The Banding Patterns of Normal Canine Chromosomes

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ABSTRACT. The chromosomal banding patterns of canine normal chromosome were analyzed by G-, Q-, C- and N-banding techniques. The chromosomes of canine normal lymphocytes consisted of 38 acrocentric autosome pairs and X-, Y-metacentric sex chromosomes. Their G- and Q-banding patterns corresponded to the those reported by Manolache *et al.* in 1976. C-bands of chromosomes were found on the centromeric regions of the 7th, 26th, 34th, 35th, 37th and 38th pairs of chromosomes. In addition, N-bands whose location had not been confirmed until now were recognized on the telomitic regions of the 7th, 9th and 22nd pairs of chromosomes.—**KEY WORDS:** banding pattern, canine karyotype, chromosome, dog.

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The chromosomes of normal canine cells consist of 38 acrocentric pairs and X-, Y-metacentric sex chromosomes [11]. However, the preparation of a well-spread metaphase and the analysis of the karyotype of canine cell are rather difficult due to a large number of acrocentric chromosomes with similar morphology. Therefore, there are few reports on canine karyotype [9, 11].

In the last 20 years, many kinds of banding techniques have been reported. Besides the conventional G- and Q-banding techniques, the C-banding technique, which can stain the constitutive heterochromatin [13], the Q-banding technique, which can identify one of the two parental chromosomes in somatic-cell hybrids [14], and the Ag-NOR technique, which can silver-stain nucleolus organizer regions (NOR) [8], were also developed and the analysis of chromosomes was greatly advanced. However, there are no reports on C- and N-banding patterns of canine chromosomes.

In the present study, chromosomal banding patterns of normal canine cells were analyzed by the G-, Q-, C- and N-banding techniques.

MATERIALS AND METHODS

Chromosome preparations: Peripheral blood was collected from clinically healthy mongrel male dogs. The blood was heparinized and lymphocytes were isolated by the method of Kawamura *et al.* [7] and washed with 0.15 M phosphate-buffered saline (PBS; pH7.2) twice. Then the cells were suspended in 5 ml of culture medium (RPMI-1640 medium containing 20% heat-inactivated horse serum) with 50 μ g of concanavalin A per ml to adjust the cell concentration to 1×10^6 cells per ml. The cell suspension was then incubated at 37°C in humidified 5% CO₂ in air for 72 hr. Fifteen minutes before terminating the culture, 50 μ l of colchicine (5 μ g/ml in distilled water, DW) were added to the cell suspension. After cultivation, the cell suspension was centrifuged at 400×g for 2 min and the supernatant was discarded. The cultivated cells were treated with 0.075 M hypotonic potassium chloride at 37°C for 15 min. After that 7 ml of Carnoy's solution were added and mixed gently. The cell suspension was

then centrifuged at $400\times g$ for 2 min and the supernatant was discarded. The cells were fixed 3 times with 10 ml of fresh Carnoy's solution and spread on a slide glass by the air-drying technique [9].

G-banding technique: G-bands were stained by the trypsin method of Seabright [10]. The preparations were first dipped in 0.025% trypsin solution in PBS for 10 to 30 sec and then in fetal calf serum diluted with PBS 3 times to stop the effect of trypsin. The preparations were rinsed with water and dipped in 50% ethanol and stained with 4% Giemsa solution diluted with PBS (pH 6.8).

Fuji Minicopy Film HR-II, Copinal (Fuji) and Fujibro WP FM3 were used for photographing, development, and printing, respectively.

Q-banding technique: Q-banding was carried out by the method of Yoshida *et al.* [14]. The preparations were stained with 33258 Hoechst solution (0.5 $\mu\text{g/ml}$ in DW) and quinacrine mustard (QM; 50 $\mu\text{g/ml}$ in McIlvaine's buffered solution) for 10 min each at room temperature. After washing with DW, the preparations were dipped in McIlvaine's buffered solution for 5 min, washed with DW and air-dried. They were mounted with a drop of McIlvaine's/glycerin mixture (1:1) and sealed with a cover slip.

A Nikon fluorescence microscope, with a 490 nm exciter and a 510nm barrier filter, was used for observation.

Kodak Technical Pan Film 2415, D-19 (Kodak) and Fujibro WP FM3 were used for photographing, development, and printing, respectively.

C-banding technique: C-banding was achieved by the technique of Sumner [13]. The preparations were treated with 0.2 N HCl for 1 hour at room temperature, rinsed in DW and placed in a 5% aqueous solution of barium hydroxide octahydroxide octahydrate at 50°C for 5 min. After thorough

rinsing in DW, the slide was incubated at 60°C for 1 hr in $2\times$ standard saline-citrate (0.3 M NaCl, 0.03 M $\text{NaH}_2\text{C}_6\text{H}_5\text{O}_7$), rinsed in DW, dipped in 70% methanol and stained with 4% Giemsa solution for 30 to 60 min.

Fuji Minicopy Film, Minidol (Fuji), Fujibro WP FM3 were used for the microscopic photography.

The preparations were then stained by Q-banding for identification of the chromosomes which had the C-band. After being photographed, they were dipped in a Carnoy's solution for 1 week to remove the Q-band and were finally re-stained by C-banding.

N-banding technique: N-banding was done following the Ag-NOR method of Kodama *et al.* [8]. The preparations were mounted with 2 drops of a 50% silver nitrate solution and covered with a nylon cloth (mesh size: 300 μm) which had been immersed in the silver nitrate solution. The slide was placed in a moist chamber and was incubated at 50°C for 1 hr. After the preparations were washed with DW and dried, they were counterstained with 1% Giemsa solution for 1 min.

Photographs of the N-banding pattern were recorded by the same method as that of C-banding.

After photographing the N-band, the slides were dipped in Carnoy's solution for 2 to 3 days to remove the Giemsa stain for identification of the N-band, and then stained by Q-banding.

RESULTS

G-and Q-band patterns: Thirty-eight acrocentric autosome pairs and X-, Y-metacentric sex chromosomes were revealed (Figs. 1 and 2).

G-bands were found as the shading of Giemsa staining on chromosomes which were treated with trypsin. Q-bands were

Fig. 1. G-banding patterns of normal male canine chromosomes.

Fig. 2. Q-banding patterns of normal male canine chromosomes.

found as a striped pattern with brightly fluorescence and dark on the chromosome. Their G- and Q-bands had almost the same banding patterns.

G- and Q-banding patterns were analyzed by the karyotype of Manolache *et al.* [9] and determined based on chromosomal numbers. The G- and Q-banding patterns are shown as karyotypic representations in Fig. 3.

C-band patterns: C-bands were found on six pairs of chromosomes. They were identified on the centromeric regions of the 7th, 26th, 34th, 35th, 37th and 38th chromosomes by counterstaining with Q-banding (Fig. 4).

N-band patterns: N-bands (or NOR) were found on three pairs of chromosomes. They were identified on the telomitic regions of the 7th, 9th and 22nd chromosomes by counterstaining with Q-banding (Fig. 5).

DISCUSSION

Thirty-eight pairs of chromosomes, that is, all except X-, Y- sex chromosomes in normal canine cells, are acrocentric and the analysis of their karyotype is difficult due to their similar morphology. For this reason, the standard karyotypes, which were confirmed in human and other animals (mouse and bovine and so on), have not been determined in the dog. There are only 2 reports showing the analysis of G- and Q-bands, those by Manolache *et al.* [9] and Selden *et al.* [11], and studies on canine chromosomes are not advanced in comparison with those of other animals. It is considered that Robertsonian translocation, which is centric fusion of 2 acrocentric chromosomes, is easily formed because most canine chromosomes are acrocentric in form. These translocations are concerned

Fig. 3. Schematic representation of canine karyotype based on G- and Q-banding patterns.

with various diseases [3, 4, 6, 12], but which chromosomes are involved with these translocations is uncertain in the dog. Karyotyping for identification of canine chromosomes is, therefore, an important problem.

G- and Q-banding techniques, which stain the characteristic bands on individual chromosomes, are widely used for studies on the identification and analysis of chromosomes. The G-banding technique stains the band by Giemsa staining after treating preparations with salt solution and proteinase, and the trypsin method [10] is the G-banding technique generally used due to its simplicity and stability. Q-banding was established by Casperson *et al.* [2], and Q-bands are recognized as clear bands with fluorescence by QM on certain regions of chromosomes.

Fig. 4. C-banding patterns of normal male canine chromosomes.

a: C-banding patterns. Arrows indicate C-bands.
b: Sequentially stained Q-banding patterns of the same metaphase.

In the present study, the analysis of normal canine chromosomes by the G- and Q-banding techniques showed that their banding patterns nearly corresponded to those presented by Manolache *et al.* [9] and Selden *et al.* [11]. Though the analysis of each chromosomal banding pattern was difficult due to their similar morphology, the individual number of chromosomes and their identification could be determined by their G- and Q-banding patterns.

The C-banding technique stains constitutive heterochromatins on centromeric and telomitic regions of chromosomes. Using this banding technique it is impossible to identify each chromosome, but it is used as a genetical marker because there are strain differences in the size and location of C-bands [15].

Fig. 5. N-banding patterns of normal male canine chromosomes.

- a: N-banding patterns. Arrows indicate N-bands.
 b: Sequentially stained Q-banding patterns of the same metaphase.
 c: N-bands were recognized on the telomitic regions of the 7th, 9th, and 22nd chromosomes based on Q-banding patterns.

In this study, C-bands were found on the centromeric regions of the 7th, 26th, 34th,

35th, 37th and 38th chromosomes. These results were similar to those reported by Manolache *et al.* [9], and the locations of C-bands were confirmed on 6 pairs of chromosomes in the canine.

N-bands were reported on 4 canine chromosomes, but their locations on the chromosomes were not identified [5]. N-banding followed the method of Giemsa staining after treatment with salt solution at high temperature, but this method had low repeatability. The silver-staining method, which was more sensitive, was reported by Bloom & Goodpasture [1].

The Ag-NOR method [8], used in this study was easier to use and N-bands were more repeatable than with the above 2 methods. N-bands were recognized on the telomitic regions of the 7th, 9th and 22nd chromosomes and the location of N-bands (NOR) on canine chromosomes was confirmed by this study.

G- and Q-banded karyotypes were presented in this study and the locations of C- and N-bands on chromosomes were identified as reported above. We hope that the present study will contribute to further studies on canine cytogenetics.

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要 約

イヌの正常染色体の各種染色法による核形解析：藤永 徹・山下 満・吉田勉弘¹⁾・水野信哉・田島誉士・岡本芳晴・大友勘十郎（北海道大学獣医学部家畜外科学講座，¹⁾理学部付属動物染色体研究施設）——正常イヌの培養白血球から染色体標本作製し，それらについてG-, Q-, C-およびN-バンド染色を施し，各々のバンドパターンを解析した。雌イヌの正常染色体は38対・76本の端部着糸型染色体と，XおよびYの性染色体からなり，そのG-およびQ-バンドパターンはManolacheら（1976）によって報告されたそれらとほぼ一致することが追認された。C-バンドは第7，26，34，35，37および38番目の6対・12本の染色体の動原体部に存在することが確認された。さらに，これまで未報告であったイヌの染色体上のN-バンド，すなわち仁形成部位は第7，9および22番目の3対・6本の染色体の末端部に存在することが始めて確認された。