

p.S143F Mutation in Lamin A/C: A New Phenotype Combining Myopathy and Progeria

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We report a young girl with a phenotype combining early-onset myopathy and a progeria. She had myopathy and marked axial weakness during the first year of life; progeroid features, including growth failure, sclerodermatous skin changes, and osteolytic lesions, developed later. We identified the underlying cause to be a hitherto unreported de novo missense mutation in the *LMNA* gene (S143F) encoding the nuclear envelope proteins lamins A and C. Although *LMNA* mutations have been known to cause Hutchinson–Gilford progeria syndrome and Emery–Dreifuss muscular dystrophy, this is the first report of a patient combining features of these two phenotypes because of a single mutation in *LMNA*.

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The progerias are a group of rare syndromes characterized by an early and accelerated aging process affecting different organ systems. Common features include growth retardation; cutaneous changes such as thin, wrinkled, or scleroderma-like skin; hair loss; premature atherosclerosis; and bone dystrophy. Hutchinson–Gilford progeria (HGPS) and Werner syndrome (WRN) are the two most frequently studied forms. HGPS manifests during the first 2 years of life when the character-

istic features of failure to thrive, delayed dentition, alopecia, and sclerodermatous skin changes begin to appear.¹ Recently, it has been shown that the classical form of HGPS is mostly caused by a dominant de novo splice site mutation (c.1824C>T) in the *LMNA* gene leading to a truncated lamin A.^{2–4} WRN is an adult-onset progeria with autosomal recessive inheritance, and patients frequently experience development of malignancies or cardiovascular disease in the course of the disease. Mutations in the *WRN* gene encoding a protein with helicase activity have been identified in the majority of patients.^{5,6} However, there appears to be clinical and genetic overlap between HGPS and WRN, as Chen and colleagues⁶ identified *LMNA* mutations in patients originally classified with atypical WRN.

Previously, a number of different phenotypes had already been associated with recessive or dominant mutations in *LMNA* including muscle diseases (Emery–Dreifuss muscular dystrophy [EDMD]; limb-girdle muscular dystrophy [LGMD1B], and dilated cardiomyopathy 1A), axonal neuropathy (Charcot–Marie–Tooth disease 2B1), and lipodystrophy syndromes (Dunnigan-type familial partial lipodystrophy, lipodystrophy with diabetes, and mandibuloacral dysplasia).⁷ Lamins are the main component of the intermediate filamentous lamina, which lines the inner nuclear membrane. They are important for nuclear architecture, but they are also involved in DNA replication and messenger RNA transcription.⁸ How various mutations in this gene can cause this striking variety of clinical phenotypes remains essentially unknown.

Here we report a young girl with a previously unrecognized combination of early-onset myopathy with marked axial weakness and progeroid features, including skin changes, loss of subcutaneous fat, and osteolytic lesions. We identified the underlying cause to be a novel dominant S143F mutation in the *LMNA* gene, thus further expanding the phenotypic spectrum of lamin A/C-associated diseases, or so-called laminopathies.

Case Report

The white patient was born at term after an unremarkable pregnancy (birth weight: 2,260g; below 3. percentile). Feeding difficulties were noted in the neonatal period, and muscular hypotonia became more evident during the subsequent months. At 10 months old, the patient had generalized hypotonia and marked axial weakness with poor head control. Motor milestones were delayed, and independent walking was achieved at 19 months of age. Poor head control warranted the use of a neck collar for support. Increased serum creatine kinase levels (four to five times the upper limit) and myopathic electromyogram findings prompted a muscle biopsy at 10 months of age, which showed marked variation in fiber size without specific structural abnormalities. There was no fiber type disproportion or grouping

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and no active degeneration or regeneration (Fig 1E). Routine immunohistochemical workup including antibodies for laminin-2 (merosin), dystrophin, and sarcoglycans did not indicate any conclusive diagnosis. Genetic testing showed a normal karyotype and a negative carrier deletion test for dystrophin. Magnetic resonance

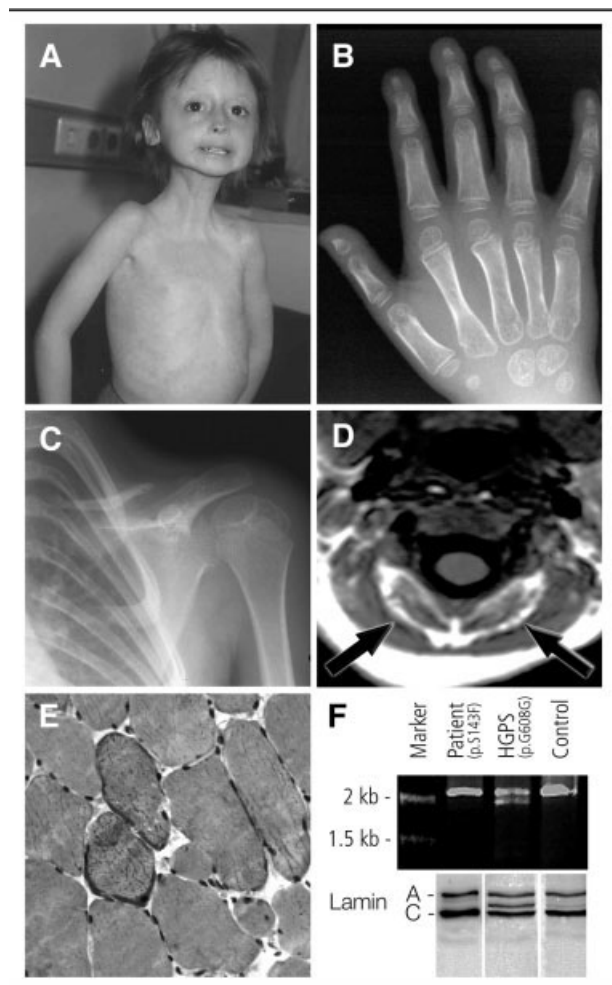


Fig 1. (A) Patient with loss of subcutaneous tissue, muscle atrophy, and facial features. (B, C) Radiographs showing osteopenia, acroosteolysis, and dystrophic short clavicle, respectively. (D) Transverse section of T1-weighted magnetic resonance imaging of the cervical spine showing generalized muscle atrophy and dystrophic changes with fatty infiltration most prominent in the paraspinal muscles (arrows). (E) Hematoxylin and eosin staining of muscle biopsy from the quadriceps femoris with variation in fiber size and small atrophic fibers. (F) Top panel shows reverse transcription polymerase chain reaction results using a primer pair spanning the entire coding region of lamin A. Our patient does not show any abnormal splicing product. The bottom panel shows immunoblot analysis from fibroblast protein with lamin A/C antibody. Again our patient shows normal protein size. For comparison, a patient with severe Hutchinson–Gilford progeria syndrome (HGPS) and the typical mutation p.G608G (GGC)GGT activating a cryptic splice site leading to a truncated protein (“progerin”) is shown. (A–C, F) Patient is 5 years old. (D, E) Patient is 10 months old.

imaging results of the brain and cervical spinal cord were normal, but severe atrophy and fatty infiltration of cervical muscles was noted (see Fig 1D). Electrocardiogram and echocardiogram results since 10 months of age were always normal. During the subsequent years, growth retardation and progeroid features (Table) became evident. Facial appearance, loss of subcutaneous tissue, and muscle atrophy are shown in Figure 1A.

At 5 years of age, radiographical evaluation showed midface hypoplasia and generalized osteopenia with osteolysis (see Fig 1B, C), which are findings typically seen in HGPS.¹ Sonography of skeletal muscles showed widespread areas of increased echogenicity most prominent in the biceps brachii and brachialis muscles of the arms and the posterior tibialis and lateral head of the gastrocnemius of the legs, consistent with a myopathic process. Clinically, distal muscle strength of the extremities was normal, and the generalized hypotonia had improved. However, axial weakness remained significant; therefore, the patient still needs a collar for head support. In addition, progressive rigidity of the spine but no contractures of Achilles tendons or elbows developed. Currently, at 7 years of age, the patient continues to show no signs of respiratory or cardiac involvement.

Results

LMNA Mutation and Protein Analysis

Genomic DNA from the index patient and both parents was extracted from blood and screened for mutations in *LMNA* by polymerase chain reaction (PCR) using intronic primers flanking each of the 12 exons of the gene, as described previously.⁹ The PCR products were tested for changes by heteroduplex analysis¹⁰ and directly sequenced by a cycle-sequencing procedure using a Taq Dye Deoxy Terminator Cycle sequencing kit (PE/Applied Biosystems, Foster City, CA) and the PCR primers. The only genomic sequence variation that we detected was a heterozygous c.428C>T missense mutation in exon 2 resulting in a p.S143F substitution. The mutation was confirmed on the messenger RNA level by reverse transcriptase PCR sequencing, but it was not detected in the parents or in 386 chromosomes of an ethnically related control population, consistent with a de novo mutation.

For protein analysis, dermal fibroblasts were eluted in radioimmunoprecipitation assay buffer, vortexed, and sonicated. A total of 15 μg total fibroblast protein was loaded on sodium dodecyl sulfate polyacrylamide gel electrophoresis and subsequently blotted on polyvinylidene difluoride membrane. Protein detection was performed with an anti-lamin A/C antibody (Santa Cruz Biotechnology, Santa Cruz, CA) using an enzyme chemiluminescence kit (Roche, Mannheim, Germany) for visualization. Reverse transcriptase PCR using a primer pair

spanning the entire coding region of lamin A and protein analysis by immunoblot did not show abnormal splicing products or truncated protein (see Fig 1F).

Discussion

The girl discussed in this article shows a unique phenotype combining early-onset myopathy with a progeroid syndrome. Her initial presentation with congenital weakness, mildly increased serum creatine kinase levels, and myopathic changes in a muscle biopsy was consistent with a myopathy or muscular dystrophy. It was only during the subsequent years that additional clinical features developed, leading to consideration of a progeroid syndrome (see the Table). Although some of the external features were not as severe as typically seen in HGPS (eg, no complete alopecia), there are many findings specific for a progeroid syndrome, and radiological investigations showed the typical osteolytic lesions (see Fig 1). Thus, our patient combines features of HGPS and a true myopathy.

Because mutations in *LMNA* have been associated with both myopathies and progeroid syndromes, we sequenced all exons of *LMNA* in our patient and identified a novel mutation in exon 2 resulting in an amino acid exchange p.S143F. Several lines of evidence support the pathogenicity of this amino acid change: (1) it is not detected in either parent; (2) it is not present in 386 chromosomes of a control population or in databases for polymorphisms; and (3) it is an evolutionary highly conserved residue.

The mutation in our patient lies within the coiled region 1B of the rod domain of lamin A/C that represents a highly conserved unique heptad repeat domain specific to nuclear lamins. The closest mutations associated with myopathies include p.R133P,¹¹ p.L140P,¹² and p.T150P¹³ changes in patients with EDMD. Interestingly, a p.S143P substitution at the same amino acid mutated in our patient leads to a distinct pheno-

type with dilated cardiomyopathy but no involvement of skeletal muscles.¹⁴ This phenomenon of clinical heterogeneity associated with a single amino acid has been described previously for other laminopathies; for example, alteration of p.R133 can either lead to EDMD (p.R133P), atypical WRN (p.R133L), or a complex phenotype with lipoatrophy, insulin-resistant diabetes, disseminated leukomelanodermic papules, liver steatosis, and cardiomyopathy (p.R133L).¹⁵

Among dominant mutations in residues close to the alteration in our patient are three cases of atypical WRN⁶ and one patient with HGPS.³ All four patients showed an intermediate phenotype. Hegele¹⁶ suggested classifying the atypical WRN group as late-onset HGPS, and Eriksson and colleagues³ described atypical features, including persistence of coarse hair over the head and ample subcutaneous tissue over the arms and legs, in their HGPS patient, suggesting a milder variant. Recently, three further dominant *LMNA* mutations (Fig 2) were identified in patients with "atypical progeroid syndromes."¹⁷ However, muscular weakness or other signs of a myopathy were not reported for any of these patients. In contrast to the typical severe HGPS mutation leading to the activation of a cryptic donor-splicing site and a truncated lamin A, we did not detect abnormal splicing or truncated protein (see Fig 1F) as a possible pathogenetic mechanism in our patient.

The muscle phenotype observed in our patient shares some characteristic features with other muscle laminopathies, including weakness in neck muscles and rigidity of the spine.⁹ However, our patient has not experienced development of any elbow or Achilles tendon contractures that are typical in EDMD. Nonetheless, it appears reasonable to conclude that the muscle involvement in our patient also is caused by the mutation in *LMNA* rather than an independent disease. Other examples of overlapping phenotypes associated with *LMNA* mutations include patients with lipodys-

Table. Clinical Findings in Our Patient Indicative of a Myopathy and a Progeria/Hutchinson–Gilford Syndrome, Respectively

Myopathy	Progeria/Hutchinson–Gilford Syndrome
Congenital/axial weakness	Growth retardation (at 5 years of age length 15cm <3 percentile, weight 5kg <3 percentile)
Muscle atrophy	Subcutaneous fat diminished
Elevated serum creatine kinase	Sclerodermatous skin lesions
Abnormal muscles on magnetic resonance imaging and ultrasound	Dystrophic nails
Myopathic muscle biopsy	Craniofacial disproportion
Rigidity of the spine	Prominent eyes
	Thin lips
	Small, beaked nose
	Sparse scalp hair and eyebrows
	Acroosteolysis
	Dystrophic, short clavicles
	Coxa valga

For characteristic features of Hutchinson–Gilford progeria syndrome compare De Busk.¹

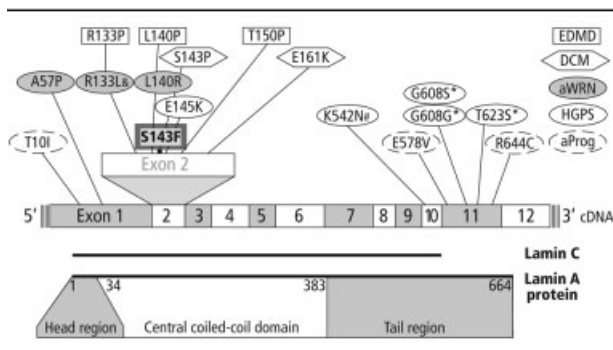


Fig 2. Schematic presentation of LMNA coding region showing all disease causing mutations in exon 2 and all mutations associated with progeroid phenotypes in the entire gene. The mutation identified in this study is in boldface. All numbers refer to amino acid positions on the protein level. *Alterations that lead to activation of a cryptic splice site and consecutive truncation of lamin A. †The same amino acid substitution also has been reported in a patient with lipoatrophy, insulin-resistant diabetes, disseminated leukomelanodermic papules, liver steatosis, and cardiomyopathy.¹⁵ #Homozygous missense mutation that has been described recently in a patient with HGPS. The heterozygous parents were asymptomatic.²⁰ aProg = atypical progeroid syndromes¹⁷; aWRN = atypical Werner syndrome²; DCM = dilated cardiomyopathy; EDMD = Emery–Dreifuss muscular dystrophy; HGPS = Hutchinson–Gilford progeria syndrome.

trophy and muscular dystrophy¹⁸ and one family with features of axonal neuropathy, muscular dystrophy, cardiac disease, and leuconychia.¹⁹

In conclusion, we present a patient with a unique phenotype combining early-onset myopathy and progeria with a de novo mutation of *LMNA*. This phenotype broadens the spectrum of laminopathies and, in particular, illustrates that additional features, such as a progeria, might evolve later during the course of the disease.

Note

At the age of eight years routine evaluation of the patient with electrocardiogram and echocardiogram revealed a mediolateral myocardial infarction.

Reference sequences, polymorphisms, and sequence variations for *LMNA* were taken from the Leiden Muscular Dystrophy pages (available online at: www.dmd.nl) if not stated otherwise.

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