Short Communication

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Surveyor assay to diagnose persistent Müllerian duct syndrome in Miniature Schnauzers

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Persistent Müllerian duct syndrome (PMDS) is a pseudohermaphroditism in males characterized by the presence of Müllerian duct derivatives. As PMDS dogs often lack clinical symptoms, a molecular diagnosis is essential to identify the syndrome in these animals. In this study, a new molecular method using DNA mismatch-specific Surveyor nuclease was developed. The Surveyor nuclease assay identified the *AMHR2* mutation that produced PMDS in a Miniature Schnauzer as accurately as that obtained by using the conventional method based on restriction digestion. As an alternative to the current molecular diagnostic method, the new method may result in increased accuracy when detecting PMDS.

Keywords: Miniature Schnauzer, persistent Müllerian duct syndrome, surveyor nuclease assay

Persistent Müllerian duct syndrome (PMDS) is an inherited autosomal recessive disorder of male pseudohermaphroditism characterized by the presence of Müllerian duct derivatives [3]. Female internal genitalia, including uterus and vagina, are observed in PMDS males because Müllerian duct regression does not occur [1]. The major etiological cause of PMDS in Miniature Schnauzers is a C to T transition (C241T) in exon 3 of the anti-Müllerian hormone receptor, type 2 gene (*AMHR2*; also known as Müllerian inhibiting substance receptor type II, *MISRII*), which mediates regression of the Müllerian duct and upper vagina [8].

Although a few cases of PMDS in Miniature Schnauzer dogs have been reported [3,4,7], it has been difficult to diagnose affected animals because carriers of the *AMHR2* mutation often show no clinical symptoms. Especially in male PMDS carriers, remnant Müllerian duct and upper vagina cannot be identified non-invasively. Since testicular tumors or pyometra could develop in affected dogs presenting with clinical symptoms of unilateral or bilateral cryptorchidism, early diagnosis is important to prevent such distressing outcomes [3,7]. Recently, a molecular diagnostic method for PMDS to identify affected dogs and carriers was developed [5]. In the present study, a new PMDS detection method using Surveyor nuclease was developed and its results compared to results of the previously reported method for determination of PMDS in Miniature Schnauzers [5].

A two-year-old male Miniature Schnauzer presented to the Dream Animal Hospital, Seoul, Korea with a sign of cryptorchidism. Unilateral cryptorchidism with external male genitalia was identified during physical examination. The smaller than normal left testis was located within the scrotum; however, the right testis was not palpable. The rest of the physical examination findings were normal. Diagnosed with cryptorchidism, a laparotomy was performed to reduce the undescended testis. The surgery revealed a uterus-like structure instead of a spermatic duct. Both testes were connected to the horns of uterus-like structure. All structures were surgically removed (Fig. 1).

Genomic DNA was isolated from the testis tissue obtained from the affected dog and from the blood of a normal Miniature Schnauzer by using DNeasy Blood & Tissue kits (Qiagen, USA). The polymerase chain reaction (PCR) was performed as previously reported [6] using the primer set forward 5′-AGC TAG GGT GGG AAA CAG GT-3′ and reverse 5′-CCT GGA

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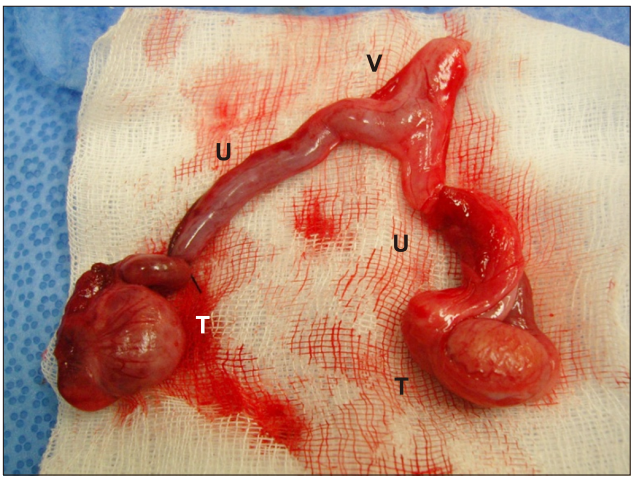

Fig. 1. Gross appearance of the uterus and testes surgically removed from a PMDS-affected Miniature Schnauzer. U, uterus; T, testis; V, vagina.

CGT TAA GCC AAG AA-3′ to amplify a 662 bp fragment within exon 3 of canine *AMHR2*, which includes the location of the C241T mutation. The reaction conditions used were: initial denaturation (95°C, 3 min), 30 cycles of denaturation (95°C, 30 sec), annealing (60°C, 30 sec), and extension (72°C, 1 min) followed by a final extension (72°C, 5 min). The 662 bp PCR products were separated by electrophoresis using 2% agarose gel, and the band was excised from the gel and purified by using NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, USA).

To detect the C241T transition of *AMHR2*, restriction digestion was performed in a reaction mixture containing 2 μL of 10× Restriction Enzyme Buffer D (Promega, USA), 2 units of *Dde* I restriction enzyme, 3 μL (100 ng) of DNA purified from the PCR band, and DNase-free water to obtain a 20 μL total reaction volume. The reaction mixture was incubated at 37°C for 2 h, after which the digestion product was separated by electrophoresis using 2% agarose gel. As described previously [5], digestion of the 662 bp PCR product with *Dde* I yields normal (CC; 333, 255, and 74 bps) or affected (TT; 333, 212, and 43 bps) DNA fragments (panel of A in Fig. 2).

In addition to examining the restriction fragment length polymorphism (RFLP) results, the Surveyor nuclease assay, a new method to detect the C241T mutation, was performed using the SURVEYOR Mutation Detection Kit (Transgenomic, USA). That assay detects a bubble originating from a point mutation within a DNA strand by using Surveyor nuclease, an enzyme that specifically cleaves a DNA mismatch [2,6]. Equal amounts of the purified PCR bands from the PMDS-affected and the unaffected normal Miniature Schnauzers were mixed, denatured, and allowed to rehybridize. The rehybridized DNA (200 ng) was incubated with 1 μL each of Surveyor nuclease S and enhancer at 42°C for 1 h. The treated samples were separated on a 2% agarose gel. The Surveyor nuclease restriction cleaved

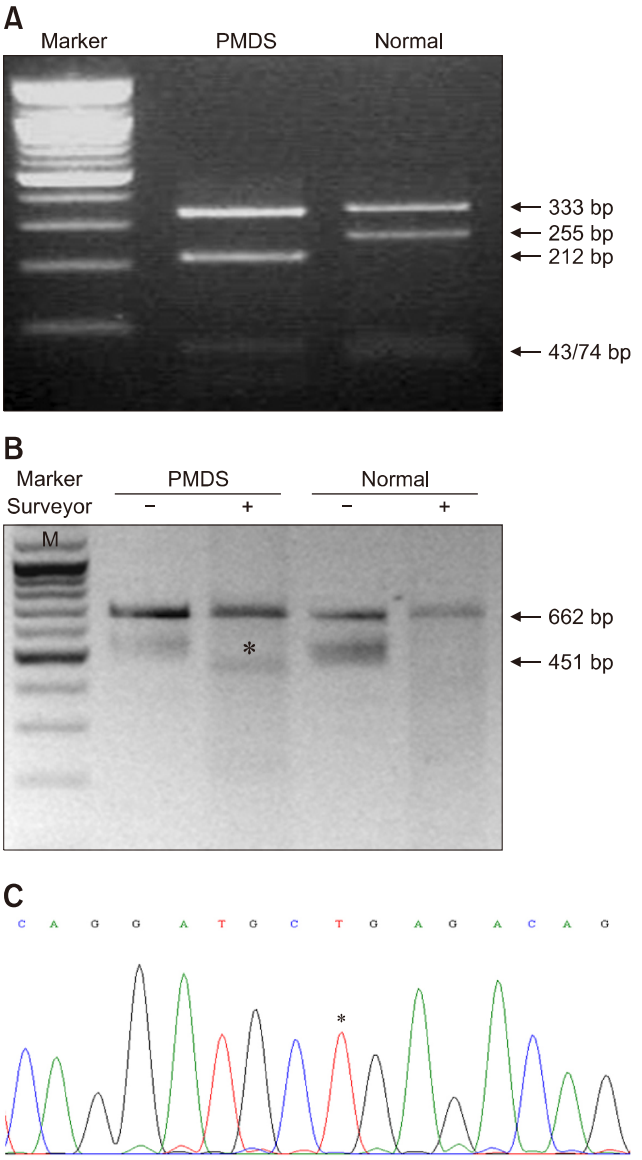

Fig. 2. (A) Restriction digestion of *AMHR2* from PMDS and normal Miniature Schnauzers. The PMDS dog had 212, 333, and 43 bp DNA fragments, whereas the normal dog had 255, 333, and 74 bp DNA fragments. (B) Surveyor nuclease assay of AMHR2 from PMDS and normal Miniature Schnauzer. The 662 bp heteroduplexed PCR amplicon is cleaved into 451 bp and 211 bp fragments in the PMDS dog, whereas no cleavage was detected in the normal dog. The 451 bp-band restricted by Surveyor nuclease is indicated by an asterisk. (C) C241T mutation detection by direct sequencing of *AMHR2* using the PCR product from the PMDS Miniature Schnauzer. The asterisk indicates a C to T conversion.

the 662 bp heteroduplex amplicon into 451 and 211 bp fragments (panel B in Fig. 2).

To confirm the genotypes identified by the restriction digestion and Surveyor nuclease assays, the 662 bp PCR products from the PMDS-affected dog were sequenced. The

resulting sequences were compared to the normal canine *AMHR2* sequence (GenBank accession No. XM_543632). As shown in panel C in Fig. 2, the sequences of the PCR products obtained from the two diagnostic methods contained the C241T mutation in exon 3 of *AMHR2*.

This study demonstrates that both molecular diagnostic methods were separately able to detect the mutation associated with PMDS in the affected Miniature Schnauzer. Both the previously reported RFLP assay [5] and the Surveyor nuclease assay can be used to identify the mutation causing PMDS. Thus, the Surveyor nuclease assay is an effective alternative to the current detection method, and, by combining the two different methods, increased accuracy of PMDS diagnosis can be expected.

Because PMDS may cause infertility and testicular cancer in Miniature Schnauzer dogs, the prevention and/or eradication of PMDS would be desirable to improve the health of the breed [5,7]. In this context, accurate diagnosis in affected and carrier dogs is critical. Nevertheless, it is difficult to clinically diagnose PMDS in Miniature Schnauzers, as carrier dogs usually do not exhibit clinical symptoms; moreover, only 50% of affected dogs exhibit cryptorchid clinical symptoms [3]. Such difficulties could potentially be solved by using molecular-based diagnostic tools. Clinical use of the Surveyor nuclease assay described in this study, either separately or in conjunction with the conventional RFLP method, may provide a simple, accurate, and convenient PMDS diagnostic tool.

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Conflict of Interest

The authors declare no conflicts of interest.

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