Published in final edited form as:

Mech Ageing Dev. 1989 January ; 47(1): 17–24.

Effects of Age, Sex and Multiple Endocrine Neoplasia Type-II on Silver Stained Nucleolar Organizer Regions

Merlin G. Butler* and John R. Lane

Division of Genetics, Department of Pediatrics, Vanderbilt University School of Medicine, T-2404 Medical Center North, Nashville, TN 37232 (U.S.A.)

Summary

Silver stained nucleolar organizer regions (AgNORs) were studied in phytohemagglutinin (PHA)-stimulated lymphocytes from 55 Caucasian control individuals (34 females with average age of 24 years and age range 19 weeks gestation to 87 years; 21 males with average age of 31 years and age range 29 weeks gestation to 72 years) and 13 individuals (7 females, 6 males; average age 38.8 years with age range 25–58 years) with multiple endocrine neoplasia-type II (MEN-II), an autosomal dominant malignancy with increased chromosome breakage. For the first time, AgNORs were examined in lymphocytes from normal fetuses and patients with MEN-II in order to determine the effects of age, sex or malignancy on the number of AgNORs. No significant difference in the average number of AgNORs were found in fetal cells ($8.2 \pm \text{S.D. } 0.7/\text{cell}$) when compared with cells from older individuals including those over 65 years of age ($8.0 \pm \text{S.D. } 0.8/\text{cell}$). There was a statistically significant negative correlation ($P < 0.05$) between the modal number of AgNORs on G but not D chromosomes in both males and females. A negative correlation was also found between the mean number of AgNORs and age but was not statistically significant. The average number of AgNORs in the MEN-II individuals was $8.5 \pm \text{S.D. } 0.7/\text{cell}$, which was not significantly different than $8.2 \pm \text{S.D. } 0.7/\text{cell}$ observed in age-matched control subjects.

Keywords

Silver stained nucleolar organizer regions (AgNORs); Multiple endocrine neoplasia-type II (MEN-II); Correlation; Age effects; Fetal lymphocytes

Introduction

Silver stain visualizes active nucleolar organizer regions (AgNORs) on the short arms of the five pairs of acrocentric chromosomes in man [1–3]. Previous studies indicated that there are variations in the size and number of AgNORs between sexes, individuals at different ages and with various disorders including malignancies [4–7]. The purpose of our cytogenetic investigation was thus to examine AgNORs in cells of a relatively large number of normal individuals of both sexes at various ages as well as patients with multiple endocrine neoplasia-type II (MEN-II), an autosomal dominant malignancy syndrome with

*To whom all correspondence and reprint requests should be addressed.

increased chromosome breakage characterized by medullary thyroid carcinomas, pheochromocytomas and parathyroid adenomas [8–10]. Therefore, we report for the first time AgNOR studies of lymphocytes from normal fetuses and patients with MEN-II and their comparison with control individuals at various ages.

Materials and Methods

Lymphocytes were harvested routinely from phytohemagglutinin (PHA)-stimulated cultures from 55 Caucasian control individuals (34 females, 21 males) from seven arbitrary age groups (fetal; 0–6 years; 7–13 years; 14–23 years; 24–44 years; 45–65 years; and greater than 65 years). The average age for the fetal group (7 females, 1 male) was 22.6 weeks gestation with a range of 19–30 weeks; average age for the 0–6-year group (5 females, 3 males) was 0.6 years with a range of 0.1–4 years; average age for the 7–13-year group (5 females, 3 males) was 10 years with a range of 7–13 years; average age for the 14–23-year group (4 females, 4 males) was 21 years with a range of 14–23 years; average age for the 24–44-year group (5 females, 3 males) was 29.5 years with a range of 24–36 years; average age for the 45–65-year group (4 females, 3 males) was 54.4 years with a range of 47–60 years; average age for the greater than 65 year group (4 females, 4 males) was 72.5 years with a range of 67–87 years. Thirteen individuals with MEN-II (7 females and 6 males with average age of 38.8 years and a range from 25 to 58 years) were studied.

Lymphocytes were obtained from eight fetuses by percutaneous umbilical blood sampling and peripheral venous blood from 47 individuals from six other age groups for comparison purposes. The chromosomes were silver stained for 90–110 s at 70 °C using a modified method developed by Howell and Black [11] to visualize the NORs then analyzed with light microscopy. Approximately 20 cells were analyzed from each individual by one observer throughout this study.

Pearsonian product moment correlation (r) of the mean and modal numbers of D and G chromosome group AgNORs compared with age in both sexes were calculated. Student t -tests of AgNORs of cells from MEN-II patients and age-matched control subjects were also calculated.

Results and Discussion

A total of 1070 metaphase spreads were analyzed from 55 control individuals with a total AgNOR mean of 8.2 with a range of 6.5–9.8. The individual and summary AgNOR data from the control individuals are shown in Tables I and II. A mean number of AgNORs for the D group chromosomes was 5.0 with a range of 3.3–5.9 and a mean number of AgNORs for the G group chromosomes was 3.3 with a range of 2.0–4.0. No significant differences were identified in the mean number of AgNORs in the D or G group chromosomes in either males or females. A negative correlation (r) value of -0.20 was found for total AgNORs of the 55 control individuals compared with age but was not significant. The correlation values of -0.24 and -0.15 , respectively for male ($n = 21$) and female controls ($n = 34$) were not significant for total AgNORs compared with age. Also, no

significant correlations were found in the average frequency of the G or D group AgNORs compared with age of either sex. No significant differences were identified in the mode number of AgNORs in the D or G group chromosomes in either males or females. A statistically significant negative correlation ($P < 0.05$) was found between the modal number of AgNORs on G but not D chromosomes and age in both males and females (Table III).

Two hundred sixty-two metaphase spreads were analyzed from 13 MEN-II individuals with the total mean number of AgNORs of 8.5 with a range 6.9–9.4 (Table II). A mean number of AgNORs for the D group chromosome was 5.1 with a range of 3–5.9. Mean number of AgNORs for the G group chromosome was 3.4 with a range of 2.8–4.0. No significant difference in the average number of AgNORs was found in comparison with age-matched controls.

Previous AgNOR studies with several malignancy syndromes (such as chronic myelocytic leukemia [5,7], Hodgkins disease [4], and meningioma [12]) and other conditions such as Down's syndrome [13] and cystic fibrosis [14] report either higher or lower mean number of AgNORs when compared with control individuals. Our study with MEN-II patients did not produce significant AgNOR differences when compared with controls. Therefore, reported AgNOR data from solid tumors, leukemia, and MEN-II patients appear to be inconclusive with regards to specific AgNOR activity.

In summary, our results indicate a lower mean number of AgNORs with advancing age but not significantly different although a significant negative correlation with age was found between the modal number of AgNORs on G but not D chromosomes in both males and females. There was no significant difference in AgNOR activity in MEN-II individuals when compared with age-matched controls. Our AgNOR data, particularly the modal number on G group chromosomes, in control individuals does support the previous findings of significantly lower AgNOR activity in older individuals by Das *et al.* [6] and Denton *et al.* [15]. Therefore, the research to date indicates that AgNOR genes may become repressed or inactivated in cells from older individuals but more research is needed to better clarify the effects on aging and development and the influence of malignancies on AgNOR activity.

References

1. Henderson AS, Warburton D, Atwood KC. Location of ribosomal DNA in the human chromosome complement. *Proc Natl Acad Sci.* 1972; 69:3394–3398. [PubMed: 4508329]
2. Hsu TC, Spirito SE, Pardue ML. Distribution of 18 S + 28 S ribosomal genes in mammalian genomes. *Chromosoma.* 1975; 53:25–36. [PubMed: 1104290]
3. Goodpasture C, Bloom SE. Visualisation of nucleolar organizer regions in mammalian chromosomes using silver staining. *Chromosoma.* 1975; 53:37–50. [PubMed: 53131]
4. Schulze B, Golinski C, Fonatsch C. Heterochromatin and nucleolus organizer regions in cells of patients with malignant and premalignant diseases. *Hum Genet.* 1984; 67:391–395. [PubMed: 6490006]
5. Sato Y, Abe S, Kubota K, Sasaki M, Miura Y. Silver-stained nucleolar organizer regions in bone marrow cells and peripheral blood lymphocytes of Philadelphia chromosome-positive chronic myelocytic leukemia patients. *Cancer Genet Cytogenet.* 1986; 23:37–45. [PubMed: 2427190]
6. Das BC, Rani R, Mitra AB, Luthra UK. The number of silver-staining NORs (rDNA) in lymphocytes of newborns and its relationship to human development. *Mech Ageing Dev.* 1986; 36:117–123. [PubMed: 3784625]

7. Trent JM, Carlin DA, Davis JR. Expression of silver-stained nucleolar organizing regions (Ag-NORs) in human cancer. *Cytogenet Cell Genet.* 1981; 30:31–38. [PubMed: 6167406]
8. Hsu TC, Pathak S, Samaan N, Hickey RC. Chromosome instability in patients with medullary carcinoma of the thyroid. *J Am Med Assoc.* 1981; 246:2046–2048.
9. Samaan NA, Hsu TC, Pathak S, Saad MF, Ordonex NG, Hickey RC. Chromosomal instability in medullary carcinoma of the thyroid. *World J Surg.* 1984; 8:487–492. [PubMed: 6148807]
10. Butler MG, Rames LJ, Joseph GM. Cytogenetic studies of individuals from four kindreds with multiple endocrine neoplasia Type II. *Cancer Genet Cytogenet.* 1987:253–260. [PubMed: 2887277]
11. Howell WM, Black DA. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: one-step method. *Experientia.* 1980; 36:1014. [PubMed: 6160049]
12. Zankl H, Huwer H, Zang KD. Cytogenetic studies on the nucleolar organizer region (NOR) activity in meningioma cells with normal and hypodiploid karyotypes. *Cancer Genet Cytogenet.* 1982; 6:47–53. [PubMed: 7104986]
13. Hansson A, Mikkelsen M. An increased tendency to satellite association of human chromosome 21: a factor in the aetiology of Down's syndrome. *ICRS.* 1974; 2:1617.
14. Ravia Y, Lieberman I, Rave A, Kremer M, Avivi L. High degree of satellite association and decreased nucleolar organizer activity in cystic fibrosis subjects. *Am J Med Genet.* 1987; 28:593–605. [PubMed: 3425629]
15. Denton TE, Liem SL, Cheng KM, Barrett JV. The relationship between aging and ribosomal gene activity in humans as evidenced by silver staining. *Mech Ageing Dev.* 1981; 75:1–7. [PubMed: 6168873]

Table 1

AgNOR Data from Control Individuals

	Age	AgNOR mean $\pm$ S.D. of D chromosomes	AgNOR mode of D chromosomes	AgNOR mean $\pm$ S.D. of G chromosomes	AgNOR mode of G chromosomes	AgNOR mean $\pm$ S.D. of D + G chromosomes	AgNOR mode of D + G chromosomes
<i>Female</i>							
1	19 weeks gestation	5.85 $\pm$ 0.49	6.0	1.95 $\pm$ 0.83	2.0	7.80 $\pm$ 0.89	8.0
2	19 weeks gestation	5.56 $\pm$ 0.52	6.0	2.44 $\pm$ 0.73	3.0	8.00 $\pm$ 1.12	9.0
3	20 weeks gestation	4.90 $\pm$ 0.71	5.0	3.95 $\pm$ 0.22	4.0	8.85 $\pm$ 0.67	9.0
4	21 weeks gestation	5.30 $\pm$ 0.73	6.0	4.00 $\pm$ 0.00	4.0	9.30 $\pm$ 0.73	10.0
5	21 weeks gestation	5.25 $\pm$ 0.44	5.0	1.95 $\pm$ 0.76	2.0	7.20 $\pm$ 1.01	7.0
6	22 weeks gestation	4.70 $\pm$ 0.73	4.0	3.85 $\pm$ 0.37	4.0	8.55 $\pm$ 0.76	8.5
7	30 weeks gestation	5.05 $\pm$ 0.83	5.5	2.50 $\pm$ 1.00	2.5	7.55 $\pm$ 1.14	8.0
8	0 years	4.80 $\pm$ 0.52	5.0	3.25 $\pm$ 0.72	3.5	8.05 $\pm$ 1.05	8.0
9	0 years	4.65 $\pm$ 0.49	5.0	3.95 $\pm$ 0.22	4.0	8.60 $\pm$ 0.60	9.0
10	0 years	5.80 $\pm$ 0.41	6.0	3.70 $\pm$ 0.57	4.0	9.50 $\pm$ 0.61	10.0
11	0 years	4.00 $\pm$ 0.46	4.0	3.90 $\pm$ 0.31	4.0	7.90 $\pm$ 0.55	8.0
12	4 years	4.80 $\pm$ 0.70	5.0	3.75 $\pm$ 0.44	4.0	8.55 $\pm$ 0.83	9.0
13	8 years	5.05 $\pm$ 0.39	5.0	3.00 $\pm$ 0.32	3.0	8.05 $\pm$ 0.51	8.0
14	9 years	3.52 $\pm$ 0.68	3.0	4.00 $\pm$ 0.00	4.0	7.52 $\pm$ 0.68	7.0
15	10 years	4.60 $\pm$ 0.60	4.5	2.55 $\pm$ 0.76	2.0	7.15 $\pm$ 0.93	7.0
16	10 years	5.71 $\pm$ 0.46	6.0	3.90 $\pm$ 0.30	4.0	9.61 $\pm$ 0.50	10.0
17	13 years	5.29 $\pm$ 0.43	5.0	3.57 $\pm$ 0.51	4.0	8.81 $\pm$ 0.75	9.0
18	21 years	5.35 $\pm$ 0.59	5.0	3.90 $\pm$ 0.31	4.0	9.25 $\pm$ 0.72	9.0
19	21 years	5.75 $\pm$ 0.44	6.0	3.10 $\pm$ 0.45	3.0	8.85 $\pm$ 0.37	9.0
20	23 years	5.62 $\pm$ 0.49	6.0	3.96 $\pm$ 0.20	4.0	9.58 $\pm$ 0.58	10.0
21	23 years	5.70 $\pm$ 0.47	6.0	3.65 $\pm$ 0.49	4.0	9.35 $\pm$ 0.81	10.0
22	24 years	5.00 $\pm$ 0.68	5.0	2.14 $\pm$ 0.66	2.0	7.14 $\pm$ 0.95	7.0
23	25 years	5.70 $\pm$ 0.47	6.0	3.13 $\pm$ 0.55	3.0	8.83 $\pm$ 0.89	9.0
24	27 years	5.05 $\pm$ 0.69	5.0	2.95 $\pm$ 0.51	3.0	8.00 $\pm$ 1.03	8.0
25	31 years	5.08 $\pm$ 0.22	5.0	2.32 $\pm$ 0.63	2.0	7.40 $\pm$ 0.71	7.0
26	36 years	5.54 $\pm$ 0.59	6.0	3.50 $\pm$ 0.51	3.5	9.08 $\pm$ 0.88	9.5
27	47 years	5.15 $\pm$ 0.74	5.0	3.35 $\pm$ 0.59	3.0	8.50 $\pm$ 0.95	9.0

	Age	AgNOR mean $\pm$ S.D. of D chromosomes	AgNOR mode of D chromosomes	AgNOR mean $\pm$ S.D. of G chromosomes	AgNOR mode of G chromosomes	AgNOR mean $\pm$ S.D. of D + G chromosomes	AgNOR mode of D + G chromosomes
28	49 years	5.25 $\pm$ 0.62	5.0	3.25 $\pm$ 0.62	3.0	8.50 $\pm$ 0.80	8.5
29	58 years	5.10 $\pm$ 0.85	6.0	3.55 $\pm$ 0.60	4.0	8.65 $\pm$ 1.04	8.5
30	60 years	4.74 $\pm$ 0.56	5.0	3.42 $\pm$ 0.61	3.5	8.16 $\pm$ 0.90	9.0
31	68 years	3.68 $\pm$ 0.67	4.0	3.16 $\pm$ 0.60	3.0	6.84 $\pm$ 0.96	7.0
32	71 years	5.85 $\pm$ 0.37	6.0	3.25 $\pm$ 0.44	3.0	9.10 $\pm$ 0.64	9.0
33	76 years	4.53 $\pm$ 0.77	5.0	3.10 $\pm$ 0.57	3.0	7.63 $\pm$ 1.06	8.0
34	87 years	4.95 $\pm$ 1.00	5.0	2.55 $\pm$ 0.61	2.0	7.50 $\pm$ 1.28	7.0
<i>Male</i>							
1	29 weeks gestation	4.45 $\pm$ 0.94	5.0	3.70 $\pm$ 0.57	4.0	8.15 $\pm$ 1.09	8.0
2	0 years	5.24 $\pm$ 0.54	5.0	3.66 $\pm$ 0.48	4.0	8.90 $\pm$ 0.77	9.0
3	0 years	4.79 $\pm$ 0.42	5.0	3.05 $\pm$ 0.23	3.0	7.84 $\pm$ 0.50	8.0
4	0 years	5.10 $\pm$ 0.64	5.0	2.00 $\pm$ 0.00	2.0	7.10 $\pm$ 0.64	7.0
5	9 years	5.00 $\pm$ 0.32	5.0	3.81 $\pm$ 0.40	4.0	8.81 $\pm$ 0.51	9.0
6	10 years	5.43 $\pm$ 0.75	6.0	3.86 $\pm$ 0.36	4.0	9.29 $\pm$ 0.72	9.5
7	11 years	4.95 $\pm$ 0.69	5.0	3.20 $\pm$ 0.52	3.0	8.15 $\pm$ 0.81	8.0
8	14 years	4.19 $\pm$ 0.93	4.0	3.14 $\pm$ 0.57	3.0	7.33 $\pm$ 1.23	8.0
9	20 years	5.90 $\pm$ 0.31	6.0	3.90 $\pm$ 0.31	4.0	9.80 $\pm$ 0.52	10.0
10	23 years	4.60 $\pm$ 0.50	5.0	3.75 $\pm$ 0.44	4.0	8.35 $\pm$ 0.67	8.5
11	23 years	5.00 $\pm$ 0.79	4.5	3.05 $\pm$ 0.69	3.0	8.05 $\pm$ 1.00	8.0
12	26 years	4.75 $\pm$ 0.79	5.0	3.75 $\pm$ 0.44	4.0	8.50 $\pm$ 0.66	9.0
13	32 years	5.06 $\pm$ 0.77	5.0	3.31 $\pm$ 0.70	3.0	8.38 $\pm$ 0.81	8.0
14	35 years	4.33 $\pm$ 0.50	4.0	3.22 $\pm$ 0.44	3.0	7.56 $\pm$ 0.53	8.0
15	48 years	3.27 $\pm$ 0.88	3.0	3.20 $\pm$ 0.41	3.0	6.47 $\pm$ 0.99	7.0
16	59 years	4.88 $\pm$ 0.45	5.0	2.83 $\pm$ 0.70	3.0	7.71 $\pm$ 0.81	8.0
17	60 years	3.83 $\pm$ 0.86	4.0	3.11 $\pm$ 0.68	3.0	6.94 $\pm$ 1.11	7.0
18	67 years	5.65 $\pm$ 0.75	6.0	2.75 $\pm$ 0.72	3.0	8.40 $\pm$ 1.19	9.0
19	68 years	5.50 $\pm$ 0.61	6.0	2.95 $\pm$ 0.22	3.0	8.45 $\pm$ 0.60	9.0
20	71 years	4.73 $\pm$ 0.59	5.0	3.87 $\pm$ 0.35	4.0	8.60 $\pm$ 0.63	8.5
21	72 years	4.73 $\pm$ 0.88	5.0	2.66 $\pm$ 0.72	2.0	7.40 $\pm$ 1.24	7.5

Table II
Data on Silver-Stained Nucleolar Organizer Regions (AgNORs) of Individuals at Various Ages With or Without Multiple Endocrine Neoplasia Type-II

Age group	No. of subjects		No. of cells	AgNOR average $\pm$ S.D. per cell	Range	AgNOR Data per chromosome group			
						D group		G group	
	(M)	(F)				Mean $\pm$ S.D.	Range	Mean $\pm$ S.D.	Range
Control subjects									
Fetal (19—30 weeks gestation)	1	7	149	8.18 $\pm$ 0.69	7.2—9.3	5.1 $\pm$ 0.5	4.4—5.8	3.0 $\pm$ 0.9	2.0—4.0
0—6 year olds	3	5	160	8.31 $\pm$ 0.74	7.1—9.5	4.9 $\pm$ 0.5	4.0—5.8	3.4 $\pm$ 0.7	2.0—4.0
7—13 year olds	3	5	165	8.42 $\pm$ 0.86	7.2—9.6	4.9 $\pm$ 0.7	3.5—5.7	3.5 $\pm$ 0.5	2.6—4.0
14—23 year olds	4	4	165	8.82 $\pm$ 0.86	7.3—9.8	5.3 $\pm$ 0.6	4.2—5.9	3.6 $\pm$ 0.4	3.1—4.0
24—44 year olds	3	5	155	8.11 $\pm$ 0.70	7.1—9.1	5.1 $\pm$ 0.4	4.3—5.7	3.0 $\pm$ 0.6	2.1—3.8
45—65 year olds	3	4	128	7.85 $\pm$ 0.85	6.5—8.6	4.6 $\pm$ 0.8	3.8—5.2	3.2 $\pm$ 0.3	2.8—3.6
> 65 year olds	4	4	148	7.99 $\pm$ 0.76	6.8—9.1	4.9 $\pm$ 0.7	3.7—5.8	3.1 $\pm$ 0.4	2.6—3.9
Multiple endocrine neoplasia-type II									
25—58 year olds	6	7	262	8.52 $\pm$ 0.73	6.9—9.4	5.1 $\pm$ 0.8	3.0—5.9	3.4 $\pm$ 0.4	2.8—4.0

Table III
AgNOR Correlation Data in Control Individuals

<i>Females (N= 34)</i>		
Age vs. mean number of G chromosome AgNORs	$r=-0.102$	$P> 0.05$
Age vs. mean number of D chromosome AgNORs	$r=-0.076$	$P> 0.05$
Age vs. mean number of D + G chromosome AgNORs	$r=-0.147$	$P> 0.05$
Age vs. mode number of G chromosome AgNORs	$r=-0.304$	$P< 0.05$
Age vs. mode number of D chromosome AgNORs	$r=-0.008$	$P> 0.05$
Age vs. mode number of D + G chromosome AgNORs	$r=-0.227$	$P> 0.05$
<i>Males (N=21)</i>		
Age vs. mean number of G chromosome AgNORs	$r=-0.218$	$P> 0.05$
Age vs. mean number of D chromosome AgNORs	$r=-0.123$	$P> 0.05$
Age vs. mean number of D + G chromosome AgNORs	$r=-0.240$	$P> 0.05$
Age vs. mode number of G chromosome AgNORs	$r=-0.293$	$P< 0.05$
Age vs. mode number of D chromosome AgNORs	$r=-0.013$	$P> 0.05$
Age vs. mode number of D + G chromosome AgNORs	$r=-0.268$	$P> 0.05$