Hutchinson-Gilford progeria syndrome: Molecular mechanisms of cardiovascular pathogenesis, role of oxidative stress and therapeutic opportunities

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KEY WORDS: A-type lamins, nuclear envelope, Hutchinson-Gilford progeria syndrome, restrictive dermopathy, laminopathies, farnesyl transferase inhibitors, oxidative stress, reactive oxygen species, cardiovascular disease.

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1. ABSTRACT

Hutchinson-Gilford Progeria Syndrome (HGPS), a rare human disease characterized by premature aging, is mainly caused by the abnormal accumulation of progerin, a mutant form of the mammalian nuclear envelope component lamin A. HGPS patients exhibit vascular alterations and die at an average age of 13 years, predominantly from myocardial infarction or stroke. Animal models of HGPS have been a valuable tool in the study of the pathological processes implicated in the origin of this disease and its associated cardiovascular alterations. Some of the molecular mechanisms of HGPS might be relevant to the process of normal aging, since progerin is detected in cells from normal elderly humans. Conversely, processes linked to normal aging, such as the increase in oxidative stress, might be relevant to the pathogenic mechanisms of HGPS. In this review, we discuss recent advances in the understanding of the molecular mechanisms underlying the cardiovascular alterations associated with HGPS, the potential role of oxidative stress, and therapeutic approaches for the treatment of this devastating disease.

2. INTRODUCTION

The mammalian nuclear envelope is formed by the outer and inner nuclear membranes, the nuclear pore complexes and the nuclear lamina (1, 2). The nuclear lamina, a thin proteinaceous mesh tightly connected with several inner nuclear membrane-associated proteins, is mainly composed of type-V intermediate filaments called lamins (3, 4) that form coiled-coil dimers and associate longitudinally to form head-to-tail polymers (5). Lamins, which are classified as A-type or B-type, are encoded by different genes (6, 7). *LMNB1* encodes lamin B1 and lamin B2, whereas *LMNB2* encodes lamin B3. *LMNA* encodes not only for the major forms lamin A and C but also for lamins AΔ10 and C2 (testis) (8-10). A-type lamins are expressed in a developmentally regulated manner (11, 12). In general, lamins A and C are expressed in most differentiated cells and are absent or expressed at low level in highly proliferating tissues (13),

and they have therefore been suggested as early markers of cell differentiation (14). A-type lamins and associated proteins play roles in various cell processes, including mechanical stabilization of the nucleus, nuclear positioning, chromatin structuring and nuclear pore complex organization, gene transcription, nuclear envelope breakdown and reassembly during mitosis, DNA replication, DNA damage response, cell cycle progression, cell differentiation, and cell polarization during cell migration (15-30).

The C-terminal ends of lamin A and B-type lamins consist of a CAAX motif: a cysteine (C), two aliphatic residues (AA), and any residue (X). The specific sequences are CSIM in lamin A and CAIM in B-type lamins. The cysteine residue can be modified by attachment of either a 15 carbon isoprenoid farnesyl or the 20 carbon geranylgeranyl moiety. Lamin A is generated as pre-lamin A and then undergoes a series of posttranslational modifications **(Figure 1).** Pre-lamin A is first farnesylated by a farnesyltransferase (FTase) at the CAAX cysteine. The AAX motif is then cleaved by the endoprotease Rce1 (31, 32), and the carboxyl methyltransferase ICMT methylates the newly accessible C-terminal cysteine residue (33, 34). Lastly, a second proteolytic cleavage is carried out by the zinc metalloendoprotease known as FACE-1 (human) or Zmpste24 (mouse) (35). Proteolytic cleavage by FACE-1/Zmpste24 removes 15 residues from the C terminus, including the farnesylated and carboxylmethylated cysteine, to yield mature lamin A (72 kDa). Zmpste24 has been detected both in the endoplasmic reticulum and in the nucleus (36). Farnesylation is proposed to facilitate the assembly of lamin A into the nuclear membrane (37), while removal of the farnesyl moiety may increase the solubility of mature lamin A (38).

3. HUTCHINSON-GILFORD PROGERIA SYNDROME AND CARDIOVASCULAR DYSFUNCTION

Mutations in the *LMNA* gene or defective processing of pre-lamin A cause a group of human diseases termed laminopathies, including the systemic disease known as Hutchinson-Gilford progeria syndrome (HGPS). The most common cause of HGPS (affecting approximately 90% of HGPS patients) is a non-inherited *de novo* heterozygous single base substitution mutation at codon 608 (G608G: GGC>GGT) in *LMNA* (39, 40). A different heterozygous missense substitution in the same codon (G608S: GGC>AGC) or in codon 145 (E145K: GAG>AAG) accounts for most other cases of HGPS (40). Both mutations at codon 608 activate a cryptic splice site within exon 11, which causes the synthesis of an aberrant protein missing 50 amino acid residues from its C-terminal region. The missing sequence includes the proteolytic cleavage site recognized by FACE-1/Zmpste24. The mutant protein, known as progerin, has an apparent molecular weight of 67 kDa and is permanently farnesylated (Figure 1).

Accumulation of progerin, which seems to exert a dominant effect over the function and structure of wild-type lamin A, causes structural abnormalities in the nuclear lamina, delays cytokinesis, hampers the DNA repair machinery and ultimately leads to the accumulation of DNA damage and increased genome instability (38, 41-46). Subjects with HGPS appear normal at birth but show a severe failure to thrive after one year of life. Clinical phenotypes of HGPS patients include sclerotic skin, alopecia, sleeping with eyes open, cyanosis, abnormalities in bones accompanied by joint contractures and prominent cutaneous vasculature, prolonged prothrombin times, high platelet counts, and elevated serum levels of phosphorous (reviewed in (47)). HGPS patients suffer from premature arteriosclerosis and die at an average age of 13-14 years, predominantly from myocardial infarction or stroke. However, the prevalence of other age-related abnormalities, such as mental deterioration, cancer, cataracts, and activation of inflammatory pathways, is not higher in HGPS patients (Figure 2A).

X-ray and electrocardiography have revealed enlargement of the left ventricle and impaired coronary function in HGPS patients (48). A recent study in 15 HGPS patients aged

between 1 and 17 years, all of whom had the G608G *LMNA* mutation, identified a progressive deterioration of cardiovascular function with age, among other alterations (47). Although some other reports detected no plaque formation in the coronary vessels (48), this association is in line with postmortem studies in HGPS patients, which reveal varying degrees of coronary arteriosclerosis (49, 50), aortic atherosclerosis with calcification (51), the presence of interstitial myocardial fibrosis (52), left ventricular hypertrophy (53) and clear signs of recent myocardial infarction (50). A recent analysis of the cardiovascular pathology in 2 children with typical HGPS disease caused by the *de novo* heterozygous mutation 1824C>T in *LMNA* revealed a spectrum of early-to-late stage plaques with phenotypic characteristics similar to typical arteriosclerosis in geriatric patients, including calcification, inflammation and evidence of plaque erosion or rupture (54). However, adventitial fibrosis was more prominent in the HGPS patients than is found in geriatric cardiovascular disease. Vascular alterations in HGPS have also been reported in other organs, such as brain (55) and kidney (52).

The mechanisms underlying the acceleration of atherosclerosis in HGPS remain unclear. For example, several factors frequently associated with increased risk of cardiovascular disease during physiological aging appear normal in HGPS patients, including serum levels of total cholesterol and low-density lipoproteins and C reactive protein (56, 57). Moreover, xanthomatous infiltrations of the vessel wall, which are characteristic of familial hypercholesterolemia, have not been reported in HGPS patients. Nonetheless, atherosclerosis in HGPS has been linked to a progressive reduction in circulating levels of HDL cholesterol and adiponectin (57). It is also possible that progerin accumulation causes vascular cell deterioration. Vascular alterations were reported in a 20-year-old progeric patient who showed unusual collagen fibrils of relatively small diameter, both in the intima and media layers, accompanied by a marked depletion of vascular smooth muscle cells (VSMCs), particularly in the aortic media, which appeared more susceptible to hemodynamic and ischemic stress (53). These observations

were supported by the case of an 11 year old HGPS patient who died of cerebral infarction and showed replacement of VSMCs by fibrous tissue (58). Increased severity of arteriosclerosis in HGPS patients might therefore be related to a greater susceptibility and vulnerability of VSMCs. Remarkably, transgenic mice carrying a bacterial artificial chromosome harboring the G608G *LMNA* mutation underwent a progressive loss of VSMCs from the medial layer of arteries, resembling the phenotype of HGPS patients (59). The arteries of these mice exhibited an abnormal accumulation of proteoglycan and collagen, associated with calcification and thickening of the adventitia layer. Given that VSMCs and endothelial cells are major sites of progerin accumulation (60), future studies in these cell types should address the effects of this mutant form of lamin A on processes that may contribute to atherosclerosis development in HGPS patients, such as senescence, cellular depletion, apoptosis and autophagy.

4. PROGERIN AND PRE-LAMIN A AS POTENTIAL NEW ELEMENTS CONTRIBUTING TO VASCULAR DYSFUNCTION DURING PHYSIOLOGICAL AGING

Evidence accumulated in the last few years suggests that lamins play a role in physiological aging (61-64) (and references therein). In the nematode *C. elegans*, major changes of nuclear architecture and loss of peripheral heterochromatin occur during aging, and reduction of the levels of lamin and lamin-associated LEM domain proteins significantly shortens its lifespan (65). Accumulation of small amounts of progerin has been also observed in cells from healthy human subjects (66-68), and is possibly due to sporadic abnormal splicing. Remarkably, a screen of 150 skin biopsies from unaffected individuals (newborn to 97 years) demonstrated accumulation of progerin protein, without changes in mRNA levels, during normal aging (69). These findings suggest a role for progerin in physiological aging. Consistently, progerin-positive fibroblasts from subjects without HGPS exhibit mitotic defects that increase with passage

number (67) and alterations of the DNA repair machinery and histone modifications (66). In the context of the cardiovascular system, Olive et al. (54) recently found that progerin is expressed in non-HGPS arteries and that vascular progerin accumulates with aging in vivo. However, unlike the situation in HGPS patients (60), progerin-positive vascular cells in non-HGPS arteries were largely free of smooth muscle α-actin and accumulated mostly in the adventitia (54). As pointed out by the authors, some of these cells maybe adventitial fibroblasts, immune cells or dedifferentiated VSMCs commonly found in atheromas. It is also noteworthy that accumulation of pre-lamin A, possibly due to age-dependent downregulation of Zmpste24/FACE-1, has been identified as a novel biomarker of VSMC aging and atherosclerosis, and might accelerate senescence during normal aging (41). Collectively, these findings suggest that progressive accumulation of progerin and pre-lamin A may contribute to age-induced vascular dysfunction (Figure 2).

5. ANIMAL MODELS OF HGPS AND THEIR CARDIOVASCULAR PHENOTYPES

The development of animal models has greatly improved our understanding of the etiology of laminopathies. Disruption of the *Zmpste24* gene generated a progeric mouse model characterized by accumulation of farnesylated prelamin A at the nuclear envelope due to defective lamin A processing (Figure 1). Embryonic fibroblasts from these mice show nuclear envelope abnormalities (35), hyper-activation of p53 signaling (70), cell senescence (70), stem cell dysfunction (71, 72) and the development of a progeroid-like phenotype (73). Homozygous *Zmpste24*-null mice die prematurely at 20 weeks on average. These animals display significant disturbances in the levels of various cardiac biomarkers (creatine kinase and glutamate dehydrogenase). Other alterations include dilated cardiomyopathy accompanied by muscle degeneration, lymphocyte infiltration, interstitial fibrosis and the presence of intracellular vesicles in the heart. Muscular alterations were not exclusive to cardiac muscle but also affected

skeletal muscle fibers, indicative of muscular dystrophy. Alterations to the lipid system were also reported (35). Similar phenotypes, including defects in pre-lamin A processing, are displayed by another *Zmpste24* loss-of-function model, in which the gene was inactivated by insertion of a neomycin resistance cassette into exon 8, which encodes the zinc binding domain (74). Curiously, however, these mice do not display the cardiomyopathy shown by the null model, even though they do present with muscle weakness and bone defects. Moreover, this second model showed no alterations in plasma levels of cardiac biomarkers (alanine and aspartate aminotransferases) relative to wild-type mice. Whether the differences between these two models are based on the alternative targeting approaches used to inactivate *Zmpste24* is unclear. Nevertheless, both mouse models represent valuable tools for investigating the molecular basis of restrictive dermopathy and progeria.

A transgenic mouse model of progeria was generated by introducing a human bacterial artificial chromosome containing the G608G mutated *LMNA* gene (59). These mice accumulate progerin and progressively lose VSMCs in the medial layer of large arteries (e.g. the descending aorta and carotid arteries), a common feature of HGPS patients. Nevertheless this animal model does not display the external phenotypes seen in human progeria, such as micrognathia and abnormal dentition (59). Among the notable features of this animal model are breakage of elastic fibers, thickening of adventitial and medial layers, proteoglycan accumulation and collagen deposition. Arterial calcification accompanied by extracellular matrix deposition is also observed in animals over 12 months old. Strikingly, these animals showed no differences from wild-type mice in life expectancy over the 20 month study period, and did not present with skin defects comparable to those seen in HGPS patients.

In all of the mouse models described so far, pre-lamin A and progerin are both farnesylated. This post-translational modification seems to be associated with the nuclear abnormalities induced by the accumulation of these proteins, since farnesyl-transferase inhibitors

(FTIs), which diminish protein farnesylation, prevent these nuclear defects (75) (see below). However, *Lmna*-nHG/+ knock-in mice expressing a non-farnesylable version of progerin display progeria-like symptoms, indicating that features of progerin other than the accumulation of farnesylated progerin underlie the severity of the disease (76). One possibility is that progerin might acquire an altered structure due to deletion of the 50 residue sequence, which would be independent of farnesylation. Another obvious candidate is the 15 C-terminal residues that are removed by FACE-1/Zmpste24 in healthy individuals (Figure 1) but are retained in the non-farnesylable version of progerin expressed by *Lmna*-nHG/+ mice. Consistent with this possibility, Davies et al. (77) very recently demonstrated that accumulation of non-farnesylated pre-lamin A leads to cardiac alterations resembling those found in progeria. The authors generated a knock-in mouse expressing a mutant form of pre-lamin A that, while it retains the 15 amino acids that are absent from mature lamin A, lacks the essential cysteine residue, rendering it non-competent for farnesylation. The results show that retention of the C-terminal 15 residues of pre-lamin A is important for the development of progeria-like symptoms in mice, and thus overturn the exclusive role in progeroid disease proposed until now for farnesylation. Mutant animals maintain near-normal body weight over a 60 week period, and bone lesions are not detected (77). However, survival curves show that mutant animals have a significantly curtailed lifespan compared with their normal littermates from 20 weeks onward. Mutant animals also show dilated cardiomyopathy, with increased left ventricle size and a reduced ejection fraction during diastolic movement. These echocardiogram findings were corroborated by diminished mRNA expression of myosin heavy chain alpha (Myh6) and increased expression of beta (Myh7), which constitutes a diagnostic marker of cardiomyopathy. Post-mortem examination detected significant fibrosis in the left ventricle. Thus the pathology of HGPS appears to arise not only from the conservation of the pre-lamin A farnesyl moiety but possibly also from the retention of the C-terminal 15 amino acids in progerin.

Other *Lmna* mutant mouse models that resemble the HGPS phenotype have been reported. In one, proline to leucine substitution at residue 530 (L530P), which is the mutation that causes the autosomal dominant form of Emery-Dreyfuss muscular dystrophy, produces severe growth retardation within the first 4-5 days of life and death within 4-5 weeks, defects reminiscent of the symptoms of HGPS patients (78). Homozygous mice (Lmna $^{L530P/L530P}$) display degeneration of cardiac muscle, with myocytes smaller than in counterpart littermates, accompanied by an increased number of fibrocytes. Even though these animals show other tissue pathologies in bone, muscle and skin, they do not have obvious defects in the aorta and small vessels (78). Levels of total triglycerides and free fatty acids were normal, while cholesterol levels were low.

6. OXIDATIVE STRESS IN HGPS

Oxidative stress triggered by the sustained production of oxidant molecules has been proposed as a major contributor to aging and its associated-degenerative pathologies, like cardiovascular disease (79, 80). These oxidants are mainly produced by mitochondria, whose integrity and function declines with age (81), resulting in the accumulation of damage to cell macromolecules, including DNA (82), proteins (83-87), and lipids (88). The accumulation of these damaged macromolecules contributes to cell, tissue and organismal aging and thus is responsible for age-related defects (89). Oxidative stress is well known to induce the accumulation of oxidized LDL, which plays an important role in the development of atherosclerosis (90, 91), and also in aging (92). Oxidative stress also induces loss of proliferative capacity, leading to irreversible cell growth arrest and premature cellular senescence (93). To counteract the effect of oxidative stress and the associated accumulation of damaged macromolecules, healthy cells activate a series of antioxidant defense mechanisms that maintain a well-balanced oxidant/antioxidant ratio (reviewed in (94)). Antioxidant molecules are produced

to decrease the levels of reactive oxygen species (ROS), proteasomal activity is increased to degrade oxidized proteins, and DNA repair systems are activated to repair oxidized DNA.

Progeroid syndromes share many features with normal aging. The first evidence that oxidative stress might contribute to the accelerated aging seen in HGPS patients was presented by Oliver et al. (95), who reported high levels of oxidatively modified proteins in the fibroblasts of individuals with genetic disorders associated with accelerated aging when compared with age-matched controls. The levels of oxidized proteins in these patients were in fact similar to those in cells from healthy 70- to 80-year-old individuals. Moreover, a recent study reported higher levels of ROS production in HGPS fibroblasts than in age-matched controls (96). Accumulation of ROS increases the level of protein oxidation and consequent protein damage. In this situation cells activate their enzymatic and non-enzymatic antioxidative defense mechanisms to reduce ROS concentrations (94). Interestingly, fibroblasts from HGPS patients express higher than normal levels of MnSOD mRNA and protein, without changes to other antioxidative enzymes, and MnSOD protein levels are also increased by cell passage in culture, which also models the aging condition (96). HGPS fibroblasts also display dysregulated mitochondrial function and proteasome activity (96). The reduced mitochondrial function is demonstrated by a decrease in ATP content, which leads to defective apoptosis and the accumulation of damaged cells. The impaired proteasomal activity of HGPS fibroblasts might underlie the deficient degradation of oxidized proteins in these cells, which impacts many important biological processes, including cell cycle progression, apoptosis and DNA repair (97). Contrasting with these findings, progerin-expressing mouse embryonic fibroblasts obtained from the transgenic mouse line carrying the G608G mutated human *LMNA* gene have similar levels of ROS, oxidatively-modified proteins and MnSOD as cells from control animals (96). It has been also shown that farnesylated pre-lamin A accumulates in fibroblasts with *LMNA* mutations and in cells from HIV patients treated with protease inhibitors that inhibit Zmpste24, and that these cells show mitochondrial

alterations, a key factor in cellular aging (98). *LMNA*-mutated fibroblasts also have higher levels of ROS production than control cells, with defective mitochondria being the most likely cause, and this leads to premature cell senescence. Both the induced oxidative stress and the associated senescent phenotype are prevented by inhibition of farnesylation (98), supporting the idea that accumulation of toxic farnesylated pre-lamin A causes this oxidative cell status, which might contribute to the aging process.

Ragnauth et al. (41) recently showed that pre-lamin A accumulates in the medial VSMCs of elderly individuals and in atherosclerotic lesions, as compared with vessels from young healthy individuals, consistent with the possibility that defective lamin A processing is associated with vascular aging in the normal population. This pre-lamin A accumulation is moreover accompanied by an induction of VSMC senescence. These authors also found that, in response to oxidative stress, FACE-1/Zmpste24 expression decreases and pre-lamin A content increases in medial VSMC, accompanied by up-regulation of the tumor suppressor p16 and the cell cycle arrest-related protein p21. These findings suggest that the oxidative stress-induced changes in the levels of FACE-1 and pre-lamin A might contribute to premature cellular senescence. The possible role of pre-lamin A in accelerating VSMC senescence is also supported by the finding that its long-term overexpression increases the levels of senescence-associated β-galactosidase. Moreover, overexpression of pre-lamin A impairs mitosis and activates DNA damage signaling, as indicated by DNA strand breakage (41).

In summary, only a few studies so far have analyzed the potential role of oxidative stress in the premature aging of HGPS patients and more work is warranted to clarify this question. More in-depth studies in mouse models of progeria may help to define the effects of progerin on oxidative stress status and its real contribution to the premature aging and associated cardiovascular disease seen in HGPS patients. For example, given the importance of proper endothelial function for preventing the development of vascular disease, it would be interesting

to determine whether or not these mouse models exhibit a degree of endothelial dysfunction comparable to that occurring in aging (99), and what effect oxidative stress in these models has on endothelial cells and their proliferation. Specifically, it would be interesting to measure the levels of nitric oxide produced by endothelial cells in these models, and to evaluate the levels of endothelial progenitor cells (EPCs), since it has been suggested that cardiovascular risk factors correlate inversely with the levels of circulating EPCs, which can therefore be used as predictors of cardiovascular events (100). Given that the endothelium regulates the proliferation of VSMCs, a cell type that is specifically targeted in HGPS, it would also be interesting to study the crosstalk between endothelial cells and VSMCs in HGPS. Moreover, information of the oxidative status of LDL in HGPS is lacking. A better understanding of these processes might lead to the development of antioxidant therapies to ameliorate the symptoms related to oxidative damage in HGPS.

7. THERAPEUTIC AGENTS FOR THE TREATMENT OF PROGERIA SYNDROMES

The pathological manifestations of progerin accumulation are ameliorated by the reduction of lamin A expression, as shown by disruption of one *LMNA* allele in *Zmpste24*-null mice, which prevents the appearance of several disease symptoms, including growth retardation (70, 101). Moreover, reduction of progerin expression in an inducible mouse model of progeria reverses the characteristic skin and tooth abnormalities (102), indicating the reversibility of the pathological phenotype induced by progerin accumulation. Some studies link the accumulation of farnesylated progerin with the observed nuclear abnormalities, and propose reduction of farnesylation as the key strategy for the treatment of the disease. Blockade of FTase activity with FTIs (for further detail see Figure 3) reverts the nuclear morphology abnormalities in several cell models of progeroid laminopathy, including fibroblasts from HGPS patients (43, 103, 104). This reversion is achieved without changes in progerin protein levels (105). Moreover, FTIs also

affect chromatin and chromatin-associated protein organization in cultured control human fibroblasts (106). It would be interesting to determine whether FTI treatment also improves chromatin and chromatin-associated protein alterations associated with progerin accumulation in HGPS patients (30, 60). Administration of FTI to *Lmna*(HG/+) mice carrying a HGPS-causing mutation increases body weight and reduces bone and muscle alterations (107). However, pre-lamin A processing is only reduced by 5%, which is less than expected (108). This has been attributed to the alternative prenylation of lamin A precursors with geranyl-geranyl groups when FTase is inhibited, which might produce similar effects to farnesylation (109) (Figure 3). It should also be remembered that features of progerin other than the accumulation of farnesylated progerin underlie the severity of the disease in mouse models, since *Lmna*-nHG/+ knock-in mice expressing a non-farnesylable version of progerin exhibit progeria-like symptoms (76). Therefore, while inhibiting the farnesylation of progerin and pre-lamin A might be therapeutically useful, this only partially reduces the accumulation of progerin accumulation and so cannot completely resolve the disease (76, 77).

Additional treatments for HGPS have been proposed, including gene therapy and pharmacological strategies. Reversion of abnormal nuclear shape in HGPS fibroblasts has been achieved with a morpholino antisense oligonucleotide directed against the alternative splice donor site in exon 11 (64) and with shRNA against progerin (101) (Figure 3). Other inhibitors of protein farnesylation such as statins, which inhibit HMG-CoA reductase (110-112), and aminobisphosphonates, which inhibit farnesylpyrophosphate synthase (113), have been demonstrated to inhibit the synthesis of both geranyl-geranyl and farnesyl groups (114, 115) (Figure 3) and have been employed in animal models. Used in combination in *Zmpste2*-null mice, statins and aminobisphosphonates efficiently inhibit both farnesylation and geranyl-geranylation of progerin and pre-lamin A, and produce an improvement in the phenotype (116). Based on these data, a clinical trial is underway in HGPS patients, targeting different points

along the pathway leading to progerin production with a combination of the statin pravastatin, the aminobisphosphonate zoledronic acid and the FTI lonafarnib (SCH 66336) (ClinicalTrials.gov identifier: NCT00879034, www.progeriaresearch.org).

Bone marrow transplantation and the use of isolated mesenchymal cells have proven therapeutic potential in patients with genetic disorders. For instance, grafts of healthy marrow-derived mesenchymal cells contribute to the formation of new tissue in children with severe bone and skeletal genetic disorders, improving the clinical symptoms of these patients (117). In progeroid laminopathies, where the progressive loss of VSMCs puts patients at increased risk of a cardiovascular incident, the use of stem cells to repopulate the damaged layers of large arteries might provide an effective treatment and should be considered for further study.

As discussed above, vascular damage in HGPS is probably linked to the protein oxidation and altered mitochondrial and proteasome function associated with the high levels of ROS in HGPS fibroblasts, which result in impaired apoptosis and accumulation of damaged cells (96). Moreover, oxidative stress downregulates FACE-1 and consequently upregulates pre-lamin A in VSMCs, contributing to premature cellular senescence (41). Interestingly, inhibition of farnesylation with FTI-277 or statins, which in addition to their cholesterol lowering effects are antioxidative molecules (118-121), prevents the increase in ROS production and the associated senescent phenotype of fibroblasts either carrying *LMNA* mutations or treated with Zmpste24-inhibiting HIV protease blockers (98). These treatments thus might prevent age-associated VSMC dysfunction. Taking these observations into account, it would be interesting to evaluate the use of antioxidant molecules, perhaps in combination with the statin–aminobisphosphonate–FTI cocktail, to prevent the vascular degeneration and accelerated aging that underlie the cardiovascular phenotypes of HGPS patients.

8. CONCLUDING REMARKS

Most cases of HGPS result from mutations in the *LMNA* gene that provoke the use of a cryptic splicing site in exon 11. This molecular alteration leads to the accumulation of progerin, a persistently farnesylated protein that causes structural abnormalities in the nuclear lamina that ultimately provoke the accumulation of DNA damage and genome instability. The importance of progerin farnesylation in the pathogenesis of HGPS is emphasized by the observation that treatment with FTIs ameliorates disease symptoms in both cellular and animal models of HGPS, although with less efficacy in the latter. The limitations of FTI treatment, as well as the mechanisms underlying their potentially beneficial effects, are not completely understood. Some beneficial actions of FTIs might result from reduced farnesylation of proteins other than progerin, or might be mediated by unrelated mechanisms. Moreover, FTI treatment appears to trigger modification of progerin with alternative prenyl groups. Animal studies provide evidence that the pathology of HGPS may arise not only from the conservation of the pre-lamin A farnesyl moiety but possibly also from the retention of the C-terminal 15 amino acids in progerin. Therefore efficient treatment of HGPS will require the development of alternative or complementary treatments. The ongoing clinical trial of a combination of statins, aminobiphosphonates and FTIs to block different enzymes involved in progerin isoprenylation promises to provide an advance in this direction. Emerging evidence suggests that oxidative stress might play a role in the premature aging associated with HGPS, and therefore antioxidative strategies might be beneficial in these patients. Importantly, recent evidence shows that both progerin and pre-lamin A progressively accumulate in the cells of healthy individuals – progerin as a consequence of the infrequent use of the aberrant splicing site in *LMNA* exon 11, and pre-lamin A as a result of the downregulation of FACE-1/Zmpste24. Research into HGPS thus has the potential not only to identify novel therapeutic strategies against this devastating disease but also to shed light into the mechanisms underlying physiological aging.

9. ACKNOWLEDGMENTS

Authors Laia Trigueros, Jose M Gonzalez and Jose Rivera equally contributed to this article. We thank Simon Bartlett for English editing and María J. Andrés for help preparing the figures. The author's lab is funded by the Spanish Ministry of Science and Innovation (MICINN) and the Fondo Europeo de Desarrollo Regional (FEDER) (grant SAF2007-62110), the Instituto de Salud Carlos III (ISCIII) (RECAVA, grant RD06/0014/0021), the Fundación Ramón Areces and Fina Biotech. J.M.G. is supported by the ISCIII. The CNIC is supported by the MICINN and the Pro-CNIC Foundation.

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FIGURE LEGENDS

Figure 1. Lamin A/C maturation and defective processing in HGPS patients and *Zmpste24*-knockout mice. Lamin A is generated as pre-lamin A and then undergoes a series of posttranslational modifications. First, pre-lamin A is farnesylated by a farnesyltransferase (FTase) at the cysteine in the C-terminal CAAX motif (CSIM). The AAX (SIM) motif is then cleaved by Rce1, and the carboxyl methyltransferase ICMT methylates the C-terminal cysteine residue. Finally, the 15 residues from the C terminus, including the farnesylated and carboxymethylated C-terminal cysteine, are cleaved by the endoprotease Zmpste24/FACE-1. In most HGPS patients, the mutation GGC to GGT in *LMNA* codon 608 (G608G) activates a cryptic splice site, causing deletion of a fifty amino acid sequence that includes the Zmpste24 cleavage site. Therefore cleavage of the 15 C terminal residues cannot occur and cells produce progerin, a mutant form of farnesylated pre-lamin A whose accumulation leads to nuclear abnormalities. In the *Zmpste24*-knockout mice, the absence of Zmpste24 causes the accumulation of farnesylated pre-lamin A whose accumulation causes a progeroid syndrome reminiscent of progerin-dependent HGPS.

Figure 2. Defective processing of lamin A in Hutchinson-Gilford Progeria Syndrome patients and during physiological aging and associated cardiovascular alterations. (A) Cells from HGPS patients accumulate progerin. (B) Cells from unaffected elderly subjects accumulate progerin and prelamin A. The accumulation of these proteins is associated with a series of cellular morphological and functional alterations that underlie several cardiovascular pathologies.

Figure 3. Pathway of biosynthesis of isoprenoid and cholesterol biosynthetic pathways, showing potential targets for the therapeutic inhibition of lamin A/C isoprenylation. Statins, amino-bisphosphonates (N-BPs), farnesyl transferase inhibitors (FTI), and geranylgeranyl transferase inhibitors (GGTI) can inhibit several steps of lamin A/C isoprenylation. Additionally, gene therapy strategies using morpholino antisense oligonucleotides and shRNA can reduce aberrant splicing of the mutated *LMNA* transcript and consequently reduce progerin accumulation. See text for details.

Figure 1

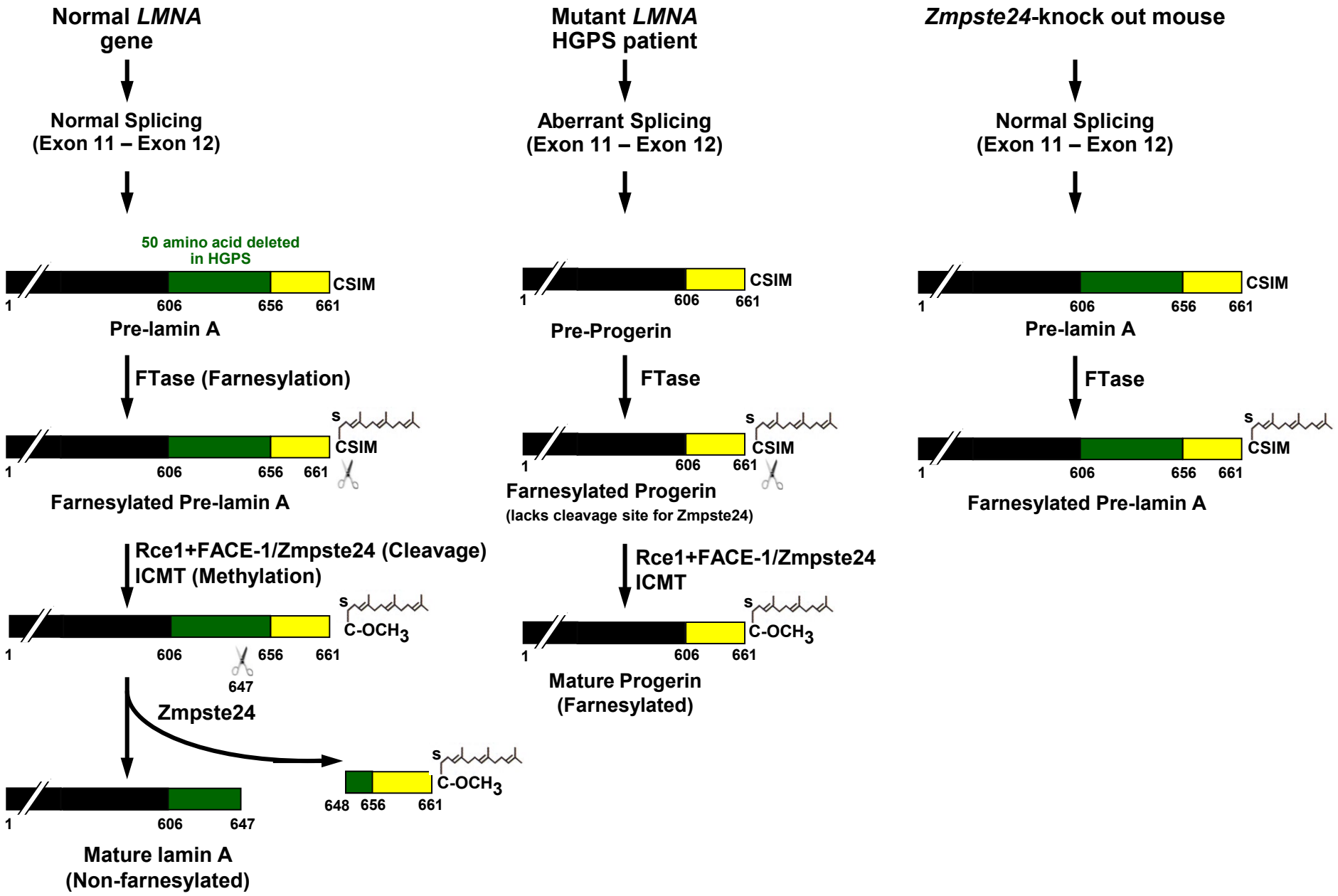

Normal *LMNA* gene
Normal Splicing (Exon 11 – Exon 12)
50 amino acid deleted in HGPS
CSIM
1
606
656
661
Pre-lamin A
FTase (Farnesylation)
S
Farnesylated Pre-lamin A
Rce1+FACE-1/Zmpste24 (Cleavage)
ICMT (Methylation)
C-OCH3
647
Zmpste24
648
Mature lamin A (Non-farnesylated)
Mutant *LMNA* HGPS patient
Aberrant Splicing (Exon 11 – Exon 12)
Pre-Progerin
FTase
Farnesylated Progerin
(lacks cleavage site for Zmpste24)
Rce1+FACE-1/Zmpste24
ICMT
Mature Progerin (Farnesylated)
Zmpste24-knock out mouse
Normal Splicing (Exon 11 – Exon 12)
Pre-lamin A
FTase
Farnesylated Pre-lamin A

Figure 2

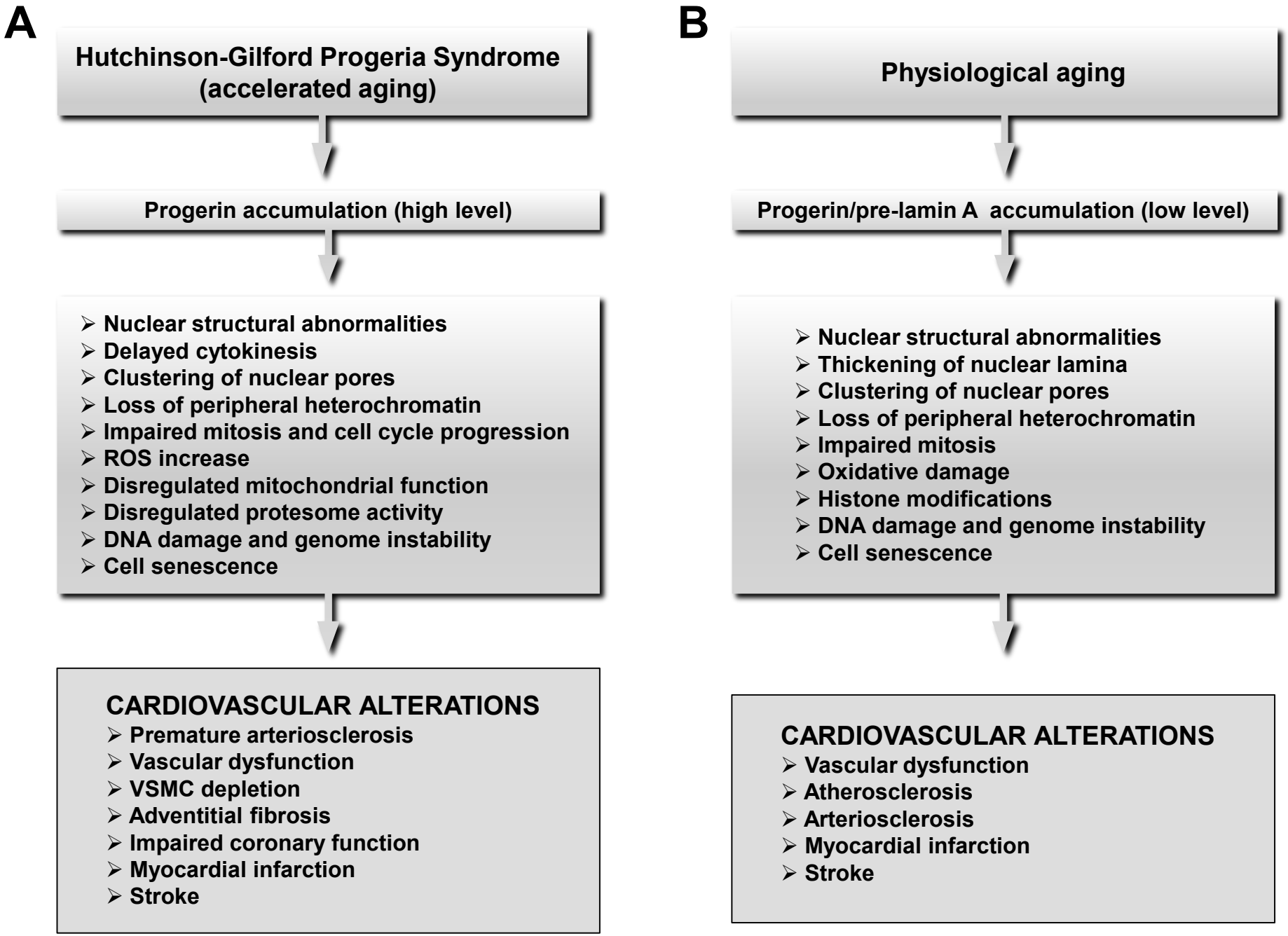
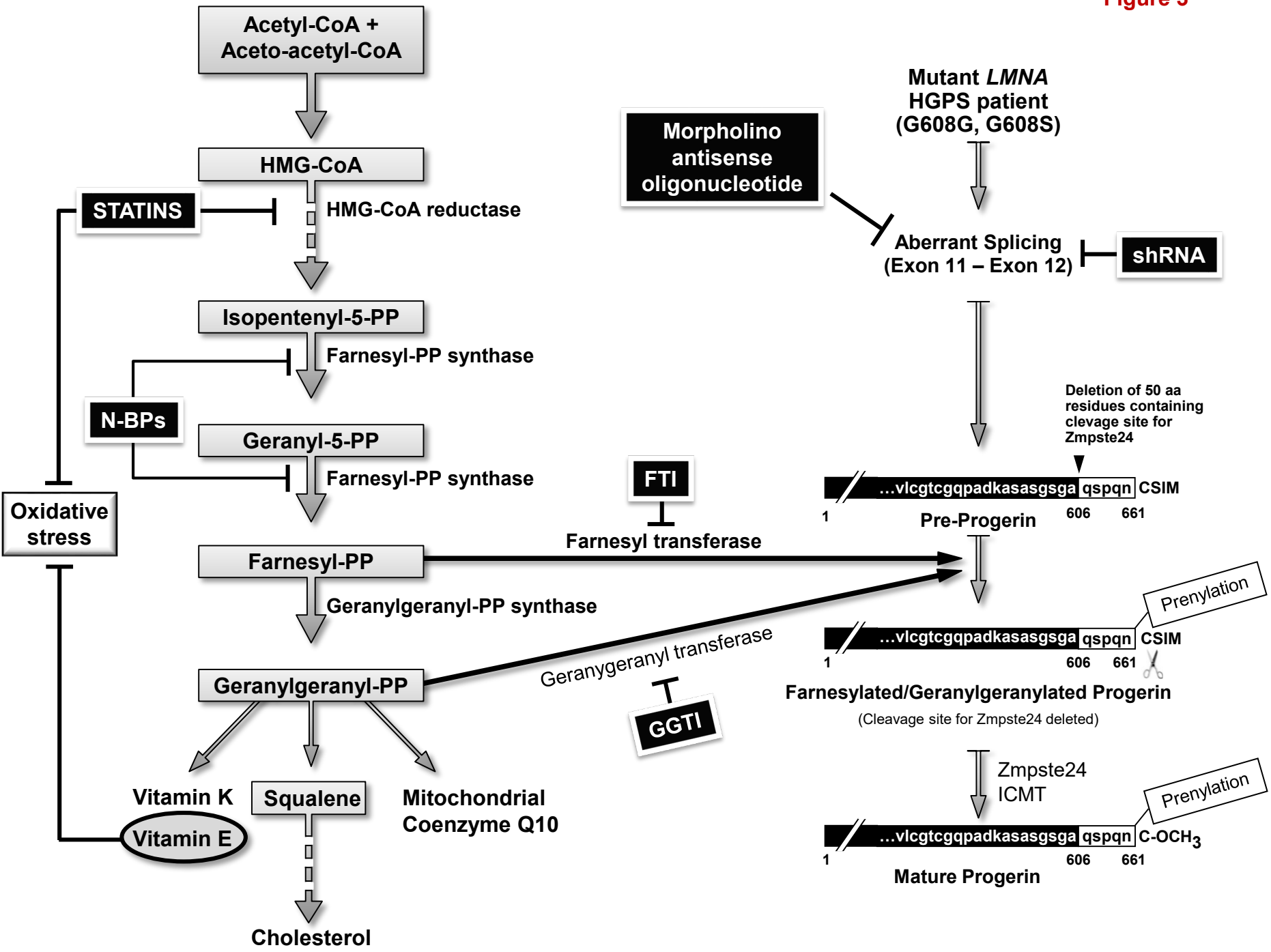

A
Hutchinson-Gilford Progeria Syndrome (accelerated aging)
Progerin accumulation (high level)
Nuclear structural abnormalities
Delayed cytokinesis
Clustering of nuclear pores
Loss of peripheral heterochromatin
Impaired mitosis and cell cycle progression
ROS increase
Disregulated mitochondrial function
Disregulated protesome activity
DNA damage and genome instability
Cell senescence
CARDIOVASCULAR ALTERATIONS
Premature arteriosclerosis
Vascular dysfunction
VSMC depletion
Adventitial fibrosis
Impaired coronary function
Myocardial infarction
Stroke
B
Physiological aging
Progerin/pre-lamin A accumulation (low level)
Nuclear structural abnormalities
Thickening of nuclear lamina
Clustering of nuclear pores
Loss of peripheral heterochromatin
Impaired mitosis
Oxidative damage
Histone modifications
DNA damage and genome instability
Cell senescence
CARDIOVASCULAR ALTERATIONS
Vascular dysfunction
Atherosclerosis
Arteriosclerosis
Myocardial infarction
Stroke

Figure 3


Acetyl-CoA +
Aceto-acetyl-CoA
HMG-CoA
STATINS
HMG-CoA reductase
Isopentenyl-5-PP
Farnesyl-PP synthase
N-BPs
Geranyl-5-PP
Farnesyl-PP synthase
Oxidative stress
FTI
Farnesyl transferase
Farnesyl-PP
Geranylgeranyl-PP synthase
Geranylgeranyl-PP
Geranygeranyl transferase
GGTI
Vitamin K
Vitamin E
Squalene
Mitochondrial Coenzyme Q10
Cholesterol
Mutant LMNA
HGPS patient
(G608G, G608S)
Morpholino antisense oligonucleotide
Aberrant Splicing
(Exon 11 – Exon 12)
shRNA
Deletion of 50 aa residues containing clevage site for Zmpste24
…vlcgtcgqpadkasasgsga qspqn CSIM
1
606
661
Pre-Progerin
Prenylation
…vlcgtcgqpadkasasgsga qspqn CSIM
1
606
661
Farnesylated/Geranylgeranylated Progerin
(Cleavage site for Zmpste24 deleted)
Zmpste24
ICMT
Prenylation
…vlcgtcgqpadkasasgsga qspqn C-OCH3
1
606
661
Mature Progerin