96. *Sister Chromatid Exchange in Human Lymphocytes in Extreme Age*

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Introduction. The phenomenon of sister chromatid exchanges (SCE) induced by 5-bromodeoxyuridine (BrdU) and consisting in isolocus exchange between sister chromatids with obligatory joining of duplexes has been first reported in 1972.¹⁾ The distribution of SCE in chromosomes is generally proportional to their relative lengths and DNA content.²⁾⁻³⁾ SCE are less frequent in telomeric and centromeric regions where heterochromatin is located.⁴⁾⁻⁶⁾ At a BrdU concentration of 7 to 15 mcg/ml their number varies age-dependent from 6.2 to 15.8 per metaphase.^{2),7)-9)}

Most investigators have detected an increase^{8),10)} or no significant age-dependent difference in SCE frequency in blood cells.¹¹⁾⁻¹⁴⁾ However, in human fibroblasts and lymphocytes SCE numbers decline both with the age of the donor and with serial cell passage.¹⁵⁾⁻¹⁷⁾

The present study was undertaken to determine the incidence of SCE per metaphase and their distribution over the length of a chromosome in lymphocyte cultures from subjects aged 80 to 88.

Methods. A total of 677 metaphase spreads were analysed in the cultures of lymphocytes from 29 subjects 18 to 88 years of age. The material included 247 metaphases from 13 subjects aged 80 to 88 and 430 metaphases from the control group aged 18 to 59.

For differential staining of sister chromatids, short-term lymphocyte cultures were used. BrdU at a concentration of 7.7 mcg was added immediately upon the initiation of cultures. The cells were incubated in darkness at 37°C for 96 hr. The chromosomes were stained without any fluorochromes according to the method.¹⁸⁾

Results and discussion. Table I shows the results of SCE assays for the two age groups. Altogether 5510 SCE were detected in 677 metaphases, averaging 8.28 per metaphase with a range of 2-23. In different subjects the SCE number per metaphase varied from 6.53 ± 1.05 to 15.80 ± 2.95 . There were no interindividual variations in some families.

Mathematical analysis was carried out as described previously.⁹⁾ When we ignored dispersal within an age group, the use of student's formula

$$t = \frac{X - Y}{\sqrt{\frac{S_{1.1}^2}{n} + \frac{S_{2.1}^2}{m}}}$$

resulted in

$$t = \frac{8.40 - 7.69}{\sqrt{0.028 + 0.03}} = \frac{0.71}{\sqrt{0.058}} = \frac{0.71}{0.24} = 2.95.$$

Thus the difference revealed between the age groups was highly significant.

To understand more clearly the trend to a decrease in SCE frequency during aging, we analyzed the data showing SCE levels for separate chromosomes. As a result of such calculations comparatively higher values were obtained for dispersal of mean SCE levels within each age group. Yet there was a significant difference in SCE number for A₁ and C chromosomes between the study group and the control subjects:

$$t_{A_1} = \frac{1.05 - 0.79}{\sqrt{0.0040 + 0.0047}} = \frac{0.26}{0.09} \approx 2.9$$

$$t_C = \frac{3.7 - 3.11}{\sqrt{0.020 + 0.021}} = \frac{0.60}{0.21} = 3.$$

The estimates for the C chromosome in subject 12 from the control group and subject 4 from the study group were not taken into account, since they deviated sharply from the group mean.

However, *t* retained significance even upon their inclusion:

$$t_C = \frac{0.52}{\sqrt{0.038 + 0.060}} = \frac{0.52}{0.31} \approx 1.7.$$

In both age groups E, F and G chromosomes showed a deficit of SCE (Table I). On the other hand, the observed values were significantly higher than those expected for A₁ and C chromosomes in control subjects, and for A₂ and B chromosomes in the older group. It can be seen that the SCE distribution in different regions of A₁ and A₂ chromosomes in blood cells from old, as well as younger donors SCE frequency peaks in the medial arm region and declines precipitously toward the telomere and the centromere, evidently in the telomeric and centromeric heterochromatin regions.^{7),16)-17),19)}

Several investigators studied SCE frequency in transplantable cell lines during early, middle and late passages, in human fibroblasts and lymphocytes (from donors aged 20 to 35 and 65 or over), and in cells from animals of various ages (both *in vivo* and *in vitro*). In experiments without the induction no age-dependent changes were revealed in these cell systems, except human fibroblasts and lymphocytes where SCE levels declined as a function of the donor age and of serial passage. Other investigators compared SCE numbers in lymphocytes from children, middle-aged people and persons at 61 or older, and also failed to detect any difference.¹⁴⁾⁻²¹⁾ The discordance between these results and our data, which show that SCE levels tend to decline during aging can probably be explained by different age distributions of the cell donors. We studied lymphocytes from subjects in the 9th decade of life, and in such cells certain chromosome regions undergo progressive heterochromatinization²⁰⁾⁻²⁴⁾ which seems to interfere with SCE.⁴⁾

A deficit of SCE in E, F and G chromosomes has been reported previously.^{2),11)} Our data agree with these observations. In both groups of our subjects there was an SCE deficit in E, F and G chromosomes, as well as in the telomeric and centromeric regions of A₁ and A₂ chromosomes. It has been proposed that deficient due to SCE levels are a high amount of heterochromatin.^{2),3)}

Summarizing, we may suggest that SCE levels are controlled by the age factor.

Table I. Frequency of sister chromatid exchanges in cultured lymphocytes and in chromosome groups by age

Case no.	Age (yr)	No. of meta-phases analysed	No. of SCE analysed	Average SCE/cell $\pm m$	No. of SCE in chromosomes								
					A ₁	A ₂	A ₃	B	C	D	E	F	G
1	18	41	305	7.44 $\pm$ 0.97	35	40	20	41	119	32	14	3	1
2	20	20	156	7.80 $\pm$ 1.82	27	13	7	14	71	18	4	2	—
3	23	25	238	9.52 $\pm$ 1.22	23	23	20	30	119	20	3	—	—
4	25	50	344	6.88 $\pm$ 0.55	35	27	30	55	149	30	10	4	4
5	26	15	98	6.53 $\pm$ 1.05	15	10	6	13	38	9	6	1	—
6	31	15	118	7.87 $\pm$ 2.09	22	9	6	13	51	10	7	—	—
7	40	25	193	7.72 $\pm$ 1.38	20	11	10	17	105	17	7	5	1
8	42	50	434	8.68 $\pm$ 0.92	50	51	29	59	191	38	13	1	2
9	47	15	131	8.73 $\pm$ 1.78	10	15	13	17	51	11	9	1	4
10	50	30	239	7.97 $\pm$ 1.19	25	19	9	30	112	20	9	2	3
11	51	22	214	9.73 $\pm$ 0.85	22	30	10	27	88	22	9	4	2
12	52	10	158	15.80 $\pm$ 2.95	13	16	15	29	63	14	6	1	1
13	53	30	316	10.53 $\pm$ 1.43	40	28	26	41	136	30	13	1	1
14	55	21	157	7.48 $\pm$ 1.15	24	14	9	27	61	15	5	1	1
15	59	26	189	7.27 $\pm$ 1.09	36	17	12	22	67	22	11	2	—
16	59	35	320	9.14 $\pm$ 1.11	39	30	29	46	128	32	15	—	1
Total					436	353	251	481	1549	350	141	28	21
1	80	30	250	8.33 $\pm$ 1.06	20	28	17	38	102	23	15	7	—
2	80	15	100	6.67 $\pm$ 2.10	10	11	4	18	38	14	3	2	—
3	80	15	110	7.33 $\pm$ 0.93	9	12	7	29	38	4	11	—	—
4	80	10	111	11.10 $\pm$ 2.49	7	9	9	8	61	13	2	1	1
5	80	20	128	6.40 $\pm$ 0.97	8	12	12	9	66	14	6	—	1
6	80	21	177	8.43 $\pm$ 1.04	18	23	15	24	67	20	9	—	1
7	80	19	114	7.57 $\pm$ 1.27	12	17	14	27	51	15	3	3	2
8	81	23	156	6.78 $\pm$ 0.84	9	17	8	41	59	10	11	1	—
9	83	27	216	8.00 $\pm$ 1.10	20	31	8	27	100	14	12	2	2
10	83	10	71	7.10 $\pm$ 1.07	8	8	6	10	24	9	4	2	—
11	87	11	84	7.64 $\pm$ 2.02	12	10	4	16	27	9	6	—	—
12	87	26	208	8.00 $\pm$ 0.68	15	28	14	27	79	25	13	3	4
13	88	20	145	7.25 $\pm$ 0.45	12	12	11	30	57	17	5	1	—
Total					160	218	129	304	769	187	100	22	11

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