Male Pseudohermaphroditism in a Raccoon Dog (Nyctereutes procyonoides)

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(Received 29 November 2004/Accepted 8 February 2005)

ABSTRACT. A wild raccoon dog (Nyctereutes procyonoides) which died due to a traffic accident on 18 October 2001, and was determined to be 4.5 years old, was examined. Visual appearance of the external genitalia in this animal showed to be female with a large penis-like clitoris protruding from the vulvar juncture. Visual examination of the internal genitalia revealed that the animal possessed both testes and uterus. Histological appearance of the removed gonads showed only Sertoli cells but no spermatogenesis. Using polymerase chain reaction with skin biopsy directed against the sex-determining region on the Y chromosome (SRY) gene, the genomic SRY gene was expressed as a single band and sequenced. Based on these findings, this raccoon dog was diagnosed as male pseudohermaphrodite.

**KEY WORDS:** Nyctereutes procyonoides, pseudohermaphroditism, raccoon dog.

Disorders of genital development occur in all mammals. The pseudohermaphroditic animal possesses the gonads of either sex but the external genitalia evidence the opposite sex: female pseudohermaphrodites have ovaries but phenotypically have a masculine appearance; male pseudohermaphrodites possess testes while having mixed or female external genitalia [7, 9]

Male pseudohermaphroditism has been described in numerous species, including humans [4, 5], dogs [2, 12], cattle [3], horses [7] and rats [1]. However, male pseudohermaphroditism of wild raccoon dogs has never been reported. This study describes a case of male pseudohermaphroditism in a wild raccoon dog which was investigated by clinical, histological and genetic methods.

Animal: A wild raccoon dog which died due to a traffic accident was collected on 18 October 2001 in Tokyo Prefecture, Japan. The animal was 4.5 years old according to the annual incremental lines in the tooth cementum of canines. The size of the clitoris, testis and uterus was expressed in mm after measuring the (length \times width \times height)^{1/3} or (length \times diameter)^{1/2} following autopsy. The testis and uterus obtained were immediately fixed in 10% formalin solution for histological observation.

Histology: The testis and uterus were dehydrated in an ethanol series and embedded in paraffin wax. Serial sections (4 µm) were mounted on slides coated with poly-L-lysine (Sigma, U.S.A.). Some sections were stained with hematoxylin-eosin (HE) for observations of general histology. The testis and uterus were stained with hematoxylin-eosin (HE) and with periodic acid Schiff’s reagent (PAS) to stain glycoproteinaceous tissue. In the uterine tissues, a few atrophic uterine glands were observed (Fig. 2).

SRY analyses: Genomic DNA was prepared from the fresh tissue of the pseudohermaphroditic raccoon dog. Control DNA was obtained from tissues of normal female and male raccoon dogs. PCR amplification was performed with the following primers: 5’-AAGCGACCCATGAACGCATT-3’ (29F) and 5’-GCTTCTGTAAGCATTTCCTCA-3’ (121R). The PCR mixture consisted of primer set (29F 10 pM, 121R 10 pM), dNTPs 0.2 mM, 0.2 µl of ampliTaq DNA polymerase (Perkin Elmer), 0.2 µl of 10 × PCR Buffer (Perkin Elmer) and 0.2 µl of template DNA in a total volume of 100 µl with 20 mM Tris-HCl (pH 8.4). The PCR condition was 94°C for 5 min to denature hybrid, 30 cycles at 94°C for 1 min, at 50°C for 1 min, and at 72°C for 1 min for amplification. The PCR product was electrophoresed in 2% agarose gel and photographs of specific bands were taken. The fresh PCR products were ligated into pCR®2.1-TOPO®, and the ligation products were transformed into the recombinant vector competent E. coli using TOPO TA Cloning® Kit (Invitrogen™). Plasmids containing the proper inserts were sequenced for both directions using Thermo Sequenase II dye terminator cycle sequencing Premix Kit (Amersham Pharmacia) and an automated sequencing system (ABI 377 instruments).

Size of clitoris, testis and uterus: Visual examination of the external genitalia showed a female. A large penis-like clitoris, which was 11.4 \times 5.3 \times 6.3 mm in size, was seen protruding from the vulvar juncture. Visual examination of the internal genitalia showed that both testes and uterus were present. The size of the right and left testes were 17.1 \times 13.9 \times 9.8 mm and 16.9 \times 13.2 \times 10.3 mm, respectively; the size of right and left uterus were 108 \times 2.5 mm and 113 \times 2.4 mm, respectively (Fig. 1).

Histology: In the testicular tissue, the seminiferous tubules were occupied only by Sertoli cells without spermatogenic cells, and there were normal Leydig cells in the interstitial tissues. In the uterine tissues, a few atrophic uterine glands were observed (Fig. 2).

SRY analyses: The results of PCR amplification using genomic DNA from the skin are shown in Fig. 3. A 131 bp fragment of SRY was amplified and sequenced from DNAs of a control male and the pseudohermaphrodite, but not those of a control female.
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Fig. 1. Visual appearance of the clitoris, testes and uterus of a male raccoon dog pseudohermaphrodite. (a): A large clitoris was seen in external genitalia (arrow); clitoris was $11.4 \times 5.3 \times 6.3$ mm in size; (b): Internal genitalia showed testis (T) and uterus (U); the size of right and left testes was $17.1 \times 13.9 \times 9.8$ mm and $16.9 \times 13.2 \times 10.3$ mm, respectively; the size of right and left uterus was $108 \times 2.5$ mm and $113 \times 2.4$ mm, respectively. B: Bladder; E: External genitalia.

Fig. 2. Histological observation of the testis and uterus of male raccoon dog pseudohermaphrodite. (a): Only Sertoli cells (←) were found in the seminiferous tubules; (c): A few atrophic uterine glands were observed in uterine tissues; (b) and (d) show the normal testis and uterus of male and female raccoon dogs, respectively. Sc: Sertoli cell.
Male pseudohermaphroditism reveals a condition of sex differentiation disorders in which the gonads are testes, but the genital ducts and/or external genitalia are incompletely masculinized [4]. In the present report, a wild raccoon dog was classified as a male pseudohermaphrodite because of the following findings: the abdominal testes which showing only Sertoli cells but no spermatogenic cells in the seminiferous tubules, the internal female genitalia (uterus), the female external genitalia (large penis-like clitoris), and the expression of SRY gene in the genomic DNA.

Sexual differentiation for the male is composed of complex mechanisms involving developmental genetics and endocrinology. Formation of the bipotential gonads and subsequent testes is dependent on a series of sex chromosome-linked and autosomal genes [6]. In mammals, the Y chromosome induces testis formation and male sexual development, while in the absence of Y chromosome, gonads differentiate into the ovaries and female development ensues. Molecular genetic studies have identified the Y-located testis determining gene SRY as well as autosomal and X-linked genes necessary for male gonadal development [10]. In this case of wild raccoon dog, a 131 bp fragment of SRY was amplified and sequenced from its skin tissues, showing the expression of SRY gene in the genomic DNA, suggesting that this case of wild raccoon dog was classified as male animal in genetics.

The raccoon dog is a typical seasonal breeder, with the breeding season in October. In this season, Sertoli cells and spermatogenesis have been observed in seminiferous tubules of normal male raccoon dogs [11]. Though the size of testes in this case of wild raccoon dog did not appear difference from that in normal wild raccoon dogs [11], only Sertoli cells but no spermatogenic cells were found in the seminiferous tubules, showing that the testes abnormally developed and did not ability to produce germ cells. In addition, a pair of uterine horns (instead of spermatic duct) with a few atrophic uterine glands and a large penis-like clitoris were also observed, showing that its reproductive ducts were female genitalia in this case of wild raccoon dog. These findings suggest that this case of wild raccoon dog was classified as pseudohermaphrodite.

In animals, pseudohermaphrodite is caused by abnormalities of the number of sex chromosomes (polyploid and mosaic) and abnormal structures (coroza, inversion, deficiency, and repetition) [8]. Unfortunately, the cytogenetic analysis could not be performed in this case of wild raccoon dog. Future studies will be investigated whether or not steroidogenesis occurs in the testes of this case of raccoon dog as well as the studies of cytogenetic analysis.

In conclusion, this wild raccoon dog, having internal male genitalia (both testes), internal female genitalia (uterus), and external female genitalia (a large clitoris), and exhibiting of SRY gene expression in genome, was diagnosed as a case of male pseudohermaphrodite.

REFERENCES