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ORIGINAL RESEARCH

Molecular Genetic Analysis of a *DMD* Frameshift Mutation in a Boy with Duchenne Muscular Dystrophy by MLPA and Sanger Sequencing

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Abstract: Duchenne muscular dystrophy (DMD) is an X-linked recessive neuromuscular disease that is characterized by progressive proximal muscle weakness and pseudohypertrophy. Currently, genetic diagnosis of DMD relies largely on multiplex ligation-dependent probe analysis (MLPA) and Sanger sequencing to identify pathogenic mutations. This study aimed to confirm the genetic etiology of a boy presenting with clinical manifestations that are highly indicative of DMD. A 14-year-old boy with heart failure and extreme muscle weakness along with his family members was recruited for this study. DNA from each participant was isolated from peripheral blood samples. We used MLPA to detect the deletion or duplication mutations of the *DMD* gene and Sanger sequencing to verify the missing region of the exon in the proband. Furthermore, the functional role of the mutation was assessed using bioinformatics. We found that the proband carried a small deletion in the *DMD* gene (c.6808_6811delTTAA). The deletion of those four nucleotides resulted in a frameshift mutation and a premature nonsense codon, which resulted in a truncated dystrophin that lost its most critical function and underwent post-transcriptional degradation. Our study demonstrated that MLPA, in combination with Sanger sequencing, is a reliable and practical approach for the genetic diagnosis of DMD, which is a significant step towards developing personalized therapy.

Keywords: DMD, MLPA, Sanger, exon skipping, personalized therapy

Introduction

Duchenne muscular dystrophy (DMD) is an X-linked recessive muscle disorder with a high incidence rate of one in 3,500–6,000 live male newborns.[1] DMD is characterized by progressive limb weakness, gastrocnemius pseudohypertrophy, waddling gait, and Gower's sign, gradually aggravating to the loss of ambulation before the age of 10–12 years old, coupled with deterioration of respiratory and cardiac functions in the final stages.[2]

Mutations in the dystrophin-encoding gene *DMD* located on the X chromosome, are the root cause of DMD. Dystrophin is a critical structural protein in myocytes that links the actin cytoskeleton to the extracellular matrix of myofibers, and plays a key role in myocyte membrane stability. The lack of normal dystrophin protein causes inaccurate localization of different proteins at the sarcolemma of muscle fibers, leading to membrane instability and myofiber degeneration.[3] There are three different types of *DMD* mutations: 1) deletion (55%-65%), 2) duplication (5%-15%), and 3) point mutations (35%).[4]

Muscle biopsy is the most commonly used diagnostic method for myopathy. Gastrocnemius biopsies of typical DMD patients reveal microscopically abnormal muscle fibers in different shapes and sizes, accompanied by fiber hypertrophy

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and atrophy and occasionally degeneration and necrosis. In addition, extensive hyperplasia was observed in the adjacent adipose and fibrous connective tissues, and immunohistochemical staining of the affected muscle with an anti-dystrophin antibody showed a lack of positively stained myolemma.[3] Although examination of muscle biopsies can help clinicians diagnose the severity of DMD, it cannot specify the number and location of mutations in *DMD*, making it inadequate for precise genetic treatment. According to the 2010 International DMD Diagnosis and management guide,[2] patients should be screened for *DMD* mutations, even after their muscle biopsies are positive. This is essential not only for designing mutation-specific treatments but also for providing prenatal genetic counseling for patients' relatives who desire healthy children. However, if a patient has not yet undergone muscle biopsy, the pathogenic mutation identified by *DMD* mutation analysis will serve as direct diagnostic evidence for DMD, thus circumventing the need for a painful biopsy. Therefore, genetic analysis of DMD patients is not only of diagnostic importance but also an important precondition for accurate treatment at the molecular level.

The clinical management of DMD requires a combination of drugs, nutritional support, and physical exercises that slow the progress of the disease, extend the lifespan of patients, and improve their quality of life, but do not cure the disease.[5] Therefore, the preferred strategy is to lower the incidence of births in affected babies through antenatal genetic screening and counseling. With the development of gene therapy technology, more and more treatment methods for DMD have gradually emerged.[6] Precision treatment is gradually rewriting the chapter of DMD treatment, and identifying the type of gene mutation is the key. At present, several methods for treating DMD at the genetic level are undergoing clinical trials, such as exon skipping mediated by antisense oligonucleotides, translational read-through mediated by ataluren, and CRISPR-Cas9 technology editing, etc.[7–9] Since 2016, eight DMD therapies have been approved by the US FDA. Among them, the therapeutic drug eteplirsen targeting exon 51 skipping of the *DMD* gene has been put into clinical application. Furthermore, more ongoing research and development efforts are continuously emerging with the aim of further enhancing both the efficacy and safety of the treatments. Those increase the hope for the cure of DMD. The size and location of the *DMD* gene mutations in patients are the most crucial data for the individualized design of these treatment methods. Therefore, genetic diagnosis serves as the entry point and a necessary stage for the precise individualized treatment of DMD patients. Currently, the most widely used genetic screening technique for DMD is multiplex ligation-dependent probe amplification (MLPA).[10] If MLPA results are negative, next generation sequencing (NGS) is needed to specifically detect point mutations and copy number mutations with high throughput. When MLPA detects a single exon deletion, Sanger sequencing is adopted to rule out false positives. Here, we used MLPA in combination with Sanger sequencing to confirm the genetic etiology of a boy presenting with clinical manifestations that are highly indicative of DMD. The boy was found to carry a small deletion in *DMD* (c.6808_6811delTTAA). The deletion of four nucleotides resulted in a frameshift mutation and a premature nonsense codon, which resulted in a truncated dystrophin that lost its most critical function and underwent post-transcriptional degradation. Our study demonstrated that MLPA, in combination with Sanger sequencing, is a reliable and practical approach for the genetic diagnosis of DMD, which is a significant step towards developing personalized therapy.

Materials and Methods

Patient

The patient was a 14-year-old boy from a non-consanguineous family (Figure 1) born after a full-term pregnancy and normal delivery without postpartum issues. The patient's tendency to walk slowly and fall frequently was noticed from an early age, and as grew older, his lower limbs became progressively weaker, making it extremely difficult for him to walk. By the age of 10, the patient was completely unable to walk. Physical examination revealed a blood pressure of 123/78 mmHg, heart rate of 119 bpm, and sinus rhythm without an obvious pathological murmur. Rough breath sounds and a few moist rales were audible over both lung bases, and the abdomen was soft without tenderness. The boy had a clear mind and showed normal mental response, a positive Gower's sign, edematous lower limbs and upper limbs with grade 1 and grade 3 strength, respectively, and a positive pathological sign and strephenopodia. Auxiliary examination revealed the following levels of certain metabolites: blood creatine kinase (CK) 2060U/L, CK-MB 40ng/mL, lactate dehydrogenase 695U/L, aspartate amino transferase 83U/L, BNP 2335.3pg/mL. Echocardiography revealed that the left

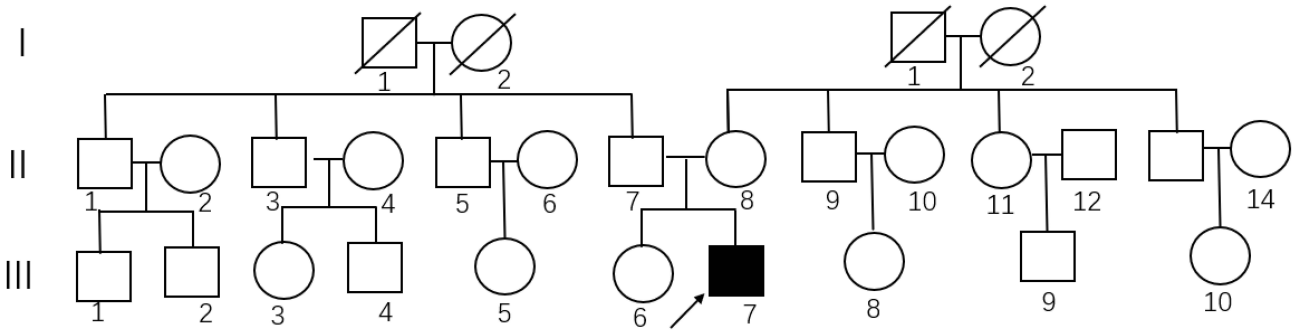

Figure 1 Family genogram. Squares represent males, circles represent females, black represents patients, arrows represent probands, and oblique lines represent deceased patients.

ventricle was enlarged and dilated, the right ventricle was slightly larger, and the bicuspid and tricuspid valves showed moderate regurgitation, all of which indicated cardiac insufficiency. Lung CT showed interstitial edema and patchy areas of fuzzy opacity in the middle lobe of the right lung and lower lobes of the bilateral lung, along with bilateral pleural effusion and a small amount of pericardial effusion. At the age of 5 years, another hospital diagnosed him with progressive muscular dystrophy without a specific classification. The patient's parents and elder sister were healthy, and none of the family members had a similar disease or phenotype (Figure 1). The clinical information of the patient was summarized in Table 1. Informed consent was obtained from the families. This study was approved by the Ethics Committee of the Union Hospital, Tongji Medical College, Huazhong University of Science and Technology and complied with the principles of the Declaration of Helsinki.

Methods

Genome DNA Extraction

Peripheral blood samples (4 mL) were collected from the proband (III7) and three family members (II7, II8, and III6), and DNA was extracted from each sample using the TIANamp Blood DNA Kit (TIANGEN BIOTECH, Beijing, China) according to the manufacturer's instructions. The concentration and purity of the extracted DNA were measured using a NanoDrop spectrophotometer, and the samples were preserved at −20°C.

Table 1 Clinical Examination for the Patient

Clinical Diagnose	**Clinical Diagnose**
Clinical symptoms	Gower's sign, edematous lower limbs and upper limbs with grade 1 and grade 3 strength, respectively, and a positive pathological sign and strephenopodia.
Vital signs	Heart rate: 119bpm Blood pressure: 123/78 mmHg Sanity
Echocardiography examination	Left heart enlargement, right heart slightly enlarged, cardiac insufficiency, moderate insufficiency of the tricuspid and bicuspid valves.
Lung CT	Interstitial edema and patchy areas of fuzzy opacity in the middle lobe of the right lung and lower lobes of the bilateral lung, along with bilateral pleural effusion and a small amount of pericardial effusion.
Other Specs	
Blood creatine kinase (CK)	2060U/L
Blood CK-MB	40ng/mL
Lactate dehydrogenase	695U/L
Aspartate amino transferase	83U/L
BNP	2335.3pg/mL

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MLPA Screening

The basic approach of MLPA is to amplify probes that can correctly hybridize and ligate with a targeted DNA sequence. Semi-quantitative analysis of the amplified product helps to rapidly analyze deletions or duplications in the gene.[11] Reaction steps for MLPA include hybridization, ligation, amplification, and capillary electrophoresis. Briefly, 100ng of genomic DNA was denatured at 98 °C for 5 min, cooled to 25 °C, and mixed with 3ul MLPA P034 or P035 probe solution in a 250ul PCR tube. The mixture was then heated to 95 °C for 5 min and incubated overnight at 60 °C for probe hybridization. After 16 h, the ligation reaction was performed using the Ligase-65 mix at 54 °C for 15 min, followed by deactivation of the Ligase-65 enzyme at 98 °C for 5 min. PCR amplification was performed using the specific SALSA FAM PCR primers. The amplified mix was separated by capillary electrophoresis and then analyzed using GeneMapper 4.0, and Coffalyser version 9.0 (http://www.mlpa.com/coffalyser/download.html). The relative peak ratio (RPR) of each exon is plotted against the corresponding bar chart.[12] Single-exon deletions were further validated using PCR amplification and DNA sequencing, and single-exon duplications were confirmed in two independent experiments. Genomic DNA from blood samples of an unaffected individual was used as a control.

PCR and Sanger Sequencing

When the MLPA results show only a single missing exon, the authenticity and accuracy of the results must be verified to exclude false positives caused by some micro mutations that can inhibit probe hybridization and ligation with the exon. Because this can result in a weakened or absent signal for a single exon, Sanger sequencing should be performed to identify these micromutations. Next, we conducted Sanger sequencing to verify the missing region of the exon. Genetool software was used to design primers for amplification of the absent exon, and TsingKe Biological Technology in Wuhan synthesized the primers. The following primers were used to amplify exon 47 of *DMD*: forward primer 5' ggccctcggtcaagtcgc 3', reverse primer 5' agccaaagcaaacggtcagg 3'. The 25ul PCR reaction mix consisted of 1ul genomic DNA, 1ul of each primer, 12.5ul master amplification mix (TsingKe Biological Technology, Beijing) and 10.5ul dH_2O. The mixture was heated to 94 °C for 3 min and the amplification reaction was performed for 35 cycles (denaturation at 94 °C for 30s, annealing for 30s, and extension at 72 °C for 35s per cycle) before the final extension at 72 °C for 10 min and standing at 4 °C. The products of PCR amplification were verified by 1% agarose gel electrophoresis, sequenced using TsingKe Biological Technology with the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems), and finally tested using the ABI PRISM 3130 genetic analyzer. After PCR amplification and Sanger sequencing, the sequence of the mutated gene was compared with that of normal controls, and the exact location and type of mutations were identified.

Bioinformatic Analysis

A BLAST search of the sequence was performed using the NCBI dbSNP database to eliminate the polymorphic loci. Thereafter, the DMD Mutation database (Leiden Open Variation Database, LOVD; http://www.dmd.nl/), ExACBrowser (http://exac.broadinstitute.org) and the 1000-genome project database (http://browser.1000genomes.org/) were searched to determine the mutation frequency. Harmful mutations were predicted using MutationTaster (http://www.mutationtaster. org/).

Results

Results of MPLA

The MLPA results of the patient showed that exon 47 of *DMD* gene completely missed the peak-map area, whereas the peak-map area of other exons was intact, suggesting that exon 47 may be a homozygous deletion (Figure 2). To distinguish between the homozygous deletion of exon 47 and other mutations in the exon causing false-positive MLPA, exon 47 was amplified by PCR and verified by Sanger sequencing.

Results of PCR and Sanger Sequencing

Exon 47 of the *DMD* gene of the patient was amplified by PCR, and the PCR products were detected by agarose gel electrophoresis, which showed that exon 47 of the *DMD* gene was not missing in the patient (Figure 3). The results were further confirmed by Sanger sequencing, which showed a small deletion mutation in the *DMD* gene and a deletion of four nucleotides at exon 47 of *DMD* gene (c.6808_6811delTTAA) in the patient (Figure 3). Exon 47 of *DMD* gene for the mother, father, and sister of the patient was amplified using the same method, and Sanger sequencing was performed, and the results showed no mutations (Figure 3). This suggests that the patient's mutation may be a new mutation or it may be inherited from the mother's germ cell chimera.

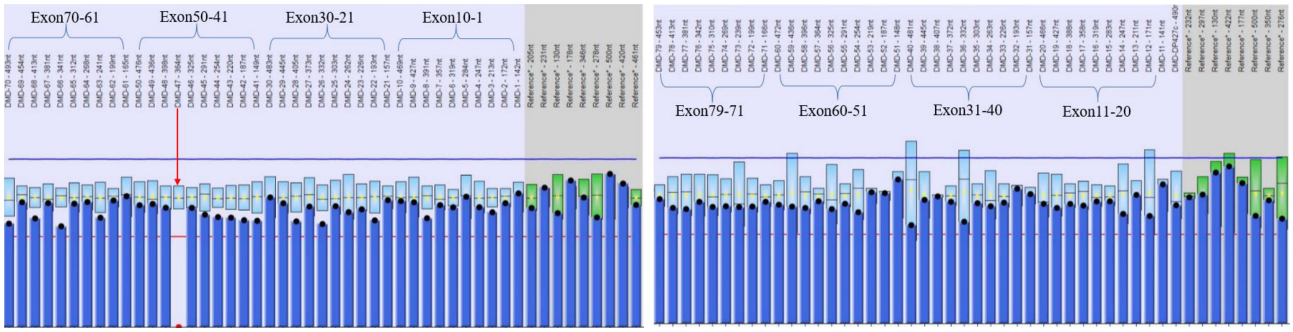

Figure 2 MLPA results of the proband. The red arrow shows the loss of exon 47 in *DMD* patients.

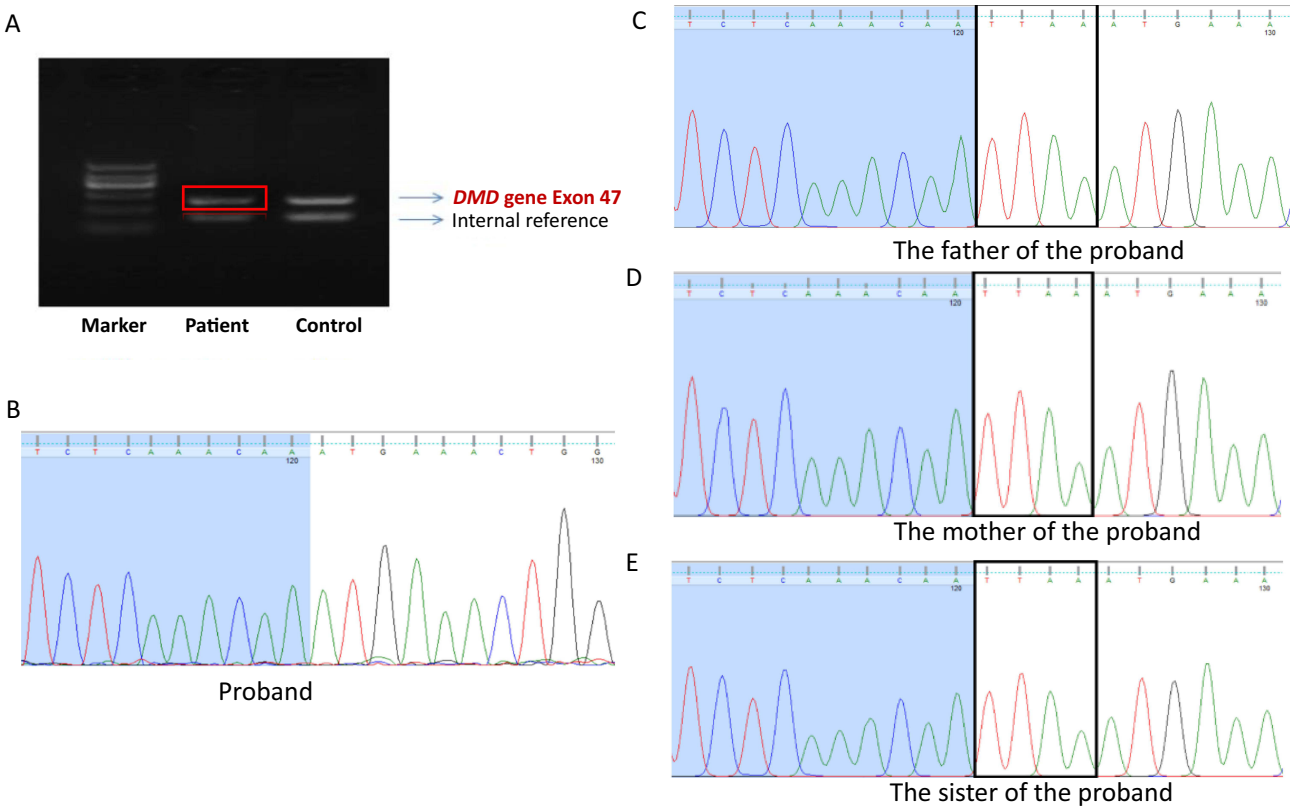

Figure 3 Results of PCR and Sanger sequencing of the proband and his families of exon 47. (**A**) Agarose gel electrophoresis showed that exon 47 of the *DMD* gene was not missing in the proband; (**B**) Sanger sequencing showed that the proband has lost 4 nucleotides at exon 47; (**C**–**E**) Sanger sequencing separately showed that the father, mother and sister of the proband do not have any deletion at exon 47.

Bioinformatics Analysis

We found that this *DMD* exon 47 deletion mutation was not found in the ExAC Browser exon group or 1000-genome project database. We performed hazard prediction for this mutation on the mutation taster website and found that Phylop (−14-+ 6) was valued at 3.183 and PhastCons (0–1) had a value of 1, indicating that the region of this mutation was conserved, which rendered any mutations in that region detrimental (Figure 4). After analyzing the sequence of exon 47, we found that the codons were rearranged owing to the deletion of 4 bp (c.6808_6811delTTAA) and terminated at the ninth codon from the mutated position after rearrangement, which likely resulted in premature termination during translation of the dystrophin protein (Figure 4). Dystrophin is composed of four structural regions: the amino-terminal, central bar, cysteine, and carboxyl-terminal regions (Figure 5). The truncated dystrophin produced as a result would have lost parts of the central bar area, the whole cysteine region, and the carboxyl-terminal region. Therefore, it can be safely

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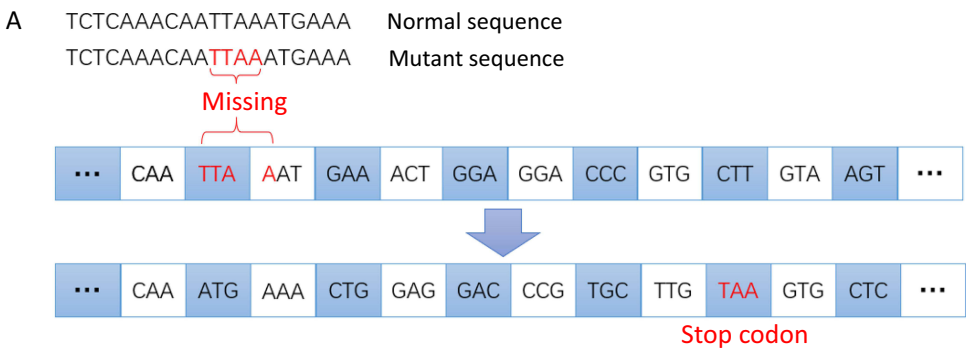

B

phyloP / phastCons	PhyloP	PhastCons
(flanking)	3.183	1
	3.183	1
	1.123	1
	3.183	1
	1.158	1
(flanking)	1.816	1

Figure 4 Bioinformatics analysis of the mutation. (**A**) Termination codon (TAA) arose at the ninth codon from the mutated position after deletion of 4 bases (c6808_6811TTAA); (**B**) Prediction of mutation harmfulness.

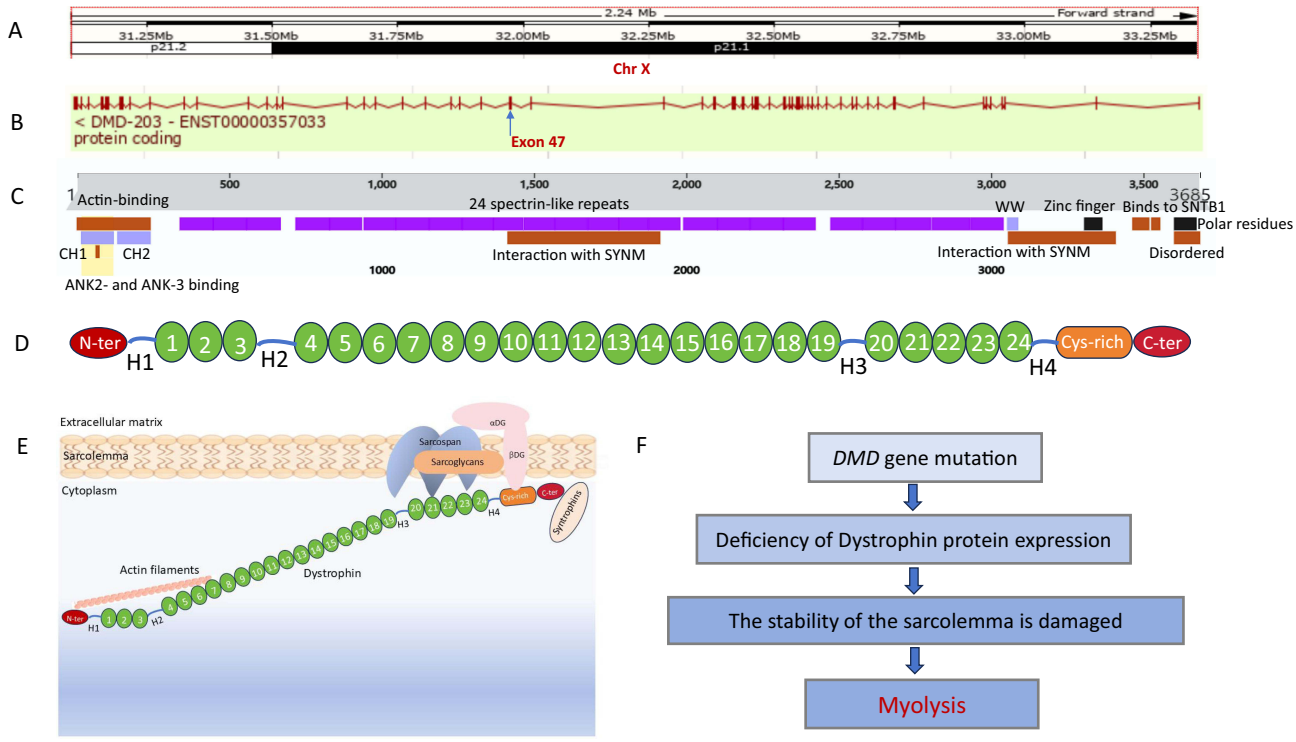

Figure 5 Structure and function of *DMD* gene and protein. A. The location of the *DMD* gene on the genome; B. *DMD* gene transcript; C. The protein domain of the *DMD* gene; D. DMD protein; E. Dystrophin associated complex; F. Pathogenic mechanism of *DMD* gene mutation.

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assumed that the microdeletion caused the *DMD* gene to be translated into a truncated protein and that the shortened dystrophin protein may lack some or all of its functions (Figure 5). The patient developed symptoms in early childhood, was unable to walk when he was 10 years old, and spent most of his time lying in bed. Therefore, the serious clinical phenotype caused by this mutation fully matched the genetic analysis, and we could conclude that the prognosis for the boy was poor.

Discussion

This study combined MLPA and Sanger sequencing to positively diagnose a highly suspected DMD patient, discover the causative gene mutation, and perform mutation hazard analysis. Considering the type and location of the mutation, especially in the context of the patient's clinical features, we predicted poor prognosis. Indeed, one month after we had diagnosed the patient at the genetic level, he died of respiratory failure.

DMD is a common X-linkage recessive disease that mainly affects the skeletal muscles. Most patients develop respiratory or heart failure at 20 years of age and often die of respiratory complications and cardiomyopathy.[13] The vast majority of DMD patients are males and females are carriers. Gene mutations in two-thirds of affected boys were inherited from their mothers. When the mother of the patient is proven to be the carrier, the mutation source can be determined immediately, and steps should be taken by the family and their relatives if any of them are planning a pregnancy. When the mother of an affected boy is sequenced negative, there is still a 10–15% possibility of the mother having germline chimerism, which manifests as the absence of any mutation in the peripheral blood cells, thus making it difficult to predict the risk of inheritance.[14] *DMD*, which encodes the dystrophin protein, is one of the largest DMD genes in humans with 79 exons and a molecular weight of 2.4Mb. It is located at Xp21.1-Xp21.3 and accounts for 1% of the X chromosome genetic material.[15] Dystrophin is composed of four structural regions: the amino-terminal, central bar, cysteine, and carboxyl terminal region.[16] The most common mutations in DMD patients are large deletions in one or more exons (55%-65% of the affected), duplication (5%-15%), and point mutations (~35%).[4] If the mutation does not cause much damage to the reading frame rule, such as base replacement in unimportant functional areas or deletion of three or more bases, the dystrophin protein retains most of its important functions, the phenotype is mild, and patients lose the ability to walk at an average age of 40 years, presenting with Becker muscular dystrophy (BMD).[17] However, when the mutation seriously damaged the reading frame rule, such as large fragment deletion/repetition, frameshift mutation or premature stop codon, the function of the translated dystrophin protein was severely damaged, the phenotype was severe, and the average age of loss of walking ability was 10 years old, manifested as DMD.[18] The patient in this study developed DMD at the age of 5 years, and gradually could not walk from the age of 10 years. Clinically, DMD was highly suspected based on the symptoms, but the patient could not be diagnosed without muscle biopsy; therefore, genetic diagnosis was the best choice.

Since MLPA can quickly and efficiently detect deletions and duplications of large fragments that comprise a large proportion of *DMD* mutations, it is one of the best options for the primary screening of DMD. If the MLPA probe peak figure shows the absence of several consecutive exons, the pathogenesis of the patient can be easily attributed to large deletions. However, if a single exon is absent in MLPA analysis, it could either be due to an actual deletion of that exon or the presence of a simple point mutation, which prevents probe hybridization and detection. Therefore, MLPA results showing one missing exon should be supplemented with Sanger sequencing to clarify whether it is a deletion or a point mutation and to identify the specific mutation in case of the latter. In our study, Sanger sequencing following MLPA revealed a loss of four bases in exon 47 of *DMD*. The DNA test of the mother's peripheral blood cells did not show any mutation, suggesting chimerism wherein certain cells of her body, such as the blood cells, did not mutate but others such as egg cells did, and thus transmitted the mutation to her progeny. Another possibility is that the patient developed a de novo mutation that was completely unrelated to his mother; however, we could not ascertain the real cause of the disease. The MutationTaster website predicted that the deletion of the four bases in exon 47 would cause a frameshift mutation. Comparison of the mutated and normal sequences revealed a termination codon in the ninth codon, starting from the deleted site, which likely resulted in premature termination during the translation of the dystrophin protein. The truncated dystrophin produced as a result would have lost parts of the central bar area, the whole cysteine region, and the carboxyl-terminal region (Figure 5). The cysteine- and carboxyl-terminal regions perform important functions and interact with the

muscle membrane glycoprotein complex and muscle cell syntropins, respectively.[19] Deficiency of both domains leads to instability of the muscle cell membrane, necrosis of muscle fiber segments, and calcium influx, finally resulting in degraded muscle bundles.[20] Our study has an obvious limitation in that we failed to obtain a suitable amount of muscular tissue to detect dystrophin in situ to verify that the mutated *DMD* produced a truncated DMD protein.

Muscular disease symptoms, which were initially diagnosed as progressive muscular dystrophy, were noticeable in the patient before the age of five years. His muscular strength gradually decreased with age, and he was unable to walk when he was 10 years of age. The patient visited our hospital at the age of 14 as an emergency case. Physical examination showed weak bilateral lower limbs with a muscle force of grade 1 and mildly hypertrophic gastrocnemius muscle. Although we could make a definite diagnosis of DMD after genetic testing, due to the lack of treatment at present, our efforts were directed towards slowing the development of the disease and improving the quality of the patient's life. Recent developments in genetic research on DMD have been encouraging; however, knowledge of individual mutations can pave the way for genetic therapy. This study jointly employed MLPA technology and Sanger sequencing to make a diagnosis for the patient at the genetic level. As the patient passed away before our genetic diagnosis results were available, we were unable to provide a treatment plan for him. As a result, this diagnosis failed to significantly promote the treatment for this patient. However, through this patient's diagnosis, we can confirm that this diagnostic strategy is reliable and worthy of clinical promotion. Therefore, children with muscular disorders that are highly suspected to have DMD should undergo genetic testing at the earliest. The first priority is to treat the affected children to protect the muscle fibers and slow disease progression. Simultaneously, based on the results of genetic tests, patients should also be encouraged to participate in clinical trials for *DMD* gene-targeted therapies. In addition, female blood relatives of patients should be screened for *DMD* mutations to identify carriers and, based on the results, offered prenatal genetic counselling.

The key to individualized medical treatment lies in identifying the type of genetic mutations of the patients. At present, several methods for treating DMD at the genetic level are in the stage of clinical trials, such as exon skipping, reading through of stop codons, CRISPR-Cas9 technology editing, etc.[6] The latest mutation-specific treatments for DMD are highly promising.[21–23] One such strategy is antisense oligonucleotide (AON)-mediated exon skipping, which employs small modified DNA or RNA molecules to manipulate the splicing process to exclude mutated exons from the mature mRNA, resulting in a shorter but partly functional dystrophin.[24] The AON approach has been successful in a recent clinical trial, and an AON targeting exon 51 has become the first of its class to be approved by United States regulators [Food and Drug Administration (FDA)] for the treatment of DMD.[25] Every AON targets a specific exon and a specific mutation, and because the causative mutation differs widely among DMD patients, every patient would require a unique AON and a personalized therapeutic schedule. Although this will certainly increase the success rate of AON treatment, it will also present a great challenge for clinicians in designing personalized AONs. Another therapeutic possibility is the drug Ataluren, which promotes read-through of a nonsense mutation to produce a full-length functional dystrophin protein. Targeted at the ribosome translation mechanism, Ataluren selectively induces ribosomal read-through of only premature and non-normal termination codons, leading to translation of a relatively complete polypeptide to rescue striated muscle function.[26] Phase 3 trials of Ataluren were conducted at 54 sites across 18 countries. Patients treated with ataluren did not show a significant improvement in 6-minute walk distance (6 MWD) compared to those in the placebo group. However, there was a significant effect of ataluren on a prespecified subgroup of patients with a baseline 6 MWD of 300m–400m; the subgroups with 6 MWD<300m or>400m were unaffected.[27] Since baseline 6 MWD values within the 300m-400m range are associated with a more predictable rate of decline over a period of 1 year, this finding has implications for future DMD trials using the 6 MWD test as the endpoint. Although the results of Ataluren are promising, they are only applicable to patients who carry premature stop codons, and their curative effect is dependent on the retained activity ability of the patient. In addition, the long-term effects of ataluren in patients with nonsense mutation DMD remain to be elucidated, limiting its widespread clinical application.

Over the past decade, a large number of studies on DMD treatment have been conducted worldwide, and remarkable progress has been made in the field of molecular therapy. Although the results of animal-level studies are promising, the clinical trials have been progressing relatively slowly due to the limitations of the drugs in targeting specific DMD mutations. The International Medical Organization suggests that all patients with highly suspected or biopsy-confirmed DMD should

undergo genetic diagnosis. Moreover, carrier screening for female family members of the patients should be conducted through MLPA or other techniques. Based on the results, genetic counseling and reproductive guidance should be provided to the families to prevent the birth of affected children. Meanwhile, patients who have been diagnosed by genetic testing have the opportunity to participate in the corresponding clinical trials of drugs or receive regular molecular-level treatment. Especially for those with nonsense mutations, they may be eligible to participate in the clinical trial of ataluren. We hope that in the future, gene diagnosis will be widely available for DMD patients in clinical practice, and individualized treatment plans can be designed for them. In cases where there are no drug treatments available, we can suggest that patients participate in clinical trials, which can also increase the number of participants for future clinical trials of new drugs, thereby accelerating the speed and efficiency of scientific research and bringing hope for health to more patients in the future.

Conclusions

The combination of MLPA and Sanger sequencing technologies can help make a definite genetic diagnosis for DMD patients. Although our findings did not play a significant role in the treatment of patients who died before the results were obtained, we have convincingly shown that this diagnostic strategy is reliable and worth applying in clinical settings. Whenever a couple's first child is positively diagnosed with DMD using a genetic test, it is necessary to screen the mother and other female blood relatives to identify carriers and provide genetic counseling for the carriers who plan to have a baby. Although DMD gene-specific treatments have greatly improved, extensive research and clinical trials are needed to design suitable therapies for all patients. As long as no cure is available for DMD, it is of great significance to perform prenatal counseling and diagnosis by MLPA and Sanger sequencing to provide crucial information for gene-specific treatment of affected children. With the continuous development of gene therapy, in the future, personalized treatment plans will be developed for DMD patients based on specific mutation types.

Acknowledgments

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Ethics Approval and Consent to Participate

This study obtained informed consent from the families and was approved by the ethics committee of the Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (UHCT-IEC-SOP-016-02-01). This study complied with the principles of the Declaration of Helsinki.

Consent to Publish

Consent for publication was obtained from all participants.

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Disclosure

The authors declare no competing interests in this work.

References

1. Emery AE. Population frequencies of inherited neuromuscular diseases--a world survey. *Neuromuscul Disord*. 1991;1(1):19–29. doi:10.1016/0960-8966(91)90039-U
2. Bushby K, Finkel R, Birnkrant DJ, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. *Lancet Neurol*. 2010;9(1):77–93. doi:10.1016/s1474-4422(09)70271-6
3. Brandsema JF, Darras BT. Dystrophinopathies. *Seminars in Neurol*. 2015;35(4):369–384. doi:10.1055/s-0035-1558982
4. Bladen CL, Salgado D, Monges S, et al. The TREAT-NMD DMD global database: analysis of more than 7,000 Duchenne muscular dystrophy mutations. *Human Mutation*. 2015;36(4):395–402. doi:10.1002/humu.22758
5. McDonald C, Camino E, Escandon R, et al. Draft guidance for industry Duchenne muscular dystrophy, Becker muscular dystrophy, and related dystrophinopathies - developing potential treatments for the entire spectrum of disease. *J Neuromuscul Dis*. 2024;11(2):499–523. doi:10.3233/JND-230219

6. Konieczny P. Systemic treatment of body-wide Duchenne muscular dystrophy symptoms. *Clin Pharmacol Ther*. 2024;116(6):1472–1484. doi:10.1002/cpt.3363
7. Chey YCJ, Arudkumar J, Aartsma-Rus A, et al. CRISPR applications for Duchenne muscular dystrophy: from animal models to potential therapies. *WIREs Mech Dis*. 2023;15(1):e1580. doi:10.1002/wsbm.1580
8. A BBA, Hosny N, Cohen H, et al. Duchenne muscular dystrophy: disease mechanism and therapeutic strategies. *Front Physiol*. 2023;14:1183101. doi:10.3389/fphys.2023.1183101
9. Ricci G, Bello L, Torri F, et al. Therapeutic opportunities and clinical outcome measures in Duchenne muscular dystrophy. *Neurol Sci*. 2022;43 (Suppl 2):625–633. doi:10.1007/s10072-022-06085-w
10. Massalska D, Bijok J, Zimowski JG, et al. Multiplex ligation-dependent probe amplification (MLPA)--new possibilities of prenatal diagnosis. *Ginekologia Polska*. 2013;84(6):461–464. doi:10.17772/gp/1605
11. Eijk-Van Os PG, Schouten JP. Multiplex Ligation-dependent Probe Amplification (MLPA(R)) for the detection of copy number variation in genomic sequences. *Methods Mol Biol*. 2011;688:97–126. doi:10.1007/978-1-60761-947-5_8
12. Schouten JP, McElgunn CJ, Waaijer R, et al. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res*. 2002;30(12):e57. doi:10.1093/nar/gnf056
13. McNeil DE, Davis C, Jillapalli D, et al. Duchenne muscular dystrophy: drug development and regulatory considerations. *Muscle Nerve*. 2010;41 (6):740–745. doi:10.1002/mus.21623
14. Fischer C, Gross W, Kruger J, et al. Modelling germline mosaicism and different new mutation rates simultaneously for appropriate risk calculations in families with Duchenne muscular dystrophy. *Ann Human Genetics*. 2006;70(Pt 2):237–248. doi:10.1111/j.1469-1809.2005.00226.x
15. Muntoni F, Torelli S, Ferlini A. Dystrophin and mutations: one gene, several proteins, multiple phenotypes. *Lancet Neurol*. 2003;2(12):731–740. doi:10.1016/S1474-4422(03)00585-4
16. Le Rumeur E, Winder SJ, Hubert JF. Dystrophin: more than just the sum of its parts. *BBA*. 2010;1804(9):1713–1722. doi:10.1016/j.bbapap.2010.05.001
17. Straub V, Guglieri M. An update on Becker muscular dystrophy. *Curr Opin Neurol*. 2023;36(5):450–454. doi:10.1097/WCO.0000000000001191
18. Aartsma-Rus A, den Dunnen JT. Phenotype predictions for exon deletions/duplications: a user guide for professionals and clinicians using Becker and Duchenne muscular dystrophy as examples. *Hum Mutat*. 2019;40(10):1630–1633. doi:10.1002/humu.23850
19. Delalande O, Czogalla A, Hubert JF, et al. Dystrophin and spectrin, two highly dissimilar sisters of the same family. *Sub-Cellular Biochem*. 2017;82:373–403. doi:10.1007/978-3-319-49674-0_12
20. Deconinck N, Dan B. Pathophysiology of duchenne muscular dystrophy: current hypotheses. *Pediatr Neurol*. 2007;36(1):1–7. doi:10.1016/j.pediatrneurol.2006.09.016
21. Wilton-Clark H, Yokota T. Antisense and gene therapy options for duchenne muscular dystrophy arising from mutations in the N-terminal hotspot. *Genes*. 2022;13(2):257. doi:10.3390/genes13020257
22. Erkut E, Yokota T. CRISPR therapeutics for Duchenne muscular dystrophy. *Int J Mol Sci*. 2022;23(3):1832. doi:10.3390/ijms23031832
23. Berling E, Nicolle R, Laforet P, et al. Gene therapy review: Duchenne muscular dystrophy case study. *Rev Neurol*. 2023;179(1–2):90–105. doi:10.1016/j.neurol.2022.11.005
24. Eser G, Topaloglu H. Current outline of exon skipping trials in Duchenne muscular dystrophy. *Genes*. 2022;13(7):1241. doi:10.3390/genes13071241
25. Aartsma-Rus A, Straub V, Hemmings R, et al. Development of exon skipping therapies for Duchenne muscular dystrophy: a critical review and a perspective on the outstanding issues. *Nucleic Acid Ther*. 2017;27:251–259. doi:10.1089/nat.2017.0682
26. Haas M, Vlcek V, Balabanov P, et al. European medicines agency review of ataluren for the treatment of ambulant patients aged 5 years and older with Duchenne muscular dystrophy resulting from a nonsense mutation in the dystrophin gene. *Neuromuscular Dis*. 2015;25(1):5–13. doi:10.1016/j.nmd.2014.11.011
27. McDonald CM, Campbell C, Torricelli RE, et al. Ataluren in patients with nonsense mutation Duchenne muscular dystrophy (ACT DMD): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. 2017;390:1489–1498. doi:10.1016/s0140-6736(17)31611-2

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