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Detection of pathogenic mutations and the mechanism of a rare chromosomal rearrangement in a Chinese family with Becker muscular dystrophy

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ABSTRACT

Objective: The objectives of this research are to genetically diagnose a family with Becker muscular dystrophy (BMD), to explore the molecular mechanism of the disease, and to predict the possibility of BMD development in two individuals who have not yet reached the age of onset (young individuals).
Methods: The multiplex polymerase chain reaction was first employed to screen dystrophin (DMD) gene deletions, and the locations of deletion breakpoints were identified using the Sequenom platform and long-range PCR. Sanger sequencing was then performed for the undeleted exons.
Results: All BMD patients and a young individual carry a deletion spanning exons 45 to 53 and an unreported missense mutation on exon 11 of the DMD gene. This point mutation was screened in 412 healthy individuals and heterozygous genotype was found in two females. Determination of deletion breakpoints demonstrated a 330-kb deletion and there was a 9-bp insertion between the breakpoints. This 9-bp could match a reference sequence located within the deleted region.
Conclusions: Two mutations of the DMD gene coexist in this family. One young child has a high disease risk. Pathogenic potential of the point mutation requires further investigation. The rare chromosomal rearrangement may be caused by short-nucleotide sequence capture or other unknown mechanisms.

1. Introduction

Duchenne and Becker muscular dystrophies (DMD and BMD) are the most common X-linked recessive childhood neuromuscular diseases. Two-thirds of the cases are inherited through carrier mothers or arise from germ line mosaicism, while the others originate through somatic mutations [1,2]. The age of BMD onset is usually later and the incidence is lower compared to DMD. It is estimated that BMD occurred in 1/18,000 newborn boys, and symptoms are usually mild, with a slow disease progression [3]. BMD begins between 5 and 20 years of age, and majority of the patients lose their ability to walk 15 to 20 years after the disease onset [3,4].

Genetically, DMD and BMD are caused by mutations in the human dystrophin (DMD) gene, the largest human gene. DMD gene spans about 2.4 Mb on Xp21.2, and consists of 79 exons. Dystrophin protein localizes to the sarcolemma of human skeletal muscle fibers, and has been demonstrated to be important in maintaining normal functions of muscle cells by linking cytoskeleton to extracellular matrix [5–7]. This protein consists of four functional domains: N-terminal region (exons 1 to 8); central rod domain (exons 9 to 63); cysteine-rich region (exons 64 to 68); and C-terminal domain (exons 69 to 79). Mutations in the DMD gene may lead to DMD or BMD where the difference can be explained by the reading frame rule [8]. Mutations destroying the DMD mRNA reading frame lead to severe DMD, while mutations preserving the reading frame produce proteins with certain functions and result in mild BMD. The reading frame rule is applicable in 92% of DMD and BMD patients [8,9].

Deletions in DMD gene have been identified in about 65% of DMD and BMD patients, and the rest are caused by gene duplications, point mutations or small indels. 5′ terminus and exons 45 to 53 are two deletion hotspots [10,11]. To date many techniques have been successfully used to detect DMD gene mutations. For deletions or duplications, southern blotting, multiplex PCR, multiplex ligation-

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dependent probe assay (MLPA) and array-comparative genomic hybridization (array CGH) can be applied [12–15]. With no gross mutation identified, sequencing of all amplified exons or cDNA can be performed to check point mutations or small indels [16,17]. As a final step, chromosome analysis is conducted [18]. Recently, Lim et al. [19] employed target region capture and second-generation sequencing with paired-end reads to detect DMD gene mutations. This method screens the whole DMD gene, including introns, and can simultaneously detect all types of mutations.

Here we report two co-inherited mutations in a Chinese family with BMD collected from Linyi, Shandong Province, China and predict the possibility of BMD development in two individuals who have not yet reached the age of onset (young individuals). The pathogenic potential and formation mechanism of the two mutations are discussed.

2. Materials and methods

2.1. Clinical data

The pedigree chart of the family with BMD is shown in Fig. 1. Diagnosis of BMD was based on clinical manifestations, physical examinations, electromyography and muscle biopsy (Supplementary text and Supplementary Fig. 1). Eight samples from three generations of this family were collected, including three affected males who developed BMD at the ages of 12, 15, and 15, respectively. In addition, 412 healthy subjects were recruited as a control group during their physical examination in Yantai Economic and Technology Development Area Hospital (Yantai ETDA Hospital). The normal controls, self-reported as Han Chinese, included 214 males and 198 females with ages ranging from 24 to 52. The possibility of DMD/BMD in these subjects was excluded clinically. Informed consent was obtained from all the subjects before the initiation of this study. The present study was approved by the Ethics Committee of the Shandong Cancer Hospital and Yantai ETDA Hospital.

2.2. Extraction of genomic DNA

Peripheral blood (2 ml) from the patients and their relatives was collected in EDTA-filled tubes. The genomic DNA was extracted using the QIAamp DNA Blood Midi Kit (QIAGEN), according to the manufacturers' instructions. The DNA concentration and purity were determined using NanoDrop 8000 UV–vis spectrophotometer, and the DNA was stored at −20 °C.

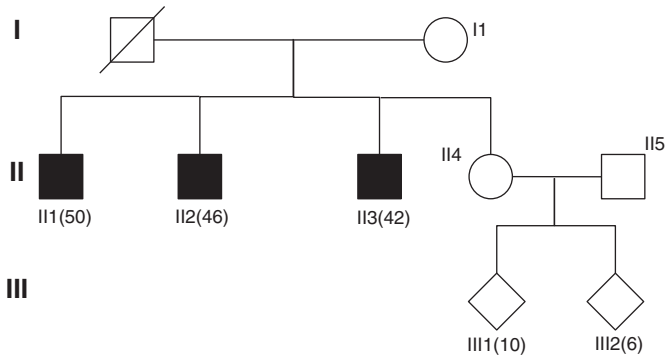

Fig. 1. Pedigree structure of the BMD family. Squares and circles denote males and females respectively; rhombi indicate young males who had not yet reached the age of BMD onset; black squares represent male BMD cases. I1, II1–II5, III1 and III2 are sample identification numbers; the number in the brackets is the subject's age when the samples were collected in 2010.

2.3. Multiplex PCR

The primers designed by Chamberlain and Beggs [12] were used to detect DMD gene deletions. In order to distinguish amplified fragments effectively, 13 exons in the deletion hotspots were amplified for four separate times. PCR amplification was performed in a total volume of 25 μl containing 5 μl of 5× PCR buffer, 3 μl of dNTP mixture, 1 μl of forward and 1 μl of reverse primers (10 μmol/l), 1 μl of template DNA, and 0.5 μl of TransStart FastPfu DNA Polymerase (TransGen Biotech), under the following conditions: pre-degeneration at 95 °C for 2 min; 30 cycles of degeneration at 95 °C for 20 s, annealing at 60 °C for 20 s, and extension at 72 °C for 15 s; and extension at 72 °C for 5 min. The PCR product was stored at 4 °C.

2.4. Sanger sequencing

PCR amplification and Sanger sequencing were performed for undeleted exons using the aforementioned protocol in Section 2.3. The primers were designed using software Primer Premier 5 according to the NCBI probe and synthesized by Sangon Biotech (Shanghai) Co., Ltd. The PCR products were sequenced by Applied Biosystems 3730XL sequencer (Applied Biosystems Inc., CA, USA).

2.5. Genotyping with Sequenom

In order to determine the breakpoints of gene deletion, we genotyped a number of loci located in deletion boundary regions. We also genotyped 412 normal subjects to evaluate the pathogenic potential of a point mutation. The candidate loci were genotyped using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS; Sequenom). All experiments were carried out at the Beijing Institute of Genomics. Primers were designed using the software MassARRAY Assay Design 3.0. Following PCR amplification and allele-specific extension, the genotyping result was analyzed using the MassARRAY Typer software (Sequenom, Inc.; San Diego, USA).

2.6. Long-range PCR

After identification of the approximate deletion region using the Sequenom platform, the genome sequence covering 1000 bp upstream of the 5′ breakpoint and 1000 bp downstream from the 3′ breakpoint was downloaded from the UCSC Genome Browser website. Primers were designed at two ends of the downloaded sequences, and long-range PCR was performed using LongAmp Taq DNA polymerase (New England Biolabs). Sanger sequencing was then carried out. The sequences were aligned using Blat in the UCSC Genome Browser to identify the breakpoint locations and the corresponding template sequences. The 100-bp reference sequences on both sides of the breakpoints were analyzed using the RepeatMasker tool (http://www.repeatmasker.org/) to find interspersed repeats and low complexity DNA sequences.

3. Results

3.1. Deletion of DMD gene exons 45 to 53 in BMD patients and a young individual

Multiplex PCR followed by agarose gel electrophoresis and ethidium bromide (EB) staining revealed a deletion of exons 45 to 53 of the DMD gene in BMD patients II1, II2 and II3. In accordance with the reading frame rule, the protein reading frame was not destroyed by the 1434-bp mRNA deletion. No deletion was detected in I1, II4 and II5, but the two females I1 and II4 may be heterozygous which could not be revealed by multiplex PCR. In addition, there was no deletion discovered in the