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FREQUENCY AND DISTRIBUTION OF SISTER CHROMATID EXCHANGES IN CULTURED HUMAN LYMPHOCYTES*

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The recently developed technique for the detection of sister chromatid exchanges (SCE) was originally described by Latt[1]. The new techniques involve labelling the two chromatids of a chromosome with 5-bromodeoxyuridine (BrdU) and subsequently staining with either fluorescent dyes[1,2], Giemsa's stain[3] or a combination of both[4]. The harlequin chromosomes yielded by this technique clearly reveal SCE (Figure 1) and provide a powerful means for their study.

The incidence of SCE is proportional to the DNA content in different mammalian species[5-8]. Studies on the incidence and distribution of SCE within and between chromosomes in man[9,10], the Chinese hamster[11] and the Indian muntjac[12] have shown that the frequency of SCE per chromosome is directly related to chromosome length. However, detailed studies in man reveal that the smaller chromosomes consistently show a lower frequency than expected[9]. The distribution of SCE within human chromosomes is non-random, with a deficiency in the centromeric and an excess in the mid-arm regions[9].

Depending upon the species studied, there are conflicting reports as to whether SCE are located on the pale G-banded regions, or at the interfaces between pale and dark G-bands. However, there is clear evidence of an excess of SCE at C-band interfaces in the Indian muntjac[12] and the kangaroo rat[13]. The distribution of SCE in many ways parallels the distribution of spontaneous gross chromosomal aberrations, but the significance of this non-random distribution in the generation of SCE events is not immediately obvious[14].

SCE have proved to be more sensitive than the conventional analysis of chromosome aberrations with regard to some mutagens and carcinogens tested[10,15,16]. The interaction between carcinogen and chromosome can sometimes cause chromosome breakage of SCE, but more often it results in a submicroscopic genetic change which may subsequently lead to malignant transformation[17].

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Figure 1.

Peripheral blood lymphocyte metaphase showing sister chromatid exchanges.

In this study we present data on both the frequency and distribution of SCE in human peripheral lymphocytes.

Material and Methods

Peripheral blood samples from 20 healthy children (10 female and 10 male) were cultured in Difco McCoy 5A medium with a concentration of 10 μg/ml BrdU (Sigma) for 72 hours. All cultures were left to grow in the dark, and slides were prepared by the air-dried technique. Differential staining was induced by the technique described by Korenberg and Freedlender[3]. Twenty well-spread metaphases from each subject were scored. The chromosome length values were calculated from those reported at the 1971 Paris Conference on Standardization of Human Cytogenetics[18].

Results and Discussion

200 cells from the 10 girls and 200 cells from the 10 boys were analyzed for the distribution of SCE between chromosomes, and the distributions in the 20 children were compared.

Distribution of SCE among chromosomes in the 20 subjects. The distribution of SCE between the nine identifiable chromosome groups was analyzed. The results

TABLE I

Chromosomal Distribution of SCE Frequencies in 10 Girls (20 metaphases per sample)

Subject	Chromosome or group								
	1	A 2	3	B 4-5	C-X 6-12-X	D 13-15	E 16-18	F 19-20	G 21-22
1	18	16	6	21	73	12	3	4	3
2	23	14	11	24	62	9	13	2	1
3	14	18	14	12	68	15	8	3	2
4	11	9	5	18	57	10	11	2	0
5	12	19	9	16	64	11	7	2	4
6	16	17	12	11	66	8	9	4	3
7	13	10	8	10	71	14	5	3	2
8	9	11	9	13	59	12	7	2	3
9	17	9	12	17	61	10	4	3	2
10	10	14	7	19	55	13	5	4	2
Total	143	137	93	161	636	114	72	29	22
$\bar{X}$	14.30	13.70	9.30	16.10	63.60	11.40	7.20	2.90	2.20
S*	4.27	3.77	2.91	4.58	5.93	2.22	3.16	0.88	1.14
$\bar{X}$/cell	0.71	0.68	0.46	0.80	3.18	0.57	0.36	0.14	0.11
S*/cell	0.95	0.84	0.65	1.02	1.32	0.49	0.70	0.19	0.25

S* = Sample standard deviation.

are shown in Tables I and II. Although there was considerable variation between subjects in the distribution of SCE among chromosomes, the difference was not statistically significant. Nor was there a significant difference between the sexes. This result shows that there is a homogeneous distribution between subjects of SCE in the same chromosome or chromosome groups, and the chromosome susceptibility to BrdU is similar.

Distribution of SCE among chromosomes. In our subjects the frequency of SCE per chromosome or chromosome group generally increased with chromosome length,

TABLE II

Chromosomal Distribution of SCE Frequencies in 10 Boys (20 metaphases per sample)

	Chromosome or group								
		A		B	C-X	D	E	F	G-Y
Subject	1	2	3	4-5	6-12-X	13-15	16-18	19-20	21-22-Y
1	10	14	7	28	52	13	8	3	2
2	14	12	10	19	64	15	8	1	4
3	8	10	13	15	48	18	10	6	3
4	19	15	9	21	79	9	11	3	3
5	20	17	11	18	76	11	6	5	2
6	14	9	8	17	53	16	9	4	5
7	6	8	7	14	58	15	7	2	4
8	17	21	14	27	65	10	8	4	2
9	15	16	11	22	69	8	12	0	3
10	16	14	12	19	68	9	9	2	3
Total	139	136	102	200	632	124	88	30	31
$\bar{X}$	13.90	13.60	10.20	20.00	63.20	12.40	8.80	3.00	3.10
S^*	4.61	3.98	2.44	4.64	10.34	3.47	1.81	1.82	0.99
$\bar{X}$/cell	0.69	0.68	0.51	1.00	3.16	0.62	0.44	0.15	0.15
S^*/cell	1.03	0.89	0.54	1.03	2.31	0.77	0.40	0.40	0.22

S^* = Sample standard deviation.

indicating that the distribution of SCE is a general function of that length (Tables III, IV). Despite the overall correlation, however, chi-square analysis of the distribution of exchange points indicated a highly significant deviation ($p < 0.001$) between the expected and observed number of exchanges in our subjects (Tables V, VI). Chromosomes 1 and 2 and the C-X group in the girls and the C-X group in the boys showed more exchanges than expected, while the D, E, F and G or G-Y groups showed fewer than expected in both boys and girls.

TABLE III

The Distribution of SCE Between Chromosomes in 10 Girls

Chromosome	Relative chromosome length[18]	Number of chromosomes scored	Number of SCE	Mean frequency of SCE/chromosome
A_1	8.44	400	143	0.357
A_2	8.02	400	137	0.342
A_3	6.83	400	93	0.232
B	6.19	800	161	0.201
C-X	4.99	3200	636	0.198
D	3.58	1200	114	0.095
E	3.18	1200	72	0.060
F	2.61	800	29	0.036
G	1.97	800	22	0.027

TABLE IV

The Distribution of SCE Between Chromosomes in 10 Boys

Chromosome	Relative chromosome length[18]	Number of chromosomes scored	Number of SCE	Mean frequency of SCE/chromosome
A_1	8.44	400	139	0.347
A_2	8.02	400	136	0.340
A_3	6.83	400	102	0.255
B	6.19	800	200	0.250
C-X	4.99	3000	632	0.210
D	3.58	1200	124	0.103
E	3.18	1200	88	0.073
F	2.61	800	30	0.037
G-Y	2.03	1000	31	0.031

TABLE V

Distribution of Exchanges Between Chromosomes in 10 Girls

Chromosome	Total relative length[18]	Percent length	Observed exchanges	Expected exchanges	X^2
A_1	16.88	0.08030	143	112.982	7.975
A_2	16.04	0.07630	137	107.354	8.187
A_3	13.66	0.06498	93	91.427	0.027
B	24.76	0.11778	161	165.717	0.134
C-X	79.94	0.38027	636	535.040	19.051
D	21.52	0.10237	114	144.035	7.911
E	19.08	0.09076	72	127.699	24.294
F	10.46	0.04976	29	70.012	24.024
G	7.88	0.03748	22	52.734	17.912
Total	210.22	1.00000	1407	1407.000	109.515 *

* $p < 0.001$.

TABLE VI

Distribution of Exchanges Between Chromosomes in 10 Boys

Chromosome	Total relative length[18]	Percent length	Observed exchanges	Expected exchanges	X^2
A_1	16.88	0.08150	139	120.783	2.748
A_2	16.04	0.07743	136	114.751	3.935
A_3	13.66	0.06594	102	97.723	0.187
B	24.76	0.11953	200	177.143	2.949
C-X	74.82	0.36119	632	535.284	17.476
D	21.52	0.10389	124	153.965	5.832
E	19.08	0.09211	88	136.507	17.237
F	10.46	0.05049	30	74.173	26.247
G-Y	10.03	0.04842	31	71.758	23.150
Total	207.25	1.00050	1482	1482.087	99.761 *

* $p < 0.001$.

The excess of exchanges in the longer chromosomes has been noted by Morgan and Crossen[19] in the B group, Crossen et al[20] in chromosomes 1, 2 and the B group, Knuutila et al[21] in the B and C groups, Cheng et al[22] in chromosomes 1 and 2 and the B, C and D groups, and by Michalová et al[23] in chromosome groups A, B and C. The excess of exchanges in the longer chromosomes may reflect differences in base composition along the length of these chromosomes[20] or their susceptibility to BrdU.

The unexpectedly fewer exchanges in the shorter chromosomes have also been noted in the literature[9,20,21,23-25]. This is compatible with the low incidence of SCE in the centromeric region, where a large number of these chromosomes are located[25].

Summary

The frequency and distribution among chromosomes of sister chromatid exchanges (SCE) was examined in a population of 20 normal children (10 girls and 10 boys) and a comparison made among the subjects. The frequency of exchanges generally increased with chromosome length. However, chromosomes 1 and 2 and the C-X group in the girls and the C-X group in the boys showed more exchanges than expected, while the D, E, F and G or G-Y groups showed fewer than expected in both boys and girls.

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