

A Chinese boy with familial Duchenne muscular dystrophy owing to a novel hemizygous nonsense mutation (c.6283C>T) in an exon of the *DMD* gene

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Abstract

Duchenne muscular dystrophy is a severe, X-linked, progressive neuromuscular disorder clinically characterised by muscle weakening and extremely high serum creatine kinase levels. A 1-year-old Chinese patient was diagnosed with early-onset Duchenne muscular dystrophy. Next-generation gene sequencing was conducted and the Sanger method was used to validate sequencing. We identified a novel nonsense mutation (c.6283C>T) in *DMD* that caused the replacement of native arginine at codon 2095 with a premature termination codon (p.R2095X), which may have had a pathogenic effect against dystrophin in our patient's muscle cell membranes. We discovered a novel nonsense mutation in *DMD* that will expand the pathogenic mutation spectrum for Duchenne muscular dystrophy.

Keywords

Duchenne muscular dystrophy, nonsense codon, gene mutation

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Introduction

Duchenne muscular dystrophy (DMD; Mendelian Inheritance in Man #310200), a severe X-linked progressive neuromuscular disorder that affects 15.9 per 100,000 live births worldwide,^{1,2} belongs to a group of inherited conditions clinically characterised by muscle weakening. Patients usually have the following clinical symptoms and signs: delayed walking, frequent falls, difficulty running and climbing stairs, proximal muscle weakness, calf hypertrophy, weak tendon reflexes, special 'duck step' gait, positive Gowers' sign and significantly elevated serum creatine kinase (CK) levels.

DMD mutations cause dystrophin deficiency. Insufficient levels of dystrophin (a protein located on muscle cell membranes) interferes with the formation of the dystrophin-glycoprotein complex, which is normally used to stabilise muscle cell membranes. This leads to defective muscle cell membrane structures. Thus, DMD patients' muscle fibres tear during contraction, resulting in muscle weakness. The *DMD* gene contains 79 exons that span 11 Kb. With 2.3 Mb of genomic DNA, *DMD* is the largest known gene in humans.³ Bladen reported that 80% of all *DMD* mutations

are large mutations (one exon or larger) and 20% of all mutations are small mutations (smaller than one exon). Furthermore, 52% of the small mutations are point mutations, of which 50% are nonsense mutations, 2% are missense mutations, 25% are small deletions, 9% are small insertions and 14% are splice sites.⁴

In this article, we report the case of a boy with early-onset DMD, in whom we identified a novel nonsense mutation in an exon of the *DMD* gene. Our finding extends the mutation spectrum of this disorder.

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Table 1. The details of the nonsense mutation site.

Gene	Location	Genomic variation	NM number	Protein defect	Variation type	Inheritance mode	Pathogenicity
DMD	chrX:32305653	c.6283C>T	NM_004006	p.R2095X	Nonsense mutation	X-linked recessive	Pathogenic PVS1 ^a , PM2 ^b , PP3 ^c

^aPVS1: pathogenic criterion is weighted as very strong, mutations lead to possible loss of gene function; ^bPM2: MAF (minimum allele frequency) < 0.005, belonging to low-frequency variation; ^cPP3: conservatism and protein structure predicted harmfulness.

Case report

Case presentation

In January 2019, a 1-year-old Chinese boy presenting with respiratory distress after 4 days of coughing and fever was admitted to the Department of Respiration at Lanzhou University Second Hospital. His mother's pregnancy, his delivery and his family history were unremarkable. His height was 75 cm and his weight was 11 kg. The child's development was slightly behind that of normal children. He could not walk independently and could stand only with support. His vital signs were normal. Physical examination revealed moist rales and wheezing in his lungs. Motor system examination showed calf hypertrophy, symmetrical proximal muscle weakness and deep tendon hyporeflexia but no sensory disturbances. Laboratory examination revealed an extremely high serum CK level of 3906 U/L (normal: 0–195 U/L). Other laboratory results were as follows: CK-myocardial band 61 U/L (normal: 0–25 U/L), lactic acid dehydrogenase 890 U/L (normal: 0–240 U/L), alanine aminotransferase 183 U/L (normal: 0–50 U/L), and aspartate aminotransferase 131 U/L (normal: 0–50 U/L). The chest X-ray showed signs of bronchopneumonia in both lungs. Echocardiography examination was normal.

The diagnosis of bronchopneumonia was unquestionable, but the diagnosis of DMD relied solely on clinical manifestations and CK levels. Muscle biopsy was refused by the patient's parents, so gene sequencing was initiated, as it was the only viable option for making a definitive diagnosis. The patient was administered ceftazidime (0.5 g intravenously every 12 h for 14 days) and continuous positive airway pressure-assisted respiratory treatments for pneumonia, as well as 1 mg/kg/d intravenous methylprednisolone for 1 week, after which the corticosteroid was changed to orally administered prednisone (0.75 mg/kg/d). The child was discharged after 17 days of treatment. A week later in the outpatient clinic, our patient was generally in good condition and his CK level was 920 U/L.

Gene sequencing

The current study was permitted by the Ethics Committee of Lanzhou University Second Hospital. Written informed consent was obtained from the legally authorised representative/parents of the minor subject for the publication of the case

report. Blood samples (2 mL) were collected from our patient and his parents for genetic analysis.

Next-generation gene sequencing was performed per the methods described in a previous study.⁵ The American College of Medical Genetics and Genomics classification criteria for pathogenic variation were used to identify the pathogenic impact of screened variants.⁶ The Sanger method was used to validate sequencing. Polymerase chain reaction amplification was performed with a reverse primer (5'-GATATAATGCTTCAACATAACGCTA-3') and a forward primer (5'-GAAGAAAAGAAGTGCAAACTACTGA-3').

Results

Gene sequence analysis identified a nonsense mutation that might have had a pathogenic effect, as defined by the American College of Medical Genetics and Genomics standards and guidelines. In particular, it revealed the presence of a hemizygote nucleotide substitution c.6283C>T in the *DMD* gene on exon 43 (Table 1). This base change led to the replacement of native arginine at codon 2095 with a premature termination codon (p.R2095X). We believe that early termination of the transcription of the gene's open-reading frames led to a truncated protein that was reduced by 1591 amino acid residues. Such a truncated protein would likely either be degraded at the mRNA level or expressed as a non-functional protein on muscle cell membranes. Thus, the genetic result provided a molecular biology basis for the diagnosis of DMD. We used Sanger sequencing to further confirm the mutation source and pattern of inheritance. As shown in Figure 1, the proband's mother carried the *DMD* heterozygous mutation c.6283C>T, and the proband's father was wild type.

Discussion

Interestingly, the child was admitted to the hospital because of pneumonia. During his diagnosis and treatment, he was found to have typical clinical manifestations of DMD and was eventually diagnosed with DMD. More specifically, the child had early-onset DMD, as his age of onset was obviously younger than the reported mean age of 4 years.^{7,8} Male children with early-onset DMD who present with calf hypertrophy and mild developmental retardation of their motor systems are seldom perceived by their parents as being ill, so

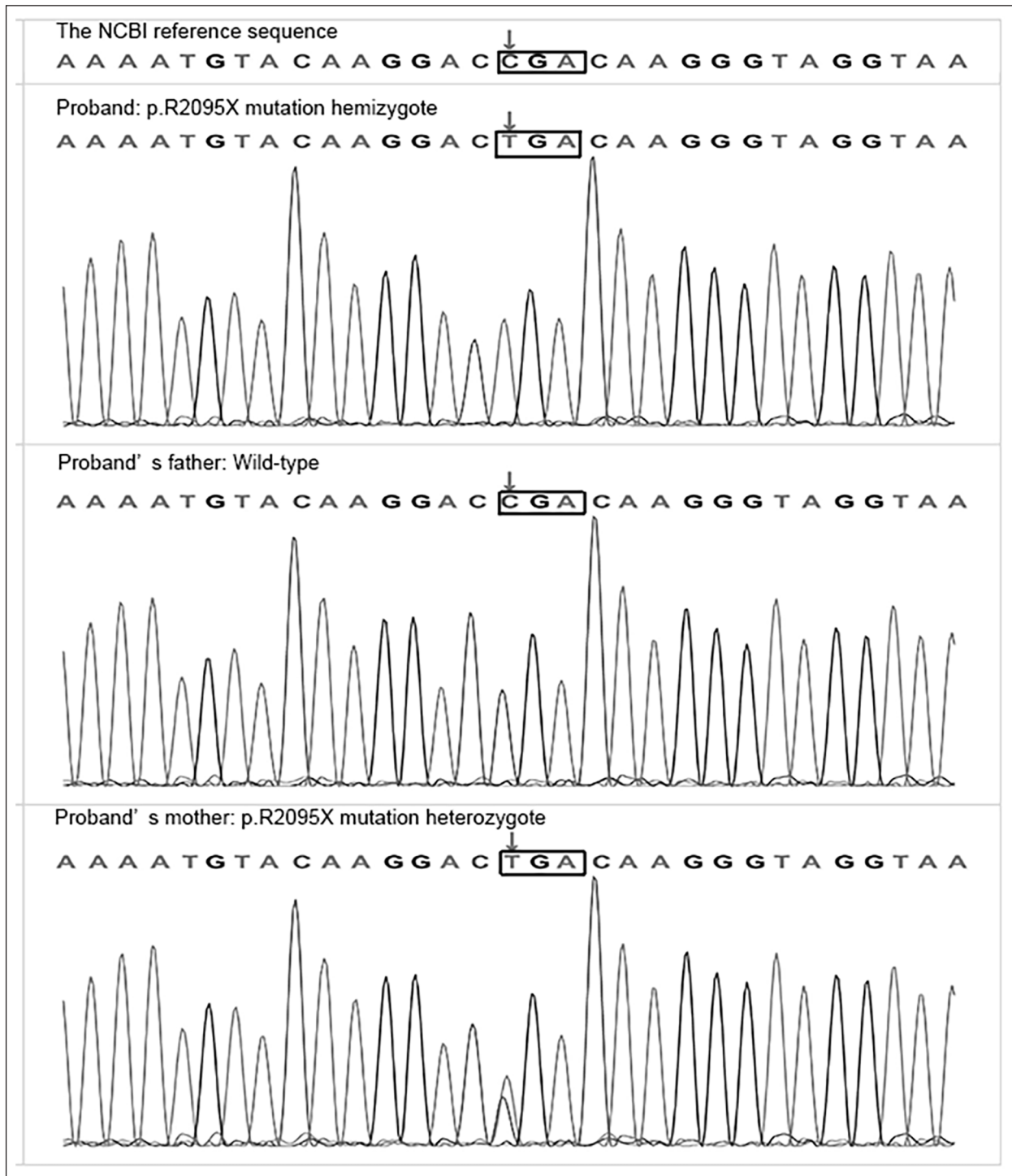


Figure 1. Results of DNA analysis. A nonsense mutation C>T substitution (c.6283C>T) changes the codon of CGA=Arginine to TGA=stop codon (see region of interest). This nonsense mutation predicts premature termination of the protein, which would result in a nonfunctional protein. The arrow indicates the new mutation site. The rectangular box represents region of interest.

they are rarely brought to their physicians for evaluation. However, under the following conditions, experienced paediatricians often suspect DMD: (1) the case follows a typical clinical presentation (including symmetrical distribution of weakness and calf hypertrophy), (2) the patient has

significantly elevated levels of serum CK (tenfold or greater) and (3) there is a family history of the illness. The diagnosis of DMD can be confirmed with genetic testing and muscle biopsy.⁹ However, in clinical practice, muscle biopsy is often rejected owing to the invasive nature of the procedure. A

muscle biopsy is not necessary if a genetic diagnosis is secured first, particularly because some families might consider the procedure traumatic.⁹ Multiplex ligation-dependent probe amplification (MLPA) and gene sequencing are the most widely used genetic testing technologies. These tests cover copy number variation and point mutations, which compose the vast majority of *DMD* mutations.¹⁰ Here, the copy number of *DMD* detected using MLPA was normal. Therefore, we performed next-generation sequencing (NGS). The diagnosis of our patient is mainly based on clinical manifestations, elevated levels of serum CK (10-fold), and NGS. This is a novel mutation, as it has not been reported in the Human Gene Mutation Database, Online Mendelian Inheritance in Man database, or ClinVar database. However, our study was limited by the parents' refusal of a muscle biopsy, which rendered us unable to perform that test for our patient. In addition, the function of the new genetic mutation must be verified via *in vitro* and *in vivo* experiments.

Nevertheless, our findings are of importance. *DMD* gene therapy has been rapidly progressing, and new drugs for specific mutation types are being used in clinical practice. Ataluren is a first-in-class, orally bioavailable drug for patients with nonsense mutation *DMD* (nmDMD). Two studies of ataluren in patients with nmDMD showed that the efficacy of ataluren is favourable and beneficial to patients.^{11,12} In China, most patients still cannot be treated with newer drugs such as ataluren, and they usually die from respiratory or cardiac failure before or during their 30s. The mutation type in our patient is nmDMD, and it may be beneficial to use ataluren. However, the drug is not available in our region. In addition, our patient is just 1 year old, and the youngest patient to have been administered the drug was a 2-year-old kid.¹³ The advantages and disadvantages of medications should be carefully evaluated. Therefore, long-term, oral, low-dose corticosteroid use has become an alternative treatment method to alleviate disease progression. Even with medical treatment, patients often need special medical care and family nursing.¹⁴ Thus, genetic counselling is strongly recommended for all families with a history of *DMD*. Indeed, prenatal diagnosis is available for carriers. The detection and counselling of female carriers are vital for disease prevention.

Conclusion

NGS was used to reveal a novel hemizygous c.6283C>T substitution in exon 43 of the *DMD* gene. Our findings expand the *DMD* pathogenic mutation spectrum and provide useful and valuable information for genetic counselling and prenatal diagnosis in families afflicted with *DMD*.

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Author contributions

All authors were involved in patient management, preparation of manuscript and approving the final version.

Declaration of conflicting interests

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Disclaimers

The views expressed in the submitted article are his or her own and not an official position of the institution or funder.

Ethics approval

Ethical approval to report this case was obtained from the Ethics Committee of Lanzhou University Second Hospital (2020A-004).

Informed consent

Written informed consent was obtained from the legally authorised representative/parents of the minor subject for the publication of the case report.

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