

Minor Physical Anomalies and Chromosomal Fragility as Potential Markers in Schizophrenia. Preliminary Report

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ABSTRACT In the present study, the authors investigated chromosomal fragility in patients with schizophrenia, and compared the patients, who were positive for minor physical anomalies (MPAs) with those who were negative for MPAs in respect to chromosome fragility. 44 patients with schizophrenia were examined and compared to 23 matched healthy controls. A modified Waldrop-scale was used for evaluation of MPAs. The patient sample was divided: 22 patients with MPAs (MPA-positive) and 22 patients without MPAs (MPA-negative). Lymphocyte cultures of the patients and normal controls were divided into parallel cultures, with one set being treated with Methotrexate (MTX) and the other set remaining untreated. From both treated and untreated cells, 100 mitoses were analysed for structural anomalies. A highly significant difference in fragile sites was found between the MPA-positive and control group in both the treated and untreated cells. The MPA-negative cells were also different from those of the control group but only in the untreated condition. The MPA-positive and MPA-negative groups of the patients differed from each other in the MTX-treated cells. The MPAs and chromosomal fragility show a positive association, such that the prevalence of MPAs correlates with a higher percentage of chromosomal fragility. Both minor physical anomalies and chromosomal fragility are related to chromosomal instability. A significant association between them supports a genetic determination of MPAs.

INTRODUCTION

Minor physical anomalies (mild errors of morphogenesis) have been frequently investigated in schizophrenic population, and their higher occurrence in these patients is indicative of a disturbed early neurodevelopment (Marengo and Weinberger 2000). Minor physical anomalies (MPAs) themselves can not give an answer on the etiology of disturbed neurodevelopment. Both genetic and nongenetic factors (viral infection, nutrition etc.) can play a role. Therefore a common occurrence with other genetically determined biological markers, such as chromosomal fragility could strengthen the genetic etiology of the MPAs and their potential usefulness as biological markers for schizophrenia.

Relatively little data are known about chromosomal fragility in schizophrenia. Recent data support a higher number of chromosomal fragile sites in patients with schizophrenia than in normal controls (Garofalo et al. 1993; Fananas et al. 1997;

Smith et al. 1997; Chen et al. 1998).

Chromosomal fragility (i.e., the number of fragile sites) was investigated in patients with schizophrenia and data were compared in respect to the presence or absence of MPAs, in order to find an association between the two nonspecific biological markers.

SUBJECTS AND METHODS

44 patients with schizophrenia (24 men, 20 women) were examined and compared to 23 matched healthy controls. The evaluation of MPAs was carried out using an extended and modified Waldrop-scale (by Méhes) described in detail in our earlier works (Trixler et al. 1997, 2001). The patient sample was divided into two groups: 22 patients with minor physical anomalies (MPA-positive), and 22 patients without MPAs (MPA-negative). The groups were matched according to age, gender and ethnicity. The patients in both schizophrenia groups received antipsychotic medication without significant differences in the duration, and dose regimen. None of them had suffered from acute infections for at least four weeks prior to blood sampling. The patients and controls all gave written informed consent in accordance with the Helsinki Declaration and local ethical requirements.

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From 72-hour lymphocyte cultures, conventionally prepared slides were stained with Giemsa without banding procedures. For inducing breaks parallel cultures were treated for 6 hours with Methotrexate (MTX) in a final concentration of 10^{-5} M (Mondello et al. 1984). From both untreated and MTX-treated preparations 100 mitoses were analysed for chromatid and chromosome breaks. Aberrations were expressed as per cent of cells with one or more aberrations. Parallely prepared lymphocyte cultures of 23 healthy subjects served as controls. The cultures of the normal control subjects were also divided between MTX-treated and untreated preparations. The slides were coded, and the examiners were not aware of the origin of the preparation.

The statistical evaluation of the results were carried out in two steps. First with the use of Kruskal-Wallis test the similarity or difference between the groups were tested, and thereafter the nonparametric Mann-Whitney test was applied.

RESULTS

The karyotype of the subjects proved to be normal in each case. The findings on chromosomal fragility are presented in table 1. The results are grouped in consistent rows; for example, one row with the number of subjects in each group

groups. According to the Mann-Whitney test a statistically highly significant difference exists

Table 1: The distribution of structural aberrations

| <i>n</i> | MPA + (22) | | MPA - (22) | | Contr. (23) | |
|----------|------------|-----|------------|-----|-------------|-----|
| | untreated | MTX | untreated | MTX | untreated | MTX |
| 0 | 2 | - | 8 | 7 | 17 | 10 |
| 1 | 4 | - | 2 | - | 2 | 5 |
| 2 | 2 | 5 | 2 | 4 | 2 | - |
| 3 | 6 | 3 | 4 | 4 | 2 | 1 |
| 4 | - | 2 | 2 | 1 | - | 4 |
| 5 | - | 4 | 1 | 2 | - | 2 |
| 6 | 2 | 1 | - | 1 | - | 1 |
| 7 | - | 1 | 1 | - | - | - |
| 10 | - | - | 2 | 1 | - | - |
| 12 | - | - | - | 1 | - | - |
| 15 | 2 | - | - | - | - | - |
| 20 | 2 | - | - | - | - | - |
| 21 | - | 2 | - | 1 | - | - |
| 22 | 1 | - | - | - | - | - |
| 25 | - | 1 | - | - | - | - |
| 28 | 1 | 1 | - | - | - | - |
| 35 | - | 1 | - | - | - | - |
| 51 | - | 1 | - | - | - | - |

n = number of aberrations/100 mitoses

with no mitoses showing chromosomal aberrations; the next row with the number of subjects in each group with 1 mitosis showing chromosomal aberrations, and so on. The details of statistical analysis are presented on table 2

Table 2: Kruskal-Wallis Test

| Groups | Ranks | | |
|--------|-------|----------|-----------|
| | | <i>N</i> | Mean Rank |
| Untr | MPA + | 22 | 46.27 |
| | MPA - | 22 | 35.77 |
| | Contr | 23 | 20.57 |
| | Total | 67 | |
| MTX | MPA + | 22 | 46.8 |
| | MPA - | 22 | 31.55 |
| | Contr | 23 | 24.11 |
| | Total | 67 | |

| | Test statistics | |
|------------|-----------------|--------|
| | Untr | MTX |
| Chi-square | 21.419 | 16.146 |
| df | 2 | 2 |
| P | .000 | .000 |

| | Frequencies | | | |
|----------|-------------|--------|-------|-------|
| | | Groups | | |
| Untr | | MPA + | MPA - | Contr |
| | > median | 16 | 12 | 4 |
| < median | 6 | 10 | 19 | |
| MTX | | | | |
| | > median | 14 | 7 | 7 |
| < median | 8 | 15 | 16 | |

| | Test statistics | |
|------------|---------------------|--------------------|
| | Untr | MTX |
| N | 67 | 67 |
| median | 1 | 3 |
| chi-square | 14.404 ^a | 6.435 ^b |
| df | 2 | 2 |
| P | .001 | .040 |

(Kruskal-Wallis test), and table 3 (Mann-Whitney test).

The Kruskal-Wallis test demonstrated that there are significant differences between the

- a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected frequency is 10.5
- b. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 9.2

Table 3: Mann-Whitney Test

| MPA + vs Contr Ranks | | | | MPA- vs Contr Ranks | | | | MPA + vs MPA - Ranks | | | | | | |
|----------------------|-------|-----------|--------------|---------------------|------|-----------|--------------|----------------------|--------|-----------|--------------|----|-------|-------|
| Groups | N | Mean Rank | Sum of Ranks | Groups | N | Mean Rank | Sum of Ranks | Groups | N | Mean Rank | Sum of Ranks | | | |
| Untr | MPA+ | 22 | 31.73 | 698 | Untr | MPA- | 22 | 28.32 | 623 | Untr | MPA+ | 22 | 26.05 | 573 |
| | Contr | 23 | 14.65 | 337 | | Contr | 23 | 17.91 | 412-00 | | Contr | 22 | 18.95 | 417 |
| | Total | 45 | | | | Total | 45 | | | | Total | 44 | | |
| MTX | MPA+ | 22 | 30.82 | 678 | MTX | MPA- | 22 | 25.52 | 561.5 | MTX | MPA+ | 22 | 27.48 | 604.5 |
| | Contr | 23 | 15.52 | 357 | | Contr | 23 | 20.59 | 473.5 | | MPA- | 22 | 17.52 | 385.5 |
| | Total | 45 | | | | Total | 45 | | | | Total | 44 | | |
| Test Statistics | | | | Test Statistics | | | | Test Statistics | | | | | | |
| | | Untr | MTX | | | Untr | MTX | | | Untr | MTX | | | |
| Mann-Whitney U | | 61.00 | 81.000 | Mann-Whitney U | | 136.00 | 197.500 | Mann-Whitney U | | 164.000 | 132.000 | | | |
| Wilcoxon W | | 337.00 | 357.000 | Wilcoxon W | | 412.000 | 473.500 | Wilcoxon W | | 417.000 | 385.500 | | | |
| Z | | -4.555 | -3.943 | Z | | -2.925 | -1.299 | Z | | -1.856 | -2.596 | | | |
| P | | .000 | .000 | P | | .003 | .194 | P | | .063 | .009 | | | |

between the MPA-positive and control groups both in the untreated and MTX-treated cells. Between the MPA-negative schizophrenia group and the control group the difference is statistically significant only in the untreated cells. The MPA-positive and MPA-negative patient groups significantly differ from each other in the MTX-treated cells.

Considering the complex statistical analysis our results support a positive association between MPAs and chromosomal fragility. The prevalence of MPAs is in correlation with a higher percentage of chromosomal fragility.

DISCUSSION

The higher prevalence of MPAs is not schizophrenia specific; rather it is indicative of a disturbed early neurodevelopment (Marenco and Weinberger 2000). With the emerging importance of the neurodevelopmental theory of schizophrenia more research has focused on MPAs in schizophrenic populations. The role of genetic and nongenetic factors in disturbed neurodevelopment has not been clarified yet. A significant part of the investigations, such as investigations extended to the family members of schizophrenia patients (Green et al. 1994; Ismail et al. 1998), or the clinical epidemiological study of O'Callaghan et al. (1991) reported a higher MPA prevalence in familial schizophrenia, which support the importance of genetic factors. In contrast, Griffiths et al. (1998) found in sporadic cases a higher MPA prevalence supporting the role of nongenetic influence.

Case reports and surveys in psychiatric wards suggested a higher than usual prevalence of chromosomal aberrations in patients with schizophrenia, and anomalies of the sex chromosomes (DeLisi et al. 1994) and the autosomes (Basset et al. 1992; Nicolson et al. 1999) have been noted. 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 minor physical anomalies (Méhés 2000).

These phenomena are related to chromosomal instability (Méhés 2000) and the increased number of chromatid and chromosome breaks refers to a defect in the chromosome repair mechanism. Data have been published about an increased chromosomal fragility in schizophrenia (Rodduck et al. 1983; Chodirker et al. 1987). Apart from the negative findings of DeLisi et al. (1988) the newer studies supported the earlier data of increased fragility in schizophrenia (Garofalo et al. 1993; Smith et al. 1997; Chen et al. 1998). Gericke et al. (1995) reported an increased fragility in Tourette patients, and increased chromosomal breakage has also been reported in association with childhood autism, (Nunez et al. 2002) Rett-syndrome, (Simonic et al. 1997; Gilberg et al. 1984) malignancy (Nowak et al. 2002). Chromosomal fragility may be connected with the formation of unstable repeat sequences at multiple sites resulting in a continuum of effects. The problem

with phenotype-genotype correlations in complex neuropsychiatric disorders may therefore, be due to the distance between a postulated breakage-enhancing effect of the primary genes and the continuum of diverse phenotypes resulting from secondary-gene involvement at a varying number of fragile sites (Gericke 1995).

Neither an increased number of MPAs, nor chromosomal fragility is schizophrenia specific; both are rather associated with chromosomal instability. According to our findings a significant association exists between the prevalence of MPAs and increased chromosomal fragility which support a genetic determination of MPAs. Recently strong efforts have emerged to define alternative phenotypes for genetic studies in schizophrenia (Tsuang, 2001). MPA and chromosomal fragility may representing features of a schizophrenia endophenotype, which is more directly determined by the genotype than the complex clinical phenotype. Detection of the existence of such type of biological markers in a subpopulation of patients with schizophrenia could be helpful to construct alternative schizophrenia phenotypes more relevant for molecular genetic studies.

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