
Hutchinson-Gilford Progeria Syndrome

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1. Introduction

Hutchinson-Gilford Progeria Syndrome (HGPS) is a lethal congenital disorder, characterised by premature appearance of accelerated ageing in children. Although HGPS was first described by Jonathan Hutchinson [1] and then by Hastings Gilford [2] more than a century ago, it was not until 2003 that the genetic basis of HGPS was uncovered [3, 4]. Approximately 90% of HGPS patients have an identical mutation in paternal allele of the *LMNA* gene – a substitution of cytosine to thymine at nucleotide 1824, c.1824C>T. Although apparently a silent mutation (that is, no change in the amino acid, G608G), it causes aberrant mRNA splicing, which leads to the production of a truncated and partially processed pre-lamin A protein called “progerin” [3, 4]. Accumulation of progerin is thought to underlie the pathophysiology of HGPS. Individuals with HGPS appear to show ageing-related phenotypes at a much faster rate than normal, consequently leaving young children with the appearance and health conditions of an aged individual. The reported incidence of HGPS is 1 in 4 to 8 million newborns and 89 patients are currently known to be alive with HGPS worldwide [5]. The observed male to female ratio of incidence of HGPS is 1.2:1 and there has been no report on ethnic-specific recurrence. HGPS affect diverse body systems including growth, skeleton, body fat, skin, hair, and cardiovascular system. However, patients show no defects in their mental and intellectual abilities [6-8]. Surprisingly, progerin has also been found in normal unaffected individuals and its level increases with age, suggesting a similar genetic mechanism in progeria as in normal physiological ageing. Thus, numerous animal models have been developed to better understand the mechanism(s) of HGPS and to develop cure for this devastating disease.

In this chapter, the main aspects of HGPS such as signs and symptoms, genetic basis, animal models, and treatments will be discussed.

2. Signs and symptoms

The median age at diagnosis of HGPS is 2.9 years [6]. The diagnosis is generally straightforward as affected patients show classical symptoms and strongly resembles one another. The affected individuals display no signs of disease at birth, but within their first years of life they gradually develop an appearance often referred to as aged-like [9, 10]. Some of the typical physical characteristics of HGPS include alopecia (loss of hair including scalp and eyebrows), prominent scalp veins and forehead, classical facial features including frontal bossing, protruding ears with absent lobes, a glyphic (broad, mildly concave nasal ridge) nose, prominent eyes, thin lips and micrognathia (small jaw) with a vertical midline groove in the chin [7, 11, 12] (Figure 1). Abnormal and delayed dentition is also common, and thin and often tight skin results from significant loss of subcutaneous fat [7, 10] (Figure 1). HGPS patients have high-pitched voices, a horse-riding stance, limited joint mobility and have short stature (median final height of 100-110 cm; median final weight of 10-15 kg). As they mature, they develop osteolysis, particularly involving the distal phalanges and clavicles [6-8, 11, 13]. On average, death occurs at the age of 13, with at least 90% of HGPS subjects dying from progressive atherosclerosis of the coronary and cerebrovascular arteries [7].



Figure 1. Photographs of a 7 year-old girl with HGPS (*LMNA* c.1824C>T, p.G608G). This patient has typical phenotypes, including alopecia, thin and tight skin, loss of subcutaneous fat, prominent scalp veins and forehead, prominent eyes, protruding ears, thin lips, and small jaw. Photos were from courtesy of The Progeria Research Foundation.

Recently, Olive *et al.* reported similarities between many aspects of cardiovascular disease in HGPS patients and normal adult individuals with atherosclerosis and suggested that progerin may be a contributor to the risk of atherosclerosis in the general population [14]. HGPS patients exhibited features that are classically associated with the atherosclerosis of ageing, including presence of plaques in the coronary arteries, arterial lesions showing calcification, inflammation, and evidence of plaque erosion or rupture. Authors speculated that

progerin accumulation in vascular cells causes nuclear defects and increases susceptibility to mechanical strain that in turn triggers cell death and inflammatory response, giving rise to atherosclerosis [14].

Interestingly, despite the presence of multiple premature ageing symptoms, many other organs, such as liver, kidney, lung, brain, gastrointestinal tract, and bone marrow, appear to be unaffected. Furthermore, not all of the ageing processes are advanced in affected children. For example, the prevalence of mental deterioration, cancer, and cataract, is not higher in HGPS patients [7]. To date, there are scarce explanations as to why only certain organs are affected in HGPS. Nevertheless, researchers have been trying to clarify some of these puzzling observations. Recently, Jung and colleagues suggested that the absence of cognitive deficits in HGPS patients may be explained by the down regulation of pre-lamin A expression in the brain [15]. Furthermore, authors hypothesised that low level of pre-lamin A in the brain may be regulated by a brain-specific microRNA (miRNA), miRNA-9. In support of the result from this study, Nissan *et al.* lately published a promising result showing that miRNA-9 inversely regulates lamin A and progerin expression in neural cells and proposed that protection of neural cells from toxic accumulation of progerin in HGPS may be due to expression of miRNA-9 [16]. Further studies, possibly using animal models, are required to investigate changes in the expression of miRNA-9 and its effects on the level of progerin in the brain.

The clinical features seen in HGPS strongly resemble several aspects of natural ageing. For this reason, HGPS has served as a useful model for deciphering some of the mechanisms underlying physiological ageing. The first evidence for changes of nuclear architecture during the normal ageing process came from work in *C. elegans* [17]. In this study, the authors demonstrated that nuclear defects accumulate during ageing and suggested that HGPS may be a result of increased rate of the normal ageing process [17]. Scaffidi and Misteli showed that cells from HGPS patients and normally aged individuals share several common nuclear defects [18]. In addition, a small amount of progerin protein was detected in protein extracts derived from elderly individuals which was absent in young samples [19]. Rodriguez *et al.* quantified the levels of progerin transcripts using real time quantitative RT-PCR and showed that the progerin transcript is present in unaffected old individuals, though at a very low level compared to HGPS patients, and this level increased with *in vitro* ageing, similarly to HGPS cells [20]. Recently, Olive and others have also reported that although the level of progerin is much higher in HGPS patients, progerin is also present in the coronary arteries of non-HGPS ageing individuals and significantly increases with advancing age [14]. On the whole, accumulation of progerin, which is formed sparsely over time as a result of the ageing process, appears to be a possible candidate and partially responsible for cellular senescence and genomic instability that is observed in ageing cells. In HGPS, this occurs at a substantially faster rate compared to normally-aged cells due to enhanced use of the cryptic splice donor site, producing higher level of progerin. The relationship between this disease of accelerating ageing and the onset of analogous symptoms during the lifespan of a normal individual is unclear. Nevertheless, the idea that progerin may play a role in general human ageing is supported by the numerous studies mentioned above.

3. Genetic basis

The *LMNA* gene is known to be a hotspot for disease-causing mutations and has gained much attention due to its association with a variety of human diseases. To date, more than 400 mutations spreading across the protein-coding region of the *LMNA* gene have been discovered (see review [21]). The *LMNA* gene is found at chromosome 1q21.2-q21.3 and is composed of 12 exons. Through alternative splicing, the *LMNA* gene encodes the A-type lamins, lamins A and C (lamin A, AΔ10, C, and C2), of which lamin A (encoded by exons 1-12) and lamin C (encoded by exons 1-10) are the major isoforms expressed in all differentiated cells in vertebrates [22, 23]. The B-type lamins, lamins B1 and B2, are another type of lamins, which are encoded by the *LMNB1* and *LMNB2* genes, respectively. The B-type lamins are found in all cells and are expressed during development. Lamin A, C, B1 and B2 are key structural components of the nuclear lamina, an intermediate filament structure that lies on the inner surface of the inner nuclear membrane and is responsible for maintaining structural stability and organising chromatin (see review [24]). The nuclear lamina determines the shape and size of the cell nucleus, and is involved in DNA replication and transcription. In addition, nuclear lamina has been shown to interact with several nuclear membrane-associated proteins, transcription factors, as well as heterochromatin itself. The nuclear lamina is required for most nuclear activities, such as chromatin organisation, DNA replication, cell cycle regulation, nuclear positioning within the cell, assembly/disassembly of the nucleus during cell division, as well as for modulating master regulatory genes and signalling pathways [25-27]. There are more than 10 different disorders that are caused by mutations in the *LMNA* gene and these disorders are collectively called laminopathies and include neuropathies, muscular dystrophies, cardiomyopathies, lipodystrophies, in addition to progeroid syndromes (see Chapter on Laminopathies).

The genetic basis for HGPS was unknown until it was found to be a single nucleotide mutation on the paternal allele with autosomal-dominant expression [3, 4]. Although numerous mutations have been reported to cause HGPS [4, 28-33], approximately 90% of cases are caused by a recurrent, dominant, *de novo* heterozygous silent amino acid substitution at c. 1824C>T, G608G (a change from glycine GGC to glycine GGT, referred to as G608G) of the *LMNA* gene [4] (Figure 2). This mutation is located in exon 11 of *LMNA* gene and results in increased activation of the cryptic splice donor site, splicing the *LMNA* gene at 5 nucleotides upstream of the mutation, leading to accumulation of aberrant mRNA transcript, missing 150 nucleotides from normal pre-lamin A. This mutated mRNA is then translated into a protein termed 'progerin', which is missing 50 amino-acid residues from its C-terminal region. It has been suggested that different mutations cause activation of the same cryptic splice site in exon 11 of *LMNA* gene, and disease severity is correlated with the usage of this splice site (Figure 2). For instance, Moulson and others described two patients with particularly severe progeroid symptoms, clearly more severe than a typical case of HGPS [30]. In both cases, the amount of progerin relative to properly processed pre-lamin A was significantly greater than that of in typical HGPS, suggesting that the severity of the disease appears to be dependent on the amount of progerin in cells [30]. Very recently, another more severe case was reported by Reunert *et al* [31]. This patient had the heterozygous *LMNA* mutation c.

1821G>A, which lead to neonatal progeria with death in the first year of life [31]. Authors showed that the ratio of progerin protein to mature lamin A was higher in this patient compared to classical HGPS and also proposed that this ratio determines the disease severity in progeria [31]. Opposite cases were also shown by Hisama and colleagues. In this study, mutations at the junction of exon 11 and intron 11 of the *LMNA* gene resulted in a considerably lower level of progerin compared to HGPS, giving rise to an adult-onset progeroid syndrome closely resembling Werner syndrome [33].

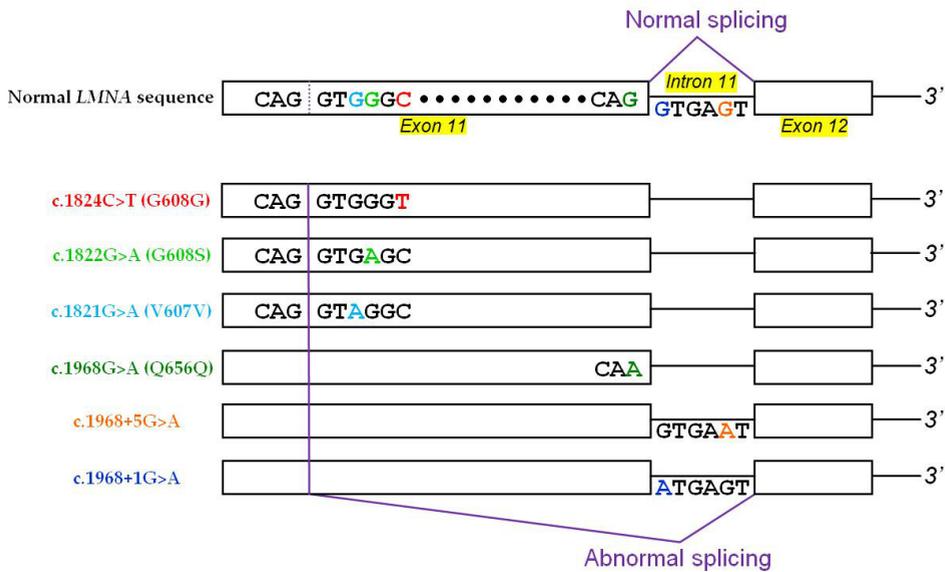


Figure 2. A schematic diagram showing point mutations leading to increased activation of a cryptic splice site within exon 11 of the *LMNA* gene [4, 30, 31, 33]. All of these mutations results in an internal deletion of 150 nucleotides of exon 11, ultimately leading to the production of an abnormally processed protein called ‘progerin’. It is interesting to note that the normal *LMNA* sequence can also be spliced abnormally, removing 150 nucleotides of exon 11, in healthy individuals and this incidence may increase with age, leading to cellular senescence [18, 20].

Under the normal condition, mature lamin A protein is produced from a precursor, pre-lamin A, via a series of post-translational processing steps, which begins at the C-terminal end. The *CaaX* motif at the C-terminal tail (where the *C* is a cysteine, the *a* residues are aliphatic amino acids, and the *X* can be any amino acids) signals for 4 sequential modifications (Figure 3A). Firstly, the cysteine of the *CaaX* motif is farnesylated by a farnesyltransferase (FTase), then the last three amino acids (*aaX*) are cleaved by a zinc metalloprotease, ZMPSTE24 (mouse) or FACE-1 (human). Following this cleavage, farnesylated C-terminal cysteine is methylated by isoprenylcysteine carboxy-methyl transferase (ICMT). Finally, the last 15 amino acids of the protein are cleaved again by ZMPSTE24, producing mature lamin A.

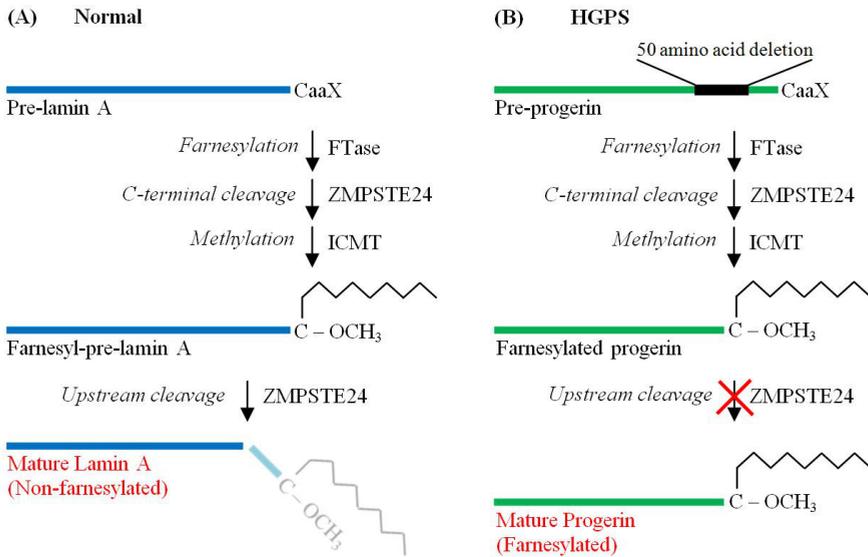


Figure 3. Post-translational processing of pre-lamin A in (A) normal condition and of truncated pre-lamin A (“pre-progerin”) in (B) HGPS. The proteolytic cleavage site (RSYLLG motif) lies within the 50 amino acid region that is lost due to HGPS mutation, and as a result, the ZMPSTE24 endoprotease cannot recognise and perform subsequent upstream cleavage. Consequently, a truncated lamin A protein (that is, progerin) remains farnesylated, which is believed to have a dominant negative effect in HGPS.

In HGPS, the first 3 steps of post-translational maturation can be performed (that is, farnesylation, cleavage, and methylation), while the fourth processing step cannot be completed as the G608G mutation eliminates the second cleavage site recognised by ZMPSTE24 of pre-lamin A resulting a permanently farnesylated form of progerin (Figure 3B) [34]. This improperly processed protein in HGPS is thought to underlie the progression of the disease phenotype [35]. Because progerin, unlike mature lamin A, remains farnesylated, it gains a high affinity for the nuclear membrane, consequently causing a disruption in the integrity of the nuclear lamina. Indeed, HGPS patient cells show a number of abnormalities in nuclear structure and function. Upon indirect immunofluorescence labelling with antibodies directed against lamins A/C, fibroblasts from individuals with HGPS were characterised by the presence of dysmorphic nuclei with altered size and shape, presence of lobules, wrinkles, herniations of the nuclear envelope, thickening of the nuclear lamina, loss of peripheral heterochromatin, and clustering of nuclear pores [4, 36, 37]. These features worsen with passages in cell culture and are correlated with an apparent intranuclear accumulation of progerin (Figure 4) [36, 38]. In addition to permanent farnesylation of the progerin, it has been hypothesised that the deletion of the phosphorylation site (Ser 625) found in the 50 amino acid-deleted region may also account for some of the HGPS phenotypes as cell cycle dependent phosphorylation of lamin A is important for its normal function [4, 39].

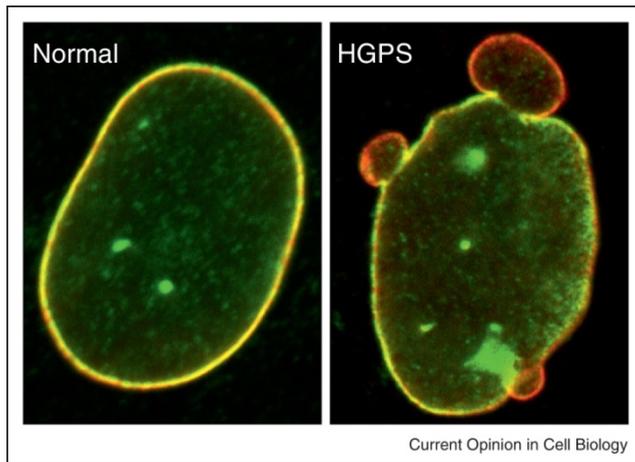


Figure 4. Immunostaining of skin fibroblasts taken from a normal individual (left) and a HGPS patient (right) showing nuclear blebbing. Lamin A/C is labelled red and lamin B1 in green. Note that the expression of lamin B1 is lost in the blebbed region. The figure has been adapted from Shimi *et al.* (2012) [40], with permission from Elsevier.

Numerous studies have addressed the senescent characteristics of HGPS cells, which intriguingly parallel with properties of fibroblasts from aged individuals. Cellular senescence is a hallmark characteristic of the ageing process, and cell nuclei from old individuals have similar defects to those of HGPS patient cells, including increased DNA damage [18, 41, 42], down-regulation of several nuclear proteins, such as the heterochromatin protein HP1 and the LAP2 group of lamin A-associated proteins [18, 37], and changes in histone modifications [18]. Heterochromatin becomes more disorganised with increased ageing in patients [43], and deregulation of chromatin organisation is a common phenomenon in HGPS, where progerin is known to alter histone methylation [44, 45]. Interestingly, the cryptic splice site that is constitutively activated in HGPS is seldom used in “normal” pre-lamin A processing in healthy aged individuals (Figure 2). To directly demonstrate that the production of progerin by sporadic use of the cryptic splice donor site in *LMNA* exon 11 is responsible for the observed changes in nuclear architecture in cells from aged individuals, Scaffidi and Mistelli used a morpholino oligonucleotide to inhibit this cryptic splice site and consequently the production of progerin and showed that the nuclear defects were reversed [18].

Although the amount of progerin in cells is considerably lower than the amount of lamin A and lamin C [46], it is obvious that this small amount of progerin is very potent in terms of causing disease phenotypes in humans and in causing misshaped nuclei in cultured cells. Supporting the hypothesis that progerin exerts dominant negative effect in HGPS, Goldman and colleagues introduced progerin into normal cells via transfection and showed that progerin is targeted to the nuclear envelope and is entirely responsible for the misshapen nuclei. Same changes were observed when progerin protein was microinjected into cytoplasm of the normal cells [36]. It was hypothesised that retention of the farnesyl group on progerin may be the key factor in the development of the HGPS phenotype. Indeed, several different

research groups showed that the nuclear abnormalities were alleviated or reversed by the inhibition of farnesylation [47-49]. Briefly, Yang and colleagues used mouse embryonic fibroblasts from a transgenic mouse expressing progerin (*Lmna*^{HG/+}) and showed that the treatment with a protein farnesyltransferase inhibitor (FTI) reduced nuclear blebbing to a baseline level observed in untreated wild-type cells [47]. Capell's group used transfection technique to demonstrate that the percentage of blebbed nuclei in HeLa cells that are transfected with progerin vector, decreased with FTI treatment in a dose-dependent manner [48]. Finally, Glynn and Glover showed a significant improvement in the nuclear morphology of HGPS cells or cells expressing mutant lamin A following FTI treatment [49].

There are several other *de novo* dominant *LMNA* mutations that are found less frequently and are known to cause atypical HGPS (see review [50]). Clinically, atypical HGPS patients exhibit additional signs and symptoms of classical HGPS or lack some of phenotypes observed in classical form. These overlapping and distinct clinical features of atypical HGPS are well described by Garg and colleagues [51].

4. Animal models

Animal models of HGPS have been a valuable tool in the study of the pathological processes implicated in the origin of this disease as well as finding a cure. Some of these mouse models are designed to express the exact mutation that is observed in human HGPS patients, or have defect in the lamin A processing. These mouse models are summarised in Table 1.

In 2006, Varga and colleagues generated a transgenic mouse model for HGPS by introducing a human bacterial artificial chromosome (BAC) c.1824C>T mutated *LMNA* gene. These animals over-expressed human lamin A/C and progerin in all tissues. Although this animal model did not display any external phenotypes seen in HGPS patients, such as growth retardation, alopecia, micrognathia and abnormal dentition, it progressively lost vascular smooth muscle cells in the medial layer of large arteries that closely resembled the most deadly aspect of the HGPS patients. Surprisingly, these animals showed no differences in their life expectancy compared to their wild-type littermates [52].

The *Zmpste24*^{-/-} model was first developed by Leung and co-workers [53]. This model is a complete knock-out model, in which animals do not have any *ZMPSTE24* enzyme. Disruption of the gene encoding *ZMPSTE24* in mice causes defective lamin A processing, which results in the accumulation of farnesylated pre-lamin A at the nuclear envelope [54, 55]. Since these *ZMPSTE24*-deficient mice have shown to have many features that resemble HGPS and other laminopathies (diseases that are caused by mutations in the nuclear lamina), this model has served as a crucial tool to explore the mechanisms underlying these diseases and to design therapies for the treatment [55, 56]. In addition to being a model for HGPS, *Zmpste24*^{-/-} mice also showed numerous characteristics of mandibuloacral dysplasia (MAD) [54], which promoted researchers to search for *ZMPSTE24* mutations in MAD patients [57]. Furthermore, loss of *ZMPSTE24* in humans has been shown to cause restrictive dermopathy, a lethal perinatal progeroid syndrome characterised by tight and rigid skin

with erosions, loss of fat and prominent superficial vasculature, thin hair, micrognathia, joint contractures, and thin dysplastic clavicles [58]. The *Zmpste24^{-/-}* mice look normal at birth, but develop skeletal abnormalities with spontaneous bone fractures. Furthermore, they show progressive hair loss, abnormal teething, muscle weakness, which ultimately lead to premature death at the age of 20-30 weeks [54, 55].

As an additional proof of the toxic effects of pre-lamin A accumulation, Fong and others compared the phenotypes of *Zmpste24^{-/-}* mice and littermate *Zmpste24^{-/-}* mice bearing one *Lmna* knock-out allele (*Zmpste24^{-/-}Lmna^{+/-}*) [59]. In this study, the authors showed that double knock-out mice carrying the *Zmpste24^{-/-}Lmna^{+/-}* genotype, expressing half the pre-lamin A of *Zmpste24^{-/-}Lmna^{+/+}* mice were completely protected from all disease phenotypes, including reduced growth rate, muscle strength and impaired bone and soft tissue development, and shortened lifespan. Furthermore, the frequency of misshapen nuclei in *Zmpste24^{-/-}Lmna^{+/-}* fibroblasts was significantly lower than fibroblasts from *Zmpste24^{-/-}Lmna^{+/+}* mice. The results from this study not only suggest that the accumulation of the farnesylated pre-lamin A is toxic, but also show that lowering the level of pre-lamin A have a beneficial effect on disease phenotypes in mice and on nuclear shape in cultured cells [59].

The *Lmna^{HG/+}* model is a progerin knock-in mouse model, in which one of the *LMNA* alleles only expresses progerin, while the other expresses lamin A/C. These animals show several HGPS-related phenotypes, including bone alterations, reduction in subcutaneous fat and premature death at around 28 weeks of age [47, 60]. Although *Lmna^{HG/+}* mice clearly show many of the early symptoms of HGPS, they do not display any signs of atherosclerosis in the intima or media of the aorta. This was surprising as most of HGPS patients die from cardiovascular complications and authors speculated that absence of these cardiovascular-related phenotypes in *Lmna^{HG/+}* mice is simply because these mice do not live long enough to develop these deficits [60]. In the homozygous *Lmna^{HG/HG}* animals, both *LMNA* alleles express progerin and therefore, lamin A/C is not produced. These animals exhibit severe growth retardation with complete absence of adipose tissue and numerous spontaneous bone fractures. They die at 3-4 weeks of age with poorly mineralised bones, micrognathia, craniofacial abnormalities [60].

In all of the mouse models described above, both pre-lamin A and progerin are farnesylated. Since the disease phenotypes in *Lmna^{HG/+}* mice were alleviated with a FTI, it was logical to suppose that the protein prenylation is important for disease pathogenesis [60-62]. To further elucidate this subject, Yang *et al.* created a knock-in mice expressing non-farnesylated progerin (*Lmna^{nHG/+}*), in which progerin's C-terminal -CSIM motif was changed to -SSIM. This single amino acid substitution eliminated protein prenylation and two following processing steps (cleavage of the last 3 amino acids and methylation, Figure 3) [63]. Yang and colleagues expected that *Lmna^{nHG/+}* mice would be free of disease, but surprisingly these animals developed all of the same disease phenotypes found in *Lmna^{HG/+}* mice and invariably succumbed to the disease [63]. Persistence of disease phenotype in *Lmna^{nHG/+}* mice, though milder than *Lmna^{HG/+}* mice, raised doubts about the primacy of the protein prenylation in disease pathogenesis suggesting that features of progerin other than the accumulation of farnesylated progerin may underlie the severity of the disease [63]. In order to

investigate the toxicity of the non-farnesylated progerin produced by the *Lmna*^{nHG} allele, Yang *et al.* generated another non-farnesylated progerin allele (*Lmna*^{csnHG}), in which the progerin's C-terminal ends with the -CSM compared to the -SSIM ending in *Lmna*^{nHG} allele [64]. CSM progerin cannot be prenylated, but it retains a C-terminal cysteine similar to the CSIM progerin that accumulates in FTI-treated *Lmna*^{HG/+} mice. Astonishingly, mice containing the *Lmna*^{csnHG} allele were free of HGPS-like disease phenotypes. Even the homozygous mice (*Lmna*^{csnHG/csnHG}), which produce exclusively progerin and no lamin A/C, were absent of all the characteristics of HGPS [64]. Furthermore, nuclear abnormalities were also milder in both types of mice. This study demonstrated that the toxicity of non-farnesylated progerin depends on the mutation used to abolish protein farnesylation [64]. The absence of HGPS-like phenotypes in mice expressing *Lmna*^{csnHG} allele is consistent in mice expressing farnesylated and non-farnesylated forms of pre-lamin A. While expression of farnesylated pre-lamin A in *Zmpste24*^{-/-} mice results in a severe HGPS-like symptoms [54, 59, 65, 66], mice expressing non-farnesylated pre-lamin A (*Lmna*^{nPLA0/nPLA0}) exhibited no HGPS-like phenotypes [67].

Although all differentiated cells express lamin A [22], there is still no clear explanation as to why the HGPS-related symptoms are limited to particular tissues and organs. Due to this segmental nature of HGPS with clinical features only present in restricted tissues, developing an ideal representative mouse model for HGPS has been a challenge. However, by using tissue-specific promoters, researchers have succeeded in designing transgenic mouse models expressing progerin in specific tissues. Unlike general knock-out or knock-in mouse models, transgenic mouse models using tissue-specific promoters provide a wealth of information about the function of specific genes, the *LMNA*, in case of HGPS. For example, Wang and others created a transgenic mouse line that expresses progerin in the epidermis by using the keratin 14 promoter [68]. Although keratinocytes of these mice showed abnormalities in nuclear morphology, their hair growth and wound healing were normal [68]. Although the advantages in using tissue-specific promoters to directly control the expression of target genes in specified tissues in transgenic animals have been acknowledged, the limitations of this system had become clear. This constitutive system had no control over the timing of the target gene expression, which depend entirely on the properties of the promoters used. The promoters in this setting is constitutively active, many starting early in the embryonic stage. In order to overcome this drawback, numerous researchers have invested time and effort in establishing conditional or inducible transgenic modelling system, one of which is regulated by tetracycline. The tetracycline-controlled transcriptional regulation system (tet-on/off) is a binary transgenic system that enables spatial and temporal regulation of gene expression [69]. By adding/removing doxycycline (a tetracycline derivative) to/from the system, it is possible to switch on/off the expression of the target gene in *in vivo*, which in turn is under the control of the tissue specific promoter. Using this system, Eriksson's group has generated a number of transgenic mouse models that express the HGPS mutation in isolated organ systems [70, 71], which served as a useful tool to study mechanism of disease progress. Briefly, transgenic mice carrying a human minigene of lamin A with the most common HGPS mutation, c.1824C>T; p.G608G, under the control of the tetracycline-regulated (tet-off) keratin 5 promoter (K5tTA) expressed the mutation in the skin, ameloblasts layer of the

teeth, salivary glands, oesophagus, stomach, tongue, nose cavity and trachea [70]. These animals showed growth retardation, hair thinning, tooth fractures and premature death, all of which are similar clinical features observed in HGPS patients [70]. In order to study skeletal abnormalities of HGPS, the osterix (Sp7-tTA) promoter was used to create a bone-specific expression model of the HGPS mutation with expression of the HGPS mutation during osteoblast development (tetop-LA^{G608G}; Sp7-tTA mice). These mice showed growth retardation, gait imbalance and abnormalities in bone structure [71]. Recently, Osorio and colleagues have designed another mouse model expressing the HGPS mutation [72]. In this knock-in mouse model, the wild-type mouse *LMNA* gene was replaced with a mutant allele that carried the c.1827C>T; p.G609G mutation, which is equivalent to the HGPS c.1824C>T; p.G608G mutation in the human *LMNA* gene (*Lmna*^{G609G/G609G}). These mice accumulate progerin and exhibit key clinical features of HGPS, such as shortened life span and bone and cardiovascular abnormalities [72].

<i>Mouse Model</i>	<i>Description</i>	<i>References</i>
BAC transgenic G608G	Over expression of human lamin A/C and progerin	[51]
<i>Zmpste24</i>^{-/-}	Knockout of the gene encoding <i>Zmpste24</i>	[53, 54]
<i>Zmpste24</i>^{-/-} <i>Lmna</i>^{+/-}	Intercross between <i>Zmpste24</i> ^{-/-} mice and <i>Lmna</i> ^{+/-} mice No expression of <i>Zmpste24</i> with only one allele expressing lamin A/C	[58]
<i>Lmna</i>^{HG/+}	One allele expresses progerin, while the other expresses lamin A/C	[46, 59]
<i>Lmna</i>^{nHG/+}	One allele expresses non-farnesylated progerin, while the other expresses lamin A/C	[62]
<i>Lmna</i>^{csnHG/csnHG}	Both alleles express non-farnesylated progerin allele (<i>Lmna</i> ^{csnHG}), in which the progerin's C-terminal ends with the -CSM compared to the -SSIM ending in <i>Lmna</i> ^{nHG} allele	[63]
K14 promoter – FLAG - progerin	Tissue specific over expression of progerin with FLAG tag	[67]
tetop-LA^{G608G}; K5-tTA	Tissue specific over expression of human lamin A and progerin by using a Keratin 5 promoter	[69]
tetop-LA^{G608G}; Sp7-tTA	Bone specific over expression of human lamin A and progerin by using a osterix promoter	[70]
<i>Lmna</i>^{G609G/G609G}	<i>Lmna</i> gene replaced with a mutant allele that carries the c.1827C>T; p.G609G mutation, which is equivalent to the HGPS c.1824C>T; p.G608G mutation in the human <i>LMNA</i> gene	[71]

Table 1. A summary table of the most relevant mouse models for HGPS.

5. Treatments

Although the cause of HGPS has been discovered nearly 10 years ago, HGPS remains incurable, with no therapy other than symptomatic treatment. Nevertheless, not long after the discovery of a mutation in *LMNA* gene as the cause of HGPS, a number of potential therapeutic strategies have emerged. A great deal of evidence suggests that the accumulation of progerin may be the key to the pathogenesis of HGPS [18, 20, 30, 31, 36, 72]. As progerin is permanently farnesylated, researchers initially turned to farnesyltransferase inhibitors (FTIs) in the search for a pathogenic treatment. FTIs were initially developed for the treatment of cancer [73]. The theory to this invention was simple: to abolish the farnesyl lipid from mutationally activated Ras proteins, thus mislocalising these signalling proteins away from the plasma membrane, where they stimulate uncontrolled cell division. Analogous concept was applied to HGPS: to mislocalise farnesylated progerin away from the nuclear envelope, with the hope that the mislocalisation would reduce the ability of the molecule to cause disease. However, potential shortcomings to the FTI treatment were recognised from the start. For example, these drugs would be expected to interfere with the farnesylation of lamin B1 and B2, possibly causing more damage to the nuclear lamina. Moreover, these molecules would be expected to disturb farnesylation of other cellular proteins, possibly loading a second insult on already compromised cells. Finally, there was a concern that pre-lamin A might be geranylgeranylated in the presence of FTI, in which case would forbid the overall strategy. Indeed, negative effects of FTI treatment were reported both *in vitro* and *in vivo* by Verstraeten and colleagues. They showed that FTI treatment caused defects in centrosome separation leading to donut-shaped nuclei [74]. However, despite these concerns, investigators cautiously raised their hopes about the possibility of testing FTIs in HGPS.

In 2004, it was first hypothesised that farnesylated progerin might be a key player in the pathogenesis of HGPS [59]. Within a year, Yang *et al.* generated a mice carrying a “progerin-only” *Lmna* allele (*Lmna*^{HG/+}) and showed that the number of *Lmna*^{HG/+} fibroblasts with misshapen nuclei was significantly decreased following the treatment of a FTI [47]. Shortly thereafter, several groups reported similar observations and demonstrated the possibility of farnesyltransferase inhibition as a therapeutic strategy for HGPS [48, 49, 75]. The finding that FTIs improve nuclear abnormalities led to testing the efficacy of FTIs in mouse models of HGPS. Fong *et al.* showed that administration of FTI restored disease phenotypes in *Zmpste24* deficient (*Zmpste24*^{-/-}) mice [76], and Yang *et al.* found that FTI significantly alleviated HGPS-related disease phenotypes (e.g. rib fractures, body weight curves, reduced bone density) and increased the survival of mice with a HGPS mutation (*Lmna*^{HG/+}) [60, 62]. Furthermore, Capell and colleagues demonstrated that treatment with FTI to HGPS mouse (BAC transgenic G608G; [52]) significantly prevented both the onset and late progression of cardiovascular disease, which is one of the most prevalent cause of death in HGPS patients [77]. However, some of enthusiasm about FTI treatment was dampened by unexpected emergence of HGPS-related disease phenotypes in mice expressing non-farnesylated progerin (*Lmna*^{nHG/+}) [63]. In order to further elucidate the fact that protein farnesylation is relevant to the pathogenesis and treatment of disease, Yang and others compared the effects of an FTI on disease phenotypes in both *Lmna*^{HG/+} and *Lmna*^{nHG/+} mice [61]. In this study, au-

thors showed that the FTI reduced disease phenotypes only in *Lmna*^{HG/+} mice, and had no effect in *Lmna*^{nHG/+} mice, which supported the idea that the beneficial effects of FTI in *Lmna*^{HG/+} mice are due to the inhibition of progerin farnesylation [61].

The encouraging results from both cell and animal studies led to the initiation of the clinical trial with FTI for HGPS patients. In 2007, 28 patients with classical HGPS were enrolled for the first clinical trial, in which children were treated with Lonafarnib (an FTI) for 2 years. Although the trial ended in December 2009, no report has been published on how effective the drug was in treating HGPS. While this first trial was in progress, the statins and aminobisphosphonates gained attention as potential therapies for the treatment of HGPS. Varela and others reported that combined treatment with statins and aminobisphosphonates effectively inhibit the farnesylation and geranylgeranylation (an alternative prenylation) of progerin and pre-lamin A, which was accompanied by an alleviation in the disease phenotype of the *Zmpste24*^{-/-} knockout mice [78]. Statins and aminobisphosphonates, both inhibit protein prenylation at different points than FTIs in the isoprenoids and cholesterol biosynthetic pathway, and are already in clinical use (Figure 5). Statins are renowned inhibitors of cholesterol synthetic pathway and are widely used in the clinic to lower cholesterol level and prescribed for cholesterol associated diseases, such as atherosclerosis. Statins inhibit the production of isoprenoid precursors involved in protein modification, thereby inhibiting lamin A maturation [79-81]. The aminobisphosphonates are currently used to treat osteoporosis. It inhibits farnesylpyrophosphate synthase, thus reducing the production of both geranylgeranyl and farnesyl group [82, 83]. In addition to results from Varela *et al.* [78], Wang and colleagues also showed that treatment of transgenic mice that express progerin in epidermis with a FTI or a combination of a statin plus an aminobisphosphonate significantly improved nuclear morphological abnormalities in intact tissue [84]. Based on these hopeful animal studies, the Triple Drug Trial to test the therapeutic effect of a combination of a statin (Pravastatin), a bisphosphonate (Zoledronic Acid), and a FTI (Lonafarnib) was initiated in August 2009, including 45 HGPS patients [5]. This trial was planned to last for 2 years, but announcement about its outcome is yet to be made.

Besides interfering the post-translational processing of mutated pre-lamin A, another major path for HGPS treatment is to reduce the expression of progerin in cells and tissue [37, 72, 85, 86]. This was first shown by Scaffidi and Misteli [37]. They used antisense morpholino oligonucleotides specifically directed against the aberrant exon 11 and exon 12 junction contained in mutated pre-mRNAs to target the splicing defect observed in HGPS, and consequently decrease the production of progerin. Authors showed that once splicing defect is corrected and the level of progerin is decreased, morphological abnormalities of HGPS fibroblasts were ameliorated [37]. More recently, Osorio *et al.* designed a 25-nucleotide morpholino that bound to the exon 10-lamin A splice donor site, and showed that its administration reduced the percentage of cells with nuclear abnormalities to wild-type levels in a dose-dependent manner [72]. It remains to be seen, however, whether these oligonucleotides can be effectively and safely administered to patients. Another approach to reduce progerin expression at mRNA level is to use a short hairpin RNA (shRNA). Huang and colleagues showed that the reduced expression of mutated *LMNA* mRNA level was associated

with amelioration of abnormal nuclear morphology [86]. However, the efficacy of shRNA in whole organism is yet to be confirmed.

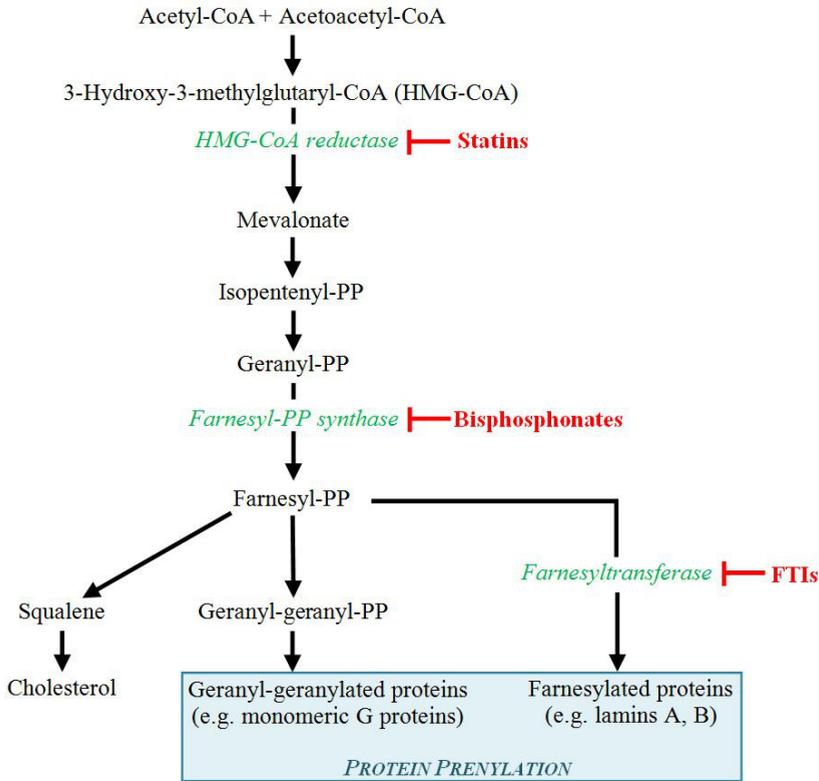


Figure 5. Isoprenoids and cholesterol biosynthetic pathway and its inhibitors for the treatment possibilities of HGPS. PP stands for pyrophosphate.

More recently, rapamycin has been gaining much attention as a new candidate for the treatment of HGPS. Rapamycin (also known as Sirolimus) is an FDA-approved drug that has been used for a long time in transplant patients as an anti-rejection drug. In addition to its historical use as an immunosuppressant, pre-clinical studies demonstrated life-span extending effect of rapamycin or rapamycin derivatives in mice [87, 88]. The effect of rapamycin is due to the inhibition of mammalian target of rapamycin (mTOR) pathway by rapamycin and is at least partly dependant on autophagy [89, 90]. Cao and colleagues have recently demonstrated that HGPS cells treated with rapamycin showed enhanced progerin degradation, slowed senescence, and reduced nuclear blebbing compared to untreated cells [91, 92]. Furthermore, similar results were reported by Cenni and others [93]. Since rapamycin is already an approved drug, its effect should be further examined in mouse models of HGPS and considered as a potential therapy for HGPS patients.

6. Conclusion

Since the discovery of the genetic basis for HGPS almost a decade ago, there has been progress in understanding the mechanism(s) of this premature ageing syndrome and its possible implications for physiological ageing. Results from numerous studies have uniformly suggested that the accumulation of an abnormally processed lamin A protein, progerin, mediates dominant-negative effects in cells from HGPS patients. Notably, over the last few years, many achievements in basic research have driven the development of potential therapies which have resulted in several clinical trials for patients with HGPS. It was inevitable to have hopes that these compounds targeting the isoprenoids and cholesterol biosynthetic pathway would alleviate the clinical course of HGPS. Nevertheless, drugs that are currently in clinical trials do not have the ability to target the cryptic splice site; therefore, additional approaches may still need to be considered.

In summary, by better understanding the mechanisms of HGPS, it may be possible to minimise the pathological process observed in HGPS, and to develop potential treatments for age-related diseases.

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