

De Novo *LMNA* Mutations Cause a New Form of Congenital Muscular Dystrophy

Susana Quijano-Roy, MD, PhD,¹⁻³ Blaise Mbieleu, MD,¹ Carsten G. Bönnemann, MD, PhD,⁴ Pierre-Yves Jeannot, MD, PhD,⁵ Jaume Colomer, MD,⁶ Nigel F. Clarke, MD, PhD,⁷ Jean-Marie Cuisset, MD,⁸ Helen Roper, MD,⁹ Linda De Meirleir, MD,¹⁰ Adele D'Amico, MD, PhD,¹¹ Rabah Ben Yaou, MD,^{2,3} Andrés Nascimento, MD,⁶ Annie Barois, MD, PhD,¹ Laurence Demay,¹² Enrico Bertini, MD, PhD,¹¹ Ana Ferreira, MD, PhD,^{2,3} Caroline A. Sewry, MD, PhD,^{13,14} Norma B. Romero, MD, PhD,^{2,3} Monique Ryan, MD, PhD,¹⁵ Francesco Muntoni, MD, PhD,¹⁴ Pascale Guicheney, PhD,^{2,3,12} Pascale Richard, MD, PhD,^{2,3,12} Gisèle Bonne, PhD,^{2,3,12} and Brigitte Estournet, MD, PhD¹

Objective: To describe a new entity of congenital muscular dystrophies caused by de novo *LMNA* mutations.

Methods: Fifteen patients presenting with a myopathy of onset in the first year of life were subjected to neurological and genetic evaluation. Histopathological and immunohistochemical analyses were performed for all patients.

Results: The 15 patients presented with muscle weakness in the first year of life, and all had de novo heterozygous *LMNA* mutations. Three of them had severe early-onset disease, no motor development, and the rest experienced development of a "dropped head" syndrome phenotype. Despite variable severity, there was a consistent clinical pattern. Patients typically presented with selective axial weakness and wasting of the cervicoaxial muscles. Limb involvement was predominantly proximal in upper extremities and distal in lower extremities. Talipes feet and a rigid spine with thoracic lordosis developed early. Proximal contractures appeared later, most often in lower limbs, sparing the elbows. Ten children required ventilatory support, three continuously through tracheotomy. Cardiac arrhythmias were observed in four of the oldest patients but were symptomatic only in one. Creatine kinase levels were mild to moderately increased. Muscle biopsies showed dystrophic changes in nine children and nonspecific myopathic changes in the remaining. Markedly atrophic fibers were common, most often type 1, and a few patients showed positive inflammatory markers.

Interpretation: The *LMNA* mutations identified appear to correlate with a relatively severe phenotype. Our results further broaden the spectrum of laminopathies and define a new disease entity that we suggest is best classified as a congenital muscular dystrophy (*LMNA*-related congenital muscular dystrophy, or L-CMD).

Ann Neurol 2008;64:177–186

Laminopathies are a highly heterogeneous group of disorders caused by mutations of the *LMNA* gene, which codes for A-type lamins, proteins of the nuclear envelope. Mutations in this gene were first identified in pa-

tients with autosomal dominant Emery–Dreifuss muscular dystrophy (EDMD2),¹ a slowly progressive myopathy with life-threatening cardiac complications.² EDMD typically presents between mid-childhood and

From the ¹Assistance Publique-Hôpitaux de Paris, Service de Pédiatrie, Hôpital Universitaire Raymond Poincaré, Centre National de Référence des Maladies Neuromusculaires Garches-Necker-Mondor-Hendaye, Garches; ²Institut National de la Santé et de la Recherche Médicale (INSERM) U582, Institut de Myologie; ³Université Pierre et Marie Curie-Paris6, Unité Mixte de Recherche S582, Institut Fédératif de Recherche, Paris, France; ⁴Division of Neurology, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA; ⁵Service de Pédiatrie, Unité de Neuropédiatrie, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; ⁶Unitat de Patologia Neuromuscular, Servei de Neurologia, Hospital Sant Joan de Déu, Esplugues, Barcelona, Spain; ⁷Institute for Neuromuscular Research, Children's Hospital at Westmead, Discipline of Paediatrics and Child Health, University of Sydney, Sydney, Australia; ⁸Service de Neurologie Pédiatrique, Hôpital Roger-Salengro, Centre National de Référence des Maladies Neuromusculaires, Centre Hospitalier Régional Universitaire de Lille, Lille, France; ⁹Department of Child Health, Birmingham Heartlands Hospital, Birmingham, United Kingdom; ¹⁰Pediatric Neurology Department, Free University of Brussels, Brussels, Belgium; ¹¹Unit of Molecular Medicine, Bambino Gesù Children's Hospital, Rome, Italy; ¹²Assistance Publique-Hôpitaux de Paris,

Groupe Hospitalier Pitié-Salpêtrière, U.F. Cardiogénétique et Myogénétique, Service de Biochimie Métabolique, Paris, France; ¹³Wolfson Centre for Inherited Neuromuscular Diseases, Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry; ¹⁴Dubowitz Neuromuscular Centre, UCL Institute of Child Health and Great Ormond Street Hospital for Children (GOSH), London, United Kingdom; and ¹⁵Neurosciences Department, Royal Children's Hospital, Parkville, Victoria, Australia.

Received Dec 24, 2007, and in revised form Mar 26, 2008. Accepted for publication Apr 4, 2008.

G.B. and B.E. contributed equally to this work.

This article includes supplementary materials available via the Internet at <http://www.interscience.wiley.com/jpages/0364-5134/suppmat>

Published online June 12, 2005, in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.21417

Address correspondence to Dr Quijano-Roy, Service de Pédiatrie, Hôpital Raymond Poincaré, 92380 Garches, France. E-mail: susana.quijano-roy@rpc.aphp.fr

early adulthood, but *LMNA* mutations have been identified in one case of fetal akinesia,³ and in three children with infantile onset of severe hypotonia and weakness,^{4,5} or isolated dropped-head syndrome.⁶ Increased creatine kinase (CK) levels in these cases were suggestive of a muscular dystrophy, but muscle biopsies showed nonspecific myopathic changes. *LMNA* mutations have been also identified in LGMD1B, a limb-girdle muscular dystrophy associated with cardiac conduction system defects, in DCM-CD, a form of dilated cardiomyopathy with conduction system disease,^{7,8} in a form of axonal neuropathy and in nonneuromuscular diseases such as partial lipodystrophy, premature aging syndromes, and restrictive dermopathy (for a review, see Broers and colleagues² article).

The congenital muscular dystrophies (CMDs) are genetic myopathies with dystrophic features on muscle biopsy and hypotonia, weakness, or delayed motor development from the first few months of life.⁹ At least nine clinical subtypes of CMD are recognized (MDC1A, MDC1B, MDC1C, MDC1D, UCMD, RSMD1, FCMD, MEB, and WWS). Mutations in 12 different genes and 2 loci have been associated with the various forms of CMD (*LAMA2*, *FKRP*, *LARGE*, *COL6A1*, *COL6A2*, *COL6A3*, *SEPN1*, *FCMD*, *POMGNT1*, *POMT1*, *POMT2*, *ITGA7*, and loci 1q42 and 3p).¹⁰ CMDs may show clinical features that overlap with EDMD, such as spinal rigidity, hyperCKemia, Achilles tendon contractures, and elbow flexion contractures. However, mutations in *LMNA* have not previously been associated with CMD.

A 2-year-old patient (Patient 1 in the Table) was first presented by one of the authors (S.Q.-R.; 2nd Workshop of the MYO-CLUSTER project GENRE on CMD, 2001) as a severe CMD with neonatal presentation, arrested motor development, increased CK levels, and with no abnormality found in the known CMD genes.¹¹ At the age of 6, he was found to have a novel de novo *LMNA* mutation. The particular pattern of muscle involvement, joint contractures, and spinal deformity prompted analysis of the *LMNA* gene in three additional children (Quijano-Roy et al., Breaking News, International Neuromuscular Diseases Congress, Istanbul, July 2006).³⁵ Comparison of these initial cases with 11 additional patients with *LMNA* mutations identified in other centers demonstrated a surprisingly consistent clinical phenotype, defining a distinct and clinically recognizable form of early-onset myopathy.

Patients and Methods

Patients

Four patients (Patients 1, 7, 14, and 15) were recruited by the French Consortium on Congenital Muscular Dystrophies and examined at Raymond Poincaré Hospital (Garches, France) (see the Table). The remaining patients were re-

cruited as part of a multicenter collaborative study: Patients 2 and 3 from London and 12 from Birmingham (England); Patient 4 from Philadelphia; Patient 5 from Sydney (Australia); Patient 6 from Lausanne (Switzerland); Patients 8, 10, and 11 from Barcelona (Spain); Patient 9 from Rome (Italy); and Patient 13 from Brussels (Belgium). Several patients have been previously described as case reports: Patient 1 (Quijano-Roy et al., Istanbul 2006),¹¹ Patients 14 and 15 (Quijano-Roy et al., Istanbul 2006), Patient 3,⁴ Patient 4,¹² Patients 8 and 10,¹³ and Patient 9.⁶ Detailed clinical, histological, and genetic data were obtained from all cases.

Histopathological and Immunohistochemical Methods

All 15 patients had at least one muscle biopsy. Number, site, and age at time of biopsies are indicated in the Table. Standard morphological and histochemical stains were performed in all patients. Because of the multicenter recruitment of the patients, immunohistochemical studies varied. Laminin α 2 staining was studied in all patients in muscle, except in one patient for whom no muscle was available and the study was performed on a skin biopsy (Patient 15). Additional immunohistochemical studies for inflammatory markers were performed in several patients: Patient 1 was analyzed with antibodies against major histocompatibility complex classes 1 and 2 (MHC-I and -II). Patient 6 was labeled with antibodies against CD3, CD20, CD4, CD8, CD68, C5b9, and MHC-I. MHC-I was also performed in Patients 2 and 3 because of the particularly severe, progressive course. Electron microscopy was performed in six patients (Patients 1, 3, 7, 9, 13, and 14). Cultured skin fibroblasts were studied for collagen VI secretion in Patients 1, 7, 11, 14, and 15, as described previously.¹⁴

Molecular Genetic Analysis

Informed consent for genetic studies was obtained for all patients. The 12 exons of *LMNA* and their intronic boundaries were analyzed using polymerase chain reaction/denaturing high-performance liquid chromatography/sequencing methods as described previously.^{15,16} In Patients 4 and 5, the coding region of *LMNA* was directly sequenced from polymerase chain reaction-amplified genomic DNA. All new *LMNA* mutations were checked in a control population of 200 unrelated control subjects. Family studies were also performed to confirm the mode of inheritance of mutations.

Results

Patients

Fifteen children were studied; eight boys and seven girls who were aged between 3 and 10 years except for one patient aged 20 years. Ethnicity was white in 12 patients and African in 3 patients (Patients 6, 7, and 13). Only one boy (Patient 7) was the offspring of consanguineous parents. CK levels were slightly or moderately increased in all patients at onset of symptoms (3–12 times reference values), and the level did not correlate with clinical severity. Nerve conduction studies were normal in all 12 patients who were tested (Patients 1, 3–8, 10, 11, and 13–15), and needle electromyography showed myopathic signs in 9 patients.

Clinical, histological, and genetic findings of the patients are shown in the Table. Variable severity was observed, and two main groups were distinguished.

GROUP I: SEVERE GROUP WITH ABSENT MOTOR DEVELOPMENT. Patients 1, 2, and 3 had decreased fetal movements, and absent or poor motor development. At presentation, they had severe hypotonia, diffuse limb and axial muscle weakness, generalized amyotrophy, and talipes foot deformities (Fig 1). Muscle atrophy was particularly severe in the neck, scapulohumeral regions, and calves. In contrast with the marked proximal upper limb weakness, hip flexion and knee extension were possible in early stages of the disease. Flexor joint contractures developed initially in distal limbs (ankles, fingers, wrists), spreading later to proximal joints of the lower extremities (knees, hips) but not to the elbows, which were even hypermobile. The spine became stiff and hyperextended at dorsal and lumbar levels, but the neck remained floppy. Patient 3 was nourished by gastrostomy after 21 months of life. All three infants required mechanical ventilation between 24 and 30 months. Syncope was observed in Patient 2 at the age of 7, and Holter electrocardiographic recording showed atrioventricular delay. Patient 1 had no cardiac symptoms but received β -blocker treatment after 7 years of age when atrial tachycardias were noted in routine studies.

GROUP II: PATIENTS WITH DROPPED-HEAD SYNDROME. Twelve children (Patients 4–15) acquired head and trunk control, and most walked independently (Fig 2). All presented with a striking loss of head control caused by neck extensor weakness. Six patients lost the ability to walk or stand unsupported between ages 2 and 5 years. The patients with a longer follow-up or more severe disease (Patients 4, 5, 6, 7, 11, 14, and 15) had a strikingly similar distribution of amyotrophy and muscle weakness to that found in Group I patients. The pattern of joint contractures and spinal stiffness was also the same, except five dropped-head patients also developed elbow contractures, although it was a mild and late feature. A few patients had only proximal weakness (Patients 10 and 12), showed a static course after the initial motor impairment (Patients 5 and 12), or had muscle weakness largely restricted to the cervical region (Patients 8, 9, and 13). These last patients were all aged younger than 5 years at assessment. Symptoms or signs of progressive restrictive respiratory failure were observed in eight children. Seven required nocturnal noninvasive ventilation; two of them were ventilated from 3 years of age and deteriorated further, requiring tracheostomies from age 5 years (Patients 7 and 15). One child died suddenly at age 3 years, but previous echocardiography and Holter electrocardiographic recording had not shown abnormalities (Patient 13).

Cardiac routine studies detected nonsustained atrial or ventricular arrhythmias in two patients at age 9 and 20 years, respectively (Patients 5 and 15). Failure to thrive was common. Three children (Patients 6, 13, and 14) required supplementary gavage feeds through a gastrostomy in the first 5 years of life, although no swallowing troubles were reported.

Histopathological and Immunohistochemical Analysis

Morphological and immunohistochemical findings are detailed in the Table.

HISTOPATHOLOGICAL ANALYSIS. Nine patients had dystrophic changes, including variability of fiber size, increased endomysial connective tissue, and/or signs of necrosis and regeneration (Figs 3A–C). All remaining patients had nonspecific myopathic features, verified in four on electron microscopy (Patients 1, 3, 13, and 14). Dystrophic features were much more evident in biopsies from the deltoid compared with the quadriceps muscle. All three biopsies taken from a deltoid muscle were markedly dystrophic, whereas only 8 of 15 quadriceps biopsies showed significant fibrosis and/or necrotic fibers. In addition, both patients who had deltoid and quadriceps biopsies showed more severe dystrophic features in the deltoid muscle, even when the upper limb biopsy was performed earlier in life (Patient 6). Dystrophic changes were often patchy within the biopsies. A frequent finding was marked variability of fiber size with zones of atrophic fibers (mainly type 1) without fiber-type grouping (see Fig 3D). Another notable finding was the presence of an interstitial mononuclear cell infiltrate in the biopsies of three patients (see Figs 3A, C).

IMMUNOHISTOCHEMICAL ANALYSIS. Expression of proteins known to be involved in congenital and progressive muscular dystrophies (including laminin- α 2, α -dystroglycan, collagen VI, dystrophin, and sarcoglycans) were essentially normal in all patients in whom they were studied. Abnormal upregulation of MHC-I was observed in the biopsies from three patients, two with an early onset (Patients 2 and 3) and another with dropped-head syndrome and a rapidly progressive course (Patient 6). This last patient also had a mononuclear infiltrate expressing T-lymphocyte markers in a deltoid biopsy at age 1 year but not in a quadriceps biopsy at age 2 years. Steroid treatment did not change the severe course of these three patients. Increased mononuclear cell infiltrates were observed in the deltoid muscle of an additional patient (Patient 1), but no upregulation of major histocompatibility antigens was observed. Collagen VI expression in cultured fibroblasts of the skin was normally expressed in the five patients where it was studied (Patients 1, 7, 11, 14,

Table. Genetic, Clinical, and Muscle Findings

Patient No./Age/Sex	Mutation	CK Level	Initial Signs	Maximal Motor Function (loss of function)	Respiratory Involvement (age)	Cardiac Involvement ^a	Joint Contractures	Muscle Weakness	Muscle Biopsy (age)	Other Muscle Findings
1/7y/M	p.L380 Sxon 6	X3	Fetal immobility Hypotonia, talipes (birth)	Antigravity limb movements (lost in arms at 1 yr; lost in legs at 3 yr) No head or trunk control	Infections MV—tracheostomy (22 mo)	paroxysmal atrial tachycardia (7 yr)	<i>Early:</i> distal limbs <i>Late:</i> knee, hip Elbows laxity	Axial>UL>LL UL—proximal LL—distal	Deltoid muscle (22 mo)—dystrophic +++	Cell infiltrate (normal MHC) Atrophic fibers
2/3 yr/M	p.R249W exon 4	X3	Fetal immobility Hypotonia, arm weakness (1-3 mo)	Rolling over at 18 mo (lost at 1.5 yr) No head or trunk control	Infections Mask MV (2.6 yr)		<i>Early:</i> distal limbs Elbows laxity	Axial>UL>LL Limbs—proximal	Quadriceps muscle (8 mo)—myopathic	MHC-I upregulated, few fibers, regenerative? Atrophic fibers Sarcolemmal damage (neomyosin +)
3/7 yr/F	p.E358K exon 6	X10	Fetal immobility Hypotonia, axial weakness (3-6 mo)	Rolling over Poor head control at 5 mo (lost at 6 mo)	Infections Mask MV (2.2 yr)	Syncope A-V delay (7 yr)	<i>Early:</i> distal limbs <i>Late:</i> hip	Axial>UL>LL Limbs—proximal	Quadriceps muscle (12 mo, 16 mo)—myopathic	MHC-I upregulated (both biopsies) Atrophic fibers Sarcolemmal damage (neomyosin +)
4/9 yr/F	p.R249W exon 4	X8	Talipes, axial weakness (3-6 mo)	Head support (lost at 9 mo) Sat when placed	Mask MV (7 yr) VC 29% (8 yr)		<i>Early:</i> ankles <i>Late:</i> knees, wrists, hips	Axial—cervical UL—proximal LL—distal Mild facial	Quadriceps muscle (9 mo)—myopathic	Atrophic fibers
5/9 yr/F	p.R50P exon 1	X10	Weakness, talipes, head drop (11-14 mo)	Pull to stand (10 mo) Steps with orthosis at 2 yr	VC 68% (9 yr)	Paroxysmal atrial tachycardia (9 yr)	<i>Early:</i> ankles <i>Late:</i> hips (4 yr), elbows (8 yr, mild)	Axial—cervical UL—proximal LL—distal	Quadriceps muscle (18 mo)—myopathic	Atrophic fibers
6/4 yr/F	p.E358K exon 6	X10	Hypotonia, axial weakness (4 mo) Head drop (17 mo)	Steps with support at 26 mo (lost at 3 yr)	Infections Mask MV (5 yr)		<i>Early:</i> distal limbs <i>Late:</i> wrist	Axial—cervical UL—proximal LL—distal	Deltoid muscle (1 yr)—dystrophic +++ Quadriceps muscle (2y)—dystrophic ++	Cell infiltrate (T lymphocyte) MHC-I upregulated (deltoid muscle) Atrophic fibres (neomyosin +, a-DG reduced)
7/10yr/M	p.L302P exon 5	X3	Axial hypotonia, head drop (6-12 mo)	Walking at 2 yr (lost at 3 yr)	Mask MV (3 yr) tracheostomy (5 yr)		<i>Early:</i> ankles <i>Late:</i> knees, wrists	Axial—cervical UL—proximal LL—distal	Quadriceps muscle (2 yr)—dystrophic +	
8/4 yr/M	p.R455P exon 7	X10	Hypotonia, head drop (6-12 mo)	Walking at 2.5 yr	—		<i>Late:</i> elbows (mild)	Cervical UL—proximal	Quadriceps muscle (11 mo)—dystrophic +	Atrophic fibers
9/3 yr/M	p.del K32 exon 1	X7	Axial hypotonia, head drop (6-12m)	Walking at 2y	—		—	Cervical UL—proximal	Quadriceps muscle (15 mo)—myopathic	
10/5yr/M	p.R453P exon 7	X12	Hypotonia, weak arms, head drop (6m)	Walking at 22 mo	—		<i>Late:</i> elbows (mild)	Cervical limbs—proximal	Quadriceps muscle (15 mo)—dystrophic +	Atrophic fibers

Table. Continued

Patient No./Age/Sex	Mutation	CK Level	Initial Signs	Maximal Motor Function (loss of function)	Respiratory Involvement (age)	Cardiac Involvement ^a	Joint Contractures	Muscle Weakness	Muscle Biopsy (age)	Other Muscle Findings
11/8yr/F	p.N39S exon 1	X4	Hypotonia, head drop (4 mo)	Walking at 18 mo (lost at 5 yr)	Mask MV (5 yr)		<i>Early:</i> ankles <i>Late:</i> hips elbows (mild)	Cervical UL—proximal LL—diffuse	Quadriceps muscle (2 yr)—dystrophic +	Atrophic fibers
12/8yr/F	p.N456D exon 7	X7	Head drop (12 mo)	Walking at 18 mo	Infections VC 30% (7 yr) Mask MV (8 yr)		<i>Early:</i> ankles <i>Late:</i> fingers	Cervical Limbs—proximal	Quadriceps muscle (2.5 yr)—dystrophic ++	Macrophages
13/3yr/F	c.1381-2a>g Int7 exon 8	X4	Hypotonia, head drop (6-12 mo)	Walking at 14 mo	—	Normal studies but sudden death (3 yr)	Elbows laxity	Cervical UL—proximal	Quadriceps muscle (2 yr)—myopathique	Atrophic fibers
14/6yr/M	p.E358K exon 6	X3	Hypotonia, head drop (6-12 mo)	Walking at 14 mo (lost at 4 yr)	Infections Mask MV (4 yr)		<i>Early:</i> ankles <i>Late:</i> knees, wrists, hips, fingers	Cervical UL—proximal LL—distal	Quadriceps muscle (2 yr)—dystrophic ++	Atrophic fibers
15/20 yr/M	p.E358K exon 6	X3	Weak arms, head drop (11 mo)	Walking at 13 mo (lost at 3 yr) Lost sitting at 6 yr	Infections Mask MV (3 yr) tracheostomy (5 yr)	Ventricular tachycardia (20 yr)	<i>Early:</i> ankles <i>Late:</i> knees, wrists, hips, elbows (20 yr)	Cervical UL—proximal LL—distal	Quadriceps muscle (2 yr)—dystrophic+ Deltoid muscle (3 yr)—dystrophic++	Cell infiltrate Atrophic fibers

^aCardiac studies (24 hour Holter-electrocardiographic recording and echocardiography) were normal when not stated otherwise, except for Patient 4, who had only echocardiography but not Holter recording. CK = creatine kinase; MV = mechanical ventilation; UL = upper limbs; LL = lower limbs; MHC = major histocompatibility complex; A-V = atrioventricular; VC = forced vital capacity; a-DG = α -dystroglycan antibodies. +: mild; ++: moderate; +++: severe.

and 15). Normal staining of laminin- α 2 was observed in the dermal junction of the skin in Patient 15.

Genetic Analysis

LMNA gene screening identified heterozygous de novo *LMNA* mutations in all patients. The Table summarizes the 11 different *LMNA* mutations identified. These mutations were absent in DNA from the patients' parents. Seven of the 11 identified mutations (p.R249W, p.L302P, p.L380S, p.R453P, p.R455P, p.N456D, and c.1381-2a>g) have not been reported previously. The remaining four mutations (p.delK32, p.N39S, p.R50P, and p.E358K) have been identified previously in a number of patients with later onset muscle laminopathies (see Supplementary Table).^{4,6,15,17-20}

Other genes were analyzed in several patients and showed no abnormalities: *ACTA1* (Patient 5), *SEPN1* (Patients 1, 6, and 7), *CAPN3* (Patient 14), and *FKRP* (Patients 1, 6, and 15). FSHD genetic analysis was normal in Patient 6. In addition, linkage analysis was performed in the only consanguineous family for the genes involved in CMD without central nervous system involvement (*SEPN1*, *FKRP*, *COL6A1*, *COL6A2*, *COL6A3*). All loci were excluded assuming autosomal recessive disease, except for *COL6A3*, because markers flanking this gene were homozygous, but a collagen VI

abnormality was excluded by immunocytochemical studies in skin cultured fibroblasts.

PHENOTYPE-GENOTYPE CORRELATIONS. To assess whether the previously reported *LMNA* mutations identified in our patients and in other with EDMD or LGMD phenotypes correlate with more severe disease, we used the UMD-*LMNA* database, which compiles all genetic and clinical data on individuals carrying a *LMNA* mutation ascertained from the literature and/or the European and French Laminopathies/EDMD research networks (database available at www.umd.be for the published mutations).^{2,21} Clinical data were available for 343 of the 833 individuals with a striated muscle laminopathy recorded so far. We found that the mean age of disease onset of the EDMD or LGMD patients with the 4 common mutations was 32 months, which is earlier than expected for these diseases.^{15,17,22} Also, of the 21 *LMNA* patients identified in the database who never walked or who lost ambulation before the age of 15 years, 10 were patients reported herein, and of the 11 remaining cases, 5 carried identical mutations to those found in our patients, that is, p.delK32 (two patients), p.N39S, p.R50P, and p.E358K.



Fig 1. Patient 1 with “severe” LMNA-related congenital muscular dystrophy (L-CMD) phenotype at 2 (A–C) and 7 years of age (D, E). Note the diffuse wasting with predominance in proximal upper and distal lower limbs (A), the spinal stiffness with head drop (B), and the talipes and calf wasting with initial preserved knee extension (C). In follow-up, thoracic hyperlordosis and knee contractures are observed (D), as well as distal contractures in upper limbs, in the absence of elbow contractures (E).

Discussion

This multicenter study defines the clinical, morphological, and genetic characteristics of *LMNA* myopathy presenting in the first year of life. The results indicate that this is a distinct nosological entity at the severe end of the spectrum of the striated muscle laminopathies. Mild-to-severe dystrophic changes were seen in many of the muscle biopsies, the serum CK level was universally increased, and there was relatively rapid disease progression. For these reasons, together with age of onset (birth to age 1 year), we propose that this entity is best classified as *LMNA*-related congenital muscular dystrophy (L-CMD). We report 15 cases, including 2 patients previously published, for whom updated clinical information was obtained.^{4,6} Two clinical phenotypes were apparent: a subgroup of patients with severe weakness and minimal or absent motor development, and a larger subgroup with milder disease who developed progressive neck weakness (dropped-head syndrome) after acquiring head control and who sat (and usually also walked). Despite some heterogeneity in clinical severity and pathological changes, all patients shared a strikingly similar pattern of muscle involvement. All children had a progressive course with an initial rapid decline in cervical/axial strength followed by a period of slower progression or stasis. Those patients with muscle weakness restricted to the

neck extensors were all younger than 5 years, which could explain the lack of other characteristic features. Progressive restrictive respiratory insufficiency was a major complication and necessitated tracheostomy in three patients. Respiratory failure was universal within the 2 first years of life in the severe group and arose before the age of 8 years in many children in the dropped-head group. Thus, these patients need close monitoring of respiratory function and gas exchange, especially after the onset of progressive motor decline. Cardiac involvement was rarely observed and was often subclinical in this series, but this may be because of the young age of most of the patients; therefore, routine studies to rule out heart dysfunction or rhythm abnormalities are highly recommended in follow-up.

Muscle biopsies were performed in the first 2 years of life. Almost half of the patients had dystrophic changes on muscle biopsy, but as was observed clinically, histological changes varied in severity in different muscles, and the site of biopsy appeared crucial in identifying focal dystrophic changes, being much more abnormal deltoid than quadriceps muscles. Interestingly, the degree of pathological abnormalities did not always correlate with the clinical severity. Scattered atrophic type 1 fibers were commonly seen and may be a useful diagnostic clue. The presence of cellular infiltrates (confirmed as T lymphocytes in one case) and

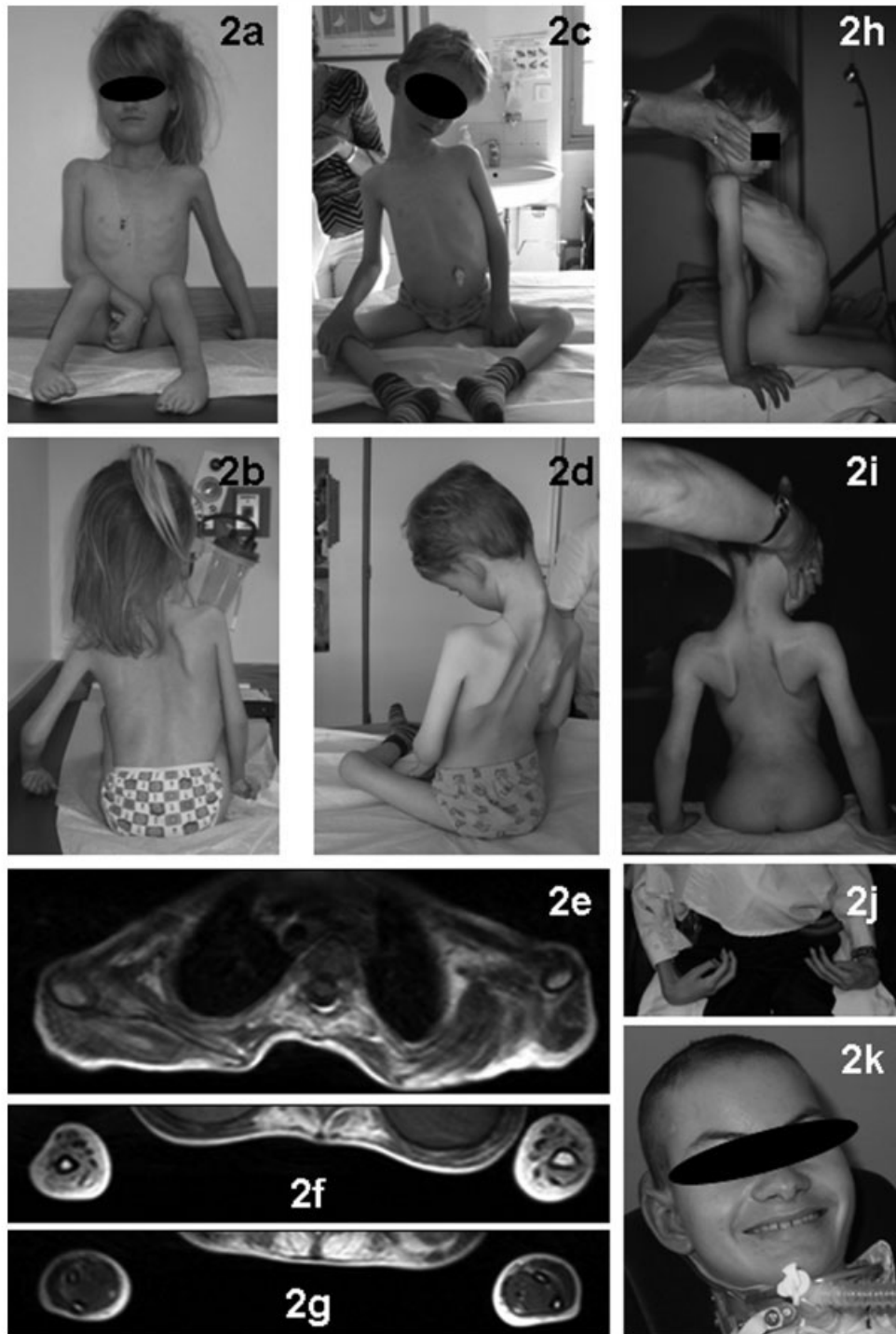


Fig 2. Patients 4 (A, B), 14 (C–G), and 15 (H–J) with “dropped-head” LMNA-related congenital muscular dystrophy (L-CMD). Note the striking neck involvement, the typical phenotype with spinal thoracolumbar lordosis, scapulohumeral wasting, and contractures in hands, feet, knees, and hips, in the absence of elbow contractures. Muscular magnetic resonance imaging of the scapular region and upper limbs (T1 sequences) showing severe abnormal signal in the biceps, triceps, and thoracic paraspinal muscles, moderate involvement of deltoid, and sparing of the distal arm muscles (E–G). Note the typical axial-cervical involvement with severe weakness and spinal hyperextension, and the diffuse wasting of limbs with relative preservation of distal arms at early stages. In the second decade of the life, there is severe diffuse weakness and contractures (D) in a tracheotomized patient with sparing of facial muscles (E). Note the striking neck involvement, the typical phenotype with spinal thoracolumbar lordosis, scapulohumeral wasting, and contractures in hands, feet, knees, and hips, in the absence of elbow contractures. Muscular magnetic resonance imaging of the scapular region and upper limbs (T1 sequences) showing severe abnormal signal in the biceps, triceps, and thoracic paraspinal muscles, moderate involvement of deltoid, and sparing of the distal arm muscles (E–G). Note the typical axial-cervical involvement with severe weakness and spinal hyperextension, and the diffuse wasting of limbs with relative preservation of distal arms at early stages. In the second decade of the life, there is severe diffuse weakness and contractures (J) in a tracheotomized patient with sparing of facial muscles (K).

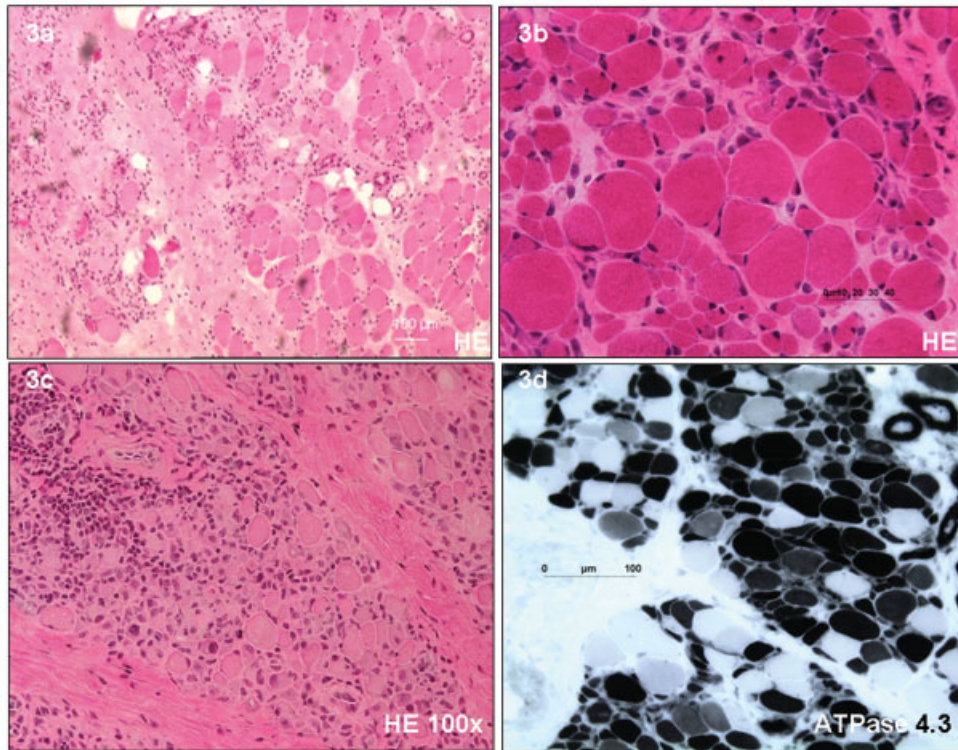


Fig 3. Muscle findings in Patients 1 (A, hematoxylin and eosin [HE]), 14 (B, HE; D, ATPase 4.3), and 6 (C, HE). Muscle biopsies from deltoid (A, C) and quadriceps muscles (B, D) showing severe or moderate dystrophic changes (A–D), cellular infiltrates (A, C), and a striking variability of the fiber size with small fibers, which are predominantly type 1 (D).

upregulation of MHC markers was another less frequent finding. It was observed early in the course of the disease of a few patients with a severe onset or a particularly rapidly progressive course. In this context, corticoids were given in some of them, but this was not followed by a change in the course of the disease. The meaning of the presence of mononuclear infiltrates and/or inflammatory markers is unknown, but it is not specific and has been observed in neonates and patients with a variety of muscular dystrophies.^{23–25}

From the nosological point of view, there is clear overlap between L-CMD and later onset striated muscle laminopathy phenotypes, especially EDMD, but several important differences exist. Both share the predominant humeroperoneal distribution of limb weakness and amyotrophy. However, the main presenting feature in L-CMD, the selective deficit of head support or the axial muscle weakness, is not characteristic of EDMD, which shows instead early spinal stiffness.^{2,15} Conversely, marked elbow contractures, a typical and early finding in EDMD patients,^{2,15} is not a feature of severe L-CMD, and patients may even show hypermobility of this joint. In addition, L-CMD patients show a much more rapid course than EDMD patients. Also, progressive restrictive respiratory insufficiency is an early and life-threatening finding in L-CMD, but this is not a major feature of EDMD. Our results and the

review of the literature indicate that laminopathies affecting the striated muscles constitute a continuous spectrum of successive phenotypes. There is a strong correlation between age of onset and the phenotype in individual patients. The L-CMD patients described in this study were weak from the first year of life. Overall, it appears that early prenatal onset may be associated with lethal fetal akinesia,³ late prenatal onset with severe L-CMD, onset before 1 year with dropped-head L-CMD,⁶ onset in childhood or young adulthood with classic EDMD,^{15,17,22,26} and in general, later onset with LGMD1B.^{16,22,27} To our knowledge, only two patients with classic signs of EDMD or LGMD have been reported to have signs before age 1 year: a patient with mild EDMD at age 67 years who in retrospect was said to have had mild elbow contractures at birth¹⁵; and a patient reported to have humeroperoneal muscle weakness, elbow, ankle, and knee contractures at birth to early childhood, and atrial fibrillation at age 53.¹⁷

Making a diagnosis in those with advanced disease is usually straightforward because of the distinct and recognizable clinical picture, not observed in other neuromuscular disorders. In early stages, however, patients may not show such specific features, and complementary investigations (histology, immunohistochemistry, CK levels, neuroimaging) and molecular studies may

be necessary to exclude other causes of floppy infant syndrome or dropped-head syndrome, especially other forms of CMD¹⁰ and congenital myopathies. In particular, SEPN1-related myopathy may be difficult to distinguish because this also has selective axial involvement²⁸ and is a reported cause of dropped-head syndrome,⁶ although CK levels are typically lower. The rapidly progressive course and increased CK levels in a child with no cognitive impairment may resemble CMD because of mutations in FKRP (MDC1C), but L-CMD patients lack muscle pseudohypertrophy, which is typically observed in MDC1C.²⁹ The development of multiple contractures may be seen in merosin-deficient and Ullrich CMD patients, but different muscle and joint involvement and specific immunohistochemical and phenotypic markers (striking brain white matter changes and distal hyperlaxity respectively) are useful in distinguishing these disorders.^{30,31} In this study, these conditions were specifically excluded in many patients using standard immunohistochemical and genetic studies.

Until now, no clear genotype/phenotype correlations have emerged from previous studies on the striated muscle laminopathies. We suspect that the specific mutations identified in our cohort are partly responsible for the severe phenotype. All the *LMNA* mutations identified in the patients were de novo, several patients sharing the same mutation. Of the 11 mutations identified, 7 are novel, and the remaining 4 (p.delK32, p.N39S, p.R50P, and p.E358K) have been previously published or submitted to the UMD-*LMNA* database in 18 patients, of a total of 334 patients with EDMD and 122 with LGMD1B. We found evidence that these 18 patients had more severe disease than is typical for EDMD or LGMD. Also, most of the patients in the database who were nonambulant or who lost ambulation before the age of 15 years were found to be either reported in our series or to share mutations with our patients. Nevertheless, the fact that a same mutation (p.E358K) was found in both severely and relatively mildly affected L-CMD patients in our cohort (Patients 3, 6, and 15) and in previously reported severe EDMD^{4,15,22} indicates that additional factors are important in determining disease severity. In this line, we recently demonstrated that digenism, that is, mutation in two separate genes, *LMNA* and *EMD* or *DES*, cosegregating within the same family could explain, in part, the wide clinical severity observed in laminopathies affecting the striated muscles.^{32,33} One can also speculate, like in most dominant disorders with incomplete penetrance and/or wide clinical variability, that modifier genes and/or environmental factors may contribute to the clinical variability. Recently, Benedetti and colleagues²² proposed that mild late-onset phenotypes may arise through loss of function secondary to haploinsufficiency, whereas dominant negative or toxic

gain-of-function mechanisms may be operating in patients with early severe phenotypes. Our results are compatible with this hypothesis, because all the L-CMD patients harbored either missense mutations or in-frame deletions that are potentially expressed. None was nonsense or frameshift mutations that would be predicted to abolish protein production from the mutant allele. It remains unclear why some missense mutations lead to severe phenotypes whereas others do not.

In conclusion, dominant de novo mutations in *LMNA* gene can be associated with a severe progressive myopathy with presentation in the first year of life, associated with a distinct pattern of weakness, invariable respiratory insufficiency, and risk for heart rhythm disturbances. The early age of onset and differences in phenotype distinguish this from EDMD. In addition, the rapidly progressive clinical course, increased CK levels, and dystrophic changes that are usually seen in clinically involved muscles are typical of a CMD. We therefore suggest that this early onset phenotype caused by *LMNA* mutations is best classified as a CMD (L-CMD). This is the first time that a nuclear envelope protein has been implicated in a CMD. This series broadens the spectrum of laminopathies affecting skeletal muscles, and opens a new chapter in the genetics and pathophysiology of congenital and early-onset muscular dystrophies.

This work was supported by the Institut National de la Santé et de la Recherche Médicale, Assistance Publique-Hôpitaux de Paris, Association Française contre les Myopathies (AFM), the GIS-Institut des Maladies Rares, European Union Fifth Framework (Euro-laminopathies contract #018690) (grant #RAS05018), AFM rare disorder network program (10722), the Muscular Dystrophy campaign (F.M.), NHMRC grant (372104, N.F.C.), and the Muscular Dystrophy Association of New South Wales, Australia (N.F.C.).

We thank the patients and their families for their participation in this study. We also thank V. Allamand, C. Bérout, I. Desguerre, V. Drouin-Garraud, M. Fardeau, C. Gartioux, E. Lacene, L. Lazaro, J.-P. Leroy, F. Leturcq, A. Lobrinus, L. Medne, K. North, V. Spehrs, B. Talim, A. Thevenon, L. Vallée, and M. Wehnert.

References

1. Bonne G, Di Barletta MR, Varnous S, et al. Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. *Nat Genet* 1999;21:285–288.
2. Broers J, Ramaekers F, Bonne G, et al. The nuclear lamins: laminopathies and their role in premature ageing. *Physiol Rev* 2006;86:967–1008.
3. van Engelen BG, Muchir A, Hutchison CJ, et al. The lethal phenotype of a homozygous nonsense mutation in the lamin A/C gene. *Neurology* 2005;64:374–376.
4. Mercuri E, Poppe M, Quinlivan R, et al. 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* 2004;61:690–694.

5. Mercuri E, Brown SC, Nihoyannopoulos P, et al. Extreme variability of skeletal and cardiac muscle involvement in patients with mutations in exon 11 of the lamin A/C gene. *Muscle Nerve* 2005;31:602–609.
6. D'Amico A, Haliloglu G, Richard P, et al. Two patients with 'Dropped head syndrome' due to mutations in LMNA or SEPN1 genes. *Neuromuscul Disord* 2005;15:521–524.
7. Muchir A, Bonne G, van der Kooij AJ, et al. Identification of mutations in the gene encoding lamins A/C in autosomal dominant limb girdle muscular dystrophy with atrioventricular conduction disturbances (LGMD1B). *Hum Mol Genet* 2000;9:1453–1459.
8. Fatkin D, MacRae C, Sasaki T, et al. Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. *N Engl J Med* 1999;341:1715–1724.
9. Dubowitz V. 68th ENMC International Workshop (5th international workshop) on Congenital Muscular Dystrophy, 9-11 April 1999, Naarden, The Netherlands. *Neuromuscul Disord* 1999;9:446–454.
10. Muntoni F, Voit T. 133rd ENMC International Workshop on Congenital Muscular Dystrophy (IXth International CMD Workshop) 21-23 January 2005, Naarden, The Netherlands. *Neuromuscul Disord* 2005;15:794–801.
11. Muntoni F, Bertini E, Bonnemann C, et al. 98th ENMC International Workshop on Congenital Muscular Dystrophy (CMD), 7th Workshop of the International Consortium on CMD, 2nd Workshop of the MYO CLUSTER project GENRE. 26-28th October, 2001, Naarden, The Netherlands. *Neuromuscul Disord* 2002;12:889–896.
12. Medne L, Glanzman A, Fliker J, et al. Rare congenital presentation of Emery-Dreifuss muscular dystrophy due to a novel de-novo LMNA mutation R249W. *Neuromusc Disord*. 2006; 16:675.
13. Nascimento A, Colomer J, Demay L, et al. Lamin A/C gene mutation as a cause of dropped head syndrome. Extending the striated muscle laminopathies. *Neuromusc Disord*. 2006;16: 675.
14. Demir E, Ferreira A, Sabatelli P, et al. Collagen VI status and clinical severity in Ullrich congenital muscular dystrophy: phenotype analysis of 11 families linked to the COL6 loci. *Neuropediatrics* 2004;35:103–112.
15. Bonne G, Mercuri E, Muchir A, et al. Clinical and molecular genetic spectrum of autosomal dominant Emery Dreifuss muscular dystrophy due to mutations of the lamin A/C gene. *Ann Neurol* 2000;48:170–180.
16. Ben Yaou R, Becane HM, Demay L, et al. Autosomal dominant limb-girdle muscular dystrophy associated with conduction defects (LGMD1B): a description of 8 new families with the LMNA gene mutations. *Rev Neurol (Paris)* 2005;161: 42–54.
17. Brown CA, Lanning RW, McKinney KQ, et al. Novel and recurrent mutations in lamin A/C in patients with Emery-Dreifuss muscular dystrophy. *Am J Med Genet* 2001;102: 359–367.
18. Vytopil M, Ricci E, Dello Russo A, et al. Frequent low penetrance mutations in the Lamin A/C gene, causing Emery Dreifuss muscular dystrophy. *Neuromuscul Disord* 2002;12: 958–963.
19. Colomer J, Sabatelli P, Columbaro M, et al. Clinical spectrum of Emery-Dreifuss muscular dystrophy 2 (EDMD2): reports of 6 patients. *Neuromusc Disord* 2004;14:591.
20. Bakay M, Wang Z, Melcon G, et al. Nuclear envelope dystrophies show a transcriptional fingerprint suggesting disruption of Rb-MyoD pathways in muscle regeneration. *Brain* 2006;129: 996–1013.
21. Bonne G, Ben Yaou R, Beroud C, et al. 108th ENMC International Workshop, 3rd Workshop of the MYO-CLUSTER project: EUROMEN, 7th International Emery-Dreifuss Muscular Dystrophy (EDMD) Workshop, 13-15 September 2002, Naarden, The Netherlands. *Neuromusc Disord* 2003;13: 508–515.
22. Benedetti S, Menditto I, Degano M, et al. Phenotypic clustering of lamin A/C mutations in neuromuscular patients. *Neurology* 2007;69:1285–1292.
23. Dubowitz V, Sewry C. *Muscle biopsy: a practical approach*. 3rd ed. Philadelphia: Saunders, 2006:612.
24. Pegoraro E, Mancias P, Swerdlow SH, et al. Congenital muscular dystrophy with primary laminin alpha2 (merosin) deficiency presenting as inflammatory myopathy. *Ann Neurol* 1996;40:782–791.
25. Nagaraju K, Rawat R, Veszelszky E, et al. Dysferlin deficiency enhances monocyte phagocytosis: a model for the inflammatory onset of limb-girdle muscular dystrophy 2B. *Am J Pathol* 2008;172:774–785.
26. Vytopil M, Benedetti S, Ricci E, et al. Mutation analysis of the lamin A/C gene (LMNA) among patients with different cardiomyopathic phenotypes. *J Med Genet* 2003;40:e132.
27. van der Kooij AJ, Ledderhof TM, de Voogt WG, et al. A newly recognized autosomal dominant limb girdle muscular dystrophy with cardiac involvement. *Ann Neurol* 1996;39:636–642.
28. Ferreira A, Quijano-Roy S, Pichereau C, et al. Mutations of the selenoprotein N gene, which is implicated in rigid spine muscular dystrophy, cause the classical phenotype of multimimicore disease: reassessing the nosology of early-onset myopathies. *Am J Hum Genet* 2002;71:739–749.
29. Quijano-Roy S, Galan L, Ferreira A, et al. Severe progressive form of congenital muscular dystrophy with calf pseudohypertrophy, macroglossia and respiratory insufficiency. *Neuromuscul Disord* 2002;12:466–475.
30. Fardeau M, Tome FM, Helbling-Leclerc A, et al. [Congenital muscular dystrophy with merosin deficiency: clinical, histopathological, immunocytochemical and genetic analysis]. *Rev Neurol (Paris)* 1996;152:11–19.
31. Camacho Vanegas O, Bertini E, Zhang RZ, et al. Ullrich scleroatonic muscular dystrophy is caused by recessive mutations in collagen type VI. *Proc Natl Acad Sci U S A* 2001;98: 7516–7521.
32. Ben Yaou R, Toutain A, Arimura T, et al. Multitissular involvement in a family with LMNA and EMD mutations: role of digenic mechanism? *Neurology* 2007;68:1883–1894.
33. Muntoni F, Bonne G, Goldfarb LG, et al. Disease severity in dominant Emery Dreifuss is increased by mutations in both emerin and desmin proteins. *Brain* 2006;129:1260–1268.
34. Muchir A, Medioni J, Laluc M, et al. Nuclear envelope alterations in fibroblasts from patients with muscular dystrophy, cardiomyopathy, and partial lipodystrophy carrying lamin A/C gene mutations. *Muscle Nerve* 2004;30:444–450.
35. Quijano-Roy S, Mbieleu B, Barois A, et al. LMNA is responsible for a new and distinct congenital muscular dystrophy associated with drop-head, spinal stiffness and progressive course. XIth International Congress on Neuromuscular Diseases. Istanbul, Turkey, 2–7 Juillet: Late Breaking News, 2006.