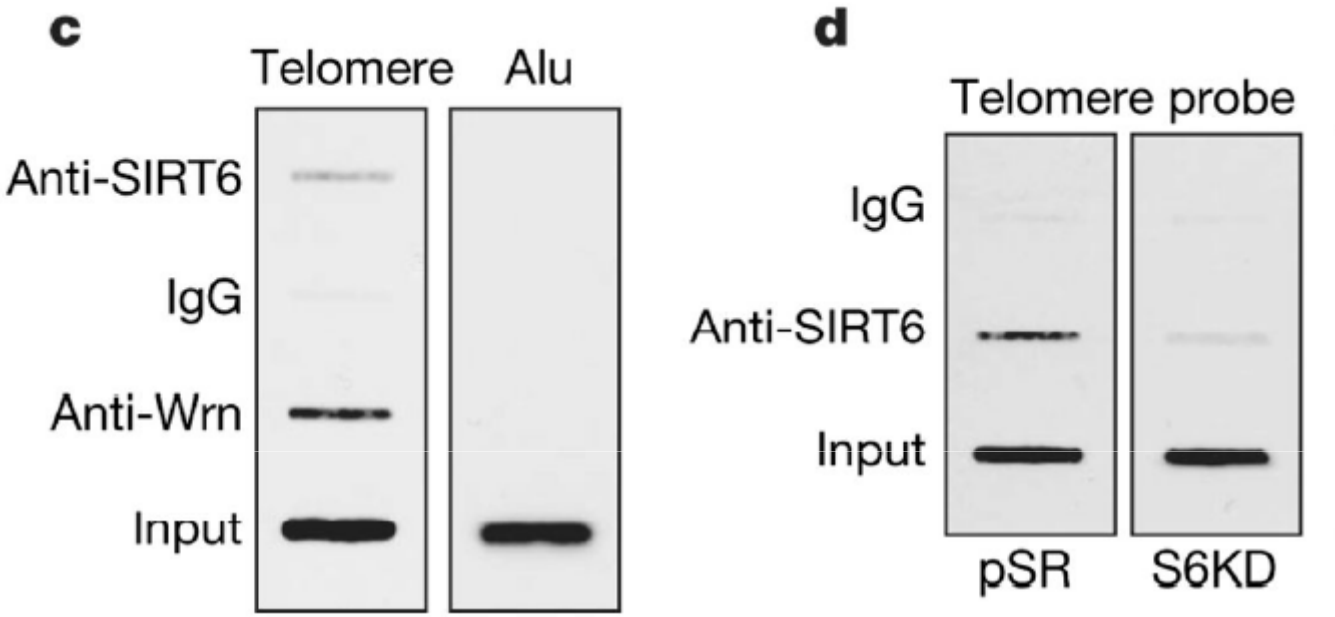
Major histone modifications *found in telomeres are*
*-H3K9 and H4K20 trimethylatio*n
-low abundance of acetylated H3 and H4

SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin

The Sir2 deacetylase regulates chromatin silencing and lifespan in Saccharomyces cerevisiae1,2.

In mice, deficiency for the Sir2 family member SIRT6 leads to a shortened lifespan and a premature ageing-like phenotype3. However, the molecular mechanisms of SIRT6 function are unclear. SIRT6 is a chromatin-associated protein3, but no enzymatic activity of SIRT6 at chromatin has yet beendetected, and the identity of physiological SIRT6 substrates is unknown. Here we show that the human SIRT6 protein is an NAD+-dependent, histone H3 lysine 9 (H3K9) deacetylase that modulates telomeric chromatin. SIRT6 associates specifically with telomeres, and SIRT6 depletion leads totelomere dysfunction with end-to-end chromosomal fusions and premature cellular senescence. Moreover, SIRT6-depleted cells exhibit abnormal telomere structures At telomeric chromatin, SIRT6 deacetylates H3K9. We propose that SIRT6 contributes to the propagation of a specialized chromatin state at mammalian telomeres, which in turn is required for proper telomere metabolism and function. Our findings constitute the first identification of a physiological enzymatic activity of SIRT6, and link chromatin regulation by SIRT6 to telomere maintenance and a human premature ageing syndrome

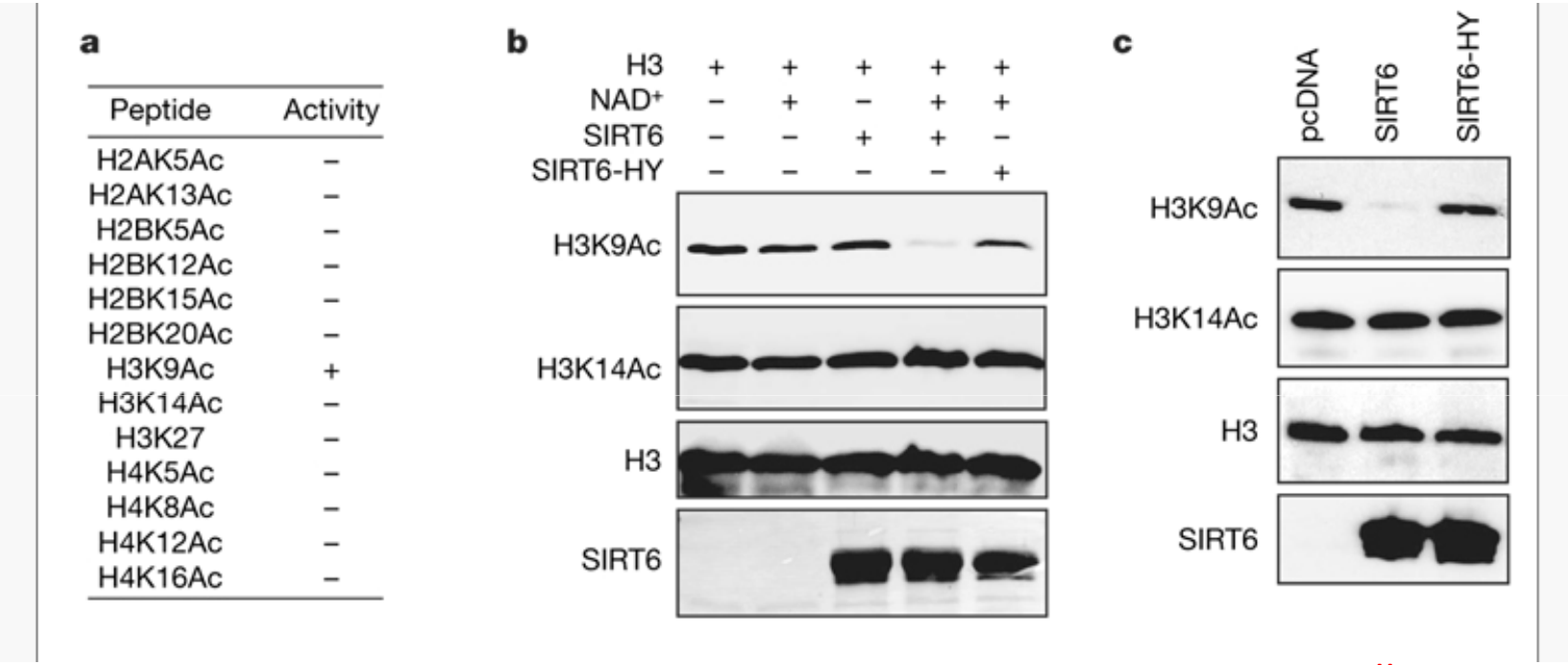
SIRT6 associates with telomeric chromatin



SIRT6 knockdown (S6KD) cells

control pSR IMR90 cells

SIRT6 (sir 2) deacetylates lysine 9 of histone H3 at telomeric chromatin



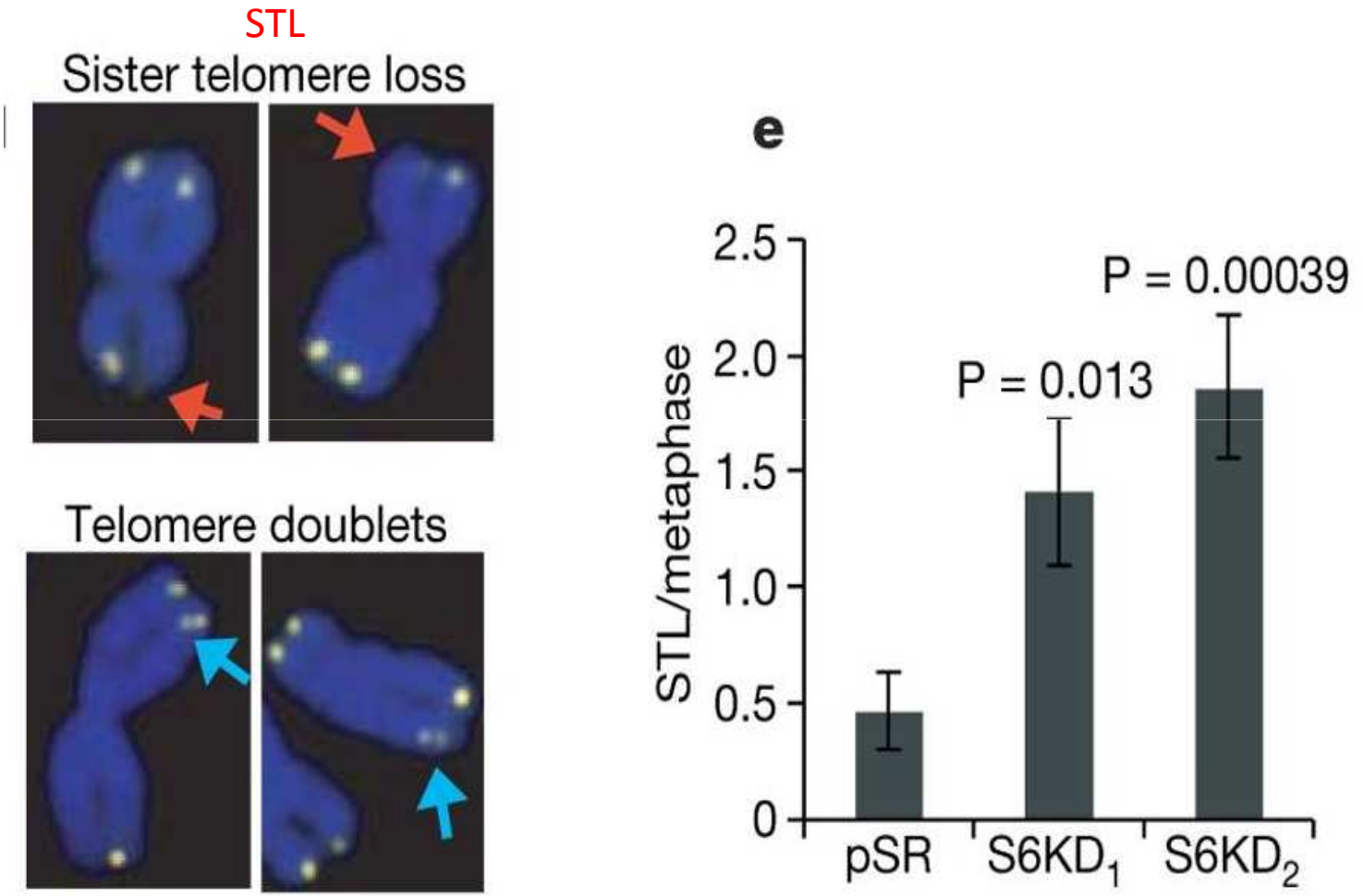
histone tail peptides

full-length histone H3

293Tcells overexpressing SIRT6

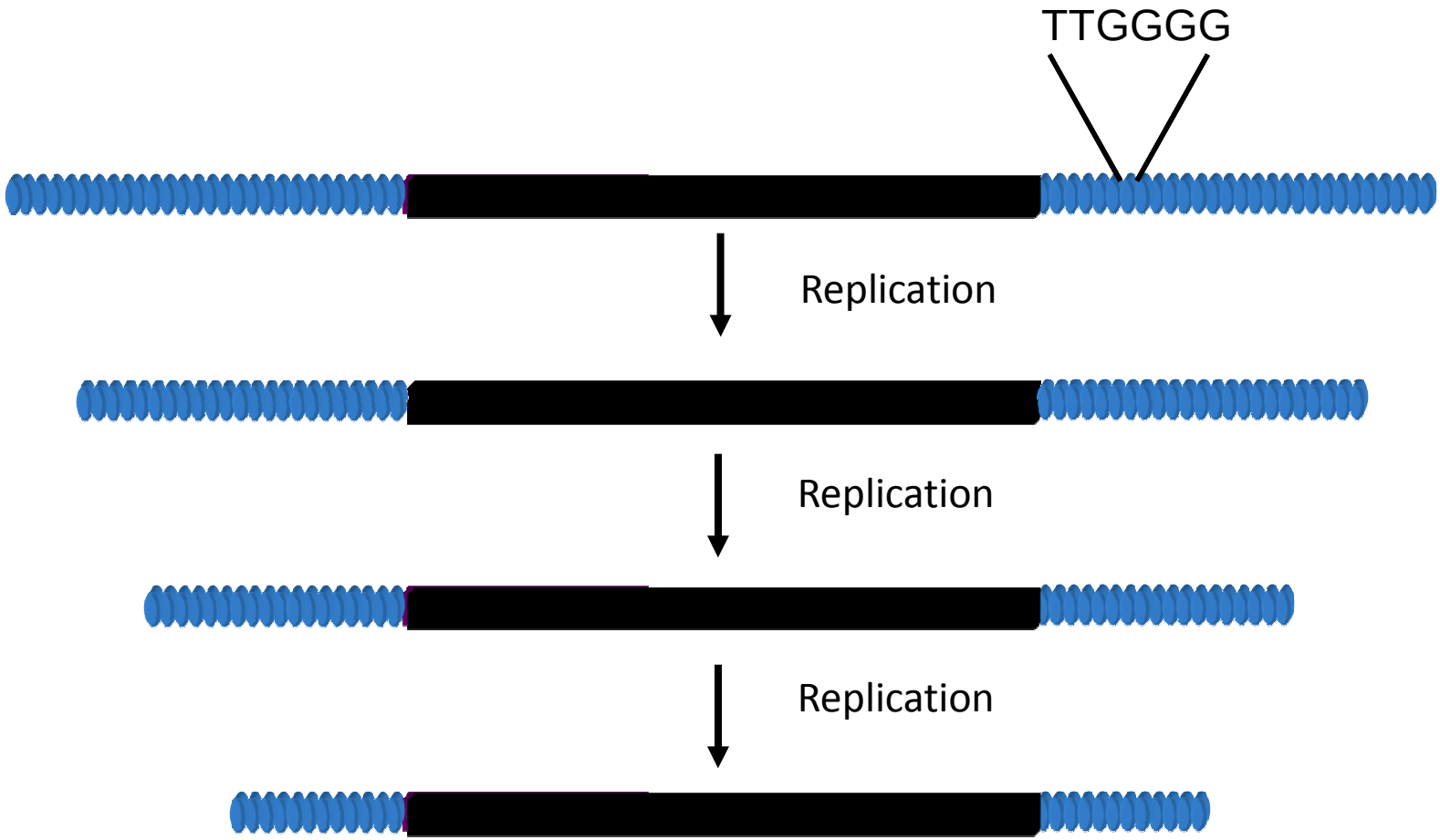
SIRT6-HY: *catalytic H133Y SIRT6 mutant protein*

SIRT6 knockdown (S6KD) cells

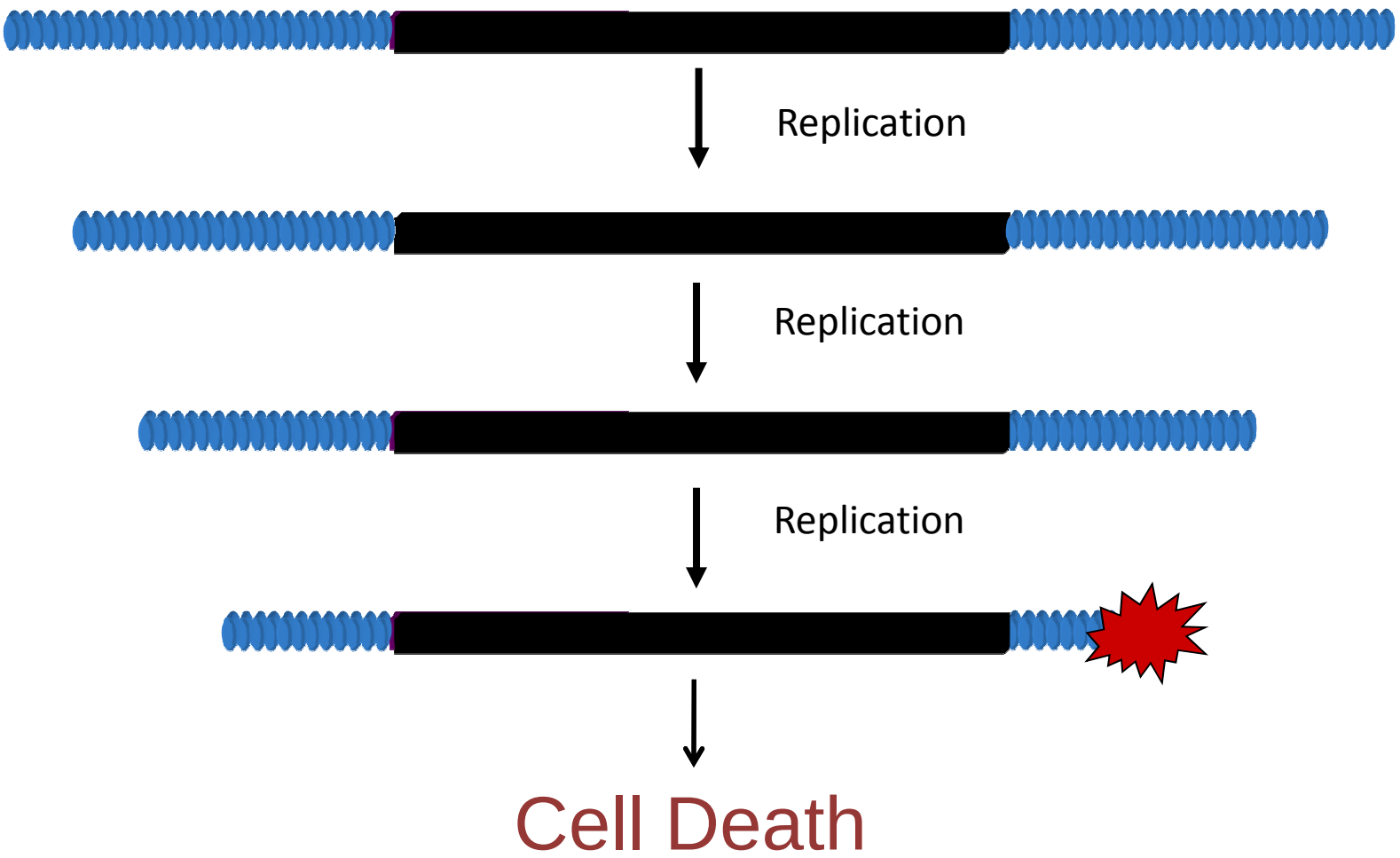


d, Representative S6KD metaphases showing aberrant telomere signals. Red arrows, sister telomere loss; blue arrows, telomere doublets. e, Quantification of sister telomere loss

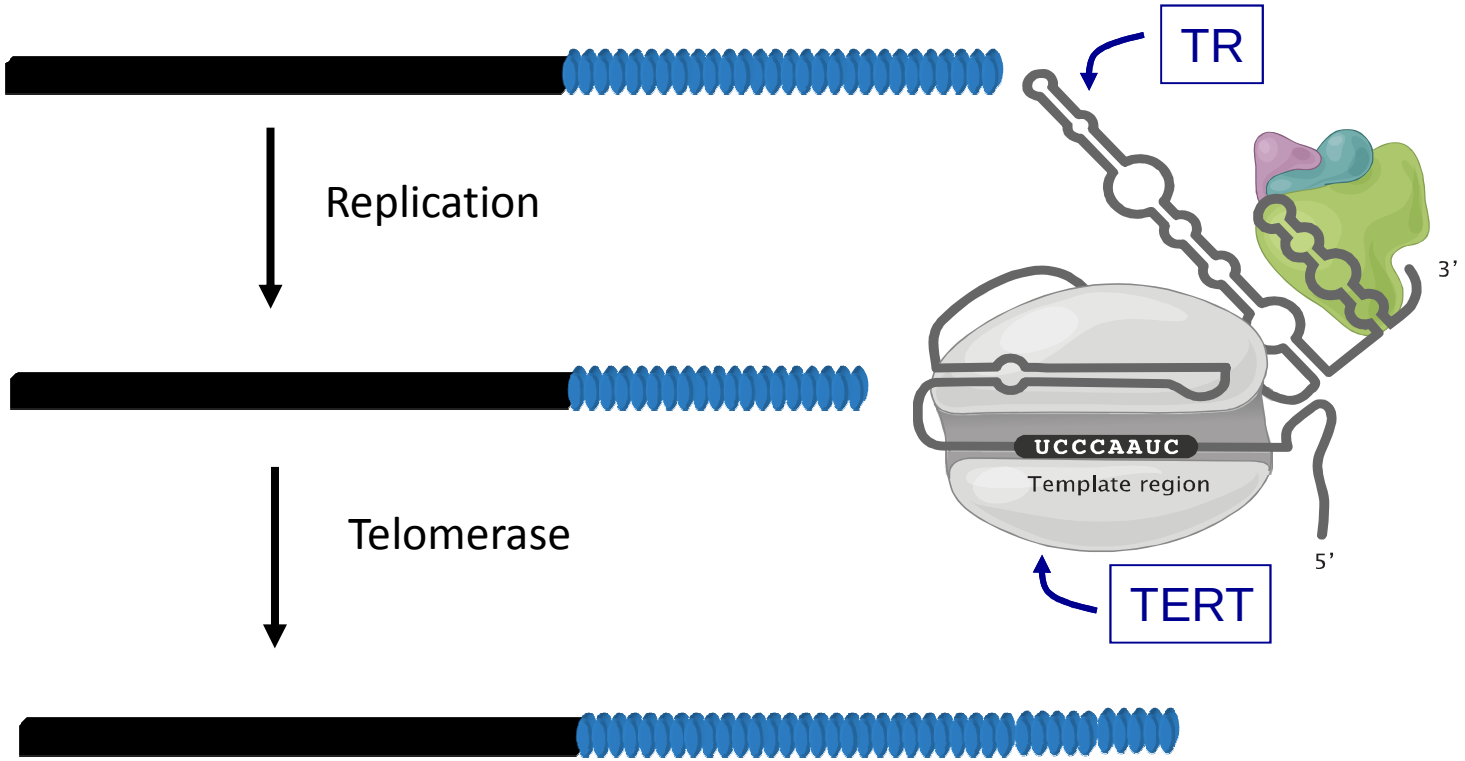
Telomeres shorten as cells divide



Telomere shortening leads to cell death



Telomerase allows telomere length equilibrium maintenance



Telomere shortening in cellular senescence

Telomeres shorten during ageing of human fibroblasts

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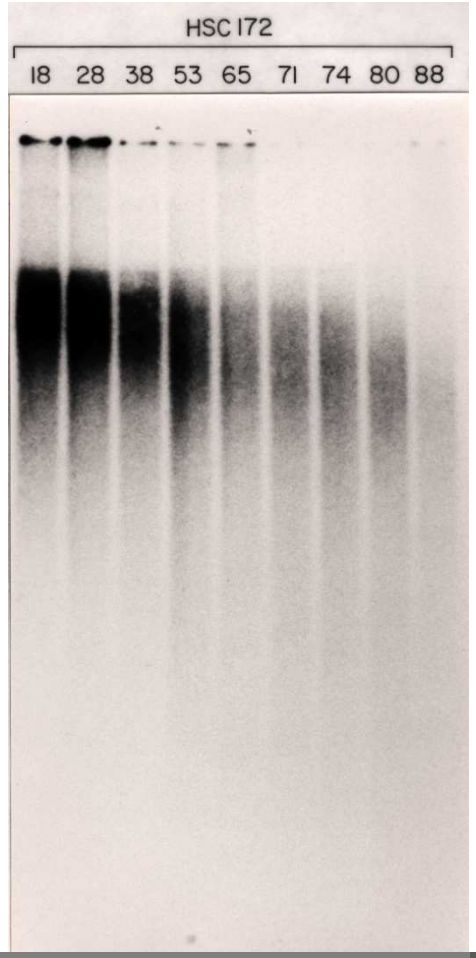
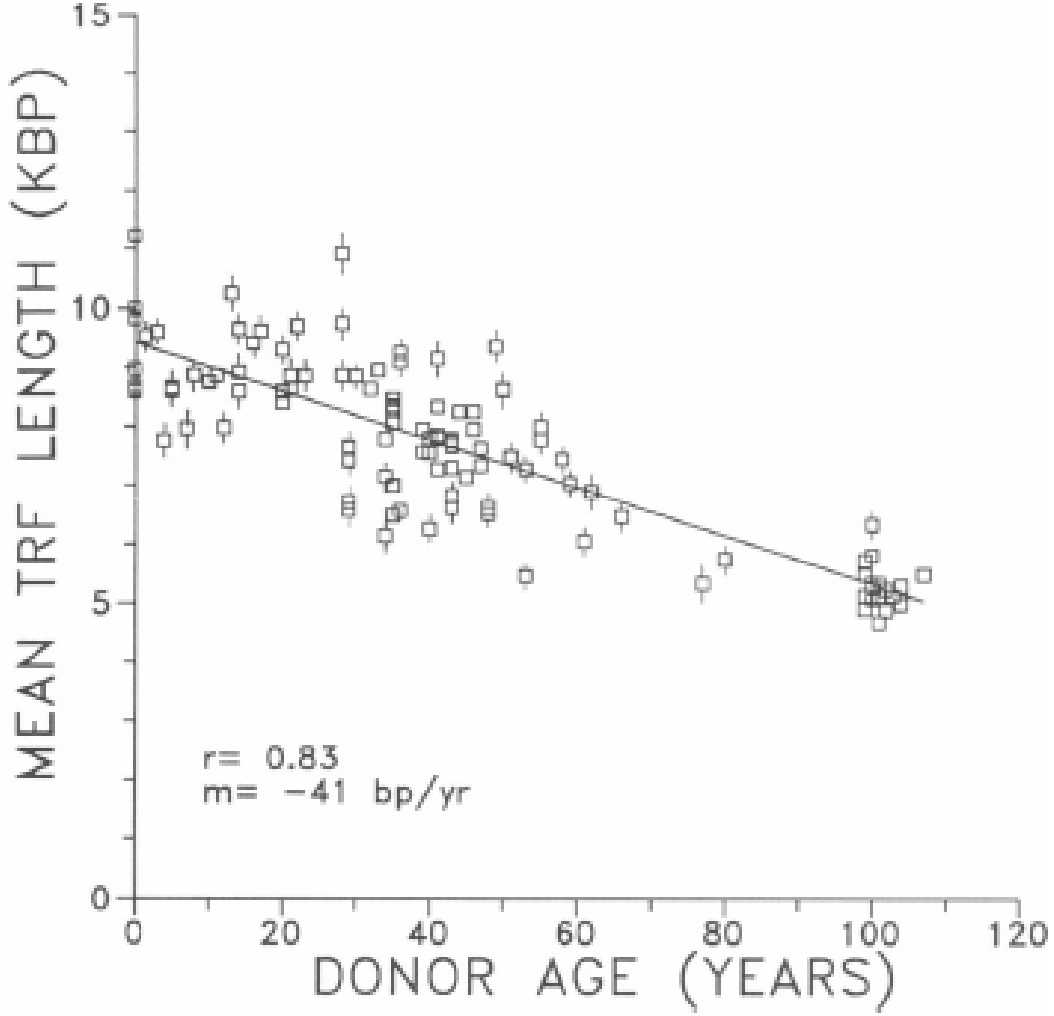
* Department of Biochemistry, McMaster University, 1200 Main Street West, Hamilton, Ontario L8N 3Z5, Canada
† Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA



Nature May 1990

Telomeres shorten with age



Telomerase is limiting in cells

Vaziri et al. *AJHG* (1995)

The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita

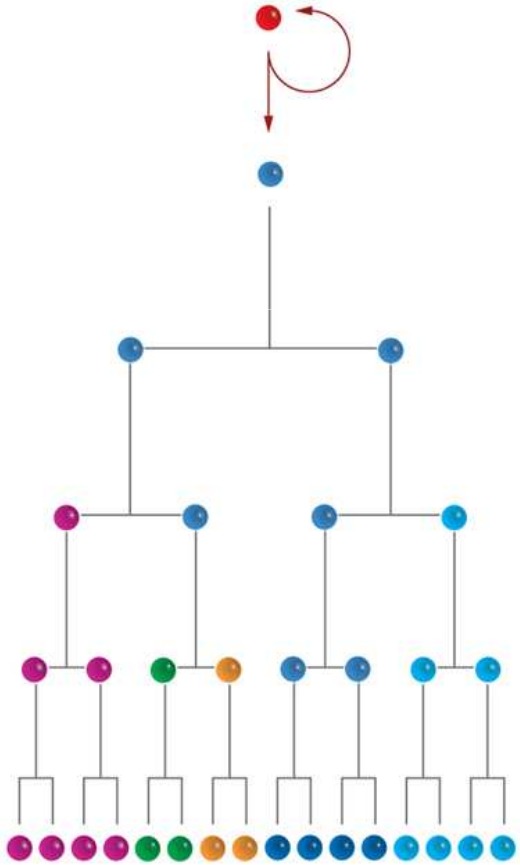
Tom Vulliamy*, Anna Marrone*, Frederick Goldman†, Andrew Dearlove‡, Monica Bessler§, Philip J. Mason* & Inderjeet Dokal*

Nature 2001

Dyskeratosis congentia causes bone marrow failure

- Skin and nail problems
  - Skin hyperpigmentation
  - Rashes
  - Abnormal nail growth

- Mortality
  - Bone marrow failure
  - Cancer
  - other?

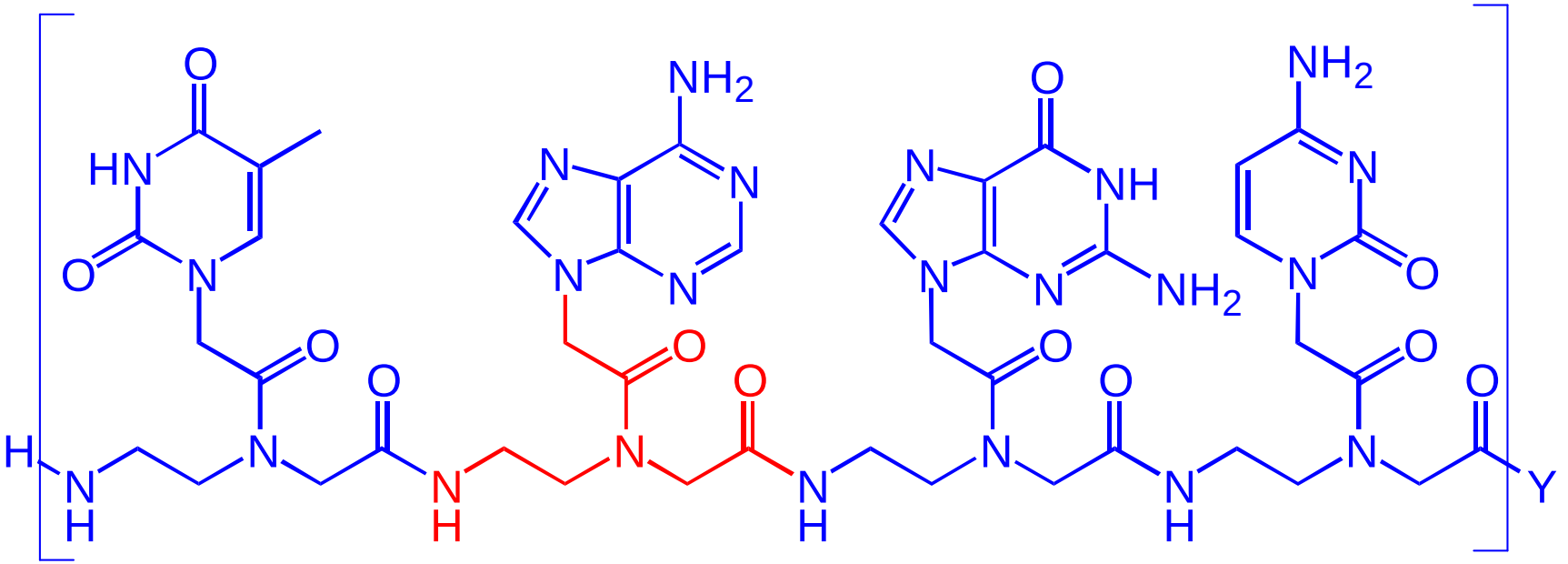


measure the average length of telomere repeats in thousands of cells

protocols that use fluorescent in situ hybridization (FISH) with labeled peptide nucleic acid (PNA) probes specific for telomere repeats

in combination with fluorescence measurements by flow cytometry (flow FISH).

PNA



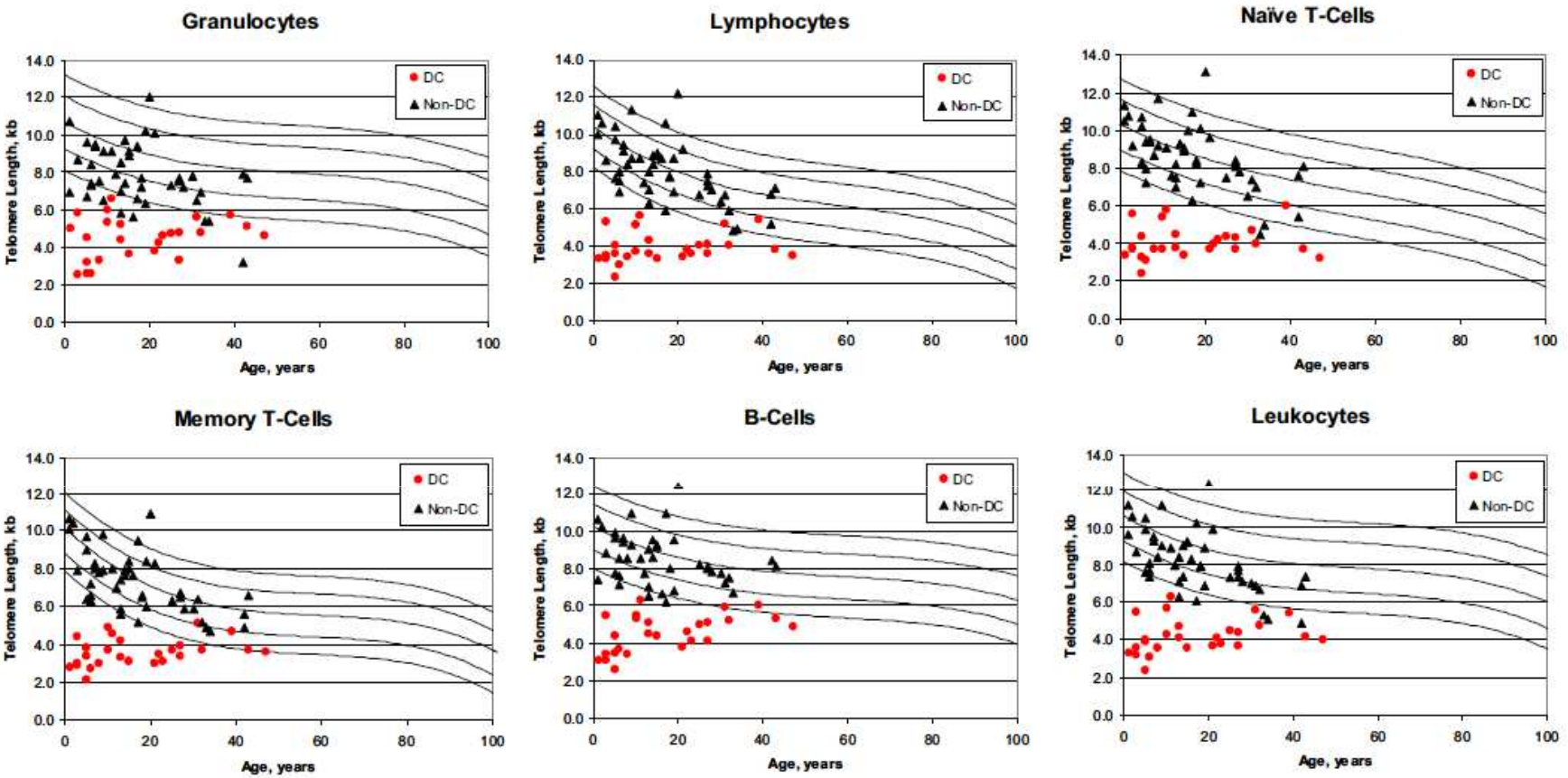


Figure 3. Telomere length according to age in dyskeratosis congenita and non–dyskeratosis congenita patients. The vertical axis represents telomere length in kilobytes. Lines in the figures indicate the first, tenth, 50th, 90th, and 99th percentiles of results from 400 normal control subjects. Symbols represent subjects: 26 patients with dyskeratosis congenita (red solid circle), 46 non–dyskeratosis congenita patients (black solid triangle).

Short telomeres in lung disease

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Telomerase Mutations in Families with Idiopathic Pulmonary Fibrosis

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ABSTRACT

BACKGROUND

Idiopathic pulmonary fibrosis is progressive and often fatal; causes of familial clustering of the disease are unknown. Germ-line mutations in the genes *hTERT* and *hTR*, encoding telomerase reverse transcriptase and telomerase RNA, respectively, cause autosomal dominant dyskeratosis congenita, a rare hereditary disorder associated with premature death from aplastic anemia and pulmonary fibrosis.

METHODS

To test the hypothesis that familial idiopathic pulmonary fibrosis may be caused by short telomeres, we screened 73 probands from the Vanderbilt Familial Pulmonary Fibrosis Registry for mutations in *hTERT* and *hTR*.

RESULTS

Six probands (8%) had heterozygous mutations in *hTERT* or *hTR*; mutant telomerase resulted in short telomeres. Asymptomatic subjects with mutant telomerase also had

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NEJM 357 p1317 (2007)

Table 1 Mutations in telomerase and telomere genes lead to a broad clinical spectrum of syndromes of telomere shortening

Gene Name	Diagnosis	Typical age of onset in years
hTR and *hTERT*	Sporadic IPF 1–3% Familial IPF[a] 8–15% Sporadic and familial aplastic anemia ~3–5% Autosomal dominant DC[b]	Broad range 5–77
DKC1	X-linked DC Hoyeraal-Hreiderasson	Less than 30 Less than 5
TINF2	Sporadic DC Autosomal dominant DC Hoyeraal-Hreiderasson	Less than 10 - Less than 5
NOP10	Autosomal recessive DC	-
NHP2	Autosomal recessive DC	-

[a]IPF refers to idiopathic pulmonary fibrosis.
[b]DC refers to dyskeratosis congenita.

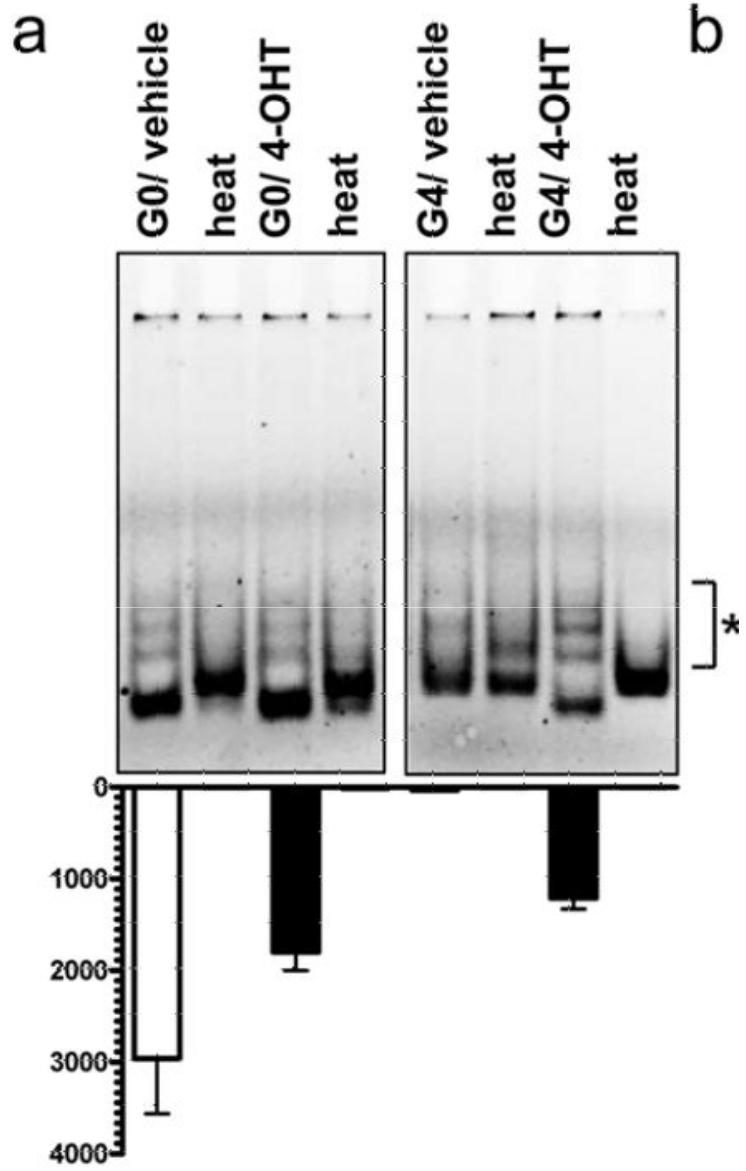
Table 2 Spectrum of bone marrow, lung, and liver disease seen in individuals with syndromes of telomere shortening

Hematologic features
Macrocytosis
Elevated hemoglobin F
Isolated cytopenias (most commonly thrombocytopenia)
Aplastic anemia
Myelodysplasia
Acute myeloid leukemia
Pulmonary fibrosis
Asymptomatic restrictive defects on pulmonary function studies
Idiopathic pulmonary fibrosis/usual interstitial pneumonia
Nonspecific interstitial pneumonia
Idiopathic interstitial pneumonia nonclassifiable on biopsy
Liver disease
Normal or mildly elevated transaminases
Atrophic nodular liver on imaging studies
Splenomegaly
Cryptogenic liver fibrosis/cirrhosis

SUMMARY POINTS

1. Mutations in telomerase and telomere components lead to a broad spectrum of disease that has clinical presentations in children and adults. The extent of telomere shortening determines the onset and severity of these disorders.
2. The study of families with mutations in telomerase components allows the identification of a distinct disease entity marked by organ failure in the bone marrow and a clustering of pulmonary and liver fibrosis. This syndrome frequently appears in adulthood and is distinct from DC, though it falls on the same spectrum.
3. IPF is the most common manifestation of a syndrome of telomere shortening. The causal role implicating short telomeres in IPF provides evidence that short telomeres are sufficient to cause common, age-related disease with its most common manifestation in the lung.
4. Syndromes of telomere shortening are unique among progeroid disorders in that they phenocopy a process that occurs in humans as they age.

Telomerase reactivation



G4 cells have no detectable telomerase activity

4-hydroxytamoxifen (4-OHT)-inducible telomerase reverse transcriptase

Telomerase reactivation reverses tissue degeneration in aged telomerase deficient mice