Original Article

Two different intragenic dystrophin deletions in a Chinese nuclear family with dystrophinopathies

Tingting Zhang¹, Feifei Hou², Qingbiao Fu³, Yanying Li⁴, Kunpeng Wang¹, Xiangyu Zhang⁵

Departments of ¹Oral and Maxillofacial Surgery, ²Pediatric Dentistry, Stomatological Hospital, Tianjin Medical University, Tianjin, P. R. China; ³Department of Stomatology, Heze Traditional Chinese Medicine Hospital, Shandong, P. R. China; ⁴Department of Endocrinology, Tianjin Nankai Hospital, Tianjin, P. R. China

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Abstract: The dystrophinopathies are caused by mutations in the dystrophin gene and are the allelic Becker and Duchenne muscular dystrophy. This study reports the presence of two independent mutations in a Chinese nuclear family with dystrophinopathies. Genomic DNA was isolated from the peripheral blood samples of 8 available family members across 3 generations. Mutation analysis of the dystrophin gene was performed using amplified polymerase chain reaction and the first and second generation sequencing. Deletions of exons 45-47 in the dystrophin gene were identified in the proband and his nephew, whereas his younger son had deletions of exons 48-52. Moreover, the proband’s mother and elder sister were heterozygous for deletions of exons 45-47. No mutation was detected in the other participants especially the proband’s wife and elder son. This molecular genetic data reveals that the mutation of the proband’s younger son was a de novo mutation. It highlights the importance of screening the entire dystrophin gene, rather than targeted mutation analysis, for additional patients who are tested in order to detect de novo mutations.

Keywords: Dystrophinopathies, DMD, BMD, dystrophin gene, deletion

Introduction

The dystrophinopathies are caused by mutations in the dystrophin gene and are the allelic Becker and Duchenne muscular dystrophy [1]. There are two major allelic diseases caused by mutations in the dystrophin gene: Becker muscular dystrophy (BMD) and Duchenne muscular dystrophy (DMD). Becker muscular dystrophy is milder and compatible with the affected man having children. Duchenne muscular dystrophy is more severe and generally incompatible with the affected man having children. The dystrophin gene is one of the largest genes (approximately 2.5 million base pairs, encoding 79 exons) identified to date, and because of its size and structural characteristics, it is susceptible to a high sporadic mutation rate and has shown a wide mutational spectrum [2, 3]. Mutations causing dystrophinopathies include deletions (60%), duplications (10%), and point mutations (30%). Deletions can occur to a various extent in the gene, from one exon to all exons, and the deletions preferentially lie on two hot spots: the major site encompassing exons 45-52 and the minor region including exons 3-19 [4-6]. Due to the X-linked recessive nature of the disorder, males carrying the mutated gene are affected while females become carriers [5]. In sporadic cases, mothers may be carriers or there may be a new mutation. It is widely believed that in 2/3 of cases, mothers were carriers and the remaining 1/3 were due to de novo mutations [7, 8]. Previous studies have demonstrated that the frequency of de novo deletions is near 60% with no preference for either hot spot [6, 7].

There has been some report with respect to two distinct mutations among nuclear family members with dystrophinopathies. Here, we share an extraordinary case that reports the presence of two independent mutations (del ex 45-47 and del ex 48-52) in the dystrophin gene
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Figure 1. Pedigree of the DMD family. The arrow indicates the proband. Roman numerals specify pedigree position in all affected members and/or individuals.

in a Chinese father and son, one causing Becker muscular dystrophy in the father and his elder sister’s son and one causing Duchenne muscular dystrophy in the son. An exon 45-47 deletion was identified in the proband and his nephew, whereas his younger son has a deletion of exons 48-52 in the dystrophin gene. In other words, we found a coexistence of two distinct intragenic dystrophin deletions among nuclear family members. In addition to analyzing family specific mutations in families with multiple affected individuals, screening all exons will provide information about different dystrophin mutations as detected in our study family.

Materials and methods

Identification and clinical assessment

The proband was initially evaluated in the Department of maxillofacial surgery, Stomatological Hospital of Tianjin Medical University. Our study was conducted with the informed consent of all 8 participants and was approved by the Ethical Committee of Tianjin Medical University, Tianjin, China. The 8 participants came from the same pedigree with 3 generations including 2 BMD patients and 1 DMD patient (Figure 1). They are the proband II2, his mother I1, elder son III1, younger son III2, his elder sister II3, brother-in-law II4 and his nephew III3. Samples of peripheral blood from the 8 family members were collected into tubes containing EDTA as an anti-coagulant.

DNA extraction

Genomic DNA was isolated from peripheral blood leukocytes using the QIAamp Blood Midi kit (Qiagen, Germany) according to the manufacturer’s instructions. The concentration and quality of genomic DNA were determined by measuring the UV absorbance at 260 nm and 280 nm (A260/280) and by gel electrophoresis.

Sequencing and data analysis

Single-read sequencing was performed by NextSeq500 (Illumina). Raw data were obtained in the format of Fastaq. Raw data was transformed into identifiable base sequences with the software CASAVA (1.8.2). Then, Align analysis, SNP analysis and DIP analysis were conducted to obtain information of mutation sites from targeted regions. Finally, protein damage analysis was conducted to qualitatively predict the probability of the results by PolyPhen-2.2.2, allowing mutation sites that need further validation to be obtained. The gene sequences of the above mutation sites were obtained from GenBank. The primers were designed by the website Primer Z and then synthesized. The mutation sites were amplified by PCR and then sequenced by first-generation sequencing. The obtained sequences were aligned with the previous results, and false positive sites obtained by NGS (next generation sequencing) were ruled out.

Results

Clinical findings

The affected patients’ muscular strength test and an MRI of the limbs were conducted at the Beijing Armed Police Hospital. We identified two patients with BMD and one with DMD in this family. The proband’s motor function has been declining for 25 years since he was 16 years old. His limb MRI suggests his bilateral gluteal muscle, vastus lateralis, vastus medialis obliquus, vastus medialis oblique, biceps femoris and adductor magnus were undergoing
atrophy. The same results occurred in his younger son. The young baby was able to move about freely until the third year after his birth. The baby became clumsy in his movement, easily fell down and walked wobbly. Both the baby and his father’s bilateral gastrocnemius showed pseudohypertrophy. The baby’s tendon reflex was weakening while his father’s disappeared. Another patient in this pedigree is a 16-year-old boy with slight clinical signs that reflected an abnormal gait in recent years. We surmise this minor defect was due to his young age. It is possible that the symptoms will be much worse as time goes on, similar to his uncle.

**Molecular analysis of the dystrophin gene**

A deletion of exons 45-47 was detected in the proband and his elder sister’s son (Figure 2). The proband’s younger son was found to have a deletion of exons 48-52 (Figure 3), which is completely different from other affected members within the family. Moreover, the proband’s mother and elder sister were heterozygous for the deletion of exons 45-47, as shown in Figure
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No mutation was detected in the other family members tested especially the proband’s wife and elder son. This result demonstrated that the intragenic deletion of the baby was due to de novo mutation in the nuclear family.

**Discussion**

More than one mutation in multiple affected members of the same does sometimes occur, but only a few families have been reported to date [8-14]. We found an interesting familial case where two independent intragenic deletions are present in the father and his son. An exons 45-47 deletion was identified in all the BMD patients and carriers. Furthermore, different mutation patterns corresponded with clinical symptoms, which is the most interesting point in this pedigree. The baby’s onset age was even younger and was much more severe compared with his cousin. According to the current situation, we infer that the youngest patient may be the most severe case.

Coexistence of two different intragenic dystrophin mutations in cross-generation individuals with dystrophinopathies is very uncommon [14]. Considering the mutation pattern with two mutations co-existing in one nuclear family, we proposed that the possible hereditary mechanism is that the proband’s younger son’s deletion mutation is a de novo mutation. The DMD gene has a high new mutation rate, meaning that there are a large number of patients with de novo mutations. Most often this will occur in a boy with no family history. Compared with other de novo mutations, it has three different points. One is that it occurred in a pedigree, whereas others usually arise as sporadic cases. Second, the two deletion patterns occurred between nuclear family members. Lastly, compared with his cousin, the baby who carried the de novo mutation had a more severe clinical phenotype. These three points are different from other de novo cases and need further explanation in regards to the hereditary mechanism.

Dystrophinopathies is a common neuromuscular disease. All reports published until now emphasize that the dystrophin gene is particularly prone to mutations and the gene has shown a wide mutational spectrum, although partial deletions represent 60-65% of all mutations [9]. Previous studies have demonstrated that the frequency of de novo deletions was near 60% with no preference for either hot spot, according to the reports of several cohorts [9, 10]. In the present study, two different mutations appear in a nuclear family with both DMD and BMD. The pathogenic potential of the unreported rare mutation and cross clinical phenotypes require further investigation and further explanation. Our findings suggest the impor-
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tance of screening the entire dystrophin gene, rather than targeted mutation analysis, for additional patients who are tested. Therefore, broad mutation screening in families with multiple affected individuals might detect additional dystrophin gene mutations and provide more accurate prenatal diagnoses.

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Disclosure of conflict of interest

None.

Address correspondence to: Xiangyu Zhang, Department of Pediatric Dentistry, Stomatological Hospital, Tianjin Medical University, 12 Qixiangtai Road, Tianjin 300070, P. R. China. Tel: 86-22-2332102; E-mail: xzhang04@tmu.edu.cn
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