Chromatid segregation analysis in native human lymphocyte anaphases using sequential fluorescence in situ hybridization

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Abstract

A sequential multiprobe fluorescence in situ hybridization technique was developed to study the 13, 18, 21, X and Y chromatid segregation in human lymphocytes anaphases cultures without antimitotic treatment. This method was used to know if exist any different chromosomes segregation in lymphocytes from Down syndrome patients and compared it with controls. The results show that the prevalent sequence of centromere separation was X, 13, 21, Y and 18 in Down syndrome patients and Y, 13, X, 21 and 18 in controls. Chromatid segregation in early anaphase was asynchronous for all chromosome pairs studied. Late anaphase showed a frequency of non-disjunction of 4.5% in the controls, affecting only chromosomes 18 and Y; in the Down syndrome patients, the frequency was higher (20.3%) and affected all chromosomes studied. This technique could be applicated to know the incidence of non disjunction in couples with repetitive abortions or in cases with different aneuploidies in the offspring.

Introduction

Several studies have been performed to detect the existence of possible abnormalities in the separation of chromatids to the spindle poles. In somatic cells the most important mechanisms in the production of aneuploid cells is premature centromere division, and the most common aneuploidies affect chromosomes 21, X and 18 (Fitzgerald and McEwan 1987; Vig and Rattner 1989; Keser et al. 1996). However, sister chromatid separation is a very fast process (Mitchinson and Salmon 1992) that is difficult to study by common cytogenetic techniques (G-bands).

To contribute to segregation sister chromatids at anaphase, we studied the sequence of centromere separation in lymphocytes from Down syndrome (DS) patients and compared it with controls, using a sequential multiprobe-FISH technique (Escudero et al. 1998) combined with a technique to obtain native anaphase and to identify the 13, 18, 21, X and Y chromosomes in the same anaphase cell. This tech-

nique allows to establish the sequence of centromere separation in early anaphases and to observe chromatid segregation and non disjunction (ND) in late anaphases.

Material and methods

Chromosome preparations

Peripheral blood samples for three SD patients (47, XY, +21) and two controls (46, XY) were cultured in RPMI-1640 medium for 72 hours according to standard techniques, but without antimitotic treatment to maintain the original arrangement of the chromosomes. Preparations were made using a modification of the technique of Ford and Congedi (1987), to obtain anaphases. Cells were treated with 1% sodium citrate for 8 min, and were fixed with 3:1 methanol:acetic acid.

Figure 1. Sequential FISH applied to the same late anaphase. a) In the first step the chromosomes labelled were 13 (green) and 21 (red). b) In the second step the chromosomes labelled were X (green), Y (red) and 18 (white in the image).

Probes for in situ hybridization

In all preparations we used five different probes detecting chromosomes 21, 13, 18, X and Y, combined in two directly labelled sets. One set included

LSI 21 Orange/13 Green probes and the other CEP 18 Aqua-Blue/X Green/Y Orange (VYSIS, Downers Grove, IL, USA) probes.

Protocol of in situ hybridization

The sequential FISH technique described by Escudero et al. (1998) was applied as follows: a first hybridization with the LSI 13 Green/21 Orange probes, capture of images, and wash at high stringency, followed by a second hybridization using the 18 Aqua-Blue/X Green/Y Orange set of probes, capturing again the image (Figure 1a, b).

Criteria of chromatid segregation

We considered that segregation was complete when the two chromatids had their centromeres clearly separated at anaphase (Figure 2a). When one of the homologues had its chromatids still held together, segregation was considered as incomplete (Figure 2b). When both chromosomes had unseparated chromatids, segregation was considered as delayed (Figure 2c).

Results

We analysed 233 anaphases, 151 from DS patients and 82 from controls. In the DS patients, 64.2% (97/151) of anaphases were early and 35.7% (54/151) were late; in the controls the percentages were 46.3% and 53.6% respectively.

Figure 2. Chromosome 18 (white) segregation types: a) Complete segregation of the two chromatids in both chromosomes. b) Incomplete segregation, one chromosome delayed (arrow). c) Segregation delayed, both chromosomes delayed (arrows). Segregation is normal for the X (green) and Y (red) chromosomes in the three anaphases.

Table 1. Degree of delay and sequence of segregation in anaphases from DS patients.

Patients	Chromosome					Sequence of centromere separation
	13	18	21	X	Y	
1	14.3% (10/70)	24.3% (17/70)	15.2% (16/105)	14.3% (5/35)	22.9% (8/35)	X and 13, 21, Y, 18
2	23.9% (21/88)	19.3% (17/88)	17.4% (23/132)	13.6% (6/44)	15.9% (7/44)	X, Y, 21, 18, 13
3	22.2% (8/36)	44.4% (16/36)	38.9% (21/54)	33.3% (6/18)	50% (9/18)	13, X, 21, 18, Y
Total	20.1% (39/194)	25.8% (50/194)	20.6% (60/291)	17.5% (17/97)	24.7% (24/97)	X, 13, 21, Y, 18

Early anaphase

Tables 1 and 2 show the degree of delay and the sequence of centromere segregation in DS patients and in controls respectively. Taking into account all the early anaphases analyzed in DS patients, the sequence of segregation was: X, 13, 21, Y and 18, while in the controls the sequence of segregation was: Y, 13, X, 21 and 18.

Table 3 shows the degree of asynchrony for chromosome pairs 13, 18 and 21 in DS patients and controls. Obviously, the X and Y were not included, and in the DS patients asynchrony was only considered when one or two chromosomes 21 had their chromatids separated. In the DS patients, chromosome 21 is significantly more asynchronic than chromosome 13 ($\chi^2=7.2549$; $P=0.0071$) and chromosome 18 ($\chi^2=4.7739$; $P=0.0289$). In the controls, no significant differences were observed among the chromosomes studied. No significant differences were found when comparing the degree of asynchrony of the 13 and 18 chromosomes in DS patients with the degree of asynchrony found in controls.

Late anaphase

In DS patients we observed a frequency of ND of 20.3% (11/54) (Table 4; Figures 3 and 4). Three anaphases had a double ND affecting one chromosome 18 and the X chromosome; in five anaphases ND affected only one chromosome: in two of them the chromosome affected was 21 and in others the affected chromosomes were 13, X and Y respectively.

In controls the frequency was 4.5% (2/44); in one case the chromosome ND affected was chromosome 18 (Figure 5), and in the other it was the Y chromosome. The frequency of ND was statistically higher in DS patients than in controls ($t=2.0153$; $P=0.0476$).

Discussion

The sequence of centromere separation is difficult to study because this process is very fast (Mitchinson and Salmon 1992). Previous studies did not show coincident results (Vig and Rattner 1989; Méhes and Kosztolányi 1992).

Table 2. Degree of delay and sequence of segregation in anaphases from controls.

Controls	Chromosome					Sequence of centromere separation
	13	18	21	X	Y	
1	16.0% (8/50)	32.0% (16/50)	32.0% (16/50)	8.0% (2/25)	16.0% (4/25)	X, 13 and Y, 18 and 21
2	19.2% (5/26)	42.3% (11/26)	38.5% (10/26)	38.5% (5/13)	15.4% (2/13)	Y, 13, 21 and X, 18
Total	17.1% (13/76)	35.5% (27/76)	34.2% (26/76)	18.4% (7/38)	15.8% (6/38)	Y, 13, X, 21, 18

Table 3. Degree of asynchrony of chromosomes 13, 18 and 21.

Patients	chro 13	chro 18	chro 21
1	17.1% 6/35	31.4% 11/35	37.1% 13/35
2	20.5% 9/44	20.5% 9/44	40.9% 18/44
3	22.2% 4/18	11.1% 2/18	33.3% 6/18
Subtotal	19.6% 19/97	22.7% 22/97	38.1% 37/97
Controls			
1	16.0% 4/25	32.0% 8/25	32.0% 8/25
2	23.1% 3/13	7.7% 1/13	15.4% 2/13
Subtotal	18.4% 7/38	23.7% 9/38	26.3% 10/38

Table 4. Non-disjunction in DS patients and in controls.

Patients	chro 13	chro 18	chro 21	chro X	chro Y	Total
1	1/23		1/23			
2		3/13		4/13	1/13	
3			1/18			
Subtotal	1/54	3/54	2/54	4/54	1/54	11/54 (20.3%)
Controls						
1		1/36				
2					1/8	
Subtotal		1/44			1/44	2/44 (4.5%)

Figure 3. Non-disjunction affecting chromosome 18 in a DS patient in late anaphase. Chromosome 18 is labelled in white, the X in green and the Y in red. The set on the right has three chromatids 18 (arrows).

The methodology used in our work, sequential multiprobe FISH, allowed to establish the sequence of centromere separation for several chromosomes. In DS patients and in controls, chromosomes 18 and 21 tend to segregate their chromatid late; in the case of chromosome 21, these results are in agreement with those obtained by Vig and Rattner (1989). Méhes and

Figure 4. Non-disjunction affecting one chromosome 18 and one chromosome 13 in a DS patient in late anaphase. Chromosome 18 labelled in white and 13 in green. The right set has three chromatids 18 (arrows) and two 13 chromatids (asterisks).

Kosztolányi (1992) obtained similar results for chromosome 18 in children with 18 trisomy. It is interesting to note that mitotic poisons such as colcemid do not seem to have any influence on the process of chromatid separation; in fact the sequence observed for some chromosomes, has been detected in studies using colcemid (Vig and Rattner 1989; Méhes and Kosztolányi 1992) and in studies that did not use it (present study). Our study shows that both in the DS patients and in controls, the X chromosome tends to segregate its chromatids early. Zijno et al. (1996) have also described the premature separation of the chromatids in the X chromosome.

We have not found any references to the synchrony of separation of the chromatids of homologous chromosomes in the literature. In our study we observed that, in DS patients, chromosomes 21 show a higher degree of asynchrony of chromatid separation than pairs 13 and 18. This finding suggests that the degree

Figure 5. Non-disjunction affecting one chromosome 18 in a control in late anaphase. Chromosome 18 labelled in red, X in green and Y in red. The anaphase group on the left has three chromatids 18.

of asynchrony may be implicated in the ND process, at least in some individuals.

Our study also shows that the proportion of early anaphases is higher in the DS group than in controls; since the frequency of ND found in late anaphases in the DS group is higher than in controls, both factors could be related, because it could reflect a tendency to early chromatid separation in the DS patients.

The methodology used in this work (multiple-probe FISH in native anaphases) allow to study the sequence of chromatid separation, the asynchronic segregation of homologues in early anaphase, and the frequency of ND in late anaphase. Moreover, this technique could be applicated to know the incidence of ND in couples with repetitive abortions or in cases with different aneuploidies in the offspring.

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