
GENETICS

Premature Centromere Division of Metaphase Chromosomes in Drug Addicts and Alcoholics

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 9, pp. 322-326, September, 1997
Original article submitted June 24, 1996

The study of metaphase chromosomes in peripheral blood lymphocytes from alcoholics and drug addicts revealed enhanced incidence of chromosomes with premature centromere division. The mean proportion of such metaphases was 14.8% in patients in the abstinent state, which is related to drastic disturbances of physiological homeostasis. The detoxification course decreased this proportion to 6%, which is comparable to the value of 5.1% in the control group. Individual differences were found in the incidence of metaphases with premature centromere division.

Key Words: *drug addiction; alcoholism; abstinent state; premature centromere division; metaphase chromosomes*

In metaphase, two chromatids of each replicated chromosomes are mutually tied in the centromere that attaches the chromosomes to the mitotic spindle microtubules. At the end of metaphase, the centromere divides, and the daughter chromosomes travel to the opposite poles of a cell. This mechanism provides correct chromosome segregation in the series of cell divisions. Sometimes during a metaphase separately located chromatids can be seen, belonging to the same chromosome, while other chromosomes of the cell are composed of two chromatids tied in the central region. This is the phenomenon of premature centromere division (PCD) [5,6], which is also referred to as C-anaphase [4].

Enhancement of metaphases with PCD chromosomes is characteristic of the Roberts syndrome and other syndromes related to chromosome abnormalities, predominantly to aneuploidy. The incidence of PCD metaphases increases with aging. There are data

on increased incidence of such metaphases in tumor cells both *in vivo* and *in vitro* [8]. In the embryonic lymphocytes of women, who were intensively treated during the pregnancy with hormones, the incidence of PCD metaphases is significantly higher than that in the control group [3]. The causes of PCD are unknown. For example, in the Roberts syndrome this phenomenon could be evoked by endogenous genetic mechanisms [7]. In addition, PCD can result from exogenous factors related to alteration of hormonal status of the organism caused by aging or hormonal therapy [3,8].

Our aim was to study PCD in metaphase chromosomes in alcoholics and drug addicts, which were in the abstinent state characterized by homeostasis disturbance [2] and stress inevitably related to hormonal imbalance or after the detoxification course.

MATERIALS AND METHODS

Twenty-eight men aged 17-40 years were enrolled in the study. Sixteen of them were alcoholics and 12

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were drug addicts (10 of them thebaic addicts and 2 patients were psychostimulant addicts). Control group consisted of 19 men of similar age, who were neither alcoholics nor drug addicts. Culturing and fixation of blood cells were performed simultaneously in the control and test groups.

Blood samples were collected immediately after admission to clinic, before medication, and two weeks after detoxification.

Peripheral blood lymphocytes were cultured during 72 h by the standard semimicropreparative procedure using Eagle's medium with 20% bovine serum and phytohemagglutinin (Paneko, Moscow). Colchicine (final concentration of 0.5 $\mu\text{g/ml}$) was added 1.5 h before fixation. The cells were exposed to 0.56% KCl hypotonic solution and fixed in methanol:acetic acid mixture (3:1). The preparations were processed according to the standard procedure with-



Fig. 1. Metaphases and metaphase fragments of peripheral blood lymphocytes in alcoholics and drug addicts in the abstinent state; 1 (a), 2 (b), 5 (c), and 8 (d) chromosomes with prematurely divided centromeres (arrows).

TABLE 1. Percentage of PCD Metaphases in Abstinent Patients and after Detoxification

Patients			Control	
subject number	% of metaphases with PCD		subject number	PCD metaphases, %
	abstinent state	after detoxification course		
Drug addiction			1	2
1	39	9	2	0
2	30	8	3	2
3	26	7	4	14
4	12	0	5	4
5	17	5	6	8
6	16	4	7	4
7	12	2	8	2
8	15	5	9	6
9	8	2	10	0
10	2	4	11	6
11	1	3	12	4
12	18	21	13	2
Mean	16.3	5.8	14	2
Alcoholism			15	14
1	30	2	16	5
2	29	3	17	10
3	26	6	18	11
4	17	1	19	0
5	16	1		
6	14	2		
7	10	0		
8	3	0		
9	13	11		
10	10	8		
11	19	18		
12	7	8		
13	6	8		
14	13	15		
15	0	5		
16	4	10		
Mean	13.6	6.1		
Overall mean	14.8	6.0		5.1

out scorching. The slides with metaphase cells were stained by the method of Giemsa (Merck). For determination of the PCD incidence, 100-300 slides with metaphase chromosomes were prepared from each patient and normal subject [1]. Analysis was carried out using encoded preparations. The data were analyzed with the help of "Statgraphics" software.

RESULTS

The metaphase cells were considered to have PCD if one or more chromosomes had separated chromatids (Fig. 1).

Table 1 shows the incidence of PCD metaphases in both abstinent and detoxicated patients in comparison with the control group. In the abstinent

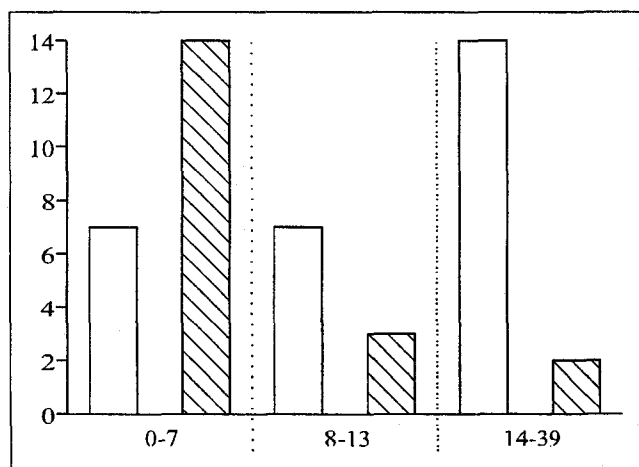


Fig. 2. Different frequencies of metaphases with PCD in the control group (dashed bars) and in abstinent patients (open bars). Abscissa: PCD metaphases, %; ordinate: number of human subjects.

patients, the proportion of PCD metaphases was 14.8%, which is significantly higher than the respective mean percentage after detoxification (6%) or in the control group (5.1%, $p < 0.05$).

The proportion of PCD metaphases varied in the control group from 0 to 14%; only two subjects had a high incidence of such metaphases (14% in both cases); 73.3% of healthy subjects had no more than 7% PCD metaphases. In abstinent patients, there were 0-39% PCD metaphases, and only in 25% of them this parameter was less than 7%, while in 50% it was 14-39% (Fig. 2).

After detoxification of 9 drug addicts (75%), the incidence of PCD metaphases decreased by several times. In alcoholics, a similar decrease was found in 43% of cases. The average incidence of PCD metaphases decreased to 6%, which virtually coincided

with the control data ($p = 0.46$). In 12 drug addicts (Nos. 1-9) and alcoholics (Nos. 1-7), the proportion of PCD metaphases was decreased considerably: 19.7% before and 3.8% after detoxification. In other 16 patients (predominantly alcoholics), the incidence of PCD metaphases virtually did not change: 8% before and 9.3% after detoxification. This fact can be explained either by the difference in individual sensitivity to detoxification or by insufficient duration of detoxification in the case of alcohol abstinence.

Interestingly, PCD was found in the same number of chromosomes irrespective of the proportion of PCD metaphases in the subjects of the test and control groups: respectively, 56.4 and 53.1% metaphases had one PCD chromosome; 43.6 and 46.9% had 2-8 (and sometimes more) such chromosomes (Table 2).

Our results show for the first time the possibility of "induced" PCD *in vivo*. The incidence of PCD metaphases does not correlate with the type of narcotic dependence (alcoholism, thebaic, or psychostimulants). At the same time, the two-week clinical study revealed the major hormonal shifts in the blood, which accompanied the abstinent state. In particular, they were pronounced for cortisol and somatotrophic hormone. However, the most important finding is a 3-fold (alcoholism) and a 5-fold (thebaic addiction) increase in the adrenocorticotrophic hormone level observed at the peak of abstinent syndrome, i.e., on days 2-4 (personal communication of Dr. I. V. Man'kovskaya, V. P. Serbsky State Research Center for Social and Forensic Psychiatry).

Such an increase in adrenocorticotrophic hormone is known to elevate corticosteroids and to underlie homeostatic disturbance in the organism at any stress conditions (in this study, after discontinuance of narcotics). Based on these findings, we will focus further studies on the effect of corticosteroids on chromosome behavior and the appearance of PCD in cultured peripheral blood lymphocytes.

TABLE 2. Incidence of PCD Chromosomes in Patients and Healthy Subjects

Number of PCD chromosomes per cell	Patients		Control	
	number of metaphases	%	number of metaphases	%
1	109	56.4	34	53.1
2	36	18.5	6	9.4
3	11	5.5	6	9.4
4	9	4.5	5	7.8
5	10	5.2	1	1.6
6	9	4.7	—	—
7-8	5	2.6	3	4.7
Virtually all chromosomes	5	2.6	9	14.0
Total	193	100	64	100

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