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Mosaic 35,X/36,XY Karyotype and Intersex in a Red Panda (*Ailurus fulgens fulgens*)

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ABSTRACT: A zoo-bred Himalayan red panda (*Ailurus fulgens fulgens*) was diagnosed as a presumptive intersex on clinical examination at 4-mo-of-age. The phenotype was predominately female but showed a large anogenital distance and bilateral ischial swellings. Based on cytogenetic evaluation, the karyotype was mos35,X/36,XY, with 50% of each cell type. A grossly normal uterus and oviducts were seen with laparoscopic examination, while the gonads were smooth-surfaced, with a ramifying vascular pattern. On histopathologic examination the bulk of the gonads consisted of clumps of poorly differentiated cells, with just a thin rim of ovarian tissue under the region covered by the fimbriae of the oviduct.

Key words: Red panda, *Ailurus fulgens fulgens*, intersex, karyotype.

The red panda (*Ailurus fulgens*) is a rare species with several distinct, physically isolated populations in the temperate forests of the Himalayas and high mountains of northern Burma and western China (Roberts and Gittleman, 1984). Several hundred are kept in zoos worldwide, and an international studbook is maintained. Over the last 10 yr the captive population has displayed a lack of growth; the main reasons are a low rate of fertility and high infant and juvenile mortality (Glatston, 1989). Chromosomal abnormalities have not been recorded previously in this species. We describe a chromosomal intersex condition in a panda cub born at Taronga Zoo, Sydney, Australia (33°51′S, 151°15′E), where red pandas have been kept and bred since 1977.

Two cubs of the Himalayan red panda (*A. f. fulgens*) were born at the zoo in December 1985. At 3-mo-of-age, one cub was noted to be much smaller than the other. One month later the cubs were each routinely immobilized with an intramuscular injection of 10 mg ketamine (Parnell Labs, Silverwater, New South Wales, Australia) and 1 mg xylazine (Rompun, Bayer Australia, Botany, New South Wales), for detailed physical examination and identification. The smaller cub (S285) weighed just 600 g, while its normal female littermate weighed 1,500 g. The anogenital distance for S285 was greater than that of the littermate, and bilateral, pea-sized, readily moveable lumps were palpable under the skin over the ischia. An apparently normal vulval opening was present. These changes were sufficient to prompt further investigation. Panda S285 was immobilized again 4 days later and blood collected aseptically into sodium heparin (15 I.U./ml) for cytogenetic examination. Blood also was collected from each of a phenotypically normal, reproductive male and female panda. Panda S285 subsequently was removed from its parents for handrearing. At 2-yr-of-age (December 1987), S285 weighed 3.75 kg and was considered sexually mature. The internal genitalia were examined laparoscopically using standard techniques (Bush et al., 1980). We noted an apparently normal bifurcated uterus and pinkish structures adjacent to the gonads which were presumed to be the fimbriae of the oviducts. In general the appearance of the uterus was remarkably similar to that of a young domestic cat. The gonads were smooth-surfaced, cream-yellow in color, oval in shape, were mostly not covered by a bursal structure, and had a finely ramifying surface vascular pattern. The animal was euthanized because of a chronic skin condition, and was necropsied on

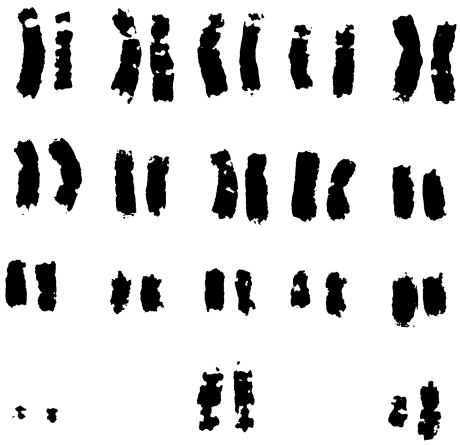

FIGURE 1. Male G-band karyotype 36,XY from panda S285. The sex chromosomes Y and X are bottom right and the arrangement is by order of descending size, bi-armed before single-armed; pairs are unnumbered in the absence of a numbering convention for this species.

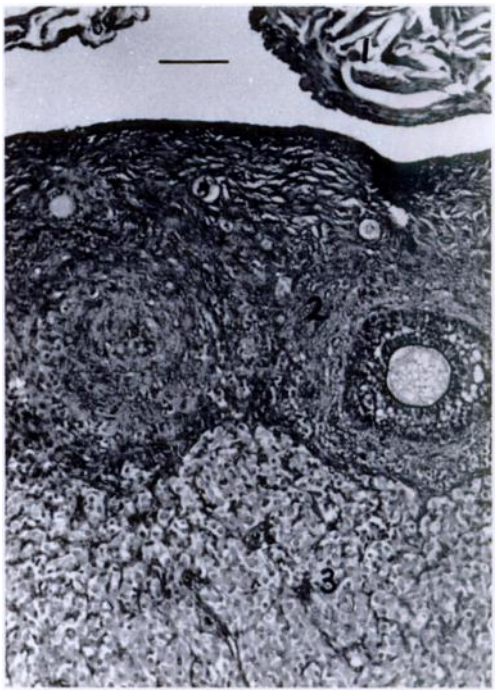

FIGURE 2. Gonad of panda S285 showing fimbriae of oviduct (1), rim of ovarian tissue with follicles (2), and clumps of poorly differentiated cells forming the bulk of the gonad (3). H&E. Bar = 100 μm.

22 February 1987 at 2-yr-and-3-mo-of-age. A record of the drug used for euthanasia was not available.

For cytogenetic examination whole blood cultures were prepared using techniques described by Halnan (1989). Evaluation of karyotype was based on orthodox stained preparations, with phase contrast examination for heterochromatin, and G-banding. The samples were assessed by counting and pairing, identifying a prominent pair of satellited chromosomes, and the sex chromosomes. The findings were subsequently confirmed by photokaryotype and cut-and-paste. Hsu and Benirschke (1967 to 1977) described the normal red panda karyotype as 2n = 36 chromosomes, comprising 16 pairs of metacentrics or submetacentrics, one pair of acrocentrics, plus the sex chromosome pair (submetacentric X and acrocentric Y). Cytogenetic evaluation of the normal male and female panda confirmed this previous work; in good quality preparations the count was consistently 36,XY or 36,XX, respectively. No aneuploid cells were found in slides from either normal animal. In addition, the G-banding patterns for the karyotypes of both normal animals were delineated by color prints. Panda S285, however, was a mosaic 35,X/36,XY with 10 of 20 cells counted being 35,X. The male G-band karyotype is given in Fig. 1. The unbanded male karyotype has been published elsewhere (Halnan, 1989).

Results of the necropsy examination corresponded to the general gross appearance seen laparoscopically 3 mo earlier. At the necropsy, however, the bursae had developed further and covered more of the gonads. The subcutaneous ischial swellings, presumed to be gubernacular appendages, were less pronounced than when first observed at 4-mo-of-age. The larger anogenital distance could be appreciated only by direct comparison with a known normal female. In fact with a casual gross observation it would have been easy to conclude at this time that S285 was a phenotypically normal female. Histologically the gonads consisted of two distinct types of tissue. A crescent of typical ovarian tissue contain-

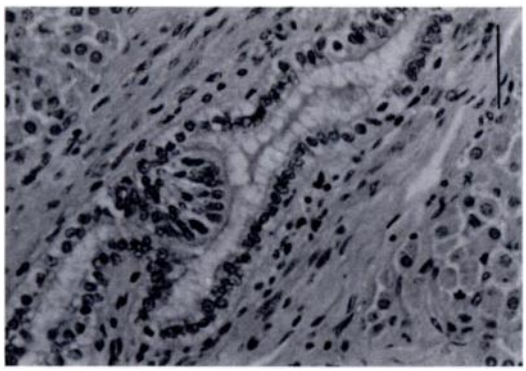

FIGURE 3. Tubule lined with simple cuboidal epithelium, suggestive of a testicular collecting duct. H&E. Bar = 50 μm.

ing numerous primitive oocytes together with a few well developed follicles was present in the region covered by the fimbriae of the oviduct (Fig. 2). The remainder of the gonads consisted of clumps of poorly differentiated cells with some fibrous inclusions. In some areas there was an impression of tubular structures, most prominent when stained with periodic acid-Schiff (PAS) reaction, but unequivocal seminiferous tubules were not present. However, there were sections of tubules lined by simple cuboidal epithelium, suggestive of testicular collecting ducts (Fig. 3). Presumably these gonadal abnormalities were a gross manifestation of the cytogenetic lesion. The oviduct, uterus, cervix and vagina were normal in structure, while bilateral, thick-walled duct-like structures, presumably mesonephric duct remnants (Patten, 1948) extended for the full length of the uterus within the broad ligament. Whether these latter structures are a normal finding in female red pandas or a reflection of this animal's intersex condition is not known.

There was a clinical impression that S285, besides being an intersex, also was immunocompromised. When first examined the animal was very underweight and had severe generalized dermatomycosis which responded to treatment. The animal later developed a recurrent pyoderma, unresponsive to treatment which finally necessitated euthanasia. A possible lymphopenia was inferred from finding low lymphocyte numbers on two occasions (Table 1). This was difficult to interpret because of the large standard deviation of reported normals, but could be consistent with a mild immunodeficiency state. At necropsy, however, the spleen and lymph nodes were quite unremarkable in structure, and no other tests were undertaken

TABLE 1. Hematological findings for red panda S285, and normal values for red pandas.

	April 1986	January 1988	Values reported by Flesness (1985)	
			$\bar{x}$	SD
Packed cell volume (%)	32	38	41.3	6.2
Red cells ($\times 10^{12}$/l)	8.45	nd[a]	8.6	1.1
Haemoglobin (g/dl)	12.0	nd	13.2	1.7
Mean corpuscular volume (fl)	37.87	nd	47.7	3.0
Mean corpuscular hemoglobin (pg)	14.2	nd	15.2	0.8
Mean corpuscular hemoglobin concentration (%)	37.5	nd	32.0	2.0
Total plasma protein (g/dl)	8.4	8.0	6.6[b]	0.7
Leucocytes ($\times 10^{9}$/l)	9.46	8.8	6.5	2.6
Segmented neutrophils ($\times 10^{9}$/l)	7.85	7.66	3.0	1.9
Band neutrophils ($\times 10^{9}$/l)	0.0	0.176	0.0	0.0
Lymphocytes ($\times 10^{9}$/l)	1.42	0.98	3.8	6.7
Monocytes ($\times 10^{9}$/l)	0.09	0.0	0.215	0.171
Eosinophils ($\times 10^{9}$/l)	0.09	0.0	0.081	0.083

[a] nd = Not determined.

[b] Reported value is serum protein.

to determine immune competence. The relationship of a possible immunodeficiency to the abnormal karyotype in this animal is unknown.

Among mammals, intersexes are rare but have been reported in many species (Halnan, 1989). The traditional system for genetic sex determination in mammals is the XX-XY system (Mittwoch, 1973). More recently accumulated data led to the revised concept of interaction of genes located on the sex chromosomes and on one or more autosome pairs (Ohno, 1979, Wachtel and New, 1981). Halnan (1989) has identified autosomal aberrations as regular findings in sub- or infertile horses and cattle, but quantitative data on G-banding in red pandas is insufficient to permit similar analysis of band deletions or inversions. In clinical diagnostic investigations a patient deficient in one of the sex chromosome pairs in all of a definitive number of cells is usually infertile or intersex (Eldridge, 1985; Halnan, 1989). Panda S285's intersex condition was consistent with this. However, the gonads did have a rim of normal appearing ovarian tissue with some well developed follicles, and the bursae, oviducts and uterus also were apparently normal. It is interesting to speculate whether conception would have occurred had the animal successfully mated. Unfortunately hormonal studies were not undertaken and reproductive behavior could not be assessed because the animal had not been kept with other pandas as an adult.

This abnormality easily could have been missed on clinical or gross necropsy examination, so it is quite possible that similar, or other, abnormal karyotypes have been overlooked in cases in infertility or juvenile mortality in captive red pandas. Inbreeding might lead to an increased incidence of such abnormalities. Yet, karyotypic differences between different races or subspecies are recognized as causes of reproductive failure in some mammalian species, for example *Aotus* spp. monkeys (De Boer, 1982), and the possibility that karyotypic differences between geographically distinct populations of red pandas might lead to genetic incompatibilities or lowered fertility of hybrid offspring should be considered. Further cytogenetic evaluation is needed in the investigation of reproductive failure and juvenile mortality in red pandas, before the significance of the findings in this animal to the genetic management of red panda stocks can be determined.

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