Duchenne Muscular Dystrophy in a Female Patient with a Karyotype of 46,X,i(X)(q10)

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Duchenne muscular dystrophy (DMD) is a severe recessive X-linked form of muscular dystrophy caused by mutations in the dystrophin gene and it affects males predominantly. Here we report a 4-year-old girl with DMD from a healthy family, in which her parents and sister have no DMD genotype. A PCR-based method of multiple ligation-dependent probe amplification (MLPA) analysis showed the deletion of exons 46 and 47 in the dystrophin gene, which led to loss of dystrophin function. No obvious phenotype of Turner syndrome was observed in this patient and cytogenetic analysis revealed that her karyotype is 46,X,i(X)(q10). In conclusion, we describe the first female patient with DMD who carries a de novo mutation of the dystrophin gene in one chromosome and isochromosome Xq, i(Xq), in another chromosome.

Keywords: Duchenne Muscular Dystrophy; de novo mutation; isochromosome Xq; karyotype; Turner syndrome

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Duchenne muscular dystrophy (DMD) is a severe recessive X-linked form of muscular dystrophy which is characterized by rapid progression of muscle degeneration, eventually leading to loss of ambulation and death. It affects approximately 1/3,500 male births (Mehler 2000). The disorder is caused by mutations in the dystrophin gene located on Xp21.2. Dystrophin gene encodes a protein of the membrane cytoskeleton in skeletal muscle (Hoffman et al. 1987). Among DMD probands affected by the pathogenic mutations, 60% have deletions and 5-10% have duplications of the exons (Den Dunnen et al. 1989). The remaining about 30% patients are caused by mutations at the nucleotide level (Den Dunnen et al. 1989; Roberts et al. 1992). A recent study revealed that in about two thirds of patients, mothers are carriers and the remaining one third patients are due to de novo mutations in dystrophin gene (Alcántara et al. 2001).

Clinical Findings

The study protocol has been reviewed and approved by the Research Ethics Committee of The Third Affiliated Hospital of Guangzhou Medical College. Informed consent was obtained from all participants.

The 4-year-old propositus is the first pregnancy, was born to a 25-year-old man and his 24-year-old wife. Both parents are healthy and nonconsanguineous with an unremarkable family history. She was referred to our institute for muscle weakness. The proposita was born at term after an uneventful pregnancy. At birth, her growth parameters were normal. Her motor development was delayed: she could sit at 10 months and walk at 15 months, but fell down easily. She shows abnormal gait, never runs and jumps, has difficulty in standing up as well as rising from the floor, she also had calf hypertrophy, muscle weakness and Gowers’ sign, but without any neurological deficits or mental abnormality. Blood examination revealed consistently elevated serum levels of creatine kinase, 9,487 U/L (normal: 15-200 U/L), creatinine kinase isoenzyme MB, 409 U/L (normal: 0-25 U/L), aspartate aminotransferase, 259 U/L (normal: 0-40 U/L), and alanine aminotransferase, 568 U/L (normal: 0-40 U/L). Neither her parents nor her younger sister has the same phenotype. Patient’s muscle biopsy is not available. Her height was 98 cm (-1SD), and weight was 20 kg, without any other clinical signs of Turner syndrome at present.

Multiplex Ligation-dependent Probe Amplification (MLPA) is a method to establish the copy number of up to 45 nucleic acid sequences in one single reaction (also called multiplex PCR reaction). MLPA reactions result in a very reproducible gel pattern with fragments ranging from 130 to 490 bp. Comparison of this gel pattern to that obtained with a control sample indicates which sequences show an aberrant copy number. By the MLPA (SALSA MLPA KIT P034/P035 DMD/Becker, MRC Holland, Amsterdam, Netherlands) analysis, we detected the deletion of exons 46 and 47 in the proposita but not in her parents or her younger
sister (Fig. 1), indicating that the patient’s deletion in the dystrophin gene was caused by de novo event. Further PCR analysis confirmed the specific deletion of these exons in the patient but not in her parents and sister (data not shown). Based on website http://www.dmd.nl, we found that the mutations are out of frame transcription, which may be susceptible to nonsense-mediated decay (NMD) process and prevent the translation. Therefore, these mutations are causative for the phenotype of DMD. The results of test for the short tandem repeat (STR) loci in the dystrophin gene and
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Xq implied that the mutations in X chromosome in the patient originated from her mother (Fig. 2A, B). Cytogenetic analysis revealed the karyotype in the proposita is 46,X,i(X)(q10) (Fig. 3). In other words, this patient is a female hemizygous for X linked genes located on the X chromosome short arm, as she has only one Xp chromosome, while her parents and younger sister have normal karyotype.

**Discussion**

It has long been proposed that i(Xq) formation is caused by centromere misdivision. However, several studies demonstrated that centromere misdivision is not a common but a rare mechanism of i(Xq) formation in humans. Instead, i(Xq) formation in human most likely results from sister chromatid breakage and reunion in proximal Xp (Callen et al. 1987; James et al. 1997). In addition, it was reported that the increasing parental age was not associated with isochromosome formation, no matter maternally or paternally derived (James et al. 1997). In our case, the patient was born when her father and mother were in their age of 25 and 24, respectively, demonstrating that isochromosome formation also occurs in the young couples’ child.

Interestingly, this index case is free from the obvious phenotype of Turner syndrome. It is possible that the girl is too young to manifest the symptoms of Turner syndrome, as a previous study concluded that the median age of female with i(Xq) to be diagnosed is 14.2 years (Stochholm et al...
The only abnormal phenotype we observed is short stature because of haploinsufficiency for SHOX gene, which is located in X-chromosome pseudoautosomal region on the distal Xp. It is important to note that this region is identical on X- and Y-chromosomes and does not undergo X-inactivation (Blaschke and Rappold 2000).

To our knowledge, several different genetic abnormalities have been documented for female DMD and the milder allelic form Becker muscular Dystrophy (BMD): (1) an X-autosome reciprocal translocation and a preferential inactivation of the normal X-chromosome (Verellen-Dumoulin et al. 1984); (2) in a classical 45, X0 karyotype of Turner syndrome, simultaneously, the only X-chromosome with a dystrophin mutation (Chelly et al. 1986); (3) skewed X inactivation in the normal X-chromosome of the female DMD mutation carriers. (Azofeifa et al. 1995); (4) uniparental disomy of female with DMD mutation in both X-chromosome (Quan et al. 1997); (5) co-occurrence of mutations in both dystrophin and androgen-receptor genes in the patient (Katayama et al. 2006); and (6) girl with homozygous dystrophin mutation caused by consanguinity, whose parents have the same mutations in the DMD gene (Fujii et al. 2009). While our patient is similar to the case of Turner syndrome with the dystrophin gene mutation in the remaining X chromosome as originally reported (Chelly et al. 1986), the karyotype of our patient is unique. We present the first female patient with DMD who carries an atypical karyotype of Turner syndrome and harbors a de novo mutation in dystrophin gene in the remaining X chromosome.

Fig. 3. Karyotype analysis of the patient. The abnormal X chromosome has two long arms compared with the normal one (indicated by arrow).

References
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