



NOTE

Internal Medicine

Dystrophin-deficient muscular dystrophy in a Toy Poodle with a single base pair insertion in exon 45 of the *Duchenne muscular dystrophy* gene

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ABSTRACT. A 10-month-old, intact male Toy Poodle was referred for a postural abnormality. Blood biochemical tests revealed a marked increase in plasma creatine phosphokinase (CPK) concentration. The isoenzyme test showed that 99% of serum CPK consisted of CPK-MM. Histopathological evaluation of muscle biopsy samples confirmed scattered degeneration and necrosis of myofibers. Immunohistochemistry for dystrophin showed an absence of staining in muscle cells. Based on these findings, the dog was diagnosed with dystrophin-deficient muscular dystrophy. Whole genome sequencing using genomic DNA extracted from blood revealed a single base pair insertion in exon 45 of the *Duchenne muscular dystrophy* (*DMD*) gene. This is the first report on muscular dystrophy in Toy Poodles and identified a novel mutation in the *DMD* gene.

KEYWORDS: duchenne muscular dystrophy, dystrophin, insertion, muscular dystrophy, Toy Poodle

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Muscular dystrophy is a group of inherited diseases characterized by the degeneration and necrosis of muscle fibers. Human patients with muscular dystrophy suffer from progressive muscle weakness, resulting in various functional disorders, affecting motor, cardiac, and respiratory functions. Although the symptoms of muscular dystrophy can be alleviated by certain treatments [1, 20, 23], there is no curative treatment for the disease. The prognosis for muscular dystrophy depends on the type and the severity of symptoms. Most individuals with muscular dystrophy eventually lose their ability to walk.

In humans, muscular dystrophy is classified into many different types based on phenotype, inheritance, genetic defects, and proteins involved. All these features are caused by mutations in genes that encode proteins necessary for the maintenance of muscle. Duchenne muscular dystrophy (DMD) is the most common and severe form of muscular dystrophy [21]. DMD is caused by mutations in the *DMD* gene on the X chromosome, which results in severe reduction or complete elimination of the dystrophin protein [12]. Dystrophin acts as a shock absorber that stabilizes the cell membrane of muscle cells against mechanical stress [10]. Since dystrophin is essential for maintaining muscle integrity, the lack of the protein causes muscle cells to be fragile and easily damaged.

Muscular dystrophy has also been reported in dogs [2–7, 9, 13, 15, 16, 18, 22]. As in humans, dystrophin-deficient muscular dystrophy is the most common type in dogs. It has been known to occur in several breeds, including the Golden Retriever,

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Labrador Retriever, Jack Russell Terrier, Norfolk Terrier, Cavalier King Charles Spaniel, and Weimaraner. Although the Toy Poodle is the most common breed of dog in Japan, muscular dystrophy in this breed has not yet been reported. Here, we describe dystrophin-deficient muscular dystrophy in a Toy Poodle and identify the underlying mutation in the *DMD* gene.

A 10-month-old, intact male Toy Poodle was referred to the Veterinary Medical Center, Osaka Prefecture University, Japan, with a postural abnormality. On physical examination, the dog's back was arched, and joint contracture in the shoulders and knees was observed (Fig. 1). Neurological examination showed abnormalities in gait (narrow stride), but not in postural response, spinal reflexes, and cranial nerve examinations. No abnormalities were identified in complete blood counts, whereas results of blood biochemical tests identified a marked increase in plasma creatine phosphokinase (CPK) concentration (34,583 IU/l; reference range [RR], 50–170 IU/l). The CPK isoenzyme test showed that 99% of serum CPK consisted of CPK-MM, which is found primarily in skeletal muscle. There were increased levels of plasma alanine aminotransferase (737 IU/l; RR, 20–99 IU/l), aspartate aminotransferase (583 IU/l; RR, 18–65 IU/l), and lactate dehydrogenase (218 IU/l; RR, 22–184 IU/l), which may have been related to leakage secondary to muscle necrosis. A decreased level of plasma creatinine (0.3 mg/dl; RR, 0.4–1.6 mg/dl) was identified and may have been related to muscle atrophy. Urine collected by cystocentesis was brown in color. Urinalysis showed urine protein (3+) and occult blood (3+), suggesting myoglobinuria. Chest and abdominal radiography and abdominal ultrasonography showed no abnormalities. On the basis of these findings, myopathy was suspected.

Serum antibody titers for *Toxoplasma gondii* and *Neospora caninum* were negative. No anti-nuclear antibodies were detected. Plain and contrast-enhanced computed tomography showed no abnormalities, whereas magnetic resonance imaging of the head and thighs revealed lesions with high signal intensity on T2-weighted and fluid attenuated inversion recovery images in the left temporalis and bilateral thigh muscles (Fig. 2). Surgical biopsies were taken from the left temporalis and sartorius muscles, and these tissue samples were fixed in 10% formalin and processed by FUJIFILM VET Systems Co., Ltd. (Tokyo, Japan) for histopathological evaluation. Hematoxylin and eosin-stained sections showed variation in myofiber size and scattered degeneration, necrosis, and regeneration of myofibers (Fig. 3A), suggesting muscular dystrophy. Immunohistochemistry for the C-terminus and N-terminus of dystrophin (DYSA and DYSB, respectively; Leica Biosystems, Nussloch, Germany) revealed a complete absence of staining in the muscle tissues (Fig. 3B). Based on these findings, dystrophin-deficient muscular dystrophy was diagnosed.



Fig. 1. External appearance of the dog. The back is arched. The knees are not extended due to joint contracture.

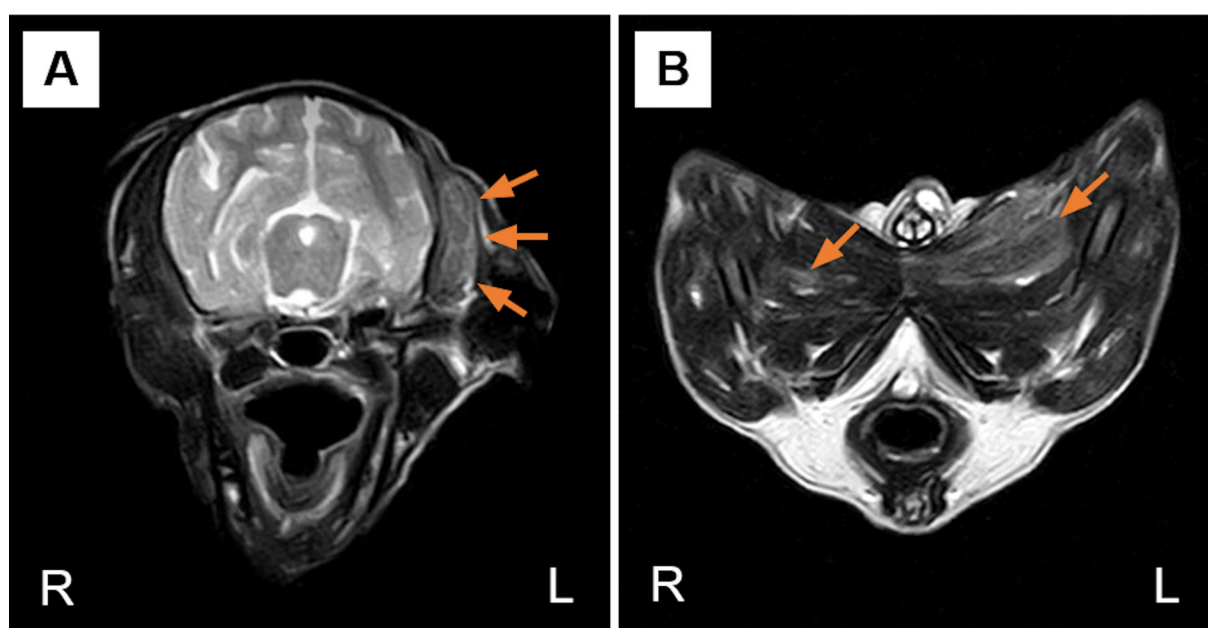


Fig. 2. T2-weighted magnetic resonance imaging of the head (A) and thighs (B). Irregularly shaped lesions with high signal intensity (arrows) were observed in the left temporalis and bilateral thigh muscles. L, left, R, right.

Whole genome sequencing analysis was performed to identify genetic abnormalities in this case. Briefly, genomic DNA was extracted from blood samples using the Qiagen DNA Blood and Tissue Kit (Hilden, Germany). DNA samples (2 µg) were prepared and sent to Takara Bio Inc. (Kusatsu, Japan), where they were clustered and sequenced (details in the online [Supplementary information](#)) on the NovaSeq 6000 (Illumina, Inc., San Diego, CA, USA). The sequence data revealed a single base pair insertion in exon 45 of the canine *DMD* gene (cfaX:g.27099343_27099344insT [ENSCAFT00000036277.5:c.6559_6560insA or ENSCAFT00000078039.1:c.6547_6548insA]), causing a frameshift and the appearance of a termination codon in exon 46. This mutation was confirmed by conventional PCR and Sanger sequencing ([Fig. 4](#)). The sequences of the primers used for these experiments were available in [Supplementary information](#).

Since polymyositis was suspected based on the MRI findings, prednisolone was administered at a dose of 2 mg/kg every 24 hr

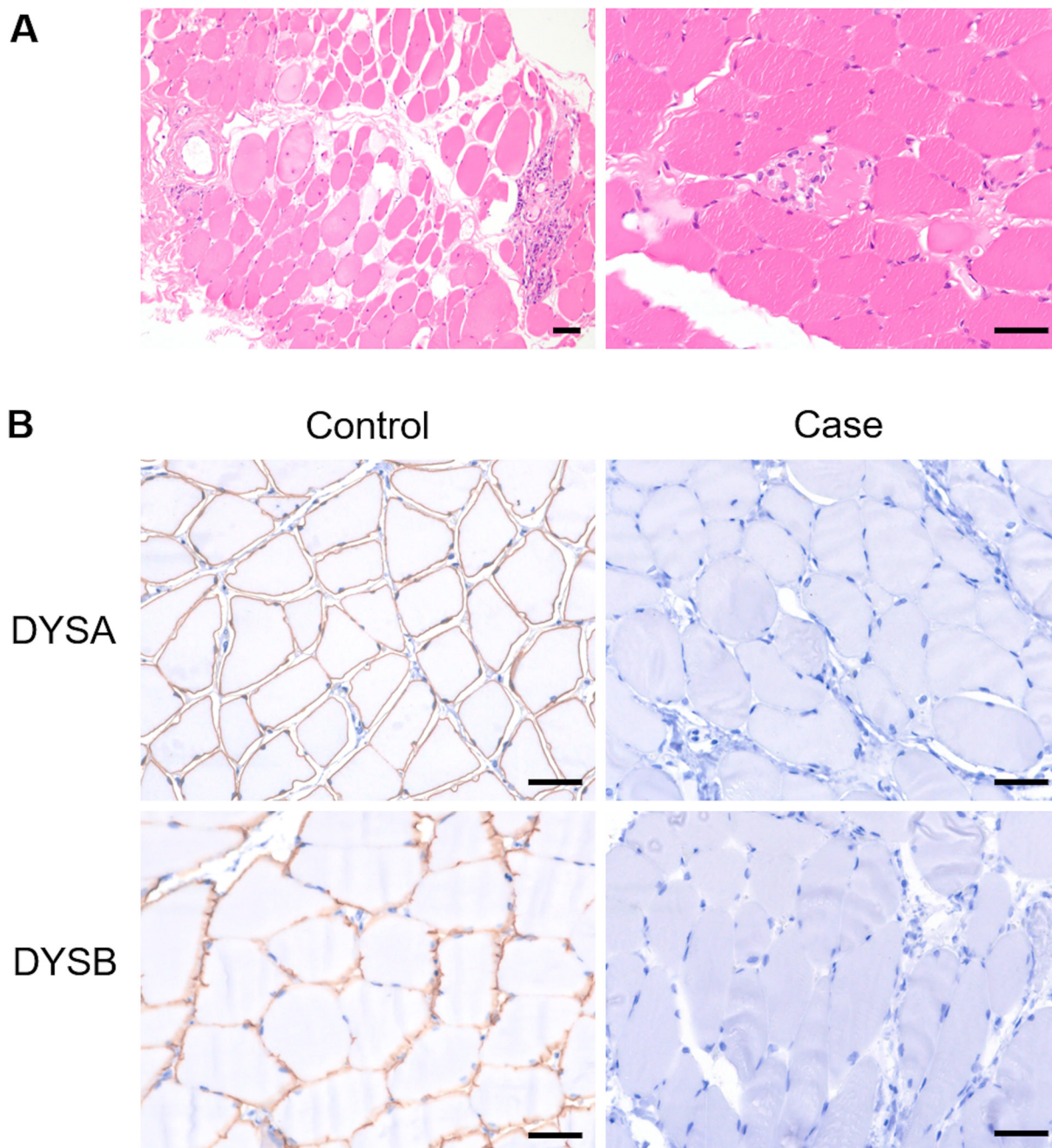


Fig. 3. Histopathological and immunohistochemical findings in muscle tissues of the dog. (A) Left side shows variation in myofiber size and myofiber degeneration. Right side shows phagocytosis of necrotic myofibers. Hematoxylin and eosin stain. Scale bars=50 µm. (B) Immunohistochemistry for C-terminus and N-terminus of dystrophin (DYSA and DYSB, respectively). Positive immunoreactivities were observed in normal controls whereas a complete absence of staining was observed in the case. Samples were counterstained with Mayer’s hematoxylin. Scale bars=50 µm.

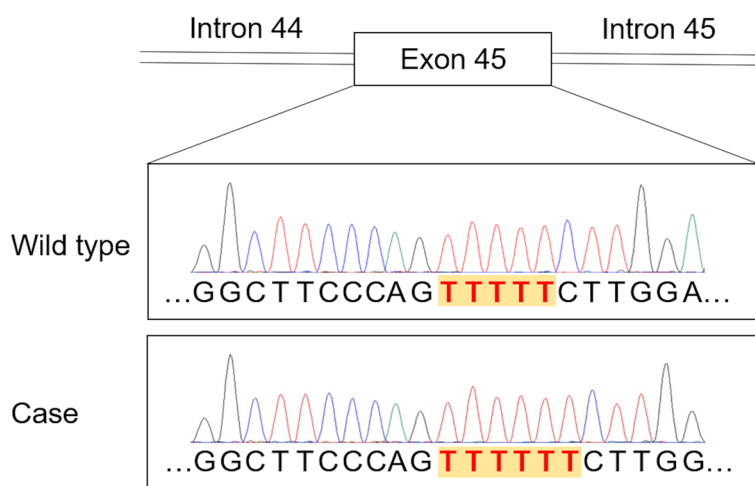


Fig. 4. Sanger sequencing of genomic DNA from Toy Poodles without muscular dystrophy (Wild type) and with muscular dystrophy (Case). A single base insertion in exon 45 of the *Duchenne muscular dystrophy* gene was confirmed in the case.

before histopathological evaluation of the muscle biopsy was completed. The owner's impression was that prednisolone mildly increased the activity of the dog. In addition, the administration of prednisolone resulted in a temporary decrease in the CPK level (3,442 IU/l); however, this was subsequently followed by a marked increase. Although the dog was ultimately diagnosed without polymyositis, and instead with muscular dystrophy, prednisolone administration was continued because it has been reported to delay loss of ambulation in human patients with DMD [20, 23]. The dose of prednisolone was gradually decreased and finally maintained at 0.5 mg/kg every 24 hr. Echocardiography, electrocardiography, and blood pressure measurements were performed after the diagnosis of muscular dystrophy. Since there were no abnormal findings in these examinations, any medical treatment for heart failure was not initiated. Simple physical therapy was performed at home, and at the time of this manuscript preparation the dog has survived for 294 days after diagnosis without any progression of gait and posture abnormalities.

To the best of our knowledge, this is the first report of muscular dystrophy in Toy Poodles. Furthermore, a mutation in the *DMD* gene was identified as a possible cause of the disease. Several reports have identified mutations in the *DMD* gene in canine dystrophin-deficient muscular dystrophy. The major mutations were deletions [9, 13, 16, 18], while the others were a substitution and an inversion [4, 22]. In this case, an insertion was identified, which is a new type of mutation in canine muscular dystrophy.

In this case, a single base pair insertion in exon 45 of the *DMD* gene caused a frameshift, leading to the appearance of a termination codon in exon 46. It was expected that this would result in the synthesis of incomplete-length dystrophin. However, immunohistochemistry did not detect the N-terminus or C-terminus of dystrophin. This discrepancy can be explained by nonsense-mediated mRNA decay, which is a translation-coupled mechanism that eliminates mRNAs containing premature translation termination codons [8, 14]. Determining *DMD* mRNA expression in muscle tissues would verify our hypothesis.

Prednisolone was administered to the case based on the treatment of human DMD [20, 23], which showed mild increase in activity. This may be due to the reduction of motor dysfunction caused by muscular dystrophy or simply the catabolic effect of this drug. Almost 300 days have passed since the initial visit of the case, and there was no progression in the disease probably owing to the therapeutic effect of prednisolone. However, it is difficult to determine the cause because no control case was set up in this study. Just as in human medicine, clinical trials are needed in veterinary medicine to investigate the therapeutic effects of prednisolone on muscular dystrophy.

DMD gene is on X chromosome, and dystrophin-deficient muscular dystrophy is inherited in an X-linked recessive pattern. Hence, this disease occurs generally in males because every male has only one X chromosome. In contrast, every female has two X chromosomes, which makes them carriers of this disease. Carriers themselves usually do not develop muscular dystrophy. However, their children may inherit the mutation or develop the diseases [11]. In human DMD, about two-thirds of patients result from transmission of a mutant *DMD* gene from a female carrier, whereas one-third of patients are due to sporadic *DMD* gene mutations [17]. Unfortunately, the cause of the development of muscular dystrophy in this case was unknown because we could not determine the genotype of the parent and sibling dogs. If a carrier is assumed to be the source of the disease, it is likely that the carrier currently exists in Japan because this Toy Poodle was purchased from a domestic breeder. In addition, considering that the Toy Poodle is the most popular breed in Japan and is actively bred, outbreaks of muscular dystrophy in this breed may occur in the near future. Since we could identify a single nucleotide insertion in exon 45 of the *DMD* gene, the detection of genetic abnormalities using genomic DNA extracted from peripheral blood may be useful for the diagnosis of muscular dystrophy and detection of carriers as a simple and non-invasive diagnostic method. Furthermore, detecting *DMD* gene mutations in dogs with muscular dystrophy may lead to new treatments such as exon skipping [19].

In conclusion, we diagnosed dystrophin-deficient muscular dystrophy in a Toy Poodle and identified the mutation in the *DMD* gene as a possible cause of the disease.

POTENTIAL CONFLICTS OF INTEREST. The authors have no conflicts of interest directly relevant to the content of this article.

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