

## Premature Centromere Division in Schizophrenia? Preliminary Report

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**KEY WORDS** Schizophrenia; chromosome instability; premature centromere division (PCD).

**ABSTRACT** Spontaneous and Methotrexate-induced chromosome instability was investigated in lymphocyte-mitoses of 20 young schizophrenic patients. As compared to controls, an increased number of chromatid and chromosome breaks and/or of premature centromere divisions (PCDs) was seen in 13 patients. No correlation between frequency of PCDs and therapy of the patients was found. In spite of the great individual variation and the fact that environmental factors could not be entirely excluded, the findings suggest a hitherto not described association of schizophrenia with PCD.

### INTRODUCTION

Case reports and surveys in psychiatric wards suggested a higher than usual prevalence of chromosomal aberrations among schizophrenic patients, already in the sixties. This led to the assumption that schizophrenia and other mental disorders may be the consequence of a high variability of gene mutations (Schultz and Andreasen 1999) that can be activated by environmental insults (Tsuang 2001) and may also lead to phenotypic alterations, such as informative morphogenetic variants [mild errors of morphogenesis, minor congenital anomalies](Trixler et al. 1997). Since these phenomena are related to chromosomal instability (Méhes 2000), we recently made an attempt to analyze the spontaneous and induced fragility in schizophrenia. The increased number of chromatid and chromosome breaks refers to a defect in chromosome repair mechanism. Sister chromatid exchange and premature division of centromeres (PCD), also regarded as a manifestation of chromosome instability (Méhes and Bühler 1995), are consequences of

other mechanisms. This is why, we also investigated the occurrence of PCDs in cultured peripheral lymphocytes of schizophrenic patients.

### PATIENTS AND METHODS

Twenty young schizophrenic patients (9 males and 11 females) aged 18 to 31 years were examined. The medication of the patients is shown in table 1. None of them had suffered from acute infections for at least four weeks prior blood sampling for lymphocyte cultures.

Since in our previous studies centromere-specific staining procedures did not enhance the objectivity of investigation, from the short-term (50-52 h) lymphocyte cultures conventionally prepared slides were stained with Giemsa without banding procedures. Parallel cultures were treated for 6 hours with Methotrexate in a final concentration of  $10^{-5}$ M. From both untreated and Methotrexate-treated preparations 100 mitoses were analyzed for the anomalies as follows: chromatid and chromosome breaks, other structural aberrations, and PCDs. PCD was defined as no centromeric connection between the two sister chromatids in at least 10 chromosomes of the same cell (Fig. 1). Aberrations were expressed as per cent of cells with one or more breaks and with multiple PCDs. Based on our previous experience and on the results of the present controls, subjects with more than 5 per cent of either of the anomalies were regarded as having an increased rate of aberrant mitoses. In four cases where remarkably high levels of aberrations were found on the first occasion, the examination was repeated after a time interval of six months.

Parallely prepared lymphocyte cultures of ten healthy subjects served as controls. The slides were coded, the examiners were not aware of the

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**Table 1: Chromosomal aberrations in schizophrenic patients (percent of cells with structural aberrations and multiple PCDs)**

Case No.	Medication	Duration	Spontaneous		MTX induced		
			Aberration	PCD	Aberration	PCD	
<i>Patients</i>							
<i>Males</i>							
1	Haloperidol	3 weeks	1	1	5	17	
2a	----	----	0	12	2	15	
b	Risperidone	6 months	1	9	2	10	
3a	Risperidone	3 months	1	20	0	32	
b	Risperidone	9 months	0	15	1	30	
4	Haloperidol + Flupenthixol	2 weeks	0	0	0	9	
5	Haloperidol + Fluphenazine	2 years 4 years	22	5	21	6	
6	Haloperidol + Clozapine	2 weeks 2 weeks	3	5	-	-	
7	Haloperidol	4 weeks	10	1	21	7	
8	Zuclopenthixol	2 weeks	6	0	-	-	
9	Haloperidol	2 years	5	0	7	1	
<i>Females</i>							
10	Clozapine	3 weeks	3	0	5	9	
11	Clozapine	4 years	2	6	2	23	
12	Haloperidol	1 year	3	1	4	27	
13	Clozapine	4 years	3	13	-	-	
14	Haloperidol + Fluphenazine	3 years 2 years	0	6	5	27	
15	Zuclopenthixol	2 weeks	0	9	0	12	
16	Haloperidol	3 weeks	2	4	-	-	
17a	Haloperidol + Flupenthixol	2 weeks 1 year	57	24	-	-	
b	Risperidone + Flupenthixol	2 years 2 years 6 mo	15	13	51	16	
18a	Risperidone + Flupenthixol	2 years 2 years 6 mo	28	5	-	-	
b	Risperidone + Flupenthixol	2 years 2 years 6 mo	18	3	-	-	
19	Flupenthixol	3 years	4	10	-	-	
20	Clozapine	1 year	7	0	10	2	
<i>Controls</i>							
<i>Males</i>							
1	-		1	1	4	2	
2	-		0	0	1	0	
3	-		0	1	5	1	
4	-		2	1	4	2	
5	-		3	0	4	2	
<i>Females</i>							
6	-		0	0	1	0	
7	-		1	0	3	3	
8	-		0	0	4	2	
9	-		3	2	5	2	
10	-		2	0	6	0	

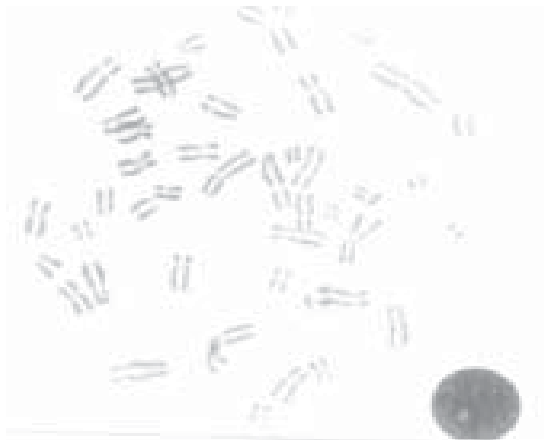
origin of the preparation.

## RESULTS

The karyotype of the subjects proved to be normal in each case.

When disclosing the codes of the slides, a

great individual variation in the prevalence of mitoses with breaks and/or PCDs was found (Table 1). This made the comparison of mean values absolutely meaningless, nevertheless, the differences between the non-homogenous data of patients and controls proved to be statistically significant ( $p < 0.01$  in any comparison).



**Fig. 1. Multiple premature centromere divisions (PCDs) in a metaphase plate from the pretreatment lymphocyte culture of patient No. 2. The sister chromatids of practically every chromosome are separated.**

When evaluating individual findings, no parallelism between the behaviour of the two main types of anomalies in the untreated cultures was seen: a significantly high rate of breakage occurred in five patients (Nos. 5, 7, 8, 18, and 20) in whom no increase of PCD prevalence was observed. In patient No.17 the frequency of both anomalies proved to be extraordinary high. In a part of the Methotrexate-treated cultures no sufficient number of mitoses could be analyzed. Out of the 13 cases that could be evaluated, significant increase of breaks was only seen in No.7 and in the second cultures of No.17. At the same time, spontaneous PCD tendency without increased fragility occurred in seven patients (Nos. 2, 3, 11, 13, 14, 15, 19), in whom an even higher prevalence of PCDs was observed after Methotrexate-induction.

It should be mentioned that apart from two dicentric chromosomes in the first untreated cultures of patient No.17, under the heading of "structural aberrations" only chromatid and chromosome breaks were registered in the whole study. In contrast to recent findings (Chen et al. 1998), no specific fragile sites were observed in this study.

The investigation was repeated in four subjects. A repeatedly high number of PCDs without structural aberrations was seen in Nos. 2 and

3. In Nos.17 and 18 an increased tendency to both breakage and PCD was registered again, however, the frequency of aberrations was somewhat lower than in the cells of their original lymphocyte cultures.

No correlation between quality and dosage of medication and frequency of aberrations was found. The freshly diagnosed patient No. 2 was without medication at the time of his first examination, when he had a high number of cells with multiple PCDs, what was also seen after 6 months of Risperidone administration. The other six patients with a high frequency of PCDs received a variety of drugs.

As also shown in table 1, the frequency of breaks and PCDs was uniformly low in both the untreated and Methotrexate-treated cells of the control subjects.

## DISCUSSION

Our study certainly has some limitations. The number of investigated subjects was relatively small and the medication of the patients could not sufficiently be standardized, in some cases also noncompliance had to be considered. The great individual variation in therapy was not related to the variation of cytogenetic anomalies. Although this speaks against a direct effect of medication, our present results permit no definite conclusions. However, it would be false to ignore these preliminary findings which may call attention to at least two possibilities:

1. In contrast to normal healthy subjects, chromosomal instability is relatively frequent in schizophrenic patients. This may be due either to a genetic predisposition or to an altered metabolism of psychoactive agents, as suggested in earlier studies (Madle et al. 1980), or both.
2. The instability may manifest itself in various ways. In some patients signs of chromosome fragility, in others PCDs prevail, referring to impaired repair mechanism and to deficient sticking of the centromeres to the kinetochore, respectively. A tendency to PCD has been described in individual cases of congenital abnormalities, immuno-deficiencies and malignancies (Plaja et al. 2001) but not in schizophrenia so far. The possibility that chromo-

somes with PCD were recorded as "acentrics" in some earlier studies can not be excluded. This may be interesting insofar as special importance has been attributed to acentrics in hypotheses concerning etiology and pathomechanism of neurobehavioural disorders (Gericke 1999). How far environmental factors could have influenced the occurrence of PCDs in this study could not be clarified, however, since no increased number of prematurely separated sister chromatids was seen in the mitoses of control subjects living under similar circumstances, environmental effects other than medication (Major et al. 1999) could not have been essential in our patients.

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