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37,X/38,XY mosaicism in a cryptorchid Bengal cat with Müllerian duct remnants

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Abstract

A 23-month-old tomcat was referred to our clinic because of male behavioral problems, cryptorchidism, and an undefined intra-abdominal organ resembling the uterus. Ultrasonography and computed tomography showed two fluid-filled tubular structures dorsolaterally to the bladder and connected to the pelvic urethra. The cat was castrated and the tubular structures were surgically removed. Histology identified them as Müllerian duct remnants. The testes were hypoplastic, the epididymes and deferent ducts were normal. Cytogenetic analyses revealed the presence of a mosaic 37,X/38,XY karyotype, which explains the clinical findings.

Introduction

Normal sexual development in mammals is a multi-step process that includes the establishment of chromosomal, gonadal and phenotypic sex. Impairment in any of these pathways leads to abnormal sexual development (Lyle, 2007; Meyers-Wallen, 2009). Recently, new classification systems for disorders of sexual development (DSD) taking molecular diagnostics into account were published for cats and dogs (Poth et al. 2010; Meyers-Wallen, 2012) following a classification proposed for humans (Pasterski et al., 2010) with the consensus terminologies of sex chromosome DSD, XY DSD and XX DSD.

Sex chromosome DSD are a result of aberrant sex chromosome structure or numbers, which arise from fusion of two zygotes in case of XX/XY chimera or non-disjunction of chromosomes within the same individual including XXY and variants, XXX and variants, monosomy X and variants and X/XY (mixed gonadal dysgenesis). An overview on different types of sex chromosome DSD in domestic cats has recently been published (Meyers-Wallen, 2012). Phenotypic male cats with 39,XXY karyotype or its mosaic variants 38,XY/39,XXY and 38,XY/39,XYY (Centerwall and Benirschke, 1975; Moran et al., 1984; Leaman et al., 1999; Schmidt-Küntzel et al., 2009; Pedersen et al., 2014) as well as tomcats with 38,XX/38,XY chimera karyotypes (Centerwall and Benirschke, 1975; Moran et al., 1984; Leaman et al., 1999; Long, 1999) may only be found if they have unusual tortoiseshell or calico coats. Cats with monosomy X and variants as well as XXX and variants are phenotypic females (Norby et al., 1974; Long and Berepubo, 1980; Johnston et al., 1983; Manna and Sarkar, 1988). Pregnancy and unilateral ovarian dysgenesis were reported in two queens with the mosaic karyotypes 37,X/38,XX and 37,X/39,XXX (Dybdahl Thomsen et al., 1987). X/XY mosaicism was reported in one dog only (Smith et al., 1989; Giger et al., 1989) and has not yet been described in cats. However, some cases of feline DSD found in the literature lack detailed cytogenetic and molecular analysis, therefore the

recently published DSD classification is not possible and X/XY mosaic cases might have been missed.

The present report describes a cryptorchid cat with Müllerian duct structures and a mosaic 37,X/38,XY karyotype.

Materials and methods

Animal

A 23-month-old Bengal tomcat was referred to our clinic with male behavioral problems i.e. aggressiveness and urine marking, suspicion of abdominal cryptorchidism, and because of an undefined intra-abdominal organ resembling the uterus noted during exploratory laparotomy by the referring veterinarian. On presentation, an inguinal right and a prescrotal left testis were found. The penis and prepuce were normal and the penis had spines. Hematology, blood chemistry and urinalysis were unremarkable, urine culture was negative. Serum was collected for testosterone (T) (IDEXX Diavet AG, Bäch, Switzerland) and AMH analysis (Laboklin, Basel, Switzerland). Azoospermia was diagnosed after semen collection by electroejaculation. The ejaculated fluid contained high numbers of inflammatory cells. Ultrasonography, plain and positive retrograde enhanced computed tomography (CT) was carried out to identify and localize the intra-abdominal structure suspected to be a uterus masculinus. Bilateral orchiectomy and laparotomy were performed, and the removed organs including both testes and the suspected uterus masculinus were fixed in 10% neutral phosphate-buffered formalin, embedded in paraffin and examined histologically.

Cytogenetic and molecular analyses

Full EDTA- and heparin-treated blood was collected and used for molecular and cytogenetic analysis, respectively. In addition, buccal cells were collected using cotton swabs. The mucosal cells were rinsed in physiological 0.9% NaCl, centrifuged, and after resuspension of the pellet, fixed three times in methanol/acetic acid. High molecular weight genomic DNA was isolated using a proteinase K/phenol extraction method. PCR amplification of the *SRY* gene from genomic DNA was performed using a primer pair described in Ciani et al. (2008), which results in a 164 bp amplification product. Chromosomes were prepared according to standard protocols from short-term lymphocyte cultures. QFQ-staining of the metaphase chromosomes followed the method described by Vosa (1971). More than 300 metaphases were analyzed and karyograms were prepared according to Ford et al. (1980). FISH analysis of the Y chromosome was carried out with an in-house developed feline whole Y chromosome painting probe according to standard protocols. The method was applied to more than 400 interphase nuclei of buccal mucosal cells.

Results

On ultrasonography, a thin-walled, fluid-filled tubular structure was visible in the caudal abdomen and suspected to be in contact with the right inguinal testis. Plain CT identified two fluid-filled tubular structures dorsolaterally to the bladder and bilaterally extending into the pelvis, converging at the pelvic inlet similar to a uterine bifurcation. In the retrograde CT urethrogram, iodinated contrast was delineated in both tubular structures, which are presumably the horns of the Müllerian duct remnants, leading each to the ipsilateral prescrotal left or inguinal right testis (Figure 1A). A connection at the level of the pelvic symphysis was identified between the pelvic urethra and the suspected Müllerian duct remnant caudal to the convergence of the horns (Figure 1B).

During surgery the fluid-filled tubular structures were identified (Figure 2) and their course was followed intra-operatively. Both horns ran through the inguinal canal and ended at the caudal pole of the left and right testis. After removal of the left testis through a small inguinal skin incision, the suspected Müllerian duct remnants were bluntly separated from the surrounding tissue using the prescrotal and abdominal approach on the left side, and the abdominal approach on the right side. The right testis and both horns of the suspected Müllerian duct remnants were brought into the abdomen through the inguinal canals. Surgical excision was performed cranial to the connection of the pelvic urethra with the converging part of the horns. Both inguinal canals, the abdomen and the inguinal incision were routinely closed. The cat was discharged from the hospital two days after surgery and made a full recovery. The behavioral problems disappeared. Histology revealed bilateral hypoplastic testes with the presence of Sertoli and Leydig cells and lack of germ cells. The epididymides and the deferent ducts were normal. On both sides, merged with the deferent duct, a tubular structure with a tunica muscularis layer and a lining consisting of a stratified epithelium was present (Figure 3), containing few to moderate numbers of degenerate neutrophil granulocytes within the lumen. The histology findings were consistent with Müllerian duct remnants and purulent inflammation. Serum T concentration was 2.2 ng/mL (laboratory reference value for mature, intact tomcats: 0.3 – 4.0 ng/mL, neutered males < 0.1 ng/mL) and AMH was 18.48 ng/mL (laboratory reference range for adult, intact tomcats: > 3.0 ng/mL, neutered males < 0.5 ng/mL).

The cat showed a mosaic 37,X/38,XY karyotype. The two cell lines were found in 96 % and 4 % of the lymphocytes, respectively. Representative metaphases after FISH with the Y chromosome painting probe are shown in Figure 4. The autosomes and both sex chromosome of the cat appeared normal with no visible structural aberrations. The result after FISH with the Y chromosome painting probe on the interphase nuclei was similar. A 164 bp fragment of the *SRY*

gene could be successfully amplified from genomic DNA of the cat (not shown). The PCR product had a 100% homology with the feline *SRY* sequence (GenBank accession no. DQ095188.1)

Discussion

In cats, DSD is most often an incidental finding at the time of spay or neuter (Dybdahl Thomsen et al., 1987; Bredal et al., 1997). Rarely, cats are presented because of fertility problems, clinical signs related to malformations of the reproductive organs or because of behavioral problems (Schulman and Levine, 1989; Pedersen et al., 2014; Pieńkowska-Schelling et al., 2015). The male aggressive behavior of the cat in our case, its penile spines and the serum T concentration within the wide reference range reported for intact, adult male cats (Johnstone et al., 1984; Johnston et al., 1996; Tsutsui et al., 2009) indicated the presence of testes. However, detection of the fluid-filled uterine horn-like structures and the bilateral cryptorchid location of the testes supported the possibility of DSD. Therefore, cytogenetic analyses and molecular analysis for the presence of the *Sry* gene were carried out. Because the intraabdominal uterus-like structure was suspicious for Müllerian duct derivatives, serum AMH was also measured.

The 37,X/38,XY mosaic karyotype found in this cat belongs to the group of sex chromosome DSD, which is reported in small companion animals only once in a dog with ambiguous external genitalia, one grossly normal and a remnant uterine horn, and only interstitial cells in the abdominal gonads (Smith et al., 1989). 77,X/78,XY gonadal mosaicism was assumed (Giger et al., 1989; Meyers-Wallen, 2012) even though the author describing the case only found 77,X karyotype. In our tomcat, FISH with a whole Y chromosome painting probe revealed a low level mosaicism with only 4% of the lymphocytes showing a 38,XY and 96% showing a 37,X karyotype. In humans with 45,X/46,XY mosaicism, varying frequencies of the cell lines are

described, i.e. 45,X cells ranging between 10-96% (Rosa et al., 2014), but no correlation between the proportion of 45,X/46,XY cell lines in the blood and the phenotype has been reported (Telvi et al., 1999; Tosson et al., 2012). The proportion of a cell line in the undifferentiated gonad determines its development (Berkovitz, 1992; Telvi et al., 1999), e.g. in human 45,X/46,XY mosaicism, in the testes and blood lymphocyte cultures both 45,X and 46,XY cell lines were consistently found in variable percentages, while in streak gonads only the 45,X cell line was present (Telvi et al., 1999; Tosson et al., 2012). The karyotype of the gonads in our case was not determined, but the finding of testes, even though in cryptorchid location and without germ cells, suggests the presence of the 38,XY cell line in the undifferentiated embryonic gonads. However, the number of 38,XY cells was probably not sufficient for normal expression of the Y-linked *SRY* gene, which must overcome a threshold during the critical time window to successfully induce the testis pathway (DiNapoli and Capel, 2008; Meyers-Wallen, 2009).

In humans, 45,X/46,XY individuals show wide phenotypic variation including normal male phenotype with normal male genitalia and testis (Telvi et al., 1999), abnormal male external and internal genitalia (e.g. hypospadias, cryptorchidism, streak or dysgenetic gonads, Müllerian duct derivatives) as well as anomalies of other organs (Chang et al., 1990; Telvi et al., 1999; Farrugia et al., 2013; Rosa et al., 2014). Persistent Müllerian structures were also found in our cat and are indicative of a failure of AMH function during the critical window of embryonic development. AMH is normally produced by the Sertoli cells of the fetal testis and initiates regression of the Müllerian (paramesonephric) ducts. Defects in its production or insensitivity to it may result in persistent ducts (Josso et al., 2001). Since AMH serum concentration in the cat (18.48 ng/mL) was within the reference range between 4.8 and 81.3 ng/mL reported for normal, adult, intact male cats (Axné and Ström Holst, 2015), insensitivity to AMH during in utero development may be possible. In a breeding experiment in Miniature Schnauzers on the inheritance of persistent

Müllerian duct syndrome (PMDS), which is an XY DSD, AMH immunoreactivity in the testes of affected pups was similar to normal littermates, and the bioactivity of the hormone was also comparable in the two groups (Meyers-Wallen et al., 1989) indicating rather a receptor insensitivity. Indeed, a malfunction of the AMH type II receptor caused by a single base pair mutation was found responsible for the failure of the Müllerian ducts to regress in the offspring of Miniature Schnauzers with PMDS (Wu et al., 2009). Hypothetically, a defect in the AMH receptor or in its downstream signaling might explain the lack of Müllerian duct regression in our X/XY mosaic case, as well. However, persistence of the Müllerian ducts can also be due to delayed or insufficient AMH secretion during the critical time of embryonic development as discussed in canine testicular DSD, i.e. XX sex reversal (Meyers-Wallen et al., 1987 and 1994).

The normal sized penis and histologically normal epididymis and vas deferens of our cat indicates androgen exposure in utero, as stabilization of the Wolffian ducts and masculinization of the urogenital sinus, genital tubercle and genital swellings during the critical embryonic period is dependent on secretion of T and its conversion to dihydro-testosterone (Lyle, 2007; Meyers-Wallen, 2009). Insufficient secretion of hormones by the hypoplastic testes may explain the cryptorchid inguinal and prescrotal location. Because transabdominal migration was complete, failure in the fetal Leydig cells' production of insulin-like 3 peptide, which appears to be the main regulator in this descensus phase, is less likely compared to testicular androgens, which have indirect and direct roles in the transabdominal and inguinoscrotal phases (Hughes and Acerini, 2008; Meyers-Wallen, 2009; Hutson et al., 2015).

The cytogenetic analysis with the finding of a 37,X/38,XY mosaic allowed clear classification of our case to the group of sex chromosome DSD. Similar clinical characteristics, i.e. ambiguous or male external genitalia, cryptorchidism and Müllerian duct derivatives were already found in cats

(reviewed Romagnoli and Schlafer, 2006, Meyers-Wallen, 2012). However, without karyotyping, they could also be explained by other DSD.

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Figure legends

Figure 1: Positive retrograde CT urethrogram. A: Transverse image showing contrast media in each horn (H) of the Müllerian duct remnants with connection to the ipsilateral testis. B: The sagittal view (thick slab reconstruction) shows both horns (H) of the remnant ducts converging into a body (B), which ends in the urethra at the level of the pelvic symphysis (white arrow).

Figure 2: Intraoperative image of the horns (H) of the Müllerian duct remnants after ligation and removal of both testes. Note that both horns (H) are dilated and they converge into a structure similar to a uterine body (B).

Figure 3: The Müllerian duct remnant is aligned by stratified epithelium (black arrows) and a muscular layer. The ductus deferens (white arrow) has normal histological structure.

Figure 4: Photograph of two metaphases after fluorescence *in-situ* hybridization with a feline whole Y chromosome painting probe. On the left metaphase the 38,XY karyotype, and on the right metaphase the 37,X karyotype is visible.