Researches Concerning the Molecular Testing of Basset Hound Dogs For Detecting Persistent Müllerian Duct Syndrome (PMDS)

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Abstract

The Persistent Müllerian Duct Syndrome (PMDS), a rare form of dog male pseudo-hermaphroditism, has been previously studied from molecular aspect in miniature schnauzers by Matsuu et al. (2009), which detected an affected case, positive to Sry gene detection, by PCR. The molecular testing for PMDS syndrome has not yet been performed in Basset Hound dogs, although affected cases exist. The aim of our research was to test the previously published protocol for Sry gene detection in an affected PMDS Basset Hound family so as to develop a possible molecular method for genotyping. The study was performed on 11 Basset Hound individuals, among which 1 affected male and 2 non-affected carriers. The protocol was applied accordingly to a previously one published by Matsuu et al. (2009). The DNA was extracted from peripheral blood samples, using a specific DNA extraction kit (Isolate II, Bioline, England). Briefly, the PCR technique was performed on 25 µl of mixture containing: 12.5 µl MasterMix (Bioline), 1 µl Forward Primer and 1 µl of Reverse Primer, 6.5 µl DNA-free nuclease PCR water (Sigma Aldrich, Germany). The PCR products were visualized in 2% agarose gel stained with EvaGreen (Sigma, England). The tested protocol has shown specificity in the case of the positive affected male, showing a light-band at 104 base pairs in the agarose gel. All the other samples were negative tested for Sry gene. Given the fact that this protocol does not use a restriction enzyme for possible detection of genotypes and carriers, we could not differentiate the carriers from the non-affected individuals. This protocol can be used only in the case of affected males but does not distinguish the carriers from the non-carriers. We should furtherly investigate other candidate genes for PMDS, such as MISRII, for the possible detection of carrier females and males.

Keywords: *PMDS, Sry gene, PCR*

INTRODUCTION

Numerous defects of sexual development in dogs were described by Meyers-Wallen (1993, 1999), who recognized anomalies of chromosomal, gonadal and phenotypic sex. In some situations, chromosomal and gonadal sex agree, but the internal and external genitalia are ambiguous or even alternative. These cases are categorized as abnormalities of phenotypic sex: male or female pseudo-hermaphrodites (Meyers-Wallen, 1993, 1999).

The Persistent Müllerian Duct Syndrome (PMDS) is a failure of Müllerian duct regression which determines the embryos to divert from the typical male developmental pathway, allowing female sexual structures such as oviducts, uterus and vagina to appear (Anatoly Ruvinsky, J. Sampson, 2001).

The Persistent Müllerian Duct Syndrome (PMDS), a rare form of dog male pseudo-hermaphroditism, (it) has been previously studied from a molecular aspect in miniature schnauzers

by Matsuu *et al.* (2009), which detected using PCR an affected case, positive to Sry gene. The molecular testing for PMDS syndrome has not been performed yet in Basset Hound dogs, although affected cases exist.

MATERIALS AND METHODS

The study was performed on 11 Basset Hound individuals, among which 1 affected male and 2 non-affected carrier. All other nine individuals had an unknown status regarding PMDS, not being related to affected and single carriers males. The male patient was diagnosed during a routine ultrasound exam, which is required before their use for breeding. His father and one of his half brother (who also sired an PMDS affected son) were negatively scanned by ultrasound, but from the genetical point of view they are single carriers (as genitors of affected males). The other eight individuals included in this study, unrelated to the first three, were subjected to ultrasound exam, being diagnosed negative, hence conclude that their genetic status can be negative or heterozygous carriers.

DNA Extraction

White cells from blood were collected by centrifugation, and genomic DNA was extracted using the Kapa blood PCR kit according to the manufacturer's instructions (KapaBiosystems, England). DNA quantity and purity of each sample were assessed on a Nanodrop ND-1000 spectrophotometer analyser (NanoDrop Technologies, Inc., Wilmington, DE, USA).

PCR-RFLP Genotyping

The PCR-RFLP genotyping was performed accordingly to the protocol previously published by Pujar *et al.* (2009). Briefly, primers were acquired according the above mentioned study: Primer Forward: 5'-AGCTAGGGTGGGAAACAGGT-3' and Reverse 5'-CCTGGACGTTAAGCCAAGAA-3'. The PCR amplification protocol used was the following: 1X PCR green Buffer, 2.5 mM MgCl₂, 5 pmol of each primer, dNTPs each at 200 µM, 2.5 U of Taq DNA Polymerase (Promega, Madison, WI, USA) and 100 ng of genomic DNA. PCR was performed under the following conditions: 94°C for 3 min followed by 35 cycles of 94°C for 30 sec, 56°C for 30sec, 72°C for 1 min and a final extension step of 72°C for 5 min.

Amplified products (15 µl) were digested with 10U *DdeI* (Fermentas, Lithuania), 4 h at

37°C and were separated in 2.5% agarose gel containing 1X SybrSafe (Invitrogen, Eugene, OR, USA). Electrophoresis was performed in a TBE buffer (pH= 8.5) at 60 V constant current for 2.5 hours. The gel was then analyzed with a Molecular Imager Gel Doc XR System (BioRad Laboratories, Hercules, CA, USA).

RESULTS AND DISCUSSION

The tested protocol has shown specificity in the case of the positive affected male, showing a light-band at 104 base pairs in the agarose gel. (Fig. 1)

All the other samples were tested negative for Sry gene.

Given the fact that this protocol does not use a restriction enzyme for possible detection of genotypes and carriers, we could not differentiate the carriers from the non-affected individuals.

At approximately 50% of positive PMDS males, in the clinical examination no external changes are observed, so they are not identified by experienced breeders or by veterinarians through the regular clinical examination. These dogs are rarely diagnosed with PMDS, only until health problems occur. At the time of the positive PMDS males are diagnosed, in most cases they or their parents and relatives have produced a large number of offspring, thereby contributing to the spread of the disease in populations of that breed. In approximately 50% of males that suffer from this syndrome are found other disorders with

Fig. 1. The electrophoretic profile of the 662 bp fragment corresponding to Sry gene. Sample 1: positive control; samples 2,3,4,5: negative samples in the DNA extracted from basset hound blood.

hereditary etiological component, unilateral or bilateral cryptorchidism, or complications of one of the retained testes into the abdominal cavity such as testicular tumors, most often Sertoli cell tumors, aspect confirmed by S. Pujari (2009) and Meyers-Wallen VN (1993).

PMDS affects males belonging to different breeds: Basset Hound, German Shepherd, Labrador Retriever. Similar studies indicate the presence of this hereditary disease in other breeds such as the Miniature Schnauzer (Pujari S., 2009, Meyers-Wallen VN, 1993), English Cocker Spaniel, Golden Retriever and Pug.

Matsuu et al. (2009) studied a case, a 10-year-old Miniature Schnauzer with bilateral cryptorchidism and after cytological, histological and bacterial examinations, the dog was diagnosed with bilateral Sertoli cell tumor with hydrometra. positive to Sry gene detection by PCR. The karyotype was 78, XY and was positive to Sry gene detection by PCR.

Wu et al. (2009) stated that the phenotype in the canine model of PMDS derived from the miniature schnauzer breed is strikingly similar to that of human patients. In the model Wu et al. (2009) described, PMDS is inherited as a sex-limited autosomal recessive trait. In this study the canine PMDS phenotype and clinical sequelae were described. Wu et al. (2009) genotyped affected and unaffected members, identifying a single base pair substitution in MISRII that introduces a stop codon in exon 3.

As described by Pujar et al. (2009) the possible genotypes are homozygous CC (normal dogs), heterozygous CT (normal dogs but carriers) and TT (PMDS affected). The study performed by Pujar et al. (2009) in the canine miniature schnauzer model, PMDS is caused by a C to T transition in exon 3 of the Müllerian inhibiting substance type II receptor (*MISRII*), which introduces a *DdeI* restriction site.

CONCLUSIONS

This protocol can be used only in the case of affected males but does not distinguish the carriers from the non-carriers. We should furtherly investigate other candidate genes for PMDS, such as MISRII, for the possible detection of female and male carriers.

The mutation causing PMDS in Basset Hound males should be searched in another location

of Sry gene or in other genes related with sex differentiation process.

To reduce the incidence of Persistent Müllerian Duct Syndrome in populations of breed dogs we recommend the ultrasound screening of males (in the absence of a genetic test capable of identifying the mutation responsible for maintaining uterine structures in males) from the age of one year in order to gain mating rights.

A target sequencing of several candidate gene could be further performed and if is not successful a full genome scan could be very successful, but in this case a pedigree information and sampling would be very important.

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