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The genetic mechanism that links Hutchinson-Gilford progeria syndrome to physiological aging

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THE GENETIC MECHANISM THAT LINKS HUTCHINSON-GILFORD PROGERIA SYNDROME TO PHYSIOLOGICAL AGING

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Dedicated to my beloved parents and life heroes:

Benito Rodríguez Rodríguez & Doris Vásquez Silva

ABSTRACT

Aging is a complex process that is not completely understood. The study of segmental progeroid syndromes such as Hutchinson-Gilford progeria syndrome (HGPS) has allowed us to connect the common genetic mechanisms that occur in normal physiological aging, with the cellular alterations presented by this severe premature aging syndrome. Since the identification of mutations in the lamin A/C coding *LMNA* gene that cause HGPS and other laminopathies, there has been an increasing interest in the potential role of lamins in the normal aging process. Progerin, a mutant form of lamin A, has attracted particular great attention. *LMNA* mutations in HGPS activate a cryptic splice site, leading to an aberrant splicing of lamin A, which results in a lamin AΔ150 transcript and progerin. Recent research data, including the results presented in this thesis, provide support for the possibility of a shared mechanism between natural physiological aging and pathological aging occurring in HGPS. This shared mechanism could contribute to solving part of the aging puzzle. The overall aim of this thesis was to gain an increased understanding of potential common genetic mechanisms behind Hutchinson-Gilford progeria syndrome and normal physiological aging. For this purpose, the research was primarily designed to study the expression of *LMNA* and the global genome differential splicing in aging cells to investigate the potential relationships that link the genetic mechanisms found in HGPS to those occurring in normal physiological aging.

In **paper I**, we develop an absolute quantification method to determine the overall expression levels of the *LMNA* gene transcripts lamin A, lamin C and lamin AΔ150 (progerin) during the *in vitro* cell aging of primary dermal fibroblasts from HGPS patients and from age-matched and parent controls. We show that lamin C is the most highly expressed transcript and that the lamin AΔ150 transcript is present in unaffected controls at an approximately 160-fold lower expression level compared with HGPS patients. While the levels of lamin A and lamin C transcripts remained unchanged during *in vitro* cell aging, the lamin AΔ150 transcript increased in late passage cells from HGPS patients and parental controls, suggesting a similar mechanism in HGPS patients and unaffected donors during cellular aging.

In **paper II**, we expand on the first study and continue to investigate the expression of progerin in unaffected cells from different age groups, evaluating progerin as an aging biomarker for cellular senescence. We utilize a newly developed progerin antibody and quantify the percentage of progerin-expressing cells in the early and late passages of cells aged *in vitro.* We found that well-defined nuclear expression of progerin in primary dermal fibroblasts that were aged *in vitro* is an extremely rare event (or below the detection limit of an immunofluorescence assay). Our results do not rule out the possibility of progerin being expressed during normal cellular aging but question progerin's contribution to physiological cellular aging.

In **paper III**, we investigate if the *LMNA* gene presents differential allelic expression, which could help to explain the phenotypic variability observed among HGPS patients and laminopathies in general. We made use of the rs4641C/T *LMNA* coding single nucleotide polymorphism (SNP) and developed an allele-specific absolute quantification method for the lamin A and lamin C transcripts. The contribution of each allele to the total transcript level was quantified in dermal fibroblasts from HGPS patients and unaffected controls. We show that the C allele is more frequently expressed, corresponding to a 70% of the total lamin A/C transcripts, and that the most common HGPS mutation, *LMNA* c.1824C>T, is found in both the C and T alleles, which could account for the phenotypic variability observed among HGPS patients.

In **paper IV**, we investigate whether aberrant splicing events, such as that occurring both in HGPS and sporadically in unaffected individuals, become more frequent and widespread on a genome-wide level during normal physiological aging, hypothetically, as a result of a declined stringency of the splicing machinery. To analyze the effect of age on splicing, we used exon microarrays to investigate the global genome differential exon expression and detect alternatively spliced genes in tissues of normal aging mice and in cells from a HGPS mouse model. We show that aging affects splicing via a considerable number of genes displaying significant differential and increased splicing with age. The most significantly enriched biological functions with alternative spliced genes during normal aging included RNA processing and the spliceosome pathway. Additionally, progeria cells had primarily differential splicing of extra cellular matrix genes, and explorative network enrichment analysis identified the NF-κB complex as a potential common network node for HGPS and normal tissue aging.

1. LIST OF PUBLICATIONS

I. Rodríguez S, Coppedè F, Sagelius H, Eriksson M. **Increased expression of the Hutchinson-Gilford progeria syndrome truncated lamin A transcript during cell aging.**
Eur J Hum Genet. 2009 Jul;17(7):928-37.

II. Rodríguez S and Eriksson M. **Evaluating progerin as a biomarker for premature and physiological aging.**
Manuscript.

III. Rodríguez S and Eriksson M. **Low and high expressing alleles of the *LMNA* gene: implications for laminopathy disease development.**
PLoS One. 2011;6(9):e25472.

IV. Rodríguez S, Grochova D, McKenna T, Borate B, Trivedi NS, Wolfsberg TG, Baxevanis AD, Erdos M and Eriksson M. **Global genome splicing analysis implicates RNA processing in physiological aging and the extra cellular matrix in progeria.**
Manuscript.

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RELATED PUBLICATION

Rodríguez S, Eriksson M. **Evidence for the involvement of lamins in aging.** Curr Aging Sci. 2010 Jul;3(2):81-9. *Review*.

Parts of the introduction have been published in the above review article [1].

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Gene symbols and abbreviation of commercial names were skipped from this list. In the text genes are indicated with *italics* (e.g. *LMNA, FACE1*), all in capital letters for human genes or with only one first capital letter for mouse genes. (e.g. *Zmpste24).*

AS	Alternative Splicing
AWS	Atypical Werner's syndrome
BAF	Barrier-to-autointegration factor
CMT2B1	Charcot-Marie-tooth disease type 2B1
CVD	Cardiovascular disease
DCM	Dilated cardiomyopathy
DDR	DNA Damage Response
DNA	Deoxyribonucleic Acid
DSB	Double Strand Breaks
Dox	Doxycycline
ECM	Extracellular Matrix
EDMD	Emery-Dreifuss Muscular Dystrophy
ER	Endoplasmatic Reticulum
FACS	Fluorescence-activated Cell Sorting
FPLD	Familial Partial Lipodystrophy
FTase	Farnesyltransferase
FTLD	Frontotemporal Lobar Degeneration
HGPS	Hutchinson-Gilford Progeria Syndrome
HP1	Heterochromatin Protein 1
Icmt	Isoprenyl carboxymethyl transferase
IF	Immunofluorescence
INM	Inner Nuclear Membrane
K5tTA	Keratin 5 Tetracycline Transactivator
LAD	Lamina-associated Domain
LAP	Lamin-associated Polypeptide
LBR	Lamin B Receptor
LGMD	Limb-Girdle Muscular Dystrophy
LINC	Linker of Nucleoskeleton and Cytoskeleton
mRNA	Messenger Ribonucleic Acid
MAD	Mandibuloacral Dysplasia
MAN1	Integral inner nuclear membrane protein MAN antigen 1
NPC	Nuclear Pore Complex
OMIM	Online Mendelian Inheritance in Man

ONM	Outer Nuclear Membrane
PFA	Paraformaldehyde
PNS	Perinuclear Space
Rb	Retinoblastoma protein
RD	Restrictive Dermopathy
rtTA	Reverse tetracycline responsive Transcriptional Transactivator
PRF	Progeria Research Foundation
ROS	Reactive Oxygen Species
SMA	Smooth Muscle Actin
SNP	Single Nucleotide Polymorphism
SREBP1	Sterol Regulatory Element-Binding Protein 1
tTA	Tetracycline responsive Transcriptional Transactivator
UV	Ultraviolet
VSMC	Vascular Smooth Muscle Cell
WS	Werner's syndrome

1. INTRODUCTION

1.1 AGING

Aging is an unavoidable and very complex biological and multifactorial process that affects all organisms, during which molecules, cells and tissues accumulate damage over time, resulting in degenerated tissue functions, an increased probability of acquiring age-related diseases and eventually senescence and death [2]. Increasing our understanding of why we age has always fascinated humans, possibly due to a hidden subconscious hope of extending youth and reaching immortality. However, medical research is primarily focused on improving health and life quality for a constantly growing elderly population and on deepening our knowledge of age-related diseases.

Due to the complexity of the aging process and despite the enormous amount of knowledge that the aging research field has produced, the molecular mechanisms causing physiological aging are not completely understood. Several theories explaining the causes of aging have been proposed, such as the mitochondrial free radical damage theory, the accumulation of random mutations, DNA polymerase errors and failures in the DNA maintenance machineries, epigenetic collapse, telomere shortening, stem cell depletion, chronic inflammation and environmental injuries [3]. However, aging is most likely caused by a couple or a variety of these factors, as initiated by endogenous and external damage-inducing agents or events. Endogenously, the DNA slowly accumulates random mutations with time due to the natural imperfection of the DNA maintenance machineries. However, as a normal by-product, our own metabolism produces reactive oxygen species (ROS) in the mitochondria that damage the macromolecules and structures of the cell. More DNA damage is caused by external agents, such as radiation (UV, x-rays or gamma-rays), hydrolysis, toxins, mutagenic chemicals and viruses. Finally, the accumulation of DNA damage contributes to aging via genomic instability, apoptosis, senescence and cancer.

There are several avenues of research in the study of aging. These include the study of cellular senescence, genes and mutations that affect lifespan in animal models, genes implicated in age-related disorders and diseases resembling premature aging (progeroid syndromes or progerias). In unimodal progeroid syndromes such as Alzheimer's disease, there is one predominant aspect of the normal aging phenotype, whereas segmental progeroid syndromes involve multiple aspects of the aging phenotype that

affect several but not all tissues [4] e.g., Down's syndrome, Werner's syndrome (WS), and Hutchinson-Gilford progeria syndrome (HGPS). Several segmental progeroid syndromes (e.g., atypical-WS and HGPS) are caused by mutations in the *LMNA* gene (table.1) coding for lamin proteins, which are constituents of the nuclear lamina. Lamin proteins comprise part of a larger group of diseases collectively named laminopathies; the study of these proteins has not only increased the understanding of these disorders but also provided insights into the functions of the nuclear lamina, its interaction with the genome and the relationship of these diseases to normal physiological aging.

1.2 HUTCHINSON-GILFORD PROGERIA SYNDROME

Hutchinson-Gilford progeria syndrome, also referred to as progeria or HGPS (Online Mendelian Inheritance in Man, OMIM, #176670), is a disease characterized by premature/accelerated aging. It is a severe, lethal and very rare genetic disease with an incidence of 1 in 4-8 million live births [5-7]; according to the Progeria research Foundation (PRF), 90 cases of living patients are currently identified worldwide (PRF 2013; http://www.progeriaresearch.org/prf-by-the-numbers.html). Children affected with HGPS appear normal at birth. However, within the first year of life, these children present growth retardation, are short in stature and have a reduced rate of weight gain. They soon develop other clinical abnormalities such as alopecia, skeletal and dental abnormalities and changes in skin with scleroderma and the loss of subcutaneous fat [6]. The average life expectancy in HGPS is 13 years, and most patients die from atherosclerosis leading to heart failure or stroke [6-8].

1.2.1 Molecular basis of HGPS

Classical HGPS is most commonly caused by *de novo* point mutations in codon 608 in exon 11 of the *LMNA* gene G608G or G608S (fig.1) [9-11]. Both mutations activate a cryptic splice site in exon 11 that leads to the deletion of 150 nucleic acids from the lamin A mRNA (fig.1). This aberrant splicing event results in a truncated prelamin A isoform that lacks 50 amino acids at its C-terminus, referred to as lamin AD Both mutations activate a cryptic splice site in exon 11 that leads to the deletion of 150 nucleic acids from the lamin A mRNA (fig.1). This aberrant splicing

eventwhich in turn is unable to remove the last 15 amino acids. This leads to an unprocessed form of lamin A that accumulates as a permanently farnesylated and carboxymethylated lamin A precursor (fig.3). More than 90% of children with classical HGPS are heterozygous for the p.G608G (c.1824C>T) point mutation in exon 11 of the *LMNA* gene [11]. However, none of the mutations in codon 608 generate a perfect splice site; therefore, the full-length lamin A protein is also expressed to a large extent by the mutant allele (fig.1).

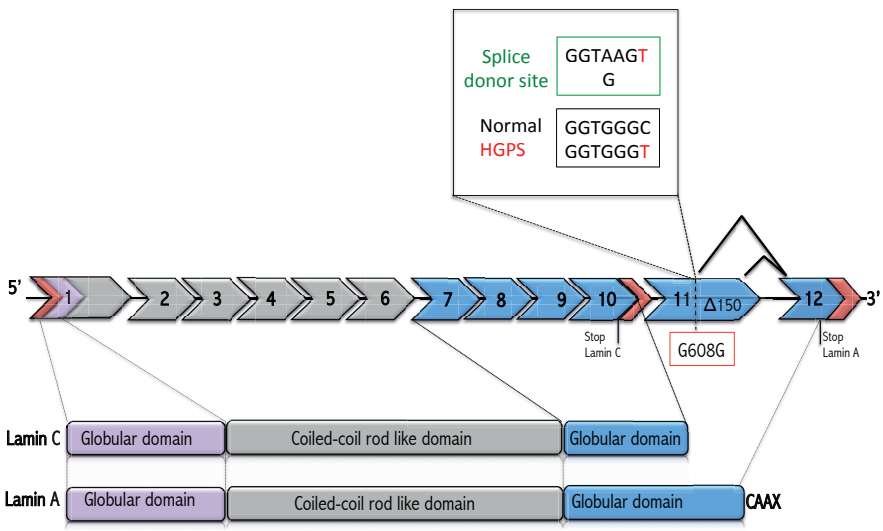

Figure 1. HGPS mutation in the *LMNA* gene: Schematic illustration of the *LMNA* with its 12 exons encoding for lamin A and lamin C domains. Exon 1-10 encodes lamin C and exons 1-12 encodes for lamin A with a terminal CAAX box. The HGPS (c.1824C>T, p.G6008G) mutation in exon 11 activates a cryptic splice site with the consequent deletion of 150 nucleotides (Δ150) from the mRNA. Untranslated region (in red).

1.2.2. Cellular phenotype in HGPS

Cells from HGPS patients present dysmorphic nuclei with aberrant and varying blebbed shapes [11-13]. These cells have a thickened nuclear lamina, a loss of peripheral heterochromatin and a clustered positioning of the nuclear pore complexes. [12; 14]. Dermal fibroblasts from HGPS patients show severe changes in nuclear shape including lobulations and a thickening of the nuclear lamina. The altered composition of the nuclear lamina in turn leads to the aberrant incorporation and localization of lamina-associated proteins to the nuclear envelope. The disorganization of the nuclear lamina and lamin A interaction with lamina-associated domains (LADs) may contribute the

observed loss of the peripheral heterochromatin in HGPS cells and clustering of the nuclear pore complexes[12] (fig.2). This also leads to the disorganization of heterochromatin, as well as to of numerous transcription factors[15] . These effects may be responsible for the highly misregulated gene expression profiles and impaired DNA damage responde (DDR) observed in HGPS cells [16; 17]. Expression of high levels of progerin in the nuclei is typical for HGPS cells. Progerin accumulation, abnormal lamin localization, the reduction of lamin B and lamin-associated polypeptide type 2 (LAP2) proteins are features that also have been observed in HGPS [12; 18]. These changes in the nuclear architecture become more severe as the cells age in culture; this severity appears to correlate with an increase in progerin expression [12]. Additionally, recent data support the notion that progerin anchors to the inner nuclear membrane through its farnesyl group, causing the characteristic blebbing of the nuclei in HGPS cells during interphase [19]. When the cell is dividing and the nuclei are disassembled, progerin localizes in cytoplasmic aggregates and membrane-like structures that interfere with chromosome segregation to generate binucleated cells [20] Many of these features can also be observed in normal senescent cells (fig.2).

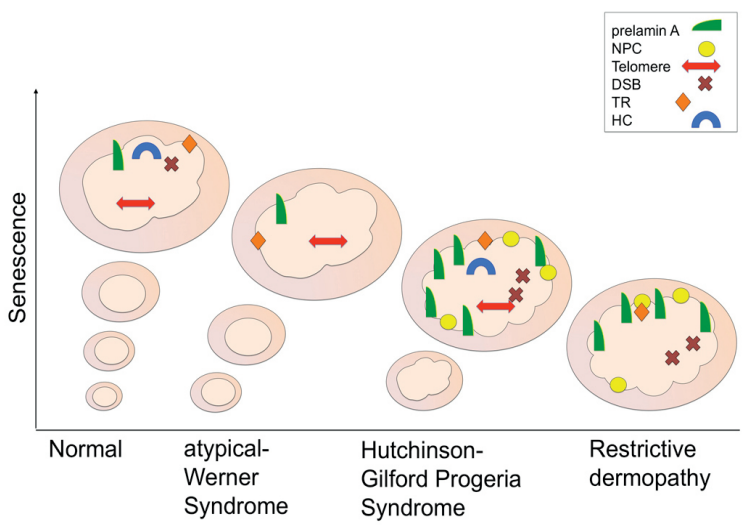

Figure 2. Premature senescense and genomic instability in segmental progeroid syndromes and normal cells. Normal senescent cells show farnesylated prelamin A (prelamin A), shortened telomeres (telomere), loss of heterochromatin (HC), altered gene-expression (transcriptional regulation, TR), and accumulation of double strand breaks (DSB). Cells from HGPS and RD patients show altered position of nuclear pore complexes or nuclear pore associated proteins with effects on nuclear import of proteins (NPC). (Rodriguez and Eriksson, 2010) [1] .

1.3 LAMINS

Nuclear lamins are type V intermediate filament proteins that are believed to be the progenitors of all intermediate filament proteins [21]. They primarily distribute in the nuclear lamina (fig.4), but can also be found in the nucleoplasm. Characteristic of intermediate filaments, lamins are able to form homopolymers that can assemble into higher order filaments, and their primary role has long been attributed to a structural function and the support of the cellular nuclei. Today, we also know that the dissociation and reassociation of lamins is necessary during mitosis and that their interaction with the chromatin and nuclear proteins plays a fundamental role during transcription and DNA replication.

Lamins are composed of a globular N-terminal head domain, a central α-helical coiled-coil rod domain, and a globular C-terminal tail domain, all of which are involved in the lamin assembly [22] (fig.1 and fig.5). The tail and rod domains are involved in molecular interactions. The rod interacts laterally with other lamins, and the tail domain binds to chromatin, lamina-associated polypeptide 2 (LAP2) and emerin [15]. The C-terminal tail domain contains a structural Ig-fold domain (411-553) [23] and a nuclear localization signal (417-422) that is required for the localization of lamins to the nucleus [23] (fig.5).

There are two types of lamins in mammals: A-type and B-type lamins. A-type lamins are encoded by the *LMNA* gene, while B-type lamins are encoded by the *LMNB1* and *LMNB2* genes. *LMNB1* encodes for lamin B1 and is located on chromosome 5q23.3-q31.1, while the *LMNB2* gene encodes for lamin B2 and lamin B3 and is located on chromosome 19p13.3 [24]. The most important distinction between A-type and B-type lamins is that B-type lamins are expressed at all developmental stages and are indispensable for cell survival[25], while A-type lamins are known to be developmentally regulated and are expressed in differentiated cells [26].

1.4 THE *LMNA* GENE

The *LMNA* gene is located on chromosome 1q21.2-q21.3 and encodes for the A-type lamins; the gene is composed of 12 exons. Exons 1-12 encode lamin A, and exons 1-10 encode lamin C [27; 28], which are the 2 main isoforms (fig.1 and fig.5).

1.4.1 A-type lamins

Besides lamin A and lamin C, there are additional minor isoforms (lamin C2 and lamin AΔ10). The lamin AΔ10 and lamin C2 isoforms have been detected in colon and several carcinoma cell lines or only in male germ cells [29; 30]. Lamin A and lamin C are the primary protein constituents of the mammalian nuclear lamina, which is a complex structure that acts as a scaffold for protein complexes that regulate nuclear structure and functions. Interest in these proteins has increased in recent years with the discovery that mutations in *LMNA* cause a variety of human diseases (termed primary laminopathies), including progeroid syndromes and disorders that primarily affect striated muscle, adipose, bone and neuronal tissues.

1.4.2 The post-translational processing of prelamin A

Lamin A is synthesized as a precursor protein, prelamin A; similar to the B-type lamins, prelamin A contains a CAAX box for farnesylation (C: Cysteine, A: aliphatic amino acid, X: any amino acid) at the carboxyl terminal end [31; 32]. The CAAX (CSIM) box is responsible for the initiation of a series of post-translational maturation steps (fig.3). First, a farnesyl group is added to the cysteine by a farnesyl transferase (FTase). In the second step, an endoprotease, Zmpste24/Rce1, cuts out the last three amino acids, AAX (SIM). Thereafter, a methyl group is fixed to the farnesylated cysteine, a process that is catalyzed by isoprenyl carboxymethyl transferase (Icmt) [33]. In the final step of prelamin A l maturation, the last 15 amino acids are cleaved away from the carboxyl end by Zmpste24 (fig. 3). The cellular localization of this process remains debated, but it most likely occurs at the endoplasmic reticulum, releasing the protein from its association with the nuclear membrane in the final step [34-36] (fig.3). Due to that the HGPS mutation activates a cryptic splice, which splice away 150 nucleotides on the prelamin A mRNA, the translated HGPS prelamin A (prelamin AΔ50) does not contain an important recognition site for the Zmpste24 enzyme (RSYLLG). This enzyme is normally needed in the last maturation step of prelamin A to cut away the 15 last amino acids, containing the farnesyl group. The result in HGPS patients, is that a truncated lamin A, which remains permanently farnesylated and carboxymethylated is produce in the HGPS cells. This abnormally processed lamin A is commonly referred to as progerin (farnesylated prelamin AΔ50 or lamin AΔ50) (fig.3).

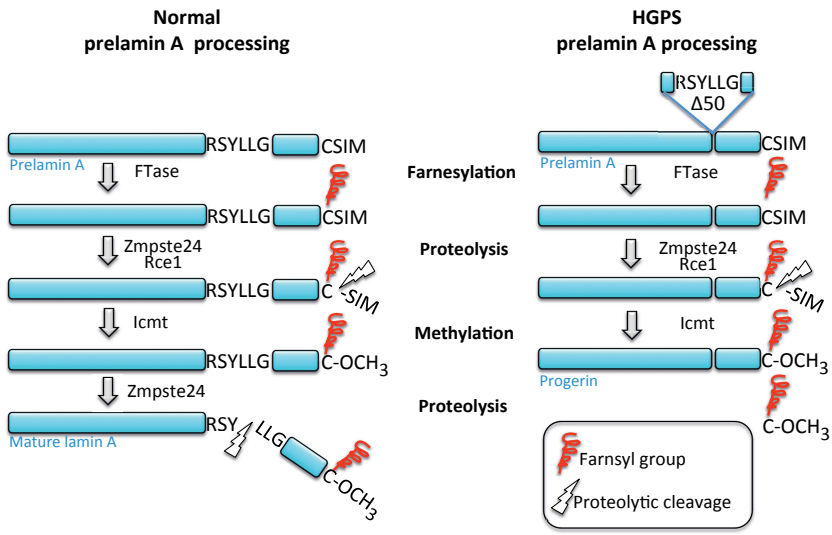

Figure 3. The post-translational processing of prelamin A. The post-translational processing of normal prelamin A and prelamin AΔ50 in HGPS are shown. The multistep processing of prelamin A to mature lamin A, involve multiple steps to obtain mature lamin A. In HGPS the recognition site RSYLLG for Zmpste24 is missing and the last proteolytic cleavage, cannot take place, resulting in a permanently farnesylated and carboxymethylated lamin AΔ50 known as progerin.

1.5 THE NUCLEAR LAMINA

The nuclear envelope separates the nuclei from the cytoplasm and is composed of the outer nuclear membrane (ONM) and the inner nuclear membrane (INM), which are separated by the perinuclear space (PNS) [37] (fig.4). The ONM and INM display periodical junctions forming the nuclear pore complexes (NPC), which are multiprotein channels that regulate macromolecular trafficking across the nuclear envelope [38]. Because the ONM has membrane continuity with the endoplasmic reticulum (ER), it contains ER proteins and ribosomes that are associated with its membrane. The INM has its own set of integral membrane proteins, many of which interact with the nuclear lamina (fig.4)

The nuclear lamina, underlying the INM, is a filamentous meshwork composed of A- and B-type lamins and nuclear lamin-associated membrane proteins. The nuclear lamina determines the nuclear shape and gives mechanical support to the nuclei. Important functions of the nuclear lamina include providing anchoring sites for chromatin domains (contributing to its impact on gene silencing), nuclear pore

complex positioning, and anchoring nuclear envelope proteins and several regulatory proteins, including signaling molecules and DNA transcription factors [39]. Additional functions are related to the lamina's involvement in DNA synthesis, DNA repair, transcription and nuclear envelope assembly.

Several studies have identified various proteins binding to the A-type lamins of the lamina, including the lamina-associated polypeptides (LAP1 and LAP2), the nuclear envelope spectrin repeat proteins (nesprin 1 and 2), the SUN proteins (SUN 1 and 2), emerin, actin, retinoblastoma protein (pRb), sterol regulatory element-binding protein 1 (SREBP1), components of the RNA polymerase II-dependent transcription complexes and DNA replication complexes. Lamin A and lamin C together interact with the SUN and nesprin proteins to form the LINC complex, which physically links the nucleoskeleton to the cytoskeleton (fig.4).

Other components of the nuclear lamina that are not known to interact with A-type lamins correspond to the lamin B receptor (LBR) and MAN1. The LBR is localized in the nuclear envelope inner membrane and contains a binding site for heterochromatin protein 1 (HP1). As with lamin A, LBR anchors the lamina and the heterochromatin to the membrane [40]. LBR is thought to mediate the interaction between chromatin and lamin B with implications in gene silencing; mutations of this gene have been associated with autosomal recessive HEM/Greenberg skeletal dysplasia [41]. MAN 1 resides in the inner nuclear membrane and shares a LEM domain with LAP2 and emerin [42]. Little is known regarding MAN1 function, with the exception of its interaction with SMAD proteins in cell signaling. Emerin is known to interact with nuclear lamins, splicing-associated and transcription-repressing factors, nesprin 1 and actin. Emerin also contains a binding site for the chromatin bridging protein barrier-to-autointegration factor (BAF); mutations in its gene *(BANF1*) have been identified as the cause of hereditary progeroid syndrome [43]. Both HP1 and BAF provide links between INM proteins and the chromatin (fig.4) .

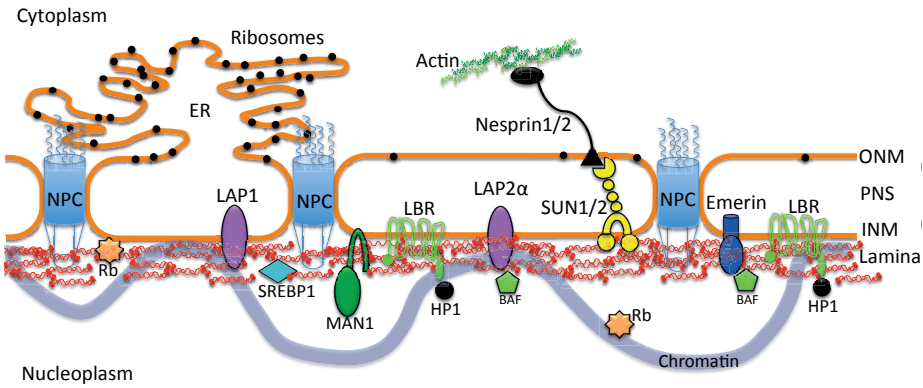

Figure 4. Components of the nuclear envelope and nuclear lamina. Underlying the inner nuclear membrane (INM) we find the nuclear lamina. (Red meshwork). The nuclear lamina is composed by lamins A, B and C. It interacts with the chromatin and several trans-membrane proteins of the INM. Through the LINC complex (Sun1/2, Nesprin ½), the lamina interacts with the cytoskeleton. The lamina plays a role in correct nuclear pore complex (NPC) positioning. The drawing was inspired by (Goldman *et al*., 2002[39]; Stewart and Burke 2007[37]).

1.6 LAMINOPATHIES

Laminopathies are a group of diseases caused by mutations in genes encoding or affecting protein components of the nuclear lamina. They can be divided into primary and secondary laminopathies. The vast majority of the identified diseases are caused by mutations in the *LMNA* gene and are therefore known as **primary laminopathies**; diseases caused by mutations in genes coding for B-type lamins (*LMNB1* and *LMNB2*), lamin-binding proteins such as *EMD, TMPO, LBR, LEMD3* and *LAP2α* or genes affecting the prelamin A processing such as *FACE1* in humans or *Zmpste24* in mice are known as **secondary laminopathies**.

1.6.1 Primary laminopathies

At present, 464 different mutations from 2,251 subjects have been identified in the *LMNA* gene (www.umd.be/LMNA/) to cause a large number of severe genetic disorders, some of which may display some phenotypic overlap [15] (fig.5). Different mutations can often cause the same disorder, but more interestingly, the same mutation can cause more or less severe phenotypes in different individuals or even different disorders. The phenotypic variability of diseases caused by mutations in the *LMNA* gene has greatly encouraged studies on the role of lamin function in disease. The

primary laminopathies constitute several autosomal dominant and recessive genetic diseases and are often, depending on the phenotype, divided into muscular dystrophies, lipodystrophies, neuropathies and segmental progeroid syndromes [44; 45] (fig.5).

1.6.1.1 Muscular dystrophies

The muscular dystrophies include Emery-Dreifuss Muscular Dystrophy (EDMD), Limb-Girdle Muscular Dystrophy (LGMD) and Dilated Cardiomyopathy (DCM). The muscular dystrophies are characterized by progressive skeletal and heart muscle weakness. Defects in muscle proteins, the death of muscle cells and the gradual loss of the muscles (replaced by scar tissue and fat) are typical clinical features. Patients with EDMD slowly show progressive contractures in their childhood and teenage years. Muscle weakness and wasting starts in the distal limb muscles. Most patients also suffer from cardiac conduction defects and arrhythmias [46; 47]. Slowly progressive shoulder and pelvic muscle weakness and wasting followed by the development of contractures and cardiac disturbances are typical for LGMD [48]. In DCM, affected individuals display impaired contractility, arrhythmias and conduction defects. The muscle weakness in DCM is a cardiac-specific dystrophy and does not affect the skeletal muscle [49; 50] (fig.5).

1.6.1.2 Lipodystrophies

Lipodystrophies are characterized by a generalized or localized loss of body fat. Within this group are Familial Partial Lipodystrophies (FPLD), generalized Lipodystrophies type 2 and Mandibuloacral Dysplasia (MAD). In FPLD, the loss of subcutaneous white adipose tissue is observed in the extremities, the trunk and the gluteal region, while the face, neck and abdominal region accumulates fat cells. FPLD [51; 52] and generalized lipodystrophy type 2[53] are associated with an increased risk of early endpoint atherosclerosis and the development of metabolic diseases such as high triglyceride levels in the blood (hyperglyceridemia) and insulin resistance, which leads to diabetes. Clinical characteristics for MAD include general lipodystrophy, the delayed closure of cranial sutures, dental crowding, joint contractures, mandibular and clavicular hypoplasia, acroosteolysis and insulin resistance. MAD patients also show short stature and alopecia [54; 55] (fig.5) and are sometimes also classified as a progeroid syndrome (table.1).

1.6.1.3 Neuropathies

The neuropathy group of the laminopathies includes Charcot-Marie-Tooth disease type 2B1 (CMT2B1). In this disease, the nerves that control the muscles are affected. CMT patients slowly lose normal use of their extremities because the nerves degenerate; the muscles in the extremities become weakened because of the loss of stimulation by the affected nerves [10].

1.6.1.4 Segmental progeroid syndromes

These syndromes are characterized to resemble several but not all phenotypic features of natural physiological aging. In recent years, the group of segmental progeroid syndromes caused by mutations in the *LMNA* gene has been expanding, and in addition to classical HGPS, this group includes atypical-HGPS, atypical-Werner syndrome (AWS), restrictive dermopathy (RD), and mandibuloacral dysplasia (MAD). While the majority of children with classical HGPS (described above) are heterozygous for the p.G608G (c.1824C>T) point mutation in exon 11 of the *LMNA* gene, there are several other less frequent mutations in the *LMNA* gene that have been found to cause atypical-HGPS. These patients have, in addition to the disease phenotypes observed in classical HGPS, phenotypes that are not observed in typical HGPS or that lack phenotypes of the classical form [11; 56-60]. Atypical-WS has features that are similar to Classical Werner syndrome (WS) (disease caused by mutations in *WNR*), but atypical-WS is caused by mutations in the *LMNA* gene and not in the same gene as classical WS [61]. Classical WS, also known as adult progeria, is not a therefore not a laminopathy because it is caused by mutations in the *WNR* gene, which encodes a RecQ helicase [62]. In WS , death usually occurs in the fifth or sixth decade of life due to atherosclerosis or neoplasia. Growth retardation manifesting from the second decade, cataracts, type 2 diabetes, osteoporosis, alopecia, sclerodermatous skin, the loss of adipose tissue and an increased likelihood for cancer are typical clinical features of WS [63; 64]. Restrictive Dermopathy (RD) is a lethal tight skin contracture syndrome. Intrauterine growth retardation, joint contractures, tight and rigid skin and prominent vessels are typical for this neonatal disease. Affected individuals die due to pulmonary hypoplasia and subsequent respiratory insufficiencies during gestation or early after birth [65-68]. RD is most commonly linked to mutations in the *ZMPSTE* gene, which leads to a loss of function; however, RD has also showed to be caused by splice mutations in the *LMNA* gene that in turn also involve exon 11.

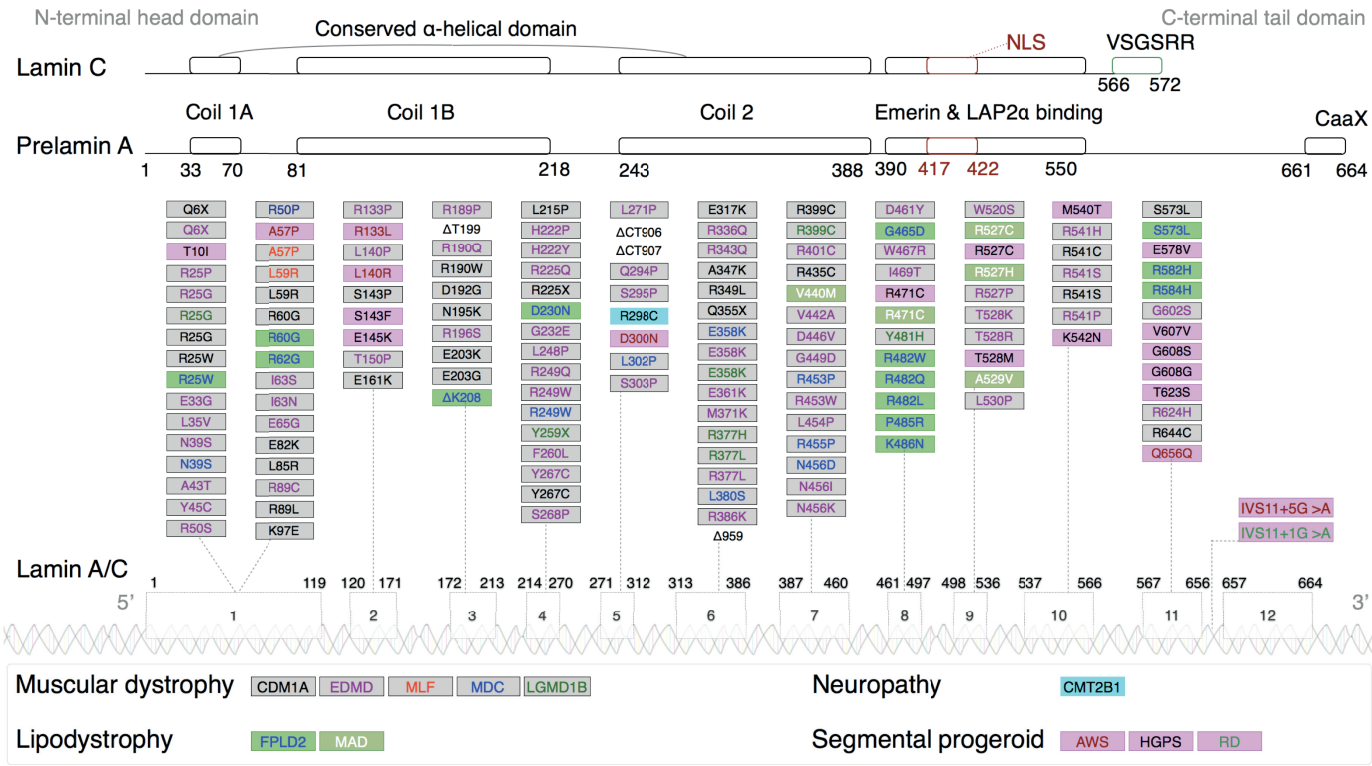
N-terminal head domain
Conserved α-helical domain
C-terminal tail domain
Lamin C
NLS
VSGSRR
566 572
Coil 1A
Coil 1B
Coil 2
Emerin & LAP2α binding
CaaX
Prelamin A
1 33 70 81 218 243 388 390 417 422 550 661 664
Q6X Q6X T10I R25P R25G R25G R25G R25W R25W E33G L35V N39S N39S A43T Y45C R50S
R50P A57P A57P L59R L59R R60G R60G R62G I63S I63N E65G E82K L85R R89C R89L K97E
R133P R133L L140P L140R S143P S143F E145K T150P E161K
R189P ΔT199 R190Q R190W D192G N195K R196S E203K E203G ΔK208
L215P H222P H222Y R225Q R225X D230N G232E L248P R249Q R249W R249W Y259X F260L Y267C Y267C S268P
L271P ΔCT506 ΔCT507 Q294P S295P R298C D300N L302P S303P
E317K R336Q R343Q A347K R349L Q355X E358K E358K E358K E361K M371K R377H R377L R377L L380S R386K Δ959
R399C R399C R401C R435C V440M V442A D446V G449D R453P R453W L454P R455P N456D N456I N456K
D461Y G465D W467R I469T R471C R471C Y481H R482W R482Q R482L P485R K486N
W520S R527C R527C R527H R527P T528K T528R T528M A529V L530P
M540T R541H R541C R541S R541S R541P K542N
S573L S573L E578V R582H R584H G602S V607V G608S G608G T623S R624H R644C Q656Q
IVS11+5G >A
IVS11+1G >A
Lamin A/C
1 119 120 171 172 213 214 270 271 312 313 386 387 460 461 497 498 536 537 566 567 656 657 664
5' 3'
1 2 3 4 5 6 7 8 9 10 11 12
Muscular dystrophy
CDM1A EDMD MLF MDC LGMD1B
Neuropathy
CMT2B1
Lipodystrophy
FPLD2 MAD
Segmental progeroid
AWS HGPS RD

Figure 5. Distribution of mutations in the *LMNA* gene causing laminopathies. The conserved α-helical segments of the central rod domain marked with coil 1a, coil 1b, and coil 2. Numbers refer to residues in the primary sequence. Lipodystrophy mutations are clustered at exon 8, which codes for an Ig-like domain. The majority of lipodystrophy cases are caused by a mutation at p.482. >90% of HGPS patients carry the *de novo* c.1824C>T, G608G mutation. CDM1A, dilated cardiomyopathy, type 1A; EDMD, Emery–Dreifuss muscular dystrophy; MLF, Malouf Syndrome; MDC, Muscular dystrophy, congenital; LGMD1B, limb girdle muscular dystrophy, type 1B; FPLD, Dunnigan familial partial lipodystrophy; MAD, mandibuloacral dysplasia; CMT2B1, Charcoot-Marie–Tooth disorder, type 2B1; AWS, atypical Werner syndrome; HGPS, Hutchinson–Gilford progeria syndrome. Picture was authorized from the authors to be used in this thesis. (McKenna *et al.,* 2013)[69]

1.6.2 Secondary laminopathies

Secondary laminopathies are caused by mutations in genes other than *LMNA* such as B-type lamin genes (*LMNB1* and *LMNB2*), genes coding for prelamin A processing proteins such as *FACE1*/*Zmpste24 (*e.g. *MAD* and *RD*), or genes coding for lamin-binding proteins such as *EMD, TMPO, LBR* and *LAMD3.*

1.7 LAMINS' INVOLVEMENT IN AGING

Lamins define the nuclear shape of the cell; thus, abnormal nuclear morphology is a general landmark for most laminopathies. However, abnormal nuclear morphology is also common in cancer cells [70 71]. Major changes in the nuclear architecture, similar to those observed in HGPS cells, also accompany aging in the nematode *C. elegans*[72]. In their study, age-related changes, primarily in non-neuronal cells, included progressive alterations of the nuclear shape with a loss of heterochromatin from the nuclear periphery and an abnormal distribution of nuclear envelope proteins as the wild-type animals became older. Additionally, the down regulation of the *C. elegans* lamin, Ce-lamin, and lamin-interacting proteins resulted in earlier death, thereby establishing a fundamental link between lifespan, nuclear morphology and the composition of the nuclear envelope [72]. More recently, the involvement of lamins in the invertebrate aging process has also been confirmed in *D. melanogaster*, where an aberrant nuclear shape and size increased with age. The same phenotype was also caused by the overexpression of the farnesylated lamin proteins, lamin B and Kugelkern, which in turn reduced the lifespan of adult flies [71; 73]. Moreover, the results from *C. elegans*, *D. melanogaster*, *Xenopus laevis* egg and cultured mammalian cells suggest that disturbances in the structure of the nuclear lamina are associated with aging, which affects fundamental cell functions such as chromatin organization, gene regulation, DNA replication, transcription, cell signaling and cell proliferation [72-74]. Since the

discovery that the *LMNA* mutations in exon 11 (which cause the most severe premature aging diseases in humans) generated the production of mutant lamin A (progerin), there has been exponentially growing interest in HGPS. The intensive study of HGPS cells has shown that progeria patients accumulate progerin and that the levels of progerin increase as cells age [12; 75]. These increasing levels coincide with more severe changes in the nuclear architecture, and the severity and progression of the disease are correlated with the amount of progerin that is expressed in the affected cell [12; 76].

1.8 PROGEROID SYNDROMES AND NORMAL AGING

HGPS and WS are likely two of the most-studied human disorders that result in symptoms of premature aging. While HGPS affects children, WS is known as the adult-onset segmental premature aging disease. Studies of these segmental progeroid syndromes have not only increased the understanding of these disorders but have also provided insights into the functions of the nuclear lamina, DNA repair, and normal physiological aging.

Progeroid syndromes caused by mutations in the *LMNA* gene (HGPS, RD, some forms of atypical-HGPS, and MAD) arise due to defects in the lamin A maturation pathway and create a common phenotypic output, which is most likely sustained by a common pathological mechanism with the accumulation of an unprocessed lamin A precursor (table 1).

As in HGPS, a mutation in the *LMNA* gene that activates the cryptic splice site, leading to the production of progerin, has been found in atypical-WS [77]. However, the levels of progerin in atypical-WS were significantly lower than what is usually observed in HGPS. In atypical-WS, similarly misshapen nuclei and an atypical localization of lamin A/C are found [61]. In normal cells, these lamins are localized to the lamina and internal foci in the nucleoplasma. However, in atypical-WS, these foci are not observed in the nucleoplasma[61] .

Abnormal lamin A/C distribution and misshapen nuclei are also observed in MAD patient cells [54; 78]. Heterochromatin loss and thickened nuclear lamina, similar to what is observed in HGPS, has also been found in MAD [79]. In cells from RD patients, the

altered expression of lamin A/C and the accumulation of prelamin A have been observed[67; 80], in addition to an abnormal nuclear structure, including an altered localization of the nuclear pore-associated proteins and altered nuclear protein importation [67; 80; 81].

Additionally, cellular characteristics of human fibroblasts expressing progerin include genome instability such as increased γ-H2AX foci, due to replication fork stalling and accumulation of double strand breaks (DSB), continuous p53 activation, and defective cell proliferation due to cell cycle arrest [82-85]. Furthermore, several studies have demonstrated the role of A-type lamins in telomere maintenance [86; 87]. In HGPS fibroblasts, DNA damage foci are localized to the telomeres in association with senescence [87]. Interestingly, it has recently been shown for normal cells, that telomere-dependent senescence activates the cryptic splice site and progerin production, and that cells with progressive telomere shortening presents extensive changes in alternative splicing [88] supporting recent suggestions for an existing interplay between telomers and lamin A both in HGPS and normal aging.

Table 1: *LMNA and ZMPSTE24* mutations causing segmental progeroid syndromes[1]

Disorder	Gene	Mutation	Effect on mRNA	Effect on Zmpste24 protein function	Affected lamin A/C domain	Effect on lamin A/C proteins
HGPS	*LMNA*	p.G608G	exon 11 Δ150		Tail	prelamin A Δ50[p]
	LMNA	p.G608S	exon 11 Δ150		Tail	prelamin A Δ50[p]
Atypical HGPS	*LMNA*	p.S143F			Rod	mutant lamin A and C
	LMNA	p.E145K			Rod	mutant lamin A and C
	LMNA	p.R527C[h]			Tail	mutant lamin A and C
	LMNA	p.V607V	exon 11 Δ150		Tail	prelamin A Δ50[p]
	LMNA	IVS11+1G >A	exon 11 Δ150		Tail	prelamin A Δ50
	LMNA	p.T623S	exon 11 Δ105		Tail	prelamin A Δ35
	LMNA	p.R644C			Tail	mutant lamin A
Atypical WS	*LMNA*	p.A57P			Head	mutant lamin A and C
	LMNA	p.R133L			Rod	mutant lamin A and C
	LMNA	p.L140R			Rod	mutant lamin A and C
	LMNA	p.D300N			Rod	mutant lamin A and C
RD	*LMNA*	IVS11+1G >A	deletion exon 11		Tail	prelamin A Δ90
	ZMPSTE24	p.I19Y fsX28[h]		PTC, loss		prelamin A accumulation
	ZMPSTE24	p.L91_L209del / p.L362 fsX18	deletion exon 3-5	PTC, loss		prelamin A accumulation
	ZMPSTE24	p.P99L fsX38 / p.L362 fsX18		PTC, loss		prelamin A accumulation
	ZMPSTE24	p.I198Y fsX20[h]		PTC, loss		prelamin A accumulation

	ZMPSTE24	p.I198Y fsX20 / p.L362F fsX19		PTC, loss		prelamin A accumulation
	ZMPSTE24	c.627 +1G >C	deletion exon 5	partial loss		prelamin A accumulation
	ZMPSTE24	p.E239X[h]		PTC, loss		prelamin A accumulation
	ZMPSTE24	p.L362F fsX19[h]		PTC, loss		prelamin A accumulation
	ZMPSTE24	p.L362 fsX18[h]		PTC, loss		prelamin A accumulation
	ZMPSTE24	p.L362 fsX18 / p.Q417X		PTC, loss		prelamin A accumulation
MAD	*LMNA*	p.V440M / p.R527H			Tail (Ig fold)	prelamin A accumulation
	LMNA	p.R471C / p.R527C[c]			Tail (Ig fold)	mutant lamin A and C
	LMNA	p.R527H[h]			Tail (Ig fold)	mutant lamin A and C
	LMNA	p.A529V[h]			Tail (Ig fold)	mutant lamin A and C
	ZMPSTE24	p.W340R / p.F361 fsX379		PTC, partial loss		prelamin A accumulation
Overlapping	*LMNA*	p.R471C[a,h]			Tail (Ig fold)	mutant lamin A and C
syndromes	*LMNA*	p.T528M / p.M540T[b]			Tail (Ig fold)	mutant lamin A and C
	LMNA	p.K542N[b,h]			Tail (Ig fold)	mutant lamin A and C
	LMNA	p.E578V[b]			Tail	mutant lamin A
	LMNA	p.G608G[c]	exon 11 Δ150		Tail	prelamin A Δ50[p]
	ZMPSTE24	p.N265S / p.L362F fsX19[d]		PTC, partial loss		prelamin A accumulation

[a]presents features of HGPS and MAD, [b]presents features of HGPS and WS, [c]presents with intermediate severe features between HGPS and RD, [d]presents features of HGPS, RD and MAD

[h] homozygote mutation, [PTC] premature termination codon, [p] progerin

Please note that this list might not be complete.

(Rodriguez and Eriksson, 2010)[1]

1.9 EVIDENCE IMPLICATING PROGERIN IN NORMAL AGING

The suggestion of a link between HGPS and normal physiological aging is due to recent reports that directly implicate progerin in normal aging.

The first evidence of this came from a study performed on fibroblasts from healthy donors [83]. In this study, it was demonstrated that the wild-type lamin A cryptic splice site in exon 11 (that when mutated leads to the expression of progerin in HGPS) was also sporadically used in healthy unaffected individuals. Additionally, the expression of the progerin transcript lamin AΔ150 in normal cells was reported for the first time and was lower (50-fold) than that of the HGPS samples. It was detected in fibroblast cultures, heart and liver tissues from young and old unaffected individuals, however, the levels of lamin AΔ150 did not increase with age. The inhibition of the cryptic splice site reversed the same age-related nuclear defects as those observed in HGPS and normal old cells such as the reduced heterochromatin-specific trimethylation of Lys9 on histone H3 (Tri-Me-K9H3) and the down-regulation of heterochromatin protein HP1γ [18; 83].

This exciting discovery encouraged researchers to search for more evidence of the possible participation and functional role of progerin during normal aging. One year later, progerin was directly detected via immunofluorescence (IF) in a very small nuclei subpopulation of 4 normal human fibroblast cell lines, and the quantification of the percentage of progerin-positive cells in 2 of these cell lines revealed an increment with the passage number up to approximately 1.5-3.0% [20]. The progerin antibody primary stained giant, binucleated and apoptotic nuclei; because several binucleated nuclei presented aberrant chromosomal segregation that was similar to that observed in HGPS, it was suggested that progerin could contribute to the normal aging process by inducing the same mitotic defects in both HGPS and normal aging [20]. In another study, the hypothesis of progerin; altering mitosis and cell cycle progression was further confirmed in HGPS cells, where it was demonstrated that the stable farnesylation and carboxymethylation of progerin caused an abnormal adherence to the membranes during mitosis, thereby impairing the re-localization of nuclear envelope/lamina components in early G1 [19]. Additionally, they showed that the required phosphorylation of Rb for the G1/S cell cycle transition was absent in HGPS blebbed cells, while HGPS cells that were normally shaped had phosphorylated-Rb.

Together, these studies suggested that progerin could contribute to HGPS and normal aging by interfering with mitosis, nuclear assembly and cell cycle progression [19; 20].

Later same year, it was demonstrated for the first time that the cryptic splice site in exon 11 of lamin A could be used *in vivo* in normal cells from human skin biopsies [89]. Progerin mRNA was detected in all samples and remained constant with age, and the progerin protein appeared to accumulate in the elderly. Progerin accumulated specifically in the primary dermal fibroblasts and terminally differentiated keratinocytes. Progerin-positive fibroblasts first appeared near the basement membrane and spread throughout the dermis with advanced age. The progerin-positive cells were not randomly distributed in the skin, and the cell cultures that were derived from samples with a high number of progerin-positive cells presented in turn few positive fibroblasts in culture; thus, the authors suggested that fibroblasts with progerin expression *in vivo* could correspond to a terminally differentiated or senescent subpopulation [89].

At this time, several questions had been raised regarding progerin expression in normal aging. While progerin had been detected in normal cells, it was not clear whether its expression increased during normal aging. The results from these few studies were somewhat contradictory. While progerin mRNA did not increase with advanced age *in vitro* or *in vivo,* the number of progerin-positive cells increased with advanced age *in vivo*. Additionally, the results regarding progerin accumulation *in vitro* were not completely convincing in some studies either due to questions regarding the amount of samples that had been tested, or whether adequate protein separation had been achieved on western blots, and regarding the specificity and sensitivity of the RT-PCR and qPCR assays.

In paper I, we addressed some of these questions by designing a powerful absolute quantification assay for *LMNA* transcripts (including progerin mRNA) and enhanced protein separation Western [75].

Evidence later came for progerin's role in the cardiovascular disease (CVD) of HGPS patients and the cardiovascular aging of unaffected individuals of advanced ages [90]. In non-HGPS subjects, the progerin-positive cells presented cytoplasmic punctate staining in some cells of the 3 layers of coronary arteries (primarily in adventicia) and were negative for vascular smooth muscle cell (VSMC) staining (SMA, smooth

muscle actin). However, HGPS patients had clear nuclear progerin staining in most VSMC, and progerin was well represented in all 3 layers (adventitial fibroblasts, VSMC of the media and intima, and endothelial cells). This study reaffirmed the hypothesis that a progerin-positive subpopulation increases in size with advanced age in additional cell types and in aging cardiovascular tissue. [90]. Additional evidence that progerin-expressing cells in vivo can correspond to a subpopulation of terminally differentiated or senescent cells can be extrapolated from another recent study in which adult stem cells in HGPS expressed low levels of progerin in comparison with non-stem cells/differentiated cells [91].

While it is known that mutations in exon 11 of the *LMNA* gene cause HGPS, it was not known until recent years what could be causing the increased progerin expression in circumstances of normal aging. In 2011, a study showed that telomere damage had a causative role activating the cryptic splice site. Progerin production was activated during the *in vitro* cell aging of normal fibroblasts in telomere-dependent cellular senescence. The authors proposed a model in senescent cells in which progressive telomere shortening together with the activation of p53 triggers a global alternative splicing, including lamin AΔ150 /progerin, which would in turn contribute to cellular senescence. The authors also presented some evidence for the extensive splicing events that occurred during senescence [88].

1.10 SPLICING DURING DISEASE AND LIFESPAN

1.10.1 Alternative splicing

Alternative splicing (AS) is a major cellular mechanism with several functions in metazoans, primarily in generating proteomic variability [92]. The majority of the genes undergo AS; it was previously thought that AS was an infrequent phenomenon, but more recent studies have reported AS to be highly widespread with approximately 74-95% of the genes being alternatively spliced [93-96]. AS has a universal role in genome evolution, developmental processes, tissue differentiation, and in regulating the transcriptome to influence the phenotype [97]. There are different types of splicing events [98] that normally occur in a regulated manner; however, as in HGPS, aberrant splicing can result in disease. More than 15% of all heritable human diseases have been known to be associated with mutations in splice sites or splicing regulatory elements [92], and a large proportion of human genetic disorders result from splicing variants [99]. Abnormal

splicing variants are thought to contribute to the development of cancer [100-102]. AS occur more often in transcripts from genes expressed in functionally complex tissues with diverse cell types, such as the brain and testis. In brain, AS is implicated in processes such as the control of synaptic plasticity associated with cognition and other neural processes [103-105]. Alterations of the splicing machinery have shown to be causative of diseases such as cystic fibrosis [106] and neuropsychiatric disorders [107; 108].

The study of the transcriptome is important because many phenotypic changes, including splicing, are not detected at the gene expression level but can be detected by investigating AS at the exon expression level [109].

1.10.2 Aging and altered global splicing

Aging in HGPS and normal physiological aging have been associated with widespread changes at the gene expression level in multiple mammalian tissues[16; 110]. Aging is known to damage several cellular components such as the genome and macromolecules (that also may form part of the splicing machinery). However, very little is known regarding how aging affects the variability of the transcriptome through alternative splicing. Recently, some studies have presented results revealing a potential association between aging and genome-wide alterations of alternative splicing. Age-related splicing has been identified in both normal individuals and patients with neurodegenerative diseases such as Alzheimer's disease and frontotemporal lobar degeneration (FTLD) [11; 111]. Additionally, another recent normal aging study in human and rhesus macaque brains using high-throughput transcriptomic sequencing, exon arrays and PCR identified widespread splicing changes with development and age in 40% of the genes (of which 30% corresponded to age-related splicing and 70% to developmental splicing)[112]. However, in a transcriptomic microarray study of blood from aging subjects, few probes (2%) were robustly associated with age; there were 7 age-associated pathways (including the processing of mRNA) [113]. It seems that recent studies points toward a potential effect of age on splicing, and most interestingly that progerin-induced senescense by telomere shortening show that normal cells with progressive telomere shortening *in vitro,* also have extensive changes in splicing [88]

2. AIMS OF THE THESIS

The overall aim of this thesis was to obtain a deeper understanding of the potential relationships that link the genetic mechanisms found in HGPS to those occurring in normal physiological aging.

The specific aims were as follows:

Paper I.
- Develop an absolute quantification method for the *LMNA* locus transcripts.
- Quantify the expression of *LMNA* locus transcripts and proteins during *in vitro* cell aging in samples from HGPS patients and unaffected donors.
- Determine the presence and expression of progerin in cells from unaffected donors during *in vitro* cell aging.

Paper II.
- Evaluate progerin as a potential biomarker for aging.
- Quantify the amount of progerin-expressing cells during *in vitro* cell aging in different age groups of unaffected donors.

Paper III.
- Develop an allele-specific absolute quantification assay for the *LMNA* gene.
- Determine whether the *LMNA* gene has differential gene expression for different alleles to test our hypothesis that high- and low-expression alleles may contribute to the phenotype variability in laminopathies.

Paper IV.
- Investigate the potential effect of physiological aging on splicing in several tissues of normal aging mice.
- Determine which pathways and biological functions are most affected by age-related splicing during physiological aging in normal aging mice.
- Investigate whether the splicing machinery presents signs of decay during the physiological aging of normal aging mice.
- Investigate the potential effect of the expression of the HGPS mutation on splicing in progeria mice.
- Determine which pathways and biological functions are most affected by HGPS-induced splicing.

3. MATERIAL AND METHODS

Material and methods used in this thesis are described in more detail and with specific information for each study in the corresponding section of each paper. Here we present an overview of these and include some extra information not available in the papers.

3.1 LABORATORY ANIMALS

3.1.1 Animal housing

Mice were bred in-house and accommodated in the experimental animal facility (Karolinska Hospital, Huddinge, Sweden). The housing conditions included a 12-hour light/dark cycle, a temperature of 20-22 °C and 50-75% air humidity. Mice were supplied with R36 pellets (Lactamin, Sweden) and drinking water *ad libitum.* Animal studies were approved by the Stockholm South Ethical review board (*Stockholms Södra Djurförsöksetiska Nämd*), Dnr S101-12, S107-09 and S141-06.

3.1.2 C57BL/6J wild-type mice

Male C57BL/6J wild-type mice were aged up to 4, 18, and 28 months (paper IV). When the desired age was reached, the mice were euthanized by cervical dislocation. Tissues from a minimum of 5 mice were collected for each age group for RNA extraction. Extra care was taken during dissection and collection of tissue to make sure that the time ranged between 30-45 minutes and tissues were immediately transferred into liquid nitrogen. Tissues were stored at -80°C prior to homogenization and RNA extraction.

3.1.3 Body weights, and tissue histopathology

C57BL/6J wild-type mice were weighted directly after cervical dislocation. Skin, skeletal muscle and femurs were fixed over night in 4% w/v paraformaldehyde (PFA), (simultaneously with tissue collection for RNA extractions). The following day, tissues were transferred into 70% ethanol for preservation until use. Femur length and thickness were measured at 3 points and then the bones were decalcified in 12.5% EDTA during a period of 3 weeks, before embedding. Tissues were embedded into paraffin, sectioned and stained with hematoxylin and eosin. Microscopic examination for pathology was performed to discard gross lesions suspected of being tumors or

representing major pathological conditions and confirm normal physiological aging pathology.

3.1.4 HGPS mice

An inducible HGPS mouse model with tissue-specific expression of the most common HGPS mutation in the skin, was created in our laboratory, FVB/N.Cg-Tg (tetop-LA^{G608G}, -EGFP) VF1-07; K5tTA[114]. The HGPS mouse model is based on the tet-ON/OFF system, which is a binary transgenic system composed of a transactivator (tTA or rtTA), under the control of a tissue-spcific promotor, and a target gene located downstream of a tet-op. When the transactivator is present it binds to the tet-op and induce expression of the target gene. A second dimension of the tet-ON/OFF system is that it is tetracycline responsive which make it possible to either turn-on or off the transgenic expression of the target gene, simply by adding doxycycline, a tetracycline derivate, to the mice drinking water [115 116]. Gene expression of the HGPS mutation in skin of our transgenice HGPS mice in paper IV was controlled via the Tet-Off system (fig.6).

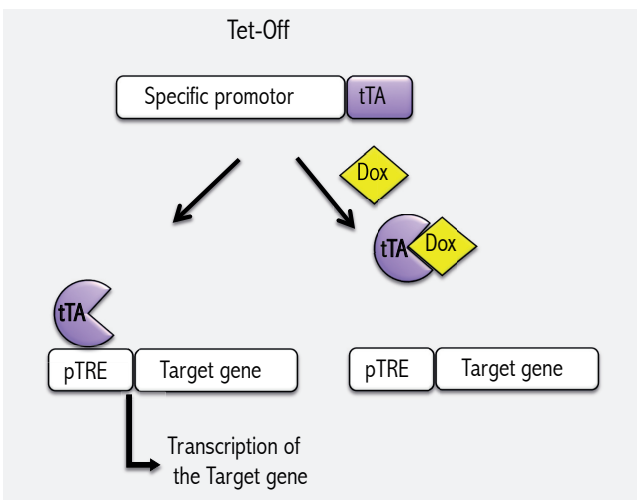

Figure 6. Schematic illustration of the regulation of the expression of the target gene with the tet-Off system.

To generate HGPS mice, transgenic mice that carried the target gene, a minigene of human lamin A with the *LMNA* c.1824C>T, p.G608G mutation (tetop-LA^{G608G}), where intercrossed with transgenic mice carrying the keratin 5 transactivator (K5tTA)[117] (fig.7). Intercrossed breedings were performed with doxycycline supplied

in the drinking water, which was removed at day of birth to induce transgenic expression, in binary transgenic offspring, of human lamin A and progerin within the first postnatal week Sagelius[114 118]. Keratinocytes were isolated from these HGPS mice [118] to be used in paper IV.

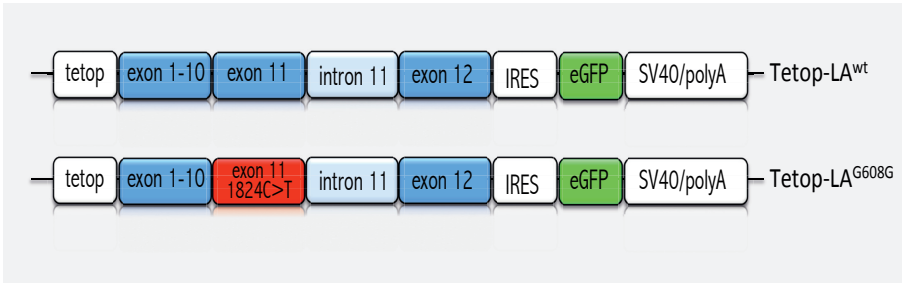

Figure 7. Constructs containing the lamin A minigenes. Schematic illustration of the lamin A constructs. Both constructs contain a responsive pTRE element (tetop), which regulates the expression of the downstream gene in the presence of a transcriptional transactivator. Construct LA, tetop-LAwt, overexpress human lamin A and construct LAG608G overexpress human lamin A and progerin. Tetop=pTRE, IRES=internal ribosomal entry site, eGFP= enhanced green fluorescent protein, poly A= polyadelination sequence.

3.1.5 Isolation of keratinocytes

Primary keratinocytes were isolated from HGPS mice at postnatal days 24 and 35, with transgenic expression induced at the day of birth [118]. Briefly, animals were sacrificed by cervical dislocation, and the fur was trimmed before the skin was taken and placed into Ca^{2+} free PBS (d-PBS; Invitrogen). Subcutaneous tissue was removed, and the skin was floated on trypsin (T4424; Sigma-Aldrich) for 2 h at 32°C. After incubation, the epidermis was scraped into S-minimal essential medium (S-MEM; Invitrogen) supplemented with 0.01% soybean trypsin inhibitor (T9128; Sigma-Aldrich) and 0.5% BSA (bovine albumin fraction V solution (7.5%); Invitrogen) and gently mixed with a magnetic stirrer at room temperature for 20 min. The cell suspension was filtered through a 70-mssenti strainer (BD Falcon, Becton Dickinson, Franklin Lakes, NJ, USA), and viable cells were counted after staining with tryphan blue.

3.2 CELL CULTURE

3.2.1 Cell cultures and growth conditions

Primary dermal fibroblasts and EBV-transformed lymphoblasts were obtained from the Coriell Cell Repositories (Camden, NJ, USA) and from the Progeria Research Foundation Cell and Tissue Bank (PRF, Peabody, Massachusetts, USA). Fibroblasts were grown in monolayer with Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 15% Fetal Bovine Serum (FBS, Biowest or Saveen & Werner), 2mM L-Glutamine (Gibco) and 1% Penicillin/Streptomycin (Gibco) in a humidified cell incubator at 37°C, 5% CO_2. Lymphoblasts were grown in cell suspension in RPMI 1640 medium supplemented with 2mM L-glutamine, 15% heat-inactivated FBS and 1% Penicillin/Streptomycin (Gibco) under same incubation conditions as fibroblasts.

3.2.2 *In vitro* cell aging

Fibroblasts were aged *in vitro* by passaging the cells for several passages until they had lost their proliferative potential. Fibroblasts cultures were grown until they reached 90-100% confluency and then splitted 1:4. Confluent cultures were trypsinated with 0.05% Trypsin-EDTA (Gibco) in the cell incubator for 5-10 minutes. RNA and proteins were extracted from cells collected at every third passage. Cells were also frozen down at about every third passage to keep a back-up of cells if needed. Cells were frozen in 10% DMSO in FBS, and stored at -140°C until use.

3.3 RNA EXTRACTION AND cDNA SYNTHESIS

3.3.1 RNA extraction

Total RNA was extracted from cells and tissues in Trizol, according to standard procedure following the manufacturers protocol (Invitrogen or Ambion). Cells were directly lysed in Trizol while tissues were homogenized in Trizol. RNA pellets were dissolved in nuclease-free water (Ultrapure, Gibco) and quantified using a spectrophotometer (BioPhotometer, Eppendorf). RNA extracts that were not being used for exon arrays were treated with DNase I according to the manufacturer's recommendations (RQ1 RNase-Free DNase kit, Promega). Thereafter, all RNA extracts were purified using RNeasy clean-up kit (Qiagen) and quantified again by regular OD spectrophotometry.

Purified RNA to be used for exon arrays were additionally quantified using a NanoDrop spectrophotometer (NanoDrop Technologies, DE, USA) and total integrity of RNA extracts was assessed by running aliquots on an Agilent 2100 Bioanalyzer (Agilent, CA, USA).

3.3.2 cDNA synthesis

Purified RNA was reverse transcribed with random hexamers using the SuperScript cDNA Synthesis Kits (Invitrogen) and cDNA samples were diluted in nuclease-free water and stored at -80°C for long term storage and -20°C for short term storage.

3.4 RT-PCR

Regular RT-PCR was used for gene walking for detection of novel *LMNA* Isoforms (paper I) and for RT-PCR validation of splicing detected with exon arrays (paper IV). cDNA was amplified by PCR using Hotstar Taq/Hot Star Taq Plus Polymerase kit (Qiagen) with primers designed specific to the gene target. PCR reaction and cycling conditions were optimized for each target. To validate the splicing data obtained from the exon arrays, the primers were designed on flanking exons of the putative spliced region and positioned to make sure that at least one primer was inside one of the spliced exon/s. PCR reactions were performed in a 25 µl reaction volume, including 1-3 µl of cDNA template with Hot Star Taq plus Polymerase (Qiagen). Quality of cDNA template was checked with amplification of β-Actin and each gene was screened on agarose gels for at least 2 different primer combinations

3.5 RELATIVE QUANTITATIVE RT-PCR USING SYBR Green

Relative quantitative real-time RT-PCR (SYBR Green qRT-PCR) was used to validate the gene expression data obtained from the exon arrays (paper IV). Random hexamers and Super-Script II Reverse Transcriptase (Invitrogen) were used for cDNA synthesis from 800 ng of RNA. cDNA was amplified by real-time PCR using SYBR Green master mix. All reactions were run in triplicate, and data was only accepted when the variation among the C_T value triplicates was < 0.3 units for C_T < 30, and < 0.5 units for C_T > 30. To calculate relative changes in gene expression, we used the comparative C_T method, the 2 ΔΔCT method [119]. Data was interpreted as the expression of the gene to be validated relative to the reference gene β-actin in progeria animals compared to wild-type animals.

3.6 ABSOLUTE QUANTITATIVE RT-PCR USING TAQMAN®

Absolute quantitative real-time RT-PCR (Taqman qRT-PCR) was used for absolute quantification of the *LMNA* transcripts (paper I) and allele-specific *LMNA* transcript quantification (paper III). Primer and Taqman MGB probe sets were designed using Primer Expresss Software version 2.0. (Applied Biosystems) and Primer version 3.24. One primer per amplicon was designed to cover an exon–exon boundary. Real-time PCR reactions were performed in a 20 µl reaction volume in triplicates, including 5 µl of cDNA template. Each plate contained a minimum of six different dilutions of cloned standards for a standard curve. All assays were performed with Taqman Universal master mix (Applied Biosystems). The concentration of oligos was initially optimized for each amplicon and the same batch of primers (Medprobe), and probes were used throughout this study. For endogenous controls, we utilized validated assays for β-glucuronidase (GUSB) and ribosomal protein large PO (RPLPO) (Applied Biosystems). The plates were loaded on an ABI7500 fast system sequence detection instrument and run according the manufacturers cycling recommendations.

3. 4 WESTERN

Western in combination with densitometry was used in paper I for characterization and quantification of *LMNA* protein isoforms. Proteins were extracted from cells using RIPA buffer (that included a cocktail of proteinase inhibitors, Roche). Samples were stored at -80°C until usage. Before loading, samples were thawed on ice and diluted in 16M urea to a final concentration of 1.3 M in a SDS loading buffer (including freshly added β-mercaptoethanol). The samples were denatured at 100°C for 5 min. Enhanced protein separation was accomplished using Protean II xi cell western blot system (Biorad) according to previously described procedure [114] where 20 cm long 4%/7.5% discontinuous Laemmli slab gels were used. The gels were run for 13 hours at 20 mA using a cooling system at 2°C. Proteins were transferred to a nitrocellulose membrane filter (Hybond-C +. Amersham Biosciences) using a Semidry Transfer Cell (Biorad) at 24 V for 40 min in 20% methanol/200 ml tris/glycine transfer buffer (Biorad). Filters were blocked in TBS-T, 5% milk for 45 min at RT. After antibody incubations and washings, ECL plus (Amersham Biosciences) was added to the filters for 5 min at RT and the filters were exposed to film.

3.5 DENSITOMETRY

Protein quantification by densitometry was used in paper I. It was performed on Western filters using the Versa Doc Imaging system (Biorad) and analyzed with the Quantity One software (Biorad). Relative band intensities were normalized to the housekeeping gene brotein quantification by densitometry was used in paper I. It was performed on Western filters using the Versa Doc Imaging system (Biorad)

3.6 IMMUNOFLUORESCENCE

To analyze protein expression directly on the cell, we used immunofluorescent stainings (paper II). Cells were stained with different primary antibodies in combination with fluorophore-conjugated secondary antibodies. Fibroblasts, fixed in 4% PFA were permeabilized with 1% NP-40 (Thermo Scientific) and blocked for 30 minutes at room temperature using blocking buffer that contained 5% serum (of the same animal type as the host for the secondary antibody) and 0.1% Brij 58 detergent (Thermo scientific) diluted in DPBS. The primary antibodies were incubated over night at +4°C and the secondary antibodies were incubated one hour RT. Cells were incubated with DAPI (4'-6-Diamidino-2-phenylindole), 3 min at room temperature for counterstaining and mounted with ProLong Gold Antifade Reagent (invitrogen).

3.7 EXON MICROARRAYS

The GeneChip® Mouse Exon 1.0 ST arrays (Affymetrix) were used in paper IV to analyze alternative splicing in aging mice. Gene Chip WT cDNA Synthesis, whole Transcript Sense Target Labeling Synthesis, exon array hybridization, staining and scan was performed by the Karolinska Institute B.E.A core facility at the department of Biosciences and Nutrition. Five point five micrograms of purified RNA was used for the exon array hybridization. Exon array quality control, data analysis and detection of alternatively spliced and differentially expressed genes were performed in Partek Genomic Suite software.

4. RESULTS AND DISCUSSION

4.1 PAPER I

To determine whether progerin was expressed during normal aging, we developed an absolute quantification method via real-time RT-PCR to measure the expression levels of the *LMNA* gene transcripts lamin A, lamin C, and lamin Δ150/progerin during the *in vitro* cell aging of primary dermal fibroblasts from HGPS patients, as well as age-matched and parent unaffected controls. Our main findings showed that the progerin transcript was expressed at very low levels in all samples from young and adult unaffected controls, which is in agreement with studies that have quantified progerin mRNA in normal fibroblast cells[83] [89]. Our results showed that the overall expression level of progerin mRNA in normal cells was 160-fold lower than the high expression levels found in the HGPS cells. However, a different finding was obtained in one study [83] that used relative qRT-PCR to demonstrate a 50-fold lower level of expression in unaffected fibroblasts. This difference could be attributed to the power of the quantification technique, sample variation or processing.

An additional finding in our study was that the progerin transcript levels increased during cellular aging in late passages both in the HGPS samples and the adult unaffected controls during *in vitro* cell aging. Previous studies quantifying progerin protein in cell cultures of aging HGPS cells had been done in few samples e.g. [12]; our results were in agreement with these studies. However, we could not confirm this result for the protein using Western blots, most likely due to a high sample variability and limited sensitivity in the assay.

Regarding the increased progerin mRNA levels during cell aging in the late passages of our unaffected controls, the increased level was only statistically significant for our adult controls but not in aged-matched controls, which could mean that it is easier for cells from elder donors to accumulate progerin transcripts; this finding suggests that mis-splicing occurs more frequently in cells from older donors and could occur even more frequently with increased age. However, in contrast with our finding, progerin mRNA quantification in cells from unaffected donors of different ages has shown both *in vitro* [83] and *in vivo* [89] that the progerin mRNA levels remain unchanged with age. This difference could be attributed to the chosen quantification method or to differences between accumulation mechanisms in cellular aging and physiological aging. However,

the model of cellular aging *in vitro* is a valuable tool for studying the molecular mechanism of physiological senescence[120].

Regarding the overall expression levels of Lamin A, C and progerin transcripts, we found that lamin C was (significantly) the most highly expressed transcript in all 3 groups. Considering that Lamin A and Lamin C share promotor, our results suggest that the splicing efficiency for lamin C is favored and/or the lamin C transcripts are more stable. An interesting result was that the lamin AΔ150 expression in HGPS did not affect the overall normal lamin A levels. The combined amounts of lamins A and AΔ150 were approximately twice to those found in the normal controls. We suggested that this could be explained by differences in mRNA stability (with consequences in the accumulation of the lamin AΔ150 transcript), differences in splicing efficiency, or the upregulation of *LMNA* gene expression in HGPS cells. There has been little research on *LMNA* mRNA expression; however, in a later study [121], we demonstrated the presence of high- and low-expressing alleles in the *LMNA* gene. Therefore, this result could also be explained if the HGPS mutation was always located in another high-expressing allele. The increased overall levels of total lamin A (A + AΔ150) in HGPS cells could also help to explain the thickened lamina observed in HGPS cells [12]. However, it is still unclear if progerin accumulates in HGPS due to increased lamin AΔ150 expression, its own accumulation or due to the accumulation of progerin.

We also quantified progerin at the protein level using Western blot and densitometry, but the progerin levels in normal cells were either absent in the cells or below the detection level; this finding has also been communicated by others [89]. Additionally, we found that lamin C migrated as a doublet band in our Western blot with enhanced protein separation. However, we did not find any evidence for a new lamin C splice form (using RT-PCR gene walking). This result could maybe be better explained by migration differences due to phosphorylation or other post-translational modifications. This observation is important to consider when quantifying progerin on Western blots with insufficient separation. In summary, we have shown in this paper that progerin mRNA is present in unaffected individuals and that the levels increase during *in vitro* cell aging both in HGPS and in unaffected parent controls, which supports the hypothesis of increased progerin expression during cellular aging and suggests a link between HGPS and normal aging. Finally, we developed an absolute quantification method that is useful for the absolute quantification of *LMNA* transcripts in HGPS and other laminopathies, as well as during aging.

4.2 PAPER II

The primary aim of this study was to on expand on the first study (paper I) to more samples from unaffected donors and different age groups, and to quantify the progerin protein directly in the cell via immunofluorescence in early and late passages of cells aged and passaged *in vitro*. We used a newly available antibody for progerin and related progerin to DNA damage and cellular senescence. Our results showed that progerin-positive cells with a clear or well-defined nuclear staining pattern is very rarely expressed in cell cultures from unaffected donors. Of 1,000 cells screened per analyzed passage, we were only able to detect 1 cell (0.1%) with a clear nuclear staining in 2 late passages from one elderly donor. The percentage of progerin-positive cells in the passaged cultures from unaffected donors had shown previously that the amount of progerin-positive cells in late passages increased up to 1.5% and 3.0%, respectively, in 2 different samples from a previous publication from a different lab and using a different antibody[20]. However, their quantification included cells that presented diffuse progerin staining surrounding small, highly condensed nuclei that were defined as apoptotic cells [20]. We also observed these diffuse progerin nuclear stainings (ranging from 0-3%) both in cells with condensed nuclei and in dividing cells that co-localized with LBR at the same time; however, we did not classify these as clear nuclear stainings. These co-localizations were most frequent (approximately 6%) in the HGPS cells, followed by young and thereafter old unaffected controls.

Apart from what we observed in the HGPS samples, we did not find any evidence for these co-localizations increasing in the late passages of unaffected controls. In this context, our results are more similar but are still in disagreement. Our progerin staining results in normal cells are more similar to those of McClintock *et al* (2007)[89], that found that fibroblast cultures derived from young subjects had 0.01% progerin-positive cells, while cultures derived from elderly subjects had an average of 0.3-0.8% progerin-positive cells; the amount of progerin-positive cells was lowered when the progerin-positive cells were quantified *in vitro* for the same subjects. Moreover, in their study, most skin sections from unaffected individuals did not have progerin-positive cells, and the distribution of positive cells in the skin was not random. Additionally, McClintock *et al* (2007)[89] presented cultured progerin-positive cells in which at least some of the cells stained could be interpreted to have cytoplasmic/diffuse nuclear staining. Considering the above findings, the amount of screened cells and the nature of the quantified progerin staining could help explain

why it was so difficult to find cells with well-defined nuclear progerin staining in our cultures of unaffected cells; this could also explain the differences observed between these studies[20; 89] and results in paper II.

Due to the extremely low level of progerin-positive cells with clear nuclear staining in the cultures of normal cells, we failed to quantify the progerin-positive cells in the early and late passages of the cells aged *in vitro*. However, the fact that the progerin-positive cells with clear nuclear staining was found in a late passage from an elderly donor is in agreement with the expected increase of progerin with age and during cellular senescence, as observed by McClintock *et al* (2007)[89], who found that this increase was slight but doubled from 0.4-0.8% within 5 passages. A very recent study failed (with the same antibody that we used) to detect progerin in the cell cultures of unaffected young and old individual, and centenarians[122], confirming that the presence of progerin-positive cells in culture is extremely rare.

Regarding progerin in HGPS cultures, we found that the average amount of progerin-positive cells increased from approximately 70% in the early passages to nearly 90% in late passages, confirming the results of previous studies[12; 14; 75]. We observed that the progerin staining was localized both to the nuclear rim and nucleoplasma in early passages, while in late passages, there was a nucleoplasmic depletion of progerin and a thickening of the lamina (with the involvement of progerin). This is agreement with what has been observed with the lamin A/C antibodies in HGPS cells [12] and suggests that the direct increased incorporation of progerin to the nuclear lamina contributes to its thickening. We also observed typical cellular phenotypes for the HGPS cells that have previously been described, such as increased nuclear blebbing, binucleated nuclei and giant cells. However, we have not yet quantified these features.

To relate our results to DNA damage in our cell cultures, we quantified the DNA damage in HGPS and in our unaffected controls. γH2AX-positive cells were detected in all passages, with the lowest amounts present in the early passages of samples from young unaffected and young HGPS samples (near 20% in both); the highest number of cells with DNA damage was unexpectedly found in late passages of our adult unaffected controls (approximately 60%). However, the amount of cells with increased DNA damage in late passages was observed in all 4 groups but was only significant in the young unaffected controls and HGPS, which was likely due to higher variability between the samples of the adults and old controls. We also quantified the cells that

had clear distinguishable DNA damage foci at the power of resolution used. Again, the amount of cells with 1-2 foci was highest (near 50%) in the late passages of the unaffected adults; the foci increased (3-5) in the late passages of HGPS and old unaffected but was not significant in the young and adult groups. And was, in turn, more frequent in the old unaffected group. In summary, the DNA damage presented a considerable variability between samples with a tendency to increase during *in vitro* cell aging.

4.3 PAPER III

The primary goal of this study was to develop an allele-specific quantification assay for *LMNA* transcripts and to determine whether the *LMNA* gene presented allele-specific expression, which could then help explain the phenotypic variability observed in HGPS patients and other laminopathies that arise from the same mutations. For this purpose, we used one of the few polymorphic *LMNA* SNPs (rs4641 C/T) that we genotyped both in the HGPS and unaffected fibroblast cultures and developed an allele-specific absolute quantification method for the lamin A and lamin C transcripts. Our results show that the *LMNA* gene presented allele-specific expression with the presence of high- and low-expressing alleles. The C allele was the most frequently expressed allele and corresponded to 70% of the total transcription for lamin A and lamin C transcripts. Studies investigating the nature of *LMNA* expression and, in particular, allelic expression have been poor; to our knowledge, there has been only one report studying the differential expression of the alleles in the *LMNA* gene[123]; Reddel and Weiss (2004) studied the wild-type and mutant allele in HGPS and controls and, via sequencing and fragment analysis RT-PCR, found no major differences between these alleles; the HGPS mutant allele was slightly less expressed (47%). The sequencing of more *LMNA* haplotype variants may help to identify new differentially expressed alleles in the *LMNA* gene. The aberrant splicing mechanism in HGPS raises questions regarding the splicing efficiency and allele-specific expression of this disease. Therefore, we analyzed the samples that were quantified for progerin from our previous study[75] (this thesis paper I) for the existence of a correlation between higher progerin transcript levels and the allelic localization of the HGPS mutation. We found that the HGPS mutation could be located in either the T or C allele. We were not able to relate the localization of the HGPS mutation to disease

severity due to a lack of clinical information for the majority of our samples. Nevertheless, personal communication with the Progeria Research Foundation confirmed that 2 of their classical HGPS samples that we had analyzed, (and in which the HGPS mutation resided in the low-expressing allele) had been graded as less severe, suggesting a possible role of allele-specific *LMNA* expression in the disease severity of HGPS. Further investigation using our method in clinically classified HGPS samples will help to answer the importance of this SNP in the pathological development of this disease. Interestingly, a recent study[124] identified this SNP as part of a haplotype that is significantly associated with longevity in long-lived humans. It would therefore be of particular interest to use our method and quantify these alleles in cDNA samples from centenarians. In summary, we have developed a powerful allele-specific assay for the *LMNA* gene that may be useful in studying and explaining the phenotypic variability, disease development and severity of laminopathies, premature aging and the possible difference in the impact of these alleles and/or progerin expression during longevity.

4.4 PAPER IV

The primary aim of this study was to investigate the effect of physiological aging splicing globally. Our results showed that there is a considerable amount of genes that are alternatively spliced in all tissues analyzed of mice. This is in agreement with other recent *in vivo* studies that have shown an effect of age on alternative splicing in human brain [111; 112] and human peripheral blood leukocytes[113]. The amount of alternatively spliced genes varied between different tissues, which is expected due to the well-known fact that splicing in general is tissue specific. We also showed that the amount of alternatively spliced genes increased with age and varied significantly between different tissues and periods of the physiological aging process. Skin was in particular interesting; while alternatively spliced genes were not found between the period of 4-18 months, on the contrary during the later aging period 18-28 months, the same tissue presented a great amount of spliced genes. These results suggested that age-dependent alternative splicing, increased with age but the start-off and magnitude of this splicing is different between tissues. Taking into consideration the total amount of alternatively spliced genes during the whole aging period 4-28 months, varying from 1247 to 1886 genes between different tissues this corresponded to 13-30% of the total amount of the

genes analyzed (non-differentially expressed genes) and to 3-9% of the total amount of genes represented on the exon arrays. This can be compared with a recent study[112] in human brain using high throughput sequencing were they found that nearly 40% of the genes that were expressed in human brain had changes in splicing over postnatal life. Of these 70% occurred during development and 30% during aging, which means that 12% of the total amount of genes expressed in brain had aging-related splicing. Considering the variation of age-related splicing that we have shown in our normal aging mice, our results could be in very well agreement with this study. To our knowledge, we are the first to show that the amount of age-related splicing also increased with advancing age, however never reaching a global effect genome-wide. That the focused nature of age-related splicing was restricted to roughly a tenth of the coding genes did not argue for a random global effect of age over splicing and might speak in part against a general decay of the splicing machinery. However, these genes could be more prone to potential acquired splicing deficiency with less stringent splice sites. One important question that remains to be answered is whether this increased splicing with age corresponded to aberrant splicing generating deleterious proteins or if it is part of a normal and regulated mechanism. The used exon arrays are not designed to detect the progerin transcript, so the *LMNA* was not detected as an alternatively spliced gene with age.

When we analyzed which biological functions and pathways that were enriched with alternatively sliced genes across all tissues, we found to our surprise that RNA processing including RNA splicing was the biological function most significantly affected with age-related splicing. In addition, the spliceosome also resulted to be significantly enriched with spliced genes. This means that the splicing machinery is in particular affected by age-related alternative splicing which speaks for that the splicing machinery could be affected. A recent study [113], using transcriptomic microarrays has shown, that in human blood there is an effect of age on RNA processing looking into pathway enrichment of genes with age-associated transcripts, which gives further support to our finding in mice. Their and our study together demonstrated that RNA processing, including RNA splicing is altered during normal physiological aging of both mice and humans. Our results directly implicate the spliceosome with alternatively spliced genes coding for important components of the spliceosome, majorly snRNAs but also spliceosome proteins (Wbp11 Prp19, and Prp43). In the spliceosome, the snRNAs, along with their associated proteins, form ribonucleoprotein

complexes (snRNPs), which bind to specific sequences on the pre mRNA substrate [125] yielding the spliceosome its catalytical competence[126]. This suggests that alternative splicing of spliceosome snRNA during aging, if not aberrant/deleterious to the splicesome, could still potentially have consequences on its specificity and splicing catalysis of specific genes. Explorative network analysis of alternatively spliced genes across all tissues identified RNA post-transcriptional modifications as the most significant network associated function. Two other less significant network associated functions were Dermatological diseases and conditions, and infectious disease with the NF-κB complex as a central node. NF-κB refers to a family of transcription factors with pro-inflammatory functions implicated in diverse disease and pathologies and biological processes. Interestingly, the IKK/NF-κB signaling pathway has been proposed to be one of the key mediators of aging. NF-κB can be activated secondarily to DNA damage via the DNA response protein ataxia telangiectasia mutated (ATM) [127; 128]. In turn, the regulation of NF-κB activation is mediated by the IKK complex of cytoplasmic kinases. In our explorative network, the RNA post-transcriptional modification functions connect to the NF-κB complex node via the nuclear RNA export factor 1 (*NXF1*) and DEAD (Asp-Glu-Ala-Asp) box helicase 3, X-linked (*DDX3X*) genes (see figure 4A, paper IV). The encoded protein of *NXF1* shuttles between the nucleus and the cytoplasm and binds *in vivo* to poly(A)+ RNA and is involved in nuclear export of mature mRNA to the cytoplasm[129]. Alternative splicing seems to be a common mechanism of this gene. *DDX3X* is a RNA helicase involved in several steps of gene expression, such as transcription, mRNA maturation, mRNA export and translation and interacts directly with *NXF1* and may be involved in nuclear export of specific mRNA [130].In turn, NXF1 expression in Jurkat T-cells have shown to activate the NF-kB. This allowed us to speculate that the age-related alternative splicing of NXF1 could potentially contribute to activate the NF-κB complex in several tissues.

We also investigated if same changes observed with alternative splicing occurred with differential expression of genes with age, but did not find any evidence for differentially expressed genes to be involved in RNA processing. Transcriptomic gene expression studies in humans have had low reproducibility du to the usage of different analysis platform, low tissue availability, low sample number and cell type heterogeneity. However, common signatures for aging across tissues and species in mice, rat and humans that have been found within the top- categories for altered gene

expression with age have been related to the immune response, including complement activation, antigen processing by the lysosome, apoptosis and anti-apoptosis pathways[131]. Although gene expression was not the main focus of our study, our results were in agreement with this study, because our top KEGG-pathways enriched with differentially expressed genes, corresponded to the complement and coagulation cascades in skeletal muscle, ECM-receptor interaction pathway in bone, and the Cytokine-cytokine receptor interaction pathway in thymus.

A secondary aim of this study was to independently analyze alternative splicing as an effect of the HGPS mutation in keratinocytes of a progeria mouse model created in our laboratories. Our results showed that the main variance in the exon arrays was represented by the age/development stage (24 days versus 35 days) and secondarily by genotype (wild-type versus HGPS mice). The highest amount of alternatively spliced genes was found during normal development. Strikingly, if compared to the results observed during normal tissue aging this corresponded at least to a double amount of genes alternatively spliced during normal development in comparison to normal aging. This could in part be due to a pure cell type analyzed in progeria mice, while in normal aging whole tissue skin was analyzed, but much probably as it has been described above, during whole life span 70% of the age-related splicing correspond to development and the rest to proper aging [112]. When comparing the same developmental period in bi-transgenic progeria keratinocytes extracted from HGPS mice, the amount of alternatively spliced genes decreased. This suggested that the expression of the HGPS mutation could have an effect on normal developmental splicing or could simply be reflected by the different phase of hair-cycle, where in the wild-type mice the majority of the skin would likely be in early anagen phase at postnatal day 24, and late anagen or early catagen phase at postnatal day 35 [132]. When we compared the amount of genes that were alternatively spliced between wild-type and progeria mice we found that as expected (since the accumulation of progerin with time is central in the disease pathology), that the amount of alternatively spliced genes as effect of the HGPS mutation was higher after 35 days of postnatal transgenic expression and alternatively spliced genes were implicated in the ECM-receptor interaction pathway. ECM (Extra Cellular Matrix) components have been described to be the second largest group of genes misregulated in cultured HGPS cells[16]. Aditionally, the inability to produce a functional ECM has ben proposed to cause proliferative arrest in a mouse model for progeria due to defective Wnt signaling that

affects affects the ECM.[133; 134] Our study gives additional evidence for alterations of the ECM composition via alternative splicing. Interestingly, as in normal aging mice, an NF-κB central node was also identified in network analysis of alternatively spliced genes for our progeria mice, suggesting that alternative splicing might contribute to aging through activation of NF-κB both in physiological aging and progeria. Our results are in agreement with previous studies that have found in fibroblasts of HGPS patients increased levels of NF-κB activation and increased inflammatory gene expression when compared to aged-matched controls[135] and in a recent study of same progeria mouse model that has been used in this thesis, but with embryonic expression of the HGPS mutation [136].

In summary our results showed that during physiological aging of normal mice, there is focused age-related splicing that increases in all tissues analyzed with age. These genes are mainly involved in RNA processing and in the spliceosome pathway, with a potential effect on activation of the NF-κB complex. In addition, we showed that the HGPS mutation reduces the amount of genes that are normally spliced in keratinocytes during mouse development, and expression of the HGPS mutation induced alternative splicing that mainly affected the ECM and potentially also the activation of the NF-κB complex.

5. CONCLUSIONS

Paper I.

- We have developed a powerful method that enables the absolute quantification of *LMNA* locus transcripts.

- We determined the presence and quantified the expression of lamin AΔ150/progerin transcript in HGPS and normal cells and showed that the levels in normal cells were 160-fold lower when compared to the levels in HGPS cells.

- We showed that the levels of the lamin AΔ150/progerin transcript increased during *in vitro* cell aging in fibroblasts from both HGPS patients and adult unaffected donors. This suggest a common genetic mechanism in HGPS and normal physiological aging.

- We showed that the lamin C transcript, was the highest expressed *LMNA* transcript.

- We showed that the expression levels of the lamin AΔ150/progerin in HGPS did not affect the level of lamin A transcripts since the total lamin A transcript levels (lamin A + progerin) were doubled in HGPS compared to normal cells.

Paper II.

- We found that progerin-expressing cells with clear nuclear staining were very rare in primary fibroblast cultures from unaffected donors, both in early and late passages.

- Our results question the physiological relevance of progerin during physiological aging since fibroblasts from unaffected donors only showed clear nuclear staining in at the most 0.1% of the cells.

Paper III.

- We have developed a powerful allele-specific absolute quantification assay for the *LMNA* gene.

- We established that the *LMNA* gene has high and low expressing alleles and showed that the SNP rs4641 C allele was expressed more frequently, which corresponded to 70% of the total lamin A/C transcripts.

- Our results might explain the differences seen in HGPS disease severity and the phenotypic variance among laminopathies

Paper IV.

- We showed that physiological aging has a focused effect on alternative splicing, when analyzed genome-wide, and that alternative splicing was increased with age in all tissues analyzed.

- Our results showed that alternative spliced genes across all tissues were mainly involved in RNA processing and the spliceosome pathways, with a potential effect on splicing specificity and catalytic activity.

- We showed that the expression of the HGPS mutation reduced the amount of genes that were alternatively spliced during normal development and induced alternative splicing of genes involved in the extra cellular matrix- receptor interaction pathway.

- We identified NF-κB as a common node in explorative networks of alternatively spliced genes, independently, in both physiological aging of normal mice and in keratinocytes from HGPS mice.

6. FUTURE PERSPECTIVES

Many studies points towards a potential link between pathological aging in HGPS and physiological aging, however many questions still remains. Increased levels of progerin transcripts during cellular senscense and an increase in the number of progerin positive cells during cellular aging and in senescense during physiological aging has been hard to demonstrate conclusively due to the low frequency of cells expressing progerin both *in vivo* and in culture. One important question is if this low frequency is relevant for physiological aging. McClintock and co-workers, have suggested that the cells in vivo corresponds to a specific subpopulation of terminally differentiated/senescent cells [89]. It would therefore be of interest to closer analyze these cells and characterize them. FACS analysis of progerin positive cell could for instance help to analyze a larger population of cells, by sorting out those few cells expressing progerin and at the same time determine their state of differentiation. Of particular interest would be if some of these cells present stem cell features, since this could maybe help to explain the effect from a small progerin-expressing cell population on physiological aging.

An other question is whether the increased progerin mRNA during cellular aging corresponds to the summary of a global low expression in all cells in culture (below the detection level for IF or if it corresponds to progerin mRNA being expressed only in the

few cells that presents nuclear progerin staining (within the detection limit for IF). FACS analysis of these cells and single cell RNA sequencing could help to answer that question and at the same time investigate if these cells express progerin due to a somatic mutation of the *LMNA* gene rather than a sporadical use of the cryptic splice site. Other studies analyzing the expression of progerin during aging of normal cells in additional tissues are needed. Another important question to resolve is if an increase in the amount of progerin-expressing cells during normal aging is a result of increased use of the cryptic splice site or a constant usage with lamin AΔ150/progerin accumulation only in a few cells. Therefore, an important aspect to investigate would therefore be to analyze progerin mRNA turnover in progerin-expressing cells. However, it seems that progerin will not help to explain aging of the brain tissue since HGPS patient do not present an aging phenotype in the brain [6 8]. This could most probably explained due to that lamin A is not preferentially expressed in the brain, showing far lower expression of lamin A in the central nervous system than in peripheral tissues[137]. (Aditionally, it has been shown that prelamin A and lamin A expression in the brain is regulated by miR-9 [137; 138] and miR-9 overexpression reduce lamin A expression in HeLa cells with no effect on lamin C [137]. Moreover, overexpression of progerin in the hippocampus with consequence of dramatic nuclear blebbings does not lead to any obvious pathological neurodegenerative phenotype in HGPS mice which suggests that post-mitotic cells are less sensitive to the toxic effect from progerin expression [139].

Addional research is needed to evaluate the significance of progerin and the lamina during normal aging. For example, to investigate progerin expression in additional tissues during normal aging, in progeria animal models and maybe even in centenarians. A recent study of centenarians showed that progerin is not expressed neither in centenarians, young or old unaffected individuals, while prelamin A increase with age and showed very high expression in centenarians[122]. This speaks for that the uncleaved farnesyl group is not so toxic as previously thought and could even be involved in longevity. The toxic effect of progerin would therefore rather come from other farnesyl-independent interactions such as with the lamina associated domains (LADs) in the chromatin, telomeres, and other lamins and proteins in the lamina. Therefore it would be of interest to investigate if pre-lamin A levels decrease during normal physiological aging *in vivo*. However, targeting isoprenylcystein methylation of the farnesylated cysteine of the terminal CAAX motif, by reducing the expression and activity of Icmt, ameliorates disease in a progeria mouse model indicating that it is not the farnesyl group

alone but also its methylation that is important in the pathology of progeria mice[140] .

We have shown that *LMNA* gene have allele-specific expression with high and low expressing alleles. Further investigation using our method in clinically classified samples from HGPS patients and individuals with other laminopathies will help to answer the importance of this SNP rs4641 or of its haplotype in the pathological development of these diseases. Interestingly, a recent study[124] identified this SNP as part of a haplotype that was significantly associated with longevity in long-lived humans. It would therefore be of particular interest to use our method and quantify these alleles in centenarians. Additionally, it also remains to be investigated if the rs4641 SNP is linked to other SNPs upstream in the same haplotype block, possibly in the promotor or enhancer region.

To further understand the common mechanisms shared by normal aging and HGPS, it is important to continue to establish common cellular and molecular pathologies that are caused by progerin and being present in both normal physiological aging as well as progeria. Whether progerin has a major role in driving senescence forward at the organismal level, or if it is a by-product of aging as a consequence of accumulated DNA damage deserves to be further explored. We have shown that splicing is altered during normal physiological aging primarily affecting RNA processing and the spliceosome, but could also include progerin. Intrestingly, a recent study showed that splicing is altered during senescense induced by telomere-dependent progerin production [88]. In addition we have shown that the HGPS mutation in mice induce altered splicing during normal development. Therefore, it would be of interest to further investigate if progerin induce age-related splicing in normal physiological aging. For instance we could extract RNA from progerin expressing cells and investigate by high-throughput sequencing altered splicing in other tissues of progeria mouse models, such as vascular smooth muscle cells, fibroblasts and endothelial cells of the arteries, osteoblasts in bone, in HGPS fibroblasts and lymphoblasts, and vascular smooth muscle cells. To investigate if age-related splicing induce progerin production, we could also further analyze our alternatively spliced genes in the spliceosome, and overexpress the different candidate splice variants in cells and then quantify progerin mRNA expression to see which splice variants make a difference, or create splice-reporter for our different splice variants and investigate if old cells activate same splicing observed with our exon arrays.

It is also of importance to determine whether altered splicing during normal aging corresponds to aberrant splicing or to natural regulated splicing with age with specific functions in the modification of the RNA processing. Therefore experiments should be designed to measure splicing efficiency and accuracy of the splicing machinery, in addition to determine if novel or aberrant splicing occur during normal physiological aging.

Finally, our and others observations that NF-κB kappa is a activated both during normal physiological and in HGPS pathological aging encourages us to direct our attention to search for common upstream NF-κB activators in HGPS and normal physiological aging, that are in turn activated by the production of the Hutchinson-Gilford progeria syndrome mutant protein: progerin. For instance it has been reported that accumulation of prelamin A isoforms at the nuclear lamina triggers an ATM- and NEMO-dependent signaling pathway that leads to NF-κB activation and treatment with NF-κB inhibitors resulted in an improved phenotype in mice with prelamin A accumulation[141; 142]. Of particular interests for our study would be to further investigate if the splice forms and increased gene expression of the alternatively spliced genes that were identified in our explorative network analysis (and that connected to the NF-κB complex DDX3X and NXF1), have an impact on NF-κB activity and inflammation.

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8. REFERENCES

1. Rodriguez, S., and Eriksson, M. (2010). Evidence for the involvement of lamins in aging. Current aging science 3, 81-89.

2. Fontana, L., Partridge, L., and Longo, V.D. (2010). Extending healthy life span--from yeast to humans. Science 328, 321-326.

3. Hekimi, S. (2006). How genetic analysis tests theories of animal aging. Nature genetics 38, 985-991.

4. Martin, G.M. (1982). Syndromes of accelerated aging. National Cancer Institute monograph 60, 241-247.

5. Brown, W.T. (1992). Progeria: a human-disease model of accelerated aging. The American journal of clinical nutrition 55, 1222S-1224S.

6. DeBusk, F.L. (1972). The Hutchinson-Gilford progeria syndrome. Report of 4 cases and review of the literature. The Journal of pediatrics 80, 697-724.

7. Hennekam, R.C. (2006). Hutchinson-Gilford progeria syndrome: review of the phenotype. American journal of medical genetics Part A 140, 2603-2624.

8. Merideth, M.A., Gordon, L.B., Clauss, S., Sachdev, V., Smith, A.C., Perry, M.B., Brewer, C.C., Zalewski, C., Kim, H.J., Solomon, B., et al. (2008). Phenotype and course of Hutchinson-Gilford progeria syndrome. The New England journal of medicine 358, 592-604.

9. Cao, H., and Hegele, R.A. (2003). LMNA is mutated in Hutchinson-Gilford progeria (MIM 176670) but not in Wiedemann-Rautenstrauch progeroid syndrome (MIM 264090). Journal of human genetics 48, 271-274.

10. De Sandre-Giovannoli, A., Bernard, R., Cau, P., Navarro, C., Amiel, J., Boccaccio, I., Lyonnet, S., Stewart, C.L., Munnich, A., Le Merrer, M., et al. (2003). Lamin a truncation in Hutchinson-Gilford progeria. Science 300, 2055.

11. Eriksson, M., Brown, W.T., Gordon, L.B., Glynn, M.W., Singer, J., Scott, L., Erdos, M.R., Robbins, C.M., Moses, T.Y., Berglund, P., et al. (2003). Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. Nature 423, 293-298.

12. Goldman, R.D., Shumaker, D.K., Erdos, M.R., Eriksson, M., Goldman, A.E., Gordon, L.B., Gruenbaum, Y., Khuon, S., Mendez, M., Varga, R., et al. (2004). Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. Proceedings of the National Academy of Sciences of the United States of America 101, 8963-8968.

13. Paradisi, M., McClintock, D., Boguslavsky, R.L., Pedicelli, C., Worman, H.J., and Djabali, K. (2005). Dermal fibroblasts in Hutchinson-Gilford progeria syndrome with the lamin A G608G mutation have dysmorphic nuclei and are hypersensitive to heat stress. BMC cell biology 6, 27.

14. McClintock, D., Gordon, L.B., and Djabali, K. (2006). Hutchinson-Gilford progeria mutant lamin A primarily targets human vascular cells as detected by an anti-Lamin A G608G antibody. Proceedings of the National Academy of Sciences of the United States of America 103, 2154-2159.

15. Capell, B.C., and Collins, F.S. (2006). Human laminopathies: nuclei gone genetically awry. Nature reviews Genetics 7, 940-952.

16. Csoka, A.B., English, S.B., Simkevich, C.P., Ginzinger, D.G., Butte, A.J., Schatten, G.P., Rothman, F.G., and Sedivy, J.M. (2004). Genome-scale expression profiling of Hutchinson-Gilford progeria syndrome reveals widespread transcriptional misregulation leading to mesodermal/mesenchymal defects and accelerated atherosclerosis. Aging cell 3, 235-243.

17. Ly, D.H., Lockhart, D.J., Lerner, R.A., and Schultz, P.G. (2000). Mitotic misregulation and human aging. Science 287, 2486-2492.

18. Scaffidi, P., and Misteli, T. (2005). Reversal of the cellular phenotype in the premature aging disease Hutchinson-Gilford progeria syndrome. Nat Med 11, 440-445.

19. Dechat, T., Shimi, T., Adam, S.A., Rusinol, A.E., Andres, D.A., Spielmann, H.P., Sinensky, M.S., and Goldman, R.D. (2007). Alterations in mitosis and cell cycle progression caused by a mutant lamin A known to accelerate human aging. Proceedings of the National Academy of Sciences of the United States of America 104, 4955-4960.

20. Cao, K., Capell, B.C., Erdos, M.R., Djabali, K., and Collins, F.S. (2007). A lamin A protein isoform overexpressed in Hutchinson-Gilford progeria syndrome interferes with mitosis in progeria and normal cells. Proc Natl Acad Sci U S A 104, 4949-4954.

21. Riemer, D., Wang, J., Zimek, A., Swalla, B.J., and Weber, K. (2000). Tunicates have unusual nuclear lamins with a large deletion in the carboxyterminal tail domain. Gene 255, 317-325.

22. Stuurman, N., Heins, S., and Aebi, U. (1998). Nuclear lamins: their structure, assembly, and interactions. Journal of structural biology 122, 42-66.

23. Verstraeten, V.L., Broers, J.L., van Steensel, M.A., Zinn-Justin, S., Ramaekers, F.C., Steijlen, P.M., Kamps, M., Kuijpers, H.J., Merckx, D., Smeets, H.J., et al. (2006). Compound heterozygosity for mutations in LMNA causes a progeria syndrome without prelamin A accumulation. Human molecular genetics 15, 2509-2522.

24. Pollard, K.M., Chan, E.K., Grant, B.J., Sullivan, K.F., Tan, E.M., and Glass, C.A. (1990). In vitro posttranslational modification of lamin B cloned from a human T-cell line. Molecular and cellular biology 10, 2164-2175.

25. Vergnes, L., Peterfy, M., Bergo, M.O., Young, S.G., and Reue, K. (2004). Lamin B1 is required for mouse development and nuclear integrity. Proceedings of the National Academy of Sciences of the United States of America 101, 10428-10433.

26. Rober, R.A., Weber, K., and Osborn, M. (1989). Differential timing of nuclear lamin A/C expression in the various organs of the mouse embryo and the young animal: a developmental study. Development 105, 365-378.

27. Fisher, D.Z., Chaudhary, N., and Blobel, G. (1986). cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filament proteins. Proceedings of the National Academy of Sciences of the United States of America 83, 6450-6454.

28. Lin, F., and Worman, H.J. (1993). Structural organization of the human gene encoding nuclear lamin A and nuclear lamin C. The Journal of biological chemistry 268, 16321-16326.

29. Furukawa, K., Inagaki, H., and Hotta, Y. (1994). Identification and cloning of an mRNA coding for a germ cell-specific A-type lamin in mice. Experimental cell research 212, 426-430.

30. Machiels, B.M., Zorenc, A.H., Endert, J.M., Kuijpers, H.J., van Eys, G.J., Ramaekers, F.C., and Broers, J.L. (1996). An alternative splicing product of the lamin A/C gene lacks exon 10. The Journal of biological chemistry 271, 9249-9253.

31. Beck, L.A., Hosick, T.J., and Sinensky, M. (1990). Isoprenylation is required for the processing of the lamin A precursor. The Journal of cell biology 110, 1489-1499.

32. Holtz, D., Tanaka, R.A., Hartwig, J., and McKeon, F. (1989). The CaaX motif of lamin A functions in conjunction with the nuclear localization signal to target assembly to the nuclear envelope. Cell 59, 969-977.

33. Sinensky, M., Fantle, K., Trujillo, M., McLain, T., Kupfer, A., and Dalton, M. (1994). The processing pathway of prelamin A. Journal of cell science 107 (Pt 1), 61-67.

34. Firmbach-Kraft, I., and Stick, R. (1995). Analysis of nuclear lamin isoprenylation in Xenopus oocytes: isoprenylation of lamin B3 precedes its uptake into the nucleus. The Journal of cell biology 129, 17-24.

35. Schmidt, W.K., Tam, A., Fujimura-Kamada, K., and Michaelis, S. (1998). Endoplasmic reticulum membrane localization of Rce1p and Ste24p, yeast proteases involved in carboxyl-terminal CAAX protein processing and amino-terminal a-factor cleavage. Proceedings of the National Academy of Sciences of the United States of America 95, 11175-11180.

36. Hennekes, H., and Nigg, E.A. (1994). The role of isoprenylation in membrane attachment of nuclear lamins. A single point mutation prevents proteolytic cleavage of the lamin A precursor and confers membrane binding properties. Journal of cell science 107 (Pt 4), 1019-1029.

37. Stewart, C.L., Roux, K.J., and Burke, B. (2007). Blurring the boundary: the nuclear envelope extends its reach. Science 318, 1408-1412.

38. Grossman, E., Medalia, O., and Zwerger, M. (2012). Functional architecture of the nuclear pore complex. Annual review of biophysics 41, 557-584.

39. Goldman, R.D., Gruenbaum, Y., Moir, R.D., Shumaker, D.K., and Spann, T.P. (2002). Nuclear lamins: building blocks of nuclear architecture. Genes & development 16, 533-547.

40. Solovei, I., Wang, A.S., Thanisch, K., Schmidt, C.S., Krebs, S., Zwerger, M., Cohen, T.V., Devys, D., Foisner, R., Peichl, L., et al. (2013). LBR and lamin A/C sequentially tether peripheral heterochromatin and inversely regulate differentiation. Cell 152, 584-598.

41. Konstantinidou, A., Karadimas, C., Waterham, H.R., Superti-Furga, A., Kaminopetros, P., Grigoriadou, M., Kokotas, H., Agrogiannis, G., Giannoulia-Karantana, A., Patsouris, E., et al. (2008). Pathologic, radiographic and molecular findings in three fetuses diagnosed with HEM/Greenberg skeletal dysplasia. Prenatal diagnosis 28, 309-312.

42. Lin, F., Blake, D.L., Callebaut, I., Skerjanc, I.S., Holmer, L., McBurney, M.W., Paulin-Levasseur, M., and Worman, H.J. (2000). MAN1, an inner nuclear membrane protein that shares the LEM domain with lamina-associated polypeptide 2 and emerin. The Journal of biological chemistry 275, 4840-4847.

43. Puente, X.S., Quesada, V., Osorio, F.G., Cabanillas, R., Cadinanos, J., Fraile, J.M., Ordonez, G.R., Puente, D.A., Gutierrez-Fernandez, A., Fanjul-Fernandez, M., et al. (2011). Exome sequencing and functional analysis identifies BANF1 mutation as the cause of a hereditary progeroid syndrome. American journal of human genetics 88, 650-656.

44. Worman, H.J., Fong, L.G., Muchir, A., and Young, S.G. (2009). Laminopathies and the long strange trip from basic cell biology to therapy. The Journal of clinical investigation 119, 1825-1836.

45. Jacob, K.N., and Garg, A. (2006). Laminopathies: multisystem dystrophy syndromes. Molecular genetics and metabolism 87, 289-302.

46. Bonne, G., Di Barletta, M.R., Varnous, S., Becane, H.M., Hammouda, E.H., Merlini, L., Muntoni, F., Greenberg, C.R., Gary, F., Urtizberea, J.A., et al. (1999). Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. Nature genetics 21, 285-288.

47. Morris, G.E. (2001). The role of the nuclear envelope in Emery-Dreifuss muscular dystrophy. Trends in molecular medicine 7, 572-577.

48. Muchir, A., Bonne, G., van der Kooi, A.J., van Meegen, M., Baas, F., Bolhuis, P.A., de Visser, M., and Schwartz, K. (2000). Identification of mutations in the gene encoding lamins A/C in autosomal dominant limb girdle muscular dystrophy with atrioventricular conduction disturbances (LGMD1B). Human molecular genetics 9, 1453-1459.

49. Fatkin, D., MacRae, C., Sasaki, T., Wolff, M.R., Porcu, M., Frenneaux, M., Atherton, J., Vidaillet, H.J., Jr., Spudich, S., De Girolami, U., et al. (1999). Missense mutations in the rod domain of the

lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. The New England journal of medicine 341, 1715-1724.

50. Fatkin, D., Christe, M.E., Aristizabal, O., McConnell, B.K., Srinivasan, S., Schoen, F.J., Seidman, C.E., Turnbull, D.H., and Seidman, J.G. (1999). Neonatal cardiomyopathy in mice homozygous for the Arg403Gln mutation in the alpha cardiac myosin heavy chain gene. The Journal of clinical investigation 103, 147-153.

51. Peters, J.M., Barnes, R., Bennett, L., Gitomer, W.M., Bowcock, A.M., and Garg, A. (1998). Localization of the gene for familial partial lipodystrophy (Dunnigan variety) to chromosome 1q21-22. Nature genetics 18, 292-295.

52. Hegele, R.A., and Pollex, R.L. (2005). Genetic and physiological insights into the metabolic syndrome. American journal of physiology Regulatory, integrative and comparative physiology 289, R663-669.

53. Csoka, A.B., Cao, H., Sammak, P.J., Constantinescu, D., Schatten, G.P., and Hegele, R.A. (2004). Novel lamin A/C gene (LMNA) mutations in atypical progeroid syndromes. Journal of medical genetics 41, 304-308.

54. Novelli, G., Muchir, A., Sangiuolo, F., Helbling-Leclerc, A., D'Apice, M.R., Massart, C., Capon, F., Sbraccia, P., Federici, M., Lauro, R., et al. (2002). Mandibuloacral dysplasia is caused by a mutation in LMNA-encoding lamin A/C. American journal of human genetics 71, 426-431.

55. Simha, V., Agarwal, A.K., Oral, E.A., Fryns, J.P., and Garg, A. (2003). Genetic and phenotypic heterogeneity in patients with mandibuloacral dysplasia-associated lipodystrophy. The Journal of clinical endocrinology and metabolism 88, 2821-2824.

56. Plasilova, M., Chattopadhyay, C., Pal, P., Schaub, N.A., Buechner, S.A., Mueller, H., Miny, P., Ghosh, A., and Heinimann, K. (2004). Homozygous missense mutation in the lamin A/C gene causes autosomal recessive Hutchinson-Gilford progeria syndrome. Journal of medical genetics 41, 609-614.

57. Kirschner, J., Brune, T., Wehnert, M., Denecke, J., Wasner, C., Feuer, A., Marquardt, T., Ketelsen, U.P., Wieacker, P., Bonnemann, C.G., et al. (2005). p.S143F mutation in lamin A/C: a new phenotype combining myopathy and progeria. Annals of neurology 57, 148-151.

58. Liang, L., Zhang, H., and Gu, X. (2009). Homozygous LMNA mutation R527C in atypical Hutchinson-Gilford progeria syndrome: evidence for autosomal recessive inheritance. Acta paediatrica 98, 1365-1368.

59. Zirn, B., Kress, W., Grimm, T., Berthold, L.D., Neubauer, B., Kuchelmeister, K., Muller, U., and Hahn, A. (2008). Association of homozygous LMNA mutation R471C with new phenotype: mandibuloacral dysplasia, progeria, and rigid spine muscular dystrophy. American journal of medical genetics Part A 146A, 1049-1054.

60. Shackleton, S., Smallwood, D.T., Clayton, P., Wilson, L.C., Agarwal, A.K., Garg, A., and Trembath, R.C. (2005). Compound heterozygous ZMPSTE24 mutations reduce prelamin A processing and result in a severe progeroid phenotype. Journal of medical genetics 42, e36.

61. Chen, L., Lee, L., Kudlow, B.A., Dos Santos, H.G., Sletvold, O., Shafeghati, Y., Botha, E.G., Garg, A., Hanson, N.B., Martin, G.M., et al. (2003). LMNA mutations in atypical Werner's syndrome. Lancet 362, 440-445.

62. Yu, C.E., Oshima, J., Fu, Y.H., Wijsman, E.M., Hisama, F., Alisch, R., Matthews, S., Nakura, J., Miki, T., Ouais, S., et al. (1996). Positional cloning of the Werner's syndrome gene. Science 272, 258-262.

63. Epstein, C.J., Martin, G.M., Schultz, A.L., and Motulsky, A.G. (1966). Werner's syndrome a review of its symptomatology, natural history, pathologic features, genetics and relationship to the natural aging process. Medicine 45, 177-221.

64. Goto, M., Miller, R.W., Ishikawa, Y., and Sugano, H. (1996). Excess of rare cancers in Werner syndrome (adult progeria). Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 5, 239-246.

65. Moulson, C.L., Go, G., Gardner, J.M., van der Wal, A.C., Smitt, J.H., van Hagen, J.M., and Miner, J.H. (2005). Homozygous and compound heterozygous mutations in ZMPSTE24 cause the laminopathy restrictive dermopathy. The Journal of investigative dermatology 125, 913-919.

66. Navarro, C.L., Cadinanos, J., De Sandre-Giovannoli, A., Bernard, R., Courrier, S., Boccaccio, I., Boyer, A., Kleijer, W.J., Wagner, A., Giuliano, F., et al. (2005). Loss of ZMPSTE24 (FACE-1) causes autosomal recessive restrictive dermopathy and accumulation of Lamin A precursors. Human molecular genetics 14, 1503-1513.

67. Navarro, C.L., De Sandre-Giovannoli, A., Bernard, R., Boccaccio, I., Boyer, A., Genevieve, D., Hadj-Rabia, S., Gaudy-Marqueste, C., Smitt, H.S., Vabres, P., et al. (2004). Lamin A and ZMPSTE24 (FACE-1) defects cause nuclear disorganization and identify restrictive dermopathy as a lethal neonatal laminopathy. Human molecular genetics 13, 2493-2503.

68. Burke, B., and Stewart, C.L. (2006). The laminopathies: the functional architecture of the nucleus and its contribution to disease. Annual review of genomics and human genetics 7, 369-405.

69. McKenna, T., Baek J-H., and Eriksson, M. (2013). Laminopathies, Genetic Disorders. In Genetic Disorders, P.M.P. (Ed), ed. (InTech), pp 27-63.

70. Dahl, K.N., Kahn, S.M., Wilson, K.L., and Discher, D.E. (2004). The nuclear envelope lamina network has elasticity and a compressibility limit suggestive of a molecular shock absorber. Journal of cell science 117, 4779-4786.

71. Beard, G.S., Bridger, J.M., Kill, I.R., and Tree, D.R. (2008). Towards a Drosophila model of Hutchinson-Gilford progeria syndrome. Biochemical Society transactions 36, 1389-1392.

72. Haithcock, E., Dayani, Y., Neufeld, E., Zahand, A.J., Feinstein, N., Mattout, A., Gruenbaum, Y., and Liu, J. (2005). Age-related changes of nuclear architecture in Caenorhabditis elegans. Proceedings of the National Academy of Sciences of the United States of America 102, 16690-16695.

73. Brandt, A., Krohne, G., and Grosshans, J. (2008). The farnesylated nuclear proteins KUGELKERN and LAMIN B promote aging-like phenotypes in Drosophila flies. Aging cell 7, 541-551.

74. Melcer, S., Gruenbaum, Y., and Krohne, G. (2007). Invertebrate lamins. Experimental cell research 313, 2157-2166.

75. Rodriguez, S., Coppede, F., Sagelius, H., and Eriksson, M. (2009). Increased expression of the Hutchinson-Gilford progeria syndrome truncated lamin A transcript during cell aging. European journal of human genetics : EJHG 17, 928-937.

76. Moulson, C.L., Fong, L.G., Gardner, J.M., Farber, E.A., Go, G., Passariello, A., Grange, D.K., Young, S.G., and Miner, J.H. (2007). Increased progerin expression associated with unusual LMNA mutations causes severe progeroid syndromes. Human mutation 28, 882-889.

77. Hisama, F.M., Lessel, D., Leistritz, D., Friedrich, K., McBride, K.L., Pastore, M.T., Gottesman, G.S., Saha, B., Martin, G.M., Kubisch, C., et al. (2011). Coronary artery disease in a Werner syndrome-like form of progeria characterized by low levels of progerin, a splice variant of lamin A. American journal of medical genetics Part A 155A, 3002-3006.

78. Miyoshi, Y., Akagi, M., Agarwal, A.K., Namba, N., Kato-Nishimura, K., Mohri, I., Yamagata, M., Nakajima, S., Mushiake, S., Shima, M., et al. (2008). Severe mandibuloacral dysplasia caused by novel compound heterozygous ZMPSTE24 mutations in two Japanese siblings. Clinical genetics 73, 535-544.

79. Filesi, I., Gullotta, F., Lattanzi, G., D'Apice, M.R., Capanni, C., Nardone, A.M., Columbaro, M., Scarano, G., Mattioli, E., Sabatelli, P., et al. (2005). Alterations of nuclear envelope and chromatin organization in mandibuloacral dysplasia, a rare form of laminopathy. Physiological genomics 23, 150-158.

80. Sander, C.S., Salman, N., van Geel, M., Broers, J.L., Al-Rahmani, A., Chedid, F., Hausser, I., Oji, V., Al Nuaimi, K., Berger, T.G., et al. (2008). A newly identified splice site mutation in ZMPSTE24 causes restrictive dermopathy in the Middle East. The British journal of dermatology 159, 961-967.

81. Busch, A., Kiel, T., Heupel, W.M., Wehnert, M., and Hubner, S. (2009). Nuclear protein import is reduced in cells expressing nuclear envelopathy-causing lamin A mutants. Experimental cell research 315, 2373-2385.

82. Liu, B., Wang, J., Chan, K.M., Tjia, W.M., Deng, W., Guan, X., Huang, J.D., Li, K.M., Chau, P.Y., Chen, D.J., et al. (2005). Genomic instability in laminopathy-based premature aging. Nature medicine 11, 780-785.

83. Scaffidi, P., and Misteli, T. (2006). Lamin A-dependent nuclear defects in human aging. Science 312, 1059-1063.

84. Kudlow, B.A., Stanfel, M.N., Burtner, C.R., Johnston, E.D., and Kennedy, B.K. (2008). Suppression of proliferative defects associated with processing-defective lamin A mutants by hTERT or inactivation of p53. Molecular biology of the cell 19, 5238-5248.

85. Musich, P.R., and Zou, Y. (2011). DNA-damage accumulation and replicative arrest in Hutchinson-Gilford progeria syndrome. Biochemical Society transactions 39, 1764-1769.

86. Gonzalez-Suarez, I., Redwood, A.B., Perkins, S.M., Vermolen, B., Lichtensztejin, D., Grotsky, D.A., Morgado-Palacin, L., Gapud, E.J., Sleckman, B.P., Sullivan, T., et al. (2009). Novel roles for A-type lamins in telomere biology and the DNA damage response pathway. The EMBO journal 28, 2414-2427.

87. Benson, E.K., Lee, S.W., and Aaronson, S.A. (2010). Role of progerin-induced telomere dysfunction in HGPS premature cellular senescence. Journal of cell science 123, 2605-2612.

88. Cao, K., Blair, C.D., Faddah, D.A., Kieckhaefer, J.E., Olive, M., Erdos, M.R., Nabel, E.G., and Collins, F.S. (2011). Progerin and telomere dysfunction collaborate to trigger cellular senescence in normal human fibroblasts. The Journal of clinical investigation 121, 2833-2844.

89. McClintock, D., Ratner, D., Lokuge, M., Owens, D.M., Gordon, L.B., Collins, F.S., and Djabali, K. (2007). The mutant form of lamin A that causes Hutchinson-Gilford progeria is a biomarker of cellular aging in human skin. PLoS One 2, e1269.

90. Olive, M., Harten, I., Mitchell, R., Beers, J.K., Djabali, K., Cao, K., Erdos, M.R., Blair, C., Funke, B., Smoot, L., et al. (2010). Cardiovascular pathology in Hutchinson-Gilford progeria: correlation with the vascular pathology of aging. Arteriosclerosis, thrombosis, and vascular biology 30, 2301-2309.

91. Wenzel, V., Roedl, D., Gabriel, D., Gordon, L.B., Herlyn, M., Schneider, R., Ring, J., and Djabali, K. (2012). Naive adult stem cells from patients with Hutchinson-Gilford progeria syndrome express low levels of progerin in vivo. Biology open 1, 516-526.

92. Maniatis, T., and Tasic, B. (2002). Alternative pre-mRNA splicing and proteome expansion in metazoans. Nature 418, 236-243.

93. B, M.P., J, M.D., and M, P.G. (2002). Mimotopes and proteome analyses using human genomic and cDNA epitope phage display. Comparative and functional genomics 3, 254-263.

94. Johnson, J.M., Castle, J., Garrett-Engele, P., Kan, Z., Loerch, P.M., Armour, C.D., Santos, R., Schadt, E.E., Stoughton, R., and Shoemaker, D.D. (2003). Genome-wide survey of human alternative pre-mRNA splicing with exon junction microarrays. Science 302, 2141-2144.

95. Wang, E.T., Sandberg, R., Luo, S., Khrebtukova, I., Zhang, L., Mayr, C., Kingsmore, S.F., Schroth, G.P., and Burge, C.B. (2008). Alternative isoform regulation in human tissue transcriptomes. Nature 456, 470-476.

96. Pan, Q., Shai, O., Lee, L.J., Frey, B.J., and Blencowe, B.J. (2008). Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. Nature genetics 40, 1413-1415.

97. Gamazon, E.R., Innocenti, F., Wei, R., Wang, L., Zhang, M., Mirkov, S., Ramirez, J., Huang, R.S., Cox, N.J., Ratain, M.J., et al. (2013). A genome-wide integrative study of microRNAs in human liver. BMC genomics 14, 395.

98. Black, D.L. (2003). Mechanisms of alternative pre-messenger RNA splicing. Annual review of biochemistry 72, 291-336.

99. Matlin, A.J., Clark, F., and Smith, C.W. (2005). Understanding alternative splicing: towards a cellular code. Nature reviews Molecular cell biology 6, 386-398.

100. David, C.J., Chen, M., Assanah, M., Canoll, P., and Manley, J.L. (2010). HnRNP proteins controlled by c-Myc deregulate pyruvate kinase mRNA splicing in cancer. Nature 463, 364-368.

101. Ladomery, M. (2013). Aberrant Alternative Splicing Is Another Hallmark of Cancer. International journal of cell biology 2013, 463786.

102. Bauer, M., Aust, G., and Schumacher, U. (2004). Different transcriptional expression of KIAA1324 and its splicing variants in human carcinoma cell lines with different metastatic capacity. Oncology reports 11, 677-680.

103. Lipscombe, D. (2005). Neuronal proteins custom designed by alternative splicing. Current opinion in neurobiology 15, 358-363.

104. Ule, J., and Darnell, R.B. (2006). RNA binding proteins and the regulation of neuronal synaptic plasticity. Current opinion in neurobiology 16, 102-110.

105. Beffert, U., Weeber, E.J., Durudas, A., Qiu, S., Masiulis, I., Sweatt, J.D., Li, W.P., Adelmann, G., Frotscher, M., Hammer, R.E., et al. (2005). Modulation of synaptic plasticity and memory by Reelin involves differential splicing of the lipoprotein receptor Apoer2. Neuron 47, 567-579.

106. Pagani, F., Buratti, E., Stuani, C., Romano, M., Zuccato, E., Niksic, M., Giglio, L., Faraguna, D., and Baralle, F.E. (2000). Splicing factors induce cystic fibrosis transmembrane regulator exon 9 skipping through a nonevolutionary conserved intronic element. The Journal of biological chemistry 275, 21041-21047.

107. Aberg, K., Saetre, P., Jareborg, N., and Jazin, E. (2006). Human QKI, a potential regulator of mRNA expression of human oligodendrocyte-related genes involved in schizophrenia. Proceedings of the National Academy of Sciences of the United States of America 103, 7482-7487.

108. Nakata, K., Lipska, B.K., Hyde, T.M., Ye, T., Newburn, E.N., Morita, Y., Vakkalanka, R., Barenboim, M., Sei, Y., Weinberger, D.R., et al. (2009). DISC1 splice variants are upregulated in schizophrenia and associated with risk polymorphisms. Proceedings of the National Academy of Sciences of the United States of America 106, 15873-15878.

109. Kwan, T., Benovoy, D., Dias, C., Gurd, S., Provencher, C., Beaulieu, P., Hudson, T.J., Sladek, R., and Majewski, J. (2008). Genome-wide analysis of transcript isoform variation in humans. Nature genetics 40, 225-231.

110. Zahn, J.M., Poosala, S., Owen, A.B., Ingram, D.K., Lustig, A., Carter, A., Weeraratna, A.T., Taub, D.D., Gorospe, M., Mazan-Mamczarz, K., et al. (2007). AGEMAP: a gene expression database for aging in mice. PLoS genetics 3, e201.

111. Tollervey, J.R., Wang, Z., Hortobagyi, T., Witten, J.T., Zarnack, K., Kayikci, M., Clark, T.A., Schweitzer, A.C., Rot, G., Curk, T., et al. (2011). Analysis of alternative splicing associated with aging and neurodegeneration in the human brain. Genome research 21, 1572-1582.

112. Mazin, P., Xiong, J., Liu, X., Yan, Z., Zhang, X., Li, M., He, L., Somel, M., Yuan, Y., Phoebe Chen, Y.P., et al. (2013). Widespread splicing changes in human brain development and aging. Molecular systems biology 9, 633.

113. Harries, L.W., Hernandez, D., Henley, W., Wood, A.R., Holly, A.C., Bradley-Smith, R.M., Yaghootkar, H., Dutta, A., Murray, A., Frayling, T.M., et al. (2011). Human aging is characterized by focused changes in gene expression and deregulation of alternative splicing. Aging cell 10, 868-878.

114. Sagelius, H., Rosengardten, Y., Hanif, M., Erdos, M.R., Rozell, B., Collins, F.S., and Eriksson, M. (2008). Targeted transgenic expression of the mutation causing Hutchinson-Gilford progeria syndrome leads to proliferative and degenerative epidermal disease. Journal of cell science 121, 969-978.

115. Gossen, M., and Bujard, H. (1992). Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. Proceedings of the National Academy of Sciences of the United States of America 89, 5547-5551.

116. Zhu, Z., Zheng, T., Lee, C.G., Homer, R.J., and Elias, J.A. (2002). Tetracycline-controlled transcriptional regulation systems: advances and application in transgenic animal modeling. Seminars in cell & developmental biology 13, 121-128.

117. Diamond, I., Owolabi, T., Marco, M., Lam, C., and Glick, A. (2000). Conditional gene expression in the epidermis of transgenic mice using the tetracycline-regulated transactivators tTA and rTA linked to the keratin 5 promoter. The Journal of investigative dermatology 115, 788-794.

118. Rosengardten, Y., McKenna, T., Grochova, D., and Eriksson, M. (2011). Stem cell depletion in Hutchinson-Gilford progeria syndrome. Aging cell 10, 1011-1020.

119. Schmittgen, T.D., and Livak, K.J. (2008). Analyzing real-time PCR data by the comparative C(T) method. Nature protocols 3, 1101-1108.

120. Hayflick, L. (1965). The Limited in Vitro Lifetime of Human Diploid Cell Strains. Experimental cell research 37, 614-636.

121. Rodriguez, S., and Eriksson, M. (2011). Low and high expressing alleles of the LMNA gene: implications for laminopathy disease development. PloS one 6, e25472.

122. Lattanzi, G., Ortolani, M., Columbaro, M., Prencipe, S., Mattioli, E., Lanzarini, C., Maraldi, N.M., Cenni, V., Garagnani, P., Salvioli, S., et al. (2014). Lamins are rapamycin targets that impact human longevity: a study in centenarians. Journal of cell science 127, 147-157.

123. Reddel, C.J., and Weiss, A.S. (2004). Lamin A expression levels are unperturbed at the normal and mutant alleles but display partial splice site selection in Hutchinson-Gilford progeria syndrome. Journal of medical genetics 41, 715-717.

124. Conneely, K.N., Capell, B.C., Erdos, M.R., Sebastiani, P., Solovieff, N., Swift, A.J., Baldwin, C.T., Budagov, T., Barzilai, N., Atzmon, G., et al. (2012). Human longevity and common variations in the LMNA gene: a meta-analysis. Aging cell 11, 475-481.

125. Guo, Z., Karunatilaka, K.S., and Rueda, D. (2009). Single-molecule analysis of protein-free U2-U6 snRNAs. Nature structural & molecular biology 16, 1154-1159.

126. Brow, D.A. (2002). Allosteric cascade of spliceosome activation. Annual review of genetics 36, 333-360.

127. Huang, T.T., Wuerzberger-Davis, S.M., Wu, Z.H., and Miyamoto, S. (2003). Sequential modification of NEMO/IKKgamma by SUMO-1 and ubiquitin mediates NF-kappaB activation by genotoxic stress. Cell 115, 565-576.

128. Wu, Z.H., Shi, Y., Tibbetts, R.S., and Miyamoto, S. (2006). Molecular linkage between the kinase ATM and NF-kappaB signaling in response to genotoxic stimuli. Science 311, 1141-1146.

129. Viphakone, N., Hautbergue, G.M., Walsh, M., Chang, C.T., Holland, A., Folco, E.G., Reed, R., and Wilson, S.A. (2012). TREX exposes the RNA-binding domain of Nxf1 to enable mRNA export. Nature communications 3, 1006.

130. Lai, M.C., Lee, Y.H., and Tarn, W.Y. (2008). The DEAD-box RNA helicase DDX3 associates with export messenger ribonucleoproteins as well as tip-associated protein and participates in translational control. Molecular biology of the cell 19, 3847-3858.

131. de Magalhaes, J.P., Curado, J., and Church, G.M. (2009). Meta-analysis of age-related gene expression profiles identifies common signatures of aging. Bioinformatics 25, 875-881.

132. Hanif, M., Rosengardten, Y., Sagelius, H., Rozell, B., and Eriksson, M. (2009). Differential expression of A-type and B-type lamins during hair cycling. PloS one 4, e4114.

133. Hernandez, L., Roux, K.J., Wong, E.S., Mounkes, L.C., Mutalif, R., Navasankari, R., Rai, B., Cool, S., Jeong, J.W., Wang, H., et al. (2010). Functional coupling between the extracellular matrix and nuclear lamina by Wnt signaling in progeria. Developmental cell 19, 413-425.

134. Mounkes, L.C., Kozlov, S., Hernandez, L., Sullivan, T., and Stewart, C.L. (2003). A progeroid syndrome in mice is caused by defects in A-type lamins. Nature 423, 298-301.

135. Adler, A.S., Sinha, S., Kawahara, T.L., Zhang, J.Y., Segal, E., and Chang, H.Y. (2007). Motif module map reveals enforcement of aging by continual NF-kappaB activity. Genes & development 21, 3244-3257.

136. McKenna, T., Rosengardten, Y., Viceconte, N., Baek, J.H., Grochova, D., and Eriksson, M. (2013). Embryonic expression of the common progeroid lamin A splice mutation arrests postnatal skin development. Aging cell.

137. Jung, H.J., Coffinier, C., Choe, Y., Beigneux, A.P., Davies, B.S., Yang, S.H., Barnes, R.H., 2nd, Hong, J., Sun, T., Pleasure, S.J., et al. (2012). Regulation of prelamin A but not lamin C by miR-9, a brain-specific microRNA. Proceedings of the National Academy of Sciences of the United States of America 109, E423-431.

138. Nissan, X., Blondel, S., Navarro, C., Maury, Y., Denis, C., Girard, M., Martinat, C., De Sandre-Giovannoli, A., Levy, N., and Peschanski, M. (2012). Unique preservation of neural cells in Hutchinson- Gilford progeria syndrome is due to the expression of the neural-specific miR-9 microRNA. Cell reports 2, 1-9.

139. Baek, J.H., Schmidt, E., Viceconte, N., Strandgren, C., Hultenby, K., Mugnaini, E., Rozell, B., and Eriksson, M. (2014). Post mitotic neuronal cells are protected from neuropathological changes induced by the common progeroid lamin A splice mutation. . In. (

140. Ibrahim, M.X., Sayin, V.I., Akula, M.K., Liu, M., Fong, L.G., Young, S.G., and Bergo, M.O. (2013). Targeting isoprenylcysteine methylation ameliorates disease in a mouse model of progeria. Science 340, 1330-1333.

141. Osorio, F.G., Lopez-Otin, C., and Freije, J.M. (2012). NF-kB in premature aging. Aging 4, 726-727.

142. Osorio, F.G., Barcena, C., Soria-Valles, C., Ramsay, A.J., de Carlos, F., Cobo, J., Fueyo, A., Freije, J.M., and Lopez-Otin, C. (2012). Nuclear lamina defects cause ATM-dependent NF-kappaB activation and link accelerated aging to a systemic inflammatory response. Genes & development 26, 2311-2324.