Mechanisms of Cardiovascular Disease in Accelerated Aging Syndromes

Brian C. Capell, Francis S. Collins, Elizabeth G. Nabel

Abstract—In the past several years, remarkable progress has been made in the understanding of the mechanisms of premature aging. These rare, genetic conditions offer valuable insights into the normal aging process and the complex biology of cardiovascular disease. Many of these advances have been made in the most dramatic of these disorders, Hutchinson–Gilford progeria syndrome. Although characterized by features of normal aging such as alopecia, skin wrinkling, and osteoporosis, patients with Hutchinson–Gilford progeria syndrome are affected by accelerated, premature atherosclerotic disease that leads to heart attacks and strokes at a mean age of 13 years. In this review, we highlight recent advances in the biology of premature aging uncovered in Hutchinson–Gilford progeria syndrome and other accelerated aging syndromes, advances that provide insight into the mechanisms of cardiovascular diseases ranging from atherosclerosis to arrhythmias. (Circ Res. 2007;101:13-26.)

Key Words: premature aging ■ progeria ■ Werner syndrome ■ genome instability

Premature aging syndromes, such as Hutchinson–Gilford progeria syndrome (HGPS), result in fatal cardiovascular disease.1 Cardiovascular features of HGPS share striking similarities and dissimilarities with atherosclerosis. In this review, we present recent discoveries in this burgeoning scientific field, focus on cardiovascular aspects, and describe how developments may lead not only to new treatments and therapies for these rare conditions but also to new mechanistic insights into the biology of cardiovascular diseases, such as atherosclerosis and arrhythmias.

The Cardiovascular Phenotype of HGPS

Since it was initially described by Hutchinson in 1886, and then described again and named by Gilford in 1904, HGPS has long been a source of fascination and curiosity. HGPS is characterized by mandibular and clavicular hypoplasia, subcutaneous fat loss, and lipodystrophy, as well as features reminiscent of normal aging such as alopecia, skin wrinkling, and osteoporosis. The most devastating aspect of this disease, however, is accelerated, premature cardiovascular disease that leads to fatal myocardial infarction (MI) or stroke by an average age of 13 years (Table).2

Although the number of autopsy reports scattered throughout the literature is limited, the description of the cardiovascular features of HGPS have proven to be quite consistent. Conducting a thorough examination of blood vessels in a 22-year-old woman with clinical features of HGPS, Stehbens...
### Accelerated Aging Syndromes and Related Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Genetic Mutation</th>
<th>Clinical/Cardiovascular Phenotype</th>
<th>Key Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hutchinson–Gilford progeria syndrome</td>
<td>90% of cases due to de novo C-to-T change at codon 608 in exon 11 of ( \text{LMNA} ), activating a cryptic splice donor site</td>
<td>Premature aging including alopecia, loss of subcutaneous fat, and premature atherosclerosis; death in early teens due to myocardial infarction or stroke</td>
<td>DeBusk (2), Eriksson et al (14)</td>
</tr>
<tr>
<td>Restrictive dermopathy</td>
<td>De novo ( \text{LMNA} ) splicing mutations leading to partial or complete loss of exon 11 (2 patients); homozygous or compound heterozygous mutations in ( \text{ZMPSTE24} ) could also be causative</td>
<td>Intrauterine growth retardation, tight and rigid skin erosions, microstomia, pulmonary hypoplasia; early neonatal lethality and thus no cardiovascular phenotype ever reported</td>
<td>Navarro et al (21), Navarro et al (22), Moulson et al (23)</td>
</tr>
<tr>
<td>Familial partial lipodystrophy, Dunnigan type</td>
<td>Autosomal dominant ( \text{LMNA} ) missense mutations cluster in exons 8 and 11, (~75%) of mutations in codon 482 of exon 8</td>
<td>Loss of adipose tissue in trunk and limbs with concomitant accumulation in the neck and face; often includes insulin-resistant diabetes, hypertriglyceridemia, and increased susceptibility to atherosclerosis</td>
<td>Hegele et al (123)</td>
</tr>
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<td>Mandibuloacral dysplasia</td>
<td>Usually autosomal recessive ( \text{LMNA} ) RS27H most common; K542N, A529N, and heterozygous RS27C/R471C (1 case) also reported; 2 cases with compound heterozygous mutations in ( \text{ZMPSTE24} )</td>
<td>Delayed closure of cranial sutures, dental crowding, short stature, lipodystrophy, joint contractures, mandibular and clavicular hypoplasia, acroosteolysis, alopecia, and insulin resistance; no cardiovascular phenotype reported</td>
<td>Novelli et al (28), Agarwal et al (29)</td>
</tr>
<tr>
<td>Werner syndrome</td>
<td>Multiple autosomal recessive mutations in the ( \text{WRN} ) gene</td>
<td>Premature aging with bilateral cataracts, type 2 diabetes mellitus, osteoporosis, short stature, hair graying and alopecia, sclerodermaous skin changes, regional atrophy of subcutaneous fat, deep ulcerations of the Achilles tendon, increased tendency for cancer, premature atherosclerosis, and death usually in the fifth or sixth decade</td>
<td>Yu et al (86), Oshima et al (87)</td>
</tr>
<tr>
<td>Atypical Werner's syndrome</td>
<td>Three reported autosomal dominant ( \text{LMNA} ) mutations: A57P, R133L, and L140R</td>
<td>Similar to Werner syndrome, although diagnosed earlier and more severe course than typical Werner syndrome</td>
<td>Chen et al (84), Adelfalk et al (98)</td>
</tr>
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<td>Emery–Dreifuss muscular dystrophy</td>
<td>Generally all autosomal dominant missense mutations reported in every codon of ( \text{LMNA} ) except 12; 1 recessive case reported; X-linked form attributable to mutations in the gene encoding emerin</td>
<td>Slowly progressive contractures and muscle weakness; wasting of skeletal muscle and cardiomyopathy with conduction disturbances</td>
<td>Bonne et al (109), Brown et al (110)</td>
</tr>
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<td>Dilated cardiomyopathy, type 1A</td>
<td>More than 20 autosomal dominant ( \text{LMNA} ) mutations described, usually missense mutations in exons 1 or 3</td>
<td>Ventricular dilatation, impaired systolic contractility, arrhythmias, conduction defects</td>
<td>Fatkin et al (118)</td>
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<td>Limb-girdle muscular dystrophy</td>
<td>Six autosomal dominant ( \text{LMNA} ) mutations described, 3 of which are missense</td>
<td>Slowly progressive shoulder and pelvic muscle weakness and wasting; later development of contractures and cardiac disturbances</td>
<td>Muchir et al (115), Todorova et al (116)</td>
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et al described a dramatic loss of vascular smooth muscle cells (VSMCs) in the medial layer of the descending aorta, other great vessels, smaller arteries, and arterioles.\(^5\) Intimal thickening was present, although the intima was acellular as well. The medial and intimal VSMCs were replaced by fibrous material extending into the adventitia and thickening of the basement membrane, spontaneous breaks in elastin structures were present, and caseous debris and calcification were observed in the intima. VSMC loss was pronounced at aortic branches and just distal to advanced vascular lesions. Collagenous tissue, especially collagen type IV, was present in a pericellular distribution throughout the media. Additional advanced vascular sclerotic features included extensive hyaline fibrosis and periodic lipid-vascularized VSMCs.\(^3\) Strikingly, inflammation and lipid deposition in the intima, characteristic of atherosclerotic plaque, were not present. Rather, the findings were suggestive of a chronic, fibrotic process driven by a primary loss of VSMCs. Interestingly, the endothelial cell layer appears to be reasonably well preserved structurally in many specimens. Stehbens concluded that medial VSMCs in individuals with HGPS are susceptible to hemodynamic and ischemic stress. Furthermore, the extension of proteoglycans and collagen from the media into the adventitia led Stehbens to postulate that the apparent loss of contractile VSMCs and replacement by a fibrous sheath leads to reduced arterial elasticity in the arterial walls. The extent of arteriosclerotic vascular changes in HGPS extend beyond the great arteries. Vascular abnormalities have also been observed in the liver, kidney, and spleen of HGPS patients,\(^4\) although these findings are not uniformly present.\(^5\) Other reports have substantiated findings of Stehbens et al and further described other vascular, myocardial, and valvular changes. Concentric left ventricular hypertrophy is present in individuals with advanced coronary disease, even in the absence of high blood pressure. Hyaline degeneration, coronary thromboses, subintimal and intussusceptum fibrosis, intracellular lipofuscin pigment,\(^6\) and calcium deposits have been described in coronary, aortic, cerebral, subclavian, and axillary arteries. Calcification and fibrosis have also been observed on the mitral annulus and aortic valve cusps.\(^5,7\) There have not been adequate descriptions of venous abnormalities to discern whether this dramatic loss of VSMCs occurs on the venous side of the circulation as well.

Individuals with HGPS are typically asymptomatic even while these structural changes are evolving within their arterial system. Symptoms suggestive of myocardial and cerebral ischemia, including angina, shortness of breath, and paroxysmal nocturnal dyspnea often occur late in the course, just before fatal MI or stroke.\(^5,8-10\) Likewise, systemic hypertension is often present but not invariably present.\(^11\) Interestingly, hyperlipidemia has not been marked nor consistent; even in individuals placed on low-fat diets, advanced vascular changes developed.\(^2\) Gordon also reported decreased HDL and adiponectin levels as children with HGPS age, whereas total cholesterol, C-reactive protein, triglycerides, and LDL all remained normal in HGPS, the same as in controls.\(^12\)

As discussed further below, HGPS is caused by single base change of a C to a T in position 608 of a gene known as \(\text{LMNA}\). The profound cardiovascular phenotype in HGPS individuals has been reproduced in a mouse model of HGPS, a transgenic line that contains this human mutant G608G \(\text{LMNA}\) gene inserted through a BAC clone.\(^13\) The large arteries in these mice also develop a progressive loss of medial VSMCs, spontaneous breaks in elastin structures, and replacement by collagen and proteoglycans as they age; this process is discernible by 5 months of age in the aorta and carotid arteries. By 12 months, these arteries are essentially void of medial VSMCs with extensive collagen and proteoglycan deposition extending into the adventitia, creating a fibrous sheath around a degenerative media. Strikingly, endothelial cells are completely preserved initially, although as the pathology in the arteries progresses, some loss in the endothelial layer is noticeable by 12 months. Inflammation and lipid deposition are absent; the pathological process is a progressive, fibrotic scarring of the arteries rather than the lipid-driven, chronic inflammatory changes typical of atherosclerosis. Interestingly, these structural changes are also associated with a blunted vasodilator response when the arterial system of these mice is challenged with the vasodilator sodium nitroprusside.\(^13\)

Although inroads have been made into understanding the pathophysiology of cardiovascular disease in HGPS, there are still a number of outstanding, important research questions. We do not understand the terminal arterial events that lead to vessel occlusion, MI, and stroke. Is there focal thrombosis that forms on an injured segment of the coronary or carotid artery? Or, is there progressive, acellular intimal thickening leading to vessel obliteration? Although the endothelium appears to be preserved until later stages of HGPS cardiovascular disease, does it function normally, and could altered function contribute to the pathophysiology? What is the role of proteolysis and the metalloproteinases in the degradation of elastin? Which collagens and proteoglycans are synthesized and by which cells? What leads to the loss of VSMCs, autophagy, apoptosis, necrosis? Is there a failure of VSMC regeneration? Or, does the synthesis of collagen and matrix proteins precede the loss of VSMCs? Does the adventitial vasa vasorum contribute to the vascular pathology? The answers to these questions are pertinent not only to HGPS but to our understanding of vascular biology in more common vascular diseases as well.

**The Molecular Genetics of HGPS**

Despite having been described more than a century ago, HGPS never received extensive attention from the scientific community because of its extreme rarity (1 in 4 million live births). This changed dramatically in 2003 when the genetic mutation behind HGPS was identified as a point mutation in the \(\text{LMNA}\) gene.\(^14,15\) In addition to encoding the A-type laminas that were well studied, essential components of the nuclear lamina, \(\text{LMNA}\) mutations had also been implicated in several other diseases collectively referred to as the lamino-pathies,\(^1\) several of which will be discussed in this review.

Lamins A and C, alternatively spliced products of \(\text{LMNA}\), are 2 of the principal components of the nuclear lamina, a dynamic protein scaffolding network lying just beneath the inner nuclear membrane. The lamina has been shown to play a role in a diverse array of nuclear processes, including...
transcriptional regulation, heterochromatin organization, and nuclear structure. Following translation, the lamin A precursor, prelamin A, undergoes a series of posttranslational modifications, including farnesylation at its C-terminal CAAX motif (CSIM in this case) by the enzyme farnesyltransferase, cleavage of the terminal 3 amino acids (SIM) by Zmpste24, and carboxymethylation by the enzyme isoprenylcysteine carboxymethyltransferase (ICMT). A second and final cleavage by Zmpste24 removes the terminal 15 amino acids and the farnesyl group, allowing the fully mature lamin A to be inserted into the lamina. In contrast, because of its 50-aa internal deletion, progerin lacks the second cleavage site and thus remains permanently farnesylated.

As mentioned above, a single de novo C-to-T mutation at position 1824 of codon 608 of the LMNA gene is responsible for more than 90% of HGPS cases. Although the mutation does not change the encoded amino acid (G608G, i.e., it remains glycine), it activates a cryptic splice donor site, creating an mRNA with a 150 nucleotide internal deletion. This in turn codes for the production of a mutant lamin A protein with a 50 amino acid internal deletion, termed progerin. This internal deletion removes the cleavage site for Zmpste24 in the lamin A processing pathway. Thus progerin remains permanently farnesylated and, as a result, permanently anchored to the nuclear envelope. This disrupts the normal organization of the nuclear lamina and apparently accounts for the characteristic downstream effects of nuclear blebbing, dysregulated gene transcription, and heterochromatin disorganization (Figure 2).

The abnormal splice donor in HGPS is not 100% efficient. In one study of cultured skin fibroblasts, progerin made up...
Figure 2. Potential mechanisms of disease. HGPS and Werner syndrome nuclei. A, It is hypothesized that the permanently farnesylated state of progerin leads to its disruption of the nuclear lamina and all of the nuclear defects observed in HGPS nuclei: blebbed nuclear morphology, altered interactions with other nuclear and cytosolic intermediate filaments, mislocalized nuclear envelope proteins, clustering of nuclear pore complexes, disorganized heterochromatin, epigenetic changes and dramatic dysregulation of gene transcription, increased sensitivity to DNA-damaging agents, and decreased DNA repair capacity. Other potential mechanisms include defective function of the endoplasmic reticulum and decreased stem cell repair and regeneration. B, In Werner syndrome, the nuclear defects are not as well characterized, although nuclear morphology deformation and chromosomal aberrations have been reported. Depending on the precise mutation, decreases in ATPase activity, exonuclease activity, helicase activity, single-strand annealing ability, DNA repair, replication initiation, foci establishment, resolution of stalled replication forks, and telomere repair and maintenance have all been reported. Despite the differing underlying mutations, in the case of both diseases, the ultimate result is similar: cell cycle abnormalities leading to premature cellular senescence, genome instability, premature atherosclerosis, and accelerated aging.
84.5% of the transcript from the mutant allele. Progerin appears to accumulate with successive cell passage number, leading to progressive nuclear envelope deformations and invaginations, the cellular hallmark of HGPS. These nuclear alterations apparently affect cell cycle progression, cell migration, and elicit premature senescence. That there might be a dosage-dependent effect with progerin is suggested by the case of a 45-year-old Japanese patient who had a different mutation (T623S) and a milder phenotype. This mutation activated a different cryptic splice site, and did so less efficiently than what is seen in typical HGPS, producing only 20% of a mutant protein with a 35-aa internal deletion.

The hypothesis that permanent farnesylation of the lamin A gene product results in a toxic protein is further supported by the identification of several patients with an even more severe HGPS-like disease known as restrictive dermopathy, characterized by intrauterine growth retardation, loss of subcutaneous fat, tight and rigid skin, bone mineralization defects, osteolytic lesions, and death usually during the first few weeks of life (Table). Many of those patients have been found to have homozygous mutations in the ZMPSTE24 gene with effectively no Zmpste24 activity, and are therefore synthesizing prelamin A protein that cannot be released from the carboxyl tail containing the farnesyl group.

A remarkable example of gene–gene interaction was the description of a patient who, despite being homozygous for null mutations in the ZMPSTE24 gene, was observed to have a milder HGPS-like phenotype, on the basis of also carrying a heterozygous LMNA mutation that resulted in a C-terminal elongation of the lamin A CAAX motif by 7 amino acids, blocking farnesylation. Thus this individual only produced half the amount of farnesylated prelamin A as would normally be expected for a ZMPSTE24 homozygote with restrictive dermopathy; the milder phenotype again supports the hypothesis that there is a dosage effect of the farnesylated protein.

Further evidence for this dosage hypothesis comes from experiments with mouse models. Mice lacking Zmpste24 display several progeroid features such as growth retardation, progressive hair loss, and craniofacial and other bone abnormalities, concomitant with a dramatic buildup of prelamin A levels. Breeding Zmpste24-null mice with mice carrying only one normal Lmna allele or one allele that produces only lamin C, which effectively reduces the levels of this farnesyl-prenylated protein by 50%, remarkably leads to a completely normal lamin C, which effectively reduces the levels of this farnesyl-prenylated protein by 50%, remarkably leads to a completely normal phenotype.

The dose effect of farnesylated protein, outlined in several examples described above, led to the proposal that reduction of this toxic protein, even if incomplete, might improve cellular function. In support of this, experiments using RNA interference and antisense morpholinos to prevent the use of the aberrant HGPS cryptic splice site, have been shown to effectively restore a normal cellular phenotype. Of greatest clinical significance, the availability of oral farnesyltransferase inhibitors, some of which are already in phase III trials for cancer, has opened a new window into potential therapy. Gratifyingly, in vitro and in vivo application of farnesyltransferase inhibitors has been shown to be effective at restoring normal nuclear shape in HGPS fibroblasts, and even in ameliorating disease symptoms in mouse models of HGPS. The sum of this work has culminated in preparation for a clinical trial in HGPS patients with farnesyltransferase inhibitors to begin by the end of 2007.

Mechanical Disturbances in HGPS

The idea of the nuclear lamina as a simple scaffolding network led many to hypothesize that it was the inability to withstand mechanical stress that led to the dramatic phenotypes seen in the laminopathies. Although there is a great deal of evidence to support these assumptions, particularly in tissues exposed to mechanical stresses such as cardiac and skeletal muscle, numerous lines of evidence, including the variability of tissue-specific phenotypes, suggest that this is not the complete story.

Some of the initial evidence for these proposed mechanical defects came from Lmna-null mice. Embryonic fibroblasts from these mice showed significantly decreased mechanical stiffness resulting in a lower bursting force. Furthermore, disturbed interactions of actin, vimentin, and tubulin-based filaments were documented, suggesting not only increased nuclear fragility but also a more general weakness in the physical interaction between the nucleus and the cytoskeleton. Cardiomyocytes from these mice exhibit contractile dysfunction and severe morphological abnormalities that are worse in the left ventricle than the right. There are also marked structural alterations and fragmented heterochromatin that are worse in the ventricles than in the atria, suggesting that increased mechanical strain can contribute to the nuclear structural defects. These findings are supported by data suggesting that lamins A and C contribute to the mechanical stiffness of nuclei, whereas others, such as lamin B1, contribute only nuclear integrity and not mechanical stiffness.

Further work in this mouse model has demonstrated that this increased nuclear fragility is also associated with transcriptional changes in the nuclear factor kB pathway. Defective mechanotransduction has been further suggested by the observations that the binding of desmin to nuclear pore complexes appears to mediate the binding of the sarcomere to chromatin via lamins. Thus, when cardiomyocytes are stretched, changes in the spatial arrangement of both the desmin–lamin intermediate filament network and the nuclear envelope-associated chromatin organization can be observed. These stretch-induced changes in the chromatin mediated by the lamin–intermediate filament connection could be a mechanism to activate hypertrophy-associated genes.
heteropolymer in which the normal segregation of lamin A and B1 into homopolymers is lost, and potentially the functions of each specific lamin are impaired.45 Further work has demonstrated that lamins A and C become trapped at the nuclear periphery in HGPS cells and have a reduced ability to rearrange under mechanical stress.44 This could certainly affect underlying gene expression and might also impair mechanotransduction in endothelial and vascular smooth muscle cells, leading to the vascular defects seen in HGPS and HGPS mice.44 The disrupted lamina, in addition to its inability to maintain its normal segregation and mobility, may also cause the mislocalization of numerous nuclear envelope proteins and may also disturb the normal function of the endoplasmic reticulum. As the endoplasmic reticulum is the site of cholesterol and fatty acid synthesis, an altered lamina might possibly disrupt lipidogenesis resulting in lipodystrophy, or in a similar fashion, disrupt Ca\(^{2+}\) release from the sarcoplasmic reticulum (Figure 2).46

Chromatin and Altered Transcriptional Regulation in HGPS

Given that the nuclear lamina directly binds heterochromatin,47,48 as well as a steadily increasing number of transcription factors,1 and that lamin A mutants have been shown to disrupt both DNA replication and RNA polymerase II–dependent transcription,49,50 it is not surprising that severe transcriptional dysregulation is seen in HGPS. The largest quantitative gene expression difference between HGPS patients and controls has been in MEOX2/GAX, a homeobox transcription factor implicated as a negative regulator of mesodermal tissue proliferation. MEOX2/GAX, which was greatly upregulated in HGPS, is also a known negative regulator of VSMC proliferation. Likewise, many genes implicated in atherosclerosis were identified as severely dysregulated in HGPS, as were several other genes involved in inflammatory processes.51 As the lamina is known to bind numerous transcription factors such as GCL (germ cell–less), MAN1, MOK2, sterol response element binding protein-1 (SREBP1) (involved in cholesterol and fatty acid biosynthesis), cFos, and pRb, these transcriptional abnormalities are not particularly surprising.52 For example, farnesylated prelamin A binds SREBP1 with a much higher affinity than mature lamin A. Thus progerin is predicted to reduce the amount of available SREBP1 and consequently to disrupt adipocyte differentiation. One possible mechanism for this regulatory role involves the binding of the lamina and C tail to nuclear actin, which is involved in complexes modifying and remodeling chromatin, thus potentially disrupting chromatin arrangement and gene regulation.52–54 In a similar manner, progerin may cause other critical nuclear envelope proteins and components of the lamina to inappropriately form complexes that disrupt proper transcription factor function (Figure 2). For example, in Zmpste24-null mice that accumulate this form of permanently farnesylated prelamin A, there is an upregulation of the downstream targets of p53, perhaps contributing to the accelerated aging phenotype seen in the mice.55

More recent evidence has detailed some additional potential causes of these transcriptional changes. HGPS cells display focal or total loss of heterochromatin and exhibit progressive alterations in epigenetic control of both facultative and constitutive heterochromatin (Figure 2).56 For example, before any signs of nuclear deformation, low levels of progerin have been shown to disrupt trimethylation of H3K27me3, a marker of facultative chromatin in the inactive X chromosome.56 Columbaro et al hypothesized that an agent affecting chromatin compaction, such as trichostatin A, which inhibits histone deacetylase activity and causes chromatin activation, could release progerin from possible chromatin binding. When used in conjunction with a farnesyltransferase inhibitor, trichostatin A was able to rescue normal heterochromatin organization while simultaneously reducing progerin levels.57 In a similar vein, a histone demethylase, JHDM3A, has been shown to reverse the reduced levels of H3K9me3 that are seen in HGPS.58

Long before the genetic cause of HGPS was elucidated, numerous reports documented extensive alterations in expression levels among several proteins in cultured skin fibroblasts. Among those with increased expression compared with normal cells are glycoprotein gp200, fibronectin, α1, and α2 type I procollagen and type IV collagen, elastin, ankyrin G, and hyaluronic acid.59–64 These changes may potentially contribute to the HGPS phenotype. For example, ankyrins are believed to link integral membrane proteins to the underlying actin-spectrin cytoskeleton.64 Hyaluronic acid, which maintains the integrity of skeletal, vascular, cutaneous, and muscular systems, accumulates in early and developing atherosclerotic lesions and has been shown to regulate proliferation and migration of VSMCs.65 Furthermore, overexpression of hyaluronic acid in a mouse model of atherosclerosis has been shown to cause an increase in lesion severity.66 Likewise, LDL receptor-null mice, lacking CD44, a major hyaluronic acid receptor, have a 50% to 70% reduction in atherosclerotic lesion size.67 Misexpression of hyaluronan synthases and the small proteoglycan decorin, both found in the artery wall and misexpressed in HGPS, have been shown to participate in atherosclerotic lesion development in mice.66,68 Other studies have shown that platelet-derived growth factor-A is elevated in fibroblasts, nucleotide pyrophosphate is decreased, and aggrecan is greatly increased and produced as a proteoglycan. Increased platelet-derived growth factor leads to decreased levels of the transcription factor cFos, which then leads to increased aggrecan and other proteoglycans. In atherosclerosis, proteoglycans are thought to contribute to the trapping of lipid in the intima, via the binding of lipoproteins to glycosaminoglycan chains. For example, the proteoglycan versican forms large complexes with the extracellular glycosaminoglycan hyaluronan and is believed to contribute to luminal narrowing.69

The first appearance of the lamin A and C proteins during differentiation suggests that they may play some role in initiating the chromatin organization necessary to determine cell fates and maintain a differentiated state, roles that could be seriously altered by LMNA mutants.1,70 The normal appearance of infants with HGPS at birth presumably reflects this pattern of expression, because cells in embryonic and fetal life largely lack lamins A and C. As the child grows older, the consequences of the HGPS mutation appear most
prominently in cell populations capable of differentiating and undergoing multiple rounds of cell division under specific stimuli, such as mesenchymal stem cells.71

**Decreased DNA Repair, Genome Instability, and Loss of Cell Cycle Control in HGPS**

The hypothesis that accumulated DNA damage may be responsible for the cardiovascular disease in HGPS was first suggested by Baker in 1981, who surmised that decreased DNA repair capacity might lead to reduced tissue regeneration, predisposing patients to the development of arteriosclerosis at an early age.7 This theory received later support when HGPS fibroblasts were found to exhibit an age-dependent reduced growth rate and lifespan in culture, as well as a delayed and hypersensitive response to heat shock.72 Liu et al demonstrated that HGPS and Zmpste24-knockout fibroblasts exhibit defective DNA repair and genome instability, as evidenced by increased chromosomal aberrations, increased sensitivity to DNA damaging agents, and increased aneuploidy. In addition, the recruitment of p53 binding protein and Rad51, both involved in DNA double-strand break repair, to sites of DNA lesions is impaired in Zmpste24-knockout and HGPS patient fibroblasts, resulting in delayed checkpoint responses and defective DNA repair.73 Correct functioning of double-stranded DNA repair is dependent on correct telomere tethering to the nuclear periphery. However, if peripheral heterochromatin is disturbed, then this could lead to inadequate DNA repair. Thus, an intact lamina may be required for the proper assembly of DNA repair machinery by serving as a scaffold.

Others have shown that the accumulation of farnesylated prelamin A leads to persistent activation of DNA damage checkpoints because of a compromise in genomic integrity.74 Progerin mislocalizes the nuclear envelope protein emerin and impairs the ability to form DNA repair foci.75 Progerin may be leading to genomic instability as evidenced by abnormal mitotic figure,14 mitotic instability,76 and other major mitotic defects. Permanently farnesylated and carboxymethylated progerin leads to its aggregate formation and abnormal association with membranes during mitosis.15,77 In addition to causing defective chromosomal segregation and binucleation,77 progerin delays the onset of cytokinesis and the targeting of nuclear envelope and lamina components into daughter cell nuclei in early G1. It also appears to be responsible for defects in the pRb-mediated transition into the S phase.17 A disorganized lamina is less able to protect the genome from the physical trauma encountered during the upheavals of mitosis and nuclear reassembly. Thus, with each round of cell division, cells are more likely to enter into senescence.77 These potential connections to premature senescence received further support recently when it was shown that p16INK4A inhibits cell cycle progression and induces senescence. Its expression rises with age in many tissues and is a marker of hematopoietic stem cell aging in the bone marrow.78 pRb is required for cell cycle arrest by p16INK4A, and given the role of the lamina in binding pRb and preventing its destruction by proteasomes,79 loss of normal pRb function attributable to a disrupted lamina might lead to unchecked p16INK4A levels, thus suppressing stem and progenitor cells needed to restore function after DNA damage.80

**The Cardiovascular Phenotype, Molecular Genetics, and Biology of Werner Syndrome**

Werner syndrome, often referred to as “adult progeria,” is characterized clinically by premature graying, loss of hair, increased cancer susceptibility, type 2 diabetes mellitus, ocular cataracts, scleroderma-like skin changes, regional atrophy of subcutaneous fat, osteoporosis, and atherosclerosis.81,82 A hypercoagulable state has also been proposed as an additional risk factor for vascular disease in Werner syndrome, including elevations in thrombin, antithrombin III complex, D-dimer, tissue plasminogen activator, and plasminogen activator inhibitor-1 and decreases in thrombomodulin.83 Death usually occurs at a median age of 54.3 years as a result of MI or, less often, stroke. The average age of diagnosis is 46.4 years for classic Werner syndrome and 23 years for atypical Werner syndrome, which is more severe clinically and caused by mutations in *LMNA*.84,85

Classic Werner syndrome is caused by autosomal recessive mutations in the *WRN* gene that encodes WRN, a 180-kDa tumor suppressor that has ATPase, helicase, exonuclease, and single-stranded DNA annealing activities.86,87 It is also involved in DNA repair, replication initiation, replication foci establishment, resolution of stalled replication forks, and recombination.88,89 WRN is 1 of 5 members of the RecQ helicase family of proteins; others, which include BLM and RECQ4, are involved in other disorders of genomic instability and increased cancer susceptibility: Bloom syndrome and Rothmund-Thomson syndrome, respectively. WRN, however, is the only member that possesses both 3’→5’ exonuclease and helicase activity.90 Even though the phenotype does not begin to appear until puberty, WRN is expressed at all ages, even during gestation, with no significant change in the levels between fetal and adult tissues.91 WRN is located primarily in the nucleus, and it relocates to form nucleoplasmic foci at sites of DNA damage.92 Most WRN mutations produce truncated mutant proteins lacking the nuclear localization signal, thus preventing WRN from reaching the nucleus and carrying out its essential functions (Figure 2).85 Proteins interacting with WRN as it carries out its exonuclease and helicase activities include replication protein A (RPA) and others with various roles in DNA replication, recombination, and repair.90

Other roles for WRN include its association with telomeres, where it is recruited by a telomeric repeat binding factor that is essential for correct telomere maintenance and repair,93 and it has also been shown to be involved in the synthesis of the lagging strand of telomeres.94 Given these diverse and extensive functions, it is clear how mutations in WRN may lead to the limited replicative capacity (a 6-fold increase in the rate of accumulation of senescent fibroblasts),95 increased genome instability, and increased nuclear deformations seen in Werner syndrome (Figure 2).96–98 Further data have implicated WRN in human cancer as WRN
function is interrupted in human cancer cells by transcriptional silencing associated with CpG island–promoter hypermethylation. This epigenetic inactivation of WRN leads to the loss of WRN-associated exonuclease activity, increased chromosomal instability, and apoptosis.99 p53 also binds and inhibits the WRN exonuclease and helicase activities, while signaling downstream transcriptional activity that regulates the initiation of apoptosis after DNA damage.100 As mentioned above, this upregulation of p53-dependent genes has been implicated as one of the mechanisms through which Zmpste24 knockout mice develop symptoms of premature aging, including cardiac fibrosis.55,101

A mouse model with a mutation in the helicase domain of WRN provides an example of how these disrupted WRN functions can lead to the phenotype, and in particular, the cardiovascular disease, seen in Werner syndrome. Before the onset of cardiomyopathy, these mice develop severe cardiac interstitial fibrosis, tumors, and abnormal increases in visceral fat deposition and fasting blood triglyceride and cholesterol levels, followed by insulin resistance, high blood glucose, and aortic stenosis.102 The adult mice also show higher levels of serum and cardiac tissue reactive oxygen species followed in time by an increase in cardiac oxidative DNA damage, all before the onset of cardiac fibrosis,102 which is not surprising, given the multiple lines of evidence for excess oxidative stress damage in vivo in Werner syndrome.103 Mouse models of Werner syndrome also support the role of telomere length in modulating the Werner syndrome phenotype as mice deficient in WRN develop normally without premature aging, whereas double-knockout mice that lack WRN and telomerase exhibit aging phenotypes in an additive manner.104–106 Furthermore, recent evidence suggests that it is the replication-associated loss of telomere length that is responsible for the chromosomal aberrations seen in Werner syndrome.107 Consistent with these findings, pharmacological inhibition of the mitogen-activated protein kinase p38 pathway, which has been implicated in senescence induced by oxidative stress and telomere shortening, significantly increased the lifespan and growth rate of Werner syndrome cells.108

**Other Related Laminopathies**

Although not considered to be true accelerated-aging or progeroid syndromes, there are several other conditions that, because of their similarity to HGPS in terms of the mutated gene involved (LMNA) and resulting cardiovascular disease, deserve mention in any consideration of mechanistic insights. It has been estimated that 2.5% to 5% of patients with congestive heart failure have LMNA mutations, indicating the significance of the problem. The cardiac disease in these patients generally starts before the fourth decade with conduction system defects or atrial arrhythmias and gradually worsens until a pacemaker is required. Approximately 50% of patients die suddenly despite pacemaker therapy, and when they survive, overt heart failure appears 15 to 20 years later.109

The first identified laminopathy was Emery–Dreifuss muscular dystrophy (EDMD),110 which, in addition to its skeletal muscle pathology, is characterized by cardiomyopathy and cardiac conduction defects (Table).111 Cardiac MRI has demonstrated that EDMD patients have abnormal cardiac function before showing any symptoms or any fibrosis.112 A mouse model with one of the common EDMD LMNA mutations (H222P) develops locomotory difficulties and develops cardiac chamber dilation and hypokinesia with conduction defects.113

Two other highly overlapping conditions caused by LMNA mutations that are similar to EDMD and prone to sudden death from cardiac arrhythmias114 are limb girdle muscular dystrophy type 1B (LGMD-1B)115,116 and dilated cardiomyopathy type 1A (DCM-1A) (Table). Although families carrying LMNA mutations tend to present quite similarly, the overlapping nature of the laminopathies and the inability to predict phenotypes based on the location or type of mutations is clear when considering the case of 3 members of the same family with the same LMNA mutation yet with different clinical phenotypes. One presented with symptoms of EDMD, another with LGMD-1B, and a third with DCM-1A, further emphasizing the influence of genetic background and modifier genes on these conditions.117 DCM-1A is characterized by 4-chamber dilation, cardiomyocyte hypertrophy, and fibrosis without inflammation.118,119 Pathologically, in DCM-1A, there is obvious fibrofatty degeneration of the atrioventricular node, remarkably deformed nuclei, and substantial glycogen deposits in the subsarcolemma,120 as well as altered desmin staining in heart biopsies from DCM-1A patients.121 Many patients show nuclear membrane damage such as focal disruptions, blebs, and nuclear pore clustering, enlarged and bizarrely shaped nuclei, irregularly distributed chromatin, and lamellar pseudoinclusions containing cytoplasmic organelles.41 A mouse model with the common DCM-1A LMNA N195K mutation results in cardiac conduction defects and death, along with misexpression of the transcription factor Hif1b/Sp4 (involved in the development of the cardiac conduction system) and the gap junction proteins, connexin 40 and connexin 43, responsible for relaying the conductive impulses of the myocardium. Desmin staining reveals loss of organization at sarcomeres and intercalated disks.122 In humans, a particular LMNA DCM-1A mutation disrupted the normal nucleoplasmic distribution of small ubiquitin-like modifier 1, which is a major regulatory protein involved in chromatin organization and gene expression,41 further emphasizing the similar observations made in these various conditions that explain the mechanisms leading to the cardiovascular phenotypes.

Another laminopathy, Dunnigan’s familial partial lipodystrophy, has been described as a monogenic form of the metabolic syndrome, as it recapitulates the key clinical and biochemical attributes of the disease and evolves slowly. Patients with familial partial lipodystrophy exhibit atherosclerosis; insulin resistance that progresses to type II diabetes; elevated plasma concentrations of free fatty acids, insulin, C-peptide, triglycerides, and C-reactive protein; depressed concentrations of HDL cholesterol, leptin, and adiponectin; and hypertension (Table).123 Female patients, especially, exhibit early coronary heart disease.124 Similar to HGPS, familial partial lipodystrophy displays numerous chromatin abnormalities and well as an accumulation of prelamin A.125

**Table**

<table>
<thead>
<tr>
<th>Condition</th>
<th>LMNA Mutations</th>
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<tr>
<td>Emery-Dreifuss muscular dystrophy</td>
<td>H222P</td>
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<tr>
<td>Limb girdle muscular dystrophy type 1B</td>
<td>N195K</td>
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<td>Dilated cardiomyopathy type 1A</td>
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1 Capell et al Accelerated Aging Syndromes and CVD 21

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Conclusions and Future Directions

Although both the underlying mutations as well as the resulting phenotypes of these syndromes might be variable, the basic root mechanisms that connect the genetic mutations and clinical phenotypes are similar. Increased DNA damage, whether caused by increased sensitivity or decreased repair, leads to genome instability and cell cycle abnormalities that cause cellular senescence. In addition to HGPS and Werner syndrome, several other progeroid syndromes are characterized by aberrant DNA repair proteins, but they do not manifest cardiovascular disease. These include Bloom syndrome, Rothmund–Thomson syndrome, Cockayne syndrome, xeroderma pigmentosum, trichothiodystrophy, and a very recently identified progeroid syndrome caused by mutations in the XPF-ERCC1 endonuclease that is required for repair of helix-distorting DNA lesions and cyotoxic DNA interstrand crosslinks.126 For example, mice with a mutation in XPD, a DNA helicase that functions in both repair and transcription and is mutated in trichothiodystrophy have many symptoms of premature aging. This unrepaird DNA damage can lead to compromised transcription, functional inactivation of critical genes, and enhanced apoptosis.127

Numerous abnormalities present in HGPS are also common occurrences in cells from aged individuals. These include nuclear blebbing, epigenetic changes, and increased levels of DNA damage. Reduced trimethylation of H3K9me3 has been observed in both HGPS and elderly patients.31 Even more surprising, however, was the observation that all individuals produced low levels of the mutant progerin product, which produces such dramatic disease when overexpressed, to normal aging or cardiovascular disease requires definition. Similarly, DNA damage, as measured by γ-H2AX foci, accumulates in senescing human cells and aging mice. The foci colocalize with double-strand break repair factors but not significantly with telomeres. These foci remain even after repair, suggesting that they may represent DNA lesions with irreparable double-strand breaks.128 In addition to H2AX, other in vitro signs of DNA damage, such as p53 binding protein, telomere dysfunction, and upregulation of p16INK4A, are seen in vivo in nonhuman primates as well and can account for >15% of the cell population in aged animals.129

Increased DNA damage and loss of cell cycle and transcriptional control, which likely contribute to the premature cardiovascular disease in HGPS and Werner syndrome, may also regulate the development of common atherosclerosis. VSMCs from fibrous atherosclerotic caps of unaffected individuals express senescence-associated β-galactosidase and the cyclin-dependent kinase inhibitors p16 and p21.130 Cells from these stable plaques also have shorter telomeres than normal vessels130; shorter telomeres have been associated with increasing severity of atherosclerosis.131 Like HGPS and Werner syndrome cells, advanced atherosclerotic plaques display premature senescence and reduced proliferation. Emerging data suggest that VSMC senescence is mediated by changes in cyclins D and E, p16, p21, and pRb and that plaque VSMCs exhibit oxidative DNA damage, leading some to conclude that human atherosclerosis is characterized by senescence of VSMCs, accelerated oxidative stress-induced DNA damage, inhibition of telomerase, and marked telomere shortening.130 Consistent with this role for telomeres in atherosclerosis, short telomeres have been observed in senescent endothelial cells and VSMCs from human atherosclerotic plaque,132 myocardial tissue from patients with end-stage heart failure and cardiac hypertrophy,133 as well as in white blood cells from patients with coronary artery disease,131 premature MI,134 hypertension,135–137 and diabetes mellitus.137–139 Mice lacking the telomerase RNA component telomerase reverse transcriptase have very short telomeres, develop hypertension, ventricular dilation, thinning of the myocardium, cardiac dysfunction, and sudden death.140–145 In contrast, heart damage induced by mechanical injury and ischemia is reduced in transgenic mice with cardiac-specific expression of telomerase reverse transcriptase. Furthermore, accelerated telomere shortening might be expected from the increased cellular turnover associated with inflammation in atherosclerosis and oxidative stress in hypertension.133

Although there is molecular and cellular evidence that the mechanisms leading to accelerated aging syndromes might reflect the pathophysiology of more common diseases, genetic studies to date have demonstrated mixed results. For instance, although a polymorphic WRN variant was reported to be associated with an increased risk for MI146 and a cysteine to arginine polymorphism was reported to be protective against MI, these associations were later found to be false positives, and the genetic variants did not change the helicase or exonuclease activities of the protein.146 Furthermore, some have reported that WRN variants do not influence MI, myocardial ischemia, intermittent claudication, arterial surgery, stroke, cognitive function, or mortality risk in the general population.147

Considering these findings, it is clear that a great deal of work remains to decipher the precise mechanisms of cardiovascular disease in HGPS, Werner syndrome, and the other laminopathies. We do not know whether the mechanisms that lead to cardiovascular disease in these rare genetic disorders also contribute to the more common forms of atherosclerosis and arrhythmias seen in the general population. Although exciting and important research remains to be conducted, enormous progress has been made in our understanding of the complex biology that underlies the laminopathies in recent years. The knowledge gained has led to substantial insights into scientific fields ranging from transcriptional regulation and chromatin organization to DNA repair and genome stability and, importantly, has brought patients with these rare conditions closer to potential treatments. Likewise, these discoveries might one day perhaps provide pertinent lessons and treatment strategies for common cardiovascular conditions, as well as aging itself.

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