

# Shared Phenotypes Among Segmental Progeroid Syndromes Suggest Underlying Pathways of Aging

Anne C. Hofer,<sup>1</sup> Rosie T. Tran,<sup>1</sup> Owais Z. Aziz,<sup>1</sup> Woodring Wright,<sup>2</sup>  
Giuseppe Novelli,<sup>3</sup> Jerry Shay,<sup>2</sup> and Marc Lewis<sup>4</sup>

<sup>1</sup>Plan II, The University of Texas at Austin.

<sup>2</sup>Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas.

<sup>3</sup>Department of Biopathology and Diagnostic Imaging, Tor Vergata University, Rome, Italy.

<sup>4</sup>Department of Psychology, The University of Texas at Austin.

Segmental progeroid syndromes are those whose phenotypes resemble accelerated aging. Here we analyze those phenotypes and hypothesize that short telomeres produce the same group of symptoms in a variety of otherwise unrelated progeroid syndromes. Specific findings are the following: (a) short telomeres in some progeroid syndromes cause an alopecia/osteoporosis/fingernail-atrophy group of symptoms; (b) fingernail atrophy in progeroid syndromes resembles the natural slowing of nail growth that occurs in normal aging and nail growth velocity, and may be a marker of replicative aging in keratinocyte stem cells; (c) alopecia and reduced hair diameter parallel the nail results; (d) osteoporosis in Dyskeratosis Congenita resembles age-related osteoporosis, but the same is not true of other progerias; and (e) gray hair is associated with short telomeres, but may also involve reactive oxygen species. On the basis of these results, we make several predictions and discuss how the segmental quality of progeroid syndromes may provide insight into normative aging.

**P**ROGEROID syndromes with very different causes sometimes share symptoms in common. Gray hair, for example, is a symptom of both Nijmegen Breakage Syndrome (NBS) and Hutchinson-Gilford Progeria Syndrome (HGP), but the two diseases are otherwise very different. In NBS, a disruption in homologous DNA repair of double strand breaks, along with failure to halt at the S-phase checkpoint, leads to symptoms such as anemia, webbed toes, and café au lait spots; in HGP, a weakened, dysfunctional inner nuclear wall leads to absence of subcutaneous fat, atherosclerosis, and wormian bones. Gray hair does not, in contrast, appear in either Bloom Syndrome or in Cockayne Syndrome, both of which have causes very different from those of NBS, HGP, and each other. Table 1 lists progeroid syndromes (which for simplicity we will hereafter call progerias), a brief description of their causes, and a representative synopsis of their symptoms. Gray hair appears in six of the eleven diseases shown. Other symptoms such as nail atrophy and osteoporosis also occur in some progeroid syndromes but not in others.

Why do shared symptoms such as gray hair occur in so many progerias, but not in all of them? Is the mechanism that causes gray hair the same in all six progerias and is it the same mechanism that causes gray hair in natural aging? In this article we will consider those questions for a number of symptoms that co-occur in a specific subgroup of progerias. We will also consider one possible explanation for the co-occurrence of those symptoms, discuss relevant mechanisms, and make predictions. Although we will mention several non-aging symptoms in passing, they lie outside of our present focus and will be discussed in depth elsewhere.

## PROGERIAS WITH SYMPTOMS IN COMMON

Figure 1 shows symptoms that the progerias have in common. For example, the darkest column shows that two progerias (Rothman-Thomson Syndrome [RTS] and Werner Syndrome) share the symptom of osteosarcoma; the lightest column shows that three progerias (Ataxia Telangiectasia, NBS, and Bloom Syndrome) share the symptom of café au lait spots. Figure 1 contains only a subset of all shared symptoms, chosen to make a point that will be clarified later. In addition, symptoms that are superficially similar or that lack explanatory power because they occur in almost all progerias (e.g., hyper-/hypo-pigmentation) are also excluded.

The third column of Figure 1 shows that four progerias share the symptoms of nail atrophy, alopecia, and osteoporosis. Atrophic nails are small and thin, and often fail to grow to the end of the finger or toe (1). Hypoplastic (underdeveloped) nails are excluded from this definition, as are dystrophic nails unless they occupy the mild end of a disease continuum that includes atrophic nails at the other end (e.g., as happens in Dyskeratosis Congenita). In trichothiodystrophy, for example, nails and hair become brittle and break due to transcription-related sulfur deficiency, but they do not progress to either nail atrophy or to alopecia and so that disease is not included in the red column of Figure 1.

Nail atrophy can occur for secondary reasons such as shoe trauma or scarring from the disease lichen planus, and as the occasional side effect of some peripheral vascular disorders (e.g., leprosy), but it is almost never a primary disease symptom. In fact, we searched more than 7000 diseases and conditions (see "Scope of Literature Review") and found nail atrophy mentioned as a symptom in just five. Four of those five diseases are progerias. The fifth disease, Cronkhite-

Table 1. Brief, Representative Descriptions of Progeroid Syndromes and Causes

Syndrome	Description/Causes
Rothmund-Thompson Syndrome (RTS):	Mutation in RECQ4 helicase in two-thirds of cases. Commonly thought to be a DNA repair defect but no evidence that RTS patients are sensitive to chemotherapy or UV. Some skeletal problems present at birth. Swelling, blistering rash appears at 3–10 months postpartum, then grows into poikiloderma. Juvenile cataracts reported in some series of patients, not in others. Other symptoms include small stature and photosensitivity, osteoporosis, alopecia (some with no hair problem though), gray hair, dystrophic/atrophic nails, osteosarcoma at median age 11.4. Life span is normal in the absence of cancer.
Werner Syndrome (WS):	Mutation in RECQ3/WRN which encodes the WRN helicase/exonuclease. Symptoms usually first noticed around puberty when no growth spurt develops. Gray hair and alopecia follow, then sclerotic skin, diabetes, and osteoporosis. Other symptoms include diabetes mellitus, cataract (variously described), hypogonadism, immunodeficiency, nail atrophy, birdlike facies, and senile dementia. Schizophrenia is ten times normal rate. Death occurs in mid to late 40s.
Hutchinson-Gilford Progeria (HGP):	LMNA mutation leads to truncated Lamin A protein and a weakened, dysfunctional inner nuclear wall. Infants look normal at birth but alopecia appears in the first year. Growth failure in the second year and the start of progressive loss of subcutaneous fat. Atherosclerosis, osteoporosis, nail atrophy, gray hair, thin skin, beaked nose, high pitched voice. Median age at death is about 13.
Dyskeratosis Congenita (DKC):	The X-linked (DKC1 mutation) and AD (TERC mutation) forms greatly reduce active telomerase levels leading to nail atrophy, osteoporosis, alopecia, gray hair, wrinkled skin, poikiloderma, hypertension, oral mucosal leukoplakia, and increased cancer, especially epithelial and hematopoietic types. Bone marrow failure is the principle cause of death. The milder AR form (cause unknown) has mainly hematopoietic symptoms. Age of onset, severity, range of symptoms, and age at death vary widely, probably due to hereditary differences in telomere length.
Ataxia Telangiectasia (AT):	ATM mutation affects downstream phosphorylation in DNA damage repair and cell cycle control. Scleral telangiectasias appear after age 3 along with photosensitivity and scleroderma. No mental problems at first, but deterioration occurs over time. Cerebellar ataxia appears when the child begins to walk. Gray hair, lymphoma and leukemia, wrinkled skin, immunodeficiency, reduced reflexes, and café au lait spots. Death from recurrent respiratory infection in adolescence or early adulthood.
Nijmegen Breakage Syndrome (NBS):	Mutations in NBS1 (in most cases), a gene phosphorylated by ATM, disrupts homologous DNA repair. Microcephaly at, or around, birth with postnatal growth retardation and decline in mental function. Also photosensitivity, immunodeficiency, predisposition to cancer, gray hair, telangiectasias, and café au lait spots. Death from respiratory infections, but greater risk of death from malignancy (lymphoma is elevated 1000 fold).
Bloom Syndrome (BS):	Caused by RECQ2/BLM helicase. Growth deficiency at birth leads to final small, proportionate stature. Telangiectasias appear on face, ears, sclerae along with café au lait spots, photosensitivity, and erythema. Long narrow face appears birdlike. Also, immunodeficiency and diabetes mellitus. Leukemias develop at mean age 22; solid tumors develop in survivors at mean age 35. Life expectancy depends on presence of malignancy and its response to treatment.
Xeroderma Pigmentosum (XP):	Defects in nucleotide excision repair (global genome repair, transcription coupled repair, or both) produces seven complementation groups (XP-A- to XP-G), all of which are components of transcription factor TFIIH. Dysfunction in DNA polymerase-eta produces the XP-V type. Symptoms depending on subtype may include photosensitivity, skin atrophy, early-onset skin cancers, poikiloderma, microcephaly, low intelligence, and mild to severe neurological features.
Trichothiodystrophy (TTD):	Transcription problem in XP-D and (rarely) XP-B (see XP entry) affects transcription in TFIIH. One reported case of third complementation group (TTD-A). Brittle (“tiger tail”) hair and nails due to sulfur deficiency, photosensitivity, ichthyotic skin, loss of subcutaneous fat, birdlike facies, aged appearance, cataracts, delayed physical and mental development, microcephaly, hypogonadism, respiratory infections, and digestive problems. Death between age 3 and 30 depending on severity.
Cockayne Syndrome (CS):	Types I (classic) and II (congenital) both caused by defects in transcription coupled repair (see xeroderma pigmentosum). Infants are normal at birth but develop early growth failure, microcephaly, a narrow face, and a beaked nose. Photosensitivity leads to thin, dry skin with rashes and telangiectasias. Demyelination in the central and peripheral nervous systems, with accompanying mental retardation and deafness. Also respiratory problems, pigmentary retinal degeneration, thin, dry hair, dental abnormalities, and delayed puberty. Death from Type I occurs in the second or third decade, death from Type II at about age 7.
Ehlers Danlos, Progeroid Form (ED):	Mutation in B4GALT7 affects glycosylation of Decorin, a collagen handling protein, and leads to failure to thrive, small face, short stature, narrow chest, psychomotor retardation, osteopenia, sparse scalp hair, loose, elastic skin, long slender fingers and toes.

Canada Syndrome (CCS), has not fully been studied or classified, but it has much in common with the progerias, and it may represent an unrecognized addition to that family of diseases. We will discuss CCS separately a little later.

Nail atrophy is not only a rare symptom, but it also co-occurs with two other less rare, but relatively uncommon symptoms, alopecia and osteoporosis. The four progerias in the third column are, in fact, the only diseases in the

literature that share these three symptoms. It is improbable that the association is coincidental, and we will argue that a single underlying process unites them. We begin with a discussion of fingernails.

#### THE FINGERNAIL SYSTEM

The fingertip (Figure 2A) is an unusually safe place for DNA. To understand just how safe it is, consider the effect of

<b>TELOMERES SHORTENED</b>	<b>Rothmund-Thomson Syndrome?</b> <b>Werner Syndrome</b> <b>Hutchinson-Gilford Progeria</b> <b>Dyskeratosis Congenita (x-linked, AD)</b>	<b>Osteo-sarcoma</b>	<b>Alopecia, nail atrophy, Osteoporosis</b>	<b>Gray hair</b>
	<b>Ataxia Telangiectasia</b> <b>Nijmegen Breakage Syndrome</b>	<b>Café au lait Spots, Scleral Telangiectasias</b>		
<b>TELOMERES NOT SHORTENED</b>	<b>Bloom Syndrome</b>			
	<b>Xeroderma Pigmentosum (seven complementation groups, one variant)</b>			
	<b>Trichothiodystrophy (three complementation groups)</b>			
	<b>Cockayne Syndrome (two complementation groups)</b>			
	<b>Ehlers-Danlos (progeroid form)</b>			

Figure 1. Shared symptoms in progeroid symptoms.

350 nm UVA light on skin versus nails. In fair skin, most UVA light is reflected from the surface, but 37% penetrates the epidermis to a depth of 60  $\mu\text{m}$  (2). There, passing through the rising columns of epidermal keratinocytes, UVA leaves a trail of damaged DNA in the form of pyrimidine dimers. By the time the light reaches the stem cells in the rete ridges of the basal layer, it still has 10% of its original damaging power (2). That much radiation is not a short-term concern in normal skin, but in progerias with DNA repair problems the resultant dimers block transcription and replication; this blockage can be lethal to cells. The result is erythema, actinic keratosis, photoaging, cancer, and other problems of photodamage.

But photodamage does not affect fingernails. Even xeroderma pigmentosum, an extremely photosensitive disease with severe skin reactions, has no fingernail symptoms. The reason is that fingernail stem cells and their transient amplifying cell (TAC) progeny are buried three times deeper in nails than they are in skin (see, for example, 3). As the nail TACs reproduce, leave the basal layer, and rise through the nail matrix, they are protected by the nail plate, which acts as a UV sunscreen (4). By the time those keratinocytes join the nail plate, they are enucleated, condensed, and immune to photodamage.

Although nail keratinocytes escape photodamage, no cycling cell can escape the dangers of replication. Fingernail TACs replicate frequently and, like all replicating cells, each time they do they lose an average of 50–100 bp of DNA

because of the end replication problem (5,6) and subsequent processing of the resulting 3' overhang. The loss is not crucial in early life because humans are born with about 15 kb of disposable TTAGGG tandem repeats (telomeres) at the ends of their chromosomes. But with replication those protective telomeres shorten. The danger is not so much in the shortening itself, but rather in the loss of the second protective role of telomeres, that of capping chromosomes and preventing the cell from mistakenly treating their ends as double strand breaks. The telomere achieves that protected state by, among other things, looping back and tucking its 3' overhang into the double stranded upstream telomeric DNA (7). The result is a large telomere loop, ending in a smaller displacement loop, which is held in place by critical proteins such as TRF2 (reviewed in 8). As telomeres shorten, this end protection is lost, possibly because critical proteins are unable to maintain the loop. The result is either senescence, or in some instances, end-to-end fusions that create a fusion-breakage-bridge cycle (9).

Some highly replicating cells mitigate the problems caused by shortening telomeres by replacing some lost repeats using the cellular reverse transcriptase enzyme, telomerase. Hair keratinocyte stem cells, for example, have weak telomerase activity, and there is strong activity in their rapidly dividing TAC progeny (10). The amount of telomerase present is not, however, sufficient to fully maintain telomeres and so keratinocyte telomeres get progressively shorter with age. Telomerase has not been studied in nails, but it is found in

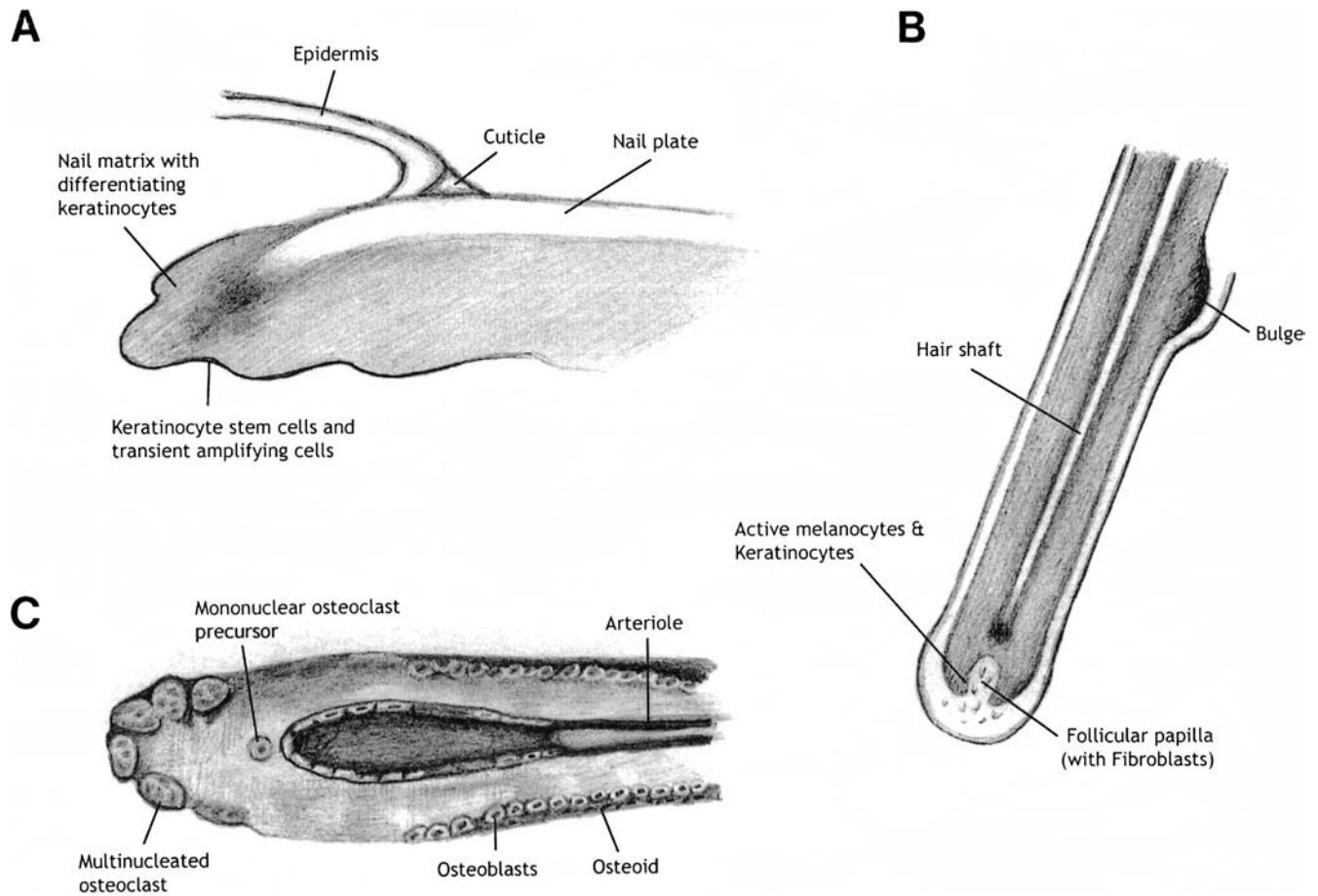


Figure 2. The fingernail system (A), hair (B), and basic multicellular unit (C).

highly replicative cells of skin (11), gastrointestinal tract-stomach-intestine (12), bone marrow (13), testes (14), and generally in all systems affected in the telomerase disorder, Dyskeratosis Congenita.

In some cases, such as Werner Syndrome, the capping loop may be lost before the telomeres become excessively short. As a result, Werner Syndrome cells senesce more quickly than normal cells, but possibly with longer telomeres (15). That scenario is not fully proven, but an emerging concept is that short-telomere problems are probably due to loop (capping) malfunctions (e.g., 16). Nevertheless, short telomeres have been established in certain of the progerias, whereas capping problems have not. For that reason, we will refer to those progerias as having a problem of short telomeres with the understanding that the final problem may be loss of capping.

It is generally believed that with each replication, the cell must transiently untie the capping loop, copy its DNA, replace some of the lost telomere repeats (if active telomerase is present), and retie the loop (reviewed in 17). If lost repeats are not replaced (as happens in Dyskeratosis Congenita), or if the mechanism that closes the displacement loop is dysfunctional, as may happen in Werner Syndrome (16), telomeres progressively shorten and eventually cause the cell to enter senescence or apoptosis. This loss of

replicative ability is the likely cause of fingernail atrophy. The pattern of diseases in Figure 1 supports the idea that nail atrophy is telomere driven. Figure 1 divides the progerias into the six that have short telomeres—Dyskeratosis Congenita (18), HGP (19), Werner Syndrome (15), Ataxia Telangiectasia (20), NBS (21)—and the five that do not. Nail atrophy occurs only in progerias that have short telomeres.

Nail atrophy does not, however, occur in every short-telomere progeria. The two exceptions, however, Ataxia Telangiectasia and NBS, strengthen the evidence for telomere involvement with nails. Unlike those in the other progerias in this category, the telomeres in Ataxia Telangiectasia and NBS do not shorten because of increased DNA replication problems. Instead, in both diseases, cells with DNA double strand breaks fail to halt for repairs at the S-phase checkpoint; this failure is a problem that eventually leads to a fusion-bridge-breakage cycle and short telomeres. Because of the repair problem in these two diseases, chromosomes that acquire normal DNA damage should have short telomeres. But, as we have already explained, there is little DNA damage in the nail system. Thus, in nails, Ataxia Telangiectasia and NBS difficulties in damage repair should not create a problem. With little damage to repair, telomeres in Ataxia Telangiectasia and NBS nail stem cell

keratinocytes should not shorten, and hence, nail atrophy should not, and does not, occur.

The short-telomere group contains one provisional entry—RTS. Two-thirds of RTS cases are caused by mutations in the RECQ4 helicase (22,23); the remaining one-third of cases have an unknown cause. RTS has the nail-alopecia-osteoporosis triad seen in other short-telomere diseases, but its telomeres have not been studied. The symptom pattern of the disease suggests that short telomeres might occur in RTS, especially in bone progenitors where the RECQ4 protein is highly expressed. Against this prediction weighs the fact that life span is shortened in all of the progerias in which short telomeres have been established, but life span is not shortened in RTS. It is possible, then, that RTS either does not have short telomeres or its effect on telomeres is more subtle, as is the case in Werner Syndrome. We are currently testing various aspects of these predictions.

The fifth disease with nail atrophy is CCS, Cronkhite Canada Syndrome. The age of diagnosis (range 31–86, mean 55) of CCS is later than that of most progeroid syndromes, but similar to that of Werner Syndrome, and many of its symptoms indicate accelerated aging. Alopecia, hyperpigmentation, vitiligo, anemia, gastrointestinal carcinoma, cataracts, loss of taste, and malabsorption (50% die of malnutrition) are common. There are more than 100 case reports of CCS in the literature, but few research studies, and the disease remains rare and mysterious.

An interesting implication of the telomere explanation for nail atrophy is that the velocity at which nails grow should be a non-invasive marker of telomere status. That is, the shorter the telomeres, the more senescent cells there should be in the system, and the more slowly the nail should grow. Moreover, nail growth velocity should be a marker that is not sensitive to the confounding effects of most DNA damage and damage repair. In the next section we elaborate on that idea.

#### FINGERNAIL ATROPHY MIRRORS NORMAL AGING

Do the nail growth velocity data tell us anything about normal aging? A literature that predates the discovery of the role of telomeres in replication suggests that it does.

Orentreich and Sharp (24) demonstrated that fingernail growth velocity changes predictably with age. In one cross-sectional study of 257 human subjects, they found that nail growth velocity increased linearly to a peak of 0.83 mm per week at about 25 years old, then decreased linearly thereafter by 0.5% per year. Orentreich and colleagues followed that study with a twelve-year longitudinal study of 27 beagles (25). Nail growth velocity in the dogs increased linearly until age 3, then decreased by 3% per year through age 15. The beagle result is notable for its parallel to the human data. Beagles live about one fifth the life span of humans; correspondingly, the dogs' nail growth velocity rose and declined 5 times faster than the human decline.

When Orentreich looked at a contemporaneous study of rat nail growth velocity (26), he found something unexpected. The velocity of nail growth did not decline in rats over their three-year life span. Why should rat nails act differently from beagle and human nails? The explanation, so elusive in its own time, is less of a puzzle now. Many

species of laboratory rat have very long telomeres (e.g., 27), and telomerase is active in many of their cells. In fact, their telomeres are so long (about four times longer than those of humans) that there is not enough shortening in the rat life span to make a difference in many aging symptoms, including, as Lavelle showed, nail growth. It is difficult to explain why rat nails differ from those of other animals by any means other than the link between nail atrophy and telomere shortening.

Oddly, fingernail growth velocity may be more than a marker of telomere length; it might also be a marker of length of life. About a decade after Orentreich's studies, Williams and colleagues (28) and Short and colleagues (29) reported a relationship between nail growth velocity and aging in pigtailed macaques. The finding inspired Coe and Ershler to measure nail growth a year later in their colony of rhesus monkeys, but that team did not immediately report their result. Thirteen years later, however, while looking at the relationship between natural killer cells and aging, the two researchers recalled the nail data. So much time had passed that 12 of their original monkeys had died and the authors were able to compare their single measurement of nail growth velocity, taken more than a decade earlier, with the number of years beyond the experiment that each animal had survived and with the age of each animal at death (30). Fingernail growth velocity was a surprisingly good predictor, correlating significantly with both years of survival ( $r = .67, p < .02$ ) and age at death ( $r = .57, p < .05$ ). Given the other links that have been established between telomeres and mortality (e.g., 31), the relationship of nail growth rate to survival is interesting.

The conclusion that follows from the forgoing discussion is that fingernail growth may be a noninvasive marker of telomere status. If so, it is a telomere marker that is not sensitive to the confounding effects of DNA damage and damage repair problems. Measuring nail growth velocity could provide information about telomere status in a remarkably short time. For example, Orentreich and colleagues (25) described a method for measuring nail growth velocity to accuracies of 0.1  $\mu\text{m}$  over periods as short as 15 minutes using a split-image range finder adapted to a trinocular microscope and a fixed reference object cemented to the nail fold. Such a non-invasive measure could have many uses. For example, the longitudinal nature of caloric restriction studies makes short-term measures of efficacy difficult to obtain. Measurement of nail growth velocity using modern equivalent of the split-image range finder might produce a non-invasive assessment of treatment effects within a few weeks after the start of a caloric restriction experiment. The simpler but slower procedure of marking the nail and measuring nail growth with calipers might also provide a continuous way to monitor telomere changes.

#### ALOPECIA

Alopecia is the second symptom shared by the short-telomere progerias. Although the genetics of hair loss are growing clearer (reviewed in 32), a link between short telomeres and baldness has not previously been noticed. The existence of such a link is not, however, surprising. Hair is similar to nails in the extent to which it is protected from

damage. Hair keratinocyte stem cells are located in the bulge (Figure 2B) (33) which is the deepest part of the “permanent” hair shaft, and are protected from most UV damage. During anagen (the growth phase of the hair cycle), these stem cells create TACs which migrate far more deeply to the basement membrane that surrounds the invaginated follicular papilla (FP). There the TACs produce cells that rise, terminally differentiate, enucleate, and join the cortex of the growing hair shaft. As with nails, these hair keratinocyte TACs are not immune to replication problems. Therefore, the fact that alopecia is found in all of the progerias in which nail atrophy occurs and in none of the progerias in which it does not occur is unsurprising and confirmatory to the short-telomere explanation.

#### **HAIR DIAMETER IS REDUCED IN AGING AND IN SHORT-TELOMERE PROGERIAS**

We propose that the equivalent to nail growth velocity is not hair growth velocity, but rather, hair shaft diameter. That is because hair growth velocity is simultaneously regulated by two opposing factors: (a) the number of differentiated hair keratinocytes generated by the TACs; and (b) the diameter of the hair.

The role of keratinocyte TACs has already been discussed. The role of hair diameter is a little more complex. Hair diameter is determined by the FP: the larger the FP, the thicker the hair shaft (34). The size of the FP is determined at the start of each anagen hair cycle when specialized fibroblasts migrate to the interior of the FP. The more those fibroblasts replicate during this growth period, the larger the final size of the FP (35), and the wider the subsequent hair shaft. Thus, hair diameter changes with each hair cycle.

With age, there is a decline in both the keratinocyte and fibroblast progenitor populations. The decline in keratinocyte progenitors slows hair growth; the decline in fibroblast progenitors and subsequent reduced hair diameter means that fewer keratinocytes are needed to lengthen the hair, so hair grows faster. Because of the simultaneous contribution of these two opposing factors, hair growth velocity varies unpredictably with age.

Although hair diameter and keratinocyte activity interact with regard to hair growth velocity, hair diameter is independent of the number of keratinocyte TACs; increasing the number of active keratinocytes does not increase the diameter of the hair. Hair diameter is, therefore, a measure of the replicative ability of the FP fibroblast progenitors.

On the basis of our results with nails, we expect that with age, shortening telomeres will lead to fewer active fibroblast progenitors in the FP and, therefore, hair diameter will decrease. Studies of the matter confirm this expectation; both the number of active fibroblast progenitors in the FP (36) and hair diameter (e.g., 37–40) decrease with age. The effect is, in fact, so strong that it consistently appears despite influences of hormones (which also affect hair diameter) and other factors (40).

Our earlier nail result suggests that hair diameter should decrease in the four short-telomere progerias with replication problems. There are no formal studies of hair diameter in any progeria, but the clinical literature often refers to hair in those four progerias as “fine” (41,42). With the

exception of the progeroid form of Ehlers-Danlos Syndrome, in which hair is described as curly and fine, no other progeria is described by that term. (The curliness in Ehlers-Danlos Syndrome suggests that the hair is being flattened as it passes through the follicle.) The single histological study looking at hair in the progerias (an RTS model mouse) (43) found that FPs were significantly reduced in the RTS mice versus controls, thereby confirming the clinical impression that hair in the short-telomere progerias is “fine.” Thus, hair diameter, like nail growth velocity, may reflect telomere shortening both in natural aging and in some progerias.

#### **OSTEOPOROSIS IN SOME PROGERIAS MAY DEPEND ON TELOMERASE**

Osteoporosis is the surprising third member of the short-telomere symptom triad—“surprising” because there is no evidence linking loss of bone mass to telomeres, and no obvious pathway from bone remodeling to hair or nail growth. Again, the symptom occurs only in the short-telomere progerias (with the single exception of a note in emedicine, <http://www.emedicine.com/>, stating that in Cockayne Syndrome “osteoporosis may occur”).

Bone remodeling is carried out by the Basic Multicellular Unit (BMU). At the front of the BMU is a team of large, multinucleated osteoclasts which dissolve existing bone in a sealed compartment on their ruffled undersides. At the back of the BMU is a trailing group of individual osteoblasts, each rebuilding some of the bone lost during the passage of the osteoclasts (Figure 2C). Osteoporosis occurs when osteoclasts resorb more bone than osteoblasts resupply (reviewed in 44). There are many ways for that to happen, but osteoporosis in old age occurs because fewer BMUs are made and because the osteoblasts secrete less osteoid, the collagen-rich bone-rebuilding material. Thus, there are fewer BMUs working to remodel bone, and the passage of each BMU produces a net loss. The result is a slow, steady erosion of bone and loss of bone microarchitecture.

Although osteoporosis in the progerias may be due to shortened telomeres (see, for example, 45 and 46), some researchers believe that telomerase itself is the problem. Telomerase is required for progenitor cells to differentiate into osteoblasts in mice (47) and possibly in humans as well. If so, then the telomerase problems in Dyskeratosis Congenita would explain osteoporosis in that disease. Problems with differentiation are more likely to affect osteoblasts than osteoclasts because the pool of immediate osteoclast progenitors is 2500 times larger than the pool of osteoblast precursors (48). Osteoblasts are, therefore, unlikely to ever be rate limiting (48). Because osteoblasts regulate entry of osteoclasts into the bone-remodeling system, it is reasonable to expect that fewer BMUs will be produced; the same situation is seen in normal aging.

The foregoing scenario is unlikely to be true for Rothmund-Thomson Syndrome, which has no obvious connection to telomerase, or for Werner Syndrome, which has a pattern of osteoporosis very unlike that seen in normal aging, including a 60%+ incidence, severe involvement of the limbs, and symmetric osteosclerosis of the hands (49,50) and again, no obvious link to telomerase. In HGP the picture is more interesting. It is our observation that symptoms in

that disease are almost entirely attributable to the progeny of mesenchymal stem cells in bone marrow (which make adipocytes, myoblasts, chondrocytes, ligament cells, and osteoblasts). It is possible then that HGP is caused by a failure of mesenchymal stem cells to differentiate resulting in fewer osteoblasts, hence fewer BMUs, and osteoporosis. This is an idea that deserves further investigation, especially into (a) the question of telomerase in differentiation of mesenchymal stem cells and (b) the potential role of the mechanical weakening of the inner nuclear wall (51–54) that occurs in HGP.

Although there is no obvious connection between short telomeres and osteoporosis, the grouping of this symptom with nail atrophy and alopecia suggests that there is some link, probably not because of a common pathway, but rather, because of a single process that independently affects the telomeres of progenitor cells in each of the three systems. The same seems to be true of gray hair as well.

#### GRAY HAIR IS ASSOCIATED WITH SHORT-TELOMERE PROGERIAS

Gray hair occurs in all six short-telomere progerias and in no others, so it too may be caused by shortened telomeres. Cross-species studies support this view. For example, *Mus musculus* has long (up to 60 kb) telomerase-active telomeres (55) that shorten only fractionally over the animal's lifetime. Blasco and colleagues (56) asked what would happen if that mouse's telomeres did shorten, and they produced a line of telomerase-null (*Terc*  $-/-$ ) knockout mice to answer the question. By the third generation, mouse telomeres had shrunk by 15 kb and, at middle age, the knockout mice had dramatically more gray hair (and alopecia) than did control mice (57). The knockouts also grayed earlier. Flow-FISH analysis confirmed that both gray hair and alopecia inversely correlated with telomere length. At the sixth generation, differences between experimental and control mice were still growing. Samper and colleagues (58) completed the logic of the original experiment by crossing late generation telomerase-null mice with *Terc*  $+/-$  mice, thereby reintroducing telomerase into the systems of some progeny. Those progeny had normal mouse telomere lengths, and many symptoms of premature aging were *rescued* (including graying; personal communication, M. Blasco, April 21, 2004).

How might short telomeres lead to gray hair? An analysis of the situation calls for a brief digression into the physiology of hair pigmentation and graying. Hair is pigmented by melanocytes located among the keratinocytes on the FPs. Each time the growth part of the hair cycle begins (anagen), a new population of melanocytes migrates to the FP from a stem cell reservoir located in the outer root sheath at about the midpoint of the hair follicle (59). Each melanocyte supplies pigment to between one and five follicular hair keratinocytes (60) throughout the entire growth period (up to 10 years) (59). That pigment, melanin, is manufactured inside the melanocyte within membrane-bounded organelles called melanosomes. The melanin, still in its melanosome, is transferred to keratinocytes via dendritic connections. Once inside the keratinocyte, the package is degraded and the pigment absorbed.

Gray hair can occur when any part of the pigmentation process is disturbed (for example, if melanogenesis fails, or if transfer of melanosomes to keratinocytes does not go smoothly). There are other ways that gray hair can occur, but oddly, the one cause not listed among them is cellular senescence. Senescence does occur in melanocytes, but senescent melanocytes produce more, not less, melanin (61,62). In skin, for example, moles are thought to be senescent melanocyte clones (62,63). Consequently, we cannot draw the otherwise obvious conclusion that short telomeres cause senescent melanocytes, less melanin, and hence, gray hair.

If telomere-driven senescence does not cause gray hair, then what does? And why are Ataxia Telangiectasia and NBS involved with gray hair when they were not involved with the nail atrophy and alopecia symptoms? The answer may lie with another suspected agent of aging, reactive oxygen species (ROS).

The most common cause of gray hair is a reduction in the active melanocyte population (61). Estimates vary, but after age 30 the number of melanocytes that supply pigment to hair drops between 10% and 20% each decade (60,64), thereby providing the basis for the rule that 50% of the population is 50% gray by age 50 (9). A widely held theory (e.g., 9,64) is that this loss of melanocytes is caused by ROS, and indeed melanogenesis produces hydrogen peroxide and reactive quinone intermediates (65,66) which can cause cross-links, single strand breaks, and other types of DNA damage (67). Existing evidence suggests ROS damage to nuclear and mitochondrial DNA leads to mutations in active melanocytes (59).

The link between ROS theory and the short-telomere progerias lies in the fact that ROSs are preferentially attracted to the GGG sequences at the 5' end on the telomere (68). In particular, hydrogen peroxide preferentially cleaves 5'-RGGG-3' (69). It is possible, therefore, that long telomeres protect against ROS damage occurring elsewhere in the DNA. As telomeres shorten, this protection is lost and stress-induced premature senescence, or apoptosis, results. According to this theory, gray hair is based entirely on the shortness of telomeres, not on why they are short. More specifically, persons with Werner Syndrome or one of the other three replication-based progerias have gray hair because their telomeres shorten due to inability to restore lost repeats, whereas those persons with Ataxia Telangiectasia or NBS have short telomeres because of breakage. But persons with any of the six progerias have short telomeres and hence are vulnerable to gray hair.

Hair melanocytes are particularly vulnerable to these problems because of their increased exposure to ROS and their long life spans. This link between ROSs and short telomeres is attractive, and a recent serendipitous finding supports it. Chronic myeloid leukemia is a hematopoietic stem cell malignancy in which the Abelson oncogene (which encodes a tyrosine kinase) transfers from chromosome 9 to the BCR region of chromosome 22. The fused gene encodes a protein thought responsible for the disease. In 1999, a drug aimed at treating the disease, imatinib mesylate, entered phase 2 clinical trials. Imatinib is a selective tyrosine kinase inhibitor that blocks phosphorylation of an ATP binding site

Table 2. Physiological Changes in Hair, Nails, and Osteoporosis and Comparative Aging Versus Short Telomere Progeroid Syndrome Phenotypes (Six Syndromes for Gray Hair, Four for all Others).

Physiological Changes in Aging	Aging Phenotype	Progeroid Syndrome Phenotype
Alopecia; reduced hair diameter: progressively fewer active fibroblast progenitors leads to smaller follicular papilla thus, smaller diameter (fine) hair; a shortened hair growth cycle (anagen) and a longer resting phase make remaining hairs less apparent.	Hair loss is among the early symptoms observed. Loss is most prominent in scalp, but occurs in other types of hair including eyebrows, eyelashes, axillary, and body. Hair diameter peaks at age 30, then declines linearly thereafter.	Hair is normal at birth, but hair loss is among the early symptoms observed. Large scale loss occurs in all types of hair generally beginning with eyebrows and eyelashes. Remaining hair is fine.
Graying: Active melanocyte population diminishes by 10%–20% per decade after age 30. Remaining melanocytes contain fewer and smaller melanosomes. Degradation of defective melanosomes by autophago-lysosomes observed.	Graying begins on the scalp, but occurs later in other locations.	Gray hair, usually first appears when hair loss begins and occurs in all types of hair more or less simultaneously.
Nails: Stuttering changes in nail growth velocity are thought to create beading. Nail thinning or possibly whirls of progenitor cells are thought to cause longitudinal ridges. Presumed reduction in the progenitor population slows growth.	Growth speed peaks at age 25, then linearly decreases by .5% per year. Longitudinal ridges and nail beading ridges and nail beading after age 40. Nails are often thin and may split or crack.	Nail dystrophy followed by dramatically slowed or completely arrested nail growth and atrophy. Nails usually thin (but sometimes thick) and may split or crack.
Bone density: Number of bone remodeling units is reduced, osteoblasts secrete less osteoid so each unit produces a net loss especially in trabecular and cortical bone. Increased fractures in hip, humerus, and pelvis.	Bone mass peaks at age 25, 1%–2% per year loss of cortical and trabecular bone mass at age 45–55, produces deficits after age 70. Hip and vertebral fractures are most common.	WS: 60%–100% lifetime incidence, unusual distribution (limbs), bilateral osteosclerosis of hands. DKC: theoretically similar to aging, no empirical data. RTC: osteoporosis with skeletal defects and extensive remodeling. HG-progeria: Apparent lack of interstitial and appositional and bone development.

of the fused gene product. But imatinib also inhibits the c-Kit tyrosine kinase receptor, which is on a pathway that activates promoter for the tyrosinase pigmentation gene (70). Mutations in the homologous c-kit gene in mice produce a white coat; mutations in c-Kit in humans produce piebaldism (71). It was, therefore, predicted that blocking the c-Kit receptor with imatinib might have an unwelcome side effect—gray hair. The two-year study showed that imatinib was indeed an effective treatment for chronic myeloid leukemia (72) and that it did indeed have a side effect. It repigmented gray hair. The result was published in the *New England Journal of Medicine* as a serendipitous finding and an interesting puzzle (73).

Shortly thereafter, Brummendorf and colleagues (74) independently reported a second peculiar finding; peripheral blood leukocytes in people who had responded to the imatinib had longer telomeres than did those in people who had not responded. It is possible that selective cloning occurred, but the authors provided convincing evidence against that possibility. It looks very much as if imatinib restored telomere length to migrating melanocyte TACs arriving at the start of a new hair cycle, and that that restoration led to repigmentation of gray hair. An interesting, albeit impractical test of this idea would be the administration of imatinib to people with Ataxia Telangiectasia. Immortalization of Ataxia Telangiectasia cells by ectopic expression of hTERT rescues the short-telomere phenotype without affecting the DNA repair/breakage problem (75). It follows, then, that if imatinib restored pigment to gray hair in Ataxia Telangiectasia, the original cause was likely to have been short telomeres, rather than the breakage problem.

#### DO SHORT-TELOMERE PROGEROID SYNDROMES RESEMBLE NORMATIVE AGING?

Table 2 shows that symptoms seen in short-telomere progerias are similar to those seen in normal aging, but there are two important differences: symptoms in the progerias usually are more severe than those in normal aging, and symptoms that appear in a specific order in aging often appear in a different order in the progerias. For example, nail growth slows in normal aging, but it arrests completely, or nearly so, in the short-telomere progerias. Eyebrow thinning follows loss of scalp hair in aging, but precedes loss of scalp hair in the progerias.

These differences between normal aging and progeria are predictable under the hypothesis that telomeres are involved in hair and nail symptoms. In nails, for example, it is reasonable to assume that a slow loss of keratinocyte progenitor cells with age will slow growth velocity. The resultant thinning nails and stuttering growth failure create beads and ridges (see Table 2, column 1). In the short-telomere progerias, where keratinocyte progenitors fail more quickly, beads and ridges are likely to be correspondingly more pronounced, and the slowed growth seen in aging is more likely to turn into complete arrest.

Differences in the order in which symptoms appear in aging and progeria are also predictable. For example, with the quick shortening of telomeres in progerias, the first hairs to thin should be the ones that cycle most rapidly rather than the ones most vulnerable to hair loss. It follows that in normal aging the vulnerable scalp hair should thin before eyebrows and lashes, but in the progerias, the faster cycling eyebrows and lashes should thin before scalp hair.

The situation with osteoporosis is less clear. Only

Dyskeratosis Congenita resembles normal aging in its rate of onset and areas affected (76). Werner Syndrome has a much higher incidence of osteoporosis than is seen in normal aging [estimates range from 60% (49) to almost 100% (50) of all cases], and it includes osteosclerosis (abnormal bone density), a symptom rarely seen in normal aging. Rothmund-Thomson Syndrome has an onset rate more in line with normal aging, but it too includes osteosclerosis and extensive bone remodeling. Osteoporosis in HGP is also different from normal aging, in that the underlying problem is bone development, not bone loss. Thus, although the co-occurrence of osteoporosis in only the short-telomere progerias is interesting, in view of the dissimilarity of the overall picture to normal aging in all but Dyskeratosis Congenita, the relationship between short telomeres and osteoporosis remains conjectural.

Finally, we note that although the short-telomere progerias have very different causes and a wide variety of symptoms, they are very similar in the particular hair loss, graying, and nail symptoms that occur (Table 2). For example, in the four progerias with short telomeres due to replication problems, nails are short and dystrophic with beading and longitudinal ridges, progressing to atrophy. Pictures depicting nail changes in the various progerias (e.g., 76,77) sometimes also show great cracking, splitting, and separation of the nail plate from the nail bed (a consequence of nail atrophy), but no other dystrophies are common. All of these symptoms can be attributed to telomere shortening. Conversely, symptoms that are not attributable to telomeres are not reported. Nail pitting, nail plate softening, spooning, clubbing, claw-like changes, white nails (leukonychia), and the many other types of symptoms that are attributable to problems with keratin, enucleation, nail plate construction, or any processes other than telomere shortening, do not normally occur. The same is true in hair. Thus, although all nail and hair symptoms increase with age, those that we have identified as belonging to the short-telomere progerias are most common, which suggests that the short telomeres are a reasonable model for hair and nail symptoms in aging.

#### **IF THE PROGERIAS ARE SEGMENTAL, IS AGING MODULAR?**

As George Martin (78) observed, the progerias are segmental diseases. Each captures some, but not all, of the symptoms of aging. But when the same group of symptoms appears together in several progerias and nowhere else, it is reasonable to hypothesize that they may have a similar underlying cause. Here we show that short telomeres are potentially such a cause for the nail atrophy, alopecia, and gray hair symptoms. We also show that at least one of those symptoms, nail growth velocity, may be tied to natural aging. Short telomeres are not the sole cause of aging and perhaps not even a major part of it—that is the lesson of Dyskeratosis Congenita, an entirely telomere-driven disease. But our analysis has shown that short telomeres contribute to at least four symptoms and probably to others and, as such, they represent a kind of module of aging. Other modules probably exist. ROSSs, for example, appear to contribute to many symptoms of aging, but they too do not explain all aging symptoms. A possible scenario then, is that

the aging phenotype is caused by a limited number of modules, each contributing its own group of symptoms, some of which are unique, and some of which overlap the symptoms of other modules. The method that we have used here is one way of untangling those interdependencies to determine which symptoms are attributable to one module, that of short telomeres.

Our approach of looking at different properties of progerias in different systems works because each progeria operating within a system of the body can be considered a natural experiment. Other natural experiments may be available. The limbus of the cornea and the pregermative zone in the lens are two populations with unique properties—the lens is especially interesting because it captures changes in a permanent sediment of cells for which developmental history is known. Cataracts are seen in several progerias but were not included in our analysis because the scenario is too complex and the symptom appears in too many diseases to consider fully. Such cross-system analyses may, however, uncover insights into aging that are not readily available from single-system studies.

#### **SCOPE OF LITERATURE REVIEW**

We searched OMIM (Online Mendelian Inheritance in Man; <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>) and emedicine (<http://www.emedicine.com/>) to obtain initial data. OMIM is the database of human genes and genetic disorders developed by the National Center for Biotechnology Information and maintained at The Johns Hopkins University. The site currently contains just over 15,000 entries, but we confined our search to well-defined diseases and syndromes by restricting ourselves to the 4535 entries that contain clinical synopses. emedicine is a large clinical knowledge base established in 1996 for physicians. Its 7000 disease and disorder entries are produced and maintained by 10,000 physician authors. Submissions go through four levels of peer review, and are continuously updated.

We supplemented those searches with numerous medical texts, in part, because diagnosis of fingernail problems is not standardized and many sources had to be consulted to get an understanding of the range and degree of the nail problems for each progeria. Major medical texts (1,79–84) and one research monograph (85) were included. Other texts were consulted on a limited basis. We obtained additional information on systems, symptoms, and diseases from several dozen major review articles and numerous original research publications. We regret that, in many cases, overlapping information and space limitation forced us to choose which of several equally incisive articles to cite.

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Address correspondence to Marc Lewis, PhD, Department of Psychology, The University of Texas at Austin, Austin, Texas, 78703. E-mail: lewis@psy.utexas.edu

## REFERENCES

- Pardo-Castillo V, Pardo O. *Diseases of the Nails*. 3rd Ed. Springfield, IL: Charles C. Thomas; 1960.
- Anderson R. Tissue optics and photoimmunology. In: Parrish J, Kripke M, Morrison W, eds. *Photoimmunology*. New York: Plenum Medical; 1983:61–71.
- Ross M, Kaye G, Pawlina W. *Histology: A Text and Atlas*. 4th Ed. New York: Lippincott Williams & Wilkins; 1995.
- Parker SG, Diffey BL. The transmission of optical radiation through human nails. *Br J Dermatol*. 1983;108:11–16.
- Olovnikov AM. [Principle of marginotomy in template synthesis of polynucleotides]. *Dokl Akad Nauk SSSR*. 1971;201:1496–1499.
- Watson J. Origin of concatameric T4 DNA. *Nat New Biol*. 1972;239:197–201.
- Griffith JD, Comeau L, Rosenfield S, et al. Mammalian telomeres end in a large duplex loop. *Cell*. 1999;97:503–514.
- de Lange T. Protection of mammalian telomeres. *Oncogene*. 2002;21:532–540.
- Keough EV, Walsh RJ. Rate of graying of human hair. *Nature*. 1965;207:877–878.
- Ramirez RD, Wright WE, Shay JW, Taylor RS. Telomerase activity concentrates in the mitotically active segments of human hair follicles. *J Invest Dermatol*. 1997;108:113–117.
- Yasumoto S, Kunimura C, Kikuchi K, et al. Telomerase activity in normal human epithelial cells. *Oncogene*. 1996;13:433–439.
- Bachor C, Bachor OA, Boukamp P. Telomerase is active in normal gastrointestinal mucosa and not up-regulated in precancerous lesions. *J Cancer Res Clin Oncol*. 1999;125:453–460.
- Hiyama K, Hirai Y, Kyoizumi S, et al. Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. *J Immunol*. 1995;155:3711–3715.
- Wright WE, Piatyszek MA, Rainey WE, Byrd W, Shay JW. Telomerase activity in human germline and embryonic tissues and cells. *Dev Genet*. 1996;18:173–179.
- Schulz VP, Zakian VA, Ogburn CE, et al. Accelerated loss of telomeric repeats may not explain accelerated replicative decline of Werner syndrome cells. *Hum Genet*. Jun 1996;97:750–754.
- Machwe A, Xiao L, Orren DK. TRF2 recruits the Werner syndrome (WRN) exonuclease for processing of telomeric DNA. *Oncogene*. 2004;23:149–156.
- Wei C, Price M. Protecting the terminus: t-loops and telomere end-binding proteins. *Cell Mol Life Sci*. 2003;60:2283–2294.
- Mitchell JR, Wood E, Collins K. A telomerase component is defective in the human disease dyskeratosis congenita. *Nature*. 1999;402:551–555.
- Allsopp RC, Vaziri H, Patterson C, et al. Telomere length predicts replicative capacity of human fibroblasts. *Proc Natl Acad Sci U S A*. 1992;89:10114–10118.
- Metcalf JA, Parkhill J, Campbell L, et al. Accelerated telomere shortening in ataxia telangiectasia. *Nat Genet*. 1996;13:350–353.
- Ranganathan V, Heine WF, Ciccone DN, et al. Rescue of a telomere length defect of Nijmegen breakage syndrome cells requires NBS and telomerase catalytic subunit. *Curr Biol*. 2001;11:962–966.
- Kitao S, Shimamoto A, Goto M, Miller RW, Smithson WA, Lindor NM, et al. Mutations in RECQL4 cause a subset of cases of Rothmund-Thomson syndrome. *Nat Genet*. 1999;22:82–84.
- Wang LL, Gannavarapu A, Kozinetz CA, et al. Association between osteosarcoma and deleterious mutations in the RECQL4 gene in Rothmund-Thomson syndrome. *J Natl Cancer Inst*. 2003;95:669–674.
- Orentreich N, Sharp N. Keratin replacement as an aging parameter. *J Soc Cosmet Chem*. 1967;18:537–547.
- Orentreich N, Markofsky J, Vogelmann J. The effect of aging on the rate of linear nail growth. *J Invest Dermatol*. 1979;73:126–130.
- Lavelle C. The effect of age on the rate of nail growth. *J Gerontol*. 1968;23:557–559.
- Makarov VL, Lejnine S, Bedoyan J, Langmore JP. Nucleosomal organization of telomere-specific chromatin in rat. *Cell*. 1993;73:775–787.
- Williams D, Short R, Bowden D. Fingernail growth rate as a biomarker of aging in the pigtailed macaque (*Macaca nemestrina*). *Exp Gerontol*. 1990;25:423–432.
- Short R, Williams DD, Bowden DM. Cross-sectional evaluation of potential biological markers of aging in pigtailed macaques: effects of age, sex, and diet. *J Gerontol*. 1987;42:644–654.
- Coe CL, Ershler WB. Intrinsic and environmental influences on immune senescence in the aged monkey. *Physiol Behav*. 2001;73:379–384.
- Cawthon R, Smith K, O'Brien E, Sivatchenko A, Kerber R. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet*. 2003;361:393–395.
- Novelli G. Genetics of Baldness. In: Reeve E, ed. *Encyclopedia of Genetics*. London: Fitzroy Dearborn Publishers; 2001:511–515.
- Cotsarelis G, Sun TT, Lavker RM. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell*. 1990;61:1329–1337.
- Van Scott EJ, Ekel TM. Geometric relationships between the matrix of the hair bulb and its dermal papilla in normal and alopecic scalp. *J Invest Dermatol*. 1958;31:281–287.
- Tobin DJ, Gunin A, Magerl M, Handijski B, Paus R. Plasticity and cytokinetic dynamics of the hair follicle mesenchyme: implications for hair growth control. *J Invest Dermatol*. 2003;120:895–904.
- Oberste-Lehn H. Effects of aging on the papillary hair follicles and on the eccrine sweat glands. In: Montagna W, ed. *Advances in the Biology of Skin*. Vol 6. Oxford, England: Pergamon Press; 1965:17–34.
- Barman JM, Astore I, Pecoraro V. The normal trichogram of the adult. *J Invest Dermatol*. 1965;44:233–236.
- Birch MP, Messenger JF, Messenger AG. Hair density, hair diameter and the prevalence of female pattern hair loss. *Br J Dermatol*. 2001;144:297–304.
- Courtois M, Loussouarn G, Hourseau C, Grollier JF. Ageing and hair cycles. *Br J Dermatol*. 1995;132:86–93.
- Sagsoz N, Kamaci M, Orbak Z. Body hair scores and total hair diameters in healthy women in the Kirikkale Region of Turkey. *Yonsei Med J*. 2004;45:483–491.
- Pesce K, Rothe MJ. The premature aging syndromes. *Clin Dermatol*. 1996;14:161–170.
- Vennos EM, James WD. Rothmund-Thomson syndrome. *Dermatol Clin*. 1995;13:143–150.
- Hoki Y, Araki R, Fujimori A, et al. Growth retardation and skin abnormalities of the Recql4-deficient mouse. *Hum Mol Genet*. 2003;12:2293–2299.
- Seeman E. Reduced bone formation and increased bone resorption: rational targets for the treatment of osteoporosis. *Osteoporos Int*. 2003;14(Suppl 3):S2–8.
- Yudoh K, Matsuno H, Nakazawa F, Katayama R, Kimura T. Reconstituting telomerase activity using the telomerase catalytic subunit prevents the telomere shortening and replicative senescence in human osteoblasts. *J Bone Miner Res*. 2001;16:1453–1464.
- Yudoh K, Nishioka K. Telomerized presenescent osteoblasts prevent bone mass loss in vivo. *Gene Ther*. 2004;11:909–915.
- Liu L, DiGirolamo CM, Navarro PA, Blasco MA, Keefe DL. Telomerase deficiency impairs differentiation of mesenchymal stem cells. *Exp Cell Res*. 2004;294:1–8.
- Parfitt AM. Osteoclast precursors as leukocytes: importance of the area code. *Bone*. 1998;23:491–494.
- Goto M. Clinical characteristics of Werner Syndrome and other premature aging syndromes: pattern of aging in progeroid syndromes. In: Goto M, Miller RW, eds. *From Premature Gray Hair to Helicase—Werner Syndrome: Implications for Aging and Cancer*. Tokyo: Japan Scientific Societies Press and S. Karger AG; 2001:27–39.
- Masuda T, Akasaka Y, Ito K, Ishikawa Y, Ishii T, Tahara H. Pathology: Werner Syndrome and normal aging. In: Goto M, Miller RW, eds. *From Premature Gray Hair to Helicase—Werner Syndrome: Implications for Aging and Cancer*. Tokyo: Japan Scientific Societies Press and S. Karger AG; 2001:41–50.
- Goldman RD, Shumaker DK, Erdos MR, et al. Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. *Proc Natl Acad Sci U S A*. 2004;101:8963–8968.
- Eriksson M, Brown WT, Gordon LB, et al. Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature*. 2003;423:293–298.

53. Lammerding J, Schulze PC, Takahashi T, et al. Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction. *J Clin Invest*. 2004;113:370–378.
54. De Sandre-Giovannoli A, Bernard R, Cau P, et al. Lamin a truncation in Hutchinson-Gilford progeria. *Science*. 2003;300:2055.
55. Zijlmans JM, Martens UM, Poon SS, et al. Telomeres in the mouse have large inter-chromosomal variations in the number of T2AG3 repeats. *Proc Natl Acad Sci U S A*. 1997;94:7423–7428.
56. Blasco MA, Lee HW, Hande MP, et al. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell*. 1997;91:25–34.
57. Rudolph KL, Chang S, Lee HW, et al. Longevity, stress response, and cancer in aging telomerase-deficient mice. *Cell*. 1999;96:701–712.
58. Samper E, Flores JM, Blasco MA. Restoration of telomerase activity rescues chromosomal instability and premature aging in Terc<sup>-/-</sup> mice with short telomeres. *EMBO Rep*. 2001;2:800–807.
59. Van Neste D, Tobin DJ. Hair cycle and hair pigmentation: dynamic interactions and changes associated with aging. *Micron*. 2004;35:193–200.
60. Tobin DJ, Paus R. Graying: gerontobiology of the hair follicle pigmentary unit. *Exp Gerontol*. 2001;36:29–54.
61. Commo S, Gaillard O, Bernard BA. Human hair greying is linked to a specific depletion of hair follicle melanocytes affecting both the bulb and the outer root sheath. *Br J Dermatol*. 2004;150:435–443.
62. Bennett DC, Medrano EE. Molecular regulation of melanocyte senescence. *Pigment Cell Res*. 2002;15:242–250.
63. Bennett DC. Human melanocyte senescence and melanoma susceptibility genes. *Oncogene*. 2003;22:3063–3069.
64. Yaar M, Gilchrist BA. Ageing and photoageing of keratinocytes and melanocytes. *Clin Exp Dermatol*. 2001;26:583–591.
65. Meyskens FL Jr, McNulty SE, Buckmeier JA, et al. Aberrant redox regulation in human metastatic melanoma cells compared to normal melanocytes. *Free Radic Biol Med*. 2001;31:799–808.
66. Nappi AJ, Vass E. Hydrogen peroxide generation associated with the oxidations of the eumelanin precursors 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid. *Melanoma Res*. 1996;6:341–349.
67. von Zglinicki T, Saretzki G, Docke W, Lotze C. Mild hyperoxia shortens telomeres and inhibits proliferation of fibroblasts: a model for senescence? *Exp Cell Res*. 1995;220:186–193.
68. Oikawa S, Kawanishi S. Site-specific DNA damage at GGG sequence by oxidative stress may accelerate telomere shortening. *FEBS Lett*. 1999;453:365–368.
69. Henle ES, Han Z, Tang N, Rai P, Luo Y, Linn S. Sequence-specific DNA cleavage by Fe<sup>2+</sup>-mediated fenton reactions has possible biological implications. *J Biol Chem* 1999;274:962–971.
70. Buchdunger E, Cioffi CL, Law N, et al. Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. *J Pharmacol Exp Ther*. 2000;295:139–145.
71. Fleischman RA, Saltman DL, Stastny V, Zneimer S. Deletion of the c-kit protooncogene in the human developmental defect piebald trait. *Proc Natl Acad Sci U S A*. 1991;88:10885–10889.
72. Kantarjian H, Sawyers C, Hochhaus A, et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med*. 2002;346:645–652.
73. Etienne G, Cony-Makhoul P, Mahon FX. Imatinib mesylate and gray hair. *N Engl J Med*. 2002;347:446.
74. Brummendorf T, Ersoz I, Hartmann U, et al. Normalization of previously shortened telomere length under treatment with imatinib argues against a preexisting telomere length deficit in normal hematopoietic stem cells from patients with chronic myeloid leukemia. *Ann N Y Acad Sci*. 2003;996:26–38.
75. Wood LD, Halvorsen TL, Dhar S, et al. Characterization of ataxia telangiectasia fibroblasts with extended life-span through telomerase expression. *Oncogene*. 2001;20:278–288.
76. Dokal I, Vulliamy T. Dyskeratosis congenita: its link to telomerase and aplastic anaemia. *Blood Rev*. 2003;17:217–225.
77. Dokal I. Dyskeratosis congenita. A disease of premature ageing. *Lancet*. 2001;358Suppl:S27.
78. Martin GM. Genetic syndromes in man with potential relevance to the pathobiology of aging. *Birth Defects Orig Artic Ser*. 1978;14:5–39.
79. Baran R, Dawber R, de Berker DAR, Haneke E, Tosti A, eds. *Diseases of the Nail and Their Management*. 3rd Ed. London: Blackwell Science Ltd; 2001.
80. Scriver C, Beaudet A, Sly W, Valle D. *The Metabolic & Molecular Bases of Inherited Disease*. 8th Ed. New York: McGraw-Hill; 2001.
81. Rich P, Scher R. *An Atlas of Diseases of the Nail. Illustrated Edition*. New York: CRC Press-Parthenon Publishers; 2003.
82. Yanoff M, Duker J. *Ophthalmology*. 1st Ed. London: Mosby International; 1999.
83. Nordlund J, Boissy R, Hearing V, King R, Ortone J, eds. *The Pigmentary System: Physiology and Pathophysiology*. 1st Ed. New York: Oxford University Press; 1998.
84. Zaias N. *The Nail in Health and Disease*. Norwalk (CT): Appleton & Lange; 1990.
85. Goto M, Miller R, eds. *From Premature Gray Hair to Helicase—Werner Syndrome: Implications for Aging and Cancer*. Tokyo: Japan Scientific Societies Press and S. Karger AG; 2001.

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