

ASSOCIATION OF ACROCENTRIC CHROMOSOMES IN SOMATIC CELLS OF ABNORMAL AND HEALTHY CALVES

S.G. Kulikova

Department of Veterinary Genetics and Biotechnology, Novosibirsk State Agrarian University, 630039 Novosibirsk, Russia

INTRODUCTION

Results of a lot of investigations, which are done in lab animals and men, testify that the location of some chromosomes in spread metaphase plate is not random and keeps definite pattern of chromosome space distribution in interphase nucleus (*e.g.*: Zakharov *et al.*, 1982; Prokofyeva-Belgovskaya, 1986). Now chromosome ability is well recognized to form associations in many Mammalian species. It is supposed, that the associations of acrocentric chromosomes can account for a chromosome nondisjunction in mitosis and meiosis and translocations formation. In this connection, it is very important to investigate the phenomenon of acrocentric chromosome associations and their correlation with somatic mutation frequencies in normal or pathological cattle.

MATERIALS AND METHODS

Research was conducted on 29 healthy and 25 abnormal calves of Black-and-White breed; they were born in state breed-farms "Tulinskoye" and "Pervomaisky" of Novosibirsk region.

The abnormal animals had different forms of congenital pathology : umbilical and inguinal hernia, deformation and paralysis of extremities, hydrocephalus, blindness, prognathia of mandible, general underdevelopment, cryptorchism, freemartinism, atresia of anal orifice and others. One-month animals without obvious phenotypic defects were selected for the group of healthy calves.

The leukocyte cultures of animal peripheral blood were incubated at 37 °C for 72h using the method of Moorhead *et al.* (1960). Air-dry slides for microscopy were made and stained with Giemsa stain (Graphodatsky and Radzably, 1988). The analysis of the chromosome preparations was carried out by microscope "Jenaval" (Carl Zeiss Jena). In each animal 50 and 100 metaphases were examined, respectively, for analysis of associative ability and spontaneous mutations of chromosomes. The studies in the associated ability of chromosomes in the healthy and abnormal calves resulted in the detailed analysis of 2,700 metaphases.

The reciprocal orientation of acrocentrics with complete chromosome identification was the criterion to identify associations in routine stained chromosomes. The disposition of two and more acrocentric chromosomes was accepted as association when the distance between the chromosome centromeres was less than the diameter of one chromatid (Begimkulov and Bakay, 1981).

Traits accounted for were the frequency of metaphases with associations, the number of associations per cell, the number of associations in metaphase and the frequency of associations different in the number of associated chromosomes. Earlier somatic chromosome mutations (polyploid and aneuploid cells, single and pair fragments, chromatid and chromosome breaks) were investigated in healthy and abnormal calves (Kulikova *et al.*, 1996 ; 1998).

All statistical analyses were performed using the program of Statistica 5.0 by StatSoft Inc. (USA). The Student's t-test was used to estimate the significance of differences between groups of the healthy and abnormal calves.

RESULTS AND DISCUSSION

During the investigation of acrocentric chromosome associations in Black-and-White calves it was observed that the animals with abnormalities have a greater chromosome ability to form association (Table 1). The frequency of cells with chromosome associations in the group of abnormal animals constituted 45.28%, that is 1.6 times more than in the animals from the healthy group - 28.14% ($P < 0.001$). A similar pattern for the number of associations per cell was observed in abnormal and healthy calves.

Table 1. Associations of autosomes for healthy and abnormal calves

Group of calves	No. of meta-phase cells	No. of cells with associations	Frequency of cells with associations, %	No. of associations	Mean no. of associations per cell	Mean no. of chromosomes in an association
Healthy	1450	408	28.14±1.82	564	0.39±0.03	2.08±0.04
Abnormal	1250	566	45.28±2.08	886	0.71±0.05	2.11±0.03

In the scientific literature, data concerning association's quantitative characteristics are contradicting. According to the report of Radzha (1986), the level of cells with chromosome associations in blood lymphocytes increases from 52.5 to 87.9% giving a mean value of 74.10% for the first year of life in Holsteinized Black-and-White young cattle. In the report (Steklenev, 1986) dealing with Grey Ukrain cows, the frequency of metaphases with associations was 49.85%. This considerable discrepancy in the results of investigations apparently can be explained by different approaches in identification of chromosome associations and age differences of the investigated animals.

In cattle the number of acrocentric autosomes is greater than in any other animal and man species. Therefore, cattle is expected to increase the number of independent associations in a cell (Begimkulov and Bakay, 1981).

Number of associations was observed in the cells of the investigated calves (Table 2). The number of associations in a cell varied from 1 to 7 in the group of the calves with congenital abnormalities and from 1 to 5 in the calves without obvious phenotypic defects. It should be noted that in the abnormal calves only 55% nucleuses did not contain associations whereas in

the healthy animals the associations were absent in 72% of cells. Cells with 1 or 2 associations were more often observed in the compared animals.

Table 2. Distribution of cells accounting for the number of associations per cell, (%)

Group of calves	No. of cells	No. of associations per cell							
		0	1	2	3	4	5	6	7
Healthy	1450	1042 (71.9)	292 (20.1)	88 (6.1)	19 (1.3)	6 (0.4)	3 (0.2)	-	-
Abnormal	1250	684 (54.7)	351 (28.1)	149 (11.9)	42 (3.4)	13 (1.0)	8 (0.6)	2 (0.2)	1 (0.1)

Non significant differences between groups were identified for the number of chromosomes involved into the association (Table 3). In the calves of the compared groups, the associations involving two chromosomes occurred more often (over 90%).

Table 3. Distribution of the number of associated chromosomes (%) for healthy and abnormal calves

Group of calves	No. of chromosomes in the association			
	2	3	4	5
Healthy	93.26	5.67	1.07	-
Abnormal	90.07	8.80	0.90	0.23

Frequency of some types of acrocentric chromosome mutations is supposed to be related to their ability to associate. In 1961 Fergusson-Smith and Handmaker, Ohno *et al.* reported that associations of acrocentric chromosomes in individuals could result in translocations and chromosome nondisjunction in mitosis and meiosis. These abnormalities give rise to newborn trisomics and monosomics for one of acrocentrics. In the present work, the dependence was investigated between the frequency change of polyploid and aneuploid cells as well as metaphases with chromosome aberrations and the frequency of acrocentric associations in healthy and abnormal calves. It was revealed that in the abnormal animals the correlation coefficient between the frequency of cells with chromosome associations and the frequency of aneuploid cells was equal to 0.44 ($P < 0.05$). While in the healthy calves not a single significant correlation coefficient for the analyzed pairs of traits was observed ($r_{x/y} = 0.03-0.11$). Pooling over the two groups, significant positive correlation coefficients were observed between the frequency of cells with associations and polyploid, hyperploid and hypoploid cells that were 0.27, 0.30 and 0.48, respectively. The correlations between frequencies of metaphases with chromosome associations and cells with different types of aberrations were low (not over 0.20) and insignificant ($P > 0.05$). The correlation between frequencies of metaphases with chromosome associations and cells with modified chromosome set (aneuploid cells: $2n \pm 1$ and $2n \pm 2$) was equal to 0.52 ($P < 0.05$). Consequently, in the calves with a high level of cells with acrocentric chromosome associations the increase in the frequency of aneuploid cells was observed.

CONCLUSION

The analysis of acrocentric chromosome associations in Black-and-White cattle demonstrated that the animals with abnormalities have a greater chromosome ability to form associations. The frequency of cells with chromosome associations in the group of the abnormal animals was equal to 45.28 ± 2.08 %, that is 1.6 times as large as in the animals from the healthy group - 28.14 ± 1.82 %. It was determined, that a significant positive correlation ($r_{x/y}=0.52$) exists between the frequencies of metaphases with chromosome associations and cells with excess and deficiency of acrocentric chromosomes. Thus, the data obtained in the paper suggest that acrocentric chromosome associations stimulate chromosome nondisjunction that results in the increase of aneuploid cells frequency.

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