Cardiac effects of the c.1583 C→G LMNA mutation in two families with Emery-Dreifuss muscular dystrophy

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Abstract. The present study aimed to examine and analyze cardiac involvement in two Emery-Dreifuss muscular dystrophy (EDMD) pedigrees caused by the c.1583 C→G mutation of the lamin A/C gene (LMNA). The clinical and genetic characteristics of members of two families with EDMD were evaluated by performing neurological examinations, skeletal muscle biopsies, cardiac evaluations, including electrocardiography, 24 h Holter, ultrasound cardiography and ⁹⁹Tc-MIBI-gated myocardial perfusion imaging, and genomic DNA sequencing. Family history investigations revealed an autosomal dominant transmission pattern of the disease in Family 1 and a sporadic case in Family 2. The three affected patients exhibited typical clinical features of EDMD, including joint contractures, muscle weakness and cardiac involvement. Muscle histopathological investigation revealed dystrophic features. In addition, each affected individual exhibited either cardiac arrhythmia, which was evident as sinus tachycardia, atrial flutter or complete atrioventricular inhibition. Cardiac imaging revealed dilated cardiomyopathy in two of the individuals, one of whom was presented with heart failure. The second patient presented with no significant abnormalities in cardiac structure or function. The three affected individuals exhibited a heterozygous missense mutation in the LMNA gene (c.1583 C→G), which caused a T528R amino acid change in the LMNA protein. In conclusion, the present study identified three patients with EDMD, exhibiting the same dominant LMNA mutation and presenting with a spectrum of severe cardiac abnormalities, including cardiac conduction system defects, cardiomyopathy and heart failure. As LMNA mutations have been associated with at least six clinical disorders, including EDMD, the results of the present study provide additional mutational and functional data, which may assist in further establishing LMNA mutational variation and disease pathogenesis.

Introduction

Emery-Dreifuss muscular dystrophy (EDMD) refers to a group of inherited muscular dystrophies, characterized by slow, progressive muscular weakness and atrophy of the humeral and peroneal distributions, with early contractures, which affect the Achilles tendon, spine and elbows (1). EDMD is also associated with cardiomyopathy and abnormalities of the cardiac conduction system (2,3). Cardiac problems are the leading cause of sudden mortality in patients with EDMD, therefore, it is important that cardiac symptoms are diagnosed and treated early to improve outcomes and prolong life expectancy in patients with EDMD (2,4).

EDMD can arise from mutations in five different genes, EMD, LMNA, SYNE1, SYNE2 and FHL1, and can be inherited as an X-linked, autosomal dominant (AD) or autosomal recessive disorder (5). The autosomal forms of the disease are predominantly caused by gene mutations in the lamin A/C gene (LMNA) (6), and patients with EDMD exhibiting LMNA mutations typically present with more severe cardiac involvement, compared with patients with other EDMD gene mutations. However, there are currently few case reports detailing the various genetic mutations according to phenotype (7-9). The structural/functional correlations are further complicated by LMNA mutations, which have been associated with several distinct clinical disorders, including Hutchinson-Gilford progeria syndrome, Charcot-Marie-Tooth disease, limb girdle muscular dystrophy, familial partial lipodystrophy and dilated cardiomyopathy, in addition to EDMD (7). To further improve knowledge regarding specific LMNA mutations and disease phenotypes, the present case study assessed the cardiac phenotypes in two families with EDMD, caused by the same c.1583 C→G (T528R) LMNA mutation.

Materials and methods

Collection of clinical data. The present case study investigated two Chinese families, which included three EDMD-affected and 12 unaffected members, ascertained by neurological examination. Clinical data and detailed family surveys were collected. The patients and family members included in the...
The following conditions were used for the subsequent histochemistry and immunofluorescence staining experiments. Morphological analysis of the muscle specimens was performed using a BX51 confocal scanning laser microscope (Olympus Corporation, Tokyo, Japan).

**Table I. Primer sequences.**

| Exon | Sense | Anti-sense |
|------|-------|------------|
| 1    | 5TCGGGACGCCAGGAGGCAAGAG3 | 5CGACTGTTTCACGCCATCCTCCA3 |
| 2    | 5CATGGGAGGAGAAGACCATACAC3 | 5GTTATGTTCTTCTCCCTCAGTG3 |
| 3    | 5GGGCAGCCTGAGTAGTACAG3 | 5AAGTCCTAATCGACTCCA3 |
| 4    | 5GAATTAGATGTCGAGACAG3 | 5TGACACCTCCTAATCTTACCTC3 |
| 5    | 5GCCACCTGTTGCTTCC3 | 5TCAGGGAGACCTGGTGAGG3 |
| 6    | 5AGGGAGGAGGAGGAGGTTTTC3 | 5TCTGAGCCTCCTAGTGACTCCG3 |
| 7    | 5CCTCCTCACCCTCCTCCTC3 | 5GCCGCAGCAGCTTACAG3 |
| 8    | 5TCAGGGTGAACTTTGGGG3 | 5ATGGAGATGATCCCTTGCTG3 |
| 9    | 5CACGGTTTCTGTGCTCTCA3 | 5GTTGAAGGACAGCACAGACCTCG3 |
| 10   | 5GTGGTGTGTGTGAGGACAGGT3 | 5TGAGGACGACGAGATGAGG3 |
| 11   | 5CTGGACACTGCGGAGGAGAG3 | 5GGCAGAAAGCGCAGAGATG3 |

The present study provided written informed consent and the study was approved by the ethics committee of the Third Hospital of Hebei Medical University (Hebei, China).

**Muscle biopsy, histochemistry and immunohistochemical analyses.** Skeletal muscle biopsies were performed for diagnostic purposes on the three affected patients. Briefly, muscle biopsy specimens (0.5 cm diameter, 1.0 cm length) were collected from the biceps brachii, under local lidocaine anesthesia (Xiamen Kang Source Biotechnology Limited, Xiamen, China). The muscle biopsy specimens were frozen in isopentane cooled in liquid nitrogen, and 7 µm cryostat sections were made using an EM UC ultrathin section machine (Leica Microsystems, Mannheim, Germany), and were used for the subsequent histochemistry and immunofluorescence staining experiments. Morphological analysis of the muscle was performed using the following routine histological stains: Hematoxylin and eosin, modified Gomori trichrome, NADH-tetrazolium reductase, succinate dehydrogenase, adenine monophosphate deaminase and cytochrome c oxidase to assess enzymatic activity, adenosine triphosphatase staining experiments. Immunohistochemical analysis was performed using antibodies targeting procoagulant and anticoagulant membrane components. The following antibodies were used in 1:50 dilutions overnight at 4°C: Anti-emerin (Novocastra, Newcastle, UK), Biotinylated anti-mouse immunoglobulin G secondary antibodies (Novocastra) were then used for 1 h at 37°C. The avidin-biotin-peroxidase complex (ABC) method was used for signal detection (ABC kit; Vector Laboratories, Inc., Burlingame, CA, USA). After staining the muscle specimens were visualized, and the images were captured, using a BX51 confocal scanning laser microscope (Olympus Corporation, Tokyo, Japan).

**Gene analysis.** Genomic DNA from nine family members from Family 1, and six family members from Family 2 were extracted from peripheral blood (500 µl) using the Genomic DNA Extraction kit 5.0 (Takara Biotechnology Co., Ltd., Dalian, China), according to the manufacturer’s instructions. Control genomic DNA samples were obtained from 50 healthy Chinese unrelated individuals. DNA concentration was determined using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Inc., Wilmington, DE, USA). Each reaction required 50 ng genomic DNA, 10 pmol primer sets (sequences in Table I; Sigma-Aldrich, Shanghai, China), dNTP and Qiagen Multiplex PCR Master Mix (Qiagen, Hilden, Germany). The following conditions were used for multiplex polymerase chain reaction (PCR): 15 min at 95°C; 40 cycles of amplification at 95°C for 30 sec, 60°C for 30 sec and 72°C for 1 min; 35 cycles of denaturation at 95°C for 45 sec, 63.5°C for 30 sec and 72°C for 45 sec; 10 min at 72°C. Briefly, 50 ng genomic DNA from the patients was amplified using the hot-start PCR method. The DNA was subjected to PCR using the Qiagen Multiplex PCR Master Mix. Each PCR reaction consisted of 50 ng genomic DNA, 16.8 µl SYBR Green PCR Master Mix, and 250 nm specific primer pairs, in a total 25 µl volume. Using a pre-sequencing kit (USB Corporation, Cleveland, OH, USA), the PCR products were purified and sequenced by dye terminator chemistry using an ABI Prism 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). The resulting sequences were subsequently aligned and mutations were determined using Sequencher version 4.9 sequence alignment software (Gene Codes Corporation, Ann Arbor, MI, USA).

**Results.**

**Clinical data.** The clinical data of the three patients with EDMD are presented and summarized in Fig. 1 and Table II. The patients from Family 1 exhibited an AD mode of inheritance, whereas the patient from Family 2 was a sporadic
case (Fig. 2A and B). The levels of serum creatine kinase were marginally increased in Patient 2, and were normal in Patients 1 and 3. The electromyogram revealed myogenic damage in each of the three patients.

Pathological analysis of muscle biopsies. Routine histological analysis revealed dystrophic features in the muscle tissues, with degeneration, polyfocal necrosis, fiber splitting and moderate to marked fibrosis. Adipose tissue infiltration was also observed. In addition, atrophic and scattered regenerating fibers were noted. The ATP enzyme staining revealed a predominance of type I fibers. Immunofluorescence staining for emerin and lamin A/C revealed normal labeling along the membrane in all three cases (Fig. 3).

Cardiac evaluation. Patient 1 (33 years old) presented with panicky, shortened breathing and dyspnea. The heart border expended to the left and a heart rate of 42 beats per min (bpm) was recorded. Patient 2 (12 years old), the daughter of Patient 1, presented with no clear cardiac abnormalities upon initial clinical examination. Patient 3 (24 years old) presented with panicky and transient amaurosis with a heart rate of 52 bpm.

Further cardiac assessments revealed a variety of abnormalities in each patient (Figs. 4 and 5). The ECG and Holter of Patient 1 demonstrated complete atrioventricular inhibition and premature ventricular contractions of 26 beats/24 h. The UCG revealed that this individual had generalized cardiac enlargement, mitral and tricuspid valve regurgitation and decreased function of the left ventricle (Table II). The $^{99}$Tc$^{m}$-MIBI GPI assessment indicated left ventricular hypertrophy and varie-
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Gated changes in the left ventricular myocardium. Patient 2 suffered from sinus tachycardia, as determined by ECG and Holter. The UCG and $^{99m}$Tc$^{99m}$-MIBI GPI scan was normal. The ECG and Holter of Patient 3 revealed atrial flutter and the UCG demonstrated mild left ventricular enlargement and decreased diastolic function of the left ventricle (Table II). The $^{99m}$Tc$^{99m}$-MIBI GPI assessment revealed that myocardial perfusion was moderately decreased in the apex of the left ventricle.

**Discussion**

The *LMNA* gene is located on chromosome 1q21 and encodes the A-type lamins, lamin A and C, and two minor isoforms, C2 and A$^{A}D_{10}$, which arise through alternative splicing. The first 566 amino acids are identical in lamins A and C (10).
Structurally, the protein is composed of a central $\alpha$-helical dimerization domain flanked by a short amino-terminal head and a larger carboxy-terminal tail (11). Lamins A and C are located in the inner nuclear lamina and are widely expressed in skeletal and cardiac muscle, fat, blood vessels, skin and nerve tissue (12). Lamins A and C are considered to be important in maintaining the size and shape of the nucleus, and contribute to DNA replication, chromatin formation, RNA splicing, cell differentiation and signal transduction (10,11,13).

The three patients with EDMD identified in the present study were members of two families. The first family presented with an AD inheritance pattern, while the single patient with EDMD in Family 2 was the only known affected member, suggesting a sporadic mutation. Patients 1, 2 and 3 all presented with the typical clinical features of EDMD, including joint contractures, muscle weakness and cardiac involvement. The contractures initially affected the shoulders, elbows or ankles and impacted the posterior cervical muscles, thereby limiting neck flexion and forward spine flexion. Muscle weakness was slowly progressive and typically targeted to the peroneal and humeral distribution. With time, a limb-girdle distribution of muscle weakness developed. Immunostaining for emerin and lamin A/C revealed normal labeling along the nuclear membrane in all three cases. Although routine histological analyses revealed dystrophic features, these phenotypes lacked specificity for EDMD and, therefore, gene analysis was required.

Figure 4. Electrocardiogram traces of patients 1, 2 and 3. (A) Patient 1 exhibited a complete atrioventricular block. (B) Patient 2 exhibited sinus tachycardia. (C) Patient 3 exhibited atrial flutter.

Figure 5. $^{99}$Tc-MIBI-gated myocardial perfusion imaging of patients 1, 2 and 3. Patient 1 exhibited variegated changes in the left ventricular myocardium. Myocardial perfusion imaging was normal for Patient 2. Patient 3 exhibited decreased myocardial perfusion in the left ventricular apex.

Figure 6. LMNA gene sequences of patients 1, 2 and 3. Chromatograms of the heterozygous c.1583 C→G (p.T528R) mutation in exon 9 of LMNA.
It was revealed that each patient possessed the same heterozygous missense mutation, c.1583C→G, in exon 9 of LMNA, which caused a T528R amino acid change in the LMNA protein. A previous study suggested that the majority (76%) of AD-EDMD cases arise from sporadic mutations (14). The mutation identified in the present study was previously described in reports of Italian and German individuals, suggesting that this change exhibits an increased incidence despite ethnic differences (15,16). In the present study, Patient 3 was the only known individual from Family 2 to exhibit the T528R LMNA mutation, which supported the suggestion that it was a sporadic mutation.

Cardiac involvement is a frequent and serious complication in EDMD, with a high risk of sudden mortality (4). The degree of cardiac involvement negatively correlates with the severity of skeletal muscle weakness, however, it demonstrates a positive correlation with age and disease course (17). The predominant cardiac manifestations are progressive conduction system defects and/or sinus node dysfunction. The incidence of atrial fibrillation and atrial flutter is the highest, followed by atrioventricular inhibition (18). A number of patients present with ventricular tachycardia, ventricular fibrillation and other malignant arrhythmias, which are the leading causes of sudden mortality in patients with EDMD (15,17). Consistent with previous reports, the present study revealed various ECG findings, which were positively correlated with age and disease course in all three patients. The ECG demonstrated only sinus tachycardia in the youngest patient (Patient 2; 12-years old). By contrast, atrial flutter was observed in Patient 3 (24-years old), supporting sinus node dysfunction and complete atrioventricular inhibition in the oldest patient (Patient 1; 33-years old). This latter finding suggested severe conduction system defects.

It has been previously reported that cardiac involvement in AD-EDMD caused by LMNA mutations may be more severe, compared with other forms of EDMD (17,19,20) and are often accompanied by dilated cardiomyopathy (DCM), left ventricular dysfunction and heart failure (15,20). Pathologically, the normal myocardium is progressively replaced by fibro-adipose tissue, resulting in atrial and ventricular enlargement with progression towards clinical heart failure (15,21). The cardiac imaging of the three patients reported in the present study revealed that myocardial damage was progressive with increasing age and course of disease. The oldest patient (Patient 1) presented with left ventricular dysfunction, enlarged heart chambers and severe myocardial perfusion defects, which suggested dilated cardiomyopathy and heart failure. Patient 3 also developed decreased myocardial perfusion in the left ventricular apex. By contrast, the youngest patient (Patient 2) revealed no abnormal findings on cardiac imaging examination, therefore, follow-up observations are required as this patient ages.

Amino acid 528 is located on the highly conserved carboxy terminal tail of lamin A/C, suggesting that the T528R mutation may negatively impact the structure of the carboxy terminal tail and decrease protein stability (22,23). It has been reported that patients with AD-EDMD caused by the R541H LMNA mutation develop severe DCM, requiring heart transplant during childhood (9,24). Analysis of protein function has revealed that the lamin A/C.p.R541H mutation causes the highly conserved sequence of the carboxy terminal tail to mis-fold, leading to severe cardiac damage, which is consistent with the observations of the present study (25,26).

In conclusion, the present study identified three patients with EDMD exhibiting the same dominant LMNA mutation. These patients presented with a spectrum of severe cardiac abnormalities, including cardiac conduction system defects, cardiomyopathy and heart failure. Since LMNA mutations have been associated with at least six clinical disorders, including EDMD, the present case study provides additional mutational and functional data, which may assist in further establishing LMNA mutational variation and disease pathogenesis.

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