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ALTERATIONS OF ACROCENTRIC CHROMOSOMES IN PERIPHERAL BLOOD LYMPHOCYTES IN PATIENTS WITH ALZHEIMER'S DISEASE

LADA ŽIVKOVIĆ[1], V. P. BAJIĆ[2], DIJANA ŽUKOVEC[1], ANDREA ČABARKAPA[1] and BILJANA SPREMO-POTPAREVIĆ[1]

[1] *Institute of Physiology, Department of Biology and Human Genetics, Faculty of Pharmacy, University of Belgrade,* 11000 Belgrade, Serbia
[2] *Institute of Biomedical Research „Galenika",* 11000 Belgrade, Serbia

Abstract - While Alzheimer's disease (AD) is primarily considered to be a neurodegenerative disorder, there is much evidence supporting the idea that it is also a systematic disease, so the occurrence of genomic instability in cells that are not neurons is equally important. Accordingly, acrocentric chromosomes play an important role in the etiology of AD, especially the presence of trisomy 21. The aim of this work was to assess the relationship between the frequency of acrocentrics included in satellite association (SA) and acrocentrics with premature centromere division (PCD) in the peripheral blood lymphocytes of AD patients and age-matched controls. Since our results showed that there are significant differences in SA frequency, as well as in the frequency of PCD in acrocentrics between AD patients and control group, we may conclude that the occurrence of acrocentric chromosome alterations presented in this study could be related to the etiology of AD.

Key words: Satellite association, premature centromere division, Alzheimer's disease

INTRODUCTION

Alzheimer's disease (AD) is a complex progressive disorder and the most common form of dementia where both genetic and environmental factors play a role in the onset of the disease (Burns et al., 2001). The majority of *cases* of Alzheimer's disease are *sporadic* forms (more than 80%) with an unknown etiology (Cruts et al., 1996; Blacker and Tanzi, 1998). Cytogenetic studies have shown that in AD there is an increased propensity to genome instability events, observed not only in the neurons of AD patients but in other cell types as well (Thomas and Fenech, 2008; Bajic et al., 2009; Moh et al., 2011). Chromosome instability, including aneuploidy, is considered as one of the mechanisms of ageing at the genomic level and was detected in neurons of the central nervous system (Yang et al., 2001; Yurov et al., 2007; Finkel et al., 2007; Spremo-Potparevic et al., 2008;) as well as in peripheral tissues of AD (Zivkovic et al., 2010; Spremo-Potparevic et al., 2004).

There are five pairs of acrocentric chromosomes in the human karyotype (pairs 13, 14 and 15 of D chromosome group and pairs 21 and 22 of G chromosome group). The Nuclear Organizer Region (NOR) represents the site of ribosomal RNA genes located at the secondary constrictions of the acrocentric chromosomes. The short arms of these chromosomes are often located in a mutual aggregation, called a satellite association (SA) (Miller, 1977). *The frequency* of human *acrocentric* chromosome *associations* in human peripheral blood lymphocytes may change with ageing (Miller, 1977; Sigmund et al., 1979). In addition, a significant decrease in the frequency of SA in AD patients when compared to age-matched

controls has been reported (Payao et al., 1994), but not always confirmed (Migliore et al., 1997).

There are numerous evidences that the supernumerary acrocentric chromosome 21 in Down Syndrome (DS) is involved in AD etiology in these patients (St. George Hyslop et al., 1987; Cruts et al., 1996; Geller and Potter, 1999). Two characteristic pathological structures are present in the brain of AD patients: the development of large numbers of neurofibrillary tangles and amyloid-based neuritic plaques. Amyloid plaques consist of β amyloid protein whose gene is located on chromosome 21q21 (Koo, 2002). Older individuals with DS develop an increased deposition of the ß amyloid protein in the brain as observed in AD patients (Holtzman and Deshmukh, 1997; Geller and Potter, 1999). The discovery that the APP gene is located on the 21st chromosome and the fact that individuals with DS develop a clinical syndrome of dementia with neuropathological characteristics almost identical to those of AD, led to the idea that trisomy 21 in DS is implicated in AD etiology in these patients (St. George Hyslop et al. 1987; Cruts et al. 1996; Geller and Potter, 1999).

The other chromosomal alteration called premature centromere division (PCD) is a phenomenon of loss of control over sequential separation and segregation of chromosome centromeres, characterized by the distinctive separation of chromosomes earlier than usual. PCD as a potential cause of improper chromosome segregation and aneuploidy has been correlated with age (Fitzgerald et al., 1986; Mehes and Buhler, 1995), but our previous data suggest that the increased frequency of PCD in the brain and peripheral tissues is a feature of AD, rather than an epiphenomenon of chronological ageing *per se* (Živković et al., 2010). Cytogenetic analysis of metaphases (Spremo-Potparević et al., 2004; Migliore et al., 1997; Migliore et al., 1999; Živković et al., 2010) and FISH analysis of interphase nuclei (Spremo-Potparević et al., 2004, Živković et al., 2006) showed increased aneuploidy and relevant expression of PCD in lymphocytes in sporadic forms of AD in comparison to controls.

Considering the overlap of some alterations that occurs during normal chronological ageing with those found in sporadic forms of AD, as well as the importance of acrocentrics in the etiology of AD, the aim of this work was to assess the number of metaphases with satellite association (SA) in peripheral blood lymphocytes of AD females and female age-matched controls, and the number of metaphases with at least one acrocentric chromosome showing PCD phenomenon in the peripheral blood lymphocytes of AD females and female age-matched controls.

MATERIALS AND METHODS

Participants

The subjects included in this study were twelve sporadic Alzheimer's disease (SAD) females and twelve elderly female age-matched controls. The *study was approved by* the Medical School Ethics Committee, University of Belgrade, and written informed consent was obtained from all participants or from their family members (2492/1). Diagnosis was based on the National Institute of Neurology criteria. Elderly female subjects without a history of neurological disorders were selected as a control group. The individuals in the two groups were matched with respect to age (± 3 years). This study was *exclusively conducted* on female *participants* because it is well known that the sporadic form of AD is more prevalent in women than in men (Henderson et al., 2000). Donors were non-smokers and had not used medication for at least 3 months before the investigations were performed. The age range of the AD participants was 60-80 years (mean 69.75±7.66), with an average *duration* of *dementia* of 2.8±0.8 years. The elderly controls' age range was 59-79 years (mean 68.92±8.16).

Blood culture

Blood samples were collected by venipuncture from the participants. Peripheral blood lymphocyte culture stimulation, cell harvests, and slide preparations were performed as previously described (Evans and O'Riordan, 1975). Briefly, heparinized whole blood

Fig. 1. G-banded metaphase chromosomes of an AD female showing satellite association

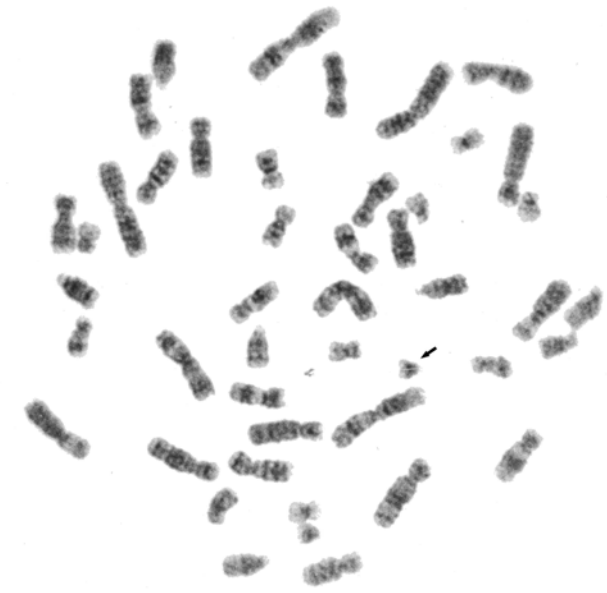

Fig. 2. G-banded metaphase chromosomes of an AD female with represented premature centromere division (PCD) on one chromosome 21. The other chromosomes have undivided centromeres

samples (0.8 mL) were added to vials with 9.2 mL RPMI 1640 medium (PAA Laboratories, Pasching, Austria), supplemented with 10% fetal calf serum (PAA Laboratories, Pasching, Austria), 5 μg/mL phytohemagglutinin (PAA Laboratories, Pasching, Austria), and a 1% cocktail of the antibiotics penicillin/streptomycin (Bio Whitaker, Barcelona, Spain). Cultures were incubated at 37°C for 72 h, and 2 h before the cultures were harvested, 0.1 μg/mL colcemid (PAA Laboratories, Pasching, Austria) was added to the media. Cells were treated with hypotonic solution (20 min), fixed in 3:1 methanol/acetic acid (3 X 20 min), and then the cell suspension was dropped onto wet, cold and grease-free microscope slides, and air-dried over a flame. The slides were aged for the next 5-7 days and then stained *using the G-banding technique (*Seabright, 1971*)* to identify and verify PCD in the chromosomes.

PCD and SA counting

The following parameters were recorded: the number of metaphases with SA, the total number of acrocentric chromosomes in SA, the total number of chromosomes 21 involved in SA, the number of metaphases with at least one acrocentric chromosome with PCD, the total number of acrocentric chromosomes with PCD and the total number of chromosome 21 with PCD. For each sample, 100 metaphase cells were analyzed. Slides were examined using an Olympus BX 50 microscope (Olympus Optical Co., GmbH, Hamburg, Germany) under oil immersion.

PCD was characterized by the distinctive separation of chromosome chromatids in regard to the majority of chromosomes in the same metaphase cell with undivided centromeres. Satellite association (SA) presents two or more chromosomes whose distance between the satellite ends is not larger than the chromatid width of each chromosome.

Data analyses and statistics

The data presents PCD frequency and SA frequency in peripheral blood lymphocytes in groups of AD patients and elderly healthy controls. Results were analyzed by using the Student's t-test. The correlation

between the frequencies of PCD and SA was tested in both AD and control patients by the Spearman's nonparametric test. $P < 0.05$ was considered as statistically significant. Statistical software GraphPad Prism (Version 5.0) was used.

RESULTS AND DISCUSSION

This study was conducted on human peripheral blood lymphocytes of AD females and age-matched healthy female controls. The examined AD patients were diagnosed with the sporadic form of the disease. The demographics and clinical characteristics of the groups are presented in Table 1.

The average frequencies of recorded parameters in the lymphocytes of both elderly controls and the AD group are reported in Table 2. Regarding these *parameters,* we established a significant decrease ($p<0.05$) in the frequency of metaphases with SA, total acrocentric chromosomes in SA and total chromosomes 21 involved in the SA in AD patients when compared to those of controls (Fig. 1).

On the other hand, the AD patients showed a significantly higher frequency of metaphases with PCD on acrocentrics, total number of PCD on acrocentrics and total number of PCD on chromosome 21 than those in the elderly control group (Fig. 2).

In both the AD group and controls the occurrence of PCD in metaphases was not related to the acrocentrics that were in the satellite association. Furthermore, no negative correlation was found between the frequencies of PCD and SA in any of the examined groups (data not shown).

Previous findings from cytogenetic studies performed to assess SA in the cells of AD patients have been controversial. Our data comply with the results of Payao et al. (1994) who showed a significant decrease in the frequency of SA in relation to the chromosome pair 21 in AD patients when compared with the elderly and young control groups. On the contrary, a study of Migliore et al. (1997) found a statistically non-significant decrease of SA in the metaphases of AD patients when compared with controls. In addition, in our results the SA frequency showed a statistically significant difference between the AD and control groups, which is opposite to that of Dönmez-Altuntaş et al. (2005). What distinguishes our findings from the above-mentioned results of Dönmez-Altuntaş et al. (2005) is a slight increase in the total number of acrocentrics with SA in AD patients when compared to the elderly controls.

Although there are certain discrepancies in the results on SA frequencies across different studies, there is a good concordance in the reports on PCD frequencies in AD. This study showed a significantly higher frequency of PCD on acrocentrics in AD patients when compared with age-matched unaffected controls, and is in agreement with the results of Migliore et al. (1997).

The ribosomal RNA genes are located within the nucleolus during active transcription (Wachtler et al., 1991). The nucleolus is the ribosome factory of the cells. This is the nuclear domain where ribosomal RNAs are synthesized, processed, and assembled with ribosomal proteins (Hernandez-Verdun et al., 2010). Ribosomal RNA genes located in the secondary constriction of acrocentrics can modulate gene expression (Payao et al., 1998; da Silva et al., 2000). Payao et al. (1994) suggested that the significantly lower frequency of silver staining and SA in AD is a consequence of a reduction in the amount and activity of ribosomal genes in acrocentrics. On the other hand, the localization of genes at the nuclear and nucleolar peripheries is associated with transcriptional repression (Fedoriw et al., 2012). Linkage is assumed between rDNA copy number, transcriptional differences as a mode of regulation, nucleolar formation and preferential association between some chromosomes (McDowell et al., 1994, Fedoriw et al., 2012). The control of nucleolar assembly is presented as well as the role of pre-existing machineries and pre-rRNAs inherited from the previous cell cycle (Hernandez-Verdun et al., 2010).

Markedly, increased PCD of acrocentrics in AD are in accordance with the well-established occur-

Table 1. General characteristics of Alzheimer's disease (AD) and control subjects. Values are means±SEM or min-max (n=12/group)

	AD	Controls
Average age (yr)	69.75±7.66	68.92±8.16
Age range (yr)	60-80	59-80
Average duration of dementia (yr)	2.8±0.8	/

Table 2. Satellite association (SA) and premature centromere division (PCD) from AD patients and elderly controls. In each individual 100 metaphases of peripheral blood lymphocytes were analyzed

	AD patients	Controls
No. of chromosome 21 in SA	13.83±3.13*	16.25±2.53
No. of total acrocentrics in SA	50.25±7.82*	58.33±6.22
No. of metaphase with SA	19.42±4.12*	23.92±6.65
No. of chromosome 21 showing PCD	1.5±1.06*	0.17±0.37
No. of PCD on acrocentrics	3.33±3.13*	0.33±0.45
No. of metaphase with at least one acrocentric with PCD	2.83±2.15*	0.25±0.35

*$P<0.05$ vs controls

rence of aneuploidies of chromosomes 21 and 13 in AD patients (Migliore et al., 1999; Boeras et al., 2008). In AD patients, aneuploidy of chromosome 21 is more frequent than that of chromosome 13 (Migliore et al., 1999). Replication, separation, and segregation of human chromosomes are highly controlled processes through the cell cycle. PCD, as a cause of improper chromosome separation, may be regarded as a manifestation of the chromosome instability (Mehes, 2000) leading to aneuploidy, giving rise to genome instability (Bajic et al., 2008; 2009; Moh, 2011). The origin and the molecular mechanisms of chromosome instability in AD are still not fully understood, although it is known that mutations in the presenilin 1 gene may lead to abnormal presenilin function, giving rise to defects in the cell cycle, an increased number of abnormal mitotic spindles and improper chromosome segregation (Boeras et al., 2008). It should be mentioned that micronutrient deficiency, for instance of folate and B12, can also have an impact on proper chromosomal segregation in AD and DS patients, and DNA integrity as well. An imbalance of folate metabolism can lead to DNA hypomethylation of the centromere region, leading to a loss of control of the centromere separation and abnormal chromosome segregation (Scarpa et al., 2003; Suzuki et al., 2002, Zivkovic in press).

We have determined that there is no PCD in acrocentrics with SA. Our study showed the significant difference in SA frequency as well as the frequency of PCD in acrocentrics between AD and control groups, and both phenomena could be closely associated with the alterations in functional organization of the nucleolus and its relation to the process of cohesion throughout interphase and during the cell cycle. We conclude that the investigations into acrocentrics considered in the present study are related to the etiology or pathology of genome instability in AD.

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