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SPONTANEOUSLY ARISING DISEASE

Sertoli Cell Tumour and Uterine Leiomyoma in Miniature Schnauzer Dogs with Persistent Müllerian Duct Syndrome Caused by Mutation in the *AMHR2* Gene

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Summary

Disorders of sex development (DSD) are a serious health problem in dogs. Different types of DSD have been described, including persistent Müllerian duct syndrome (PMDS), for which the molecular background has been identified in miniature schnauzers. Human patients with PMDS are at increased risk for cancers of the gonads (predominantly) or the Müllerian duct structures (rarely). This report describes two miniature schnauzer dogs with PMDS caused by a known nonsense mutation in the *AMHR2* gene, with concurrent development of genital neoplasia. The first case (78,XY and *SRY*-positive) had unilateral cryptorchidism and a Sertoli cell tumour in the abdominal testicle. The second case (mosaic karyotype 77,XY,rob/78,XY and *SRY*-positive) had both gonads descended in the scrotum and developed an abdominal mass derived from the uterine wall, which showed histological features typical of leiomyoma.

Keywords: dog; leiomyoma; persistent Müllerian duct syndrome; Sertoli cell tumour

Persistent Müllerian duct syndrome (PMDS) is a monogenic disorder of sex development (DSD) observed in mammals with male sex chromosomes. It manifests as the presence of testes, epididymides, ductus deferentes and male external genitalia in combination with a uterus and oviducts. The persistence of these Müllerian structures in males is caused by the disturbed functioning or secretion of Müllerian-inhibiting substance (MIS), also called anti-Müllerian hormone (AMH), or by altered structure of its receptor, anti-Müllerian hormone receptor type 2 (AMHR2).

In man, 64 mutations in *AMH* and 58 in *AMHR2* have been identified to date (Picard *et al.*, 2017). The incidence of this disorder is low in man, although no reliable estimate of its frequency is available. However, at least several hundred affected patients have been described in the literature (Picard *et al.*, 2017). In domestic animals, PMDS has been reported mainly in dogs, especially in the miniature schnauzer breed, and a causative mutation in the *AMHR2* gene has been identified in this breed only (Wu *et al.*, 2009).

A characteristic feature of PMDS is the increased risk of cancer of the reproductive system. It is known that a third of human PMDS patients develop tumours, mainly derived from the gonads and rarely from Müllerian duct structures (Picard *et al.*, 2017).

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In dogs with PMDS, only gonadal tumours have been observed to date (Table 1). Moreover, since the identification of the causative *AMHR2* mutation by Wu *et al.* (2009), there has been no report of tumour development in miniature schnauzers that are homozygous for this mutation. Here we present data from two miniature schnauzer dogs with PMDS caused by the known *AMHR2* gene mutation (C > T transition in exon 3 causing premature STOP codon), in which Sertoli cell tumour or uterine leiomyoma was found.

Two miniature schnauzer dogs were examined clinically due to abnormal sex development or the presence of an abdominal mass. Further studies were carried out with the use of histological, cytogenetic and molecular techniques. In brief, sections of fixed tissue samples were stained with haematoxylin and eosin (HE) for light microscopical examination. For the cytogenetic analysis, an in-vitro culture of lymphocytes was used (Iannuzzi and Di Berardino, 2008). The chromosome preparations were stained with Giemsa stain and the sex chromosomes were identified due to their unique, bi-armed morphology, as compared with autosomes (Switonski *et al.*, 1996). Molecular analysis focused on the detection of two genes (*SRY* and *ZFY*) located on the Y chromosome, as well as on DNA sequencing of the *AMHR2* fragment, where the causative mutation was found by Wu *et al.* (2009). To amplify the examined fragments, polymerase chain reaction (PCR) was performed with primers specific for *SRY*, *ZFY* and *AMHR2* (Wu *et al.*, 2009; Switonski *et al.*, 2012; Szczerbal *et al.*, 2014). Electrophoretic visualization in agarose gel (1.5%) of the *SRY* and *ZFY* genes and Sanger sequencing of the *AMHR2* gene fragment with an automatic DNA sequencer (Genetic Analyzer, Applied Biosystems, Foster City, California, USA) were performed.

Case 1 was a 9-year-old phenotypical male, presented for clinical investigation due to unilateral cryptorchidism. Abdominal palpation followed by ultrasonography revealed a 10 cm diameter mass in the mid-abdomen. On surgical investigation, the mass was identified as gonadal tissue located caudal to the right kidney and connected to the right uterine horn. The left gonad, fully descended into the scrotum, was connected to the left uterine horn and was retracted into the abdomen during surgery (Supplementary Fig. 1). Both uterine horns were connected to the uterine body, which ended in the cervix. The uterus and both gonads were removed during surgery and fixed in formalin for further investigation.

Histopathology identified the gonads as two atrophic testes. The abdominal testicle had a multinodular appearance and was highly cellular, with a population of elongated vacuolated cells in a palisade arrangement, indicative of a Sertoli cell tumour (SCT), with compression of the normal tubuli seminiferi (Fig. 1). The tumour consisted of solid zones and zones with cystic dilation. The testis was surrounded by a defined tunica and subdivided by prominent fibrous septa. In the scrotal testicle, several sections of tubuli seminiferi were observed, lined by Sertoli cells and inactive germ cells. No interstitial cells were visible in the testicle. The epididymis and plexus pampiniformis were identified. The presence of an oviduct was suspected, based on the presence of highly folded mucosa resembling fimbria; however, this oviduct was underdeveloped. The whole uterus

Table 1
Reports of cryptorchidism and gonadal tumours in dogs with PMDS.

Breed	*Age (years)*	*Cryptorchidism*	*Tumour*	*Reference*
Maltese	7	Unilateral	SCT	Park *et al.*, 2017
Crossbred	17	Unilateral	Seminoma	Park *et al.*, 2017
Miniature schnauzer	9	Unilateral	SCT and seminoma	Madureira *et al.*, 2017
Miniature schnauzer	6	Bilateral	SCT	Johnston and Johnston, 2016
Miniature schnauzer	8	Unilateral	SCT	Cahua *et al.*, 2015
Miniature schnauzer	7	Unilateral	Seminoma	Breshears and Peters, 2011
Yorkshire terrier	2	Bilateral	Seminoma	Hagel *et al.*, 2010
Miniature schnauzer	5	Bilateral	SCT	Vegter *et al.*, 2010
Miniature schnauzer	10	Bilateral	SCT	Matsuu *et al.*, 2009
Miniature schnauzer	5	Unilateral	SCT	Schmerbach *et al.*, 2005
Miniature schnauzer: Case 1	9	Unilateral	SCT	This study
Miniature schnauzer: Case 2	11	None (gonads in scrotum)	Uterine leiomyoma	This study

SCT, Sertoli cell tumour.