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Novel mutations in LMNA A/C gene and associated phenotypes

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Mutations in the lamin A/C gene (LMNA) have been associated with several phenotypes ranging from systemic to prevalent of muscle, heart, skin, nerve etc. More recently they have been associated with dilated cardiomyopathy (DCM) and severe forms of arrhythmogenic right ventricular cardiomyopathy (ARVC).
We report four novel mutations - 3 missense and 1 deletion – in 4 unrelated patients showing different phenotypes, ranging from the early onset congenital form of laminopathy to classical LGMD phenotype, to LGMD and heart involvement. All these newly identified variants were not found in 300 ethnically-matched control subjects.
The variant c.103-105del CTG was described post-mortem in a young patient with congenital muscular dystrophy who presented at the age of 9 a first degree A-V block and subsequently several episodes of supraventricular parossystic tachycardia. Two patients presented as onset symptom lower limbs muscle weakness, and developed heart conduction defects requiring pacemaker implantation at the age of 26 and 38 years, respectively. One of them who carried the mutation c.1339G>C died at the age of 40 by intractable heart failure; the second one carrying the mutation 265C>T died at the age of 30, for a trmboembolic event. A classical LGMD phenotype without heart involvement was observed in the patient with the mutation 1579C>T, who died at the age of 68 years for respiratory insufficiency.

Key words: LMNA A/C gene, lamin A/C, Emery-Dreifuss muscular dystrophies, Laminopathies

Introduction

Laminopathies are a heterogeneous group of genetic disorders caused by mutation in LMNA gene encoding A and C lamins. Lamins are ubiquitous nuclear intermediate filament proteins that form a scaffold, termed nuclear lamina, at the nuclear periphery. Binding to a growing number of nuclear protein complexes, they provide nuclear stability, help connect the nucleus to the cytoskeleton, and contribute to modulate chromatin organization, gene regulation and expression, genome stability, differentiation, and tissue-specific functions (1-3).

Alterations of the nuclear envelope have been associated with several disorders including autosomal dominant (AD) forms of Emery-Dreifuss muscular dystrophy (EDMD2), dilated cardiomyopathy with conduction system defects (DCM-CD), limb girdle muscular dystrophy 1B (LGMD1B) with atrioventricular conduction disturbances, Dunnigan-type familial partial lipodystrophy (FPLD), mandibuloacral dysplasia (MAD), autosomal recessive (AR) forms of axonal Charcot-Marie-Tooth (ARCMT2,CMT2B), as far as progeroid syndromes (4-13).

To date, more than 450 LMNA mutations have been reported in locus-specific databases (http://www.umd.be/LMNA/;http://www.dmd.nl/), but few genotype/phenotype correlations have been defined, suggesting the presence of genetic modifiers. For this reason laminopathies represent a good example of "allelic heterogeneity" as mutations in the same gene can cause different phenotypes according to the site of the mutation along the gene (14, 15).

Of particular interest for cardio-myologists are the groups of LMNA-related myopathies (LM) and cardiomyopathies (LCM). LMNA-related myopathies (LM) repre-

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Table 1. Clinical features of the reported patients.

Patient	Muscle phenotype	Cardiac phenotype	Other signs	Gene Mutation	Protein	LMNA A/C exon
1	Congenital	Yes	Dropped head	c. 103-105del CTG	L35del	1
2	Mild	Yes		c.265C>T	R89C	1
3	Severe	Yes		c.1339G>C	E447Q	7
4	Severe LGMD	No	Respiratory failure	c.1579C>T	R527C	9

sent the more consistent subgroup of diseases due to mutations in LMNA gene. Three main different phenotypes have been reported based on distribution of muscle weakness or age at onset: LGMD1B, EDMD2, and a form of congenital muscular dystrophy (MDCL) (16, 17). A considerable clinical overlap exists among the three phenotypes suggesting they should be considered as a continuum in the clinical spectrum. Indeed, heart is involved in all three entities, with similar features (16, 18). Interestingly, heart involvement may precede onset of muscle weakness or sometimes be isolated. The phenotypic spectrum of muscle laminopathies ranges from severe congenital forms of muscular dystrophy to limb-girdle forms with adult onset and much milder weakness, often associated to a high risk of cardiovascular morbidity and mortality (19-22).

Here we described four novel mutations in LMNA gene in 4 unrelated patients showing phenotypes ranging from an early onset congenital muscular dystrophy to severe classical LGMD phenotype.

Patients and methods

All the reported patients are or were followed at the Cardiomiology and Medical Genetics of the Second University of Naples, between the last 10 years. All patients showed pronounced cervical muscle weakness, elbow retractions and elevation of serum creatine kinase, suggesting a Emery-Dreifuss muscular Dystrophy phenotype. The clinical features of the five patients are summarized in Table 1.

Blood samples for genetic analysis were collected after informed consent of patients or their tutors when minors, according to the Declaration of Helsinki. DNA was extracted following the standard operating procedures and analyzed trough PCR analysis of the entire coding region of LMNA A/C gene.

Results

Patients

Patient 1.

Patient 1 came to our observation for gait disturbances and frequent falls by the age of 3 years. Clinical examination revealed elbow, knee and heel retractions, and generalized muscular atrophy. until he lost ambulation by the age of 10 years for a delay in the acquisition of motor milestones. Ambulation was acquired at the age of 18 months.

Muscle biopsy showed a dystrophic pattern with a positive staining for LAMA2. From the age of 3 years to the age of 9 years he was stable. At the age of 9 years, he presented a first degree AV block and several episodes of supra-ventricular paroxysmal tachycardia, despite the treatment with beta-blockers. He died few months after for a sustained supraventricular tachycardia. The variant c.103-105del CTG (L35del) in LMNA A/C gene was found post-mortem.

Patient 2.

Patient 3 presented lower limbs muscle weakness as onset symptom, since the age of 12 years. Clinical examination revealed elbow retractions, gait disturbances and proximal muscle atrophy. Clinical Emery Dreifuss muscular dystrophy was diagnosed and LMNA A/C analysis showed the mutation c.265C>T (R89C) in exon 1. He developed heart conduction defects (hyperkinetic arrhythmia), requiring pacemaker implantation at the age of 26 years; he presented heart failure at the age of 30 and died for a tromboembolic event.

Patient 3.

Patient 2 presented since the age of 8 years a progressive weakness and gait disturbances. Elbow retractions were found at clinical examination as well as atrial fibrillation on ECG. ECG Holter revealed the presence of sinuatrial pauses, requiring pacemaker implantation at the age of 38. He lost ambulation at the age of 39 years and died at the age of 40 years by intractable heart failure. The mutation c.1339G>C (E447Q) in the LMNA gene, changing a glutamate in glutamine, at 447 position, was found.

Patient 4.

Patient 4 presented the classical phenotype of a limb girdle muscular dystrophy, at his first examination at our Service, at the age of 50. He complained of lower limbs

weakness by the age of 21, with progressive worsening in the daily motor performance. The loss of ambulation occurred at the age of 40. Clinical examination revealed proximal muscle wasting and weakness. No heart involvement was present and death occurred at the age of 68 years for respiratory failure. Later, a mutation analysis of the LMNA gene disclosed the novel missense mutation c.1579C>T, and we diagnosed him as EDMD2 (laminopathy).

Genetic analysis

All the reported mutations were novel, not previously described (transcript isoform LMNA-004, corresponding to Ensemble transcript ENST00000368299) and not found in 300 normal individuals.

The deletion occurring in the first exon of the transcript (103-105 del) determines the loss of the leucine at position 35, which is fundamental for the folding of the mature protein and the formation of the intermediate filament protein. The C to T transition (265 C>T) on the same exon determines the substitution of an arginine with a cysteine lying in the myosin rod fragment domain (R89C).

The G to C transversion (1339 G>C) and C to T transition (1579 C>T) in exons 7 and 9 respectively, determine changes in the aminoacidic composition of the Lamin A/C globular tail domain (E447Q and R527C) (Fig. 1).

Furthermore we translated the mutated transcript into its corresponding protein and annotated the functional domains through the InterProScan software (Mitchell A et al, 2015. Nucleic Acids Research).

We found that the deletion of the leucine at position 35 in particular, produces a mutated protein with the complete loss of the 30-62 SSF64593 domain which is part of the coiled coil region (Fig. 2) necessary for the structural support of the nucleus.

Discussion

Primary laminopathies caused by mutations in the LMNA gene typically display an extremely pleiotropic clinical presentation including cardiac, muscular and metabolic phenotypes. Additionally, many atypical laminopathies have been described combining features of two or more of the distinctive disorders or syndromes associated with LMNA mutations (15, 18).

Arrhythmic disorders are infrequent in young adults

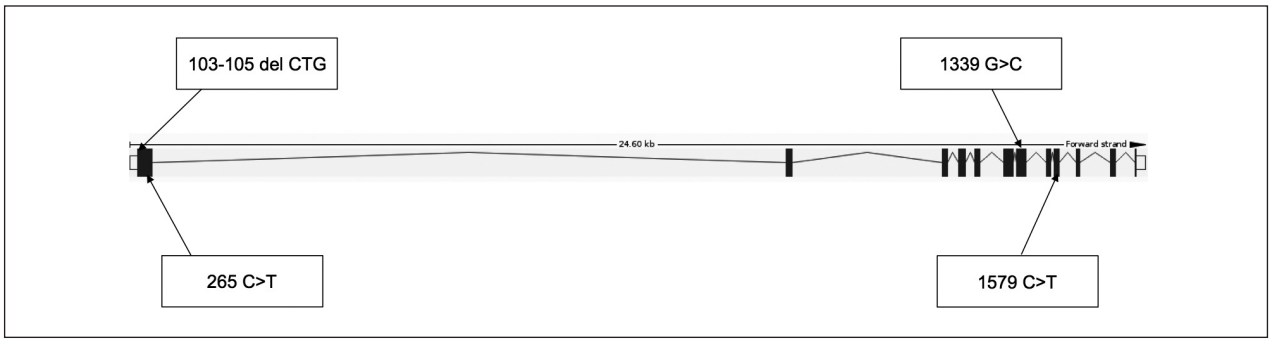

Figure 1 . Schematic representation of the LMNA A/C gene and position of novel mutations.

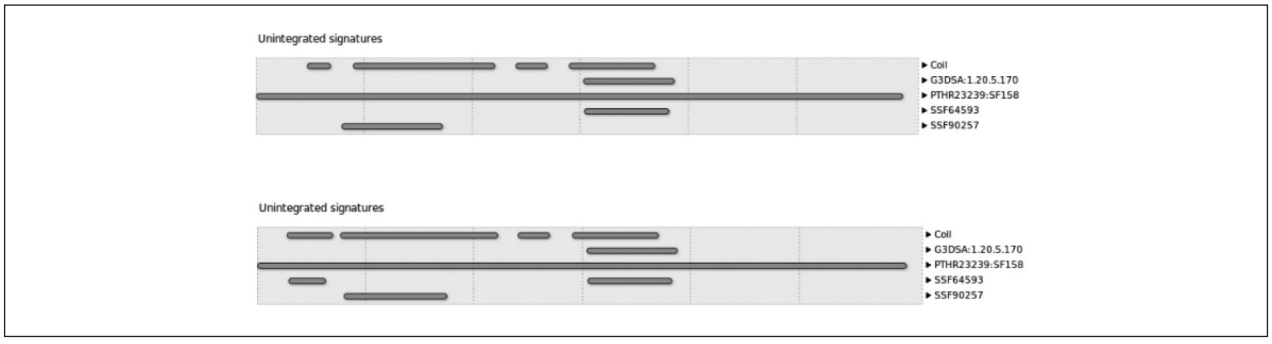

Figure 2. Comparative wild and mutated Lamin protein domains. Protein domains harboring the mutation L35del (top) and protein domains of LMNA-004 transcript (bottom). It is possible to note that some domains are missed in the mutated protein (see text). Domain annotations follows InterPro database nomenclature.

and should evoke myopathy associated cardiomyopathy, even though muscular symptoms are moderate or absent (23). Cardiac involvement is responsible for syncope, thromboembolic events and sudden death and often requires early cardioverter defibrillator implantation (24, 25).

Dropped head in children and contractures could be the clue to diagnose EDMD and indicate the need for a careful cardiological evaluation (26-29).

The reported cases further expand the role of the LMNA A/C gene in the pathogenesis of cardiac laminopathies, suggesting that LMNA should be included in mutation screening of all patients with droppen head and/or suspected arrhythmogenic cardiomyopathy, particularly when they have ECG evidence for conduction defects.

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