Mutation analysis of the lamin A/C gene (LMNA) among patients with different cardiomyopathic phenotypes


Laminopathies represent a heterogeneous group of genetic disorders characterised by mutations in the LMNA gene, which encodes two lamins, A and C, by alternative splicing of the primary transcript. Lamins belong to the intermediate filament multigene family and form the nuclear lamina, a mesh-like structure adjacent to the nucleoplasmic side of the inner nuclear membrane. They interact with emerin, the proteins encoded by the gene for the X-linked (X EDMD) form of EDMD, with several nuclear envelope proteins and with chromatin. Despite their widespread distribution and their role in nuclear architecture, alterations of lamin A/C are responsible for a number of very specific but quite heterogeneous disorders.

The first laminopathy was the autosomal dominant form of Emery-Dreifuss muscular dystrophy (EDMD), a genetic disorder characterised by the clinical triad of early onset contractures, progressive muscular wasting and weakness with humeroperoneal distribution and cardiac conduction defects. The finding that emerin, an inner nuclear envelope protein, and LMNA were both involved in EDMD suggested that the lamins may represent specific and relevant factors in cardiac and skeletal muscle and that integrity of the nuclear membrane and associated structures is specifically required for muscle function.

However, later on it was found that besides autosomal dominant Emery-Dreifuss muscular dystrophy (AD-EDMD), mutations in LMNA are responsible for six other disorders: limb girdle muscular dystrophy 1B, (LGMD1B),3–5 dilated cardiomyopathy with conduction system disease, (DCM-CD),4–6 Dunningan-type familial partial lipodystrophy,7–9 one recessive axonal form of Charcot-Marie-Tooth neuropathy,10 mandibuloacral dysplasia,11 and Hutchinson Gilford progeria.12 13

Despite the very different phenotypic consequences of mutations in LMNA, and the quite large number of mutations identified, no genotype/phenotype correlation has been demonstrated, pointing to the role of factors other than lamins A and C in determining the different tissue specific phenotypes. Mutation analysis of the LMNA gene has shown that mutations are predominantly missense and do not cause a reduction in the amount of lamin A/C at the nuclear lamina. However these mutations must lead to perturbations of the nuclear lamina responsible for the phenotype through alterations of interactions with emerin and with other proteins that can confer the tissue specificity of the disorders.

Three of the autosomal dominant disorders affect skeletal and cardiac muscle to a various degree. LGMD1B (MIM 159001) belongs to a heterogeneous group of muscular dystrophies. It is inherited as an autosomal dominant trait, characterised by slowly progressive limb girdle weakness and wasting, atrioventricular cardiac conduction defects and dilated cardiomyopathy. Patients affected with LGMD1B differ from patients with AD-EDMD as they lack early contractures and show predominant proximal myopathy. The heart involvement in DCM-CD (MIM 115200) is clinically similar to the cardiac disease in AD-EDMD and LGMD 1B and takes the form of conduction system disease and dilated cardiomyopathy. Patients do not present with contractures or muscle wasting or weakness. The overlapping features of these disorders suggest that the three autosomal dominant disorders affecting skeletal and cardiac muscle may represent one disease entity with variable expression of symptoms and that the boundaries between the three phenotypes are unclear. At least in one case, a mutation in LMNA gene was shown to account for all three phenotypes within a single family. Among the LMNA disorders, dilated cardiomyopathy is the disease of highest sociomedical importance since it

Key points

- The aim of the work was to study the frequency of mutations in the LMNA gene associated to the different cardiac and skeletal muscle phenotypes described and that seem to form a group of overlapping entities with marked clinical variability.
- We collected 166 patients who could be divided into four groups: Emery Dreifuss muscular dystrophy (EDMD), isolated heart disease, isolated muscular dystrophy, and idiopathic hyperCKaemia. All patients were positive for emerin immunohistochemistry.
- Mutation analysis of LMNA demonstrates that the presence of heart involvement and age of onset are distinguishing features separating patients with EDMD and LMNA mutation from patients with EDMD who are LMNA/STA negative, while distribution of myopathy is not a reliable diagnostic criterion in EDMD.
- The frequency of LMNA mutations in isolated heart disease is very low (3–5%) but similar to that of other genes identified, underscoring the high heterogeneity of the cardiac phenotype and a role of LMNA in this group of disorders.
- Patients carrying a wild type LMNA gene did not carry missense mutations in the X-linked gene STA.
- At least one other gene will have to be identified to characterise the EDMD phenotype fully.

Abbreviations: AD-EDMD, autosomal dominant Emery-Dreifuss muscular dystrophy; AV, atrioventricular; CK, creatine kinase; DCM-CD, dilated cardiomyopathy with conduction system disease; EDMD, Emery-Dreifuss muscular dystrophy; LGMD1B, limb girdle muscular dystrophy 1B; X EDMD, X-linked Emery-Dreifuss muscular dystrophy
Table 1 Groups of patients analysed

<table>
<thead>
<tr>
<th>Clinical syndromes</th>
<th>Clinical subgroups</th>
<th>Number of patients</th>
<th>Number of mutations (%)</th>
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<tbody>
<tr>
<td>1 EDMD</td>
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<tr>
<td>2 Isolated heart disorders</td>
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<tr>
<td>3 Myopathy</td>
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<tr>
<td>4 Idiopathic hyperCKaemia</td>
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<td>5 Lipodystrophy</td>
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METHODS

Genetic analysis of LMNA and STA genes

All exons and exon-intron junctions of the LMNA and STA genes were amplified by PCR and analysed by DHPLC (Transgenomic) as described previously. To analyse putative homozygote mutations, the PCR products from probands were mixed with equimolar amounts of PCR products from a normal individual. The PCR products with peak profiles different from the wild type were sequenced directly using a Perkin Elmer 373A Automated Sequencer. Primer, PCR and DHPLC conditions are listed at www.sanraffaele.org/research/onioloo/

RESULTS

The LMNA gene was screened for mutations in a set of 166 patients presenting with EDMD or the isolated cardiac or muscular phenotype. Three patients whose parents were first degree cousins were analysed for homozygous mutations in the LMNA gene. No recessive mutations were found.

EDMD-like phenotypes

Fifteen mutations (39.5%) were identified in 38 unrelated patients clinically defined as AD-EDMD, based on their muscle phenotype and presence of normal emerin (table 2). Twenty one patients who did not have a mutation in the LMNA gene (table 3) were screened for mutations in the coding sequence of the X-linked gene STA gene. No mutations were detected in the X-linked gene.

Eleven different mutations were identified. The mutations R453W and R249Q, repeatedly reported, were found three times and twice respectively among the group of patients. A third mutation n (T528R) changes a T residue previously reported in a different mutation. It was found twice. Finally, the E112del mutation was also previously reported. The remaining seven mutations were novel. One mutation was found in the portion of first exon encoding the N-terminal tail. Six were found within LMNA exons encoding the central rod domain. Four mutations altered amino acid residues in the C terminal tail.

One mutation (patient CM) was a heterozygous 103C>G substitution, causing the L35V amino acid change, not found among 200 chromosomes of control subjects. The nucleotide change results in a conservative substitution. However, L to V substitutions were previously shown to cause phenotypic changes and structural alterations and may possibly change the structure and the interactions of lamin A/C as well. Patient CM had multiple contractures (elbows, knees, ankles) with onset in early childhood, spine rigidity, diffuse muscular wasting and serious supraventricular tachyarrhythmias at age 9. Healthy parents did not carry the mutation.

Patient AA (described in greater detail elsewhere) is worth mentioning here. She carried a three nucleotide deletion (94–96delAAG, K32del) and presented with severe EDMD at age 16. The mutation was inherited from her father who at age 52 was healthy. The presence of incomplete penetrance of dominant heterozygous mutations in LMNA gene was repeatedly reported and suggests that healthy parents of sporadic cases should be tested since they may be asymptomatic carriers possibly at high risk of sudden death.

Isolated cardiac disease

Two novel mutations (1303C>T in patient JIR, and 1039G>A in patient NE5) were identified among 71 patients with isolated cardiac disease (table 1). Thirty patients had dilated
cardiomyopathy, 25 had cardiac conduction defects or arrhythmia and 16 a combination of both.

The patient JiR had severe isolated dilated cardiomyopathy. The first symptom was dyspnea at age 24. Echocardiography, at age 27, revealed marked left ventricular dysfunction with an ejection fraction of 22%. No significant heart rhythm disturbances were detected. Thus from this point of view, the clinical phenotype of the patient is quite different from previous descriptions of heart disease in EDMD or DCM-CD as in these disorders conduction disturbances or arrhythmias occur first and left ventricular dysfunction, if present, follows. Clinical neurological examination showed no abnormalities but needle electromyography revealed mild a “myogenic” pattern in the anterior tibialis. The muscle biopsy showed mild fibre type disproportion, a non-specific alteration frequently described in EDMD. The father died of heart failure at age 55, the mother and sister were reported as healthy. None was available for analysis. JiR carried a missense heterozygous mutation causing a R435C change. The mutation affects the initial portion of C terminal tail of the protein and was not found among 200 chromosomes of control subjects.

Patient NE5 had had a pacemaker implanted for severe conduction defects with onset at age 44. He had no clinical signs of either skeletal muscle involvement or dilated cardiomyopathy. The parents were first degree cousins. His paternal uncle and maternal aunt both died from stroke, presumably of cardioembolic origin due to arrhythmia. The proband had two sisters and two brothers. Both sisters died suddenly at ages 25 and 32. The latter had had a pacemaker and suffered from elbow contractures. One of the brothers has mildly elevated levels of CK. The other brother was diagnosed with an atrioventricular (AV) conduction defect. The proband, the maternal aunt and the brother was diagnosed with an atrioventricular (AV) conduction defect. The proband, the maternal aunt and the brother were emerin-positive and did not carry mutations in the LMNA gene. No mutations were found among 200 chromosomes of control subjects.

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Other disorders
No mutations were found among 23 cases of idiopathic hyperCKaemia or in two cases of lipodystrophy (table 1).

DISCUSSION
We report the mutation analysis of a large group of patients presenting a variable set of cardiac muscle phenotypes, characteristics of laminopathies, ranging from typical EDMD to isolated dilated cardiomyopathy: the inclusion criteria were more permissive than those previously established for EDMD, due to the marked variability of clinical presentation repeatedly described in previous studies. 18 19

We identified 18 mutations in the LMNA gene. No mutations were found in the X-linked STA gene in patients who were emerin-positive and did not carry mutations in LMNA, thus confirming the diagnostic value of the absence of emerin. The group more frequently presenting mutations was that defined as EDMD, where 15 mutations were identified, corresponding to 39.5% of the patients in the group. The analysis distinguished the patients tentatively diagnosed as EDMD into two groups, one carrying LMNA heterozygote mutations and the second presenting with wild type LMNA and STA genes. The two groups could not be distinguished based on their muscular phenotype. The clinical feature distinguishing the two groups is the cardiac involvement as 10 out of 15 (66%) patients with LMNA mutations suffered from cardiac disease, while among patients not presenting LMNA/STA mutations only 5 out of 23 (21%) had cardiac disorders. The mean age of the two groups is similar (21 and 26 years respectively), but, in the LMNA group, patients not presenting with cardiac involvement are the youngest (7–16 years of age, mean age 13 years) and they may still develop cardiac disease later in life. These results confirm that cardiomyopathy is a distinctive diagnostic feature of AD-
EDMD, and can be used to distinguish EDMD from other muscular disorders with muscle contractures. Some of the patients in our group may be affected by Bethlem myopathy and the genetic analysis is underway to confirm the hypothesis.

Two mutations were found among the 71 patients presenting isolated heart involvement, one among 30 cases (3.5%) of pure idiopathic dilated cardiomyopathy and the other in 25 cases (5%) of cardiomyopathy with conduction defects and arrhythmia. The result supports the view that the two conditions are genetically highly heterogeneous. On the other hand, since the frequency of LMNA mutations in this group of patients is similar to that of other genes responsible for cardiac disorders, our results indicate that mutations in LMNA have to be taken into account in the diagnosis of both groups of disorders.

No mutations were found among 23 cases of idiopathic hyperCKaemia, showing that the LMNA gene is not commonly involved in idiopathic hyperCKaemia.

Missense LMNA mutations are distributed along the entire LMNA gene but tend to recur within certain confined regions in exon 1, 4, 7, and 9. No genotype-phenotype correlation could be made. Moreover our study indicates that mutation spanning few amino acids of evolutionary highly conserved regions result in phenotypes with strikingly different interactions.

In conclusion our results show that myopathy with contractures—either isolated or associated with heart disease—is a genetically heterogeneous disease. LMNA mutations occur in almost 40% of the patients presenting muscle contractures and in approximately 70% of adult patients presenting cardiac disorders as well. They also occur in other groups of patients and in a quite unpredictable way. Finally, the identification of patients like DAP, Oar and NM13 presenting an EDMD phenotype with cardiac conduction defects and no mutations in either LMNA or STA indicates that other genes have to be identified to fully explain the EDMD phenotype.

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Authors’ affiliations

M Vytopil, R Ricotti, D Toniolo, Institute of Molecular Genetics-CNR, Via Abbiategrasso 207, Pavia, Italy

M Vytopil, S Vohanka, Department of Neurology, University Hospital Brno, Brno, Czech Republic

J Toman, Department of Internal Medicine, St Anne’s University Hospital, Brno, Czech Republic

S Benedetti, D Toniolo, DIBIT-San Raffaele Scientific Institute, Milan, Italy

E Ricci, G Galluzzi, Institute of Neurology

A Dello Russo, Institute of Cardiology, Catholic University, Rome, Italy

L Merlino, Neuromuscular Unit, Istituto Ortopedico Rizzoli, Bologna, Italy

O Boriani, M Gallina, Institute of Cardiology, University of Bologna, Italy

L Morandi, Besta Neurological Institute, Milan, Italy

L Politano, Department of Medical Genetics, University of Naples, Naples, Italy

M Moggio, L Chiveri, Department of Neurology, University of Milan, Milan, Italy

I Hausmanowa-Petrusewicz, Medical Research Centre, Polish Academy of Science, Warsaw, Poland

E Ricci, Centre for Neuromuscular Diseases, UILDM-Rome Section, Rome, Italy

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Correspondence to: D Toniolo, PhD, DIBIT, San Raffaele Scientific Institute, via Olgettina 58, I-20132 Milan, Italy; daniela.toniolo@hsr.it

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