

## SATELLITE ASSOCIATION AND NUCLEOLAR ORGANIZER REGIONS IN THE ELDERLY

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### ABSTRACT

Satellite association frequency and positive staining of nucleolar organizer regions in human acrocentric chromosomes both of which are assumed to depend on the presence of functional genes were studied in lymphocyte cultures from a group of newborns and from a group of 70 - 90 year old subjects, by the Ag-staining technique. We found similar frequencies of Ag-positive staining in both groups but a higher frequency of satellite association in the older age group.

### INTRODUCTION

The nucleolar organizer regions (NORs) of humans are situated in the secondary constriction regions of the five pairs of acrocentric chromosomes. NORs are specifically stained by a silver nitrate staining procedure (Goodpasture and Bloom, 1975). The silver stainability of these regions carrying the structural genes for rRNA is determined by their transcriptional activity (Miller *et al.*, 1976; Croce *et al.*, 1977; Hubbell, 1985; Jimenez *et al.*, 1988).

The cooperation of several NORs which form a common nucleolus at interphase can be observed at metaphase as a close association of the short arms of the acrocentric chromosomes, termed "satellite association" (Ferguson-Smith and Handmaker, 1961).

The frequency of satellite association (SA) appears to be related to the silver stainability of the NOR regions (Miller *et al.*, 1977) and to the intensity of silver deposition (Galperin-Lemaître *et al.*, 1980). Only acrocentric chromosomes with active NORs (i.e. Ag-staining) have been found in association, whereas unstained (in-

active NORs) chromosomes have never been seen in satellite association (Verma *et al.*, 1983).

The distribution of number and size of Ag-stained NORs in a given individual is not random but is relatively constant from cell to cell (Miller *et al.*, 1977; Ray and Pearson, 1979; Jotterand-Bellomo and van Melle, 1981). This characteristic modal number and variation in size of Ag-stained NORs occurring in each individual appears to be of Mendelian inheritance (Varley, 1977; Martin, 1985).

The frequencies of Ag-stained NORs and of satellite associations have been studied as a function of subject sex and age. Most studies have demonstrated that the mean number of satellite associations and of Ag-stained NORs is similar in men and women, with no significant sex differences (Rosenkrans and Holzer, 1972; Ray and Pearson, 1979; Denton *et al.*, 1981; Kumagai, 1982).

In an initial study on lymphocyte cultures from 38 individuals aged 0 to 92 years and divided arbitrarily into four age groups, in which they evaluated the frequency of metaphases with associations, the number of associations per metaphase and the frequency of different types of acrocentric associations, Prokofieva-Belgovskaya *et al.* (1968) noted that the associating ability of the acrocentrics changed with subject age, at first increasing with age and later decreasing again with senescence. Similar results were obtained by Liem *et al.* (1977) for a sample of 167 individuals ranging in age from newborn to 86 years and divided into four age groups. These investigators observed fewer associations among newborn infants and among individuals aged 50 or older than among the groups of intermediate age.

In a study conducted on individuals aged 10 to 13 and 62 to 69 years, Mattevi and Salzano (1975) detected a lower frequency of D/D and D/G associations in the older group. In another study, Vormittag (1980) detected a lower mean frequency of satellite associations among older people (76-85 years) than among young people (15-20 years). Even though he did not detect a difference in satellite association between men and women in a sample of 60 individuals ranging in age from newborn to 62 years, Kumagai (1982) detected a tendency towards a slightly lower frequency among women in the older group. In a sample of 10 individuals aged 20 to 50 years, compared with 10 individuals aged 80 to 93 years, Lezhava (1984) detected a lower frequency of associations formed by chromosomes 13 and 22 in the older group even though the number of associated chromosomes did not differ between groups.

In an investigation of the relationship between aging and ribosomal gene activity demonstrated by Ag-staining, Denton *et al.* (1981) detected a progressive decrease in Ag-stained NORs as a function of age in lymphocyte cultures from 127 individuals. Similarly, Lezhava (1984) observed that the silver stainability of certain acrocentrics was significantly decreased in the older age group.

In the present study we evaluated the frequency of satellite associations and positive NOR staining in chromosomes from a group of newborns and a group of elderly individuals.

## MATERIAL AND METHODS

Lymphocyte cultures were set up from cord blood from 10 newborns (5 males and 5 females) and peripheral blood samples from 10 normal healthy individuals (5 males and 5 females) aged 70-90 years (mean 75.6 years). Cells were cultured for 72 hours in RPMI 1640 medium supplemented with 20% calf serum and phytohemagglutinin P. The slides were stained with silver nitrate by the method of Howell and Black (1980) (Figure 1).

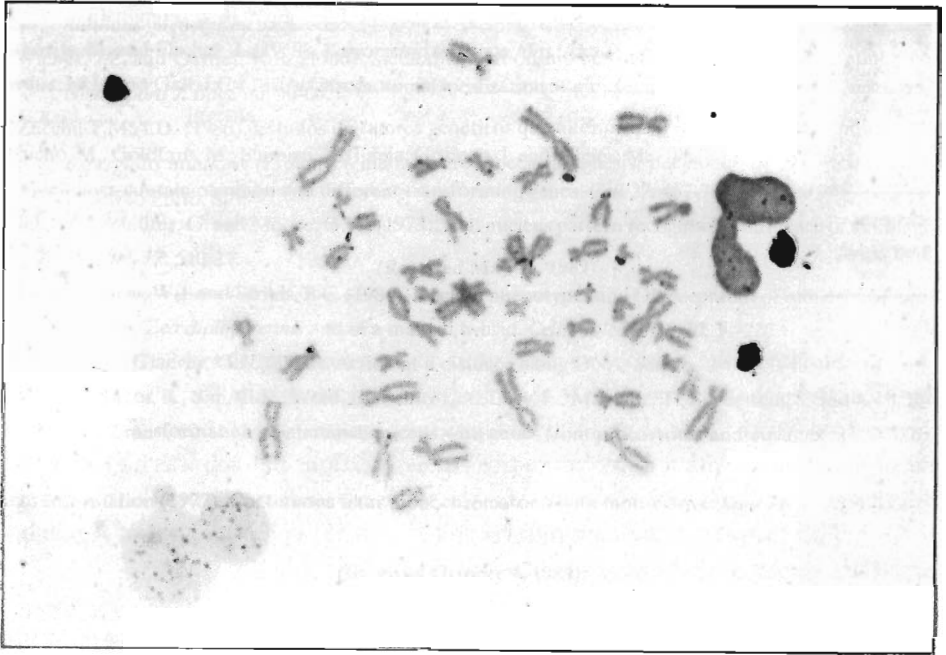


Figure 1 - Satellite associations and Ag<sup>+</sup> NORs as revealed by silver staining technique.

The frequencies of satellite association and Ag-stained NORs were analyzed in 20 metaphases for each culture on coded slides.

A satellite association was scored if the chromosomes were connected by an Ag-stained region or if the satellite regions of the chromosomes were within a chromatid width of each other but one of them lacked Ag-stain (Miller *et al.*, 1977).

The sizes of Ag-positive segments were classified visually according to Lezhava (1984): 0 = no silver deposition; grade 1 = small segment (less than the width of the chromatid); grade 2 = large segment (equal to or larger than the width of the chromatid).

## RESULTS AND DISCUSSION

The frequency of satellite associations is given in Table I. The number of metaphases with SA in the elderly group was significantly higher ( $\chi^2 = 6.29$ ) than in the newborn group. D/G was the most frequent type of association and most associations (79.4%) consisted of two chromosomes.

Table I - Satellite association (SA) analyzed in 200 metaphase cells from newborns and in 200 from elderly subjects.

Age group	Metaphases with SA No.	Type of SA				No. of chromo- somes in SA	Mean No. of chromo- somes per SA	Mean No. of SA per metaphase	No. of Metaphases with this No. of SAs				
		D/D	D/G	G/G	others				0	1	2	3	4
Newborns	137	38	96	19	34	415	2.21	0.93	62	90	44	3	0
70-90 years	159	49	92	42	53	544	2.31	1.18	41	94	55	8	2

Similarly, the number of associated chromosomes in the elderly group was significantly higher ( $\chi^2 = 22.82$ ) than in the newborn group for a total of 2000 chromosomes with associating ability analyzed per group. Table I shows that the number of metaphases with a different number of associations per cell was higher in the elderly group and that four associations in the same cell were noted only in this group.

There was no significant difference ( $\chi^2 = 0.55$ ) in the number of Ag-staining NORs between newborns and elderly individuals (Table II).

Table II - Number of chromosomes with different sizes of Ag-stained NORs and correlation of Ag-size and frequency of satellite association.

Age group	Size of Ag-stain	No. of chromosomes with Ag-stain	No. of chromosomes in SA with Ag-stain
Newborns	0 (absent)	258	6
	1 (small)	1,104	248
	2 (large)	638	161
70-90 years	0 (absent)	274	2
	1 (small)	945	293
	2 (large)	781	249

According to the literature, a correlation between the frequency of satellite associations and the number of Ag-staining NORs would be expected (Miller *et al.*, 1977; Lernia *et al.*, 1980; Lezhava, 1984). This, however, was not the case in the present study, even though cytological analysis showed that unstained chromosomes were rarely associated. Indeed, only 8 of 532 chromosomes with negative NORs (1.5%) were found in association, as opposed to 951 of 3468 chromosomes with positive NORs (27.4%).

Regarding NOR size as a function of associating ability, we noted that chromosomes with grade 1 NORs were associated at a frequency of 26.40%, whereas chromosomes with grade 2 NORs were associated at a frequency of 28.89%.

In general, the present results indicate that the frequency of Ag-stained NORs was similar in the elderly and newborn groups, though there was a significantly higher frequency of satellite associations in the elderly group. The literature has reported decreased or unchanged satellite association or Ag-stained NOR frequency as a function of aging. Even though there is no report of increased satellite association frequency with aging, in two studies conducted on newborns and elderly individuals the percentage of metaphases with associations was numerically higher in the elderly group (Prokofieva-Belgovskaya *et al.*, 1968; Liem *et al.*, 1977).

Few studies have been published on these parameters as a function of aging and there are great differences among the samples studied. Some investigators compare young individuals with elderly individuals (Mattevi and Salzano, 1975; Vormitag, 1980; Lezhava, 1984), others study individuals of different ages (Denton *et al.*, 1981; Kumagai, 1982), while still others study individuals divided into four age ranges, i.e., newborn infants, children and teenagers, young adults, and elderly individuals (Prokofieva-Belgovskaya *et al.*, 1968; Liem *et al.*, 1977).

In view of the opposite trends reported in studies on the expression of nucleolar organizer regions, i.e., decreased, unchanged or increased expression, we believe that there is no significant change with aging in the frequencies of satellite association and Ag-stained NORs.

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## RESUMO

A expressão dos genes ribossômicos representada pelas frequências de associações de satélites e de regiões organizadoras de nucléolo (RONs) ativas foi avaliada em culturas de linfócitos de um grupo

de recém-nascidos e um grupo de indivíduos com idades entre 70-90 anos, através da técnica de coloração pela prata. Observamos frequências semelhantes de RONs ativas em ambos os grupos ao lado de uma frequência mais elevada de associações de satélites no grupo dos idosos.

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