Aging in the Cardiovascular System: Lessons from Hutchinson-Gilford progeria syndrome

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Abbreviations

53BP1 - 53 binding protein-1

BAC - bacterial artificial chromosome

Bmp2/BMP2 - bone morphogenetic protein 2

CVD - cardiovascular disease

DNA-PK - DNA-dependent protein kinase

DNA-PKcs - DNA-dependent protein kinase catalytic subunit

EC(s) - endothelial cell(s)

ECM - extracellular matrix

ePPi - extracellular inorganic pyrophosphate

FTI(s) - farnesyl transferase inhibitor(s)

HF – heart failure

HGPS - Hutchinson-Gilford progeria syndrome

ICMT - isoprenylcysteine carboxyl methyltransferase

iPSC(s) - induced pluripotent stem cell(s)

LV – left ventricle

LVH - left ventricular hypertrophy

mTOR - mammalian target of rapamycin

NHEJ - non-homologous end joining

PWV - pulse wave velocity

Runx2 - Run-related transcription factor-2

SMC(s) - smooth muscle cell(s)

VC – Vascular calcification

VSMC(s) - vascular smooth muscle cell(s)

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Abstract

Aging, the main risk factor for cardiovascular disease (CVD), is becoming progressively more prevalent in our societies. A better understanding of how aging promotes CVD is therefore urgently needed in order to develop new strategies to reduce disease burden. Atherosclerosis and heart failure contribute significantly to age-associated CVD-related morbimortality. CVD and aging are both accelerated in patients suffering Hutchinson-Gilford progeria syndrome (HGPS), a rare genetic disorder caused by the prelamin A mutant progerin. Progerin causes extensive atherosclerosis and cardiac electrophysiological alterations that invariably lead to premature aging and death. This review summarizes the main structural and functional alterations to the cardiovascular system during physiological and premature aging and discuss the mechanisms underlying exaggerated CVD and aging induced by prelamin A and progerin. Since both proteins are expressed in normally aging non-HGPS individuals, and most hallmarks of normal aging occur in progeria, research into HGPS can identify mechanisms underlying physiological aging.

1. Learning about physiological cardiovascular aging from Hutchinson-Gilford progeria syndrome (HGPS)

Cardiovascular disease (CVD) is strongly associated with aging and is the leading cause of morbimortality worldwide (1, 2). The increasing prevalence of CVD is due in part to significant improvements in treatments, which by extending lifespan have contributed to progressive societal aging. Population aging is already one of the most important demographic phenomena of our times in developed countries and is advancing rapidly in much of the developing world, bringing with it a major medical, social and economic impact. For example, >20% of Europeans will be 65 or older by 2025, and by 2050 19 countries are projected to have at least 10% of their population aged 80 years or over, with many having CVD and other age-associated disorders and dependent on the work of others (3). The economic cost of treating CVD patients is huge and is projected to increase substantially in the coming years. For example, every day EU member states collectively spend more than €4 billion on health care (4). Moreover, between 2010 and 2030, total direct medical costs of CVD in the United States are projected to triple, and a 61% increase in indirect costs is predicted due to CVD-related productivity loss (5). There is therefore an urgent need to define the mechanisms by which aging induces deterioration in the cardiovascular system independently of other risk factors, most of which are modifiable. This knowledge is essential for the provision of sustainable health care to a rapidly ageing population.

Animal and human studies have identified four main causes of accumulated damage that are proposed to drive mammalian aging: genomic instability, telomere attrition, epigenetic alterations, and loss of proteostasis (6). These primary hallmarks of aging trigger deregulated nutrient sensing, mitochondrial dysfunction, and cellular senescence (antagonistic aging hallmarks), processes that lead to stem cell exhaustion and altered intercellular communication, the main culprits of aging (integrative hallmarks). These aging mechanisms have been identified through the comparison of young and normally aging animals or human subjects or through interventional studies assessing how alterations to specific genetic pathways and biochemical processes affect lifespan during physiological aging. However, there is evidence that research into HGPS (OMIM 176670) may shed light

on the cellular and molecular mechanisms driving normal aging and associated CVD. HGPS patients exhibit premature aging associated with excessive atherosclerosis and the development of cardiac electrical defects, which lead to death typically in the early teens. The disease is caused by progerin, a variant of the precursor protein prelamin A produced because of a *de novo* mutation in the *LMNA* gene (7, 8) (see below). Other human progeroid syndromes have been linked to loss-of-function mutations in *ZMPSTE24*, causing abnormal accumulation of prelamin A (9). Remarkably, all the hallmarks of normal aging proposed by López-Otín et al. (6) have been described in animal models of progeria, and some have also been reported in HGPS patients (reviewed in (6, 10-12)). Moreover, normal aging in non-HGPS individuals features low level prelamin A and progerin expression in cells and tissues (reviewed in (10-13)), including cells within the adventitia, media, and coronary atherosclerotic lesions (14). Oxidative stress and telomere shortening, both of which are thought to contribute to normal aging (6), have been reported to promote the expression of prelamin A (15) and progerin (16) in normal cells. In this review, we summarize the main structural and functional alterations in the cardiovascular system during physiological and premature aging, and discuss cellular and molecular mechanisms implicated in the acceleration of CVD and aging induced by abnormal expression of prelamin A or progerin.

2. CVD in physiological aging

2.1. Cardiac alterations

Heart failure (HF)

HF is a complex clinical syndrome strongly associated with aging and results from any structural or functional impairment of ventricular filling or ejection that causes insufficient perfusion of peripheral tissues (17, 18). Clinical manifestations of HF are dyspnea and fatigue, limited capacity for exercise, and fluid retention, which may lead to pulmonary and/or peripheral edema. In a large proportion of aged patients, ejection fraction remains unaltered, indicating that overall systolic function is preserved (19, 20). Therefore, age-related HF in otherwise healthy individuals is attributed to a dysfunction in the diastolic filling of the left ventricle (LV), rather than an impairment of systolic function (21). Left ventricular dysfunction occurs when the LV loses its ability to relax normally, caused by stiffening of the cardiac muscle as a result of fibrosis (22).

Echocardiographically determined early (E) and late (A) ventricular velocities are both altered in aged patients. Aging is associated with a decline in early diastolic LV filling (23-25). Early diastolic filling occurs after closure of the aortic valve and depends mainly on the active relaxation of the myocardium, myocardial compliance, and the pressure gradient from the atrium to the ventricle (26). The delay in early emptying is compensated by a more vigorous end-diastolic atrial contraction, increasing the A wave and therefore reducing the E/A ratio (23-25). As a result, aging does not affect end-diastolic volume at rest. Mechanisms

proposed for the reduced early diastolic LV filling rate during aging are fibrosis and stiffening of the ventricle (reduced compliance), as well as incomplete myofilament Ca^{2+} recruitment in the preceding systole (reducing active myocardial relaxation) (21, 27).

Left ventricular hypertrophy (LVH)

The prevalence of LVH, defined as an increase in echocardiographically-measured LV wall thickness, increases dramatically with age in men and women (28-30), and stands out as a powerful independent predictor for mortality and morbidity, especially in the elderly (28, 31). Age-associated LVH is the result of a progressive pathologic cardiac remodeling involving cardiomyocyte hyperthrophy and death, and collagen deposition (21, 32). Blood pressure, obesity, valve disease, and myocardial infarction are independent identified conditions that can induce the development of LVH (29).

Cardiac fibrosis

Aging is associated with increased collagen content in the extracellular matrix (ECM), leading to cardiac fibrosis and the development of LVH (33). However, the precise mechanisms underlying age-related cardiac remodeling remain largely undefined. Cardiac fibrosis during aging has been attributed to the downregulation of matrix-degrading pathways and increased collagen crosslinking, rather than to increased collagen synthesis, the main cause of cardiac fibrosis in hypertension (34). Fibrosis is also activated to replace dead cardiac tissue (34, 35). Fibrosis is a major predisposing factor for mechanical and electrical dysfunction in HF, since it reduces the ability of the myocardium to conduct electrical impulses, to relax and stretch properly, and to diffuse oxygen, thus increasing the age-related incidence of LV and heart-valve dysfunction and arrhythmias (36). The fibrosis-induced increase in cardiomyocyte workload combines with the impairment of the physiological environment to promote additional cardiomyocyte cell death and the replacement of lost cells by fibrotic material, generating a vicious cycle that aggravates cardiac dysfunction (37). Furthermore, enhanced collagen deposition and cardiomyocyte hypertrophy are thought to be consequences of increased LV workload induced by vessel stiffening (34), highlighting the importance of vascular dysfunction in the development of age-associated CVD.

Cardiac valve disease

Mitral and aortic valves become thicker and stiffer with age, leading to valve dysfunction and regurgitation (36, 38). Age-related functional impairment of heart valves due to calcification and connective tissue degeneration might be caused by progressive alterations to the ECM, resulting in loss of elasto-mechanical force, weakness, and stiffening and driving inflammation and further calcification (36, 38).

Hearth rhythm alterations

Cardiac output at rest is not modified by aging, since both stroke volume and heart rate are maintained. However, the aged heart has reduced capacity to increase heart rate, and therefore cardiac output, in response to physical exercise. Hence, the maximum acute cardiac output reserve decreases by around 30% between the ages of 20 and 85 years (39). This effect may account for the age-associated reduced physical performance capacity and increased fatigue after exercise.

The prevalence of atrial fibrillation also increases with age, and is epidemiologically associated with hypertension, heart-valve disease, and HF (40). Factors suggested to contribute to atrial fibrillation are overt sinus node dysfunction and altered autonomous nervous regulation (41, 42). Aging reduces the intrinsic pacemaker activity of the sinoatrial node, likely due to fibrosis, alterations to electrical conduction, and changes in the sinoatrial node action potential (43).

Defective autonomic cardiovascular regulation

Alterations in the autonomic regulation of the heart and blood vessels during aging have a major impact on cardiac and vascular functions, such as heart rate, myocardial contractility, myocardial relaxation, and vascular regulation of blood flow. Aging-associated autonomic cardiovascular dysregulation includes elevated sympathetic activity linked to increased plasma levels of catecholamines, decreased β-adrenergic sensitivity, and decreased baroreflex sensitivity. The causes of these aging-related alterations remain poorly understood; however, increased sympathetic activity has been suggested as a mechanism to compensate the decreases in baroreflex sensitivity and β-adrenergic postsynaptic activity (21, 44-46).

2.2. Vascular alterations

Hypertension

The classic assumption that aging causes hypertension is today disputed (47). Data from longitudinal studies led some authors to propose that the aging-associated sympathetic activity increases blood pressure (44); however, whether this increase crosses the threshold to hypertension depends upon the initial blood pressure values in youth (48). Sun et al. (49) emphasize the etiological involvement of vascular stiffening in blood pressure elevation, suggesting that large artery stiffening underlies the drop in sympathetic baroreflex sensitivity, which is subsequently compensated by sympathetic activation. In human, the baroreflexes rapidly and reflexively modulate blood pressure, and their impairment with aging is therefore also associated with a decline in blood pressure variability (50, 51).

Vascular stiffening, endothelial dysfunction, and atherosclerosis

Two major age-related vascular alterations are arterial stiffening and endothelial dysfunction (1, 2, 52). Arterial stiffening is noninvasively calculated from the pulse wave velocity (PWV) and is a highly reliable, blood pressure-independent predictor of cardiac events in a variety of adult populations, including the elderly (53). Carotid-femoral PWV increases progressively from around the age of 50 and arterial stiffness can reach an incidence of 64% to 74% in the geriatric population (54, 55). Increased stiffness of the large arteries places a strain on the heart, leading to cardiac fibrosis and HF. Vessel stiffening also sets a fertile stage for the initiation and progression of hypertension and atherosclerosis in the elderly by promoting cellular dysfunction in the vessel wall (56). The mechanisms underlying age-induced arterial stiffening include ECM alterations and associated increases in fibrosis and inflammation (49). Age-induced alterations in ECM structure and composition in the artery wall are due to increased collagen deposition and crosslinking, accumulation of advanced glycation end-products, and elastin fiber breakage. Progressive fibrosis in the tunica media is one of the suspected underlying mechanisms predisposing to inflammation and atherosclerosis during aging (45, 56, 57).

Aging is also associated with endothelial dysfunction, which alters homeostatic EC function, including the control of vascular tone, vascular permeability, and inflammation (52). Age-related accumulation of dysfunctional ECs is associated with decreased bioavailability of the cellular messenger nitric oxide (52), which increases permeability and inflammation and triggers a positive feedback that aggravates the phenomenon in the long term (58). Endothelial dysfunction is a major determinant of both the initiation and progression of atherosclerosis, a degenerative process that occurs within large elastic arteries during aging (2, 47, 59). Age-associated vascular-wall remodeling includes luminal enlargement as well as intimal and medial thickening (60). Intima-media thickening is an early sign of human atherosclerosis (47) and is an independent predictor of future cardiovascular events (61). Intimal thickening is initiated and sustained by the recruitment of blood-borne leukocytes, which is triggered by the activation of adhesion molecules on dysfunctional ECs (62). Neointimal leukocytes trigger a complex local immune response that further promotes leukocyte recruitment, and induces the migration of vascular smooth muscle cells (VSMCs) from the tunica media to the growing atherosclerotic lesion. Activated neointimal VSMCs switch from a ‘contractile’ to a ‘synthetic’ phenotype characterized by dedifferentiation, proliferation, and abundant secretion of ECM components (63, 64).

There is persistent controversy about whether atherosclerosis results from the accumulation of risk factors with aging or, conversely, aging itself promotes atherosclerosis independently of other factors. The important influence of risk factor exposure is attested by the lack of atherosclerosis in elderly members of isolated tribal societies (65) and by its presence in children with a high risk exposure (66), suggesting that it is possible to age without atherosclerosis. However, signs of atherosclerosis have been detected in ancient mummified human remains from societies not exposed to modern risk factors (67). This, together with the incidence of atherosclerosis in individuals with HGPS and other premature aging syndromes, supports the idea that the strongest atherosclerosis risk factor is aging itself.

3. CVD in HGPS patients

HGPS is an ultra-rare human genetic disease, with an estimated prevalence of 1 in 20 million people (www.progeriaresearch.org). The disease is characterized by accelerated aging caused by a *de novo* mutation in the *LMNA* gene (7, 8). In normal cells, alternative splicing of *LMNA* transcripts gives rise to two major A-type lamin variants (lamin A and lamin C) as well as minor variants (lamin AΔ10 and the germline-specific lamin C2) (68-70). The precursor protein prelamin A undergoes several posttranslational modifications to yield mature lamin A (Figure 1A). First, a farnesyltransferase farnesylates the cysteine residue at the C-terminal cysteine-serine-isoleucine-methionine (CSIM) motif. The 3 C-terminal amino acids are then removed, enabling methylation of the new C terminus by isoprenylcysteine carboxyl methyltransferase (ICMT). Finally, the zinc metalloprotease ZMPSTE24/FACE-1 removes the farnesylated and carboxymethylated 15 C-terminal residues. Mature lamin A is then incorporated into the nuclear lamina, a protein scaffolding network that underlies the inner nuclear membrane, providing mechanical strength to the nucleus and regulating many cellular functions, including DNA replication and repair, chromatin organization, signal transduction, and gene transcription (71).

'Classic' progeria in most HGPS patients is caused by a heterozygous *de novo* c.1824C>T (p.Gly608Gly) point mutation in the *LMNA* gene (7, 8). This synonymous mutation creates an aberrant splice site in exon 11 that deletes 150 nucleotides, resulting in the synthesis of a truncated prelamin A variant called progerin (Δ50 prelamin A) (Figure 1B). Lack of the 50 amino acids at the progerin C-terminus impedes cleavage of the terminal 15 amino acids, causing the accumulation of permanently farnesylated and carboxymethylated progerin. *ZMPSTE24/FACE-1*-inactivating mutations provoke the accumulation of farnesylated prelamin A and are also linked to human progeroid syndromes (9). Abnormal prelamin A and progerin expression causes multiple structural and functional alterations that affect signal transduction, gene transcription, and chromatin organization, ultimately provoking growth arrest, cell senescence, cell death, and the acceleration of organismal aging (11, 71) (Figure 1B). Remarkably, most of the processes affected in HGPS are implicated in normal aging (6).

HGPS patients appear normal at birth but start to develop symptoms during the first 12-18 months of life. The disease is characterized by failure to thrive, abnormal dentition, alopecia, lipodystrophy, skin abnormalities, joint contractures, osteoporosis, and osteolysis, progressively impairing walking and other motor activities. However, the most severe medical problem in HGPS patients is atherosclerosis and cardiac electrical abnormalities, causing premature death at an average age of 14.6 years, mainly from myocardial infarction or stroke (72-76).

Many of the cardiovascular alterations in HGPS patients also feature in physiological aging (Table 1). However, unlike the physiologically elderly, HGPS patients have an elevated platelet count and a prolonged prothrombin time (75), and typically lack or are only mildly affected by most traditional cardiovascular risk factors. For example, HGPS patients and healthy children have similar levels of mean plasma cholesterol, LDL and HDL cholesterol, triglyceride, and median C-reactive protein (74, 75, 77). Moreover, ~30% HGPS patients show only slight elevation of systolic and diastolic blood pressures compared with age-

matched healthy children (74, 75, 78). The study of CVD in HGPS therefore offers a unique opportunity to identify mechanisms that cause age-associated cardiovascular damage in the absence of other risk factors or aging-associated chronic diseases that can secondarily influence cardiovascular health.

As with normal aging, noninvasive imaging in HGPS patients detects patent carotid plaques only at later stages (74, 75, 78). However, arterial stenosis affects HGPS patients of all ages and may be an early indicator of atherosclerotic plaque formation (74). Atherosclerosis in HGPS patients is accompanied by alterations typically seen in normal aging, such as inflammation, VSMC loss, and plaque erosion and rupture (74). Nevertheless, HGPS vessels also uniquely feature prominent adventitial thickening and fibrosis (14, 79). Another feature of physiological aging also found in HGPS is vascular calcification (VC), which is associated with augmented CVD-associated morbimortality in the general population (80); VC affects the aorta and aortic and mitral valves of some HGPS patients, and can cause aortic and/or mitral regurgitation (14, 75, 81-84). Neuroimaging studies in a 25-patient cohort identified early and clinically silent stroke as a prevalent characteristic of HGPS (85). Stroke in HGPS patients can also leave neurologic sequelae (86).

HGPS is also characterized by vessel stiffening, an alteration associated with physiological aging which independently predicts the incidence of future cardiovascular events. Analysis of a 21-patient cohort identified vascular stiffening as an early and pervasive feature of the disease, detecting PWV values comparable to those typically seen in adults older than 60 years (74). While carotid intima-media thickness is normal in HGPS patients, their carotid arteries have an above-normal echodensity, especially in the adventitia, consistent with elevated vascular fibrosis seen on autopsy (14, 74, 75, 87). Altered ankle-brachial index in HGPS is an indicator of peripheral artery disease and vascular dysfunction (74, 82); nevertheless, endothelial vasodilator function seems to be preserved, since there is no alteration to flow-mediated dilation, an indicator of endothelial vasodilator function (75). Further studies are warranted to identify the mechanisms underlying progerin-induced vascular dysfunction.

A subset of HGPS patients show electrocardiographic alterations, including repolarization abnormalities, such as ST depression/elevation and negative and biphasic T waves; these abnormalities are especially evident in patients with LV hypertrophy, diastolic dysfunction, or cardiac valve dysfunction at advanced disease stages (74-76, 81, 84). Cardiac rhythm in HGPS patients is in the normal range; however, heart rate tends to be below normal in older patients (76).

In summary, the key features of CVD in HGPS patients are vascular stiffening and remodeling, with prominent medial and adventitial fibrosis, VSMC loss, accelerated atherosclerosis, and premature death from myocardial infarction or stroke. Premature death in HGPS might also be linked to arrhythmias resulting from cardiac electrical defects. Additional studies are needed to define the precise mechanisms through which progerin expression accelerates CVD and to elucidate the relative contribution of cardiac and vascular alterations to premature death in HGPS.

4. Mouse models of progeria

An estimated 350 to 400 children live with HGPS worldwide (www.progeriaresearch.org). This very low number presents many challenges, both for research to identify mechanisms underlying premature aging and associated CVD and for conducting clinical trials to assess new therapies. To facilitate HGPS research, a number of strategies have been used over the past 15 years to create mouse models of progeria (Table 2). Although none of these models fully recapitulates HGPS symptoms, probably due to interspecies differences, they have been extremely useful in identifying molecular and cellular mechanisms underlying progeria and testing therapeutic strategies. This section summarizes the main characteristics of available progeroid mouse models.

4.1. *Lmna^{HG}*

Yang and colleagues produced the first HGPS-like mouse model, creating a knock-in mouse line carrying a mutant progerin-expressing allele referred to as *Lmna^{HG}* ('Hutchinson-Gilford') (88, 89). Heterozygous *Lmna$^{HG/+}$* mice express progerin together with lamin A and C and start losing weight at 6-8 weeks of age, and either die or require euthanasia by 4-6 months of age (compared with >2 years average lifespan in wild-type mice). Homozygous *Lmna$^{HG/HG}$* mice exclusively express progerin and have a more severe phenotype, dying by 3-4 weeks of age. Both, *Lmna$^{HG/+}$* and *Lmna$^{HG/HG}$* present osteoporosis, loss of subcutaneous fat, and alopecia, features observed in HGPS patients. Nevertheless, these models show no signs of CVD (89).

4.2. *G608G BAC*

Varga et al. (90) generated *G608G BAC* transgenic mice using a bacterial artificial chromosome (BAC) harboring a version of the human *LMNA* gene containing the HGPS-causing c.1824C>T mutation (p.G608G). These mice express human progerin and endogenous mouse lamin A/C but show no overt progeroid features (90). However, autopsy studies revealed progressive loss of VSMCs in the large arteries of *G608G BAC* mice starting at 5 months of age. This is accompanied by collagen and proteoglycan deposition in the media, broken elastic fibers, and thickened adventitia and medial layers (90), all of which are vascular pathologies described in human patients (14, 79, 91). Consistent with the aortic phenotype of *G608G BAC* mice, these animals show impaired vascular responsiveness after sodium nitroprusside administration; however, *G608G BAC* mice present no signs of atherosclerotic plaque formation.

4.3. *Lmna$^{G609G/G609G}$*

Osorio et al. (92) generated *Lmna^{G609G}* knock-in mice carrying a c.1827C>T (p.G609G) mutation in the endogenous mouse *Lmna* gene, equivalent to the HGPS-causing human mutation *LMNA* c.1824C>T (p.G608G). The *Lmna^{G609G}* allele gives rise to progerin (via

aberrant splicing), lamin C, and some residual lamin A, mimicking the situation in HGPS patients. Homozygous *Lmna*$^{G609G/G609G}$ mice appear normal at birth, but from 3 weeks of age they develop progeroid symptoms, including failure to thrive, loss of subcutaneous fat, hair follicle attrition, and bone alterations, and die at an average age of 15 weeks. These mice also exhibit hypoglycemia and altered plasma concentrations of metabolic hormones (increased GH and adiponectin and reduced IGF-1, insulin, and leptin). *Lmna*$^{G609G/G609G}$ mice exhibit VSMC loss in the aortic arch, but not in the thoracic aorta. Longitudinal studies revealed normal blood pressure, but *Lmna*$^{G609G/G609G}$ mice progressively developed QRS wave prolongation—consistent with altered ventricular depolarization—and bradycardia. On the other hand, heterozygous *Lmna*$^{G609G/+}$ mice appear normal until ~32 weeks of age, when they rapidly develop a severe phenotype similar to that of homozygotes, and die shortly thereafter (92). *Lmna*$^{G609G/+}$ mice show extensive calcification of the aortic media (93), an important feature of human HGPS. Although the *Lmna*$^{G609G/G609G}$ mouse model recapitulates most clinical features of HGPS, there have been no reports of atherosclerosis in this model.

Lee et al. (94) recently generated a new *Lmna*$^{G609G/G609G}$ model, which carries a HGPS-causing mutation in codon 609 of *Lmna* and produces progerin via abnormal splicing. The phenotype is similar to other HGPS mouse models, including severe VSMC loss in the media of the ascending aorta and adventitial fibrosis by 4 months of age. However, the effect of the mutation on longevity has not been reported.

4.4. *Zmpste24*$^{-/-}$

The final step in prelamin A maturation is the cleavage of the farnesylated C-terminus by the zinc metalloproteinase ZMPSTE24 (Fig. 1A). *ZMPSTE24* deficiency in humans results in farnesylated prelamin A accumulation, causing various progeria-like syndromes, such as restrictive dermopathy (95-97) and mandibuloacral dysplasia (98). *Zmpste24*-deficient mice expressing farnesylated prelamin A thus present an appropriate preclinical model for studying premature aging. *Zmpste24*$^{-/-}$ mice generated by Bergo et al. (99) show postnatal growth retardation, alopecia, reduced subcutaneous fat, muscle weakness, and bone abnormalities and die by 6-7 months of age. The *Zmpste24*$^{-/-}$ mouse model generated by Pendas et al. (100) has a slightly more severe phenotype, including postnatal growth retardation, alopecia, lipodystrophy, skeletal and muscular atrophy, cardiac alterations (dilation of both ventricles, interstitial fibrosis, and ventricular wall thinning), and death at an average age of 5 months. Recent studies in this progeroid model revealed electrical cardiac alterations associated with connexin 43 mislocalization (see below) (76).

5. HGPS: General mechanisms and treatments

Lamin A/C play major roles in a broad range of cell functions, including maintenance of nuclear mechanical stability, signal transduction, gene transcription, chromatin organization, DNA damage repair, cell-cycle progression, and cell differentiation and migration (71). By affecting multiple pathways, progerin and prelamin A accumulation may therefore trigger premature aging through a number of non-mutually exclusive mechanisms. Moreover, the profile of activated mechanisms might differ between different tissues depending on

differences in the amount of lamin A (and therefore progerin) produced, which is related to tissue stiffness (101).

Mouse and human studies suggest that HGPS severity is determined by both the total amount of progerin and the ratio of farnesylated progerin to mature lamin A. Indeed, different human *LMNA* point mutations are associated with major differences in progerin levels and disease severity, ranging from neonatal progeria (high progerin level) to late-onset progeria (low progerin level) (102-104). Accordingly, in $Lmna^{G609G/G609G}$ mice, the aging phenotype is ameliorated and survival prolonged by the reduction in progerin expression with antisense morpholinos targeting aberrant *Lmna* exon 11-exon 12 splicing (92). Progerin production, adventitial fibrosis, and VSMC loss in $Lmna^{G609G/G609G}$ mice are also reduced by antisense oligonucleotides designed to shift alternative splicing from lamin A toward lamin C; however, the effect of this strategy on longevity was not reported (94).

Unlike mature lamin A, progerin remains permanently farnesylated (Figure 1). The hypothesis that persistent farnesylation is a chief cause of progeria was supported by mouse and human studies demonstrating progeroid symptoms associated with farnesylated prelamin A accumulation caused by *ZMPSTE24* deficiency (95, 96, 99, 100). Moreover, a patient with both a homozygous loss-of-function *ZMPSTE24* mutation and a heterozygous *LMNA* mutation resulting in C-terminal elongation of the final lamin A had a milder-than-usual progeroid phenotype, possibly due to reduced levels of farnesylated prelamin A (105). Supporting this conclusion, $Zmpste24^{-/-}$ mice with *Lmna* haplodeficiency display no overt aging phenotype (106). The importance of farnesylation in HGPS pathogenesis was confirmed by the generation of $Lmna^{csmHG/csmHG}$ mice, which produce nonfarnesylated progerin and do not age prematurely (107). Likewise, $Lmna^{nPLAO/nPLAO}$ mice, expressing only non-farnesylated prelamin A, develop cardiomyopathy but not progeria (108). Moreover, treatment with farnesyl transferase inhibitors (FTIs) diminishes nuclear defects in progerin-expressing cells (88, 109), prevents CVD onset and late progression in progeroid *G608G BAC* mice (110), and prolongs the survival of progeroid $Zmpste24^{-/-}$ and $Lmna^{HG/+}$ mice (89, 111).

Later work showed that FTI treatment results in alternative geranylgeranyltransferase-induced prenylation of prelamin A and progerin (112), similar to the effect of FTIs on some oncoproteins in cancer therapy (113). Combined treatment of $Zmpste24^{-/-}$ mice with statins and aminobisphosphonates to block both prelamin A farnesylation and geranylgeranylation improved the progeriod phenotype and prolonged lifespan (112). Based on findings in progerin- and prelamin A-expressing mice and cells (reviewed in (10)), clinical trials have been conducted with HGPS patients to test the effect of treatment with an FTI (lonafarnib) alone or in combination with statins (pravastatin) and bisphosphonates (zoledronate) (72, 78, 87). Lonafarnib monotherapy provided some improvement in vascular stiffness, bone structure, and audiological status and was estimated to increase mean survival by 1.6 years (72, 87). Triple-drug therapy with lonafarnib, pravastatin, and zoledronate showed an additional improvement in bone mineral density, but there was no cardiovascular improvement compared with lonafarnib monotherapy (78). Thus, although farnesylated progerin appears to play a major role in HGPS, current therapies to prevent progerin farnesylation appear to provide only a modest benefit.

The observation that unfarnesylated and farnesylated progerin both form aggregates at the nuclear membrane prompted Kalinowski et al. (114) to suggest that progerin association with the inner nuclear membrane also involves increased electrostatic interactions and aggregation. In addition, the less heterogeneous and more compact tail of progerin compared with normal lamin A may affect its interaction with DNA and other proteins (115).

Another factor that might contribute to progerin toxicity is altered protein structure, due to the deletion of 50 amino acids near the C-terminal region. Abnormal interactions of progerin with other nuclear components cause nuclear blebbing, increased thickness and stiffness of the nuclear lamina, heterochromatin mislocalization, and alterations to nuclear pore complexes (116, 117). Recently, Lee et al. (118) found that progerin binds strongly to lamin A/C and that chemical disruption of progerin-lamin A/C heterodimers reduces nuclear aberrations, prevents cell senescence, ameliorates progeroid features, and extends lifespan of $Lmna^{G609G/G609G}$ mice.

ICMT-catalyzed carboxymethylation of the progerin C-terminal farnesylcysteine residue might also play a role in progeria. Reducing ICMT expression and activity by 70-90% in hypomorphic $Zmpste24^{-/-}Icmt^{hm/hm}$ mice improved body weight, grip strength, and bone structure and extended survival compared with control $Zmpste24^{-/-}Icmt^{+/+}$ littermates with intact ICMT (119). Diminished ICMT activity in $Zmpste24^{-/-}Icmt^{hm/hm}$ mice was associated with prelamin A mislocalization and activation of signaling through AKT and mTOR (mammalian target of rapamycin), in turn delaying cell senescence. However, it is noteworthy that the mTOR inhibitor rapamycin activated autophagic clearance of progerin and reduced nuclear abnormalities (120, 121). These results clearly show that ICMT and mTOR are implicated in premature aging, but further studies are needed to define the precise underlying mechanisms and relationship between ICMT, AKT, and mTOR.

6. Mechanisms underlying CVD in progeria

This section summarizes current knowledge of the cellular and molecular mechanisms through which prelamin A and progerin damage the cardiovascular system. This knowledge is of major interest for understanding the mechanisms implicated in CVD during normal aging, since both prelamin A and progerin are expressed at low level in cells and tissues of non-HGPS individuals, including medial VSMCs and atherosclerotic lesions (14, 15, 122).

6.1. VSMC loss

Progressive VSMC loss is a characteristic of HGPS patients (14, 79, 91) and progeria mouse models (90, 92, 94), suggesting an important role in progeroid vascular disease. Although less severe, depletion of VSMCs in the media also occurs in physiological aging (123).

VSMCs are subject to high mechanical stress related to blood flow. In normal conditions, cells respond to increased shear stress by increasing the expression of lamin A/C and changing their nuclear localization (101, 124, 125). Abnormal responses to physical stress in progerin-expressing cells may lead to cell damage and death (117, 126). Consistent with this notion, sustained mechanical stress applied to HGPS fibroblasts reduces cell

viability and increases apoptotic cell death (127). Progerin-induced alterations in mechanotransduction might be explained by changes in the expression of proteins controlling cytoskeleton organization, mechanotransduction, and ECM production (128, 129). Supporting this view, the ascending aorta of progerin-expressing *G608G BAC* transgenic mice have reduced expression of vimentin (128), a cytoskeletal protein attached to the nucleus, endoplasmic reticulum, and mitochondria that is essential for maintaining cellular integrity (130). This correlation between mechanotransduction protein downregulation and high shear stress might partially explain VSMC loss in HGPS.

The mechanisms underlying progerin-induced VSMC loss can be explored in human SMCs differentiated from induced pluripotent stem cells (iPSCs) derived from healthy individuals and HGPS patients. Liu et al. (131) reported premature senescence associated with vascular aging in iPSC-derived progerin-expressing SMCs, and identified the interaction between progerin and the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), a catalytic subunit of nuclear DNA-PK that participates in DNA repair by non-homologous end joining (NHEJ). Conflicting with these findings, Kinoshita et al. (132) reported that progerin, unlike wild-type lamin A, cannot interact with DNA-PK or other proteins implicated in the DNA damage response. They also found that expression of progerin in VSMCs, but not in ECs, causes DNA-PK activation, leading to VSMC growth arrest and senescence. Further studies are thus warranted to clarify the interaction between progerin and DNA-PK and its subunits in different cell types, and to map out its pathophysiological consequences.

Zhang et al. (133) reported caspase-independent severe proliferative defects in SMCs derived from HGPS-iPSCs. They also found that progerin expression in SMCs inhibits poly(ADP-ribose) polymerase 1 (PARP1), an important DNA repair regulator, and activates the error-prone NHEJ response, causing prolonged mitosis, mitotic catastrophe, and cell death. Prelamin A also induces DNA damage and increases the DNA damage response in aged VSMCs (15, 134). This response might be a consequence of impaired recruitment of 53 binding protein-1 (53BP1) to DNA damage sites, resulting from defective nuclear import related to nucleoporin 153 mislocalization (135). Defective DNA damage repair has also been described in non-vascular HGPS cells and progeria mouse models (136-138). These accumulated findings confirm that a defective DNA damage response contributes to progerin-driven VSMC death. Remarkably, DNA damage plays an important role in normal aging (139).

6.2. Vascular calcification

Like HGPS patients (14, 81-83), progeroid *G608G BAC* and $Lmna^{G609G/+}$ mice develop aortic calcification (90, 93). In the calcified aortas of $Lmna^{G609G/+}$ mice, Villa-Bellosta et al. (93) found abnormally high expression of the osteogenic markers bone morphogenetic protein 2 (*Bmp2*) and Run-related transcription factor-2 (*Runx2*), without alterations in the anti-calcification agents matrix Gla-protein and fetuin A. Moreover, $Lmna^{G609G/+}$-derived primary VSMCs showed a reduced capacity to inhibit calcium deposition *in vitro*, which was associated with lower extracellular concentration of inorganic pyrophosphate (ePPi), the major endogenous inhibitor of VC. Reduced ePPi levels in VSMC cultures was associated with impaired ePPi synthesis due to decreased ATP production (the main substrate for ePPi

synthesis) and upregulation of both tissue-nonspecific alkaline phosphatase (TNAP, the main enzyme causing PPi hydrolysis) and ectonucleoside triphosphatase diphosphohydrolase 1 (eNTPD1, an enzyme that hydrolyzes ATP to release Pi). Compared with *Lmna*$^{+/+}$ littermates, *Lmna*$^{G609G/+}$ mice had lower plasma concentrations of ePPi and ATP, and treatment with exogenous PPi prevented VC in *Lmna*$^{G609G/G609G}$ mice (93).

Prelamin A expression in VSMCs also promotes VC through a mechanism involving the activation of signaling via the DNA damage-related ataxia telangiectasia mutated (ATM)/ataxia telangiectasia and Rad3-related (ATR) pathway (134). Activation of this pathway induces the senescence-associated secretory phenotype in VSMCs, which release pro-calcification factors such as BMP2 that can trigger calcification both locally and at remote sites (134). Moreover, exposure of VSMC cultures to calcifying medium leads to upregulation of lamin A and prelamin A expression, accompanied by augmented expression of pro-calcifying factors such as Runx2, osteocalcin, and osteopontin and increased calcium deposition (140). Remarkably, human mesenchymal stems cells expressing progerin also have elevated levels of osteopontin and show enhanced osteogenic differentiation (141).

In summary, lamin A and its mutant or unprocessed forms participate in osteoblastic VSMC differentiation and VC, underlying the need to deepen our knowledge about the role of progerin and prelamin A in VC during premature aging.

6.3. Endothelial dysfunction

EC dysfunction plays a key role in all stages of atherosclerosis, which is the life-threatening symptom of HGPS. ECs sense and respond to different types of blood flow, and aortic regions subjected to turbulent blood flow and high sheer stress, such as the ascending aorta, are more susceptible to atherosclerosis (142, 143). Song et al. (128) observed intact EC monolayers in regions of the ascending aorta of *G608G BAC* mice that were almost completely devoid of VSMCs. Compared with ECs in regions with preserved VSMCs, progerin-expressing ECs near regions with massive VSMC loss have more-than 8-fold higher vimentin expression, which might make them more resistant to shear stress, thus explaining the presence of well-preserved endothelium in HGPS vessels (14).

Elevated adhesion molecule expression in dysfunctional ECs triggers monocyte adhesion, an important step in atherosclerosis initiation and progression (62). Recent studies show that prelamin A accumulation in ECs, by blocking lamin A maturation, induces cell senescence and promotes intercellular adhesion molecule 1 (ICAM1)-dependent monocyte adhesion (144). Further studies are needed to determine the connection between ECs, progerin and prelamin A expression and atheroma build-up.

6.4. Cardiac electrical alterations

Consistent with the observed repolarization abnormalities in HGPS patients, progeriod *Zmpste24*$^{-/-}$ mice progressively develop T-wave flattening (75, 76). Moreover, both progerin-expressing *Lmna*$^{G609G/G609G}$ mice and prelamin A-expressing *Zmpste24*$^{-/-}$ mice develop severe bradycardia with aging (76, 92). Aging is also associated with QRS prolongation in

Lmna$^{G609G/G609G}$ mice and with PQ and QRS prolongation in *Zmpste24*$^{-/-}$ mice, indicating defective cardiac conduction. These alterations may reflect intercellular connectivity defects, since the gap junction protein connexin 43 is mislocalized in myocardial tissue of HGPS patients and *Zmpste24*$^{-/-}$ mice. These results suggest that cardiac alterations in HGPS patients and progeroid mice are a characteristic of progeria that could increase the risk of arrhythmias and lead to premature death. Moreover, some of the alterations in the progeorid heart are also frequently observed during normal aging (145, 146), suggesting the existence of common mechanisms underlying heart alterations in HGPS patients and in the geriatric population.

7. Concluding remarks and perspectives

Aging is the main risk factor for CVD. Since societies are progressively aging and CVD is the main cause of morbimortality worldwide, it is urgent to improve our knowledge of the mechanisms underlying tissue and organismal aging. This information is critical to the development of new strategies to reduce disease burden in the elderly and thus promote healthy aging. Intense efforts in basic, clinical, and epidemiological research have identified general mechanisms implicated in aging. These were recently classified into primary hallmarks of aging (genomic instability, telomere attrition, epigenetic alterations, and loss of proteostasis) that trigger antagonistic hallmarks (deregulated nutrient sensing, mitochondrial dysfunction, and cellular senescence), leading to integrative hallmarks (stem cell exhaustion and altered intercellular communication). A challenge in aging research is to identify which of the aging hallmarks contribute mainly to explain the high interindividual variability in human biological aging, as well as their relative contribution to age-associated cardiovascular damage. This knowledge should help to develop new therapies and improve prevention by identifying individuals at higher risk of suffering age-related diseases before symptoms appear, thus promoting healthy aging and reducing the health care and socio-economic impact of aging.

Aging and CVD are strongly accelerated in patients with HGPS, a rare genetic disorder caused by the unprocessed form of lamin A called progerin. Human progeria is also linked to abnormal accumulation of prelamin A caused by inactivating mutations in *ZMPSTE24*. Remarkably, both prelamin A and progerin are expressed at low level in cells and tissues from normally aging individuals, including cells of the artery wall. Progeria research can therefore shed light on the cell and molecular mechanisms driving normal aging and associated CVD. Traditional cardiovascular risk factors such as hypercholesterolemia, diabetes, obesity, hypertension, and smoking are typically absent or only mildly expressed in HGPS patients; therefore research into this disease offers a unique opportunity to isolate mechanisms that directly cause age-dependent cardiovascular damage from modifiable risk factors that progressively deteriorate cells and tissues during aging and can secondarily influence cardiovascular health.

The identification of specific and shared mechanisms involved in normal and premature aging will require high-throughput genomic, epigenomic, transcriptomic, proteomic, and metabolomic studies. Moreover, loss-of-function and gain-of-function studies targeting candidate factors identified in 'omic' studies will permit the establishment of causal relationships. Bearing in mind the large number of cell types that participate in

CVD and normal and premature aging, it will be of great interest to generate new conditional and/or tissue-specific mouse models, with special emphasis on cells known to play a major role in atherosclerosis (e.g., monocytes/macrophages, lymphocytes, ECs, and VSMCs). There is a specific need to create small and large animal models of progeria that develop atherosclerosis, one of the main causes of death in HGPS patients. In control cells, oxidative stress and telomere shortening have been proposed to induce prelamin A and progerin expression, respectively (15, 16). Further research should focus on understanding how physiological aging leads to the accumulation of unprocessed forms of lamin A, and whether nuclear abnormalities induced by these proteins contribute to normal aging.

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Table1. Age-associated structural and functional changes in the cardiovascular system: Physiological vs premature aging

	Physiological aging	Premature aging
Heart failure	YES	YES
Left ventricular diastolic dysfunction	YES	YES
Ventricular hypertrophy	YES	YES
Cardiac fibrosis	YES	YES
Heart valve dysfunction	YES	YES
Increased sympathetic activity	YES	YES
Decreased baroreflex sensitivity	YES	YES
Intima/media thickening	YES	YES
Vascular stiffening	YES	YES
Vascular fibrosis	YES	YES
Endothelial dysfunction	YES	NO
Atherosclerosis	YES	YES
Vascular calcification	YES	YES
Hypertension	YES	YES
Elevated platelet count	NO	YES
Prolonged prothrombin time	NO	YES

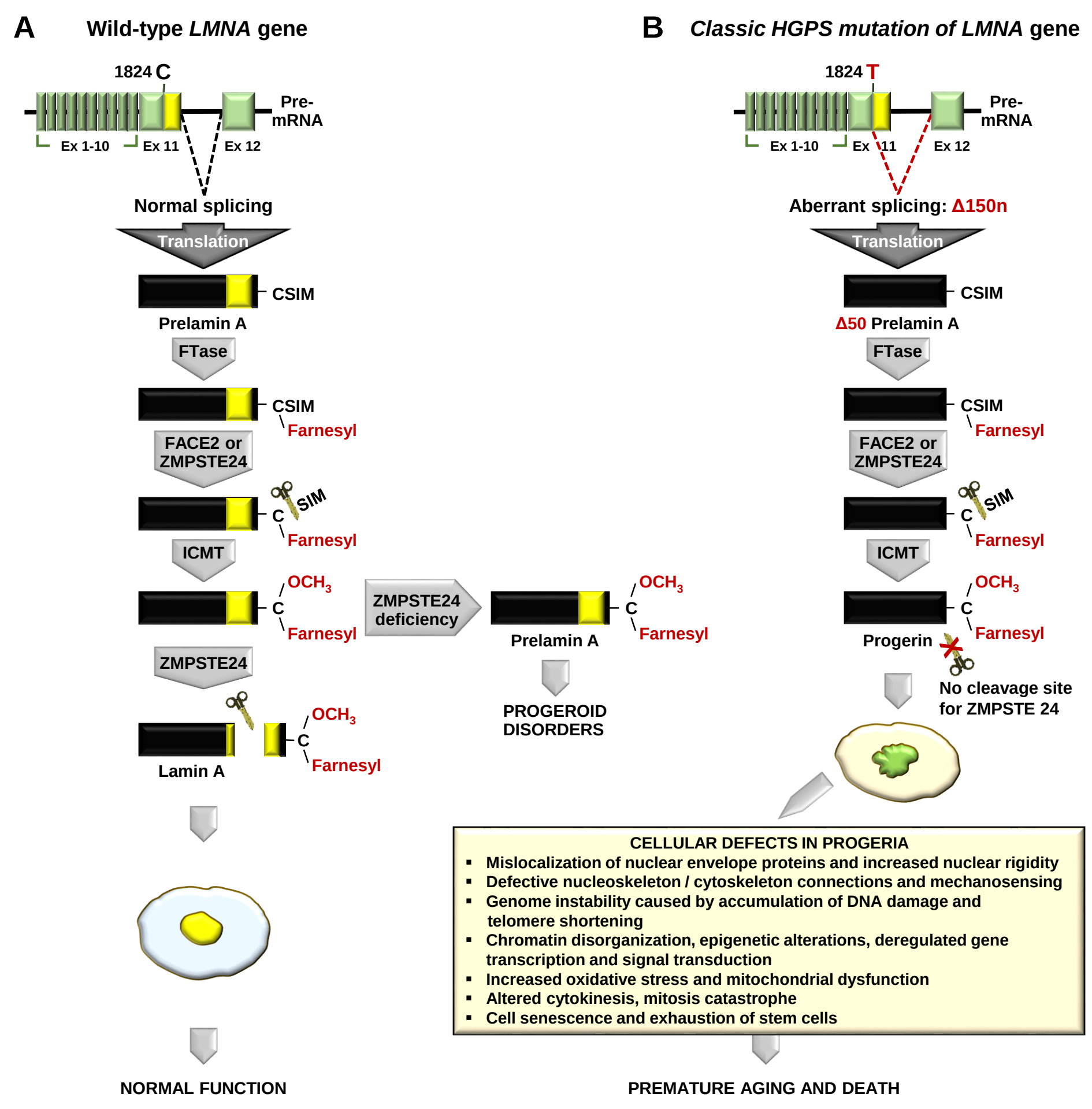
Table 2. Mouse models of HGPS

Model	Gene or Transgene	A-type lamin expression	Vascular alterations	Cardiac alterations	Premature aging	References
Lmna$^{HG/+}$	*Lmna*	progerin, lamin A/C	NO	NO	YES	(88, 89)
Lmna$^{HG/HG}$	*Lmna*	progerin	NO	NO	YES	(88, 89)
BAC-G608G	Tg(*LMNA** G608G)	lamin A/C, human progerin	YES	N	NO	(90)
Lmna$^{G609G/+}$	*Lmna*	progerin, lamin A/C	YES	N	YES	(92, 93)
Lmna$^{G609G/G609G}$	*Lmna*	progerin, lamin C, residual lamin A	YES	YES	YES	(92)
Lmna$^{G609G/G609G}$	*Lmna*	progerin, lamin C, residual lamin A	YES	NR	YES	(94)
Zmpste24$^{-/-}$	*Zmpste24*	prelamin A, lamin C	NO	NO	YES	(99)
Zmpste24$^{-/-}$	*Zmpste24*	prelamin A, lamin C	NO	YES	YES	(76, 100)

NR – not reported

FIGURE 1: PRELAMIN A PROCESSING IN NORMAL AND HGPS CELLS.

(A) In control cells carrying the wild-type *LMNA* sequence, normal splicing between exons 11 and 12 gives raise to prelamin A, which undergoes sequential post-translational modifications to yield mature lamin A. Final cleavage by the protease ZMPSTE24 removes the farnesylated and carboxymethylated C-terminus. *ZMPSTE24*-inactivating mutations lead to accumulation of permanently farnesylated and carboxymethylated prelamin A, which accelerates aging. **(B)** Classic HGPS is caused by a heterozygosis *de novo* synonymous mutation in the *LMNA* gene (c.1824C>T; p.G608G), which results in aberrant splicing between exon 11-12 and the synthesis of progerin. Lack of the 50-aminoacid residues encompassing the ZMPSTE24 cleavage site prevents removal of the progerin C-terminus, which remains permanently farnesylated, causing multiple cellular alterations and premature aging and death.


A
Wild-type LMNA gene
1824 C
Pre-mRNA
Ex 1-10
Ex 11
Ex 12
Normal splicing
Translation
CSIM
Prelamin A
FTase
CSIM
Farnesyl
FACE2 or ZMPSTE24
SIM
C
Farnesyl
ICMT
OCH3
C
Farnesyl
ZMPSTE24
OCH3
C
Farnesyl
Lamin A
NORMAL FUNCTION
ZMPSTE24 deficiency
Prelamin A
OCH3
C
Farnesyl
PROGEROID DISORDERS
B
Classic HGPS mutation of LMNA gene
1824 T
Pre-mRNA
Ex 1-10
Ex 11
Ex 12
Aberrant splicing: Δ150n
Translation
CSIM
Δ50 Prelamin A
FTase
CSIM
Farnesyl
FACE2 or ZMPSTE24
SIM
C
Farnesyl
ICMT
OCH3
C
Farnesyl
Progerin
No cleavage site for ZMPSTE 24
CELLULAR DEFECTS IN PROGERIA
Mislocalization of nuclear envelope proteins and increased nuclear rigidity
Defective nucleoskeleton / cytoskeleton connections and mechanosensing
Genome instability caused by accumulation of DNA damage and telomere shortening
Chromatin disorganization, epigenetic alterations, deregulated gene transcription and signal transduction
Increased oxidative stress and mitochondrial dysfunction
Altered cytokinesis, mitosis catastrophe
Cell senescence and exhaustion of stem cells
PREMATURE AGING AND DEATH