A novel phenotype with splicing mutation identified in a Chinese family with desminopathy

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Abstract

Background: Desminopathy, a hereditary myofibrillar myopathy, mainly results from the desmin gene (DES) mutations. Desminopathy involves various phenotypes, mainly including different cardiomyopathies, skeletal myopathy, and arrhythmia. Combined with genotype, it helps us precisely diagnose and treat for desminopathy.

Methods: Sanger sequencing was used to characterize DES variation, and then a minigene assay was used to verify the effect of splice-site mutation on pre-mRNA splicing. Phenotypes were analyzed based on clinical characteristics associated with desminopathy.

Results: A splicing mutation (c.735+1G>T) in DES was detected in the proband. A minigene assay revealed skipping of the whole exon 3 and transcription of abnormal pre-mRNA lacking 32 codons. Another affected family member who carried the identical mutation, was identified with a novel phenotype of desminopathy, non-compaction of ventricular myocardium. There were 2 different phenotypes varied in cardiomyopathy and skeletal myopathy among the 2 patients, but no significant correlation between genotype and phenotype was identified.

Conclusions: We reported a novel phenotype with a splicing mutation in DES, enlarging the spectrum of phenotype in desminopathy. Molecular studies of desminopathy should promote our understanding of its pathogenesis and provide a precise molecular diagnosis of this disorder, facilitating clinical prevention and treatment at an early stage.

Keywords: Desminopathy; Cardiomyopathy; Desmin gene; Splicing mutation

Introduction

Desminopathy is a largely heterogeneous group of conditions involving inherited or sporadic myofibrillar myopathy, also called desmin-related myopathy or desmin myopathy, among others. In terms of its mode of inheritance, the autosomal dominant form is predominant. Although the incidence and prevalence of desminopathy are currently unclear, the data reported thus far suggest that it is a rare disease with a prevalence of no more than 0.05%.1,2 The disease onset ranges from childhood to late adulthood. Clinically, it is characterized as skeletal and cardiac myopathy.1,3 The most typical symptoms manifest as progressive skeletal muscle weakness from the distal lower extremities and cardiomyopathy, as well as severe arrhythmia and respiratory problems.1,4,5 It is progressive and may shift from its initial clinical presentation with age. The prognosis is poor, with a meta-analysis revealing a mortality rate of 26% (27/104) among desmin mutation carriers.1 The causes of death include sudden cardiac death, heart failure, respiratory insufficiency, chest infection, and iatrogenic complications of cardiac treatment. The manifestation of cardiac disease is the major cause of premature death in desminopathy.6 The pathology of myopathy is characterized by desmin-positive protein aggregates and degenerative changes of the myofibrillar apparatus.1,7 Electron micrography of muscle tissue

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shows disruption of the cytoskeleton, and the accumulation of aggregated proteins and autophagy vacuoles.[7,8]

Desminopathy is caused by destruction of the structure of desmin, which is a class III intermediate filament protein. The mature desmin molecule contains 470 amino acids and is composed of alpha-helical rods consisting of 308 amino acid residues flanked by globular N-terminal (Head) and C-terminal (Tail) structures, which include four highly conserved alpha-helical subdomains (1A, 1B, 2A, 2B) linked by non-helical linkers.[12,5,9] Desmin is encoded by DES (OMIM #125660), which is located on chromosome 2q35 and consists of 9 exons within an 8.4-kb region.[10]: DES mutations, including missense, non-sense, and splicing mutations, play a critical role among the pathogenic inherited factors associated with desminopathy.[11-13]

Over the past 2 decades, since the 1st discovery of a pathogenic mutation in DES,[14] over 60 DES mutations have been reported, predominantly in Caucasian populations and mostly missense mutations.[9] Phenotypes of cardiomyopathy mainly involved hypertrophic, dilated, restrictive, and arrhythmogenic right ventricular cardiomyopathies. Moreover, no splicing variations in DES have been studied in the Chinese population. Desminopathy with a phenotype of non-compaction of ventricular myocardium (NVM) has not been reported before.

Methods

Ethical approval

This study was approved by the Ethics Committee of Fuwai Hospital and carried out in accordance with the Declaration of Helsinki. Each participant provided written informed consent.

Subjects

The proband (individual III 14), a 46-year-old male with symptoms of chest distress, shortness of breath, and syncope, was admitted to Fuwai Hospital, Beijing, China. The index patient had been diagnosed as having complete left bundle branch block at the age of 8 during a physical examination [Figure 1A], but did not receive any further treatment at that time. At the 1st medical consultation in September 2007, the patient presented symptoms of syncope, loss of consciousness, palpitation, and chest tightness, and an electrocardiogram showed 3rd-degree atrioventricular block (AVB) [Figure 1B]. Therefore, a pacemaker was implanted for symptom relief by ventricular pacing [Figure 1C]. He initially experienced fatigue of the distal extremities at age of 30 years. With disease progression, the symptom of fatigue spread from distal to proximal limbs. He was also admitted to a neurology department, but did not receive specific treatment there, and was diagnosed with desminopathy by neuropathologic examination of skeletal muscle from the right bicep. The pathologic results showed abnormal myofibril deposits, which were positive for desmin immunohistochemical staining, accompanied by myofibril hypertrophy and atrophy. Ultrastructural analysis of skeletal muscle revealed typical pathologic features of myopathy, such as myofibril hypertrophy, broken structure of desmin, and high-electron-density deposits. In recent years, the symptom of abdominal distension and diarrhea repeatedly developed and persisted for several days each time.

During hospitalization, he underwent further examination including echocardiography, cardiac computed tomography (CT), and 24-h dynamic electrocardiography. A total of 9 family members (III 1, III 3, III 14, IV 1, IV 2, IV 3, IV 6, IV 8, and IV 9) were recruited in this study. The pedigree chart is shown in Figure 2A. Peripheral venous blood was collected from each subject for biochemical and genetic analyses. A clinical survey was also conducted for each participant.

Genetic testing

Genomic DNA was extracted from all subjects’ blood using the QIA amp DNA Blood Midi kit (QIAGEN, Hilden, Germany). Based on the patient’s phenotype of desminopathy, DES was chosen as the target gene for analysis.[9] Therefore, polymerase chain reaction (PCR) was used to amplify all exons of the DES (NM_001927) using 9 pairs of primers (Supplementary material, http://links.lww.com/CM9/A1). All PCR products were sequenced using an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA). The identification of variants was performed using Chromas software (version 2.22, Technelysium, Brisbane, Australia).

In silico analysis

To predict the potential to affect splicing, the following splice-site prediction programs were used: (1) Splice Site Finder[15] (http://www.inter-activebiosoft-ware.com); (2) MAXENT[16] (http://genes.mit.edu/burgelab/maxent/Xmaxentscan-scoreseq.html); and (3) GENESPLICER[17] (http://www.interactivebiosoftware.com/doc/ala-mutvisual/2.5/splicing.html).

Minigene splicing assay

A minigene splicing assay was carried out to validate whether this variant affects splicing products. A splicing reporter minigene assay was established by PCR amplification of wild type (WT) and mutant genomic DNA sequences, as described previously.[18,19] The amplified target sequences contained exons 2 to 4 of DES, together with 520 base pairs (bp) from the 5’ and 3’ flanking intronic sequences. DES minigene fragments were digested with the restriction endonucleases BamHI and MluI (New England Bio Labs) and cloned into pcAS2 reporter vectors (provided by Mario Tosi, Rouen Institute for Biomedical Research, France). Sanger sequencing was performed to evaluate whether the WT and mutant type (MT) expression vectors had been constructed successfully. Two kinds of expression vector were transfected into HEK293T cells (Cell Resource Center, Peking Union Medical College, China). All cellular RNAs were extracted using TRIzol reagent (Life Technologies, Grand Island, NY). Reverse-transcription PCR (RT-PCR) was performed using the GoScript Reverse Transcription System (Promega Corporation, Madison, WI, USA). Complementary
Figure 1: Electrocardiograph shows the aggravation of arrhythmia in the proband. (A) Complete left bundle branch block. (B) Third-degree atrioventricular. (C) Ventricular pacing after implanted a pacemaker.

Figure 2: A splicing mutation in $\text{DES}$ in a Chinese family with desminopathy. (A) Pedigree of this family. The black arrow indicated proband. (B) Sanger sequencing chromatogram shows a heterozygous $\text{c.735+1G>T}$ splicing mutation in $\text{DES}$. $\text{DES}$: desmin gene.
DNA sequences, including WT and MT, were amplified using the following primer pairs (forward primer: 5'-CTGACCCCTGCTGACCCCTCT-3'; reverse primer: 5'-CTCTACCCAGCCTTCTCAGCAA-3'). The resulting PCR products were separated by electrophoresis on a 1% agarose gel and analyzed by Sanger sequencing.

**Results**

**Clinical features**

In the recent events focused on here, upon hospitalization, 24-h dynamic electrocardiogram revealed atrial flutter, ventricular tachycardia, and AVB in the proband. Hence, he was treated with an implantable cardioverter defibrillator (ICD) to prevent sudden death. Echocardiography showed biaatrium enlargement (left atrial diameter: 42 mm; right atrial diameter: 38 mm) and restriction of cardiac diastolic function (E/A > 2) [Figure 3A]. CT scan also confirmed biaatrium enlargement, but no abnormality of myocardial density [Figure 3B]. All the progress of desminopathy in the proband is shown in Table 1.

The proband’s father (II 5), who had been implanted with a pacemaker at the age of 42, had also experienced cardiomyopathy, severe arrhythmia, and muscle weakness and died of heart and renal failure 14 years later. Moreover, both the proband’s aunt (II 3) and his cousin (III 10) had died after giving birth to their 4th (III 12) and 2nd (VI 8) children at the ages of 34 and 29 years, respectively. In addition, his nephew (VI 8) had been diagnosed with NVM by magnetic resonance imaging [Figure 3C] and 3rd-degree AVB with the symptoms of chest distress and edema of lower extremity, which were treated with a pacemaker when he was 22 years old. Currently 2 patients were detected with elevated serum creatine kinase level (normal range: 0–200 IU/L), 399 IU/L (II 14) and 320 IU/L (VI 8), respectively. These abnormal clinical features were not observed in others in this family.

**Analysis of DES mutation**

The results of Sanger sequencing of DES are shown in Figure 2B. The proband and his nephew (VI 8) were heterozygous for a nucleotide G>T transversion in the donor splice site of intron 3 (c.735+1G>T). Among the rest of the subjects, there were no carriers of this mutation.

In silico analysis predicted the splice-site mutation (c.735+1G>T), using splicing prediction software including Splice Site Finder, MAXENT, and GENESPLICER. It also indicated that it was likely to influence splicing products of pre-mRNA.

**Exon skipping identified by minigene assay**

To examine the functional effect of the c.735+1G>T mutation in DES on pre-mRNA splicing, a mutant DES minigene was transfected into HEK293T cells for transcription. Upon 1% agarose gel electrophoresis, transcripts generated by WT and MT minigenes were located near the 500-bp marker (DL2000; Takara, Japan).

Table 1: Desminopathy progression with age in the proband

| Age     | Symptoms or signs                          | Therapies                          |
|---------|--------------------------------------------|------------------------------------|
| 8 years | Complete left bundle branch block          | 0                                  |
| 30 years| Muscle weakness of the distal extremities  | Pacemaker                          |
| 36 years| Syncope, loss of consciousness, palpitation, chest tightness, third-degree AVB and muscle weakness of the distal extremities | Avoid heavy activities |
| 40 years| Lower limb weakness, abnormal gait         | Domperidone, montmorillonite powder and bifidobacteria |
| 41 years| Upper limb weakness                        | ICD and amiodarone                 |
| 44 years| Abdominal distension and diarrhea          |                                    |
| 46 years| Syncope, atrial flutter, ventricular tachycardia, AVB, developed skeletal muscle weakness and RCM |                                    |

AVB: Atrioventricular block; ICD: Implantable cardioverter defibrillator; RCM: Restrictive cardiomyopathy.
in the gel. The RT-PCR sequencing results showed that the WT and MT minigenes produced 524- (expected size) and 428-bp bands, respectively [Figure 4B]. By sequencing the 2 different products, the WT transcript was shown to contain exons arranged sequentially from exon 2 to exon 4, while in the MT transcript, exon 2 was followed by exon 4, but with exon 3 missing. This deletion of exon 3 resulted in a reduction of 96 bp (32 codons) in the length of the MT transcript [Figure 4C].

Discussion

In the present study, we investigated a desminopathy-associated pedigree and identified a novel phenotype with desminopathy and the possibility of pathogenicity of a splice-site mutation using Sanger sequencing and minigene assay. Our results demonstrated that the proband and his nephew (VI 8) are carriers of a heterozygous splice-site mutation (c.735+1G>T) of DES, for which functional assessment revealed abnormality of the RNA splicing process and exon 3 skipping. However, clinical phenotypes were totally different in the 2 affected individuals. These findings are significant because they not only provide possible genetic pathogenic evidence for desminopathy associated with a splice site of DES, but also enlarge the spectrum of phenotype.

Desmin is a critical component of the extra-sarcomeric cytoskeleton. Its biologic functions include structural and functional alignment and anchoring of myofibrils, as well...
as the positioning of cell organelles and conduction of cell signaling pathways. An abnormal structure of desmin could thus produce a range of dysfunctions. DES, a single-copy gene, encodes desmin and is mainly expressed in muscle cells. DES mutation is the most common etiologic factor of desminopathy at the genetic level. Pathogenic mutations can impact upon the mRNA transcription and translation of desmin, generating structurally and functionally abnormal copies of the desmin protein. In addition to damage to the cytoskeletal network, mutant desmin impairs protein interactions, cell signaling cascades, mitochondrial functions, and protein quality control mechanisms. In the case of DES splicing mutation, this causes defects in the function of desmin, which may increase the fragility of myofibrils and impair their contraction.

Several lines of evidence indicated that DES splice-site mutations were responsible for the desminopathy exhibited by our patients. First, a heterozygous splice-site mutation in DES was identified by Sanger sequencing. Moreover, in silico analysis predicted that this mutation would affect splicing at the pre-mRNA level. Third, by expressing genomic DNA fragments, we showed that this kind of mutation inactivated the splice site and caused exon 3 skipping [Figure 4A]. Exon 3 consists of 96 bp and encodes a total of 32 codons. Although deletion of exon 3 would not disrupt the reading frame, it would result in the translation of a truncated desmin protein lacking 32 amino acids. The heptad repeat pattern is in turn disrupted by this translation of a truncated desmin protein lacking 32 amino acids. This would not disrupt the reading frame, it would result in the expression of genomic DNA fragments, we showed that this kind of mutation inactivated the splice site and caused exon 3 skipping [Table 2], which results in the deletion of 32 amino acids from Asp214 to Glu245, instead of in-frame fusion between exon 2 and exon 4. Unlike most missense mutations affecting the highly conserved YRKLLEGGE motif of the 2B helix, all of the reported splicing mutations only disrupt the structure of the 1B subdomain in desmin. Cetin et al found a special splice-site mutation (c.1289–2 > G) in DES, which causes a limb girdle muscular dystrophy phenotype instead of desminopathy. Therefore, research performed to date indicates that nearly all pathogenic splice-site mutations in DES are gathered upstream or downstream of exon 3.

Table 2: Splice-site mutations resulting in deletion of 32 codons encoded by exon 3 in desmin gene

| Sequence  | Acceptor site (end of intron 2) | Exon 3 | Donor site (start of intron 3) | Reference |
|-----------|---------------------------------|--------|--------------------------------|-----------|
| Wild type | ... tccca GAC ... GAG          | gtatac | ... Dunand et al (2009)         |
| c.640-2A>C| ... tccca GAC ... GAG          | gtatac | ... Kostareva et al (2006)      |
| c.640-2A>G| ... tccca GAC ... GAG          | gtatac | ... Goldfarb et al (2004)       |
| c.640-1G>A| ... tccca GAC ... GAG          | gtatac | ... Park et al (2000)           |
| c.734A>G  | ... tccca GAC ... GAG          | gtatac | ... Nalini et al (2013)         |
| c.735+1G>A| ... tccca GAC ... GAG          | atatac | ... Goldfarb et al (2004); Gudkova et al (2013) |
| c.735+1G>T| ... tccca GAC ... GAG          | atatac | ... Ojrzyńska et al (2017)      |
| c.735+3A>G| ... tccca GAC ... GAG          | gtatac | ... Dalakas et al (2000); McDonald et al (2012); Pugh et al (2014) |

Nucleotide replacements are bolded.
proband’s father (II 5), and at 18 years of age in the proband’s nephew (VI 8). With the development of desminopathy, the index patients also underwent the implantation of an ICD against ventricular tachycardia. Therefore, diagnosis and therapy for severe arrhythmia earlier should be pay high attention to prevent sudden death. Furthermore, the proband occasionally manifested diarrhea, so we suspect that this splicing mutation had affected his gastrointestinal smooth muscle.

DES mutations are pleiotropic, causing different phenotypes such as various cardiomyopathies, skeletal myopathies, smooth muscle myopathy, and respiratory dysfunction, even among mutations located in close vicinity to each other. Moreover, 2 kinds of splicing mutations are pleiotropic, causing different phenotypes. Affected his gastrointestinal smooth muscle.

Diarrhea, so we suspect that this splicing mutation had earlier should be pay high attention to prevent sudden cardiac arrest, and other complications. This study extends our knowledge of the spectrum of phenotype and their geographic distribution in desminopathy.

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**Conflicts of interest**

None.

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