

A Single Base Pair Mutation Encoding a Premature Stop Codon in the MIS Type II Receptor Is Responsible for Canine Persistent Müllerian Duct Syndrome

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ABSTRACT: Müllerian inhibiting substance (MIS), a secreted glycoprotein in the transforming growth factor- β family of growth factors, mediates regression of the Müllerian ducts during embryonic sex differentiation in males. In persistent Müllerian duct syndrome (PMDS), rather than undergoing involution, the Müllerian ducts persist in males, giving rise to the uterus, fallopian tubes, and upper vagina. Genetic defects in MIS or its receptor (*MISRII*) have been identified in patients with PMDS. The phenotype in the canine model of PMDS derived from the miniature schnauzer breed is strikingly similar to that of human patients. In this model, PMDS is inherited as a sex-limited autosomal recessive trait. Previous studies indicated that a defect in the MIS receptor or its downstream signaling pathway was likely to be causative of the canine syndrome. In this study, the

canine PMDS phenotype and clinical sequelae are described in detail. Affected and unaffected members of this pedigree are genotyped, identifying a single base pair substitution in *MISRII* that introduces a stop codon in exon 3. The homozygous mutation terminates translation at 80 amino acids, eliminating much of the extracellular domain and the entire transmembrane and intracellular signaling domains. Findings in this model could enable insights to be garnered from correlation of detailed clinical descriptions with molecular defects, which are not otherwise possible in the human syndrome.

Key words: Dog, anti-Müllerian hormone, AMH type II receptor (AMHR2), Müllerian duct regression.

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During sexual differentiation of male embryos, Müllerian inhibiting substance (MIS), also called anti-Müllerian hormone (AMH), plays an important role in mediating regression of the Müllerian ducts, the embryologic precursors of the uterus, fallopian tubes, and upper vagina (Jost, 1953). MIS is a secreted glycoprotein in the transforming growth factor- β (TGF- β) family of growth factors, which was identified initially for its fetal role in Müllerian duct regression but has been found to have additional postnatal roles in gonad development (Racine et al, 1998; Durlinger et al, 1999; Lee et al, 1999; Wu et al, 2005). Like other members of the TGF- β family, MIS

signals through a receptor complex comprised of type I and II serine-threonine kinase receptors. MIS binds to its type II receptor (MISRII), which has a cysteine-rich extracellular domain that confers ligand specificity and an intracellular kinase domain (Baarends et al, 1994; di Clemente et al, 1994; Teixeira and Donahoe, 1996). Upon ligand binding, MISRII recruits and phosphorylates a shared, ligand-independent type I receptor, which initiates downstream activation of intracellular mediators such as Smad1, 5, and 8 (Guedard et al, 2000; Visser et al, 2001; Jamin et al, 2002) other signaling pathways, or both, such as beta-catenin (Allard et al, 2000) and nuclear factor κ B (Segev et al, 2002). The ability of MIS to induce Müllerian duct regression is thought to be mediated via a paracrine mechanism involving mesothelial-epithelial interactions. Although MISRII is expressed in the mesenchymal cells, it is the adjacent epithelial cells that undergo apoptotic cell death during Müllerian duct regression (Allard et al, 2000; Xavier and Allard, 2003). The exact signaling pathway has not been delineated; nevertheless, the expression of biologically active MIS and its receptors

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during a critical embryonic window is essential for normal male-specific internal reproductive tract development.

A defect in MIS signaling causes retained rudimentary Müllerian structures or an infantile uterus and Fallopian tubes, a condition found in humans (Brook et al, 1973; Sloan and Walsh, 1976) as well as a number of other species such as dogs (Brown et al, 1976), cattle (Jost, 1965), goats (Haibel and Rojko, 1990), and cats (Schulman and Levine, 1989). In infants and children, this is typically identified at the time of surgery for cryptorchidism or inguinal hernia and is termed persistent Müllerian duct syndrome (PMDS; Brook et al, 1973; Sloan and Walsh, 1976; Josso et al, 1983). The testes can be bilaterally undescended, or one can descend and carry the contralateral testis into the same scrotum, a condition called transverse testicular ectopia.

In patients with PMDS, the mode of inheritance is primarily autosomal recessive. Molecular studies have identified genetic defects in either *MIS* or *MISRII* in over 80% of patients with PMDS (Guerrier et al, 1989; Imbeaud et al, 1994, 1995; Messika-Zeitoun et al, 2001; Belville et al, 2004). Those patients with *MIS* gene defects have unmeasurable or low serum concentrations of MIS and have been found to have a number of different mutations spanning the gene. In contrast, mutations of *MISRII* are more conserved, with a common 27-bp deletion in exon 10 in the serine-threonine kinase domain, accounting for 25% of known receptor defects (Imbeaud et al, 1996). The *MISRII* mutations identified thus far either affect ligand binding or abrogate kinase activity of the receptor with no mutations of the transmembrane region (exon 4) identified as yet.

Canine PMDS has been reported as an inherited disorder in 2 breeds: the miniature schnauzer in the United States (Brown et al, 1976; Marshall et al, 1982; Meyers-Wallen et al, 1989) and the basset hound in Europe (Nickel et al, 1992). The causative genetic defect has not been identified in either breed. The canine model derived from the PMDS miniature schnauzer has a phenotype that is strikingly similar to that of human PMDS (Meyers-Wallen et al, 1989). In the PMDS model, the expression of MIS mRNA and protein were no different in testes of PMDS embryos than those of normal embryos during the critical period for Müllerian duct regression (Meyers-Wallen et al, 1991, 1993). Furthermore, in studies for which a semi-quantitative urogenital ridge organ culture bioassay for MIS activity was used (Donahoe et al, 1976, 1977a,b,c; Meyers-Wallen et al, 1989), testes from affected neonates and embryos were found to have comparable MIS bioactivity during Müllerian duct regression as age-matched

testes of normal dogs, confirming that the MIS was biologically functional. These findings indicated that target organ insensitivity caused by a mutation in either *MISRII* or a downstream gene in its signaling pathway was likely to be causative (Meyers-Wallen et al, 1993). In this study, we delineate the phenotype and clinical sequelae of this canine PMDS model in greater detail and identify the causative molecular defect in the pedigree with sex-limited autosomal recessive PMDS.

Methods

All animal investigations were conducted in accordance with NIH guidelines for vertebrate animal research and were approved by the Institutional Animal Care and Use Committees (University of Pennsylvania and Cornell University).

Pedigree

The canine PMDS model pedigree was derived at the University of Pennsylvania from 1 purebred miniature schnauzer litter containing 2 affected males, as previously described (Meyers-Wallen et al, 1989). In experimental matings, 2 PMDS miniature schnauzer males from that initial litter (A8 and A9, Figure 1) were outcrossed to beagle females to produce F1 generations, which then produced F2 and F1 backcross (F1BC) generations (Meyers-Wallen et al, 1989). Thereafter, the model was maintained by inbreeding within the pedigree. As previously reported, χ^2 analysis of affected dogs in the F2 and F1BC generations excluded autosomal dominant inheritance of the trait ($P = .003$). No PMDS males were produced when a proven carrier miniature schnauzer female (A2) was bred to males of a different breed, rejecting X-linked inheritance ($P < .001$, χ^2 analysis). However, sex-limited autosomal recessive inheritance, in which only homozygous mutant males express the PMDS phenotype, could not be rejected (F1BC, $P = .92$; F2, $P = .53$).

For this study, an informative subset from the large PMDS model pedigree maintained at Cornell University was chosen for mutational analysis on the basis of availability of samples for DNA extraction (Figure 1). This subset included normal females and males, PMDS males, and proven carriers identified by production of PMDS offspring in experimental matings. Diagnosis of PMDS was established by a normal male karyotype (78, XY) and the presence of bilateral oviducts, uterus, and cranial vagina, identified during surgery or necropsy as previously described (Meyers-Wallen et al, 1989). The subset also included normal and PMDS males whose testes were previously tested in the organ culture assay and found to contain biologically active MIS (A141, A181, A185, A216, A217, A218, A265; Meyers-Wallen et al, 1989). An unrelated beagle female was also sequenced as a normal control (A66).

Sequencing Canine PMDS MISRII

Canine *MISRII* is homologous to the human gene; therefore, the exons (2, 3, 5, 6, 10) encoding the ligand binding and kinase

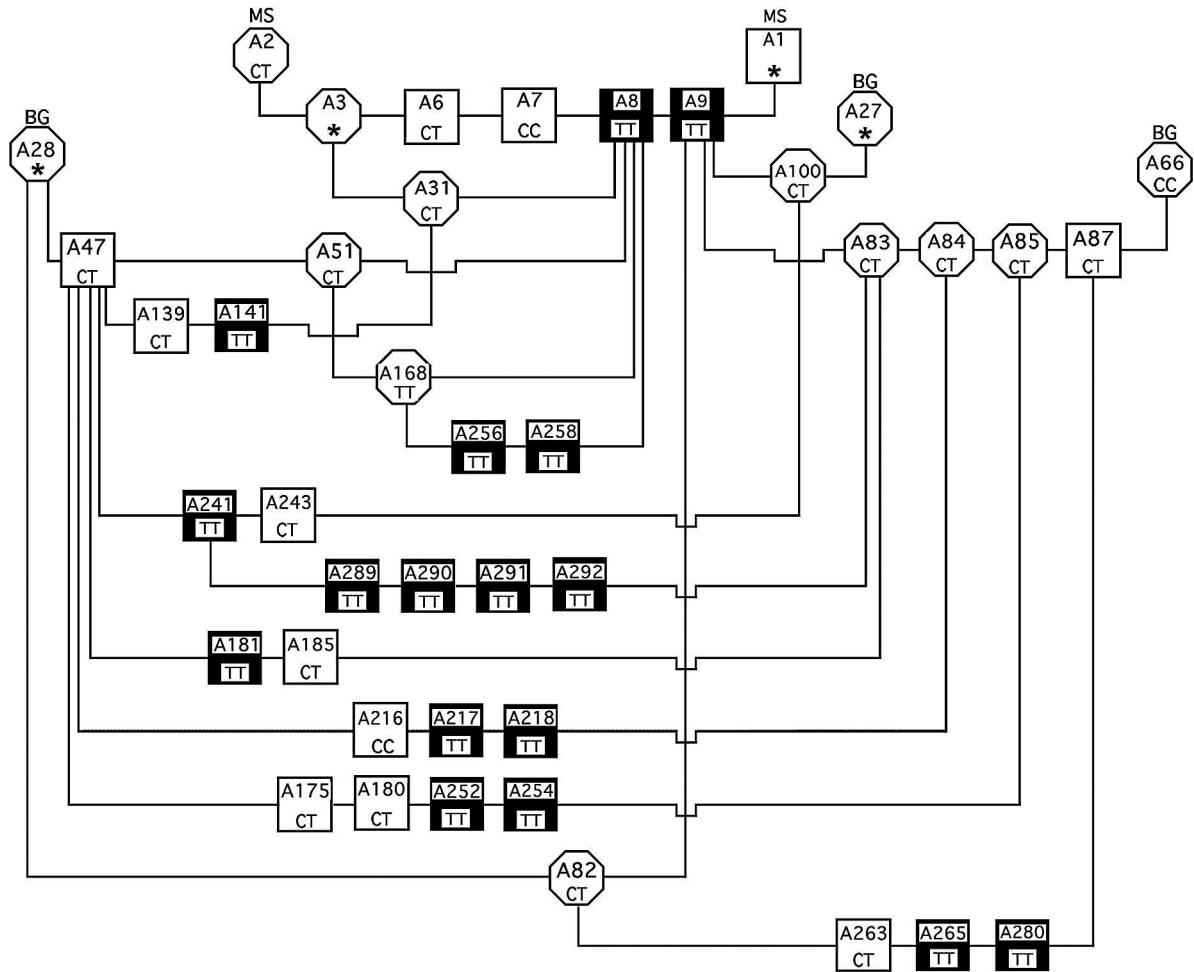


Figure 1. The canine persistent Müllerian duct syndrome (PMDS) pedigree subset was screened for the C241T mutation in exon 3 of *MISRII* (nucleotide 241 in predicted mRNA sequence, <http://genome.ucsc.edu>, May 2007 assembly). This subset contains 42 dogs selected from a pedigree derived from the miniature schnauzer (MS) breed and outcrossed to beagles (BG). PMDS males are symbolized by filled squares, normal males by open squares, and females by open circles. Within each symbol is the animal identification number (top) and genotype at the 241 base position (bottom). The asterisks indicate that DNA was unavailable for those dogs.

domains that have been reported to have a high frequency of mutations in human PMDS patients were initially targeted for screening (Imbeaud et al, 1995, 1996; Belville et al, 1999; Messika-Zeitoun et al, 2001; Hoshiya et al, 2003; Zenteno et al, 2004; Josso

et al, 2005). Primer pairs (Table 1) were designed from the *Canis familiaris* chromosome 27 genomic contig, containing whole genome sequence (<http://www.ncbi.nlm.nih.gov>, accession NW139903; range, 1800250–1806150), using Primer3 ([Table 1. Primer pair sequences, designed to amplify canine MISRII exons 2, 3, 5, 6, and 10](http://</p>
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MISRII Primer	Sequence 5' to 3'	Product Size, bp
Exon 2F	TACCTCTGCTGATCCTGTTTG	429
Exon 2R	ACACATTCCATTTCTCCTCCT	
Exon 3F	TTCCTAGACCCAGTTATGCTCGCT	330
Exon 3R	AACCAGCCTTGGTTCTACCTCTCA	
Exon 5F	GCAGCATCATCTGGGTACT	452
Exon 5R	AGATTACCTGGCGAAACAC	
Exon 6F	TGTGTTTCTCCAGGTGCCT	449
Exon 6R	CTGAAGCGGATGAATAAGCA	
Exon 10F	CTCACCCTCTCTATGTCC	437
Exon 10R	TGGAAGAGGATGAGTTGAGGT	

Abbreviations: MIS, Müllerian inhibiting substance; MISRII, MIS type II receptor.

frodo.wi.mit.edu). Each primer pair was designed to amplify the entire exon and splice junctions in the predicted *C familiaris* homolog of the human AMH receptor, type II mRNA (<http://www.ncbi.nlm.nih.gov>, accession XM_543632) identified by the Basic Local Alignment Search Tool (Altschul et al, 1990).

Genomic DNA was extracted from stored whole blood samples of 38 dogs by standard phenol and chloroform extraction with ethanol precipitation (Sambrook et al, 1989) then quantified by spectrophotometry (NanoDrop ND-1000 spectrophotometer; NanoDrop Products, Wilmington, Delaware). Polymerase chain reaction (PCR) amplification of exons 2, 3, 5, 6, and 10 from genomic DNA was performed in the Mastercycler ep realplex PCR detection system (Eppendorf, Hamburg, Germany). Each PCR reaction (50 μ l) contained 100–400 ng of DNA in reaction buffer with 1.5 mM MgCl₂, 200 μ M dNTPs, 200–300 nM of the primers, and Taq DNA polymerase (Roche Applied Science, Indianapolis, Indiana, or Denville Scientific INC, Metuchen, New Jersey). Reaction conditions were: initial denaturation (94°C, 5 minutes); then 30 cycles of denaturation (94°C, 20 seconds), annealing (50.6°C, 20 seconds), and extension (72°C, 30 seconds); with a final extension stage (72°C, 5 minutes).

PCR products were separated by electrophoresis in 1.5% agarose gels, and PCR-amplified products were purified with a MinElute Gel Extraction Kit (Qiagen, Valencia, California). The primer pairs (Table 1) were used for bidirectional sequencing with the Applied Biosystems BigDye V1.1 Cycle Sequencing Kit (Applied Biosystems 3130xl Genetic Analyzer; Applied Biosystems, Foster City, California; DNA Sequencing Facility, UMass Medical School) with the use of 15–25 ng of purified PCR products. The sequence output was processed with the FinchTV program (version 1.4; Geospiza Inc, Seattle, Washington) and compared with the predicted mRNA sequence in the canine genome database (<http://www.ncbi.nlm.nih.gov/blast>).

Results

Canine PMDS Males: Phenotype and Clinical Sequelae

PMDS males in this model have a normal male karyotype (78, XY) and bilateral testes but develop bilateral oviducts (uterine tubes or fallopian tubes), a complete bicornuate uterus and uterine body, a cervix, and the cranial portion of the vagina (upper vagina), which enters the dorsal prostate (Meyers-Wallen et al, 1989; Figure 2). Histological sections in PMDS neonates revealed that some PMDS males have small-diameter connections, either single or paired, between the cranial vagina and the prostatic urethra (data not shown). These are likely remnants of the Müllerian duct junction with the embryonic urogenital sinus. The uterine horns and vasa deferentia lie parallel to each other along their entire course (Figures 3 and 4). PMDS dogs also have complete male internal genitalia, including bilateral epididymides, vasa deferentia (adja-

cent to the uterine horns), and a prostate. Grossly apparent unilateral segmental aplasia of the body of the epididymis was present in 1 affected dog (A8; Table 2); however, epididymides were grossly normal in all other PMDS males examined.

Externally, PMDS dogs have a normal male phenotype, except that approximately 50% are either unilaterally or bilaterally cryptorchid (Meyers-Wallen et al, 1989). Late descent of the testis into the scrotum has been observed in some PMDS males of this model, for example A8 (Table 2). The cranial end of the uterine horn is firmly attached to the caudal pole of the testis, which can impair testis descent. Transverse testicular ectopia has not been identified in affected dogs of this model. Histological features of cryptorchid testes in PMDS dogs include absence of germ cells, whereas scrotal testes appear normal (Figures 3 and 4). Although PMDS males with bilateral scrotal testes were fertile, sperm counts were frequently lower than expected for body weight (Table 2). In 1 case, this was explained by unilateral segmental aplasia of the body of the epididymis (A8). The cause remains unknown in others, as no barrier to sperm transport was identified in the epididymis or vas deferens. Dogs with bilateral cryptorchidism were sterile. As in dogs that are not affected by PMDS but are unilaterally cryptorchid, PMDS males with unilateral cryptorchidism were subfertile, in that sperm counts were less than those of normal males of similar body weight (Table 2). However, by using timed breeding management, in which insemination was performed at the optimal period for fertilization, litters were obtained from all PMDS males with unilateral cryptorchidism that were bred ($n = 3$).

As in other cryptorchid dogs, Sertoli cell tumor (Figure 5) is a common sequela to cryptorchidism in aged PMDS males (Brown et al, 1976; Marshall et al, 1982). Pyometra is also reported in older PMDS miniature schnauzers (Marshall et al, 1982; Figure 5). A vaginal-urethral connection is the likely route for ascending infection in such cases. For example, at 14 months of age, a breeding PMDS male (A241; Table 2) developed pyometra, which was treated with a combination of systemic antibiotics and surgical therapy. Briefly, through a ventral approach into the prostatic urethra, the orifice of the vaginal-urethral connection was identified in the dorsal urethral wall, isolated by catheterization, and ligated. The uterine body was drained by marsupialization. Four weeks postoperatively, infection had resolved. Spermatozoa were not identified in monthly semen collections until 4 months postoperatively, after which the sperm count stabilized in the normal range. This male sired litters until retirement (4 years postoperatively; Table 2) and, to

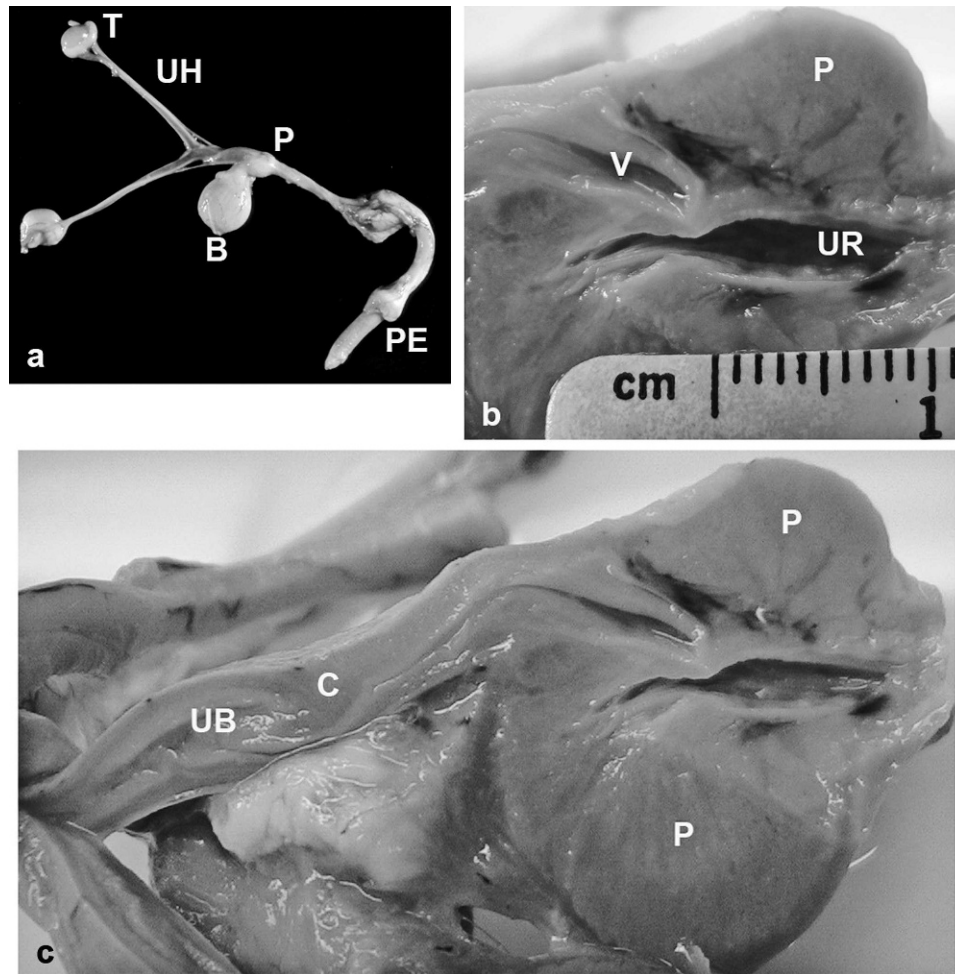


Figure 2. Gross features of the reproductive tract in PMDS males: (a) complete reproductive tract, age 60 days. (b, c) A portion of the formalin-fixed reproductive tract from a 2.5-year-old PMDS male: (b) cranial vagina entering the dorsal prostate, longitudinal cut bisecting the prostate—note the thin separation between the vagina and the prostatic urethra; (c) longitudinal cut that is lateral and parallel to that shown in panel b, showing additional Müllerian duct derivatives lying cranial to the prostate. T indicates testis; UH, uterine horn; P, prostate; B, urinary bladder; PE, penis; V, cranial vagina; UR, urethra; UB, uterine body; C, cervix.

the authors' knowledge, is the only case of pyometra in a PMDS male dog in which fertility has been restored.

Canine PMDS MISRII Sequence Analysis

The PCR-amplified sequences of canine *MISRII* exons 2, 5, 6, or 10 were identical among PMDS affected, unaffected, and heterozygous dogs. A single base pair substitution (C241T) in exon 3 of the *C familiaris MISRII* mRNA (<http://www.ncbi.nlm.nih.gov>, accession XM_543632) was identified (Figures 6 and 7). The nucleotide at this position is cytosine in the predicted mRNA sequence from the canine genome database and in unaffected dogs in this pedigree, as well as the unrelated control beagle (Figure 1). Homozygous thymidine substitution at this position alters the reading frame by changing the codon from arginine to a stop codon (TGA) at nucleotides 241–243 in Exon 3

(Figure 7). Genotypes at the nucleotide 241 position are concordant with the phenotypes of all dogs tested in the pedigree subset. Specifically, all affected males are homozygous for the mutation (TT), and all males with a normal phenotype are either wild type (CC) or heterozygous (CT) (Figure 1). Furthermore, the genotypes identified were consistent with the number of PMDS offspring produced by individuals involved in experimental matings. For example, when bred to PMDS males (TT), female A168 (TT) produced 9 PMDS males but no normal males. Similarly, when bred to 2 proven carrier females, normal male A7 (CC) produced 2 normal males but no PMDS males (Figure 1; Table 2). These results provide the following genotypes at nucleotide 241 for the 38 dogs sequenced: 17 PMDS males (TT), 11 phenotypically normal males (C/T or CC), 1 homozygous female (TT), 8 heterozygous

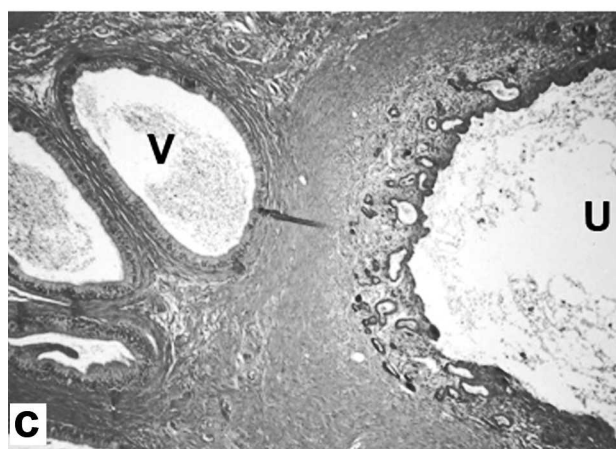
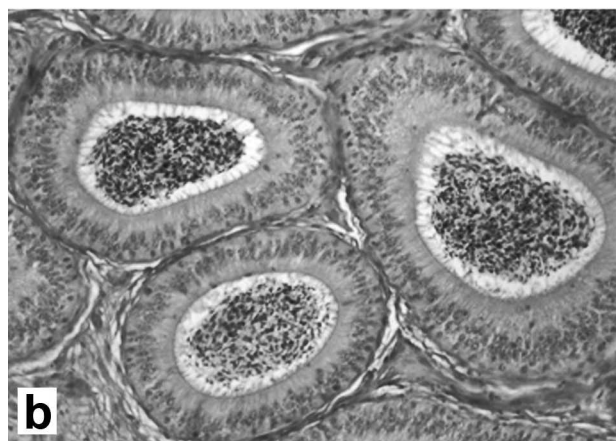
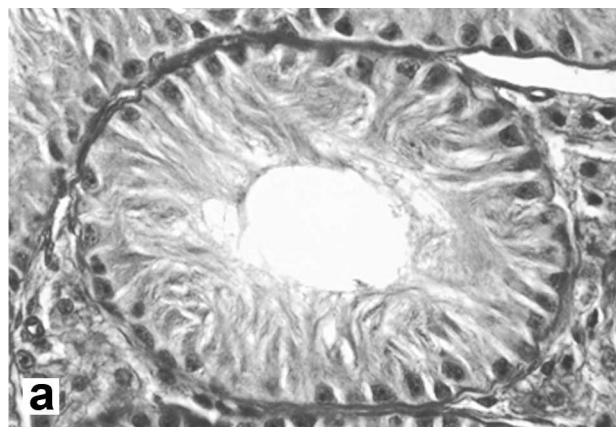
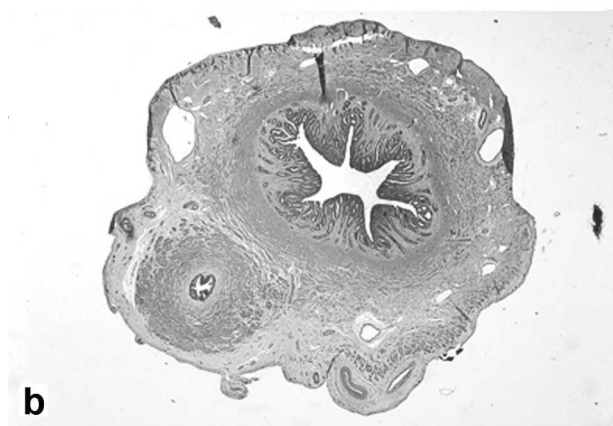
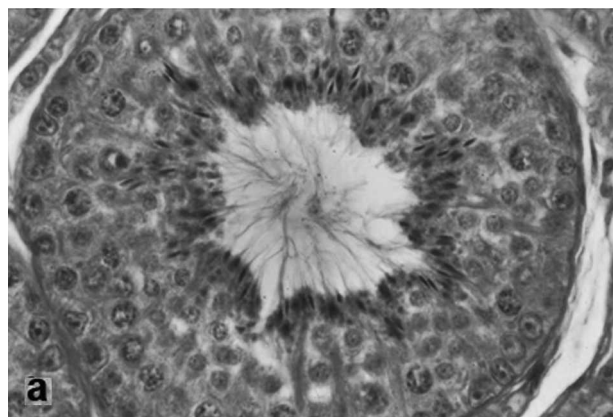


Figure 3. Selected histological features of the reproductive tract in PMDS male A8, age 6 years (hematoxylin and eosin stain): **(a)** seminiferous tubule of left scrotal testis showing normal stages of spermatogenesis (original magnification $\times 400$), **(b)** cross section of uterine horn and adjacent vas deferens in parallel that, except for their presence together, have normal characteristics (original magnification $\times 250$).

females (CT), and 1 homozygous normal female (CC). Therefore these genotypes are not only concordant with the observed phenotypes but are also consistent with sex-limited autosomal recessive inheritance of the PMDS trait.

Discussion

Canine PMDS: Phenotype and Clinical Sequelae

This article provides a detailed description of the canine PMDS model phenotype, providing insights on management of clinical complications that could be relevant to the human disorder, in that the phenotype closely resembles that of human PMDS. All affected dogs have normal virilization of the external genitalia but internally develop fully differentiated Müllerian and Wolffian duct systems. All layers of the uterus develop, despite the absence of ovarian hormonal stimulation.

Figure 4. Selected histological features of the reproductive tract of PMDS male A9, age 3 years (hematoxylin and eosin stain): **(a)** absence of spermatogonia in seminiferous tubule of the abdominal cryptorchid testis (original magnification $\times 400$); **(b)** cross section, spermatozoa in the lumen of the epididymis adjacent to the scrotal testis (original magnification $\times 250$); **(c)** cross section, caudal uterine horn (U) and spermatozoa in the lumen of the vas deferens (V) ipsilateral to the scrotal testis (original magnification $\times 250$).

The cranial tip of the uterine horn is attached to the caudal pole of the testis, whereas the upper vagina inserts into the dorsal prostate. In contrast to reports

Table 2. Characteristics of breeding males in the PMDS pedigree

ID	BW, lbs	No. of Litters Sired	No. of Offspring Sired	Sperm, million per ejaculate ^a	Position of Testes
Normal males					
A7	18	2	8	366	2 s
A47	20	11	71	350	2 s
A87	35	35	195	416–690	2 s
PMDS males					
A8	18	9	52	50	2 s ^b
A9	18	5	19	40–140	1 c, 1 s
A241 prepyometra	30	3	19	226	2 s
A241 postrecovery	30	24	159	253–640	2 s
A280	32	12	72	145–195	2 s

Abbreviations: BW, body weight; c, cryptorchid testis; PMDS, persistent Müllerian duct syndrome; s, scrotal testis.

^a For BW = 10–34 lbs, after sexual rest, the normal range for total sperm per ejaculate is 400 ± 110 million (Amann, 1986).

^b Unilateral segmental aplasia of epididymis, delayed testis descent into scrotum.

describing PMDS in humans, the testes can be descended or cryptorchid (unilaterally or bilaterally), and spontaneous late descent has also been observed. As expected for any cryptorchid dog, cryptorchid PMDS dogs had sperm counts that were below the normal range. However, this was also observed in some PMDS dogs that were not cryptorchid and had no gross defects in the epididymis or vas deferens. Although functional studies have not been conducted in PMDS dogs, there are apparently no physical barriers to sperm transport or semen delivery during ejaculation. Therefore, it is unclear whether the canine *MISR11* defect has a direct effect on sperm count. In vitro, MIS has been shown to arrest the maturation of murine gonocytes to A-type spermatogonia (Zhou et al, 1993). Moreover, MIS is present in human seminal plasma and binds to human sperm (Fallat et al, 1996, 1998); thus, it is conceivable that MIS has a direct role in spermatogenesis.

Because cryptorchidism was identified in only 50% of PMDS dogs in this pedigree, it is not a consistent finding in this canine model as it has been in humans. This raises the question of whether the human association of PMDS with cryptorchidism is due, in part, to ascertainment bias. Most human patients with PMDS are diagnosed coincidentally at the time of surgical procedures to correct undescended testis or transverse testicular ectopia (Brook et al, 1973; Sloan and Walsh, 1976). Milder phenotypes associated with retained Müllerian structures but descended, fertile testes and normal genitalia might remain undetected. The etiology of cryptorchidism associated with PMDS has been a matter of debate as to whether MIS plays a

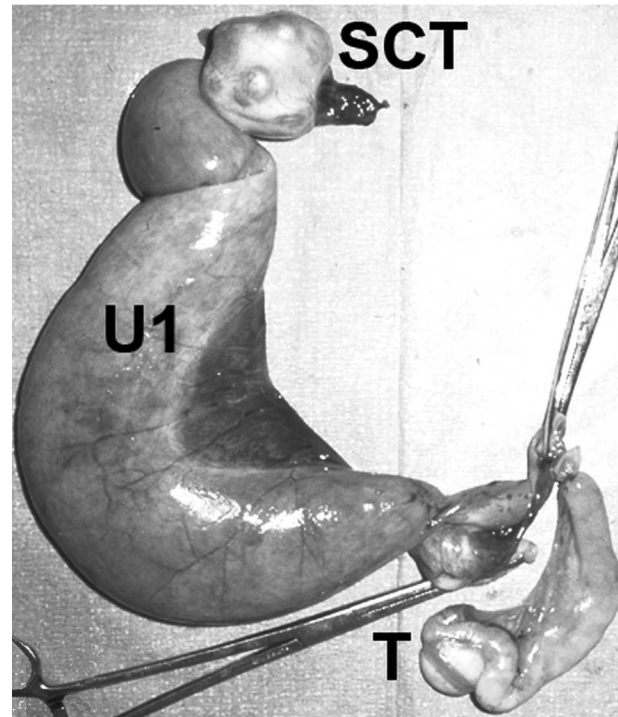


Figure 5. Sequelae to canine PMDS and cryptorchidism. The excised uterine horns, cryptorchid testis, and scrotal testis from an aged PMDS male are shown. The uterine horn (U1) attached to the cryptorchid testis is grossly dilated by pyometra. The remaining uterine horn, attached to the scrotal testis (T), is mildly dilated. Nodules in the markedly enlarged cryptorchid testis were identified by histology as Sertoli cell tumor (SCT).

role in normal descent of the testis (Hutson, 2003) or whether the retained Müllerian structures physically compromise the ability of the testis to descend (Imbeaud et al, 1996). Imbeaud and colleagues noted that several patients with PMDS had a short vas deferens that is adherent or embedded within the uterine wall, which would physically prohibit testicular descent. Findings in the canine model support the latter hypothesis, as the lower pole of the testis remains physically attached to the cranial end of the uterine horn. In canine PMDS males with descended testes, the cranial uterine horn is drawn into the scrotum with the testis. Thus it is possible that the 50% of PMDS dogs with failure of testis descent result from the physical encumbrance of the uterus in inguinoscrotal descent.

Sequelae that occur in canine PMDS might provide some insight for human patients. Patients with persistent Müllerian remnants have been reported to develop urinary obstruction and urogenital tract infections (Tank and Hatch, 1986; Krstic et al, 2001; Lima et al, 2004), as well as gonadal tumors (Melman et al, 1981; Kazim, 1985; Snow et al, 1985). In addition to development of Sertoli cell tumors in cryptorchid testes, PMDS dogs can develop pyometra, as reported previ-

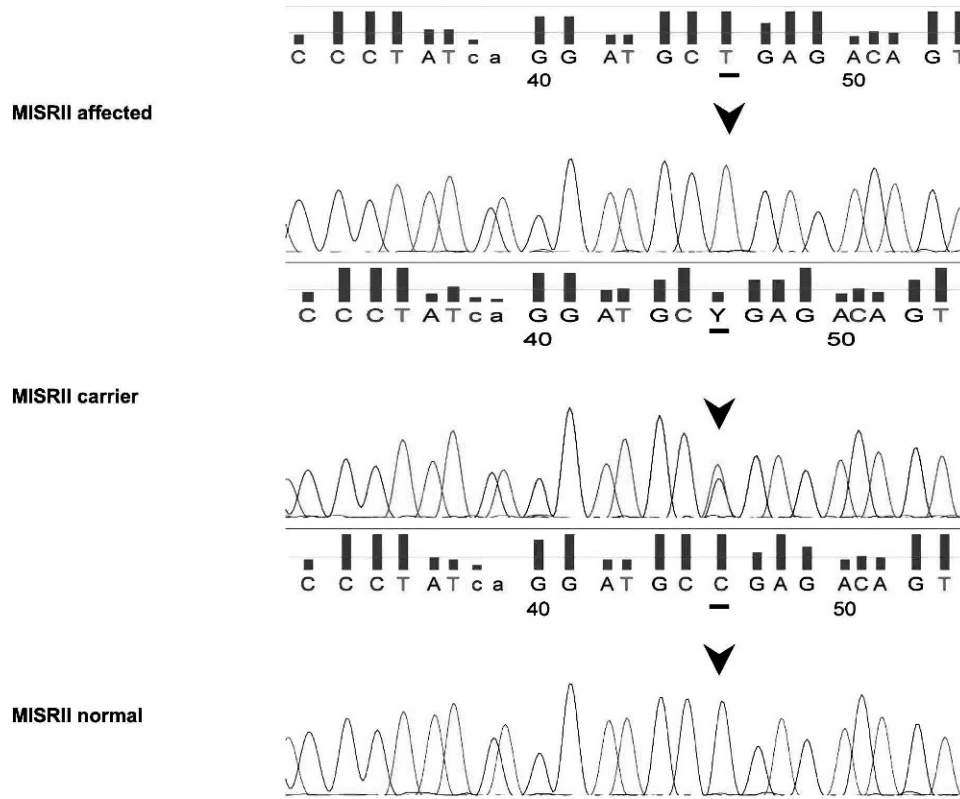


Figure 6. Selected electrophoretograms from a PMDS affected dog (A9) (top panel), a carrier (A85) (middle panel), and a normal noncarrier male (A7) (bottom panel). Arrows indicate nucleotide 241 of canine *MISRII*.

ously (Marshall et al, 1982). The vaginal-urethral connection between the cranial vagina and the prostatic urethra that we describe in PMDS dogs is of clinical significance. When present, this connection provides a route for ascending infection from the urethra to the uterus but is too narrow for adequate purulent outflow. Although routine treatment for canine pets with PMDS,

with or without pyometra, is gonadectomy and hysterectomy, retention of fertility is of greater concern for human PMDS patients. Restoration of fertility subsequent to treatment for pyometra in the canine PMDS case described in this report indicates that sterility need not be a final outcome for human patients who develop secondary infection.

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1  AUGAUGCUGGGGACUCUGGGCCUUUGGGUACUACUUCCACAGCUGUGCAAGCACCCCCA 60
   M M L G T L G L W V L L P T A V Q A P P
61  AGCAGGCGGACCUGUGUGUUCUUUGAGGCCCCUGGAGUACGGGGAAGCACAAAGACACUG 120
   S R R T C V F F E A P G V R G S T K T L
121 GGAAGCUGCUAGAUGCAGGACCAGGGCCCCCAGGGUUAUCCGCUGCCUCUACAGCCGC 180
   G K L L D A G P G P P R V I R C L Y S R
181 UGCUGUUUUGGGAUCUGGAACCUGACCCAAGACCAGGCACAGGUGGAGAUGCAAGGAUGC 240
   C C F G I W N L T Q D Q A Q V E M Q G C
241 CGAGACAGUGAUGAGCCAGACUGUGAGUCCCCCACUGUGACCUGAGCCCCGAGCCAC 300
   R D S D E P D C E S P H C D L S P R A H
301 CCCAGCCCCGGUCCACUCUCUUCACCUGCUCCUGUGGCACUGACUUCUGCAAUGCCAAU 360
   P S P G S T L F T C S C G T D F C N A N
361 UACAGCCAU.....
   Y S H
    
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Figure 7. Predicted mRNA and amino acid sequence of canine *MISRII*; partial sequence showing the first 369 nucleotides of the predicted coding region (XM_543632, <http://www.ncbi.nlm.nih.gov/entrez>, <http://bio.lundberg.gu.se/edu/translat.html>). The highlighted single nucleotide change at position 241 (exon 3) and affected amino acid changes the codon at 81 from arginine (R) to a premature stop codon (TGA). The resultant mRNA transcript would be 243 rather than 1806 bp, and might be unstable. Should the truncated transcript be translated, the resultant protein would consist of 80 rather than 602 amino acids, such that the extracellular domain would be disrupted and the entire transmembrane and intracellular signaling domains would be absent, resulting in a nonfunctional *MISRII*.

Canine PMDS MISRII Mutation

Molecular analysis of the *MISRII* gene in this canine pedigree identified a C241T mutation in exon 3 of the *MISRII* gene that is the genetic defect in PMDS miniature schnauzers. The genotype, normal (CC), carrier (TC), or PMDS (TT) at this position is concordant with all phenotypes in the pedigree subset and consistent with the sex-limited autosomal recessive mode of inheritance. The mutation would cause premature termination of translation at nucleotide 243; thus, the predicted protein product would contain 80 amino acids instead of the normal 602. The truncated protein would consist of a partial extracellular domain (exons 1–3) and lack the entire transmembrane and intracellular signaling domains. As a result of this mutation, we predict that the MISRII would be either rapidly degraded or nonfunctional.

The canine *MISRII* gene is highly homologous to its human counterpart, *AMHR2* (Imbeaud et al, 1995) and shares sequence similarity of approximately 30% with other TGF-beta type II receptors (Salhi et al, 2004). Human *AMHR2* has 11 exons: the first 3 encode the signal sequence and extracellular domain, exon 4 encodes most of the transmembrane domain, and exons 5–11 encode the highly conserved intracellular serine-threonine kinase domains (Imbeaud et al, 1995). Mutations of human *MISRII* identified thus far have all been localized to exons encoding the extracellular ligand-binding domain or intracellular kinase domains (Imbeaud et al, 1995, 1996; Messika-Zeitoun et al, 2001; di Clemente and Belville, 2006). Human mutations in exon 3, similar to the one identified here in the canine PMDS model, lead to a stop codon, resulting in a truncated nonfunctional protein (Imbeaud et al, 1996). The most common human mutation, found in 25% of patients suspected to have a receptor defect, is a 27-bp deletion in exon 10, which deletes 9 amino acids from a critical kinase domain. Other reported mutations include deletions, splice mutations, and single base substitutions, causing nonsense and missense mutations. An *in vitro* system in which engineered constructs of human mutations are overexpressed in COS cells has been used to evaluate effects of mutations on ligand binding and downstream signaling (Messika-Zeitoun et al, 2001; Josso et al, 2005; di Clemente and Belville, 2006).

We have identified the genetic mutation responsible for PMDS in the canine model derived from the miniature schnauzer. The genotypes at this newly discovered mutation site are consistent with the clinical phenotype of a large, carefully characterized pedigree, and with previous reports on the normal function of the *MIS* gene (Meyers-Wallen et al, 1989, 1993). In analogy to similar mutations reported in humans, the mutation

predicts a nonfunctional truncated protein that would not be anchored in the cell membrane and has no kinase domain. Therefore, we conclude that the C241T mutation is not a nucleotide polymorphism, but rather, the causative genetic defect for PMDS in this canine model. Knowing the specific genetic defect responsible for PMDS in this pedigree should enable the development of a simple screening strategy to detect this mutation in other members of the miniature schnauzer breed. Identification of the genetic mutation in this canine model might also enable insights to be garnered from correlation of detailed clinical descriptions with molecular defects, which are difficult to study in the human condition.

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