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## Review Article

# Phenomics and lamins: From disease to therapy

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### ABSTRACT

Systematic correlation of phenotype with genotype is a key goal of the emerging field of phenomics, which is expected to help define complex diseases. Careful evaluation of phenotype–genotype associations in monogenic disorders, such as laminopathies, might provide new hypotheses to be tested with molecular and cellular studies and might also suggest potential new intervention strategies. For instance, evaluation of the clinical features of carriers of mutant LMNA in kindreds with familial partial lipodystrophy suggests rational, staged intervention using established pharmaceutical agents to prevent cardiovascular complications not just for patients with lipodystrophy but by extension for patients with the common metabolic syndrome. Careful non-invasive imaging shows phenotypic differences between partial lipodystrophy due to mutant LMNA and not due to mutant LMNA. Furthermore, hierarchical cluster analysis detects systematic relationships between organ involvement in laminopathies and mutation position in the LMNA genomic sequence. However, sometimes the same LMNA mutation can underlie markedly different clinical phenotypes; cellular and molecular experiments can help to explain the mechanistic basis for such differences. Finally, promising novel treatment modalities for laminopathies, such as farnesyl transferase inhibition and gene-based therapies, might help not only to illuminate mechanisms that link genotype to phenotype, but also to provide hope for patients suffering with laminopathies, since these treatments are designed to modulate key early or proximal steps in the pathogenesis of these disorders.

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## Introduction

Modern genomic tools are revolutionising the prediction, prevention and treatment of human diseases. However, non-quantitative, non-specific, insensitive phenotypes are weak links in the discovery chain. The critical importance of well-characterised phenotypes – observable structures and functions arising from the effects of molecules, cells, tissues and organs – was underscored by the introduction of the concept of “phenomics” [1–4]. Phenomics can be defined as integrated multidisciplinary research to understand the complex consequences of genomic variation through systematic evaluation and cataloguing of standardized phenotypes [3,5]. Sensitive phenomic tools can reveal previously unseen phenotypic markers, also called “early” or “intermediate” phenotypes. Herein, we present examples of phenomics applied to define laminopathies at the levels of pathogenesis and potential clinical intervention. Specifically, we will discuss: (1) phenomic assessment of the stages of partial lipodystrophy and how this both suggests staged interventions using existing treatments and serves as a potential model for the common “metabolic syndrome” (MetS); (2) phenomic differences between familial partial lipodystrophy (FPLD) subtypes caused by LMNA mutations and not caused by LMNA mutations; (3) how phenotypic analogy between genetic and acquired partial lipodystrophy led to discovery of mutations in the re-annotated LMNB2 gene; (4) phenomic–genomic analysis showing a non-random relationship between LMNA mutations and clinical phenotypes; (5) phenotypic heterogeneity among some patients with identical genomic mutations; (6) mechanistic insights into phenomic–genomic relationships from cellular and molecular investigations; and (7) the role of novel therapies – such as farnesyl transferase inhibition and gene-based approaches – in further elucidating phenotype–genotype relationships in laminopathies, which for the purpose of this review will refer to diseases caused by mutations in genes encoding lamins (LMNA, LMNB1 and LMNB2) and also genes encoding lamin-associated proteins of the inner nuclear membrane, although we will focus on LMNA-associated diseases. Our review begins with lipodystrophies, which serve as monogenic models for the common MetS.

### The common metabolic syndrome (MetS)

The constellation of disturbed carbohydrate and insulin metabolism, with central obesity, dyslipidemia (elevated triglycerides [TG] with depressed HDL cholesterol), hypertension, and type 2 DM (T2DM) is called the “metabolic syndrome”

(MetS) or “insulin resistance syndrome” [6]. MetS affects ~30% of North Americans and results from the interaction of environmental factors, such as caloric excess and physical inactivity, with largely unknown genetic susceptibility factors [5,6]. Altered serum concentrations of inflammatory markers are a recognized part of MetS [7–9]. The World Health Organization [10], the National Cholesterol Education Program [11] and the International Diabetes Federation [12] have proposed clinical definitions for MetS. Despite debate as to whether MetS is a discrete phenotype, the MetS concept has been useful for clinical and research applications [5]. Genetic studies have revealed novel etiologies for MetS [5]. Some molecularly characterized monogenic forms, such as the lipodystrophies, have provided important insights for understanding common MetS.

### Lipodystrophies: monogenic models of MetS

Lipodystrophy refers to rare conditions that are characterized by fat loss in some anatomical sites, with fat accumulation in non-dystrophic sites, and unusual sites such as liver and muscle. Increased fatty acid flux through plasma and liver is associated with development of dyslipidemia, insulin resistance and atherosclerosis. Lipodystrophies can be either inherited or acquired, and either partial or generalized. Some molecularly defined types of partial lipodystrophy include: familial partial lipodystrophy type 2 (FPLD2; MIM 151660) caused by mutations in LMNA [13–15] and type 3 (FPLD3; MIM 604367) caused by mutations in PPARG [16], and acquired partial lipodystrophy (APL, “Barraquer–Simons syndrome” MIM 608709) caused in some cases by mutations in LMNB2 [17]. These partial lipodystrophies each display features of common MetS, including dysglycemia, dyslipidemia (elevated TG and depressed HDL cholesterol), hypertension and central obesity. Although the expression of MetS in rare monogenic syndromes could simply be secondary to fat redistribution, the causative gene products might produce insulin resistance directly, and thus might illuminate new causative mechanisms for insulin resistance in such common disorders as T2DM and obesity.

### Metabolic evolution of Dunnigan-type familial partial lipodystrophy type 2 (FPLD2)

Dunnigan and Kobblerling independently described patients who were normal at birth but later lost subcutaneous fat from extremities and the gluteal region, resulting in prominent,

well-defined musculature with excess fat deposition around the face and intra-abdominally [18]. Non-invasive imaging studies showed preservation of inter- and intramuscular, intra-abdominal, intrathoracic and bone marrow fat [19]. The biochemical hallmark of FPLD2 is insulin resistance, with dyslipidemia, hypertension and type 2 diabetes (T2DM) presenting later in life. Other findings include acanthosis nigricans, hirsutism, menstrual abnormalities and polycystic ovaries. In Canadian subjects we showed that FPLD2, which has some features of MetS, was caused by a missense mutation in *LMNA* [13]. Phenomic studies in showed FPLD2 subjects, especially women, were at very high risk for early coronary heart disease (CHD) [20] and had many of the same metabolic disturbances found in common MetS [21]. By genotyping 86 subjects from Canadian FPLD2 families, we determined the evolution of the metabolic disturbances (Fig. 1).

Fig. 1 schematically shows the temporal progression of the clinical and biochemical features seen among patients with FPLD2. The germ line heterozygous *LMNA* mutation is present at birth. The first manifestation in early adolescence is fat redistribution. Future treatments might be directed towards impeding adipose tissue loss, stimulating re-growth or replacing lost tissue.

Mid-stage disease has a characteristic biochemical profile, including elevated plasma concentrations of FFA, insulin and C-peptide, TG and CRP, with depressed plasma concentrations of high-density lipoprotein (HDL) cholesterol, leptin and adiponectin. Physical signs of insulin resistance, such as acanthosis nigricans, fatty liver and polycystic ovaries, appear. Hypertension emerges as a problem at this stage. Treatment now focuses on vascular disease prevention, with control of hypertension and dyslipidemia using existing drugs. Future treatments may include pre-emptive use of insulin-sensitizing drugs and/or leptin injections with or without adiponectin.

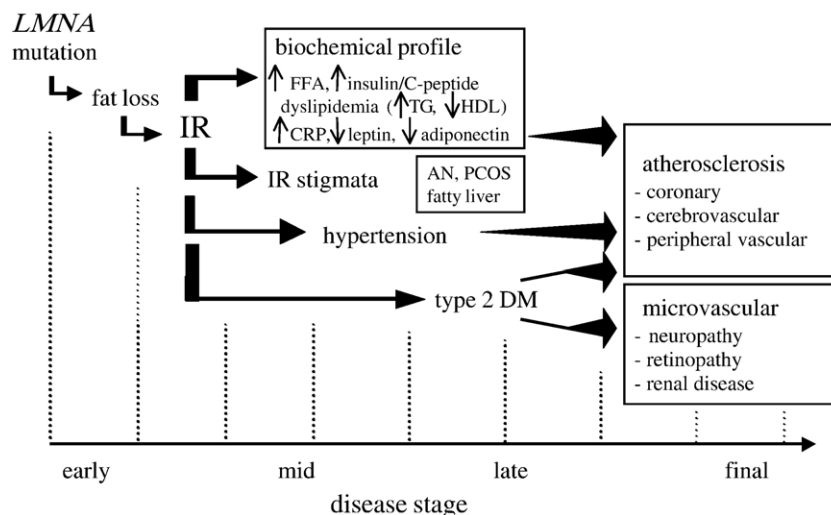
Late-stage disease is characterized by development of diabetes, which causes profound metabolic changes. Treatment at this stage is focused on intensive control of glycemia, dyslipidemia and hypertension with existing pharmacotherapies in order to prevent complications. Future treatments may include leptin with or without adiponectin.

Final stage disease is characterized by the development of diabetic complications. Premature atherosclerosis is seen in FPLD2 patients [20], although most subjects with CHD were diabetic, suggesting that diabetes is necessary for expression of vascular disease. Therapies include stabilisation of metabolic variables, palliation and secondary prevention of vascular disease, again using established evidence-based therapies.

Finally, there are some theoretical therapeutic options in treatment of partial lipodystrophy. For instance, the central role of hepatic stearoyl-coenzyme A desaturase-1 (SCD-1) in hepatosteatosis implicates this enzyme as a potential drug target [22]. Treatments might also come from among new agents under development, including new agonists for peroxisome proliferator-activated receptors (PPARs) and target gene-selective PPAR receptor modulators (SPPARMs) that might selectively affect adipose differentiation and viability [23] in subjects with FPLD2. Newer drugs for dyslipidemia [24] might also be appropriate for managing hypertriglyceridemia, prophylaxis of pancreatitis and secondary prevention of vascular disease in partial lipodystrophy.

### Phenomic evaluation of two forms of familial partial lipodystrophy

Despite superficial clinical similarities, phenomic evaluation showed substantial differences between familial partial lipodystrophy due to mutant *LMNA* (FPLD2) and not due to mutant *LMNA* (FPLD3). Subjects of European ancestry from a Canadian



**Fig. 1 – Disease stages in partial lipodystrophy.** Schematic temporal progression of the clinical and biochemical features seen among patients with the FPLD2 (*LMNA* subtype). The abscissa depicts passage of time moving left-to-right. Tapered arrows indicate worsening of the specific traits with time. Small arrows up or down indicate elevation or depression, respectively. Abbreviations: IR, insulin resistance; FFA, free fatty acid; TG, triglyceride; HDL, high-density lipoprotein; CRP, C-reactive protein; AN, acanthosis nigricans; PCOS, polycystic ovarian syndrome; DM, diabetes mellitus.

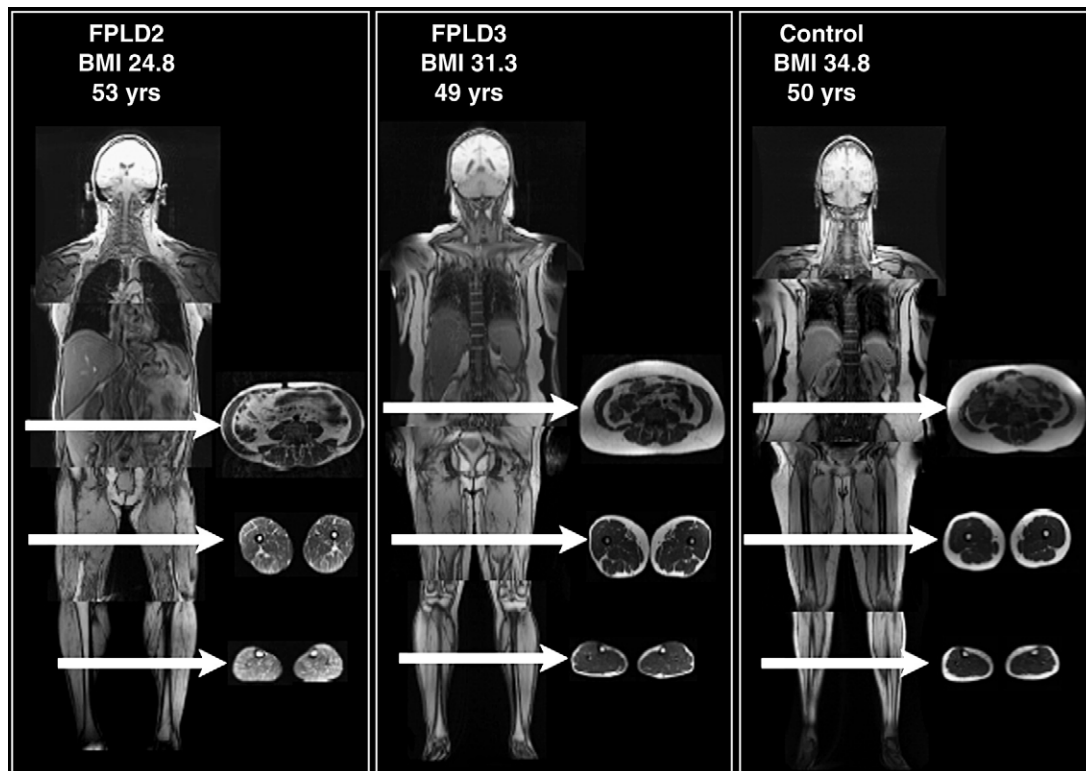
FPLD2 family with *LMNA* R482Q [13,20] and FPLD3 patients with various mutations in *PPARG* [16] were studied. Careful phenomic assessment using magnetic resonance imaging indicated subcutaneous abdominal and gluteal fat stores were completely absent in *LMNA* mutation carriers, but were present although reduced in *PPARG* mutation carriers (Fig. 2). Furthermore, the extent of subcutaneous fat loss on extremities was markedly greater among *LMNA* mutation carriers compared to *PPARG* mutation carriers [25]. The sum of historical, physical and biochemical features indicated that partial lipodystrophy due to mutant *LMNA* compared to that due to mutant *PPARG* was associated with: (1) more extensive subcutaneous adipose tissue loss; (2) less severe and/or later onset of clinical endpoints such as acanthosis nigricans, hepatic steatosis, polycystic ovarian disease and hirsutism; (3) less severe hypertension; (4) later onset of type 2 diabetes; (5) less severe biochemical insulin resistance; (6) less severe depression of adipocytokines in some cases; and (7) a more consistent biochemical response to oral hypoglycaemic agents. However, early atherosclerosis was more evident among women with *LMNA* R482Q [20]. Thus compared to FPLD2 subjects, the clinical and biochemical derangements in FPLD3 subjects were disproportionate to the extent of lipody-

strophy, implying that *PPARG* mutations have additional effects on metabolism.

### ***LMNB2* mutations found in patients with acquired partial lipodystrophy (APL)**

APL (MIM 608709), also known as Barraquer–Simons syndrome, does not segregate as a classical simple mendelian trait in families [26]. In addition, the presence of dermatomyositis, systemic lupus erythematosus, C3 hypocomplementemia and membranoproliferative glomerulonephritis in some APL patients [26] suggests that disease expression might require a trigger. APL thus represents a complex phenotype, possibly with a component of genetic susceptibility that requires the presence of environmental factors or acquired disorders to be expressed.

We studied nine patients with APL; these individuals had onset of their disease in the second decade of life [17]. Most had features of common MetS, including hypertension, dyslipidemia and T2DM. Two APL patients had early CVD: a stroke in the fourth decade and CHD in the fifth decade of life, respectively. Since APL showed phenotypic similarity with



**Fig. 2 – Phenomic analysis using magnetic resonance imaging (MRI). Fat stores appear as a bright white signal. Total body MRI scans showing fat distribution in a subject with FPLD2 (heterozygote for *LMNA* R482Q mutation), a subject with FPLD3 (heterozygote for *PPARG* F388L mutation) and a non-lipodystrophic moderately obese subject. The age and body mass index (BMI) of each subject is shown in each panel. Three selected trans-axial slices are shown for comparison of fat distribution between the three subjects: the slices through the abdomen correspond to the level of the fourth lumbar vertebrae (L4), also with mid-thigh and mid-calf levels as shown. The L4 slice for the FPLD2 subject shows a paucity of subcutaneous fat with excess intra-abdominal or visceral fat, while both the FPLD3 and control subject have prominent concentric subcutaneous fat stores. The mid-thigh and mid-calf slices for the FPLD2 subject show virtually non-subcutaneous fat, while the slices at the same levels for the FPLD3 subject show attenuated, but not absent, fat stores compared to the normal control subject.**

another laminopathy, FPLD2, we hypothesized that APL might also be due to mutation in other nuclear lamina proteins. We sequenced *LMNB2* encoding lamin B2 in nine patients with APL. We found three rare *LMNB2* mutations in four APL patients: intron 1 –6G>T, exon 5 p.R215Q [in two patients] and exon 8 p.A407T. The relative risk of APL for carriers of these mutations was 110.1 [95% confidence interval [CI] 35.9 to 271.0;  $P < 0.00001$ ]. The findings: (1) were consistent with molecular genetic heterogeneity for APL, since five of nine patients did not have a *LMNB2* mutation; (2) indicated how phenotypic analogies could generate hypotheses for studies to define the genetic basis of human disease; (3) extended the range of phenotypes associated with mutations affecting the nuclear envelope; (4) increased the number of nuclear envelope components that are associated with disease; (5) implicated a second nuclear lamin gene in a human lipodystrophy syndrome; and (6) suggested that re-annotation of known genes will disclose new coding sequences that should be examined by genomic sequence analysis to search for new disease-associated mutations.

### Correlation of *LMNA* mutation position with clinical phenotypes

Numerous distinct disease phenotypes have been shown to result from scores of different *LMNA* mutations, including 14 autosomal dominant (AD) and three autosomal recessive (AR) phenotypes (see Table 1). The hypothesis that *LMNA* mutation position determines the laminopathy phenotype was tested by classifying laminopathies according to their phenotypic similarity [27] using hierarchical cluster analysis (HCA). Fig. 3 shows a grid with the columns specifying organ and system involvement in *LMNA*-type laminopathies and the rows specifying the laminopathy type. AR and AD Emery–Dreifuss muscular dystrophy (EMD2) clustered to the same rank and were considered to be the same phenotype for this analysis. This grid was analysed by HCA and a single dendrogram explained the data, with the horizontal branch lengths indicating the correlation coefficient distances between the *LMNA*-type laminopathies defined by the organ system involvement. The HCA identified two main classes of laminopathies: “class 1 laminopathies” included those with cardiac, skeletal muscle and neurological involvement, while “class 2 laminopathies” included simple partial lipodystrophy, progeria syndromes and mandibuloacral dysplasia [27].

To identify a relationship between HCA-defined laminopathy class and the mutation site within the primary nucleotide sequence, *LMNA* was next divided into two regions based upon nuclear localization signal (NLS) sequence (residues 416 to 423) within which no mutations have yet been found. The region upstream of codon 416 encoded the central alpha-helical rod domain, while the region downstream of codon 423 encoded the “homology box”, including the all-beta immunoglobulin-like fold and a DNA-binding region [27]. For each *LMNA*-type laminopathy, missense, nonsense and splicing mutation positions from human disease mutation databases were mapped along the linear cDNA sequence (Fig. 3). 91 *LMNA* mutations were analysed. HCA laminopathy class was significantly associated with mutation site in the

**Table 1 – *LMNA*-related laminopathies studied using phenomic hierarchical cluster analysis**

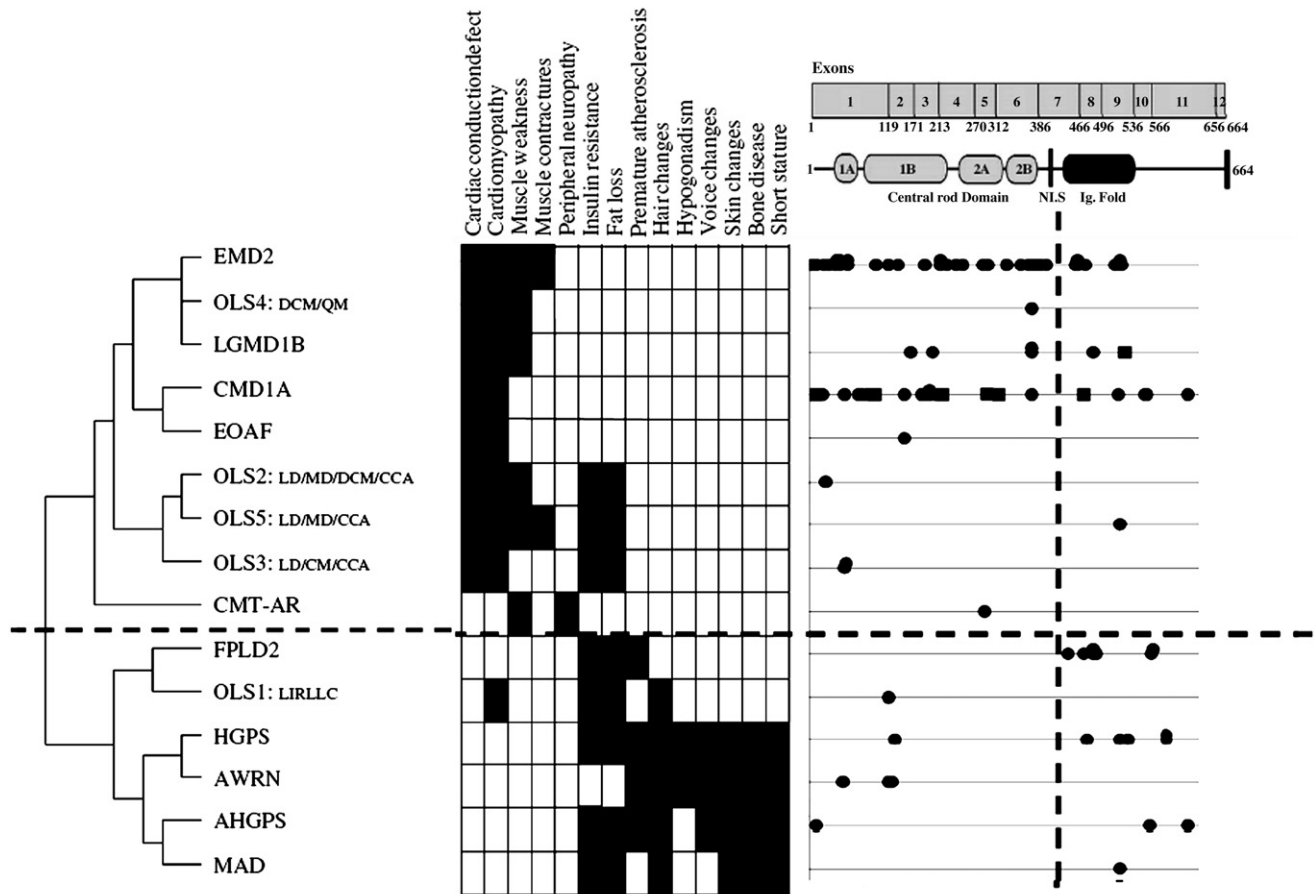
|  | MIM number  |
|--|-------------|
| Autosomal dominant (AD) disorders                      |             |
| Emery–Dreifuss muscular dystrophy (AD-EMD2)            | 181350      |
| Dilated cardiomyopathy with CCA (CMD1A)                | 115200      |
| Early onset atrial fibrillation (EOAF)                 | 607554      |
| Limb–girdle muscular dystrophy (LGMD1B)                | 159001      |
| Familial partial lipodystrophy – Dunnigan type (FPLD2) | 151660      |
| Hutchinson–Gilford progeria syndrome (HGPS)            | 176670      |
| Restrictive dermopathy (RD)                            | 150330.0036 |
| Atypical progeria syndromes                            |             |
| Atypical HGPS (AHGPS)                                  | 150330      |
| Atypical Werner syndrome (AWRN)                        | 150330.0030 |
| Overlapping syndromes (OLS)                            |             |
| OLS1 (LIRLLC)  | 608056      |
| OLS2 (LD+MW+DCM+CCA)                                   | *R28W       |
| OLS3 (LD+DCM+CCA)                                      | 150330.0005 |
| OLS4 (CMD1A+QM)  | 150330.0017 |
| OLS5 (LD+MD+CCA)                                       | 150330.0003 |
| Autosomal recessive (AR) disorders                     |             |
| Emery–Dreifuss muscular dystrophy (AR-EMD2)            | 604929      |
| Charcot Marie Tooth disease (AR-CMT)                   | 605588      |
| Mandibuloacral dysplasia (MAD)                         | 248370      |

Abbreviations: MIM, Mendelian Inheritance in Man; LD, lipodystrophy; MW muscle weakness; DCM, dilated cardiomyopathy; CCA, cardiac conduction abnormalities; QM, quadriceps myopathy; MD, muscular dystrophy; LIRLLC, syndrome of lipodystrophy, insulin-resistant diabetes, disseminated leukomelanodermic papules, liver steatosis and cardiomyopathy. Specific individual mutations can be found under MIM numbers, except \*R28W (see Hegele [27]).

*LMNA* nucleotide sequence. The odds for association between a HCA class 1 laminopathy and a mutation upstream of the *LMNA* NLS was 8.4 (95% CI 2.9 to 24.7;  $P < 0.0001$ ). Thus, *LMNA*-type laminopathies assembled into two HCA phenotype classes based upon organ system involvement. Furthermore, HCA laminopathy class had a strong, non-random association with the *LMNA* mutation position relative to the NLS and this phenomic–genomic relationship was systematic. Different associations with primary structure could underlie differences in pathogenic mechanisms and abnormal function in specific diseases. HCA can thus help deconvolute phenomic relationships between different clinical entities, in turn detecting phenomic–genomic associations and generating hypotheses for mechanistic studies.

### Phenotypic heterogeneity of *LMNA* mutants

Phenomic–genomic correlation in laminopathies can be undermined by phenotypic heterogeneity at both the clinical and cellular levels among some patients who carry identical or similar mutations [28,29]. For instance, we reported two female patients, one African-American and one Caucasian, who shared the same R133L mutation but had vastly different body fat distribution [30]. The extent of the lipodystrophy appeared to be correlated with the severity of the metabolic complications and also the degree of abnormal nuclear morphology. The difference could not be explained by the



**Fig. 3** – Schematic diagram of laminopathy–*LMNA* phenotype–genotype association. The grid shows organ system involvement in laminopathies, with darkened cells indicating presence of the sub-phenotype. Horizontal dendrogram branch lengths are proportional to correlation coefficient distances between the laminopathies. “Class 1” and “class 2” laminopathies are above and below the thick horizontal dashed line, respectively. *LMNA* cDNA sequence map is at the top right; thin vertical lines demarcate exon boundaries and numbers refer to amino acid sequence. Lamin A/C protein structure is indicated, together with the rod domain, nuclear localization signal (NLS) and immunoglobulin (Ig) fold. Circles and squares show positions of missense/in-frame deletion and missense/splicing mutations, respectively. For mutations, vertical position is collinear with the associated laminopathy, while horizontal position indicates linear distance from the cDNA transcription start site. Thick dashed vertical line divides mutations into those upstream or downstream of the NLS. There are 54, 16, 6 and 15 mutations in the upper left, upper right, lower left and lower right quadrants, respectively, formed by intersection of the two dashed lines. See [Table 1](#) for abbreviations.

presence of coding single nucleotide polymorphisms (SNPs) [28].

A related complication is the issue of overlapping syndromes comprising various combinations of muscular dystrophy, cardiac myopathy, lipodystrophy and progeroid features [31,32]. The ontological emphasis or diagnostic classification of such overlapping syndromes often depends on both the most prominent sub-phenotype(s) at the time of diagnosis and the subspecialty expertise or clinical acumen of the attending physicians. For instance, progeroid features of atypical Werner syndrome include atrophic skin, subcutaneous fat loss and muscular atrophy, but these manifestations are also part of the spectrum of lipodystrophy and muscular dystrophy [30]. HGPS and restrictive dermopathy (RD) are somewhat unique among laminopathies in that each is caused by a splicing mutation producing an in-frame deletion of the C-terminal region, including the proteolytic sites; the disorders

share some phenotypic similarities and overall are much more severe than other laminopathies caused by missense mutations. However, general features of HGPS such as small jaw, slanted shoulders and small hands are also seen in MAD [33]. Careful cross-sectional documentation and longitudinal follow-up of component phenotypes would enhance phenotype–genotype correlation.

Gender and age can also affect phenomic–genomic analysis. For instance, FPLD is less easily recognized in males [34]. Also, the prevalence of arrhythmia and cardiac complications among EDMD patients increases with age [28]. In addition, a study examining the three MAD homozygotes for the R527H mutation and the same clinical phenotype demonstrated that the extent of nuclear and heterochromatin irregularities were correlated with prelamin A accumulation and, interestingly, patient age [35]. Finally the recent finding of the age-related appearance of the HGPS  $\Delta 50$  mutant lamin A, progerin, in

normal fibroblasts [36], indicates that the age of the skin fibroblast donor must be accounted for in cell biological studies of lamins.

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### Postulated mechanisms of phenotypic variations of LMNA mutant cells

There are several hypotheses to explain how LMNA mutations cause laminopathies at the cellular level and also why different mutations present a wide variety of phenotypes. Missense mutations underlying laminopathies have been predicted to cause misfolding of lamin A/C or failure of dimerization and assembly of the lamina, which would be expected to result in structural abnormalities and/or fragility of nuclei [31,37]. This structural model was recently augmented by findings that fibroblasts from *Lmna* knockout mice had increased sensitivity to mechanical stress [38] and cells from HGPS patients had reduced deformability [39]. Differential responses to mechanical stress depending on the mutations and cell types may underlie phenotypic variability. However this structural model does not fully explain the heterogeneous tissue-specific and often non-overlapping phenotypes caused by LMNA missense mutations.

A second, non-exclusive model for pathogenesis of laminopathies is that lamins regulate gene expression and/or other nuclear activities. In addition to its structural function, the nuclear envelope probably contributes to global patterns of gene expression. Since heterochromatin is generally transcriptionally silent, changes in heterochromatin structure could alter gene expression patterns, perhaps unique to each LMNA mutation. Changes in lamina structure may result in reorganization of heterochromatin [40]. Disorganization and loss of heterochromatin have been observed in HGPS cells [41,42] and in MAD [35], and restoration of heterochromatin-rescued nuclear dysmorphism [41].

Furthermore, nuclear lamins directly affect gene expression. Transcriptional regulators such as the retinoblastoma gene product (pRB) – a tumour suppressor – interact with lamin A *in vitro* and are tightly associated with the nuclear matrix in cells [43]. When an array of LMNA mutants were introduced to mouse *Lmna*<sup>-/-</sup> cells, not all mutants could rescue cell cycle function attributed to the lamin A–pRB interaction [44]. Lamin A also interacts with sterol response element binding protein, a transcriptional factor that is involved in adipocyte differentiation [45]; indeed this interaction could be a key determinant in the development of lipodystrophy. Regulation of these transcriptional factors may further differ depending on the position of the LMNA mutations.

Earlier studies of subnuclear distribution indicated how various disease mutations result in the different expression of nuclear structural proteins. Some lamin A mutants have dramatically abnormal intranuclear localization. In one study, 4/15 overexpressed LMNA mutants showed decreased nuclear rim staining and formation of intranuclear aggregates [46]. Another study showed that 3/4 LMNA mutant proteins overexpressed in HeLa cells form nuclear aggregates and cause the relocation of endogenous wild type lamin [47]. Abnormal localization of mutant lamins is also associated

with loss of lamina-associated emerin. LMNA mutations associated with EDMD and DCM1A cause abnormal lamin localization and loss of emerin, while in contrast, lamin A/C-carrying mutations in FPLD behave in a manner indistinguishable from wild type lamins [46,47]. Such observations indicate the complexity underlying phenomic–genomic relationships.

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### Potential pharmacological treatment of laminopathies

Presently, laminopathies cannot be cured; the symptoms can only be treated as they occur [48,49]. Patients are often managed by a multidisciplinary medical team. The strategic use of established medications might delay the cardiovascular complications in certain laminopathies like FPLD2, as discussed above. But such treatments are mechanistically removed from the root causes – very early and proximal cellular pathways and networks – of the disease phenotypes. Treatments for the root causes are exemplified by strategies to treat HGPS, which is arguably the most severe laminopathy. Two approaches are being explored as treatments for HGPS: pharmacological using farnesyl transferase inhibitors (FTIs) and gene therapy using RNA interference (RNAi).

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### Farnesyl transferase inhibitors

The initial processing step of prelamin A is the farnesylation of the cysteine residue at amino acid 661, followed by cleavage of the last three amino acids by the zinc metalloprotease, Zmpste24, or Rce1 [50]. The C-terminal end becomes methylated and the C-terminal 18 amino acid is cleaved by Zmpste24 to generate mature lamin A [50]. In HGPS, mutant lamin A retaining the C-terminal end appears to primarily target vascular cells, leading to atherosclerosis [51]. Zmpste24 knockout mice expressed only prelamin A that retains C-terminal fragments instead of mature lamin A and presented a phenocopy of HGPS [52,53]. These findings suggested that accumulation of unprocessed prelamin A is part of the pathogenesis of HGPS. By blocking the initial step of the maturation of mutant prelamin A, FTIs prevent accumulation of the progerin mutant [54].

FTIs have been shown to reverse the characteristic nuclear blebbing in transformed cell lines expressing progerin [55–57], primary fibroblasts derived from HGPS patients [55,56,58,59] and mouse cells carrying the HGPS mutation [60]. Treatment of HGPS cells with an FTI together with a deacetylase inhibitor showed more dramatic improvement of nuclear morphology and chromatin organization than treatment with an FTI alone [41]. Systemic administration of FTIs ameliorated cellular and disease phenotypes of HGPS mice [61]. An FTI also improved the nuclear shape of Zmpste24-deficient mouse embryonic fibroblasts in culture [59] and various disease phenotypes including improved survival in Zmpste24 knockout mice [62]. These results suggest that FTIs could be used as a treatment for HGPS; in fact a clinical trial has been mobilized under the aegis of the Progeria Research Foundation ([www.progeriaresearch.org](http://www.progeriaresearch.org)).

The potential utility of FTIs in laminopathies caused by missense mutations is unclear. FTIs improved the nuclear morphology of primary fibroblasts isolated from two progeroid patients heterozygous for LMNA mutations R644C and E578V [59]. These mutations reside at the C-terminal regions present in lamin A, but not lamin C. If lamin A is dispensable as long as lamin C is expressed, as observed in mice [62], elimination or reduction of mutant lamin A while concurrently retaining wild type lamin C would improve the disease phenotype. On the other hand, fibroblasts from a compound heterozygote for LMNA mutations T528M and p.M540T with progeroid features but no prelamin A accumulation did not respond to FTI treatment [63]. While it is theoretically possible that simply reducing mutant lamin A regardless of the mutation type may modulate disease phenotypes, the pathogenicity of some missense mutations underlying milder phenotypes may be unrelated to prelamin A accumulation and farnesylation. Further research will clarify target specificity of FTIs and potential adverse effects [64].

### Gene therapy for laminopathy

The second experimental therapeutic approach in HGPS is gene therapy based on RNA interference (RNAi) in which mutant LMNA mRNAs are selectively destroyed [65,66]. In RNAi, the 21–23 mers of double-stranded RNAs introduced to cells hybridize to target mRNA and recruit machineries to destroy the target mRNAs. Double stranded RNA can be delivered to cells utilizing expression of short hairpin RNA (shRNA) [65]. Alternatively, synthetic oligonucleotides with long half-lives can be employed [65]. Both approaches were shown to effectively restore various cellular and nuclear phenotypes. In HGPS fibroblasts and lymphocytes, Scaffidi and Misteli [66] introduced morpholino oligonucleotides targeted to the activated cryptic splicing site and observed that progerin reached <5% of the pretreatment level while several cell biological parameters returned to normal. Our group generated shRNA targeted to the mutant mRNA with a lenti-viral system to introduce it to patient fibroblasts [65]. The mutant proteins were reduced to ~25% compared to untransfected cells, and cellular disease phenotypes including nuclear morphology and replicative life spans were partially restored in the short term.

The above proof-of-principle experiments show potential for clinical applications. However, systemic delivery of shRNA or synthetic oligonucleotide is a major hurdle for any gene therapy. Progerin has been shown to accumulate in patient-derived vascular cells from skin [51]. Pathological studies of HGPS mice suggest that progressive loss of vascular smooth muscle cells may cause of fatal cardiac complications [67]. These issues necessitate the development of efficient delivery methods, particularly to the vascular cells. If elimination of lamin A in certain mutation types consistently alleviates the disease phenotypes under experimental conditions, it is possible that simple reduction of the toxic mutant protein using RNAi might become a treatment consideration for selected LMNA mutations causing specific phenotypes in which accumulation of the toxic mutant lamin is the predominant mechanism of disease.

### Conclusions

Thus, we demonstrate how careful evaluation of phenotype–genotype associations in monogenic phenotypes, such as laminopathies, might provide new hypotheses to be tested through molecular and cellular studies and might also suggest potential new intervention strategies. We showed how phenomic assessment of the stages of FPLD suggests clinical interventions taken from the existing pharmacological armamentarium for prevention of the cardiovascular complications. We also showed how phenomic differences between FPLD2 and FPLD3 were defined using careful, high-resolution phenomic analysis. Phenotypic analogy between FPLD2 and APL led to discovery of mutations in LMNB2. Also, a phenomic–genomic analytic strategy could demonstrate a non-random relationship between LMNA mutations and clinical phenotype. However, straightforward study of phenotype–genotype correlation in laminopathies is complicated by phenotypic heterogeneity among some patients with the identical genomic mutation. Cellular and molecular investigations have provided understanding of some of the mechanisms linking phenotype to genotype in laminopathies. Finally, novel therapies – such as farnesyl transferase inhibition and gene-based approaches – might help to further elucidate the nature of phenotype–genotype relationships in laminopathies while concurrently providing hope for patients with laminopathies and their families.

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### REFERENCES

- [1] N. Freimer, C. Sabatti, The human phenome project, *Nat. Genet.* 34 (2003) 15–21.
- [2] R. Gerlai, Phenomics: fiction or the future? *Trends Neurosci.* 25 (2002) 506–509.
- [3] R.A. Hegele, Phenomics, lipodystrophy, and the metabolic syndrome, *Trends Cardiovasc. Med.* 14 (2004) 133–137.
- [4] N.J. Schork, Genetics of complex disease: approaches, problems, and solutions, *Am. J. Respir. Crit. Care Med.* 156 (1997) S103–S109.
- [5] R.L. Pollex, R.A. Hegele, Genetic determinants of the metabolic syndrome, *Nat. Clin. Pract. Cardiovasc. Med.* 3 (2006) 482–489.
- [6] G.M. Reaven, Pathophysiology of insulin resistance in human disease, *Physiol. Rev.* 75 (1995) 473–486.
- [7] I. Juhan-Vague, M.C. Alessi, P.E. Morange, Hypofibrinolysis and increased PAI-1 are linked to atherothrombosis via insulin resistance and obesity, *Ann. Med.* 32 (Suppl. 1) (2000) 78–84.



- [8] W. Koenig, Insulin resistance, heart disease and inflammation. Identifying the 'at-risk' patient: the earlier the better? The role of inflammatory markers, *Int. J. Clin. Pract., Suppl.* (2002) 23–30.
- [9] H.M. Lakka, D.E. Laaksonen, T.A. Lakka, L.K. Niskanen, E. Kumpusalo, J. Tuomilehto, J.T. Salonen, The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men, *JAMA* 288 (2002) 2709–2716.
- [10] K.G. Alberti, P.Z. Zimmet, Definition, diagnosis and classification of diabetes mellitus and its complications: Part 1. Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation, *Diabet. Med.* 15 (1998) 539–553.
- [11] Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III), *JAMA* 285 (2001) 2486–2497.
- [12] K. Alberti, Conclusions from the 2004 IDF Consensus on the Metabolic Syndrome, 1st International Congress on "prediabetes" and the Metabolic Syndrome, Epidemiology, Management, and Prevention of Diabetes and Cardiovascular Disease, Berlin, Germany, 2005.
- [13] H. Cao, R.A. Hegele, Nuclear lamin A/C R482Q mutation in Canadian kindreds with Dunnigan-type familial partial lipodystrophy, *Hum. Mol. Genet.* 9 (2000) 109–112.
- [14] S. Shackleton, D.J. Lloyd, S.N. Jackson, R. Evans, M.F. Niermeijer, B.M. Singh, H. Schmidt, G. Brabant, S. Kumar, P.N. Durrington, S. Gregory, S. O'Rahilly, R.C. Trembath, LMNA, encoding lamin A/C, is mutated in partial lipodystrophy, *Nat. Genet.* 24 (2000) 153–156.
- [15] R.A. Speckman, A. Garg, F. Du, L. Bennett, R. Veile, E. Arioglu, S.I. Taylor, M. Lovett, A.M. Bowcock, Mutational and haplotype analyses of families with familial partial lipodystrophy (Dunnigan variety) reveal recurrent missense mutations in the globular C-terminal domain of lamin A/C, *Am. J. Hum. Genet.* 66 (2000) 1192–1198.
- [16] R.A. Hegele, Lessons from human mutations in PPARgamma, *Int. J. Obes. (Lond.)* 29 (Suppl. 1) (2005) S31–S35.
- [17] R.A. Hegele, H. Cao, D.M. Liu, G.A. Costain, V. Charlton-Menys, N.W. Rodger, P.N. Durrington, Sequencing of the reannotated LMNB2 gene reveals novel mutations in patients with acquired partial lipodystrophy, *Am. J. Hum. Genet.* 79 (2006) 383–389.
- [18] J. Kobberling, M.G. Dunnigan, Familial partial lipodystrophy: two types of an X linked dominant syndrome, lethal in the hemizygous state, *J. Med. Genet.* 23 (1986) 120–127.
- [19] A. Garg, R.M. Peshock, J.L. Fleckenstein, Adipose tissue distribution pattern in patients with familial partial lipodystrophy (Dunnigan variety), *J. Clin. Endocrinol. Metab.* 84 (1999) 170–174.
- [20] R.A. Hegele, Premature atherosclerosis associated with monogenic insulin resistance, *Circulation* 103 (2001) 2225–2229.
- [21] R.A. Hegele, M.E. Kraw, M.R. Ban, B.A. Miskie, M.W. Huff, H. Cao, Elevated serum C-reactive protein and free fatty acids among nondiabetic carriers of missense mutations in the gene encoding lamin A/C (LMNA) with partial lipodystrophy, *Arterioscler. Thromb. Vasc. Biol.* 23 (2003) 111–116.
- [22] P. Cohen, J.M. Ntambi, J.M. Friedman, Stearoyl-CoA desaturase-1 and the metabolic syndrome, *Curr. Drug Targets Immune Endocr. Metabol. Disord.* 3 (2003) 271–280.
- [23] A.R. Miller, G.J. Etgen, Novel peroxisome proliferator-activated receptor ligands for type 2 diabetes and the metabolic syndrome, *Expert Opin. Investig. Drugs* 12 (2003) 1489–1500.
- [24] H. Bays, E.A. Stein, Pharmacotherapy for dyslipidaemia – current therapies and future agents, *Expert Opin. Pharmacother.* 4 (2003) 1901–1938.
- [25] S.A. Al-Attar, R.L. Pollex, J.F. Robinson, B.A. Miskie, R. Walcarus, C. Harper Little, B.K. Rutt, R.A. Hegele, Quantitative and qualitative differences in subcutaneous adipose tissue stores across lipodystrophy types shown by magnetic resonance imaging, *BMC Med. Imaging* 7 (2007) 3.
- [26] A. Garg, Acquired and inherited lipodystrophies, *N. Engl. J. Med.* 350 (2004) 1220–1234.
- [27] R. Hegele, LMNA mutation position predicts organ system involvement in laminopathies, *Clin. Genet.* 68 (2005) 31–34.
- [28] K.N. Jacob, F. Baptista, H.G. dos Santos, J. Oshima, A.K. Agarwal, A. Garg, Phenotypic heterogeneity in body fat distribution in patients with atypical Werner's syndrome due to heterozygous Arg133Leu lamin A/C mutation, *J. Clin. Endocrinol. Metab.* 90 (2005) 6699–6706.
- [29] E. Mercuri, M. Poppe, R. Quinlivan, S. Messina, M. Kinali, L. Demay, J. Bourke, P. Richard, C. Sewry, M. Pike, G. Bonne, F. Muntoni, K. Bushby, Extreme variability of phenotype in patients with an identical missense mutation in the lamin A/C gene: from congenital onset with severe phenotype to milder classic Emery–Dreifuss variant, *Arch. Neurol.* 61 (2004) 690–694.
- [30] L. Chen, L. Lee, B.A. Kudlow, H.G. Dos Santos, O. Sletvold, Y. Shafeghati, E.G. Botha, A. Garg, N.B. Hanson, G.M. Martin, I.S. Mian, B.K. Kennedy, J. Oshima, LMNA mutations in atypical Werner's syndrome, *Lancet* 362 (2003) 440–445.
- [31] J.L. Broers, F.C. Ramaekers, G. Bonne, R.B. Yaou, C.J. Hutchison, Nuclear lamins: laminopathies and their role in premature ageing, *Physiol. Rev.* 86 (2006) 967–1008.
- [32] K.N. Jacob, A. Garg, Laminopathies: multisystem dystrophy syndromes, *Mol. Genet. Metab.* 87 (2006) 289–302.
- [33] M. Plasilova, C. Chattopadhyay, P. Pal, N.A. Schaub, S.A. Buechner, H. Mueller, P. Miny, A. Ghosh, K. Heinemann, Homozygous missense mutation in the lamin A/C gene causes autosomal recessive Hutchinson–Gilford progeria syndrome, *J. Med. Genet.* 41 (2004) 609–614.
- [34] A.K. Agarwal, A. Garg, Genetic basis of lipodystrophies and management of metabolic complications, *Annu. Rev. Med.* 57 (2006) 297–311.
- [35] I. Filesi, F. Gullotta, G. Lattanzi, M.R. D'Apice, C. Capanni, A.M. Nardone, M. Columbaro, G. Scarano, E. Mattioli, P. Sabatelli, N.M. Maraldi, S. Biocca, G. Novelli, Alterations of nuclear envelope and chromatin organization in mandibuloacral dysplasia, a rare form of laminopathy, *Physiol. Genomics* 23 (2005) 150–158.
- [36] P. Scaffidi, T. Misteli, Lamin A-dependent nuclear defects in human aging, *Science* 312 (2006) 1059–1063.
- [37] C.J. Hutchison, H.J. Worman, A-type lamins: guardians of the soma? *Nat. Cell Biol.* 6 (2004) 1062–1067.
- [38] J. Lammerding, P.C. Schulze, T. Takahashi, S. Kozlov, T. Sullivan, R.D. Kamm, C.L. Stewart, R.T. Lee, Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction, *J. Clin. Invest.* 113 (2004) 370–378.
- [39] K.N. Dahl, P. Scaffidi, M.F. Islam, A.G. Yodh, K.L. Wilson, T. Misteli, Distinct structural and mechanical properties of the nuclear lamina in Hutchinson–Gilford progeria syndrome, *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006) 10271–10276.
- [40] V. Raz, F. Carlotti, B.J. Vermolen, E. van der Poel, W.C. Sloos, S. Knaan-Shanzer, A.A. de Vries, R.C. Hoeben, I.T. Young, H.J. Tanke, Y. Garini, R.W. Dirks, Changes in lamina structure are followed by spatial reorganization of heterochromatin regions in caspase-8-activated human mesenchymal stem cells, *J. Cell Sci.* 119 (2006) 4247–4256.
- [41] M. Columbaro, C. Capanni, E. Mattioli, G. Novelli, V.K. Parnaik, S. Squarzone, N.M. Maraldi, G. Lattanzi, Rescue of heterochromatin organization in Hutchinson–Gilford progeria by drug treatment, *Cell. Mol. Life Sci.* 62 (2005) 2669–2678.
- [42] D.K. Shumaker, T. Dechat, A. Kohlmaier, S.A. Adam, M.R. Bozovsky, M.R. Erdos, M. Eriksson, A.E. Goldman, S. Khuon,

- F.S. Collins, T. Jenuwein, R.D. Goldman, Mutant nuclear lamin A leads to progressive alterations of epigenetic control in premature aging, *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006) 8703–8708.
- [43] B.K. Kennedy, D.A. Barbie, M. Classon, N. Dyson, E. Harlow, Nuclear organization of DNA replication in primary mammalian cells, *Genes Dev.* 14 (2000) 2855–2868.
- [44] R.T. Nitta, S.A. Jameson, B.A. Kudlow, L.A. Conlan, B.K. Kennedy, Stabilization of the retinoblastoma protein by A-type nuclear lamins is required for INK4A-mediated cell cycle arrest, *Mol. Cell. Biol.* 26 (2006) 5360–5372.
- [45] D.J. Lloyd, R.C. Trembath, S. Shackleton, A novel interaction between lamin A and SREBP1: implications for partial lipodystrophy and other laminopathies, *Hum. Mol. Genet.* 11 (2002) 769–777.
- [46] C. Ostlund, G. Bonne, K. Schwartz, H.J. Worman, Properties of lamin A mutants found in Emery–Dreifuss muscular dystrophy, cardiomyopathy and Dunnigan-type partial lipodystrophy, *J. Cell Sci.* 114 (2001) 4435–4445.
- [47] W.H. Raharjo, P. Enarson, T. Sullivan, C.L. Stewart, B. Burke, Nuclear envelope defects associated with LMNA mutations cause dilated cardiomyopathy and Emery–Dreifuss muscular dystrophy, *J. Cell Sci.* 114 (2001) 4447–4457.
- [48] G. Bonne, D. Rian-Budirtha, R.B. Yaou, Emery–Dreifuss muscular dystrophy, GeneReviews at GeneTests-GeneClinics: Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle. 1997–2006, 2004, Available at <http://www.genereviews.org>.
- [49] W.T. Brown, L.B. Gordon, F.S. Collins, Hutchinson–Gilford progeria syndrome, GeneReviews at GeneTests-GeneClinics: Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle. 1997–2006, 2006, Available at <http://www.genereviews.org>.
- [50] D.P. Corrigan, D. Kuszczak, A.E. Rusinol, D.P. Thewke, C.A. Hrycyna, S. Michaelis, M.S. Sinensky, Prelamin A endoproteolytic processing in vitro by recombinant Zmpste24, *Biochem. J.* 387 (2005) 129–138.
- [51] D. McClintock, L.B. Gordon, K. Djabali, Hutchinson–Gilford progeria mutant lamin A primarily targets human vascular cells as detected by an anti-Lamin A G608G antibody, *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006) 2154–2159.
- [52] M.O. Bergo, B. Gavino, J. Ross, W.K. Schmidt, C. Hong, L.V. Kendall, A. Mohr, M. Meta, H. Genant, Y. Jiang, E.R. Wisner, N. Van Bruggen, R.A. Carano, S. Michaelis, S.M. Griffey, S.G. Young, Zmpste24 deficiency in mice causes spontaneous bone fractures, muscle weakness, and a prelamin A processing defect, *Proc. Natl. Acad. Sci. U. S. A.* 99 (2002) 13049–13054.
- [53] A.M. Pendas, Z. Zhou, J. Cadinanos, J.M. Freije, J. Wang, K. Hulthenby, A. Astudillo, A. Wernerson, F. Rodriguez, K. Tryggvason, C. Lopez-Otin, Defective prelamin A processing and muscular and adipocyte alterations in Zmpste24 metalloproteinase-deficient mice, *Nat. Genet.* 31 (2002) 94–99.
- [54] A.E. Rusinol, M.S. Sinensky, Farnesylated lamins, progeroid syndromes and farnesyl transferase inhibitors, *J. Cell Sci.* 119 (2006) 3265–3272.
- [55] B.C. Capell, M.R. Erdos, J.P. Madigan, J.J. Fiordalisi, R. Varga, K.N. Conneely, L.B. Gordon, C.J. Der, A.D. Cox, F.S. Collins, Inhibiting farnesylation of progerin prevents the characteristic nuclear blebbing of Hutchinson–Gilford progeria syndrome, *Proc. Natl. Acad. Sci. U. S. A.* 102 (2005) 12879–12884.
- [56] M.W. Glynn, T.W. Glover, Incomplete processing of mutant lamin A in Hutchinson–Gilford progeria leads to nuclear abnormalities, which are reversed by farnesyltransferase inhibition, *Hum. Mol. Genet.* 14 (2005) 2959–2969.
- [57] M.P. Mallampalli, G. Huyer, P. Bendale, M.H. Gelb, S. Michaelis, Inhibiting farnesylation reverses the nuclear morphology defect in a HeLa cell model for Hutchinson–Gilford progeria syndrome, *Proc. Natl. Acad. Sci. U. S. A.* 102 (2005) 14416–14421.
- [58] Y. Liu, A. Rusinol, M. Sinensky, Y. Wang, Y. Zou, DNA damage responses in progeroid syndromes arise from defective maturation of prelamin A, *J. Cell Sci.* 119 (2006) 4644–4649.
- [59] J.I. Toth, S.H. Yang, X. Qiao, A.P. Beigneux, M.H. Gelb, C.L. Moulson, J.H. Miner, S.G. Young, L.G. Fong, Blocking protein farnesyltransferase improves nuclear shape in fibroblasts from humans with progeroid syndromes, *Proc. Natl. Acad. Sci. U. S. A.* 102 (2005) 12873–12878.
- [60] S.H. Yang, M.O. Bergo, J.I. Toth, X. Qiao, Y. Hu, S. Sandoval, M. Meta, P. Bendale, M.H. Gelb, S.G. Young, L.G. Fong, Blocking protein farnesyltransferase improves nuclear shape in mouse fibroblasts with a targeted Hutchinson–Gilford progeria syndrome mutation, *Proc. Natl. Acad. Sci. U. S. A.* 102 (2005) 10291–10296.
- [61] S.H. Yang, M. Meta, X. Qiao, D. Frost, J. Bauch, C. Coffinier, S. Majumdar, M.O. Bergo, S.G. Young, L.G. Fong, A farnesyltransferase inhibitor improves disease phenotypes in mice with a Hutchinson–Gilford progeria syndrome mutation, *J. Clin. Invest.* 116 (2006) 2115–2121.
- [62] L.G. Fong, D. Frost, M. Meta, X. Qiao, S.H. Yang, C. Coffinier, S.G. Young, A protein farnesyltransferase inhibitor ameliorates disease in a mouse model of progeria, *Science* 311 (2006) 1621–1623.
- [63] V.L. Verstraeten, J.L. Broers, M.A. van Steensel, S. Zinn-Justin, F.C. Ramaekers, P.M. Steijlen, M. Kamps, H.J. Kuijpers, D. Merckx, H.J. Smeets, R.C. Hennekam, C.L. Marcelis, A. van den Wijngaard, Compound heterozygosity for mutations in LMNA causes a progeria syndrome without prelamin A accumulation, *Hum. Mol. Genet.* 15 (2006) 2509–2522.
- [64] A.D. Basso, P. Kirschmeier, W.R. Bishop, Lipid posttranslational modifications. Farnesyl transferase inhibitors, *J. Lipid Res.* 47 (2006) 15–31.
- [65] S. Huang, L. Chen, N. Libina, J. Janes, G.M. Martin, J. Campisi, J. Oshima, Correction of cellular phenotypes of Hutchinson–Gilford progeria cells by RNA interference, *Hum. Genet.* 118 (2005) 444–450.
- [66] P. Scaffidi, T. Misteli, Reversal of the cellular phenotype in the premature aging disease Hutchinson–Gilford progeria syndrome, *Nat. Med.* 11 (2005) 440–445.
- [67] R. Varga, M. Eriksson, M.R. Erdos, M. Olive, I. Harten, F. Kolodgie, B.C. Capell, J. Cheng, D. Faddah, S. Perkins, H. Avallone, H. San, X. Qu, S. Ganesh, L.B. Gordon, R. Virmani, T.N. Wight, E.G. Nabel, F.S. Collins, Progressive vascular smooth muscle cell defects in a mouse model of Hutchinson–Gilford progeria syndrome, *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006) 3250–3255.