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),11 dilated cardiomyopathy with conduction system disease, (DCM-CD),12 Dunningan-type familial partial lipodystrophy,13 recessive axonal form of Charcot-Marie-Tooth neuropathy,14 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 perturbances 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, atroventricular 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.14 Among the LMNA disorders, dilated cardiomyopathy is the disease of highest sociomedical importance since it
occurs with high population frequency, many affected individuals are young and they frequently face dismal prognosis. To reach a more exact estimation of the frequency of LMNA mutations in different laminopathies and to try to refine the clinical and genetic boundaries between individual phenotypes, a large, clinically heterogeneous set of patients was analysed, and here we report the results of the mutation analysis of LMNA and emerin genes in this group of patients.

**PATIENTS**

One hundred and sixty six patients belonging to five clinical subgroups were collected for the analysis (table 1).

Thirty eight patients were diagnosed with EDMD based on the following criteria: (a) contractures of elbows or ankles, or rigid spine; (b) progressive muscular dystrophy with humero- or proximal, axial, or generalised distribution and onset of muscular symptoms before age 20. Patients with the muscle phenotype but not presenting with cardiac disorder after the age of 20 were also included in this group.

Seventy one unrelated patients with isolated heart disease were divided into three clinical subgroups as shown in table 1. The following inclusion criteria were applied: (a) DCM with ejection fraction less than 45% and no limit in age of onset, (b) presence of permanent or recurrent supraventricular tachyarrhythmias before age 45, (c) presence of atrioventricular conduction defects requiring insertion of pacemaker before age 45. Patients with diabetes mellitus, atherosclerosis, heart disease, or alcoholism were excluded.

Thirty two patients with diagnostically unclassified muscular dystrophies were analysed. Twelve of them had heart disease and twenty had isolated muscular dystrophy.

Twenty three patients aged between 3 and 70 and presenting with idiopathic hyperCKaemia were screened for mutations. Creatine kinase (CK) levels ranged from 246 U/l to >9000 U/l. Patients were all asymptomatic. The most frequent causes of muscle disorder—dystrophin, dysferlin, sarcoglycans and laminin—were excluded by analysing muscle biopsies.

Two patients with lipodystrophy were analysed. One patient presented with partial lipodystrophy of forearms and hands and contractures of elbows. The second patient was described as having generalised Berardinelli-type lipodystrophy, hyperCKaemia, weakness of bilateral peroneals, scoliosis and arrhythmia. His parents were first degree cousins.

**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/toniolo/

**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 head. 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 introducing the A347K change in a residue highly conserved in different species. The sequence mutation 1039G affected with AV conduction defect were carrying the mutation that was not found among 130 chromosomes of control subjects. The mutation affects the portion of exon 1 which encodes the central rod domain.

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 phenotypes, characteristics of laminopathies, ranging from typical EDMD to isolated dilated cardiomyopathy: the inclusion criteria were more permissive than those previously established for EDMD,18,19 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 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 defined as EDMD, where 15 mutations were identified, corresponding to 39.5% of the patients in the group. The group more frequently presenting mutations was that defined as EDMD, 15 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 disorder later in life. These results confirm that cardiomyopathy is a distinctive diagnostic feature of AD-

### Table 2 Patients with EDMD cases and LMNA mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation</th>
<th>Protein change</th>
<th>Age</th>
<th>I</th>
<th>Distribution of clinical myopathy</th>
<th>Contractures</th>
<th>Cardiac involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SS</td>
<td>73C-&gt;G</td>
<td>36</td>
<td>nk</td>
<td>proximal, axial</td>
<td>elbow, ankle</td>
<td>CD</td>
</tr>
<tr>
<td>2</td>
<td>AAg</td>
<td>94A-&gt;96del AAG</td>
<td>16</td>
<td>fam</td>
<td>proximal, axial</td>
<td>elbow, ankle</td>
<td>A, DCM</td>
</tr>
<tr>
<td>3</td>
<td>CM</td>
<td>103C-&gt;G</td>
<td>9</td>
<td>spor</td>
<td>generalised</td>
<td>elbow, knee, ankle, rigid spine</td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td>CaA</td>
<td>334-336 delGAG</td>
<td>15</td>
<td>nk</td>
<td>proximal</td>
<td>elbow, rigid spine</td>
<td>none</td>
</tr>
<tr>
<td>5</td>
<td>SD</td>
<td>743T-&gt;C</td>
<td>15</td>
<td>fam</td>
<td>proximal in lower limbs</td>
<td>elbow</td>
<td>none</td>
</tr>
<tr>
<td>6</td>
<td>MLG</td>
<td>746G-&gt;A</td>
<td>28</td>
<td>spor</td>
<td>generalised</td>
<td>elbow, knee, ankle, rigid spine</td>
<td>A, CD, PM</td>
</tr>
<tr>
<td>7</td>
<td>AAT</td>
<td>746G-&gt;A</td>
<td>22</td>
<td>nk</td>
<td>proximal</td>
<td>elbow, knee, ankle, rigid spine</td>
<td>CD</td>
</tr>
<tr>
<td>8</td>
<td>CE</td>
<td>800A-&gt;G</td>
<td>27</td>
<td>spor</td>
<td>proximal</td>
<td>elbow, ankle, spine</td>
<td>CD</td>
</tr>
<tr>
<td>9</td>
<td>AP</td>
<td>1337A-&gt;T</td>
<td>7</td>
<td>nk</td>
<td>proximal</td>
<td>elbow, ankle, neck</td>
<td>none</td>
</tr>
<tr>
<td>10</td>
<td>Ulu</td>
<td>1357C-&gt;T</td>
<td>16</td>
<td>nk</td>
<td>none</td>
<td>shoulder, hip, elbow, ankle, rigid spine</td>
<td>none</td>
</tr>
<tr>
<td>11</td>
<td>Kuch</td>
<td>1357C-&gt;T</td>
<td>10</td>
<td>fam</td>
<td>none</td>
<td>rigid spine</td>
<td>none</td>
</tr>
<tr>
<td>12</td>
<td>Cat</td>
<td>1357C-&gt;T</td>
<td>46</td>
<td>nk</td>
<td>axial, proximal</td>
<td>rigid spine</td>
<td>CD, PM</td>
</tr>
<tr>
<td>13</td>
<td>NMC</td>
<td>1583G-&gt;G</td>
<td>28</td>
<td>nk</td>
<td>generalised</td>
<td>elbow, ankle, rigid spine</td>
<td>A, CD, PM</td>
</tr>
<tr>
<td>14</td>
<td>28</td>
<td>1583G-&gt;G</td>
<td>23</td>
<td>spor</td>
<td>humeroperoneal, axial</td>
<td>elbow, rigid spine</td>
<td>AF</td>
</tr>
<tr>
<td>15</td>
<td>Cal</td>
<td>1622G-&gt;A</td>
<td>20</td>
<td>nk</td>
<td>humeroperoneal</td>
<td>rigid spine</td>
<td>A</td>
</tr>
</tbody>
</table>

I, inheritance; nk, not known; fam, familial; spor, sporadic; A, arrhythmia; AF, atrial fibrillation; CD, conduction defects; DCM, dilated cardiomyopathy; PM, pacemaker;
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 with 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 evolutionarily highly conserved regions result in phenotypes with strikingly different symptoms and varying severity and point to the role of other factors in determining phenotype.

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.

**ACKNOWLEDGEMENTS**

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