CASE REPORT

Emery-Dreifuss muscular dystrophy (EDMD) is a rare disorder characterized by the clinical triad of early-onset contractures, slowly progressive weakness, and muscle wasting in humeroperoneal muscles, and adult-onset cardiomyopathy with conduction block. In 1994, Bione et al. first reported the X-linked heredity and mapped the locus to Xp28, which encodes a 34 kD nuclear membrane protein designated "emerin". The nuclear lamin A/C (LMNA) gene was identified to be responsible for autosomal dominant EDMD (AD-EDMD) in 1999. Subsequently, different mutations in the LMNA gene were confirmed to cause AD and autosomal recessive EDMD in 2000. Mutations in LMNA are now known to be responsible for AD-EDMD, limb-girdle muscular dystrophy 1B (LGMD1B), dilated cardiomyopathy with conduction system disease (DCM-CD), Dunningan-type familial partial lipodystrophy, one recessive axonal form of Charcot-Marie-Tooth neuropathy, mandibuloacral dysplasia, and Hutchinson-Gilford progeria; even atypical Werner's syndrome, restrictive dermopathy, and metabolic syndrome are thought to be associated with LMNA gene mutation.

In this report, we describe the diagnosis of AD-EDMD in a mother and two daughters based on the family history, clinical manifestations, and pathologic findings. Due to the wide clinical spectrum, significant intrafamilial variability of severity, and the need for hereditary consultation, we used

Key Words: Emery-Dreifuss muscular dystrophy, LMNA gene

Emery-Dreifuss muscular dystrophy (EDMD) is a rare disorder characterized by the clinical triad of early-onset contractures, progressive weakness, and muscle wasting in humeroperoneal muscles, and cardiomyopathy with conduction block. We analyzed blood samples from an EDMD family, including a mother and two daughters, and found a novel mutation in codon 520 in exon 9 of the lamin A/C (LMNA) gene, resulting in a substitution of tryptophan (W) by glycine (G) in all three patients. The mother died after a stroke-like episode at the age of 43. The elder sister received pacemaker implantation, which improved symptoms of exercise intolerance and dizziness. These cases illustrate the necessity of correct diagnosis, evaluation, and follow-up of cardiac problems due to the wide clinical spectrum and high prevalence of cardiac conduction block in patients with autosomal dominant EDMD. [J Formos Med Assoc 2007;106(2 Suppl):S27–S31]

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mutation analysis to detect LMNA gene mutation in these three clinically diagnosed AD-EDMD patients and in other family members.

Case Reports

Case 1
An 18-year-old girl had suffered from progressive muscle weakness since early childhood. She complained of occasional palpitation, easy dizziness, and mild chest discomfort. Physical and neurologic examinations showed intact, symmetric facial expression, complete eyelash burying, limited neck flexion, waddling gait, shoulder and pelvic girdle muscular atrophy and weakness, and joint contracture over elbow and ankle joints. No tongue fasciculation, calf muscle hypertrophy, or myotonia was noted. Laboratory tests showed mildly elevated creatine kinase (CK) at 549 IU/L (normal, <174). Nerve conduction velocity was within normal limits and electromyogram was indicative of myopathy. A muscle biopsy was then taken over the left biceps brachii muscle, and showed myopathic change with moderate variation in fiber size, internal nuclei, occasional necrotic and regenerating fibers, and focal connective tissue proliferation (Figure 1A). Based on the above findings, EDMD was suspected. Further cardiac surveys were arranged. Twenty-four-hour Holter ECG showed frequent atrial premature contraction and intermittent first degree atrioventricular (AV) block; cardiac echo demonstrated fair systolic function but mild mitral regurgitation. No obvious progression was noted and cardiac function was still fair during 6 years of follow-up.

Case 2
A 20-year-old young woman, the elder sister of the proband, visited our hospital due to generalized muscle weakness and limited neck flexion. Serum CK was mildly elevated at 676 IU/L and muscle biopsy showed marked variation in fiber size, internal nuclei, and occasional necrotic fibers (Figure 1B). Twenty-four-hour Holter ECG showed a markedly prolonged PR interval with paroxysmal atrial fibrillation; cardiac echo demonstrated fair systolic function but mild mitral regurgitation. In the following 2 years, dizziness became more frequent and she complained of exertional dyspnea. Follow-up 24-hour Holter ECG demonstrated atrial fibrillation combined with complete AV block. A rate-responsive single chambered ventricular pacemaker (Medtronic Sigma SSR 303, Minneapolis, MN, USA) was implanted in May 2004. Postoperatively, 24-hour Holter ECG showed atrial fibrillation with ventricular pacing rhythm, and clinical symptoms including frequent dizziness and exertional dyspnea were much improved.

Case 3
A 43-year-old woman, the mother of the above two sisters, was brought to our hospital due to...
a 20-year history of Gowers’ sign and exercise intolerance. Twenty-four-hour Holter ECG showed frequent ventricular premature contraction. Cardiac echo showed dilated left atrium and left ventricle with mild mitral regurgitation. Left ventricular systolic and diastolic functions were normal. She refused muscle biopsy and was then lost to follow-up at our outpatient department. Two years later, she was sent to our emergency room due to sudden loss of consciousness with apnea. Junctional bradycardia with intermittent bigeminal ventricular premature contraction was also noted. Brain computed tomography showed an infarction-like lesion over the pons. During hospitalization, hemodynamically unstable ventricular tachycardia and ventricular fibrillation developed requiring electrical cardioversion to resuscitate. She died due to hypoxic ischemic encephalopathy with heart and respiratory failure.

**Methods**

Peripheral blood samples were collected for mutation analysis from these three symptomatic patients and four other asymptomatic family members after approval was obtained from the Human Experiment and Ethics Committee of Kaohsiung Medical University Hospital. Genomic deoxyribonucleic acid (DNA) was isolated from peripheral blood mononuclear cells using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions. Polymerase chain reaction (PCR) was performed using specific primer sets for each of the 12 exons of *LMNA*. The primer sequences are listed in the Table. Single-strand conformation polymorphism analysis of the PCR products was performed using the GenePhor DNA Separation System and GeneGel Excel 12.5/24 Kit (Amersham Biosciences Co., Tokyo, Japan) under each of three different temperatures of 5°C, 10°C, and 15°C. The aberrant PCR products were purified by solid-phase extraction and bidirectionally sequenced with the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s instructions. Sequencing reactions were separated using an ABI 3100 genetic analyzer. The T6293G mutation of *LMNA* introduces a new *Hpa*II restriction site. Genomic DNAs from the patients and normal individuals were amplified using the primers 5′-GCTTGGGACCTTGGGGAGA-3′ and 5′-ATGGCTCTGCTCCCTTTAAACATCC-3′ under the following cycling conditions: 95°C for 3 minutes; 40 cycles of 95°C for 30 seconds, 58°C for 1 minute, 72°C for 1 minute, followed by 72°C for 5 minutes. The PCR products were digested with *Hpa*II (New England Biolabs, Beverly, MA, USA) and analyzed by agarose gel electrophoresis.

<table>
<thead>
<tr>
<th>Exon</th>
<th>Sense primer</th>
<th>Antisense primer</th>
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<tbody>
<tr>
<td>1</td>
<td>ACTCCGAGCACGTCTCCTGTCC</td>
<td>CTTCTCCACTCCCGGCA</td>
</tr>
<tr>
<td>2</td>
<td>CTTCTCTAAACTCTACCTCC</td>
<td>CTTCTCCACTCCCGGCA</td>
</tr>
<tr>
<td>3</td>
<td>CCTCTCAGCTTCCCTCAAGTTC</td>
<td>CGAGCCCAGCTCTGTCATC</td>
</tr>
<tr>
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<td>GCCCTCCAGGAACTCCTCCTG</td>
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<tr>
<td>5</td>
<td>CTGTAGCAGTGTGCAACT</td>
<td>ATCCCTCCTCCCTGAGGAA</td>
</tr>
<tr>
<td>6</td>
<td>CCTTGGAGAGCTCACAAAC</td>
<td>GCCAGGAGGACAGTGGCA</td>
</tr>
<tr>
<td>7</td>
<td>GGCAGCTGGCCTTGACTAGA</td>
<td>CATCCCTCCTGGCCACT</td>
</tr>
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<td>8</td>
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<td>GACACTTACCCAGGGAGCTCC</td>
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<tr>
<td>11</td>
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<td>TCAGGAAGCCACCCCTGCTCA</td>
</tr>
<tr>
<td>12</td>
<td>GTGTGTCAACCTCCTCCTCCTG</td>
<td>CAGATGTTGGAGGTTTCCCTGGA</td>
</tr>
</tbody>
</table>
Results and Discussion

PCR amplification and direct sequencing (Figure 2) revealed a novel mutation in codon 520 in exon 9 of the \textit{LMNA} gene, resulting in a substitution of tryptophan (W) by glycine (G) (missense mutation) in all three patients (II-1, III-1, III-2). These patients were all heterozygous for the W520G mutation. Restriction endonuclease analysis of the W520G mutation showed that \textit{HpaII} treatment yielded two fragments, 274 bp and 138 bp, of the W520G mutant gene and an undigested 412 bp fragment of the normal gene (Figure 3). Restriction endonuclease analysis confirmed the mutation and showed that the four asymptomatic relatives (II-2, 3, 4, 5) and 200 unrelated normal individuals did not bear this mutation. Based on molecular analyses and clinical presentations, AD-EDMD was diagnosed in these three family members.

The \textit{LMNA} gene encodes two different proteins, lamins A/C, produced by alternative splicing. Lamins A/C are components of the nuclear envelope and expressed in a wide range of tissues, including adult heart and skeletal muscle. There are many theories regarding the effects of \textit{LMNA}, but the real mechanism by which \textit{LMNA} gene mutations cause EDMD and cardiomyopathy is still uncertain\textsuperscript{4,8}. More than 70 nucleotide substitutions, predominantly missense mutations, have been reported in the \textit{LMNA} gene, among which 37-point

![Figure 2](image1.png)

**Figure 2.** Pedigree and partial nucleotide sequence of \textit{LMNA} gene show the heterozygous T to G mutation at nucleotide position S20 in patients II-1, III-1 and III-2. This change is absent in normal control.

![Figure 3](image2.png)

**Figure 3.** Restriction endonuclease analysis of the W520G mutation: the size of the PCR product is 412 bp; \textit{HpaII} treatment yielded two fragments, 274 bp and 138 bp, of the W520G mutant gene and an undigested 412 bp fragment of the normal gene.
mutations are responsible for AD-EDMD. W520S (TG→TG) was previously detected by Bonne et al,9 but W520G is a novel finding. The correlation between the phenotype and type or localization of the mutations within the gene remains unclear. Vytopil et al10 indicated that mutation spanning only a few amino acids of evolutionary highly conserved regions results in phenotypes with strikingly different symptoms and varying severity. These studies have demonstrated a high proportion of de novo mutations and marked inter- and intrafamilial variabilities in the clinical expression of LMNA mutations. The broad spectrum of both LMNA mutations and of clinical expression should prompt screening for LMNA in familial and sporadic cases of both EDMD and dilated cardiomyopathy associated with conduction system disease.9

Based on previous literature, nearly half of EDMD patients died suddenly and the outlook seemed improved with pacemaker implantation.11 But other studies have shown that the risk of sudden death in EDMD patients, even those without manifest cardiac abnormalities, may be unrelated to heart failure and unresponsive to pacemaker therapy.12,13 These findings show the importance of regular cardiac evaluation and illustrate the need for further study of the mechanism of sudden death. Further, the indication for implantable cardioverter-defibrillator use thus becomes a new important question.14 Although the risk of sudden death still exists, the elder sister in this family received pacemaker implantation, which substantially improved her clinical symptoms and life quality. However, further electrophysiologic examination and long-term follow-up are necessary.

In conclusion, this is the first report of a novel W520G mutation in Taiwanese patients with AD-EDMD. The findings suggest the need for wider screening of asymptomatic family members of sporadic cases. Early intervention for conduction block is crucial to identify cases and improve life quality.

References