Title: MANDIBULOACRAL DYSPLASIA: A PREMATURE AGEING DISEASE WITH ASPECTS OF PHYSIOLOGICAL AGEING

Authors: Vittoria Cenni, Maria Rosaria D'Apice, Paolo Garagnani, Marta Columbaro, Giuseppe Novelli, Claudio Franceschi, Giovanna Lattanzi

PII: S1568-1637(17)30175-7
DOI: https://doi.org/10.1016/j.arr.2017.12.001
Reference: ARR 800

To appear in: *Ageing Research Reviews*

Received date: 28-7-2017
Revised date: 9-11-2017
Accepted date: 1-12-2017

Please cite this article as: Cenni, Vittoria, D'Apice, Maria Rosaria, Garagnani, Paolo, Columbaro, Marta, Novelli, Giuseppe, Franceschi, Claudio, Lattanzi, Giovanna, MANDIBULOACRAL DYSPLASIA: A PREMATURE AGEING DISEASE WITH ASPECTS OF PHYSIOLOGICAL AGEING.Ageing Research Reviews https://doi.org/10.1016/j.arr.2017.12.001

MANDIBULOACRAL DYSPLASIA: A PREMATURE AGEING DISEASE WITH ASPECTS OF PHYSIOLOGICAL AGEING

Vittoria Cenni[1,2*], Maria Rosaria D'Apice[3], Paolo Garagnani[4,5], Marta Columbaro[2], Giuseppe Novelli[3,6], Claudio Franceschi[4], Giovanna Lattanzi[1,2*].

[1]CNR Institute of Molecular Genetics, Unit of Bologna, Via di Barbiano 1/10 I-40136, Bologna, Italy.

[2]Rizzoli Orthopedic Institute, Bologna, Via di Barbiano 1/10 I-40136, Bologna, Italy.

[3]Tor Vergata University Hospital, Viale Oxford 81, I-00133 Rome, Italy.

[4]Department of Experimental, Diagnostic and Specialty Medicine, Alma Mater Studiorum, University of Bologna, Via Massarenti 9, I-40148 Bologna, Italy.

[5]Clinical Chemistry, Department of Laboratory Medicine, Karolinska Institutet at Huddinge University Hospital, S-141 86 Stockholm, Sweden.

[6]University of Rome Tor Vergata, Via Montpellier 1, I-00133 Rome, Italy.

*Correspondence to:

Vittoria Cenni: vittoria.cenni@cnr.it

Giovanna Lattanzi: giovanna.lattanzi@cnr.it

HIGHLIGHTS

- Mandibuloacral dysplasia (MAD) is a premature ageing disease caused by *LMNA* and *ZMPSTE24* mutations.
- MAD causes severe abnormalities in bone, skin and adipose tissue.
- Prelamin A accumulation in MAD affects adipogenesis, bone differentiation and cellular response to stress.
- Potential therapeutic approaches for MAD can provide new tools to treat ageing-associated diseases.
- Genetic defects in lamin A or lamin-A processing enzyme elicit determinants of ageing.

Abstract

Mandibuloacral dysplasia (MAD) is a rare genetic condition characterized by bone abnormalities including localized osteolysis and generalized osteoporosis, skin pigmentation, lipodystrophic signs and mildly accelerated ageing. The molecular defects associated with MAD are mutations in *LMNA* or *ZMPSTE24* (*FACE1*) gene, causing type A or type B MAD, respectively. Downstream of *LMNA* or *ZMPSTE24* mutations, the lamin A precursor, prelamin A, is accumulated in cells and affects chromatin dynamics and stress response. A new form of mandibuloacral dysplasia has been recently associated with mutations in *POLD1* gene, encoding DNA polymerase delta, a major player in DNA replication. Of note, involvement of prelamin A in chromatin dynamics and recruitment of DNA repair factors has been also determined under physiological conditions, at the border between stress response and cellular senescence. Here, we review current knowledge on MAD clinical and pathogenetic aspects and highlight aspects typical of physiological ageing.

Keywords: Progeroid syndromes; Mandibuloacral dysplasia (MAD); Lamin A/C gene (*LMNA*); ZMPSTE24; Prelamin A; Aging.

1. Introduction

In 2002, the discovery that a single amino acid substitution in lamin A/C can cause Mandibuloacral Dysplasia (MAD), a progeroid disease with lipodystrophy and bone abnormalities (Novelli et al., 2002), suggested for the first time a key role of the nuclear lamina in organism development and ageing and paved the way to the discovery of *LMNA* mutations causing Hutchinson-Gilford Progeria (HGPS) (De Sandre-Giovannoli et al., 2003; Eriksson et al., 2003). Since then, a plethora of studies have deepened the link between lamin A/C and the ageing process (Camozzi et al., 2014; Lattanzi et al., 2014; Liu et al., 2013; Misteli and Scaffidi, 2005; Porter et al., 2016) and new findings have provided an unexpected view of mechanisms related to cellular and organism senescence. Among all the progeroid syndromes, those linked to *LMNA* mutations are of utmost interest as traits of those diseases faithfully recapitulate ageing aspects. These diseases, known as progeroid laminopathies, feature accelerated ageing, ranging from mildly accelerated as in type A MAD (MADA) to premature and severely accelerated as in HGPS (Evangelisti et al., 2016). More recently, another *LMNA*-linked progeroid phenotype, recapitulating all aspects of MADA, but with variable involvement of diverse tissues, has been characterized and called atypical progeria syndrome (APS) (Garg et al., 2009). Interestingly, while bone, skin and adipose tissue are severely affected in all forms of *LMNA*-linked progeria and cardiovascular system is selectively involved in HGPS pathology, brain is always spared due to downregulation of mutated transcripts in this organ (Evangelisti et al., 2016). Progeroid laminopathies can be also linked to mutations of other genes contributing to lamin A/C processing and to structure and function of the nuclear lamina, such as *ZMPSTE24*, which is mutated in B type MAD (MADB) (Agarwal et al., 2003) , and *BANF1*, mutated in the Nestor-Guillermo Progeria Syndrome (NGPS) (Soria-Valles et al., 2015). Additionally, an unexpected discovery in 2013 identified DNA polymerase delta gene (*POLD1*) mutations as the cause of a new progeroid disorder featuring mandibular hypoplasia, deafness and generalized lipodystrophy (MDPL) (Weedon et al., 2013). So far, mutations in *POLD1* gene have been identified in 16 patients with MDPL syndrome (Elouej et al., 2017; Lessel et al., 2015; Pelosini et al., 2014; Reinier et al., 2015). Based on accumulated evidence after the discovery of *POLD1* pathogenetic mutations (Elouej et al., 2017; Lessel et al., 2015; Pelosini et al., 2014), we may consider MDPL as a variant of MAD phenotype, with generalized lipodystrophy, osteopenia, accelerated ageing, hair abnormalities and short stature as recurring phenotypes.

Since the discovery of MAD and HGPS mutations, prelamin A, the precursor protein of lamin A, has been recognized as a new player in ageing-related pathways . In fact, accumulation of prelamin A to toxic levels has been found in MADA (Agarwal et al., 2003; Capanni et al., 2005; Cenni et al., 2014; Evangelisti et al., 2015), while accumulation of a prelamin A truncated form, called progerin,

has been determined in HGPS (Columbaro et al., 2005; Eriksson et al., 2003). Prelamin A involvement in APS is still matter of debate (Mattioli et al., in preparation). Moreover, it has been demonstrated that prelamin A accumulation may also occur in the absence of any *LMNA* mutation, can be triggered by oxidative or replicative stress stimuli (Lattanzi et al., 2014) and, when accumulated to toxic levels, may induce cellular senescence (Porter et al., 2016). However, fine tuning of prelamin A levels is requested to keep cells in healthy state. In fact, while prelamin A is accumulated in smooth muscle cells from healthy individuals subjected to stress or during ageing (Liu et al., 2013; Porter et al., 2016), low levels of farnesylated prelamin A ensure rapid stress response in centenarian fibroblasts (Lattanzi et al., 2014) and are even required in differentiated muscle cells (Mattioli et al., 2011).

Actually, the nuclear lamina as a whole undergoes remodeling during cellular senescence, and other nuclear envelope/lamina constituents, besides A type lamins, have been implicated in the ageing process. Modulation of lamin B1 or lamin B2 during cellular senescence has been demonstrated (Barascu et al., 2012; Butin-Israeli et al., 2011; Dreesen et al., 2013) and the lamin A/C binding partner BAF has been implicated in a bone disease featuring accelerated ageing, the NGPS (Loi et al., 2016; Soria-Valles et al., 2015).

MAD cells show typical morphological abnormalities resembling those observed in cells subjected to stress as well as in cells from old individuals, consisting of nuclear dysmorphism, loss of peripheral heterochromatin and nuclear lamina thickening (Filesi et al., 2005; Lattanzi et al., 2014). Importantly, while in normal cells both prelamin A levels and chromatin organization are restored upon recovery from oxidative stress (Lattanzi et al., 2014), mutations in *LMNA* may cause progressive prelamin A accumulation and heterochromatin loss in a sort of unscheduled and dysregulated stress response (Angori et al., 2017) (Mattioli et al., in preparation).

Here, we review current knowledge on MAD clinical features and pathogenetic pathways, and highlight MAD phenotypes also occurring in physiological ageing.

2. Clinical aspects of MAD

Overall phenotype

MAD is a rare autosomal recessive condition first reported by Young et al. (Young et al., 1971). The first cases have been mistakenly diagnosed as Cleidocranial Dysplasia (Cavallazzi et al., 1960). Additional reports of MAD patients defined two types of MAD associated with two types of body fat distribution pattern that will be later linked to different genetic defects: MADA (OMIM #248370) associated to type A pattern of lipodystrophy and mutations in *LMNA* gene (Novelli et al.,

2002) and MADB (OMIM #608612), caused by mutations of the zinc metalloproteinase *ZMPSTE24* gene, presenting with type B generalized lipodystrophy (Agarwal et al., 2003).

Clinical signs of MADA and MADB present usually in early childhood (at 4-5 years), with bone, skin, and adipose tissue alterations (Pallotta and Morgese, 1984; Tenconi et al., 1986; Tudisco et al., 2000). The signs and symptoms of MADB can develop by the age of 2, defining a more severe phenotype than MADA (Cunningham et al., 2010) (Fig. 1).

Mandibular hypoplasia, dental crowding, clavicular resorption, acral osteolysis, skin abnormalities and partial lipodystrophy are typical of MADA (Novelli et al., 2002). Mildly accelerated ageing becomes visible in the second decade in MADA patients (Avnet et al., 2011). Mandibular hypoplasia, dental crowding, premature tooth eruption, clavicular resorption, acral osteolysis, skin abnormalities including absent or sparse hair (total or subtotal alopecia), generalized lipodystrophy and short stature are clinical signs of MADB, which is also associated with more pronounced accelerated ageing (Agarwal et al., 2003). Accelerated ageing appears in the first or second decade in MDPL patients (Lessel et al., 2015; Weedon et al., 2013), although a severe premature ageing phenotype resembling HGPS has been recently described (Elouej et al., 2017). MAD patients' cognition and social behavior are normal, allowing them to attend regular schools. A schematic representation of onset of clinical manifestations of typical MADA and MADB is reported in Figure 1.

As all progeroid laminopathies, MAD is a segmental form of progeria (Evangelisti et al., 2016), with several organs, including brain, completely spared and others, such as bone, skin and adipose tissue severely affected. Myocardium is not a target of MAD pathology, although secondary effects on the cardiovascular system are observed in some patients (https://rarediseases.org/rare-diseases/mandibuloacral-dysplasia/).

Bone abnormalities

MAD patients have a distinctive facial appearance presenting in most cases with prominent scalp veins, ocular proptosis, beak-like thin nose, slow and progressive osteolysis of mandible, terminal phalanges and clavicles (Fig. 2), and small mouth with malocclusion and overlapping teeth (Cunningham et al., 2010; Novelli et al., 2002).

Delayed closure of the fibrous joints of the skull (cranial sutures) in affected infants is common. The chest appears bell-shaped and very narrow due to severe bilateral clavicular hypoplasia due to post-natal onset osteolysis and thin ribs due to bone resorption (Fig. 2). The distal phalanges of all digits are short and club-shaped (acroosteolysis), and the interphalangeal joints are prominent (Fig. 2). Finger and toe nails are dystrophic, brittle, broad, and curved downward. MADA patients also

have generalized joint stiffness with limited extension at the right elbow, knees, and ankles (Pallotta and Morgese, 1984). Bone abnormalities show an age-related progression (Garavelli et al., 2009) and osteolysis occurs in all MAD patients. In addition, MADB patients have several unusual skeletal abnormalities including breaking of vertebrae, development of amorphous subcutaneous calcium deposits, progressive submetaphyseal changes at the proximal ends of long bones and severe osteoporosis with fractures and delayed healing (Cunningham et al., 2010). Bone resorption, especially at phalanges and in young individuals, is often associated with pain. Osteopenia and mandibular hypoplasia, beaked nose, crowded teeth are characteristic of MDPL (Pelosini et al., 2014; Weedon et al., 2013).

Skin abnormalities

In MAD patients, the skin appears thin and wrinkled on neck and abdomen, with mottled brown hyperpigmentation area (Acanthosis nigricans), and it is atrophic over acral regions, with visible veins and tendons because of lack of subcutaneous fat (Novelli et al., 2002; Pallotta and Morgese, 1984). Skin rigidity may limit joint movements (Pallotta R., personal communication). Thigh facial skin or scleroderma-like skin and skin thinning have been described in MDPL (Lessel et al., 2015).

Lipodystrophy

In MADA, the distribution pattern of fat throughout the body is altered with degeneration of subcutaneous adipose tissue in torso and limbs and accumulation on the face, neck and trunk (Novelli et al., 2002). Some variability in the distribution of fat around the neck is observed among patients, with cases of normal fat or slightly excess in the neck, others with accumulation in the back of the head (occiput) and upper back causing a buffalo hump. This pattern of partial lipodystrophy, called type A lipodystrophy, is similar to that described in patients with Dunningan-type familial partial lipodystrophy (FPLD2; OMIM #151660), caused by other mutations in the *LMNA* gene (Araujo-Vilar et al., 2009). Lipodystrophy may be associated with clinical features of metabolic syndromes including insulin resistance, impaired glucose tolerance, and diabetes mellitus (Simha and Garg, 2002), which are treated according to conventional therapies. Some MADA female patients present lack of breast development with normal or irregular menstrual periods (Ozer et al., 2016). Contrary to what observed in MADA, there is a specific association of MADB with generalized lipodystrophy (Type B lipodystrophy) with almost complete loss of subcutaneous fat (Simha and Garg, 2002). However, hyperinsulinemia and insulin resistance associated with its metabolic consequences are reported in most MADA and MADB cases (Simha and Garg, 2002). Partial or generalized lipodystrophy is observed in APS (Garg et al., 2009), while generalized

lipodystrophy is a common feature of all so far described MDPL patients (Elouej et al., 2017; Lessel et al., 2015; Pelosini et al., 2014; Weedon et al., 2013).

Renal disease

The presence of renal disease known as focal segmental glomerulosclerosis (FSGS) is a distinguishing feature of MADB patients. This kidney disorder can potentially progress to cause severe complications such as nephrotic syndrome and, ultimately, kidney failure (Agarwal et al., 2006).

Muscle involvement

Skeletal and cardiac muscle are not affected in most cases of MADA and MADB. However, muscle phenotypes overlapping with other laminopathies have been described, usually associated with mutations other than the R527H *LMNA* hot spot mutation. In these cases, muscle weakness may occur (Lombardi et al., 2007). Muscle wasting has been also reported in MDPL (Lessel et al., 2015).

3. Gene mutations associated with MAD

3.1 LMNA gene and products

Lamin A and C are nuclear lamina proteins produced by alternative splicing from the same transcript encoded by *LMNA* gene (cytogenetic location: 1q22), which also encodes lamin A Δ10 and the germ cell-specific lamin C2. In the nucleus, lamin A/C are the major constituents of the nuclear lamina and play fundamental roles in the regulation of several cellular events.

Mature lamin A is produced at the end of the maturation of its precursor, prelamin A, which is 18 amino acids longer (Davies et al., 2011; Lattanzi et al., 2007). In particular (Fig. 3), prelamin A, which presents a -CAAX group at its C-terminus, undergoes hierarchical processing steps, including farnesylation of the Cys-residue of the –CAAX group by a farnesyl-transferase, followed by a proteolytic cleavage of the farnesylated region by an endoprotease (which can be RCE1 or ZMPSTE24). The cleaved intermediate is then methylated by the Isoprenylcysteine Carboxyl Methyltransferase **(**ICMT) and once again cleaved by ZMPSTE24, giving rise to the final product, lamin A. It has been calculated that prelamin A is processed with a half-time of about 30 minutes (Lehner et al., 1986). Accumulation of prelamin A processing intermediates due to lamin A/C mutations or altered ZMPSTE24 activity have deleterious consequences on the physiology of cells and tissues, as observed in many laminopathies, in which severity of disease is tightly related to

prelamin A abundance (Agarwal et al., 2003; Denecke et al., 2006; Filesi et al., 2005; Wang et al., 2016).

This is the case of degenerative or deadly diseases as MADA and MADB, or HGPS (OMIM #176670) and Restrictive Dermopathy (RD, OMIM #275210), respectively, where accumulation of prelamin A is responsible for disease onset (Columbaro et al., 2010; Denecke et al., 2006; Wang et al., 2016). In particular, in HGPS, the truncated form of farnesylated prelamin A, called progerin, is accumulated due to a silent point mutation in the *LMNA* gene (De Sandre-Giovannoli et al., 2003; Eriksson et al., 2003). In RD cells, farnesylated prelamin A is accumulated due to homozygous mutations in *ZMPSTE24* gene and mature lamin A is completely absent (Navarro et al., 2004). However, not all MADA and progeria forms linked to *LMNA* gene mutations feature prelamin A accumulation and the molecular mechanisms causing these diseases warrant further investigation (Garg et al., 2009; Soria-Valles et al., 2015).

3.2. LMNA mutations associated with MADA

MADA is caused, in most cases, by homozygous mutations of *LMNA* gene and it is associated with accumulation of mutated prelamin A along with mutated lamin A/C (Camozzi et al., 2012; Capanni et al., 2005; Filesi et al., 2005). The most common lamin A/C mutation causative of MADA is the homozygous missense substitution of c.1580G>A mapping in the exon 9 of *LMNA* gene, resulting in p.Arg527His mutation falling in the C-terminal tail of the protein (Garavelli et al., 2009; Novelli et al., 2002; Shen et al., 2003; Simha et al., 2003). However, other *LMNA* mutations albeit less frequent and some heterozygous compound conditions (Lombardi et al., 2007), have been identified, as detailed in Table 1. Fig. 4 shows clustering of so far identified MADA pathogenetic variants in the C-terminal globular domain common to prelamin A and lamin C.

3.3 ZMPSTE24 mutations associated with MADB

As outlined above, mutations of *ZMPSTE24* have been associated with severe progeroid syndromes, as MADB (Agarwal et al., 2003) and RD , as well as a severe metabolic syndrome with abnormal fat accumulation and dilated cardiomyopathy (Galant et al., 2016).

The product of the gene *ZMPSTE24* (genetic location: 1p34), encoding the Zinc Metalloproteinase STE24 (ZMPSTE24) previously indicated as FACE-1, from Farnesylated Proteins Converting Enzyme-1, is a multispanning integral membrane protease consisting of seven transmembrane domains, involved in the maturation of lamin A. In particular, ZMPSTE24 cleaves farnesylated prelamin A at the carboxyl end of the Cysteine residue of the CSIM motif (Agarwal et al., 2003; Barrowman and Michaelis, 2009). After initial evidence suggesting that prelamin A processing

occurred in the cytosol at the endoplasmic reticulum followed by import into the nucleus (Navarro et al., 2006; Wu et al., 2014), there is now evidence that prelamin A maturation also occurs within the nucleus (Barrowman et al., 2008) and that non farnesylated prelamin A is efficiently imported in the nucleus (Lattanzi et al., 2007; Mattioli et al., 2008). Mutations of *ZMPSTE24* gene result in a total or partial loss of function of the enzymatic activity of the encoded protein, resulting in accumulation of farnesylated prelamin A, the only known substrate of the enzyme (Fong et al., 2004). *ZMPSTE24* mutations give rise to diverse clinical conditions depending on the degree of prelamin A processing impairment. As a whole, ten different *ZMPSTE24* mutations have been hitherto reported in 12 MADB patients from ten independent families (Table 1 and Fig. 5).

4. Cellular functions of *LMNA* products

4.1 Cellular functions of lamin A/C

The nuclear lamina is an elastic meshwork underlying the inner nuclear membrane (Turgay et al., 2017), to which it is attached through integral membrane protein binding. By virtue of the integrity of this structure, cell nucleus performs structural and molecular activities. In fact, the nuclear lamina is able to withstand robust mechanical stress (Shah et al., 2017), modulates shuttling of proteins from the cytosol to the nucleus and *vice versa* (Capanni et al., 2005; Cenni et al., 2014; Columbaro et al., 2013) and it is involved in chromosome positioning (Bridger et al., 2014; Lund et al., 2013; Robson et al., 2017), while chromosome segregation is affected by mutated lamin A (Cao et al., 2007). Importantly, under physiological conditions, chromatin tethering at the nuclear periphery is finely tuned by lamin A/C in cooperation with diverse nuclear lamina/nuclear envelope constituents (Solovei et al., 2013), and localization of structural and enzymatic proteins throughout chromatin is mediated by lamin A/C in response to specific stimuli (Camozzi et al., 2014).

LMNA gene products regulate indeed gene transcription through diverse mechanisms (Meaburn et al., 2007). By the first one, lamin A/C directly binds to transcription factors, affecting their availability and thus their transcriptional potential. This has been demonstrated for SREBP1, cFos (Gonzalez et al., 2008), Sp1 (Ruiz de Eguino et al., 2012), Oct-1 (Cenni et al., 2014; Columbaro et al., 2013) and recently for NF-Y (Cicchillitti et al., 2017). *LMNA* products also interfere with histone post-translational modifications (Lattanzi et al., 2007; Mattioli et al., 2008), so that lamin mutations alter chromatin accessibility to transcription factors during cell cycle and/or differentiation (Oldenburg et al., 2017). Moreover, lamins modulate polycomb group of proteins, which are epigenetic repressors (Cesarini et al., 2015).

Lamin A/C is also connected to cytoskeletal proteins in the so-called LINC (Linker of Nucleoskeleton and Cytoskeleton) complex, a protein platform also including nuclear membrane

proteins SUN1, SUN2, nesprins and emerin, which establishes and regulates interactions with actin, tubulin and other cytoskeleton constituents (Camozzi et al., 2012; Mattioli et al., 2011; Meinke et al., 2014; Mejat and Misteli, 2010). The involvement of lamins in diverse cellular functions might explain the fact that mutations in *LMNA* gene have deleterious consequences leading to diverse diseases (Camozzi et al., 2014).

4.2 *Cellular functions of prelamin A*

While altered tuning of prelamin A/lamin A ratio may elicit severe pathogenetic effects, multiple findings point to modulation of prelamin A processing rate as necessary to the execution to physiological events. Previous evidences reported by us have indeed demonstrated that prelamin A is accumulated in differentiating skeletal muscle cells (Capanni et al., 2008). In this context, farnesylated prelamin A functions to ensure the proper alignment of nuclei along the nascent myotubes through recruitment of the LINC protein SUN1 to the nuclear envelope of differentiated muscle cells (Mattioli et al., 2011). On the other hand, in cultured skin fibroblasts, transient chromatin relaxation and changes in nuclear shape, which are associated with oxidative stress conditions, parallel transient accumulation of prelamin A (Lattanzi et al., 2014), hinting to a role for prelamin A in the regulation of stress-associated chromatin dynamics. Loss of peripheral heterochromatin and prelamin A accumulation are in fact observed in skin fibroblasts subjected to oxidative stress, as in cells from old or centenarian subjects even in the absence of any stimulus (Fig. 6) (Lattanzi et al., 2014). Of note, in fibroblasts from centenarian donors, a low amount of farnesylated prelamin A persists at the nuclear lamina under basal conditions due to down-regulation of *ZMPSTE24* gene (Lattanzi et al., 2014). This unexpected pattern of prelamin A modulation in cells from very old individuals, favors recruitment of 53BP1 in the nuclear interior and rapid response to oxidative stress, a mechanism that most likely delays cellular senescence (Lattanzi et al., 2014). In support of this conclusion, we observed that knockdown of *LMNA* by siRNA reduced resistance of centenarian cells to stress and increased mortality, while expression of a prelamin A mutant, which accumulates as farnesylated prelamin A enhances the rate of DNA repair in cells subjected to oxidative stress (Lattanzi et al., 2014).

However, prelamin A is a potentially toxic protein, so that improper amount and/or timing of protein accumulation may cause pathogenetic effects Farnesylated prelamin A accumulation has been reported in vascular smooth muscle cells (VSMC) exposed to oxidative stress. Under these conditions, prelamin A increase, that correlates with the down-regulation of lamin A endoprotease ZMPSTE24, acts to accelerate cellular senescence (Liu et al., 2013; Ragnauth et al., 2010). The latter mechanism may have deleterious consequences especially for development of cardiovascular disorders (Porter et al., 2016), but can also contribute to shift cells that could potentially undergo

transformation towards a beneficial senescence program. Along this line, the in vivo demonstration of a protective role of prelamin A against cancer invasiveness has been provided by de la Rosa et al. (de la Rosa et al., 2013). The authors demonstrated that mosaic mice accumulating prelamin A in a percentage of cells were less prone to infiltrating oral carcinomas, and down regulation of Zmpste24 in cells reduced tumor invasiveness (de la Rosa et al., 2013).

5. Pathogenetic pathways affected in MAD

Prelamin A processing

Despite these considerations, the main molecular defect so far associated with MAD pathogenesis is accumulation of anomalous levels of prelamin A (Capanni et al., 2005; Cenni et al., 2014; Filesi et al., 2005). As outlined in paragraph 3.1, prelamin A is the main splicing product of the *LMNA* gene and requires a complex post-translational processing to yield mature lamin A. The reason why prelamin A levels are increased in MADA cells is still unknown, while increase of prelamin A in MADB is obviously associated with loss of functionality or haploinsufficiency of the prelamin A endoprotease ZMPSTE24, which inefficiently cleaves prelamin A in patient cells (Corrigan et al., 2005). *In vitro* studies with recombinant mutated ZMPSTE24 forms have demonstrated that the severity of the pathology caused by *ZMPSTE24* mutations correlates with the residual enzymatic activity of the mutated enzyme (Barrowman et al., 2012). As a matter of fact, RD patients which show the most severe phenotype and die within a few hours to a few weeks of birth, harbor null c.1085dupT *ZMPSTE24* mutations on both alleles that entirely abrogate enzymatic activity; on the contrary, most patients with MADB are heterozygotes with a null mutation on one allele and a missense mutation on the other allele, resulting in a partially active ZMPSTE24 mutant. Interestingly, a particular clinical condition demonstrates the direct correlation between prelamin A farnesylation and severity of MADB phenotype. In that case, a *ZMPSTE24* null mutation was associated with a heterozygous c.1960C4T mutation in *LMNA* gene leading to production of a truncated non-farnesylable prelamin A from the mutated allele (Table 1) (Denecke et al., 2006). Thus, the patient showed a mild phenotype, due to accumulation of a lower amount of farnesylated prelamin A (Denecke et al., 2006).

As demonstrated in skin fibroblasts (Camozzi et al., 2012), both the unprocessed (non farnesylated) lamin A precursor and farnesylated prelamin A can be accumulated in MADA. The whole organization of the nuclear envelope is affected by prelamin A accumulation, as shown in cultured fibroblasts from affected individuals, which present anomalous distribution of emerin, SUN1 and SUN2 (Camozzi et al., 2012; Filesi et al., 2005; Lattanzi, 2011). In MADA fibroblasts, SUN2 aberrant honeycomb appearance at the nuclear envelope is directly linked to accumulation of

farnesylated prelamin A, as demonstrated by rescue of proper organization in cells treated with farnesylation inhibitors (Camozzi et al., 2012). Overexpression of wild-type lamin A in MADA cells, which only express mutated *LMNA* products, will provide further insights on the role of prelamin A and/or specific MADA-linked *LMNA* mutations in aberrant SUN2 localization.

Thus, the first pathogenetic event downstream of mutated prelamin A accumulation in MADA is disruption of nuclear envelope organization. However, other processes, affecting tissue-specific phenotypes, are triggered, as outlined below.

Cellular differentiation

In specific tissues, accumulation of prelamin A may have deleterious effects on cellular differentiation. In MADA cells and in preadipocytes that accumulate prelamin A, cellular differentiation is hampered due to impaired import of transcription factors, including SREBP1 (Capanni et al., 2005) and Oct-1, that are required for adipogenic gene activation or stress response (Cenni et al., 2014; Infante et al., 2014; Infante and Rodriguez, 2016). In osteoblast-like cells expressing the most common R527H *LMNA* mutation associated with MADA, TGFbeta 2 increase occurs downstream of mutated prelamin A accumulation (Evangelisti et al., 2015). In fact, MADA osteoblasts produce elevated levels of TGFbeta 2 (Avnet et al., 2011), that can be recovered also in patient serum (Evangelisti et al., 2015), and this secreted factor activates a non-canonical pathway of osteoclast differentiation (Avnet et al., 2011; Evangelisti et al., 2015). Further, in osteoclast precursors derived from blood, prelamin A accumulation accelerates differentiation, though inefficient osteoclasts are formed (Zini et al., 2008). Consistent with those observations, aberrant osteoclastogenesis determined in laminopathic cells can be prevented either by using specific TGFbeta 2 neutralizing antibody or by reducing accumulation of farnesylated prelamin A (Evangelisti et al., 2015).

Cytokine regulation

Of note, accumulation of TGFbeta 2 in MADA appears to be part of a mechanism aimed at degradation of mutated lamin A through the TGFbeta 2-dependent activation of Akt-mTOR axis (Evangelisti et al., 2015). Our data suggest the existence of a feedback loop between lamin A and TGFbeta 2, whereby lamin A regulates cytokine secretion, while elevated TGFbeta 2 levels occurring in the presence of mutated lamins contribute to degradation of non-functional proteins. Data obtained in a mouse model of muscular laminopathy (Chatzifrangkeskou et al., 2016) support this view. However, the reported increase of TGFbeta 2 (Avnet et al., 2011) in cells and serum from MADA patients (Evangelisti et al., 2015), suggests that systemic effects are also relevant for MADA (and most likely MADB) pathogenesis. Data from experimental models of progeroid laminopathies, including *Zmpste24* null mice, strongly support this hypothesis (de la Rosa et al.,

2013). In fact, in *Zmpste24* null mice (Osorio et al., 2012), as in a human cellular model overexpressing R527H *LMNA* (Evangelisti et al., 2015), increased secretion of interleukin 6 (IL6), a cytokine widely associated with cellular senescence, has been observed. Moreover, increase of Matrix metalloproteinase 9 (MMP9), but not IL6, was observed in patient serum (Lombardi et al., 2008). These data as a whole delineate a complex scenario, which warrants further investigation.

Deacetylase activity

Epigenetic defects were initially observed in MADA cells and linked to accumulation of prelamin A (Filesi et al., 2005). In fact, we reported that histone marks of heterochromatin, including trimethylated H3K9, are altered in MADA and peripheral heterochromatin is lost in a significant percentage of fibroblast nuclei (Filesi et al., 2005). Of note, these defects can be partially rescued by reducing prelamin A farnesylation, showing that farnesylated prelamin A drives chromatin epigenetic changes (Camozzi et al., 2012). More recently, we found that MADA cells show impairment of deacetylases, a possible cause of altered chromatin dynamics. In MADA fibroblasts, SIRT1 is not properly anchored to the nuclear matrix (Cenni et al., 2014), while histone deacetylase 2 (HDAC2) fails to bind lamin A/C (Mattioli et al., in preparation). Loss of lamin A/C-HDAC2 interaction impacts on stress recovery mechanisms in MADA cells, so that, in cells subjected to oxidative stress, senescence associated heterochromatin foci (SAHF), a hallmark of senescent cells (Narita et al., 2006), are formed. This suggests that repeated stress stimuli and failure to properly manage this condition is a mechanism leading to senescence in MAD. Of note, the finding that a new form of MAD, the MDPL, is caused by mutations in *POLD1*, encoding DNA polymerase δ catalytic subunit (Weedon et al., 2013), strongly suggests that mechanisms regulating DNA replication, especially during DNA damage response, may be also involved in MAD pathogenesis. In agreement with this hypothesis, altered DNA damage check point (di Masi et al., 2008) and elongation of S phase in MADA cells (Cenni et al., 2014) have been reported. A scheme of the pathogenetic pathways of MADA and MADB is represented in Fig. 7.

6. Translational approaches to MAD

As reported by our previous studies (Camozzi et al., 2012), cultured MADA fibroblasts feature unprocessed prelamin A accumulation. In particular, by the use of antibodies directed against the farnesylated or the full length (non-farnesylated) form of prelamin A, we have demonstrated that prelamin A post-translational modifications in MADA depend on the passage number of cultured cells: while farnesylated prelamin A is accumulated at low passage, the non-farnesylated form is more abundant at high passage number (Camozzi et al., 2012). Therefore, with the aim of improving the phenotype of cultured MADA cells, different cellular treatments have been set up to

reduce the amount of accumulated prelamin A. This has been reached by two different approaches. The first one consists in the targeting of prelamin A maturation, while the second approach triggers unprocessed prelamin A intermediates versus degradation.

Statins and FTIs (farnesyl transferase inhibitors) inhibit farnesylated prelamin A accumulation. Treatment of MADA cultured cells with statins was effective in the recovery of the chromatin phenotype, which is altered in MADA cells, but only in low passage cultures (Camozzi et al., 2012). Combined treatment with mevinolin and Trichostatin A (TSA, inhibitor of class I and II mammalian histone deacetylase) improved the effects observed with mevinolin alone (Camozzi et al., 2012). The evidence that those drugs are mostly effective in low passage cells, when only the farnesylated form of prelamin A is accumulated, could in part explain the efficacy of current clinical trials with statins in MADA and the less satisfactory response observed in older patients (Camozzi et al., 2012).

In an attempt to enhance the proteolysis of prelamin A forms accumulated in MADA cells, our group unexpectedly found that prelamin A forms present different susceptibility to degradation (Cenni et al., 2011). In particular, while non-farnesylated prelamin A undergoes spontaneous lysosomal degradation, farnesylated prelamin A does not, revealing an unusual protein stability (Bertacchini et al., 2013). To the aim of reducing this stability and addressing farnesylated prelamin A to degradation, autophagic cellular processes were induced by pharmacological treatment. In cells overexpressing R527H mutated lamin A and in MADA cells, we were able to demonstrate the efficacy of rapamycin, a known inhibitor of the mTOR signaling pathway in triggering autophagy and authopagic-dependent degradation of farnesylated prelamin A (Cenni et al., 2014). Intriguingly, in MADA fibroblasts, rapamycin-triggered autophagic degradation of farnesylated prelamin A elicited improvement of cell cycle dynamics (Cenni et al., 2014).

As already described, patients affected by MADA suffer from an osteolytic process, due to an excessive amount of TGFbeta 2 released by both MADA osteoblasts (Avnet et al., 2011) and osteoblast-like cells overexpressing lamin A R527H or farnesylated prelamin A (Evangelisti et al., 2015). By triggering the Akt/mTOR pathway, TGFbeta 2 activates osteoclastogenesis and extracellular matrix resorption. By the use of TGFbeta 2-neutralizing antibodies, as well as statins or RAD001 (a rapalog that inhibits the mTOR pathway), we were able to block the osteolytic process triggered by mutated lamin A, indicating rapalogs and TGFbeta 2-neutralizing antibodies as new therapeutic tools for MADA treatment (Evangelisti et al., 2015). In this context, it is worthy to consider that cytokines or other circulating molecules warrant further investigation as potential therapeutic targets for MAD.

Being characterized by the same retention of unprocessed farnesylated prelamin A in the nucleus, a reduced number of studies have been specifically performed in MADB cells. Nonetheless, a study performed in skin fibroblasts from MADB patients demonstrated that cellular treatment with morpholino antisense oligonucleotides (AON) directed against prelamin A promotes the reduction of prelamin A levels, resulting in a reduction of senescence-associated β-galactosidase activity as well as an improvement of nuclear shape abnormalities (Harhouri et al., 2016). According to the authors, in the future, other therapeutic strategies for premature and accelerated ageing diseases should be considered, such as gene therapy approach (CRISPR), cell-based replacement therapies, particularly targeting the vascular tissue, or RNA therapy based on RNA interference (Harhouri et al., 2016).

In an effort to limit acral bone resorption, in 2010, a patient affected of MADB was treated with intravenous pamidronate infusions. However, albeit well tolerated and effective in increasing bone density, pamidronate treatment failed to avoid osteolysis or increase cortical thickness (Cunningham et al., 2010).

7. Cellular aspects of MAD resembling physiological ageing

Among defects typical of MADA fibroblasts, prelamin A increase, nuclear enlargement and heterochromatin loss (Fig. 6) are those more closely recapitulating features of cells from old individuals. In cells from very old individuals, reduction of prelamin A processing rate and accumulation of farnesylated prelamin A is associated with downregulation of *ZMPSTE24* elicited by oxidative or replicative stress (Lattanzi et al., 2014). In these physiological conditions, as well as in the presence of *LMNA* mutations, prelamin A drives chromatin reorganization. Prelamin A accumulation is now recognized as a trigger of chromatin reorganization, as demonstrated by inhibition of protein maturation using peptides targeted to the ZMPSTE24 cleavage site (Mattioli et al., 2008), by site directed mutagenesis targeting either the prelamin A cleavage site or the C-terminal farnesylable cysteine (Lattanzi et al., 2007) or in the *Zmpste24* null mouse model (Osorio et al., 2010). Such chromatin effects are most likely mediated by different anchorage or activity of epigenetic factors in the presence of diverse levels of prelamin A. Thus, not surprisingly, such mechanisms are affected by mutated prelamin A (as in MADA and HGPS) or by toxic levels of wild-type prelamin A (as in MADB). In fact, epigenetic enzymes, such as HDAC2 (Mattioli et al in preparation) or SIRT1, which are considered major players in the regulation of stress response and anti-ageing processes (Hekmatimoghaddam et al., 2017) are affected in MADA cells. Nuclear matrix anchorage of SIRT1 is reduced in MADA (Cenni et al., 2014). SIRT1 is a deacetylase targeting acetylated histones and its inactivation leads to increased histone H4K16 and H3K9

acetylation, as well as decreased H3K9 trimethylation, all age-associated epigenetic marks (Lopez-Otin et al., 2013), which in fact are defective in MADA (Cenni et al., 2014; Filesi et al., 2005). Consistent with the altered regulation of H3K9 trimethylation, solubilisation and mislocalization of heterochromatin protein 1 beta (HP1β) are also observed in MADA (Filesi et al., 2005; Lombardi et al., 2007). Interestingly, reduced mobility of HP1β has been determined in senescent cells, while "rejuvenation" of the same cells by the four reprogramming transcription factors increased HP1β solubility (Manukyan and Singh, 2014). Thus, we cannot rule out the possibility that not all chromatin modifications observed in MADA drive cellular senescence, while we can speculate that some epigenetic changes, as increased HP1β solubility, could even counteract cell ageing caused by *LMNA* mutations. Further, prolonged S phase, an hallmark of replication stress has been determined in MADA cells and shown to be rescued by rapamycin-induced reduction of mutated prelamin A accumulation, a result again linking prelamin A functionality to efficient cell response to stress stimuli (Cenni et al., 2014; Venkatachalam et al., 2017). We previously observed in MADA nuclei, 53BP1 is severely reduced with respect to controls, while γH2AX foci are well detectable (Cenni et al., 2014). While suggesting a reduction of 53BP1 recruitment to γH2AX foci, this situation could reflect an unrepairable DNA damage (di Masi et al., 2008; Richards et al., 2011), but may also be linked to senescence, since increase of γH2AX in the absence of 53BP1 foci has been demonstrated in senescent cells even in the absence of DNA breaks (Gibbs-Seymour et al., 2015; Kreienkamp et al., 2016; Leontieva et al., 2012).

Finally, the secretome of MADA cells could share aspects of senescence-associated secretory phenotype (SASP) (Coppe et al., 2010), as indicated by the increase of osteoprotegerin in MADA osteoblasts (Avnet et al., 2011) and by dysregulation of IL6 and IL15 suggested by experimental models (Evangelisti et al., 2015). These observations, if further explored, may open relevant perspectives for MADA and other ageing-related disorders, based on therapeutic targeting of altered cytokines.

8. Conclusions

MAD is an invalidating disease with the most severe disabilities being caused by bone disorders and accelerated organism ageing. Psychological aspects associated with changes in body shape are also relevant. The pathogenesis of MAD is linked to altered modulation of lamin A precursor levels in cells and involves defective chromatin dynamics that in turn affects cellular differentiation and DNA damage response. Systemic effects are also relevant, so far mainly associated with altered cytokine secretion and TGFbeta 2 – dependent activation of osteoclast differentiation and bone resorption. Both the molecular mechanisms and clinical features of MAD appear to occur

downstream of altered prelamin A-dependent pathways that regulate stress and DNA damage response in healthy individuals. This implies that MAD cellular models and pathways can be studied as a paradigm of physiological ageing. Most importantly, this means that targeting effectors of prelamin A, including TGFbeta 2 or signalling molecules in the mTOR pathway, can represent a suitable approach to counteract accelerated ageing. Mutations in nuclear lamina genes affect DNA organization, thus altering the normal epigenetic architecture of the cells (Camozzi et al., 2014; Chen et al., 2017). In ageing, one of the most studied and interesting phenomena is the degeneration of the epigenetic integrity in all the tissues and organs. Of all the epigenetic agents, DNA methylation is one of the most studied in ageing. The age related DNA methylation changes are among the most promising sources of biological ageing biomarkers (Stubbs et al., 2017). Accordingly, it is of extreme interest to investigate the effect of the pro-ageing *LMNA* mutations associated with progeroid laminopathies on the epigenetic architecture and specifically on DNA methylation patterns that change with age. To date, only few studies investigated the effect of mutations in nuclear lamina genes on the epigenomic structure (Osorio et al., 2010; Perovanovic et al., 2016). Some genome-wide DNA methylation changes in HGPS were reported, with a fraction of CpG sites gaining methylation (in a healthy unmethylated context) and loosing methylation in control hypermethylated regions (Heyn et al., 2013) and some specific hypermethylated sequences, for instance in the ribosomal DNA of *Zmpste24* null cells have been identified (Osorio et al., 2010). We predict that deepening the epigenetic landscape of slowly progressing forms of progeria like MADA or MADB and even MDPL may provide unbiased understanding of lamin-dependent ageing-related epigenetic pathways.

As a whole, looking at lamin-linked progeroid disorders, it becomes clear that determinants of ageing may also originate from an altered genetic background (Fig. 8). To contextualize the progeroid syndromes in the perspective of ageing determinants (Capri et al., 2014) could help understanding to which extent those diseases mimic the ageing process and provide new hints to discover genes contributing to physiological ageing. This review reinforces the notion that genetics has profound effects in determining the ageing process.

Acknowledgements

The authors thank patients and their families for contributing pictures and cells. This work was supported by grants from: “5 per mille 2013 e 2014” - Rizzoli Orthopaedic Institute, to VC and GL, AIProSaB Italy Prot.3/2016 to GL, PRIN MIUR 2015FBNB5Y to GL, and Fondazione Del Monte di Bologna e Ravenna grant 2015-2016 to GL. The authors thank all the members of the Italian Network for Laminopathies (*www.igm.cnr.it/1/laminopatie/*) for helpful discussion.

References

Agarwal, A.K., Fryns, J.P., Auchus, R.J., Garg, A., 2003. Zinc metalloproteinase, ZMPSTE24, is mutated in mandibuloacral dysplasia. Human molecular genetics 12, 1995-2001.

Agarwal, A.K., Zhou, X.J., Hall, R.K., Nicholls, K., Bankier, A., Van Esch, H., Fryns, J.P., Garg, A., 2006. Focal segmental glomerulosclerosis in patients with mandibuloacral dysplasia owing to ZMPSTE24 deficiency. Journal of investigative medicine : the official publication of the American Federation for Clinical Research 54, 208-213.

Angori, S., Capanni, C., Faulkner, G., Bean, C., Boriani, G., Lattanzi, G., Cenni, V., 2017. Emery-Dreifuss Muscular Dystrophy-Associated Mutant Forms of Lamin A Recruit the Stress Responsive Protein Ankrd2 into the Nucleus, Affecting the Cellular Response to Oxidative Stress. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology 42, 169-184.

Araujo-Vilar, D., Lattanzi, G., Gonzalez-Mendez, B., Costa-Freitas, A.T., Prieto, D., Columbaro, M., Mattioli, E., Victoria, B., Martinez-Sanchez, N., Ramazanova, A., Fraga, M., Beiras, A., Forteza, J., Dominguez-Gerpe, L., Calvo, C., Lado-Abeal, J., 2009. Site-dependent differences in both prelamin A and adipogenic genes in subcutaneous adipose tissue of patients with type 2 familial partial lipodystrophy. Journal of medical genetics 46, 40-48.

Avnet, S., Pallotta, R., Perut, F., Baldini, N., Pittis, M.G., Saponari, A., Lucarelli, E., Dozza, B., Greggi, T., Maraldi, N.M., Capanni, C., Mattioli, E., Columbaro, M., Lattanzi, G., 2011. Osteoblasts from a mandibuloacral dysplasia patient induce human blood precursors to differentiate into active osteoclasts. Biochim Biophys Acta 1812, 711-718.

Barascu, A., Le Chalony, C., Pennarun, G., Genet, D., Zaarour, N., Bertrand, P., 2012. Oxydative stress alters nuclear shape through lamins dysregulation: a route to senescence. Nucleus (Austin, Tex.) 3, 411-417.

Barrowman, J., Hamblet, C., George, C.M., Michaelis, S., 2008. Analysis of prelamin A biogenesis reveals the nucleus to be a CaaX processing compartment. Molecular biology of the cell 19, 5398-5408.

Barrowman, J., Michaelis, S., 2009. ZMPSTE24, an integral membrane zinc metalloprotease with a connection to progeroid disorders. Biological chemistry 390, 761-773.

Barrowman, J., Wiley, P.A., Hudon-Miller, S.E., Hrycyna, C.A., Michaelis, S., 2012. Human ZMPSTE24 disease mutations: residual proteolytic activity correlates with disease severity. Human molecular genetics 21, 4084-4093.

Bertacchini, J., Beretti, F., Cenni, V., Guida, M., Gibellini, F., Mediani, L., Marin, O., Maraldi, N.M., de Pol, A., Lattanzi, G., Cocco, L., Marmiroli, S., 2013. The protein kinase Akt/PKB regulates both prelamin A degradation and Lmna gene expression. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 27, 2145-2155.

Bridger, J.M., Arican-Gotkas, H.D., Foster, H.A., Godwin, L.S., Harvey, A., Kill, I.R., Knight, M., Mehta, I.S., Ahmed, M.H., 2014. The non-random repositioning of whole chromosomes and individual gene loci in interphase nuclei and its relevance in disease, infection, aging, and cancer. Advances in experimental medicine and biology 773, 263-279.

Butin-Israeli, V., Ben-nun-Shaul, O., Kopatz, I., Adam, S.A., Shimi, T., Goldman, R.D., Oppenheim, A., 2011. Simian virus 40 induces lamin A/C fluctuations and nuclear envelope deformation during cell entry. Nucleus (Austin, Tex.) 2, 320-330.

Camozzi, D., Capanni, C., Cenni, V., Mattioli, E., Columbaro, M., Squarzoni, S., Lattanzi, G., 2014. Diverse lamin-dependent mechanisms interact to control chromatin dynamics. Focus on laminopathies. Nucleus (Austin, Tex.) 5, 427-440.

Camozzi, D., D'Apice, M.R., Schena, E., Cenni, V., Columbaro, M., Capanni, C., Maraldi, N.M., Squarzoni, S., Ortolani, M., Novelli, G., Lattanzi, G., 2012. Altered chromatin organization and SUN2 localization in mandibuloacral dysplasia are rescued by drug treatment. Histochem Cell Biol 138, 643-651.

Cao, K., Capell, B.C., Erdos, M.R., Djabali, K., Collins, F.S., 2007. A lamin A protein isoform overexpressed in Hutchinson-Gilford progeria syndrome interferes with mitosis in progeria and normal cells. Proceedings of the National Academy of Sciences of the United States of America 104, 4949-4954.

Capanni, C., Del Coco, R., Squarzoni, S., Columbaro, M., Mattioli, E., Camozzi, D., Rocchi, A., Scotlandi, K., Maraldi, N., Foisner, R., Lattanzi, G., 2008. Prelamin A is involved in early steps of muscle differentiation. Experimental cell research 314, 3628-3637.

Capanni, C., Mattioli, E., Columbaro, M., Lucarelli, E., Parnaik, V.K., Novelli, G., Wehnert, M., Cenni, V., Maraldi, N.M., Squarzoni, S., Lattanzi, G., 2005. Altered pre-lamin A processing is a common mechanism leading to lipodystrophy. Human molecular genetics 14, 1489-1502.

Capri, M., Santoro, A., Garagnani, P., Bacalini, M.G., Pirazzini, C., Olivieri, F., Procopio, A., Salvioli, S., Franceschi, C., 2014. Genes of human longevity: an endless quest? Current vascular pharmacology 12, 707-717.

Cavallazzi, C., Cremoncini, R., Quadri, A., 1960. [On a case of cleidocranial dysostosis]. Rivista di clinica pediatrica 65, 312-326.

Cenni, V., Capanni, C., Columbaro, M., Ortolani, M., D'Apice, M.R., Novelli, G., Fini, M., Marmiroli, S., Scarano, E., Maraldi, N.M., Squarzoni, S., Prencipe, S., Lattanzi, G., 2011. Autophagic degradation of farnesylated prelamin A as a therapeutic approach to lamin-linked progeria. European journal of histochemistry : EJH 55, e36.

Cenni, V., Capanni, C., Mattioli, E., Columbaro, M., Wehnert, M., Ortolani, M., Fini, M., Novelli, G., Bertacchini, J., Maraldi, N.M., Marmiroli, S., D'Apice, M.R., Prencipe, S., Squarzoni, S., Lattanzi, G., 2014. Rapamycin treatment of Mandibuloacral dysplasia cells rescues localization of chromatin-associated proteins and cell cycle dynamics. Aging 6, 755-770.

Cesarini, E., Mozzetta, C., Marullo, F., Gregoretti, F., Gargiulo, A., Columbaro, M., Cortesi, A., Antonelli, L., Di Pelino, S., Squarzoni, S., Palacios, D., Zippo, A., Bodega, B., Oliva, G., Lanzuolo, C., 2015. Lamin A/C sustains PcG protein architecture, maintaining transcriptional repression at target genes. The Journal of cell biology 211, 533-551.

Chatzifrangkeskou, M., Le Dour, C., Wu, W., Morrow, J.P., Joseph, L.C., Beuvin, M., Sera, F., Homma, S., Vignier, N., Mougenot, N., Bonne, G., Lipson, K.E., Worman, H.J., Muchir, A., 2016. ERK1/2 directly acts on CTGF/CCN2 expression to mediate myocardial fibrosis in cardiomyopathy caused by mutations in the lamin A/C gene. Human molecular genetics 25, 2220-2233.

Chen, Z., Chang, W.Y., Etheridge, A., Strickfaden, H., Jin, Z., Palidwor, G., Cho, J.H., Wang, K., Kwon, S.Y., Dore, C., Raymond, A., Hotta, A., Ellis, J., Kandel, R.A., Dilworth, F.J., Perkins, T.J., Hendzel, M.J., Galas, D.J., Stanford, W.L., 2017. Reprogramming progeria fibroblasts re-establishes a normal epigenetic landscape. Aging cell.

Cicchillitti, L., Manni, I., Mancone, C., Regazzo, G., Spagnuolo, M., Alonzi, T., Carlomosti, F., Dell'Anna, M.L., Dell'Omo, G., Picardo, M., Ciana, P., Capogrossi, M.C., Tripodi, M., Magenta, A., Rizzo, M.G., Gurtner, A., Piaggio, G., 2017. The laminA/NF-Y protein complex reveals an unknown transcriptional mechanism on cell proliferation. Oncotarget 8, 2628-2646.

Columbaro, M., Capanni, C., Mattioli, E., Novelli, G., Parnaik, V.K., Squarzoni, S., Maraldi, N.M., Lattanzi, G., 2005. Rescue of heterochromatin organization in Hutchinson-Gilford progeria by drug treatment. Cellular and molecular life sciences : CMLS 62, 2669-2678.

Columbaro, M., Mattioli, E., Maraldi, N.M., Ortolani, M., Gasparini, L., D'Apice, M.R., Postorivo, D., Nardone, A.M., Avnet, S., Cortelli, P., Liguori, R., Lattanzi, G., 2013. Oct-1 recruitment to the nuclear envelope in adult-onset autosomal dominant leukodystrophy. Biochimica Et Biophysica Acta-Molecular Basis of Disease 1832, 411-420.

Columbaro, M., Mattioli, E., Schena, E., Capanni, C., Cenni, V., Levy, N., Navarro, C.L., Del Coco, R., Squarzoni, S., Camozzi, D., Hutchison, C.J., Wehnert, M., Lattanzi, G., 2010. Prelamin A processing and functional effects in restrictive dermopathy. Cell cycle 9, 4766-4768.

Coppe, J.P., Desprez, P.Y., Krtolica, A., Campisi, J., 2010. The senescence-associated secretory phenotype: the dark side of tumor suppression. Annual review of pathology 5, 99-118.

Corrigan, D.P., Kuszczak, D., Rusinol, A.E., Thewke, D.P., Hrycyna, C.A., Michaelis, S., Sinensky, M.S., 2005. Prelamin A endoproteolytic processing in vitro by recombinant Zmpste24. The Biochemical journal 387, 129-138.

Cunningham, V.J., D'Apice, M.R., Licata, N., Novelli, G., Cundy, T., 2010. Skeletal phenotype of mandibuloacral dysplasia associated with mutations in ZMPSTE24. Bone 47, 591-597.

Davies, B.S., Coffinier, C., Yang, S.H., Barnes, R.H., 2nd, Jung, H.J., Young, S.G., Fong, L.G., 2011. Investigating the purpose of prelamin A processing. Nucleus (Austin, Tex.) 2, 4-9.

de la Rosa, J., Freije, J.M., Cabanillas, R., Osorio, F.G., Fraga, M.F., Fernandez-Garcia, M.S., Rad, R., Fanjul, V., Ugalde, A.P., Liang, Q., Prosser, H.M., Bradley, A., Cadinanos, J., Lopez-Otin, C., 2013. Prelamin A causes progeria through cell-extrinsic mechanisms and prevents cancer invasion. Nature communications 4, 2268.

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

Denecke, J., Brune, T., Feldhaus, T., Robenek, H., Kranz, C., Auchus, R.J., Agarwal, A.K., Marquardt, T., 2006. A homozygous ZMPSTE24 null mutation in combination with a heterozygous mutation in the LMNA gene causes Hutchinson-Gilford progeria syndrome (HGPS): insights into the pathophysiology of HGPS. Human mutation 27, 524-531.

di Masi, A., D'Apice, M.R., Ricordy, R., Tanzarella, C., Novelli, G., 2008. The R527H mutation in LMNA gene causes an increased sensitivity to ionizing radiation. Cell cycle 7, 2030-2037.

Dreesen, O., Ong, P.F., Chojnowski, A., Colman, A., 2013. The contrasting roles of lamin B1 in cellular aging and human disease. Nucleus (Austin, Tex.) 4, 283-290.

Elouej, S., Beleza-Meireles, A., Caswell, R., Colclough, K., Ellard, S., Desvignes, J.P., Beroud, C., Levy, N., Mohammed, S., De Sandre-Giovannoli, A., 2017. Exome sequencing reveals a de novo POLD1 mutation causing phenotypic variability in mandibular hypoplasia, deafness, progeroid features, and lipodystrophy syndrome (MDPL). Metabolism 71, 213-225.

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., Dutra, A., Pak, E., Durkin, S., Csoka, A.B., Boehnke, M., Glover, T.W., Collins, F.S., 2003. Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. Nature 423, 293-298.

Evangelisti, C., Bernasconi, P., Cavalcante, P., Cappelletti, C., D'Apice, M.R., Sbraccia, P., Novelli, G., Prencipe, S., Lemma, S., Baldini, N., Avnet, S., Squarzoni, S., Martelli, A.M., Lattanzi, G., 2015. Modulation of TGFbeta 2 levels by lamin A in U2-OS osteoblast-like cells: understanding the osteolytic process triggered by altered lamins. Oncotarget 6, 7424-7437.

Evangelisti, C., Cenni, V., Lattanzi, G., 2016. Potential therapeutic effects of the MTOR inhibitors for preventing ageing and progeria-related disorders. British journal of clinical pharmacology 82, 1229-1244.

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

Fong, L.G., Ng, J.K., Meta, M., Cote, N., Yang, S.H., Stewart, C.L., Sullivan, T., Burghardt, A., Majumdar, S., Reue, K., Bergo, M.O., Young, S.G., 2004. Heterozygosity for Lmna deficiency eliminates the progeria-like phenotypes in Zmpste24-deficient mice. Proceedings of the National Academy of Sciences of the United States of America 101, 18111-18116.

Galant, D., Gaborit, B., Desgrouas, C., Abdesselam, I., Bernard, M., Levy, N., Merono, F., Coirault, C., Roll, P., Lagarde, A., Bonello-Palot, N., Bourgeois, P., Dutour, A., Badens, C., 2016. A Heterozygous ZMPSTE24 Mutation Associated with Severe Metabolic Syndrome, Ectopic Fat Accumulation, and Dilated Cardiomyopathy. Cells 5.

Garavelli, L., D'Apice, M.R., Rivieri, F., Bertoli, M., Wischmeijer, A., Gelmini, C., De Nigris, V., Albertini, E., Rosato, S., Virdis, R., Bacchini, E., Dal Zotto, R., Banchini, G., Iughetti, L., Bernasconi, S., Superti-Furga, A., Novelli, G., 2009. Mandibuloacral dysplasia type A in childhood. Am J Med Genet A 149A, 2258-2264.

Garg, A., Subramanyam, L., Agarwal, A.K., Simha, V., Levine, B., D'Apice, M.R., Novelli, G., Crow, Y., 2009. Atypical progeroid syndrome due to heterozygous missense LMNA mutations. The Journal of clinical endocrinology and metabolism 94, 4971-4983.

Gibbs-Seymour, I., Markiewicz, E., Bekker-Jensen, S., Mailand, N., Hutchison, C.J., 2015. Lamin A/C-dependent interaction with 53BP1 promotes cellular responses to DNA damage. Aging cell 14, 162-169.

Gonzalez, J.M., Navarro-Puche, A., Casar, B., Crespo, P., Andres, V., 2008. Fast regulation of AP-1 activity through interaction of lamin A/C, ERK1/2, and c-Fos at the nuclear envelope. The Journal of cell biology 183, 653-666.

Harhouri, K., Navarro, C., Baquerre, C., Da Silva, N., Bartoli, C., Casey, F., Mawuse, G.K., Doubaj, Y., Levy, N., De Sandre-Giovannoli, A., 2016. Antisense-Based Progerin Downregulation in HGPS-Like Patients' Cells. Cells 5.

Hekmatimoghaddam, S., Dehghani Firoozabadi, A., Zare-Khormizi, M.R., Pourrajab, F., 2017. Sirt1 and Parp1 as epigenome safeguards and microRNAs as SASP-associated signals, in cellular senescence and aging. Ageing research reviews 40, 120-141.

Heyn, H., Moran, S., Esteller, M., 2013. Aberrant DNA methylation profiles in the premature aging disorders Hutchinson-Gilford Progeria and Werner syndrome. Epigenetics 8, 28-33.

Infante, A., Gago, A., de Eguino, G.R., Calvo-Fernandez, T., Gomez-Vallejo, V., Llop, J., Schlangen, K., Fullaondo, A., Aransay, A.M., Martin, A., Rodriguez, C.I., 2014. Prelamin A accumulation and stress conditions induce impaired Oct-1 activity and autophagy in prematurely aged human mesenchymal stem cell. Aging 6, 264-280.

Infante, A., Rodriguez, C.I., 2016. Pathologically Relevant Prelamin A Interactions with Transcription Factors. Methods in enzymology 569, 485-501.

Kreienkamp, R., Croke, M., Neumann, M.A., Bedia-Diaz, G., Graziano, S., Dusso, A., Dorsett, D., Carlberg, C., Gonzalo, S., 2016. Vitamin D receptor signaling improves Hutchinson-Gilford progeria syndrome cellular phenotypes. Oncotarget 7, 30018-30031.

Lattanzi, G., 2011. Prelamin A-mediated nuclear envelope dynamics in normal and laminopathic cells. Biochemical Society transactions 39, 1698-1704.

Lattanzi, G., Columbaro, M., Mattioli, E., Cenni, V., Camozzi, D., Wehnert, M., Santi, S., Riccio, M., Del Coco, R., Maraldi, N.M., Squarzoni, S., Foisner, R., Capanni, C., 2007. Pre-Lamin A processing is linked to heterochromatin organization. Journal of cellular biochemistry 102, 1149-1159.

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

Lehner, C.F., Furstenberger, G., Eppenberger, H.M., Nigg, E.A., 1986. Biogenesis of the nuclear lamina: in vivo synthesis and processing of nuclear protein precursors. Proceedings of the National Academy of Sciences of the United States of America 83, 2096-2099.

Leontieva, O.V., Lenzo, F., Demidenko, Z.N., Blagosklonny, M.V., 2012. Hyper-mitogenic drive coexists with mitotic incompetence in senescent cells. Cell cycle 11, 4642-4649.

Lessel, D., Hisama, F.M., Szakszon, K., Saha, B., Sanjuanelo, A.B., Salbert, B.A., Steele, P.D., Baldwin, J., Brown, W.T., Piussan, C., Plauchu, H., Szilvassy, J., Horkay, E., Hogel, J., Martin, G.M., Herr, A.J., Oshima, J., Kubisch, C., 2015. POLD1 Germline Mutations in Patients Initially Diagnosed with Werner Syndrome. Human mutation 36, 1070-1079.

Liu, Y., Drozdov, I., Shroff, R., Beltran, L.E., Shanahan, C.M., 2013. Prelamin A accelerates vascular calcification via activation of the DNA damage response and senescence-associated secretory phenotype in vascular smooth muscle cells. Circ Res 112, e99-109.

Loi, M., Cenni, V., Duchi, S., Squarzoni, S., Lopez-Otin, C., Foisner, R., Lattanzi, G., Capanni, C., 2016. Barrier-to-autointegration factor (BAF) involvement in prelamin A-related chromatin organization changes. Oncotarget 7, 15662-15677.

Lombardi, F., Fasciglione, G.F., D'Apice, M.R., Vielle, A., D'Adamo, M., Sbraccia, P., Marini, S., Borgiani, P., Coletta, M., Novelli, G., 2008. Increased release and activity of matrix metalloproteinase-9 in patients with mandibuloacral dysplasia type A, a rare premature ageing syndrome. Clinical genetics 74, 374-383.

Lombardi, F., Gullotta, F., Columbaro, M., Filareto, A., D'Adamo, M., Vielle, A., Guglielmi, V., Nardone, A.M., Azzolini, V., Grosso, E., Lattanzi, G., D'Apice, M.R., Masala, S., Maraldi, N.M., Sbraccia, P., Novelli, G., 2007. Compound heterozygosity for mutations in LMNA in a patient with a myopathic and lipodystrophic mandibuloacral dysplasia type A phenotype. The Journal of clinical endocrinology and metabolism 92, 4467-4471.

Lopez-Otin, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., 2013. The hallmarks of aging. Cell 153, 1194-1217.

Lund, E., Oldenburg, A.R., Delbarre, E., Freberg, C.T., Duband-Goulet, I., Eskeland, R., Buendia, B., Collas, P., 2013. Lamin A/C-promoter interactions specify chromatin state-dependent transcription outcomes. Genome research 23, 1580-1589.

Manukyan, M., Singh, P.B., 2014. Epigenome rejuvenation: HP1beta mobility as a measure of pluripotent and senescent chromatin ground states. Scientific reports 4, 4789.

Mattioli, E., Columbaro, M., Capanni, C., Maraldi, N.M., Cenni, V., Scotlandi, K., Marino, M.T., Merlini, L., Squarzoni, S., Lattanzi, G., 2011. Prelamin A-mediated recruitment of SUN1 to the nuclear envelope directs nuclear positioning in human muscle. Cell Death Differ 18, 1305-1315.

Mattioli, E., Columbaro, M., Capanni, C., Santi, S., Maraldi, N.M., D'Apice, M.R., Novelli, G., Riccio, M., Squarzoni, S., Foisner, R., Lattanzi, G., 2008. Drugs affecting prelamin A processing: effects on heterochromatin organization. Experimental cell research 314, 453-462.

Meaburn, K.J., Cabuy, E., Bonne, G., Levy, N., Morris, G.E., Novelli, G., Kill, I.R., Bridger, J.M., 2007. Primary laminopathy fibroblasts display altered genome organization and apoptosis. Aging cell 6, 139-153.

Meinke, P., Mattioli, E., Haque, F., Antoku, S., Columbaro, M., Straatman, K.R., Worman, H.J., Gundersen, G.G., Lattanzi, G., Wehnert, M., Shackleton, S., 2014. Muscular dystrophy-associated SUN1 and SUN2 variants disrupt nuclear-cytoskeletal connections and myonuclear organization. PLoS genetics 10, e1004605.

Mejat, A., Misteli, T., 2010. LINC complexes in health and disease. Nucleus (Austin, Tex.) 1, 40-52.

Misteli, T., Scaffidi, P., 2005. Genome instability in progeria: when repair gets old. Nature medicine 11, 718-719.

Narita, M., Narita, M., Krizhanovsky, V., Nunez, S., Chicas, A., Hearn, S.A., Myers, M.P., Lowe, S.W., 2006. A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation. Cell 126, 503-514.

Navarro, C.L., Cau, P., Levy, N., 2006. Molecular bases of progeroid syndromes. Human molecular genetics 15, R151-R161.

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., Faivre, L., Verloes, A., Van Essen, T., Flori, E., Hennekam, R., Beemer, F.A., Laurent, N., Le Merrer, M., Cau, P., Levy, N., 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.

Novelli, G., Muchir, A., Sangiuolo, F., Helbling-Leclerc, A., D'Apice, M.R., Massart, C., Capon, F., Sbraccia, P., Federici, M., Lauro, R., Tudisco, C., Pallotta, R., Scarano, G., Dallapiccola, B., Merlini, L., Bonne, G., 2002. Mandibuloacral dysplasia is caused by a mutation in LMNA-encoding lamin A/C. American journal of human genetics 71, 426-431.

Oldenburg, A., Briand, N., Sorensen, A.L., Cahyani, I., Shah, A., Moskaug, J.O., Collas, P., 2017. A lipodystrophy-causing lamin A mutant alters conformation and epigenetic regulation of the anti-adipogenic MIR335 locus. The Journal of cell biology 216, 2731-2743.

Osorio, F.G., Barcena, C., Soria-Valles, C., Ramsay, A.J., de Carlos, F., Cobo, J., Fueyo, A., Freije, J.M., 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.

Osorio, F.G., Varela, I., Lara, E., Puente, X.S., Espada, J., Santoro, R., Freije, J.M., Fraga, M.F., Lopez-Otin, C., 2010. Nuclear envelope alterations generate an aging-like epigenetic pattern in mice deficient in Zmpste24 metalloprotease. Aging cell 9, 947-957.

Ozer, L., Unsal, E., Aktuna, S., Baltaci, V., Celikkol, P., Akyigit, F., Sen, A., Ayvaz, O., Balci, S., 2016. Mandibuloacral dysplasia and LMNA A529V mutation in Turkish patients with severe skeletal changes and absent breast development. Clin Dysmorphol 25, 91-97.

Pallotta, R., Morgese, G., 1984. Mandibuloacral dysplasia: a rare progeroid syndrome. Two brothers confirm autosomal recessive inheritance. Clinical genetics 26, 133-138.

Pelosini, C., Martinelli, S., Ceccarini, G., Magno, S., Barone, I., Basolo, A., Fierabracci, P., Vitti, P., Maffei, M., Santini, F., 2014. Identification of a novel mutation in the polymerase delta 1 (POLD1) gene in a lipodystrophic patient affected by mandibular hypoplasia, deafness, progeroid features (MDPL) syndrome. Metabolism: clinical and experimental 63, 1385-1389.

Perovanovic, J., Dell'Orso, S., Gnochi, V.F., Jaiswal, J.K., Sartorelli, V., Vigouroux, C., Mamchaoui, K., Mouly, V., Bonne, G., Hoffman, E.P., 2016. Laminopathies disrupt epigenomic developmental programs and cell fate. Science translational medicine 8, 335ra358.

Porter, L.J., Holt, M.R., Soong, D., Shanahan, C.M., Warren, D.T., 2016. Prelamin A Accumulation Attenuates Rac1 Activity and Increases the Intrinsic Migrational Persistence of Aged Vascular Smooth Muscle Cells. Cells 5.

Ragnauth, C.D., Warren, D.T., Liu, Y., McNair, R., Tajsic, T., Figg, N., Shroff, R., Skepper, J., Shanahan, C.M., 2010. Prelamin A acts to accelerate smooth muscle cell senescence and is a novel biomarker of human vascular aging. Circulation 121, 2200-2210.

Reinier, F., Zoledziewska, M., Hanna, D., Smith, J.D., Valentini, M., Zara, I., Berutti, R., Sanna, S., Oppo, M., Cusano, R., Satta, R., Montesu, M.A., Jones, C., Cerimele, D., Nickerson, D.A., Angius, A., Cucca, F., Cottoni, F., Crisponi, L., 2015. Mandibular hypoplasia, deafness, progeroid features and lipodystrophy (MDPL) syndrome in the context of inherited lipodystrophies. Metabolism: clinical and experimental 64, 1530-1540.

Richards, S.A., Muter, J., Ritchie, P., Lattanzi, G., Hutchison, C.J., 2011. The accumulation of un-repairable DNA damage in laminopathy progeria fibroblasts is caused by ROS generation and is prevented by treatment with N-acetyl cysteine. Human molecular genetics 20, 3997-4004.

Robson, M.I., de Las Heras, J.I., Czapiewski, R., Sivakumar, A., Kerr, A.R.W., Schirmer, E.C., 2017. Constrained release of lamina-associated enhancers and genes from the nuclear envelope during T-cell activation facilitates their association in chromosome compartments. Genome research 27, 1126-1138.

Ruiz de Eguino, G., Infante, A., Schlangen, K., Aransay, A.M., Fullaondo, A., Soriano, M., Garcia-Verdugo, J.M., Martin, A.G., Rodriguez, C.I., 2012. Sp1 transcription factor interaction with accumulated prelamin a impairs adipose lineage differentiation in human mesenchymal stem cells: essential role of sp1 in the integrity of lipid vesicles. Stem cells translational medicine 1, 309-321.

Shah, P., Wolf, K., Lammerding, J., 2017. Bursting the Bubble - Nuclear Envelope Rupture as a Path to Genomic Instability? Trends in cell biology 27, 546-555.

Shen, J.J., Brown, C.A., Lupski, J.R., Potocki, L., 2003. Mandibuloacral dysplasia caused by homozygosity for the R527H mutation in lamin A/C. Journal of medical genetics 40, 854-857.

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

Simha, V., Garg, A., 2002. Body fat distribution and metabolic derangements in patients with familial partial lipodystrophy associated with mandibuloacral dysplasia. The Journal of clinical endocrinology and metabolism 87, 776-785.

Solovei, I., Wang, A.S., Thanisch, K., Schmidt, C.S., Krebs, S., Zwerger, M., Cohen, T.V., Devys, D., Foisner, R., Peichl, L., Herrmann, H., Blum, H., Engelkamp, D., Stewart, C.L., Leonhardt, H., Joffe, B., 2013. LBR and lamin A/C sequentially tether peripheral heterochromatin and inversely regulate differentiation. Cell 152, 584-598.

Soria-Valles, C., Osorio, F.G., Gutierrez-Fernandez, A., De Los Angeles, A., Bueno, C., Menendez, P., Martin-Subero, J.I., Daley, G.Q., Freije, J.M., Lopez-Otin, C., 2015. NF-kappaB activation impairs somatic cell reprogramming in ageing. Nature cell biology 17, 1004-1013.

Stubbs, T.M., Bonder, M.J., Stark, A.K., Krueger, F., Team, B.I.A.C., von Meyenn, F., Stegle, O., Reik, W., 2017. Multi-tissue DNA methylation age predictor in mouse. Genome biology 18, 68.

Tenconi, R., Miotti, F., Miotti, A., Audino, G., Ferro, R., Clementi, M., 1986. Another Italian family with mandibuloacral dysplasia: why does it seem more frequent in Italy? American journal of medical genetics 24, 357-364.

Tudisco, C., Canepa, G., Novelli, G., Dallapiccola, B., 2000. Familial mandibuloacral dysplasia: report of an additional Italian patient. American journal of medical genetics 94, 237-241.

Turgay, Y., Eibauer, M., Goldman, A.E., Shimi, T., Khayat, M., Ben-Harush, K., Dubrovsky-Gaupp, A., Sapra, K.T., Goldman, R.D., Medalia, O., 2017. The molecular architecture of lamins in somatic cells. Nature 543, 261-264.

Venkatachalam, G., Surana, U., Clement, M.V., 2017. Replication stress-induced endogenous DNA damage drives cellular senescence induced by a sub-lethal oxidative stress. Nucleic acids research 45, 10564-10582.

Wang, Y., Lichter-Konecki, U., Anyane-Yeboa, K., Shaw, J.E., Lu, J.T., Ostlund, C., Shin, J.Y., Clark, L.N., Gundersen, G.G., Nagy, P.L., Worman, H.J., 2016. A mutation abolishing the ZMPSTE24 cleavage site in prelamin A causes a progeroid disorder. Journal of cell science 129, 1975-1980.

Weedon, M.N., Ellard, S., Prindle, M.J., Caswell, R., Lango Allen, H., Oram, R., Godbole, K., Yajnik, C.S., Sbraccia, P., Novelli, G., Turnpenny, P., McCann, E., Goh, K.J., Wang, Y., Fulford, J., McCulloch, L.J., Savage, D.B., O'Rahilly, S., Kos, K., Loeb, L.A., Semple, R.K., Hattersley, A.T., 2013. An in-frame deletion at the polymerase active site of POLD1 causes a multisystem disorder with lipodystrophy. Nature genetics 45, 947-950.

Wu, D., Flannery, A.R., Cai, H., Ko, E., Cao, K., 2014. Nuclear localization signal deletion mutants of lamin A and progerin reveal insights into lamin A processing and emerin targeting. Nucleus (Austin, Tex.) 5, 66-74.

Young, L.W., Radebaugh, J.F., Rubin, P., Sensenbrenner, J.A., Fiorelli, G., McKusick, V.A., 1971. New syndrome manifested by mandibular hypoplasia, acroosteolysis, stiff joints and cutaneous atrophy (mandibuloacral dysplasia) in two unrelated boys. Birth defects original article series 7, 291-297.

Zini, N., Avnet, S., Ghisu, S., Maraldi, N.M., Squarzoni, S., Baldini, N., Lattanzi, G., 2008. Effects of prelamin A processing inhibitors on the differentiation and activity of human osteoclasts. Journal of cellular biochemistry 105, 34-40.

Figure Legend

Figure 1: Comparison of age of onset of clinical symptoms in MADA and MADB.
The majority of clinical cases of MADA and MADB patients so far reported were screened for some of the most recurrent symptoms of the diseases. Lamin A/C and ZMPSTE24 pathogenetic mutations are indicated by specific colors (see text for bibliographic reference).

Figure 2: Common phenotypical hallmarks of patients affected by MAD.
A) Hands and **B)** feet presenting bulbous finger tips, particularly evident in A). **C)** X-Rays of the hands showing the loss of bone from the distal phalanges (acroosteolysis). **D)** X-rays of the thorax showing a severe bilateral clavicular hypoplasia. All the images are from of a unique MADA patient (homozygous *LMNA* mutation p.Arg527His) of age 25, and show some of the most macroscopic phenotypical abnormalities observed in patients suffering from MADA and MADB.

Figure 3: Maturation of lamin A.
Under non pathological conditions, lamin A is released in the nucleus as a result of post-translational and hierarchical modifications of its precursor, prelamin A. Translated prelamin A (non farnesylated prelamin A) is farnesylated by a farnesyl transferase (Ftase), which adds a farnesyl isoprenoid group to Cysteine 661 (C661) of the CaaX motif (CSIM), producing the farnesylated prelamin A. Farnesylated prelamin A is next subjected to a proteolytic cleavage by ZMPSTE24 or RCE1 (Ras converting Enzyme 1), which cleave the last 3 amino acids. Later on, an isoprenylcysteine carboxyl methyltransferase (ICMT) adds a carboxymethyl group to the farneyslated Cysteine 661, producing farnesylated and carboxymethylated prelamin A. The protease ZMPSTE24 next recognizes this intermediate, cleaving it at Leucine 647 (L647) finally releasing mature lamin A. At the end of these maturation steps, mature lamin A terminates at Tyrosine 646 (Y646) and is 18 amino acids shorter than its precursor.

Figure 4: Schematic representation of MADA pathogenic mutations in *LMNA* gene products.
Known genetic missense mutations on *LMNA* gene resulting in pathogenetic mutations causative of MADA disease. All the major *LMNA* products, that is prelamin A, lamin A and C are represented. MADA-pathogenic mutations identified so far have been reported in homozygosis, except for those marked with an asterisk, found in heterozygosis (see text for references). NLS: Nuclear Localization Signal.

Figure 5: Schematic representation of MADB pathogenic mutations in ZMPSTE24 enzyme.
Schematic representation of ZMPSTE24 protein, in which seven transmembrane domains lie, indicating the mutations causative of MADB so far identified. Many of the mutations lie in the catalytic domain and are responsible for a complete or partial abolishment of the functions of the enzyme, resulting in a more or less severe phenotype.

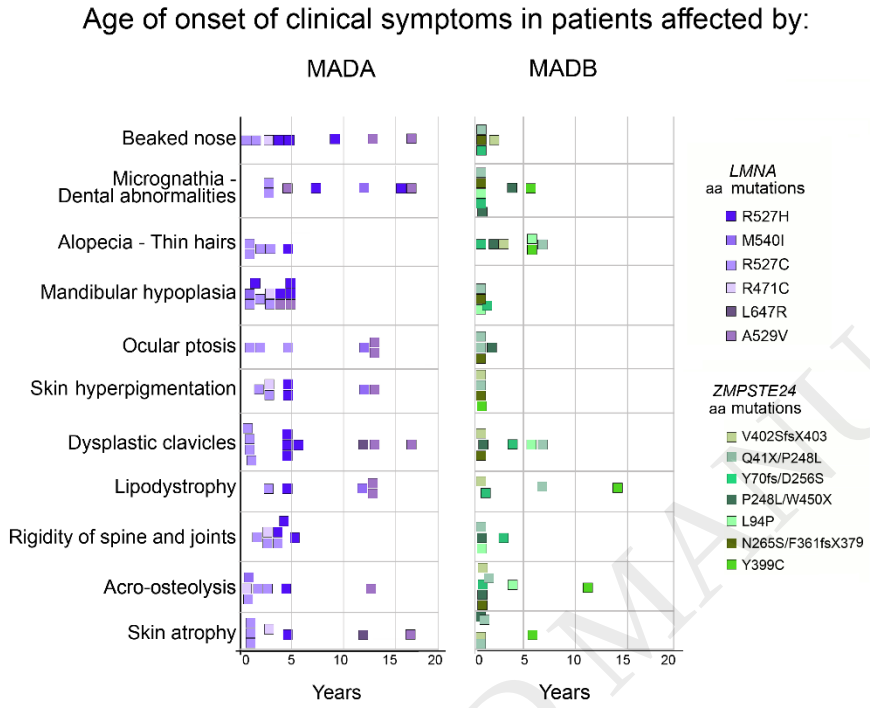
Figure 6: Heterochromatin loss and prelamin A accumulation in aged nuclei.
A) Cultured skin fibroblasts from control young, old (age 60-80 years) and centenarian donors (age 95-105 years), and from MADA patients (*LMNA* R527H mutation, age 25 years) were subjected to ultrastructural analysis. Representative pictures of more than 3 diverse subject samples at low and high magnification are shown. Heterochromatin loss is detectable in fibroblast nuclei from young donors subjected to oxidative stress for 24 hours (+ H_2O_2) (see Lattanzi et al. 2014 for details) and in cells from old, centenarian and MADA donors. **B)** Same samples as in A) were subjected to immunofluorescence analysis to detect prelamin A accumulation. Nuclei from young donors exposed to oxidative stress (+ H_2O_2) show transient accumulation of non-farnesylated prelamin A (green), while permanent accumulation of non-farnesylated prelamin A is observed in old donor nuclei, permanent accumulation of farnesylated prelamin A (red) is observed in centenarian nuclei and accumulation of both prelamin A forms is found in MADA nuclei. DAPI staining (blue) highlights nuclei.

Figure 7. Pathogenetic pathways for MAD.
Accumulation of prelamin A, due to MADA and MADB pathogenetic mutations in *LMNA* or *ZMPSTE24* gene, occurs at the nuclear lamina. This leads to an altered distribution of nuclear envelope proteins, as emerin, and SUN1 and SUN2, causing multiple anomalous events as: *i.* an altered transcription factor import in the nucleus; *ii.* altered chromatin dynamics resulting in loss of heterochromatin and SAHF formation upon stress; *iii.* failure to regulate TGF beta 2 levels that triggers an altered TGFbeta2 release; *iv*. altered SIRT1 and HDAC2 interplay with lamin A/C *v.* altered release of some cytokines involved in osteoclastogenesis, as OPG, cathepsin K and RANKL, besides TGFbeta2.

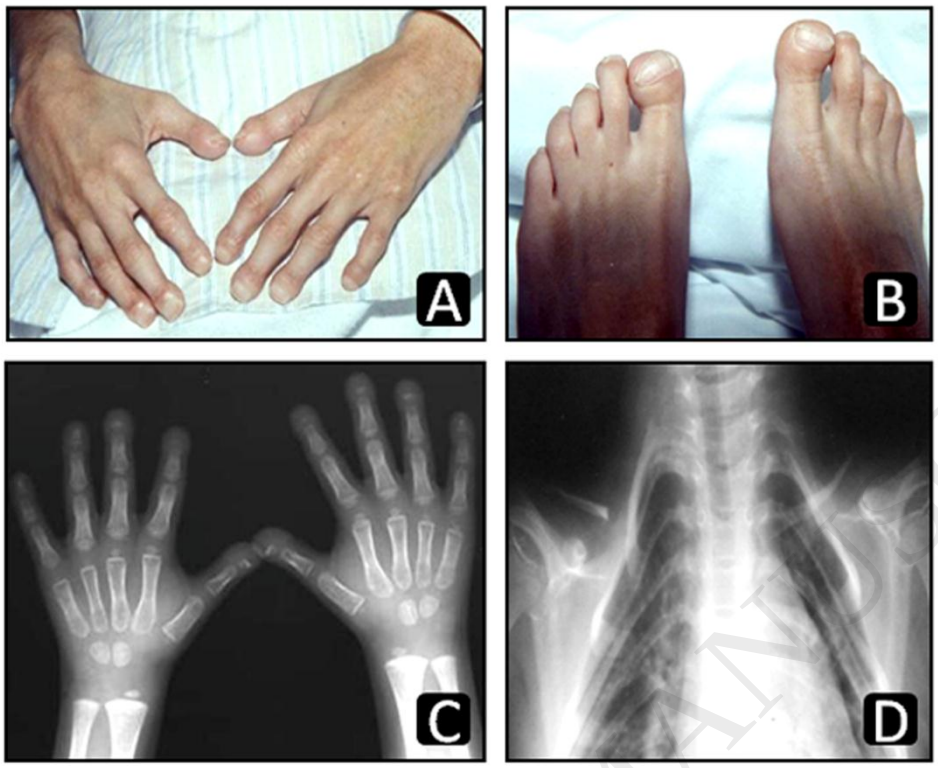
Figure 8. Proposed effect of lamin A on genetic determinants on ageing.

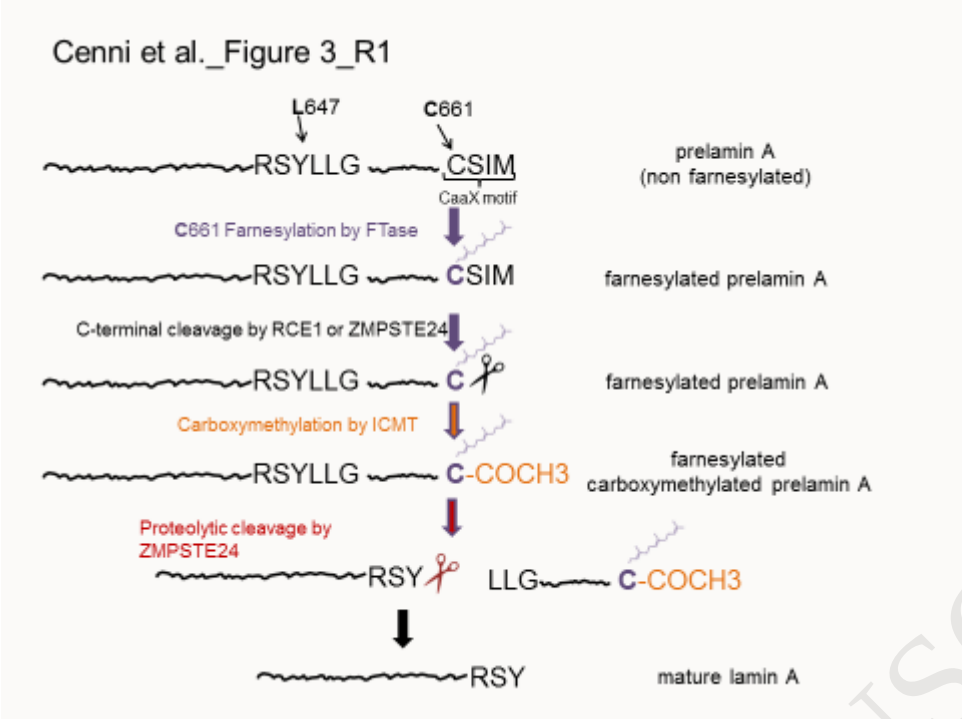
As summarized in this review, defects of lamin A or prelamin A accumulation due to *LMNA* or *ZMPSTE24* mutations may trigger determinants of ageing. References to studies showing a link between lamin-dependent processes and each ageing determinant are reported in the cartoon.

Cenni et al._Figure 1_R1

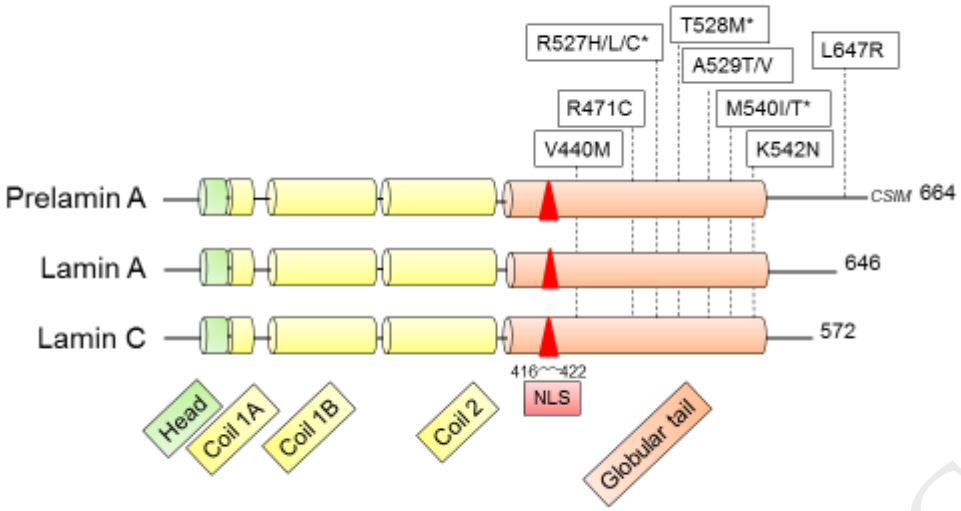


Cenni et al._Figure 2_R1

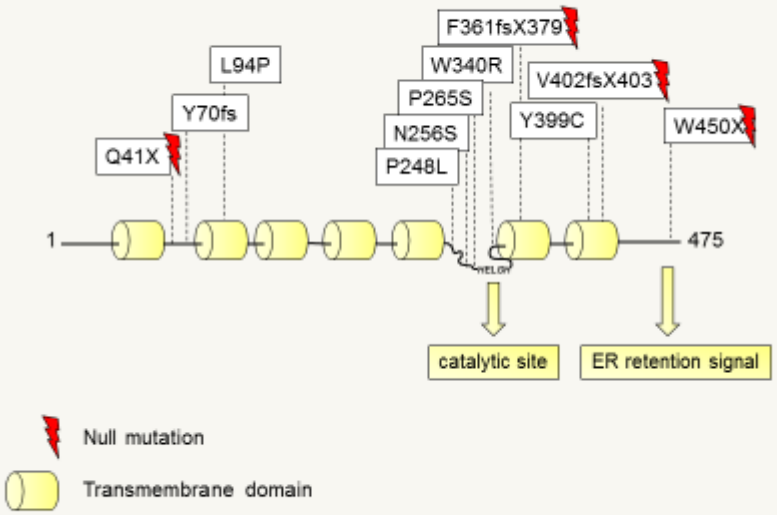




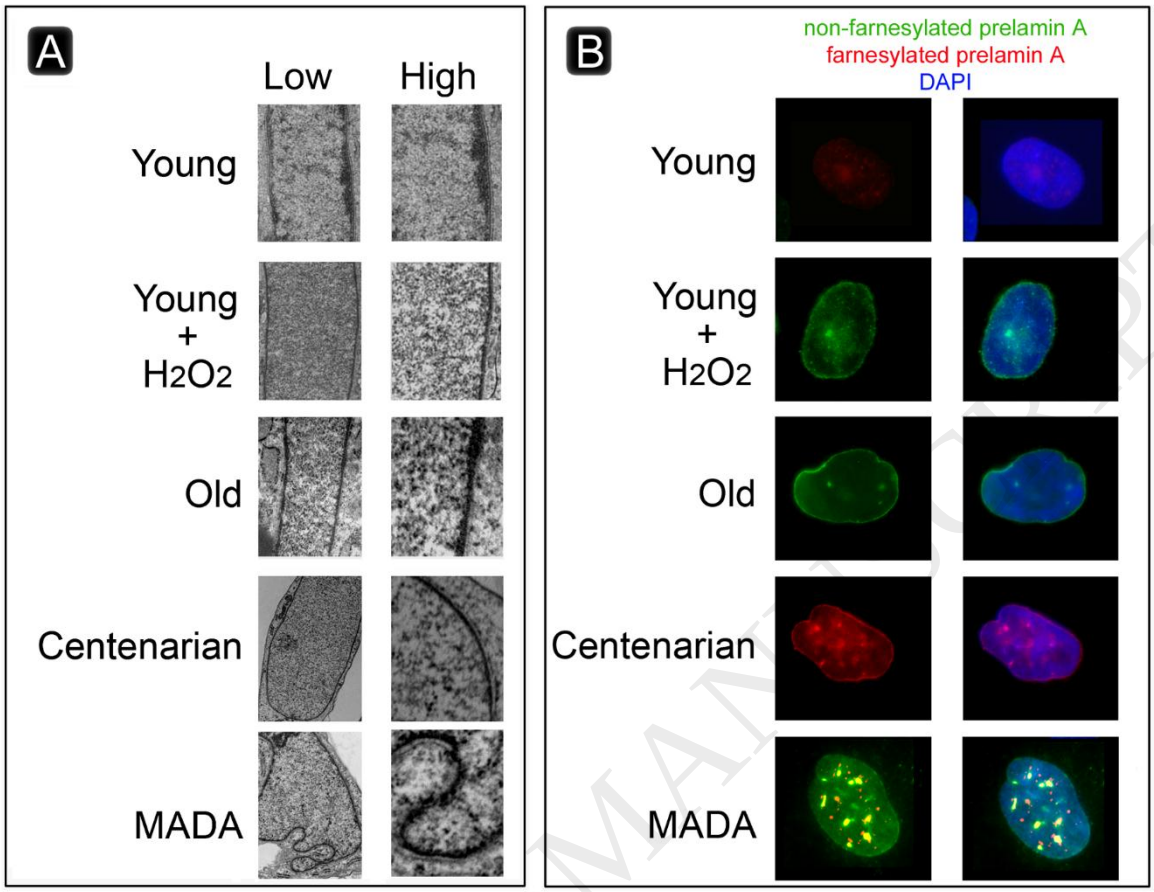
Cenni et al._Figure 4_R1



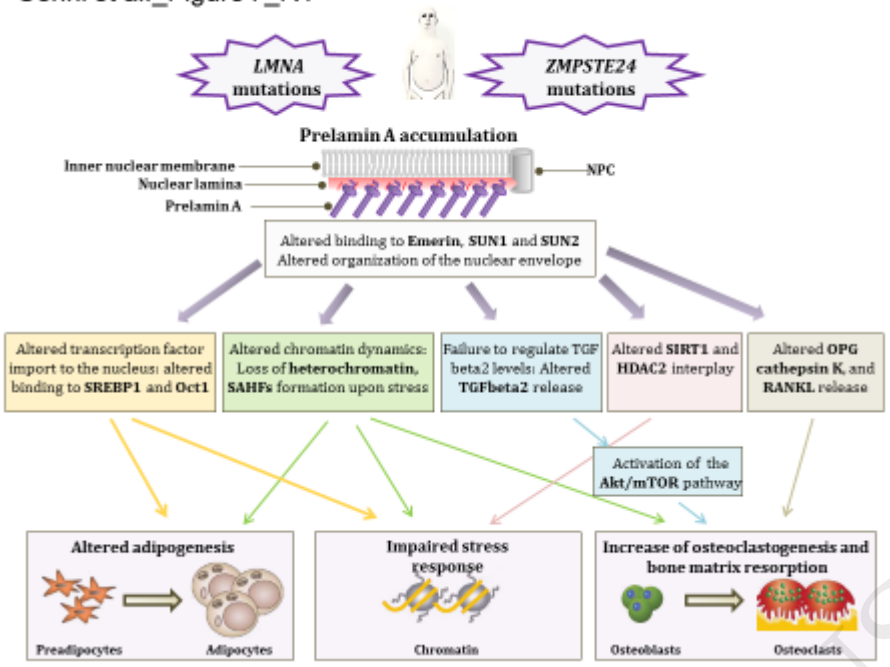
Cenni et al._Figure 5_R1



Cenni et al._Figure 6_R1



Cenni et al._Figure 7_R1



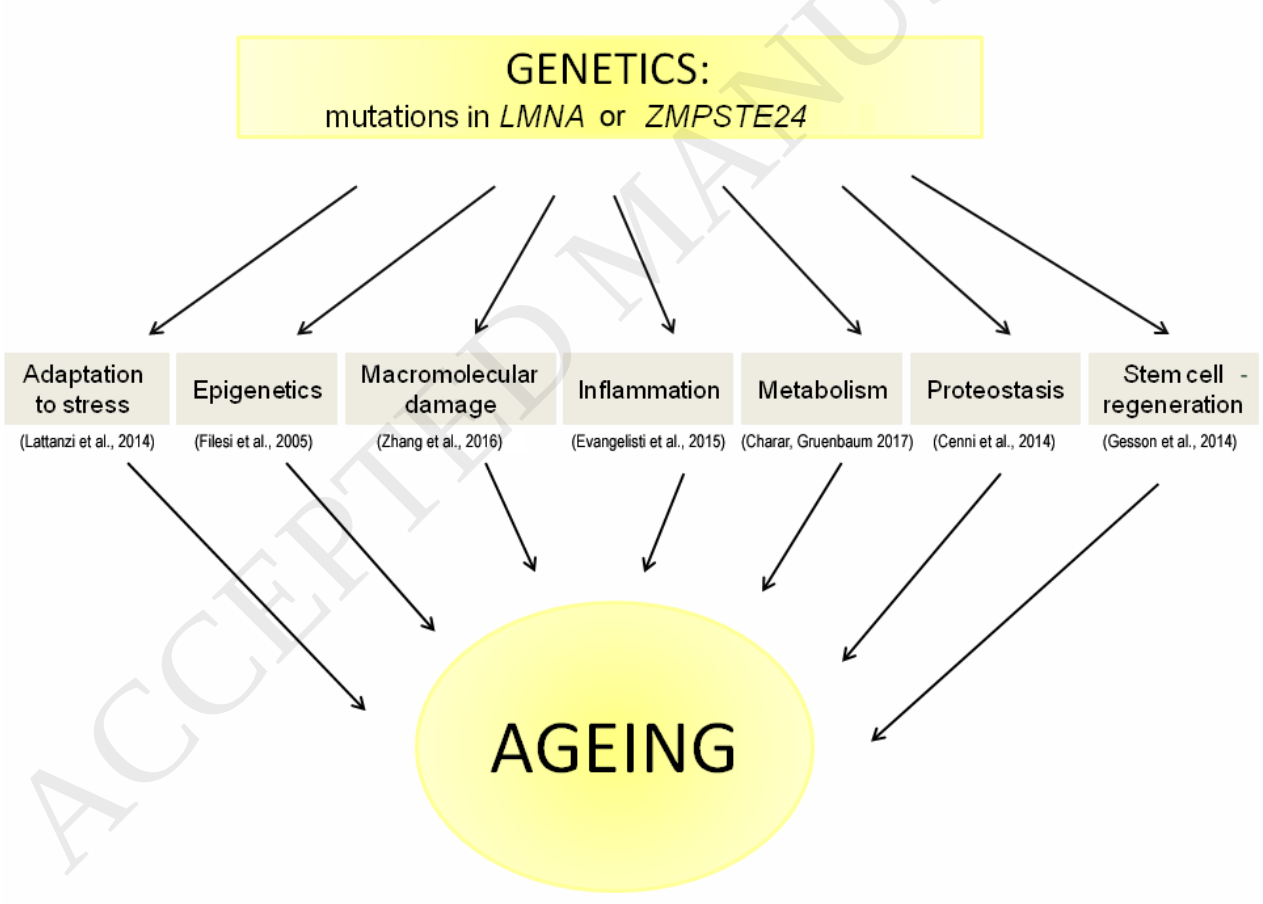


Table Legend

Table 1. Full list of the pathogenetic mutations identified so far in *LMNA* and *ZMPSTE24* and corresponding phenotypes. For each gene mutation, the nucleotidic and the respective amino acidic mutation are shown. Phenotypes are also indicated. Abbreviation used are: fs frameshift; del deletion; dup duplication; X stop codon.

Cenni et al._Table 1_R1

Form	Gene	Mutation	Mutation	Genotype	Phenotype	Refs.
MADA	*LMNA*	c.1580G>A	p.Arg527His	Homoz.	Typical MADA.	(Filesi et al., 2005; Garavelli et al., 2009; Shen et al., 2003; Simha et al., 2003)
MADA	LMNA	c.1585G>A	p.Ala529Thr	Homoz.	Typical MADA.	(Kosho et al., 2007)
MADA	LMNA	c.1586C>T	p.Ala529Val	Homoz.	Typical MADA.	(Cogulu et al., 2003; Garg et al., 2005; Ozer et al., 2016)
MADA	LMNA	c.1623C>T	p.Arg471Cys	Homoz.	Typical MADA.	(Zirn et al., 2008)
MADA	LMNA	c.1626G>C	p.Lys542Asn	Homoz.	Typical MADA.	(Plasilova et al., 2004)
MADA	LMNA	c.1579C>T	p.Arg527Cys	Homoz.	Typical MADA.	(Agarwal et al., 2008; Luo et al., 2014)
MADA	LMNA	c.1940T>G	p.Leu647Arg	Heteroz.	Typical MADA.	(Wang et al., 2016)
MADA	LMNA	c.1620G >A	p.Met540Ile	Homoz.	Typical MADA.	(Yassaee et al., 2016)

MADA	LMNA	c.1623C>T c.1579C>T	p.Arg471Cys p.Arg527Cys	Heteroz.	Atypical MADA with progeroid features.	(Cao and Hegele, 2003)
MADA	LMNA	c.1580G>T	p.Arg527Leu	Homoz	Atypical MADA with progeroid features.	(Al-Haggar et al., 2012)
MADA	LMNA	c.1580G>A c.1318G>A	p.Arg527His p.Val440Met	Heteroz.	Typical MADA with skeletal muscle involvement.	(Lombardi et al., 2007)
MADA	LMNA	c.1626G>C c.1619T>C	p.Thr528Met p.Met540Thr	Heteroz.	Atypical MADA with progeroid features.	(Verstraeten et al., 2006)
MADB	ZMPSTE24	c.1085dupT c.1018T>C	p.Phe361fsX379 p.Trp340Arg	Heteroz.	Typical MADB.	(Agarwal et al., 2003)
MADB	ZMPSTE24	c.1085dupT c.794A>G	p.Phe361fsX379 p.Asn265Ser	Heteroz.	Typical MADB with glomerulosclerosis.	(Agarwal et al., 2006)
MADB	ZMPSTE24	c.1085dupT c.794A>G	p.Phe361fsX379 p.Asn265Ser	Heteroz.	Atypical MADB displaying features of HGPS, MAD and RD.	(Shackleton et al., 2005)
MADB	ZMPSTE24	c.121C>T c.743C>T	p.Gln41X p.Pro248Leu	Heteroz.	Typical MADB.	(Miyoshi et al., 2008)
MADB	ZMPSTE24	c.207_208delCT c.794A>G	p.Tyr70fs p.Asp256Ser	Heteroz.	Typical MADB with severe bone involvement.	(Cunningham et al., 2010)
MADB	ZMPSTE24	c.743C>T c.1349G>A	p.Pro248Leu p.Trp450X	Heteroz.	Typical MADB.	(Ahmad et al., 2010)
MADB	ZMPSTE24	c.281T>C	p.Leu94Pro	Homoz.	Typical MADB with a congenital myopathy.	(Ben Yaou et al., 2011)

MADB	ZMPSTE24	c.794A>G c.1085dupT	p.Asn265Ser p.Phe361fsX379	Heteroz.	Typical MADB.	(Kwan, 2015)
MADB	ZMPSTE24	c.1196A>G	p.Tyr399Cys	Homoz.	Typical MADB.	(Haye et al., 2016)