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Micronucleated lymphocytes in parents of Down syndrome children

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Abstract

Down syndrome (DS) is the most common disease due to an autosomal aneuploidy in live born children and also the major known genetic cause of mental retardation. The risk of a DS pregnancy increases substantially with increasing maternal age. However, several women aged less than 35 years at conception have a child with DS. The micronucleus (MN) assay can identify chromosome breakage or chromosome malsegregation and is an ideal biomarker to investigate genomic instability. The aim of the present study was to determine the frequency of peripheral lymphocytes with MN in the parents of DS individuals. The subjects were 17 couples, 1 father and 9 mothers, and 24 couples who had at least one healthy child formed the control group. For each individual we evaluated the frequency of binucleated micronucleated lymphocytes (BNMN%) as number of binucleated lymphocytes containing one or more MN per 1000 binucleated cells. The mean age of DS parents and controls was 32.6 and 29.8 years, respectively. The frequency of MN in DS parents was significantly higher compared to controls. The higher frequency of MN in DS parents suggests a higher predisposition of DS parents to aneuploidy events in this sample.

Key words: Micronuclei; Parents of Down syndrome children; Chromosomal nondisjunction; Down syndrome

Introduction

Down syndrome (DS) is the most common disease due to an autosomal aneuploidy in live born children and the major known genetic cause of mental retardation. DS results from an extra chromosome 21 as the result of a nondisjunction in approximately 95% of cases during maternal meiosis I (1,2).

The risk of a DS pregnancy is a function of maternal age, and after age 35 years it increases substantially with increasing maternal age (3,4). However, women aged less than 35 years at conception have had children with DS, suggesting a predisposition to early abnormal chromosome segregation events in these women (5,6).

Indeed, an increased meiotic nondisjunction in parents of trisomic children and in couples with recurrent abortions has been demonstrated, supporting the hypothesis that errors of chromosome segregation may be due to spindle defects, to chromosome breakage, or to abnormal chromosome associations (7-9).

Although many specific tests exist that allow the detection of loss or gain of whole chromosomes (10,11), there is an apparent lack of reports on the evaluation of a possible predisposition to nondisjunction processes (9).

The cytokinesis-block micronucleus assay is a widely used technique for measuring DNA damage in human populations. The micronucleus (MN) test is an efficient assay of chromosomal instability, chromosome malsegregation, DNA hypomethylation, DNA misrepair, chromosome breaks, and asymmetrical rearrangement. Thus, MN should be ideal biomarkers for the investigation of genomic instability (12,13).

Micronuclei are acentric chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division and are excluded from the nucleus (14). Different mechanisms may be involved in the formation of MN (15), including chromosome breakage, due to unrepaired or misrepaired DNA lesions, or chromosome loss and nondisjunction due to mitotic malfunction (16,17). The objective of the present study was to examine the frequency of lymphocytes with MN in parents of DS individuals.

Subjects and Methods

Peripheral blood samples were collected from 17 couples, 1 father and 9 mothers who had a DS child with

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karyotypically confirmed free trisomy 21. Two couples who had two DS children confirmed by free trisomy 21 by karyotype were also included in this study group. Couples with a DS child whose karyotype resulted from mosaicism or translocation were excluded from the present investigation. The mean age of parents with DS children was 32.6 years, 31.3 years for mothers of DS children and 34 years for fathers of DS children. The control group consisted of 24 couples who had at least one healthy child before 35 years, and no experience of miscarriages, abnormal pregnancies or children affected by genetic disorders in their life. The mean age of control subjects was 29.8 years and the mean maternal and paternal ages were 28.2 and 31.4 years, respectively. The study was approved by the Ethics Committee of Universidade Federal do Triângulo Mineiro (CEP/UFTM, No. 1023) and written informed consent was obtained from all subjects before participation.

Micronucleated lymphocyte assay

The MN assay was performed by the method of Fenech (14). Two paired independent lymphocyte cultures were prepared for each subject. The medium used was RPMI 1640 (Gibco BRL, Brazil) supplemented with 20% fetal bovine serum (Gibco BRL) and 4% phytohemagglutinin. Cytochalasin B (Sigma, USA) at a final concentration of 6 µg/mL, was added to each tube 44 h after the beginning of the cultures in order to block the cytokinesis of dividing cells. The lymphocyte cultures were harvested after 72 h. Cells were then treated with a hypotonic solution (1% sodium citrate) and fixed in 3:1 methanol:acetic acid solution. The cell suspension was dropped onto clean slides and stained with Giemsa. Two thousand binucleated cells were examined for each individual and we evaluated the frequency of binucleated micronucleated lymphocytes (BNMN%) as the number of binucleated lymphocytes containing one or more MN per 1000 binucleated cells. The ratio of the percentage of binucleated cells to the total cells scored was also evaluated (5,14).

The chi-square test was used to assess the significance of the presence of MN in couples with a DS child. Differences between groups were considered to be statistically significant if $P < 0.05$.

Results

The frequency of MN in the lymphocytes of the 17 couples, 1 father and 9 mothers with a DS child and of the control group of 24 couples is shown in Table 1. The frequency of MN in mothers and fathers with a DS child was clearly higher than in the control group. DS fathers and DS mothers had similar values (Figure 1).

The mean MN number was 18.05 (range: 3-77) in DS fathers and 17.92 (range: 3.5-82) in DS mothers. In the control group, the mean MN number was 4.73 (range: 1.5-12.5) in fathers and 4.44 (range: 1-10) in mothers. Figures 2 and 3 represent the individual variability in DS fathers and control fathers, and DS mothers and control mothers, respectively. The two couples with two DS children had a higher MN number than the parents with one DS child; the first couple had 29 and 33 MN and the second 33 and 24 MN for mother and father, respectively.

Table 1. Micronucleus (MN) frequency in 1000 binucleated (BN) cells from parents of Down syndrome (DS) children and controls.

	BN cells	MN	%	MN total	%
DS mothers	26,000	466	1.79	793	1.81
DS fathers	18,000	327	1.81		
Control mothers	24,000	107	0.44	220	0.46
Control fathers	24,000	114	0.47		

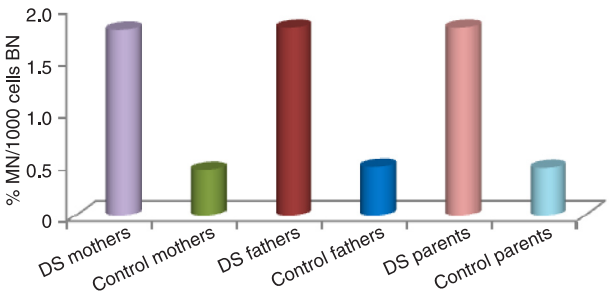

Figure 1. Frequency of micronuclei (MN) in 1000 binucleated (BN) cells from parents of Down syndrome (DS) children and controls.

The median MN number in binucleated lymphocytes was calculated based on control group data. Considering that the median found was six, the MN number of parents with a DS child was clearly higher compared to controls ($\chi^2 = 26.083$; $P < 0.0001$). In addition, MN number was higher in mothers of DS children compared to control mothers ($\chi^2 = 17.045$, $P < 0.0001$), and the same was observed for fathers ($\chi^2 = 9.058$, $P < 0.0026$; Table 2).

Discussion

Down syndrome is the most common human aneuploid condition. Several reports about this question associate nondisjunction with increased maternal age, which is the only incontrovertible factor known to be involved in the DS (7,9,18). However, genetic factors may be involved in the chromosome nondisjunction causing aneuploidy since young parents can have DS progeny, a fact indicating susceptibility to abnormal chromosome segregation in these cases.

The results of the present study suggest that parents of DS children have a significant increase in MN frequency

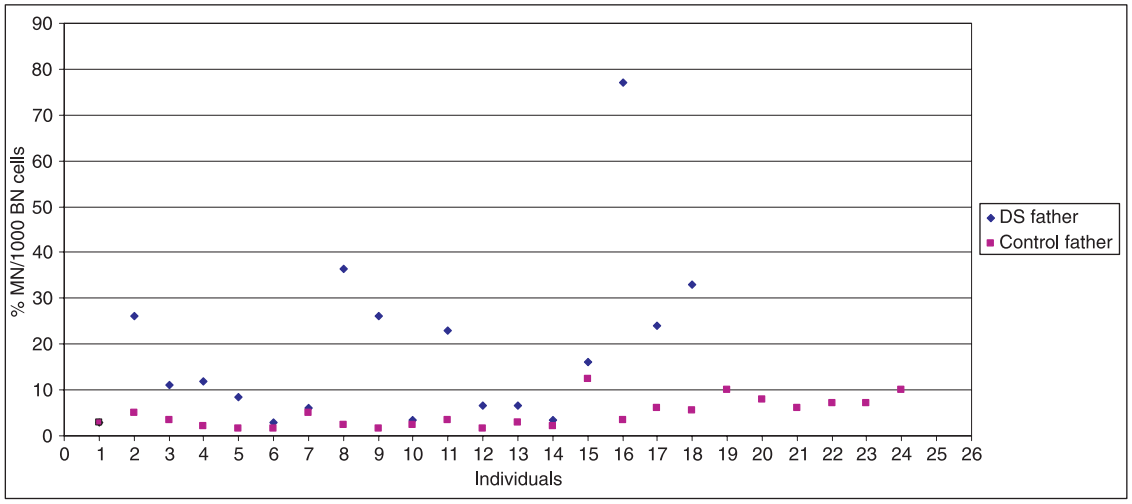

Figure 2. Number of micronuclei (MN) per 1000 binucleated (BN) cells of Down syndrome (DS) fathers and control fathers.

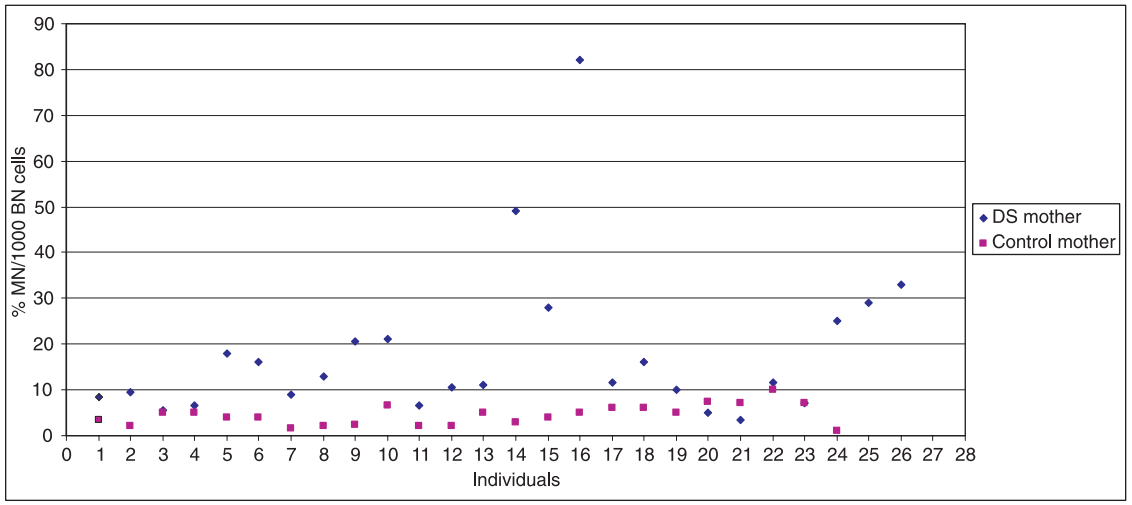

Figure 3. Number of micronuclei (MN) per 1000 binucleated (BN) cells of Down syndrome (DS) mothers and control mothers.

Table 2. Number of mothers and fathers with a Down syndrome (DS) child and control parents according to median micronucleus number.

	Median micronucleus number		χ^2	P	Total (%)*
	≥6 (%)	<6 (%)			
DS mother	23 (88.5)	3 (29.2)	17.045	<0.0001	26 (100)
Control mother	7 (29.2)	17 (70.8)			24 (100)
DS father	14 (77.8)	4 (22.2)	9.058	<0.0026	18 (100)
Control father	8 (33.3)	16 (66.7)			24 (100)

Data are reported as number of individuals with percent in parentheses. *χ^2 = 26.083; P < 0.0001.

in binucleated lymphocytes. This study differs from other studies in that it analyses both mothers and fathers of Down syndrome babies. Migliore et al. (6) observed a significantly increased frequency of binucleated cells with micronuclei in a group of women who had a DS child at a young age compared to a control group (mothers who had a healthy child), suggesting that young mothers have a generalized tendency to chromosome malsegregation events. The susceptibility to abnormal segregation was observed in young women during MN studies using probes 13 and 21 (5).

DS individuals have higher frequencies of MN compared to the group of parents of DS children and to the control group. Both DS individuals and parents of DS children exhibit higher frequencies of MN induced by genotoxicants compared to controls (9), showing genomic instability, as observed in the present study. The increased MN frequency is a sign of cytogenetic damage, probably associated with spindle disruption leading to nondisjunction. There are several possible molecular mechanisms causing abnormal chromosome segregation that result in MN formation. One of these mechanisms is related to hypomethylation of cytosine in centromeric and pericentromeric repetitive sequences. It is possible that mutations leading to defects in the dynamics of interaction between kinetochore and microtubules could also be a cause of MN formation due to chromosome loss in anaphase (19). The persistence of abnormal microtubule attachment to kinetochores is the main mechanism for chromosomal instability (20). Folate deficiency can lead to heterochromatin demethylation causing defects in the structure of the centromere, which could induce an abnormal distribution of replicated chromosomes during nuclear division (21,22). However, Wang et al. (23) observed that there seems to be no strong relationship between methylation status of folate and aneuploidy rate.

Considering that MN is a biomarker of chromosome breakage and/or whole chromosome loss, an increased frequency of MN in parents of a DS child suggests a higher predisposition to aneuploidy.

Not only maternal aging is involved in Down syndrome, but biochemical pathways could also promote maternal and paternal meiotic nondisjunction and the risk of having a DS child. In chromosome segregation, an important role should be attributed to the microtubular system. A misregulation of the microtubule assembly could be implicated in an increased risk of aneuploidy (6). Ford (24) proposed that a mechanism controlling microtubular polymerization and/or alteration in the microtubular structures is responsible for the formation of trisomic cells during mitosis and meiosis. In fact, the structural integrity of the microtubule spindle apparatus is fundamental for the correct attachment to kinetochores and is also crucial for chromosome segregation fidelity. It is known that failure of any of these processes is related to a high probability of whole chromosome nondisjunction. In addition, the reduction of folate intake by parents and maternal grandmothers seems to be relevant in chromosome nondisjunction and consequently could be associated with a higher risk of DS progeny (6,25). Beetstra et al. (26) observed that folate deficiency or inherited defects in folate metabolism may lead to increased chromosome 21 mosaicism *in vivo* during the fetal stage.

There is no evidence for a paternal age effect on nondisjunction of chromosome 21; therefore, identification of risk factors related to recombination remains inconclusive (27). In fact, despite the large number of studies about DS and maternal risk, little is known about the association of DS with paternal nondisjunction. Extrinsic and intrinsic risk factors regarding nondisjunction, recombination and segregation are still unclear.

In the present study, we observed an increased frequency of MN in fathers and mothers of DS children. Since MN is considered to be a biomarker of chromosome breakage and/or chromosome loss, the increase of MN in parents of DS children suggests that these individuals seem to have a predisposition to chromosomal nondisjunction.

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