Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Buccal micronucleus frequency is associated with age in Down syndrome

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Genet. Mol. Res. 8 (4): 1231-1237 (2009)
Received May 14, 2009
Accepted June 18, 2009
Published October 13, 2009

ABSTRACT. Down syndrome has been linked to premature aging and genomic instability. We examined the frequency of micronucleus (MN) and binucleated cells in the oral mucosa of Down syndrome patients and healthy controls matched by age and gender, addressing the effect of age and family income. Down syndrome individuals had an increased number of MN (14.30 ± 9.35 *vs* 4.03 ± 1.71; $P < 0.001$) and binucleated cells (0.97 ± 1.3 *vs* 0.33 ± 0.66; $P < 0.05$) per 2000 cells. Micronucleus frequency of Down syndrome individuals correlated positively with age ($r = 0.437$; $P = 0.009$), and the older (≥21) Down syndrome age group (30.8 ± 8.4 years old) had about 2-fold more micronuclei ($P \leq 0.05$) than did the younger group (<21). Average family income did not correlate with MN frequency in controls ($r = -0.948$; $P = 0.183$), but a borderline negative correlation was seen in DS subjects ($r = -0.9484$; $P = 0.0516$). Individuals whose average income was ten times minimum wages had about 2-fold less MN than those receiving around minimum wage. We conclude that the buccal MN assay is a useful and minimally invasive method for monitoring genetic damage in humans and could be used as a tool to evaluate age-associated genomic instability in Down syndrome.

Key words: Genomic instability; Down syndrome; Micronucleus; Buccal cells

INTRODUCTION

Down syndrome (DS) is by far the most common genetic syndrome of chromosomal origin (Caria et al., 1997). DS affects up to 1 in 800 live births (Migliore et al., 2006), has an estimated perinatal mortality rate of 30% in developing countries (Pedrollo et al., 1999) and is associated with a decreased life expectancy (Leonard et al., 2000). Besides mental retardation and several congenital malformations, DS individuals show increased risk of malignant disease and have symptoms of premature aging (e.g., sensory, motor, cutaneous, biochemical, and neurological alterations) (Lott and Head, 2005), including increased oxidative stress markers (Garcez et al., 2005), genome instability (Franceschi et al., 1992; Thomas et al., 2008), apoptosis misregulation (Anderson et al., 2000), cancer (Sullivan et al., 2007), and early progression of Alzheimer's disease (AD) (Brugge et al., 1994).

The level of genome instability depends on three groups or factors: the environment, genetics and age. Older individuals typically show more DNA damage than the younger ones (Fenech, 1998). Environmental pollutants and occupational factors can increase genomic instability and cancer risk (Ames, 1989). A socioeconomic gradient is seen for many genetic diseases such as teratogenesis (Yang et al., 2008). However, there is a lack of information about the effect of this socioeconomic gradient at the DNA level. Therefore, we aimed in this study to evaluate the frequency of micronucleus (MN) in exfoliated buccal cells, a useful and minimally invasive method for monitoring genetic damage in humans (Holland et al., 2008), in DS carriers and healthy controls matched by age and gender, addressing the effect of age and family income.

MATERIAL AND METHODS

Approval for this study was obtained from the Catholic University of Pelotas Ethics Committee. All individuals or their relatives signed a post-informed consent before inclusion in the study. Thirty randomly selected DS carriers and thirty healthy controls were selected according to gender and age intervals (≤10 years of age, 11-20 years of age and ≥21 years of age). Each age interval consisted of five females and five males for either controls or DS carriers. Demographic characteristics of the groups are shown in Table 1.

Table 1. Age and gender of subjects studied.

	Controls	Down's syndrome
Age (years)	16.80 ± 11.90	17.07 ± 12.28
Gender		
Male	15	15
Female	15	15
Family income (minimum wages)	3.83 ± 3.39	4.23 ± 3.02

Data are reported as means ± SD for 30 individuals in each group.

The medical records of the DS carriers were collected using information belonging to the Monitoring Program of Congenital Malformations of Pelotas, RS, Brazil. Only DS carriers with free trisomy were included in the study.

Cell sampling, slide preparation and scoring

Collection, staining and analysis of the micronuclei were performed as described else-

where (Roth et al., 2008). Briefly, an oral mucosa scraping was carried out to collect exfoliated cells using a wooden, water-soaked tongue depressor. The cells were then transferred to two clean slides which, after drying, were placed in an oven for 30 min and then fixed with methanol and stained with Schiff reagent and fast green according to Stich et al. (1985). Two thousand cells were scored per individual under 1000X magnification. The slides were coded and the same scorer analyzed all slides. The criteria of Tolbert et al. (1992) were used for analyses. Only cells that were not fragmented, heaped or overlapping and with their nuclei intact and not karyorrhectic or karyolitic were analyzed. The micronucleus definition of Picker and Fox (1986) was used. Results are reported as the number of micronucleated or binucleated cells per 2000 cells ± standard deviation.

Statistical analysis

Statistical analysis was carried out using the Mann-Whitney U-test to compare the frequency of MN cells between controls and DS and between gender. The Kruskal-Wallis test, followed by the Dunn multiple comparison test, was used to compare the frequency of MN cells between age groups. Pearson's correlation analysis was used to evaluate the association between the age or average family income and the frequency of MN cells in controls and DS. Values are reported as means ± standard deviation. The level of significance was set at $P \leq 0.05$.

RESULTS

Micronucleus frequency was significantly higher in DS than controls (about 3.5-fold; $P < 0.001$) and did not differ significantly between gender either in control or DS individuals (Figure 1A). Binucleated (BN) cells were significantly higher in DS than controls only among females (about 3.25-fold; $P < 0.05$) and among overall subjects (about 2.5-fold; $P < 0.05$), and did not differ significantly among males (Figure 1B).

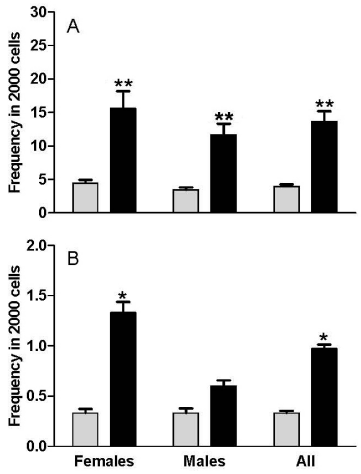

Figure 1. Frequency of micronucleated (A) and binucleated (B) cells in buccal cells of controls (grey) and Down syndrome individuals (black) according to gender (15 male and 15 female controls or Down syndrome carriers). Statistical significance according to the Mann-Whitney U-test at *$P \leq 0.05$ or at **$P \leq 0.001$.

Micronucleus frequency was not associated with aging in controls, regarding either age group analyses (P = 0.0721, Kruskal-Wallis; Figure 2Ai) or Pearson's correlation analyses (r = 0.336; P = 0.069). For DS carriers, a significant positive correlation (r = 0.437; P = 0.009) was observed between age and MN frequency, and the older age group (30.8 ± 8.4 years old) showed about 2-fold more MN ($P \leq 0.05$) than the younger DS groups (Figure 2Aii). Regarding BN cells, a different pattern was seen between controls and DS individuals, although without statistical significance (Figure 2B).

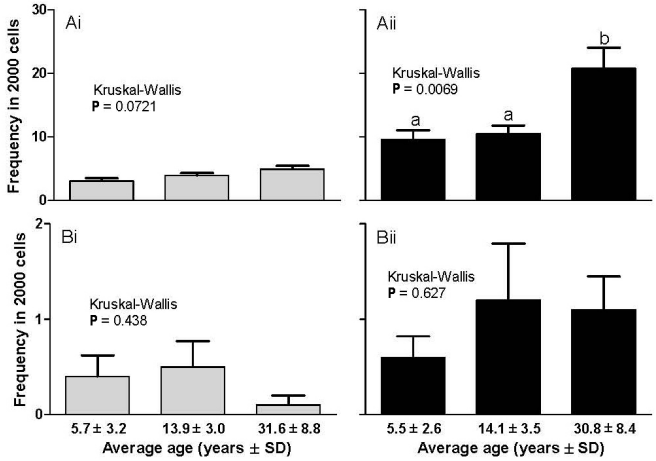

Figure 2. Frequency of micronucleated (A) and binucleated (B) cells in buccal cells of controls (i) and Down syndrome individuals (ii), according to age intervals (10 controls or Down syndrome carriers per age interval). Groups sharing the same letters do not differ according to the Kruskal-Wallis test followed by the Dunn multiple comparison test.

Average family income did not correlate with MN frequency in controls (r = -0.948; P = 0.183), but a borderline negative correlation was seen in DS subjects (r = -0.9484; P = 0.0516). Individuals whose average income was 10 times minimum wages had about 2-fold less MN than those receiving around minimum wage, although not significantly. No association between the frequency of BN cells and income was seen.

DISCUSSION

In the present study, DS individuals showed between 2.7- and 4.4-fold more MN than controls, depending on age and up to 3.25-fold more BN cells, according to gender (Figures 1 and 2).

DS is associated with lower DNA repair efficiency and an accelerated decline in DNA repair capacity with age (Raji and Rao, 1998). Genomic instability is one of the major causes of aging (Busuttil et al., 2004). DS shares several clinical signs with other premature-aging syndromes, such as the Werner and Cockaine syndromes and ataxia telangiectasia, and particularly an increased risk of malignancy (Maluf and Erdtmann, 2001).

Another feature of Down syndrome is impaired cell proliferation, which can be noticed as an increased frequency of BN cells observed in the study. It has been hypothesized that cells that fail to complete cytokinesis show a higher frequency of nondisjunction than healthy counterparts (Shi and King, 2005), indicating that the frequency of BN cells could be

used as a marker of aneuploidy risk (Thomas et al., 2008). We lack an explanation why only females with DS showed higher BN cell frequency.

In agreement with the present results, Thomas et al. (2008) showed that MN frequency in the buccal cells of DS individuals (22.5 ± 2.2 years old) was about 7-fold higher than that of younger healthy controls (10.4 ± 5.8 years old) and about 2 times higher than in older controls (67.1 ± 2.6 years old). On the other hand, Scarfì et al. (1990) observed a generally lower frequency of MN in cytokinesis-blocked lymphocytes of DS subjects than controls, but observed that mitomycin-C was able to cause more DNA damage in cells from older than younger DS subjects (Scarfi et al., 1990). In another study, Maluf and Erdtmann (2001) found no significant difference between the DS subjects and controls, as also evaluated by MN frequency in cytokinesis-blocked lymphocytes. Two explanations would be differences in metabolism and/or apoptosis levels between buccal cells and lymphocytes. Accordingly, it has already been demonstrated that different cell lines respond differently in terms of micronucleus induction and apoptosis in response to genotoxic insults (Simko et al., 1998). Notwithstanding, further research is still needed to understand the cell dynamics in the oral mucosa and its relation to DNA damage end-points in this test model (Holland et al., 2008; Thomas et al., 2008). The analysis of the different cell populations of the oral mucosa proposed in the cytome version of the oral MN assay by Thomas et al. (2008) would be an alternative. Such parameters were not included in this paper because data analysis was performed before the publication of the cytome protocol.

There are genetic, biochemical, neuropathological, and oxidative stress analogies between DS and AD (Zana et al., 2007). In the present study, DS individuals aged ≥21 (average 30.8 ± 8.4 years old) showed 2-fold more micronucleus than the younger DS carriers (Figure 2). In fact, virtually all individuals with DS develop sufficient neuropathology for a diagnosis of AD by the age of 40 years, and the accumulation of β-amyloid plaques and neurofibrillary tangles has been reported in DS children as young as 8 years old (Lott and Head, 2005). Several epidemiological studies have indicated an increased frequency of AD in families with DS subjects (Lott and Head, 2005). Other studies have shown that chromosome 21 aneuploidy is increased about 2-fold in AD (Migliore et al., 1999; Thomas and Fenech, 2008). Mutations in genes mapped to chromosome 21 are also linked to AD risk (Thomas and Fenech, 2007), such as genes coding for antioxidant enzymes, several genes related to AD (Kimura et al., 2007), some genes involved in folate and methyl group metabolism (Migliore et al., 2006), and other genes of DNA repair and synthesis (Caria et al., 1997). Considering the parallel between the increase in MN frequency and early Alzheimer symptoms, one could hypothesize that the increase in MN frequency observed could be related to early progression of AD in the DS individuals evaluated, which warrants further study. Given this fact, alterations in the cell kinetics or structural profile of the buccal mucosa, including MN frequency changes, have been shown in AD (Migliore et al., 1997; Trippi et al., 2001; Petrozzi et al., 2002; Thomas et al., 2007), and may be useful as potential biomarkers in identifying individuals with a high risk of developing AD (Thomas et al., 2007). Therefore, the possibility to apply the MN assay in the detection of AD risk, opening a new application for the MN assay, has already been suggested for identifying individuals with high risk of cancer (Bonassi et al., 2007).

The stepwise decrease in MN frequency in parallel to family income increase has not yet been reported in the literature. Notwithstanding, we (Friedrisch et al., 2008) and others (Betancourt et al., 1995; Ortiz et al., 1995) have already reported that nutrition factors can affect genomic stability either in diseased or healthy states. Antioxidant and folic acid deficiency has been extensively shown to increase DNA damage in children and adults. Polymorphisms in

folic acid metabolism genes in mothers have been shown to increase the risk of DS in offspring (Coppede et al., 2009). Folic acid deficiency is the leading cause of neural tube defects during embryogenesis (Fenech, 2005). In favor to this hypothesis, there is consistent evidence of neural tube defects in populations with lower socioeconomic status as measured by education, occupation and income (Yang et al., 2008), indicating that the socioeconomic gradient seen for many diseases (Adler and Ostrove, 1999) could be demonstrated at the DNA level. Moreover, the present results are "tuned" to the concept of Zana et al. (2007) that "a balanced up-regulation of endogenous antioxidants, together with multiple exogenous antioxidant supplementation, may be expected to be one of the most promising treatment methods for DS". Therefore, further studies should be performed to better understand the relation between income and genomic stability, which could play an important role in increasing the life expectancy of DS individuals.

ACKNOWLEDGMENTS

The authors are very grateful to the DS individuals and to the control subjects, who spontaneously took part in this study. Research supported by the Catholic University of Pelotas.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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