LMNA-linked lipodystrophies: from altered fat distribution to cellular alterations

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Abstract
Mutations in the LMNA gene, encoding the nuclear intermediate filaments the A-type lamins, result in a wide variety of diseases known as laminopathies. Some of them, such as familial partial lipodystrophy of Dunnigan and metabolic laminopathies, are characterized by lipodystrophic syndromes with altered fat distribution and severe metabolic alterations with insulin resistance and dyslipidaemia. Metabolic disturbances could be due either to the inability of adipose tissue to adequately store triacylglycerols or to other cellular alterations linked to A-type lamin mutations. Indeed, abnormal prelamin A accumulation and farnesylation, which are clearly involved in laminopathic premature aging syndromes, could play important roles in lipodystrophies. In addition, gene expression alterations, and signalling abnormalities affecting SREBP1 (sterol-regulatory-element-binding protein 1) and MAPK (mitogen-activated protein kinase) pathways, could participate in the pathophysiological mechanisms leading to LMNA (lamin A/C)-linked metabolic alterations and lipodystrophies. In the present review, we describe the clinical phenotype of LMNA-linked lipodystrophies and discuss the current physiological and biochemical hypotheses regarding the pathophysiology of these diseases.

Introduction
In the last decade, several genes have been identified whose disruption causes different human syndromes of partial or generalized loss of AT (adipose tissue), i.e. lipodystrophies. Although the role of some of these genes in adipocyte function has been identified, for others relatively little was known about their molecular function at the time of their identification [1]. The aim of the present review is to focus on partial lipodystrophic syndromes due to mutations in the LMNA (lamin A/C) gene, an unexpected regulator of AT development, function and aging.

Structure and roles of A-type lamins
Alternative splicing of the LMNA gene produces lamins A and C, the two main isoforms of A-type lamins. These nuclear intermediate filament proteins have a typical tripartite organization, comprising a short N-terminus, a C-terminal globular domain and a central α-helix responsible for dimerization [2].

Together with B-type lamins, lamins A and C form the nuclear lamina, a filamentous network underlying the INM (inner nuclear membrane), involved in nuclear organization and function. Lamin filaments are important for the assembly, structure, shape and mechanical stability of the nucleus, and bind several NE (nuclear envelope) transmembrane proteins. Among them, proteins with LEM (lamin-associative polypeptide 2/emerin/MAN) domain are involved in nuclear architecture and chromatin organization, whereas lamin-binding SUN domain proteins participate in the structural nuclear–cytoskeleton link that plays roles in cell polarization and migration (for a review, see [3]).

A-type lamins are also present in the nucleoplasm, where they contribute to chromatin organization, and gene expression and signalling. Indeed, they bind to DNA replication factors and to several transcription factors [4,5] and are involved in DNA repair [6].

Lamin A is first translated as prelamin A containing a C-terminal consensus CSIM (Cys-Ser-Ile-Met) motif that is matured in the endoplasmic reticulum. First, a 15-carbon farnesylation residue is added to the cysteine residue of the CSIM motif, then the SIM residues are cleaved and the cysteine residue is carboxymethylated. These modifications appear to be necessary for targeting prelamin A to the INM, where the ZMPSTE24 (zinc metalloproteinase Ste24 homologue) cleaves the 15 last C-terminal amino acids. Therefore mature lamin A is not farnesylated. Lamin C has six specific amino acids, does not contain any CSIM box and is not post-translationally matured.

Laminopathies: from LMNA gene to tissue-specific diseases
Since the discovery of the first naturally occurring LMNA mutation in humans, a wide spectrum of tissue-specific...
diseases has been described, collectively called laminopathies. Among them, lipodystrophic syndromes are characterized by AT defects and metabolic alterations. Other laminopathies are cardiomyopathies with cardiac conduction defects, muscular dystrophies, neuropathies, premature aging diseases and rare overlapping phenotypes [7].

FPLD2 (Dunnigan-type familial partial lipodystrophy)

Patients with FPLD2 (OMIM #151660) have a normal fat distribution in childhood. After puberty, scAT (subcutaneous AT) gradually disappears from limbs, and gluteal and truncal areas. Simultaneously, AT accumulates in the face and neck. Imaging studies show the subcutaneous lipodystrophy with preservation of inter- and intra-muscular, intra-abdominal, and bone marrow fat [8] (Figure 1).

After fat redistribution, metabolic manifestations of FPLD2 include severe insulin resistance with hyperinsulinemia and acanthosis nigricans, leading to diabetes, major hypertriglyceridaemia and decreased levels of HDL (high-density lipoprotein)-cholesterol, leptin and adiponectin [7–10]. PCOS (polycystic ovary syndrome) with hyperandrogenism and infertility is often present in women [11], in whom the disease is usually more severe [10,12]. Liver steatosis, which can lead to cirrhosis [13], and premature atherosclerosis [14] are also part of the FPLD2 phenotype. In addition to the lipodystrophic phenotype, several patients show muscular alterations [15], which are reminiscent of laminopathic muscular dystrophies. The transgenic mouse model of FPLD2 is also characterized by insulin resistance and enlarged fatty liver, although with a generalized lipoatrophy [16].

Some 85% of FPLD2 patients present heterozygous missense substitutions at LMNA codon 482, substituting a basic amino acid for a neutral one [17,18]. Most other mutations are also located in the C-terminal Ig-like domain.

Metabolic laminopathies

Metabolic laminopathies are characterized by severe insulin resistance with metabolic alterations, similar to those observed in FPLD2, but minor or absent lipoatropathy [19]. The diagnosis could be difficult since they are close to a classical metabolic syndrome. As compared with FPLD2, the body mass index of patients with metabolic laminopathies is higher. To note, some patients associate metabolic alterations with myopathy and/or cardiomyopathy [19–21] (Figure 1).

LMNA mutations responsible for metabolic laminopathies can be localized in the entire coding sequence and affect both lamin A and C, but, as for typical FPLD2, some of them are lamin A-specific. Interestingly, we described recently the first homozygous LMNA mutation responsible for a lipodystrophic syndrome. This LMNA p.T655fsX49 mutation leads to the synthesis of a longer and non-farnesylated prelamin A, lacking the C-terminal consensus CSIM motif [22].

Other laminopathies with AT alterations

HGPS (Hutchinson–Gilford progeria syndrome)

HGPS is a rare and severe premature aging disorder (OMIM #176670). Children with HGPS are normal at birth, but develop growth retardation in the first year of life. Other clinical features include skin abnormalities, hair loss, bone and cartilage alterations, generalized subcutaneous lipoatrophy with insulin resistance and low HDL-cholesterol [23–25]. Premature atherosclerosis leads to cardiovascular diseases, generally causing death at around 13 years of age [25]. This observation suggests the presence of common pathophysiological mechanisms in laminopathic lipodystrophies and HGPS.

The typical HGPS mutation is a de novo heterozygous LMNA substitution within exon 11 (p.G608G) that activates a cryptic donor-splicing site, resulting in the production of a shorter prelamin A called progerin, which lacks its endoprotease cleavage site and is therefore constitutively farnesylated [23,24].

Other progeroid syndromes

MAD (mandibuloacral dysplasia) is a rare autosomal recessive disorder caused by LMNA (OMIM #248370) or ZMPSTE24 (OMIM #606480) mutations and characterized by postnatal growth retardation, mandibular and clavicular acro-osteolysis, mottled cutaneous pigmentation and partial or generalized lipoatrophy and insulin resistance [26,27].

Several observations of patients with complex phenotypes, including signs of FPLD2 or metabolic laminopathy and progeria or MAD, have also been described [28,29].

Pathophysiology of LMNA-linked lipodystrophies

Laminopathies and insulin resistance

The classical hypothesis to explain insulin resistance in lipodystrophies is that the inability of AT to store triacylglycerols leads to an increased level of circulating...
NEFAs (non-esterified fatty acids), resulting in ectopic fat storage and lipotoxicity. This leads to decreased glucose uptake in muscle, increased hepatic glucose production and decreased insulin production by the pancreatic β-cells with increased production of triacylglycerols by the liver.

However, as only minor or absent lipodystrophy is observed in patients with metabolic laminopathies, other mechanisms could be involved in metabolic alterations. In agreement with this hypothesis, the skin fibroblasts from a patient with insulin resistance without obvious clinical lipodystrophy showed several defects in the insulin transduction pathway, suggesting that LMNA mutations could directly impair insulin signalling [30].

**AT investigations in LMNA-linked lipodystrophies**

The cause of lipodystrophic regional differences in FPLD2 has not been identified. Prelamin A accumulation and altered adipogenic transcription factors such as PPAR (peroxisome-proliferator-activated receptor) γ could contribute to lipoatrophy [31].

Interestingly, we showed recently that accumulated cervical scAT in lipodystrophic patients with LMNA mutations is not due to a simple compensatory increase in triacylglycerol stores, but rather to a peculiar structural remodelling. We observed an altered AT architecture with non-inflammatory stores, but rather to a peculiar structural remodelling. We vical scAT in lipodystrophic patients with lipoatrophy [31].

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**Cellular alterations in LMNA-linked lipodystrophies**

**Nuclear organization defects**

LMNA mutations are associated with several cellular alterations such as disorganization of nuclear lamina, nuclear blebbing and decreased resistance to mechanical stress, mainly observed in cultured skin fibroblasts [23,33,34] and in myoblastic and pre-adipocyte murine models overexpressing mutated lamin A.

It is likely that FPLD2-linked mutations induce changes in A-type lamin interactions with chromatin-associated proteins or with DNA, thus modifying the chromatin organization [33,35]. However, all of these cellular alterations are not specific to FPLD2 and are common features in laminopathies [23,34]. The lamin A/C sequence around Arg482 contains a DNA-binding domain, and mutations of codon 482 should lower the affinity of lamin A for DNA [5]. This region could be involved directly in the general mechanism regulating chromatin organization that controls transcription during tissue-specific differentiation processes.

**Prelamin A**

A number of findings point to the role of the farnesyl group in the pathophysiology of premature aging. Indeed, progerin remains farnesylated, and mutations in the ZMPSTE24 gene, leading to the constitutive farnesylation of prelamin A, are associated with premature aging syndrome in both mice and humans [27,36]. Moreover, progerin or farnesylated prelamin A accumulation is associated with nuclear shape defects [23,24,36,37], and the level of farnesylated prelamin A seems to be a major factor determining the severity of the phenotype [38]. In mice, pharmacological inhibition of farnesylation leads to partial amelioration of the phenotype [39], but alternative prenylation of prelamin A could occur with a geranylgeranyl group instead of a farnesyl one. Treatment with pravastatin and aminobisphonates, blocking all prelamin A prenylations, produced encouraging results in mice [40].

Abnormal cellular prelamin A accumulation also occurs in lipodystrophic laminopathies [37,41]. Interestingly, prelamin A accumulation is also observed in HIV-linked lipodystrophic patients under antiretroviral treatment [32,37], due to inhibition of ZMPSTE24 activity by HIV PIs (protease inhibitors) [42]. We showed that inhibition of farnesylation in fibroblasts from patients with LMNA mutations or treated with PIs prevents cellular senescence and oxidative stress [37]. This suggests a role for farnesylation in the pathophysiological mechanisms of LMNA-linked lipodystrophic syndromes.

However, a homozygous LMNA p.T655fsX49 mutation leading to the expression of a mutated non-farnesylated prelamin A [22] is responsible for lipodystrophy and insulin resistance, with patients’ fibroblasts presenting typical laminopathic nuclear dysmorphies, oxidative stress and premature senescence. This shows that prelamin A farnesylation is not the unique pathophysiological mechanism in metabolic laminopathies.

**The gene expression and altered signalling hypothesis:** SREBP1 (sterol-regulatory-element-binding protein 1), ERK (extracellular-signal-regulated kinase) and c-Fos

The mutational hotspot observed in FPLD-linked phenotypes suggests that specific alterations of lamin A/C could induce adipocyte-specific dysfunction. The alteration of the charge of Arg482 in the C-terminal domain could lead to specific alterations of the binding of lamin A/C with other partners [3].

SREBP1, a transcription factor that controls the expression of several proteins implicated in lipid homeostasis, adipogenesis and insulin sensitivity, has been identified as a prelamin A-binding protein [41,43]. In 3T3-L1 pre-adipocytes, farnesyltransferase inhibitors, which lead to prelamin A accumulation, induce the sequestration of SREBP1c at the NE, associated with a down-regulation of PPARγ expression [41]. Similar data were previously obtained in 3T3-F442A murine pre-adipocytes treated with PIs [44]. Sequestration of SREBP1 to the NE could reduce PPARγ activation and therefore inhibit adipogenesis [41,44]. Finally, a prelamin A accumulation associated with decreased PPARγ expression was observed in thigh [31] and cervical subcutaneous AT [32] from lipodystrophic patients with LMNA mutations. Taken as a whole, these data suggest
Figure 2 | Gene expression and altered signalling hypothesis in LMNA-linked lipodystrophies

(A) SREBP1 is cleaved in response to insulin and translocated to the nucleus, resulting in lipid homeostasis and adipogenesis gene expression. The insulin signalling pathway results in ERK1/2 phosphorylation and nuclear localization. At the NE, ERK1/2 phosphorylates c-Fos, resulting in its release from the NE and in insulin target gene transcription. (B) Lamin A mutations and/or prelamin A accumulation could retain SREBP1c, ERK1/2 and c-Fos at the NE and impair their transcriptional activities, leading to lipid homeostasis, adipogenesis and insulin signalling defects.

that SREBP1c sequestration by accumulated prelamin A could play a role in the altered AT development and could participate to the cellular insulin resistance observed in patients.

The MAPK (mitogen-activated protein kinase) pathway is implicated in insulin signalling, involving activation by phosphorylation of several proteins such as ERK and c-Fos, a member of the AP-1 (activator protein 1) family complex. Interestingly, in an LMNAH222P/knockin mouse with muscular dystrophy, an abnormal ERK1/2 activation was observed [45]. Moreover, A-type lamins regulate the AP-1 pathway by interacting either with ERK1/2 or c-Fos, leading to c-Fos release or sequestration at the nuclear rim [4]. One hypothesis could be that, in LMNA-linked lipodystrophies, mutated prelamin A could retain c-Fos at the NE and block its activity on insulin signalling. A gene expression and altered signalling hypothesis is presented in Figure 2.

Treatment of FPLD2

To date, there is no specific treatment for LMNA-linked lipodystrophies, and patients are prone to develop acute pancreatitis, long-term complications of diabetes, liver steatosis and cirrhosis, and accelerated atherosclerosis. Pathophysiological treatments might stimulate re-growth or replacing lost AT with normal white AT, improve the ability of AT to store triacylglycerols, reducing the caloric intake and the dietary lipid burden seems also rational.

At the moment, the insulin-sensitizing strategy uses first lifestyle modifications (diet, physical activity), then metformin, which also reduces appetite. However, many patients do not achieve adequate glycaemic control with metformin.

As LMNA mutations and/or prelamin A accumulation are thought to lead to SREBP1c inactivation and a decrease in PPARγ levels [31,32], treatment with glitazones (PPARγ ligands) should rescue the adipogenic process. However, their effect is equivocal. Glitazones do not reverse fat loss and can worsen excess cervical fat deposition [46]. In most patients, high doses of insulin are required.

Hypertriglyceridaemia can lead to pancreatitis and is often resistant to conventional therapy. Fibric acid derivatives (PPARα agonists) or high-dose ω-3 polyunsaturated fatty acids can be helpful. In some patients, low-dose statins, which also inhibit prelamin A farnesylation, can be added to reduce non-HDL-cholesterol levels.

Regarding adipocytokine replacement, the use of recombinant leptin in hypoleptinaemic patients with lipodystrophies gave very promising results with marked improvement in hyperglycaemia, hypertriglyceridaemia, and hepatic steatosis [47,48]. However, the effects of leptin were less significant in patients with laminopathies than in those with generalized lipodystrophies [48]. Leptin therapy reduced
appetite and caused weight loss, which participated in improving metabolic complications. In addition to its central effects, leptin also reduced ectopic lipid deposition in the liver and muscle [49]. Adiponectin replacement is not currently available in humans, but has been reported to lower plasma glucose and NEFA levels in mouse models of lipodystrophy.

Finally, given the high cardiovascular risk associated with laminopathies, the treatment needs to be multifactorial, avoiding as early and as effective as possible each cardiovascular risk factor.

Conclusions
Lipodystrophies due to LMNA mutations represent a heterogeneous group of diseases, with insulin resistance being the common feature in these diseases. Many studies investigated whether mutations in A-type lamins could result in AT alterations and metabolic defects. Prelamin A accumulation and its farnesylation state is linked to premature aging syndromes and/or lipodystrophies, but its impact in AT remain unclear. The gene expression hypothesis, and alterations in signalling pathways, gives other insights into the pathophysiology of LMNA-linked lipodystrophies. Nonetheless, these hypotheses need further study, notably regarding the consequences on the insulin signalling pathways.

Funding

References


Received 27 July 2011
doi:10.1042/BST20110675