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Case Report

A novel mutation of *dystrophin* in a Becker muscular dystrophy family with severe cardiac involvement: from genetics to clinicopathology

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ABSTRACT

Background: Dystrophin gene defects are the pathogenic molecular basis of Becker muscular dystrophy (BMD), characterised by skeletal myopathy and cardiomyopathy. Because of the broad phenotype spectrum, it was difficult to use the traditional diagnostic method to achieve an early accurate diagnosis of BMD-associated cardiomyopathy.
Methods: We applied an in-house gene panel testing and a gene-filtering strategy to investigate the genetic defect in a four-generation family with 73 members. The proband had a heart transplant due to heart failure; the explanted heart was subjected to a careful pathological analysis.
Results: A novel small in-frame mutation (c.4998_5000 del CAG, p.1667 del Ala) of the *dystrophin* gene was identified and co-segregated in the affected family members. By using the image segmentation technology, we found the left ventricular free wall demonstrated severe fibrofatty replacement of cardiac myocytes from the epicardium to the endocardium.
Conclusion: We identified a novel *dystrophin* mutation (p.1667 del Ala), resulting in BMD-associated cardiomyopathy that demonstrated the pathological features of significant fibrofatty replacement in the sub-epicardial layer of the ventricle; further, the high-throughput sequencing is helpful for making an early diagnosis of BMD.

1. Introduction

Becker muscular dystrophy (BMD) is a hereditary disorder characterised by skeletal muscle and myocardial dysplasia. The estimated prevalence of BMD is about 1:12,000 population [1]. Compared with Duchenne muscular dystrophy (DMD), BMD has a relatively later onset and milder symptoms but a higher incidence with respect to the myocardial damage. About 70% of patients with BMD will gradually progress to cardiomyopathy in their 30s and eventually develop congestive heart failure [2].

The *dystrophin* gene is one of the largest genes, with 79 exons located on the X chromosome [3]. The gene encodes the protein dystrophin, a major intermediate protein of skeletal muscle and myocardium. By interacting with membrane proteins to form the dystrophin–glycoprotein complex, dystrophin plays a crucial role in structural and functional maintenance, including the coordination of mechanical stress transmission, organisation, signal transduction, and others [4]. Hence, mutations in the *dystrophin* gene may lead to dysfunction of skeletal and cardiac muscle [5]. Previous work has shown that different *dystrophin* mutations were confirmed to cause DMD and BMD, as well as X-linked dilated cardiomyopathy [3,6].

In this study, we investigated a four-generation Chinese family that included eight male members with a history of cardiomyopathy or muscular dystrophy; a novel small in-frame *dystrophin* mutation at exon 35 was identified by targeted next-generation sequencing. It is especially interesting that a pathological feature of the explanted heart (BMD-associated cardiomyopathy) was obvious fibrofatty replacement from the epicardium to the endocardium.

2. Methods

2.1. Patients and subjects

This study was approved by the Ethics Committee of Fuwai Hospital. All participants provided written informed consent. The probands provided written informed consent for donating the explanted heart for scientific research. This study investigated the medical history of family members (Fig. 1). The proband (III-33) and his nephew (IV-23) underwent examination with 12-lead electrocardiography (ECG), cardiac magnetic resonance (CMR), echocardiography, and blood work in our hospital. Information on the remaining six patients was collected from the local hospital. The proband received a heart transplant in our hospital, and the explanted heart was carefully examined.

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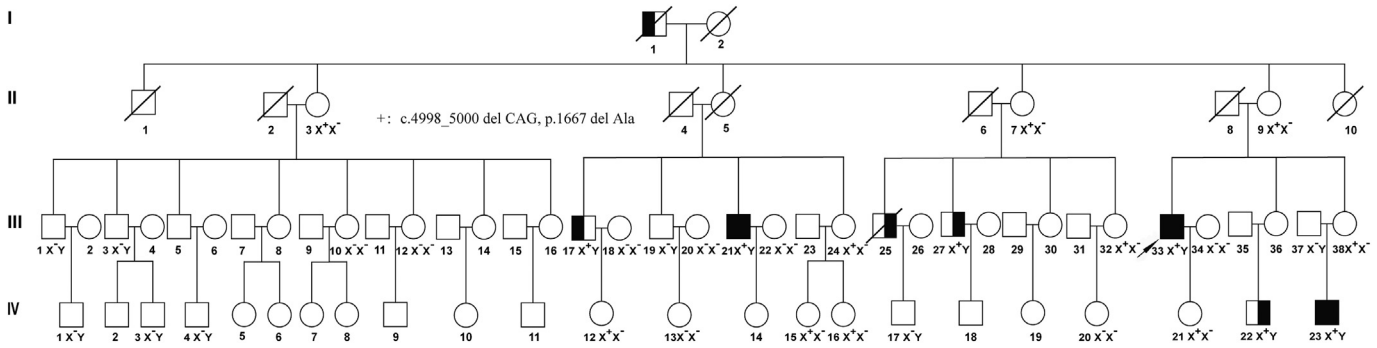

Fig. 1. Pedigree of the family with BMD. The generations (I-IV) are indicated at the left, and each individual is identified with a pedigree number; Squares indicate males and circles indicate females. Shadows indicate the phenotype in the individuals, i.e., cardiomyopathy (right half-filled), skeletal myopathy (left half-filled). Arrow denote the proband and slants denote dead individuals, + indicates *dystrophin* p.1667 del Ala mutation positive, and − indicates negative.

2.2. Genetic testing

The whole genome DNA was extracted from peripheral blood cell by using the DNeasy Blood and Tissue Kit (Qiagen, USA). The gene was first screened using multiplex ligation probe amplification (MLPA) on the proband, but no large intragenic deletion or duplication was detected. Next, we performed an in-house gene panel screening, containing 259 cardiomyopathy-related genes (Table S1). The variants were annotated by ANNOVAR and subsequently filtered according to the following criteria: (1) variants with an allele frequency>0.005 in the East Asian population according to the 1000 Genomes Project (http://browser.1000genomes.org) and Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org) were removed; (2) several types of variants in the coding region, including frameshift, non-frameshift indel, stop-gain, and splicing mutations, were retained; (3) for missense mutation, the pathogenicity of amino acid alteration was evaluated and filtered by SIFT, Polyphen-2, and MutationTaster programs. By comparing distinct species of amino acid sequences, the conservation analysis was performed in the UniProt database.

2.3. Histopathological examination of the explanted heart

The proband received a heart transplant in our hospital. We first observed the gross pathological characteristics of the explant and then dissected six representative specimens from the basal ventricular myocardium. The tissue was then dehydrated, embedded in paraffin, and cut into 3-μm-thick sections, which were stained with haematoxylin and eosin (H&E) and Masson's trichrome.

2.4. Immunofluorescence

First, the sections were incubated with a primary antibody and then with anti-rabbit or anti-mouse IgG. Primary antibodies included anti–C-terminal (ab15722; Abcam, UK), anti–rod domain (D8168; Sigma, USA) of dystrophin, and anti-sarcomeric α-actinin antibodies (ab9465 and ab137346; Abcam). The secondary antibodies: Alexa Fluor 488 goat anti-mouse IgG (A28175; Thermo Fisher, USA), Alexa Fluor 488 goat anti- rabbit IgG (A21206; Thermo Fisher), Alexa Fluor 594 goat anti-mouse IgG (A21203; Thermo Fisher) and Alexa Fluor 594 goat anti-rabbit IgG (A11012; Thermo Fisher), were diluted 1:500 in PBS and then incubated for 1 h at room temperature. The immunofluorescence preparations were analysed with the use of confocal microscopy (Zeiss LSM710/780; Zeiss, Germany).

3. Results

3.1. Clinical features

The proband (III-33), a 52-year-old male, was afflicted as a child by gastrocnemius muscle pseudohypertrophy. The height, weight of the proband were 174 cm, 78 kg separately, with a body mass index of 25.67 kg/m^2. Since 2003, he has shown exertional myalgia of the thighs

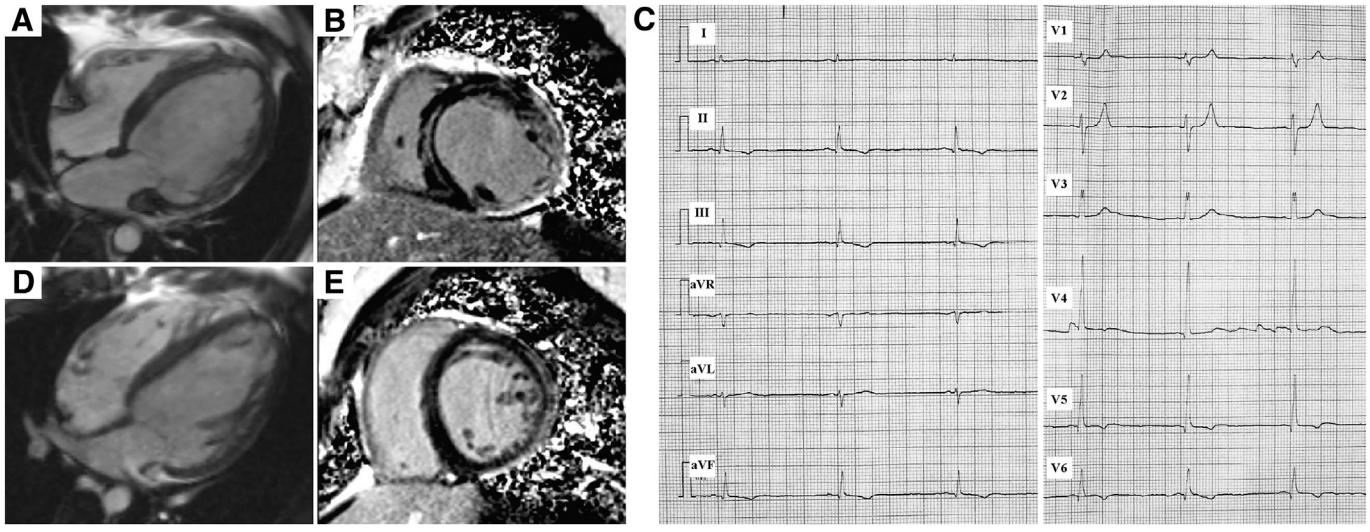

Fig. 2. ECGs and CMR of proband. (C) ECGs showing t wave inversion ($V_{5–6}$), prolonged PR segment, and incomplete left bundle branch block; (A, B, D, E) CMR showing left ventricular enlargement and thickening of regional ventricular wall.