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NOR association in *Canis familiaris*

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INTRODUCTION

The domestic dog has 78 chromosomes (Gustavsson, 1964). Because of the large number of chromosomes of almost similar size and morphology, a precise analysis of the karyotype is rather difficult (Howard-Peebles and Pryor, 1980; Manolache *et al*, 1976; Wurster-Hill and Centerwall, 1982). However, a G-banded karyotype of *Canis lupus* (Wayne *et al*, 1987), which is supported to be homologue to that of *Canis familiaris*, displayed detailed information about banding patterns and served as a guideline for the numbering system used in the RBG-banded karyotype (Poulsen *et al*, in press) and in the present study. Several authors have reported on the nucleolar organizer regions (NORs) and NOR associations in the karyotype of the domestic dog (Kopp *et al*, 1982a, b; Pathak *et al*, 1982). In this paper, the frequencies of NOR associations and the localization of NORs in the female karyotype of *Canis familiaris* are presented using sequential RBG-banding and the silver (Ag)-NOR staining technique.

MATERIALS AND METHODS

The donor animals were selected at random from the laboratory *Beagles* at the Institute of Biomedicine, Odense University. Peripheral blood samples from 3 female dogs were cultured and processed for RBG-band induction as previously described (Rønne 1985; Poulsen *et al*, in press). Ag-NOR staining was modified after Howell and Black (1980) and used to stain previously RBG-banded metaphases.

From each donor animal, 10 selected RBG-banded metaphase and prometaphase plates with good spreading and well-defined bands were photographed, registered and karyotyped according to Poulsen *et al* (in press). The selected metaphase and prometaphase plates were subsequently silver-stained to display active NORs and rephotographed after counter-staining with 3% Giemsa solution in Sørensen's phosphate buffer (pH 8.0) for 5 min. Comparison between donor animals showed the same NOR pattern for all 3 animals. Forty RBG-banded metaphase and prometaphase plates selected at random from all 3 donor animals were photographed and registered. These cells were then silver-stained, counter-stained with Giemsa and rephotographed. Metaphase and prometaphase plates with corresponding R-band and NOR-staining were compared. The locations of active NORs and NOR associations were determined.

RESULTS

A total of 40 randomly selected metaphases were sequentially examined with RBG-banding and Ag-NOR staining. Eight autosomes, 5, 8, 14, 16, 19, 21, 32, 37, carry NORs in the telomeric regions (fig 1). The late-replicating X chromosome also showed an active telomeric NOR (Xq) in 10% of the examined metaphases (fig 2). Twenty-one metaphases displayed NOR association among the autosomes at a range of 1–3 associations per metaphase. The X chromosomes were not involved in any NOR association. As shown in table I, chromosomes 14 (38.46%) and 16 (19.23%) have remarkably high levels of involvement in NOR association. Typical NOR associations are shown in figure 3.

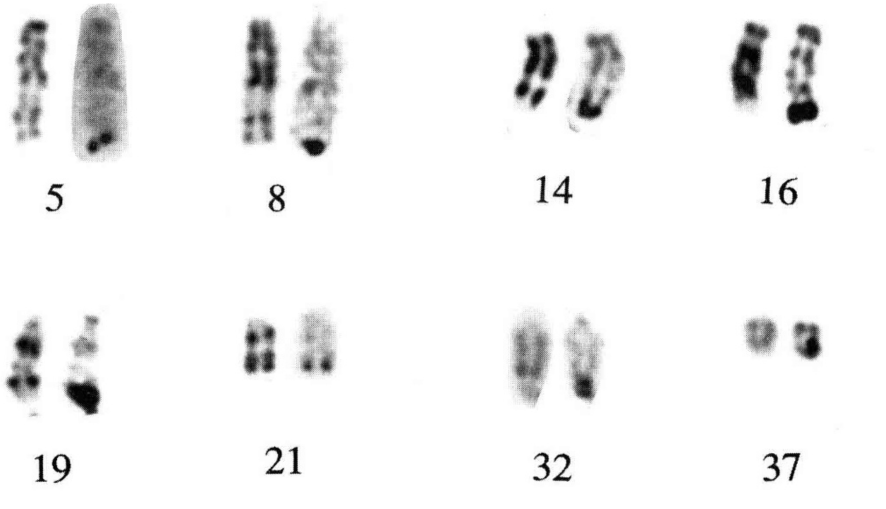

Fig 1. The 8 representative autosomes of the dog carrying active NORs after fluorescence plus Giemsa (FPG)-staining (left side) and subsequent Ag-NOR staining (right side).

DISCUSSION

Several authors have described NORs in domestic dog (Kopp *et al*, 1982a, b; Pathak *et al*, 1982; Howard-Peebles and Howell, 1983). Since the dog karyotype is not standardized, the reported positions of NORs on dog chromosomes were inconsistent. Howard-Peebles and Howell (1983) published that the maximum number of NORs in the dog karyotype was 8, including the NOR on the Y chromosome. Using high-resolution RBG-banding (Poulsen *et al*, in press), NORs

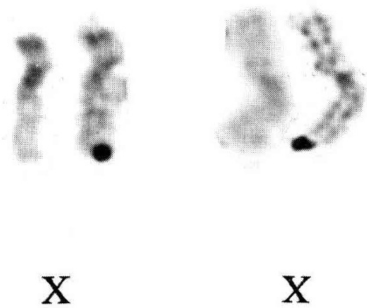

Fig 2. The late-replicating X chromosomes of the domestic dog showing silver-positive NOR (right side) on the telomere of the q-arm.

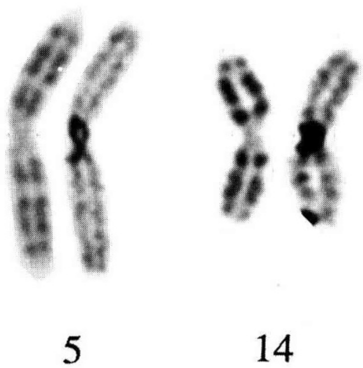

Fig 3. Typical NOR associations observed in dog karyotypes (right side). When stained only with Giemsa (left side), they look like metacentric chromosomes.

Table I. The number and frequency of 26 identified NOR associations between autosomes.

Chromosome number	*5*	*8*	*14*	*16*	*19*	*21*	*32*	*37*	*Total*	*Frequency*
5	1			1				1	3	5.77
8			2		1	1	1		5	9.62
14		2	3[a]	8[a]		3	2	2	20	38.46
16	1		8[a]			1			10	19.23
19		1				1			2	3.85
21		1	3	1	1				6	11.54
32		1	2						3	5.77
37	1		2						3	5.77
Total									52	100.01

[a] NOR association (14–14–16) was counted as 14–14, 14–16, 14–16.

on 8 different autosomes were observed (fig 1). In males, Pathak *et al* (1982) and Kopp *et al* (1982b) reported that the Y but not the X chromosome displayed active NORs. Late-replicating X chromosomes bearing silver grains were observed at a low frequency (10%) after Ag-NOR staining (fig 2). However, at this stage, further investigation of a larger population is needed to determine the presence and role of NORs on sex chromosomes in the dog.

As previously reported by Kopp *et al* (1982a), we observed a high incidence (50%) of metaphases with NOR association. NOR-associated chromosomes may look like metacentrics as shown in figure 3. Translocations in normal dog (Larsen *et al*, 1978, 1979; Mayr *et al*, 1986; Ma and Gilmore, 1971; Welling and Strandström 1988), dog cancer (Grindem and Buoen, 1986; Benjamin and Noronha, 1967; Oshimura *et al*, 1973; Else *et al*, 1982; Welling *et al*, 1988) and other abnormalities (Shive *et al*, 1965; Hare *et al*, 1967) were reported after using conventional Giemsa-stained or G-banded chromosomes. Especially in cancer studies (Mellink *et al*, 1989), metacentric chromosomes have been used as markers of neoplastic development. Without using NOR staining, however, there is a high risk of confusing NOR association with true translocation.

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