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Chromosomal Study in Ageing

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KEY WORDS Ageing; satellite association; fragile site.

ABSTRACT Chromosomal study on satellite association and fragile site was made in ageing. Satellite association was studied on 68 cases and fragile site was studied on 91 cases from newborn to over 90 years by blood culture. Satellite association increased in ageing. The frequency of fragile sites was statistically high in only 20-30 years group, but no difference was found in newborn, 60-76 and over 90 years group.

INTRODUCTION

It has been well known that the birth of infants with trisomy is related to nondisjunction in aged mother, and the nondisjunction is considered relating with the satellite association. On the other hand, fragile site is very interesting subject on the clinical disorders, such as the fragile X syndrome (Sutherland 1983). However, those studies in ageing have been still meager. This study was made researches into the cytogenetical effects of ageing based on satellite association and on fragile site.

MATERIALS AND METHODS

Satellite Association: Satellite association was studied on 68 cases divided in 5 groups, that is, the cases of newborn were ten, of from 20 to 25 (20-25) years were 9, of 40-45 years were 21, of 60-76 years were 18 and over 90 years were 10.

The chromosomal preparations were made by standard blood culture procedures (Kadotani 1986). Phytohemagglutin-stimulated peripheral blood was cultured in RPMI 1640 medium (Nissui Pharmaceutical Co., Ltd., Japan) for 72 hours, and slides were made by means of the flame-drying technique. The conventional Giemsa and G-banding differential staining were routinely employed for chromosome identifications. Satellite associations were counted with 50 -100 well-delineated metaphases per case.

Fragile Site: Chromosome fragile site was studied on 91 cases divided in 4 groups, that is, the cases of newborn were 29, of 20-30 years were 22, of 60-76 years were 30 and of over 90 years were 10 cases.

The chromosomal preparations were made by standard blood culture procedures (Kadotani 1986). Phytohemagglutin-stimulated peripheral blood was cultured in Eagle's MEM medium without folic acid (Nissui pharmaceutical Co., LTD., Japan) for 72 hours (Sutherland 1979; Sugio and Kajii 1985) and slides were made by means of the flame-drying technique. The conventional Giemsa and G-banding differential staining were routinely employed for chromosome identifications. Chromosome observations were made on 50~100 well-delineated metaphases per case. The break point with occurrence of 4.0 % or more at the same point was considered as the fragile site (Takimoto et al. 1985; Sutherland and Mattei 1987).

RESULTS

Satellite Association: Satellite association was surveyed in ageing (Fig.1). Satellite associations in each age group were shown in Table 1. The frequencies of total satellite association per cell were 0.62 in newborn, 1.01 in 20-25 years, 1.47 in 40-45 years, 1.47 in 60-76 years, 1.2 in over 90 years group. The frequency of satellite associations was statistically higher (α=0.05) in over 20-25 years group than newborn.

Details of the type of satellite association in ageing were shown in Table 2. Satellite association was studied on associated number and on associated chromosomes, that is, they were divided 11 types of satellite association in each age- group: in the association with two chromosomes, one of D-group chromosomes with one of D-group chromosomes (DD), one of D-group chromosomes with one of G-group chromosomes (DG), and one of G-groups chromosomes and one of G-group chromosomes (GG), in the association with 3 chromosomes; DDD, DDG and/or

Table 1: Satellite association in ageing

Age (years)	*Cases*	*Observed cells*	*Sat. ass.*	*Sat. ass./ cell*
0	10	874	541	0.62
20-25	9	567	573	1.01*
40-45	21	1728	2548	1.47*
60-76	18	1412	2071	1.47*
>90	10	500	600	1.2*

Sat. ass.: Satellite association
*: α=0.05

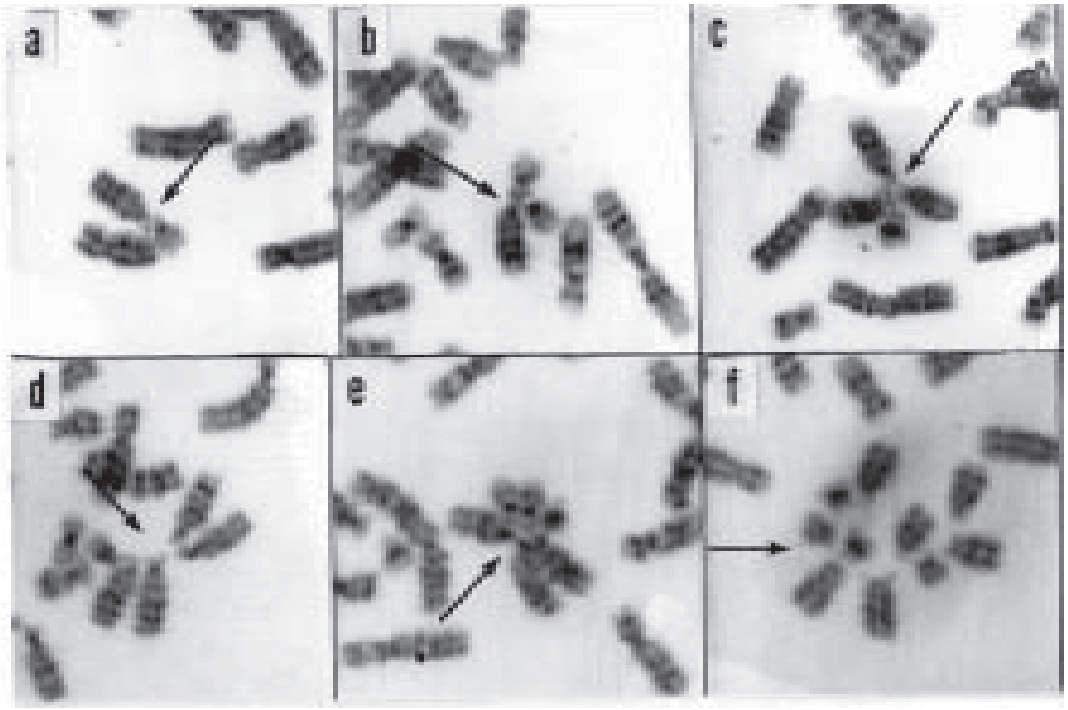

Fig. 1. Photographs of satellite association; a: two chromosomes association, b: three chromosomes association, c: four chromosomes association, d: five chromosomes association, e: six chromosomes association, f: ten chromosomes association

DGG, and GGG, in the association with 4 chromosomes; DDDD, DDDG, DDGG, and/or DGGG, and GGGG. The satellite association with over 5 chromosomes was consisting of D- and G-group chromosomes. The satellite association consisting of 2 chromosomes occupied 86.7 % in newborn, 78.2 % in 20-25 years old group, 70.8 % in 40-45 years, 75.9 % in 60-76 years and 73.8 % in over 90 years (Table 2). Total percentage of the satellite association consisting of two chromosomes was much the same in any age group. Total percentage of over three chromosomes was relatively but not statistically higher in over 20-25 years group than newborn. No difference was found statistically between each of over 20-25 years groups. Satellite association with over 6 chromosomes was observed only in over 40-45 years groups.

The frequency and the type of satellite association in ageing were shown in figure 2. They were shown almost same tendency in all groups.

Table 2: The frequency of satellite association in ageing (%)

Age	*Type of satellite association*													
(years)	*2 chrms*				*3 chrms*				*4 chrms*				*5 chrms*	*over 6 chrms*
	DD	*DG*	*GG*	*Total*	*DDD*	*D-G*	*GGG*	*Total*	*DDDD*	*D-G*	*GGGG*	*Total*	*D-G*	*D-G*
0	17.9	47.7	21.1	86.7	1.6	8.9	1.3	11.8	0	1.3	0.2	1.5	0	0
20-25	21.3	40.5	16.4	78.2	1.8	12.4	2.3	16.5	0.5	3.8	0	4.3	1	0
40-45	21.5	38	11.3	70.8	2	16.2	1.2	19.4	0.3	6.9	0.1	7.3	1.9	0.6
60-76	20.1	41.8	14	75.9	2.1	14.4	0.8	17.3	0.3	4.8	0	5.1	1.4	0.2
>90	16.8	43	14	73.8	1.5	16.8	1.8	20.1	0.2	4	0	4.2	1.3	0.5

chrms: chromosomes, D-G: the association consisting of D- and G-group chromosomes.

Table 3: Associated acrocentric chromosomes in over 90 years group

2 chrms		*3 chrms*		*4 chrms*		*5 chrms*		*over 6 chrms*	
21:22	59	13:21:22	14	13:14:21:22	3	13:15:21:22:22	3	14:14:22:15:13:21:13:22:21:15	1
13:21	54	14:21:22	14	13:15:21:22	3	14:15:21:22:22	2	13:13:15:21:22:22	1
13:22	52	13:15:22	11	14:21:21:22	3	13:13:14:15:21	1	13:13:14:14:15:22	1
15:21	45	13:14:21	9	13:21:22:22	2	13:14:14:15:22	1		
14:21	39	21:21:22	9	14:15:22:22	2	13:14:21:21:22	1		
15:22	36	14:15:21	8	13:13:21:22	1				
14:22	32	14:15:22	7	13:13:14:15	1				
13:15	28	15:21:22	7	13:13:14:21	1				
13:14	25	13:15:21	6	13:14:15:21	1				
14:15	25	13:14:15	5	13:14:21:21	1				
21:21	17	13:13:22	3	13:14:22:22	1				
15:15	10	13:14:22	3	13:15:15:22	1				
14:14	8	14:22:22	3	14:14:21:22	1				
22:22	8	15:15:22	3	15:15:21:22	1				
13:13	5	15:22:22	3	15:15:22:22	1				
		13:13:15	2	15:21:21:22	1				
		14:21:21	2	15:21:22:22	1				
		15:21:21	2						
		21:22:22	2						
		13:13:21	1						
		13:14:14	1						
		13:21:21	1						
		13:22:22	1						
		14:14:15	1						
		14:14:21	1						
		14:14:22	1						
		15:15:21	1						
	443		121		25		8		3

chrms: chromosomes

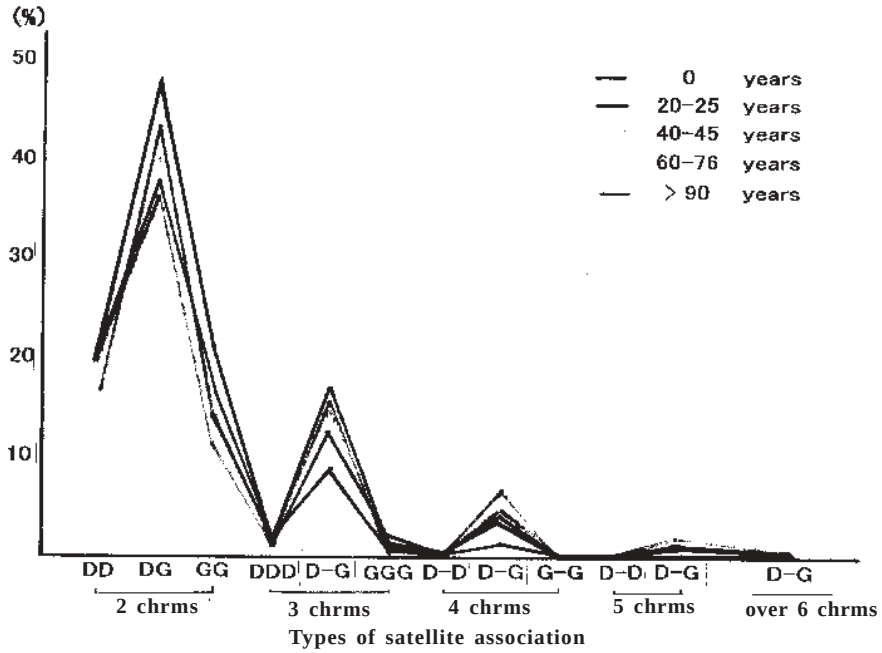

Fig. 2. The frequency of satellite association in each age group. Chrms: chromosomes, D– D: DDDD in 4 chrms and DDDDD in 5 chrms, D– G: the association consisting of D- and G-group chromosomes.

Table 4: Chromosome break points and frafgile sites in ageing

Age (years)	*No. of Cases*	*Ob. Cells*	*Cells with B.P.(%)*	*No. of B.P.(%)*	*Fra.Sit.*	*Fra.Sit./Cases*
0	29	1450	143(9.9)	160(11.0)	6	0.2
20-30	22	1100	294(26.7)**	394(35.8)**	48	2.2**
60-76	30	1500	125(8.3)	143(9.5)	8	0.3
>90	10	500	58(11.6)	64(12.8)	4	0.4

No.: Number, Ob.: Observed, B.P.: Break Point, Fra.Sit.: Fragile Sites
**: α=0.01

The types of satellite association and their frequencies in over 90 years group were shown in Table 3. The association of chromosomes 21 and 22 was most frequently.

Fragile Site: The number of chromosome break points and fragile sites was counted in each age-group (Table 4), and they were statistically higher in 20-30 years than other three groups (α=0.01).

Chromosome break points and fragile sites were completely same tendency for frequency. No difference was found between newborn, 60-76 and over 90 years group in the frequencies of fragile site. Fragile site was not observed in increasing with ageing.

The details of fragile site in ageing were shown in Table 5. Twenty kinds of fragile sites were observed in 20-30 years, and the site at 12q13 was folic acid sensitive heritable fragile site. The site at 3p14 was shown in all age group and most frequently in newborn, 20-30 and 60-76 years group, which was one of common fragile sites.

Table 5: Fragile sites in ageing

0 year		*20-30 years*		*60-76 years*		*>90 years*	
3p14	4	3p14	16	3p14	6	6p21	2
1p31	1	1p32	6	2q33	1	1p11	1
14q22	1	16q22	5	14q24	1	3p14	1
		1q25	2				
		3q27	2				
		6q26	2				
		14q24	2				
		1p22	1				
		1p31	1				
		3p25	1				
		4q31	1				
		6p21	1				
		7q11	1				
		7q21	1				
		7q32	1				
		8q24	1				
		9p13	1				
		9q12	1				
		*12q13	1				
		15q15	1				
	6		48		8		4

*12q13 : Folic acid sensitive heritable fragile site

CONCLUSION AND SUMMARY

It has been reported on the chromosome study connecting with ageing in some fields, that is, satellite association and ageing reported by Kadotani et al. (1978a, 1978b) and Lezhava (1999), fragile site and ageing by Kadotani (1990) and Kadotani and Watanabe (1998), adaptive response to X-rays and ageing by Gadhia (1998), aneuploidy in human sperm and ageing by Shi and Martin (2000), chromosome aberrations and ageing by Kadotani et al. (1978a) and Tawn and Whitehouse (2001). It was already reported on the satellite association of chromosomes in ageing from 0 to 76 years consisting of four groups (Kadotani et al. 1978a). Additionally, the group of over 90 years was studied in this report. The association consisting of over 6 chromosomes was observed in the groups of over 40 years, and the association consisting of ten chromosomes was observed in over 90 years group. Satellite association was increased and statistically high in ageing comparing with newborn. Two chromosomes association, especially DG association, was most frequently in all age groups. The same as our study has not been reported yet. Further study in this field is expected.

This study was surveyed on the folic acid sensitive fragile site in ageing. Ninety-one cases aged from 0 to over 90 years consisting of four groups were studied on fragile sites. The increasing of the frequencies of fragile sites with ageing was not observed, but the frequency of fragile sites was remarkably high in 20-30 years group. No difference was found between newborn, 60-76, and over 90 years group in the frequencies of fragile site. The frequency of fragile site was statistically high in 20-30 years group. This remarkably high frequency was considered the affection with some kinds of exogenous factors (Sbrana and Musio 1995; Kadotani and Watanabe 1998). Most frequently fragile site was

the site at 3p14: common fragile site, but no difference was found between newborn, 60-76 and over 90 years groups.

Satellite association and fragile site in chromosomes were surveyed in ageing. As a summary, satellite association was tending to increase in ageing, however, fragile site did not increase with ageing. The increasing of satellite association with age was suggested one genesis of trisomy of chromosome, especially the trisomy of chromosome 21.

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