Chromosome 17 and 21 aneuploidy in buccal cells is increased with ageing and in Alzheimer’s disease

Philip Thomas1,2,* and Michael Fenech1
1CSIRO Human Nutrition, PO Box 10041, Adelaide BC, Adelaide, South Australia 5000, Australia and 2Discipline of Physiology, School of Molecular and Biomedical Sciences, The University of Adelaide, Adelaide, South Australia 5005, Australia

Alzheimer’s disease (AD) is a premature ageing syndrome characterized by cognitive impairment arising from neuropathological changes occurring within specific areas of the brain. We report a 1.5-fold increase in trisomy 21 (P < 0.001) and a 1.2-fold increase in trisomy 17 (P < 0.001) in buccal cells of Alzheimer’s patients compared to age- and gender-matched controls. Chromosome 17 and chromosome 21 monosomy and trisomy increase significantly with age (P < 0.001). Down’s syndrome, which exhibits similar neuropathological features to those observed in AD also showed a strong increase in chromosome 17 monosomy and trisomy compared to matched controls (P < 0.001). These results suggest that an increased incidence of aneuploidy for both chromosomes 17 and 21 may contribute to the aetiology of AD. We also investigated aneuploidy rates in hippocampal brain cells, which have been shown to have a low rate of cell division, which may be relevant in potential incidence of non-disjunction. However, aneuploidy rate for chromosomes 17 and 21 in the nuclei of hippocampus cells of brains from Alzheimer’s patients and controls were not significantly different. These results are suggestive that the aneuploidy events investigated which are increased beyond the incidence in normal ageing may be influenced by genetic factors that may predispose to AD, but are unlikely to be a primary cause of AD brain pathology.

Introduction

Alzheimer’s disease (AD) is a complex progressive neurodegenerative disorder of the brain and is the commonest form of dementia (1). Neurodegenerative changes appear firstly within the entorhinal cortex and then progress to the hippocampus eventually disrupting learning and short-term memory (2,3). The disease is clinically defined by progressive memory loss, visuospatial and language impairment and various psychiatric and behavioural changes (1,4–6). The two histopathological structures present within the brain that positively identify AD conclusively at post-mortem are the neurofibrillary tangles and the amyloid-based neuritic plaques (5,6).

Neurofibrillary tangles are composed of the microtubule-associated protein tau, the gene of which is located on chromosome 17q21.1 (7). Tau is important as it associates with tubulin in the formation of microtubules. Microtubules impart shape and structure to cells and also form the basis of a cellular transport network allowing the movement of neurotransmitters, micronutrients and organelles that are essential for normal cellular function (8). Microtubules also provide points of attachment for chromosomes during cell division, which if disrupted may result in an increased incidence of chromosome malsegregation (9). The neurofibrillary tangles contain structures known as paired helical threads comprised of tau which are hyperphosphorylated. This hyperphosphorylation leads to a dissociation between tubulin and tau, resulting in a breakdown of the brain transport system leading to loss of biological activity, cell death and potential microtubule dysfunction leading to chromosome malsegregation (7,8). The second histopathological feature in the brain of AD patients is the amyloid-based neuritic plaques. These consist of the 42 amino acid β amyloid peptide (Aβ42), which originates from the aberrant proteolysis of the amyloid precursor protein (App), the gene (APP) of which is located on chromosome 21q21 (10).

Down’s syndrome (DS) like AD is a premature ageing syndrome characterized by various degrees of chromosome 21 trisomy and an elevated incidence of genome instability (11,12). DS individuals develop dementia and manifest brain changes that are histopathologically indistinguishable from AD, usually during the third or fourth decade of life (13,14). For this reason it has been suggested that both AD and DS share underlying mechanisms involving susceptibility to chromosome malsegregation events, particularly those involving chromosome 21. Alteration in gene dosage and expression for the TAU and APP gene resulting from aneuploidy may be an important contributor to the aetiology of AD. It has not yet been determined whether aneuploidy of chromosome 17 has a higher incidence in AD and DS and whether gene dosage and altered gene expression of the TAU gene may play a potential contributory role in AD and DS pathology.

There is much evidence from previous studies to show that both AD and DS share an increased susceptibility to genomic instability events. Micronuclei (biomarkers of chromosome malsegregation and breakage) are elevated in AD lymphocytes and were shown to be centromere positive indicating whole chromosome loss (15). Migliore et al. (16) also showed a significant increase in micronuclei in the lymphocytes of young mothers of Down’s syndrome individuals (MDS) and showed they were more prone to non-disjunction events for chromosome 13 and 21. A similar predisposition to increased aneuploidy events for chromosome 13 and 21 has been shown in lymphocytes of AD patients (17,18). Fibroblast cultures from sporadic and familial AD patient’s that carry mutations within the presenilin 1, presenilin 2 and the App were found to...
have an ~2-fold increase in the number of trisomy 21 cells compared to cultures from normal individuals (19). These findings suggest that aneuploidy may have a contributing role to early changes in disease development.

The aim of this study was to use fluorescent in situ hybridization (FISH) and fluorescently labelled DNA probes to determine the incidence of aneuploidy of chromosomes 17 and 21 in the buccal mucosa and hippocampal brain tissue of Alzheimer’s patients compared to age- and gender-matched healthy controls. It was also our intention to investigate any age-related effects on aneuploidy involving these two chromosomes between a young and old cohort, and whether aneuploidy for chromosome 17 in the buccal mucosa of DS was significantly different from controls. Buccal cells (BCs) were selected for this study because they can be collected non-invasively and originate from the neuroectoderm from which brain tissue is derived (20). BCs may therefore exhibit genetic defects common to brain tissue acquired during the early stages of development.

### Methods

#### Recruitment and characteristics of participants

Approval for this study was obtained from CSIRO Human Nutrition, Adelaide University, Ramsay Health Care and Southern Cross University Human Experimentation Ethics Committees. Participants in this study consisted of four distinct groups: 30 younger controls (age 18–26 years), 26 older controls (age 64–75 years), 21 DS individuals (age 5–20 years) and 54 clinically diagnosed Alzheimer’s patients (age 58–93 years). None of the participants recruited to the study were receiving anti-folate therapy or cancer treatment. Alzheimer’s patients were recruited at the College Grove Private Hospital, Walkerville, Adelaide, South Australia, following their initial diagnosis and prior to commencement of therapy. Diagnosis of AD was made by experienced clinicians according to the criteria outlined by the National Institute of Neurological and Communicative Disorders and Stroke-AD and Related Disorders Association (21). These are recognized standards used in all clinical trials. Mini Mental State Examination (MMSE) scores which are a recognized measure of cognitive impairment were only available for the Alzheimer’s cohorts. Participants did not receive any remuneration for their participation.

Alzheimer’s patients were separated into two distinct groups. One group (younger AD group) was age matched to the older control, while the second group was classified as the older AD group. Gender ratio differences between the groups were not significant. MMSE scores between the two AD groups were not significantly different. The age of the control group compared to the younger AD group was significantly different but the younger AD group had a significantly lower age relative to the older AD group ($P < 0.0001$). The younger and older controls were ‘normal’ functioning healthy individuals, who self-volunteered and consented to the study and did not report a history of cognitive impairment. Demographic characteristics of the cohorts are shown in Table I. Consent from AD and DS volunteers was provided by either partners or guardians.

#### BC collection and slide preparation

BCs were collected using a modified version of the method used by Beliën et al. (22). Prior to BC collection, the mouth was rinsed thoroughly with water to remove any unwanted debris. Small headed toothbrushes (Supply SA, Camden Park, Adelaide, Australia, code 85300012) were rotated 20 times in a circular motion against the inside of the cheek, starting from a central point and gradually increasing in circumference to produce an outward spiral effect. Both cheeks were sampled using separate brushes. The heads of the brushes were individually placed into separate 30-ml yellow-top containers (Sarstedt, Australia Pty. Ltd, Technology Park, Adelaide, Australia, code, 60.9922.918) containing BC buffer [0.01 M Tris–HCl (Sigma T-3253), 0.1 M ethylenediaminetetraacetic acid tetra sodium salt (Sigma E5391) and 0.02 M sodium chloride (Sigma S5886)] at pH 7.0 and agitated to dislodge the cells. Cells from both right and left cheeks were transferred into separate TV-10 centrifuge tubes (Sarstedt, code 60.9921.829) and spun for 10 min at 1500 r.p.m. (MSE Mistral 2000). Supernatant was removed and replaced with 10 ml of fresh BC buffer. The BC buffer helps to inactivate endogenous DNAases and aids in removing bacteria that may complicate scoring. Cells were spun and washed twice more, with a final volume of 5 ml of BC buffer being added to the cells. The cell suspension was vortexed and then homogenized for 2 min in a hand homogenizer (Wheaton Scientific 0.1- to 0.15-mm gauge) to increase the number of single cells in suspension. Left and right cell populations were pooled into a 30-ml yellow-top container and drawn into a syringe with a 21-gauge needle and expelled to encourage cellular separation. Cells were passed into a TV-10 tube through a 100-µm nylon filter (Millipore Australia Pty. Ltd, North Ryde, NSW, Australia, code MILNYH02500) held in a swinnex filter (Millipore, code MILSX000250) to remove large aggregates of unseparated cells that hinder slide preparation and cell analysis. Cells were further spun at 1500 r.p.m. for 10 min and the supernatant was removed. Cells were re-suspended in 1 ml of BC buffer and the cell concentration was determined by a Coulter counter (Beckman Coulter Model ZB1; settings: attenuation, 1; threshold, 8, aperture, 1/4; manometer, 0.5). Cell suspensions were prepared containing 80 000 cells/ml after initial readings from a 1:50 dilution (300 µl/15 ml isoton). Dimethyl sulphoxide (50 µl/ml; Sigma 2650) was added to help clarify cellular boundaries by further separating the cells. One hundred and twenty microlitres of cell suspension was added to cytospin cups and spun at 600 r.p.m. for 5 min in a cytocentrifuge (Shandon cytocentrifuge 3). Slides containing two spots of cells were air-dried for 10 min and then fixed in ethanol:acetic acid (3:1) for 10 min. Slides for FISH were air-dried for 10 min prior to being stored at −70°C in a sealed desiccated slide box.

#### Brain tissue collection and slide preparation

Frozen non-fixed hippocampal brain tissue from histopathologically confirmed Alzheimer’s patients (positive for the presence of amyloid plaques and neurofibrillary tangles) was provided by the Brain Bank of South Australia, Centre of Neuroscience, Flinders University. Control hippocampal tissue from histopathological normal individuals was provided by both the New South Wales Tissue Resource Centre, Department of Pathology, University of Sydney and the Brain Bank of South Australia. Tissue provided from the hippocampus was taken from slices. Cores were taken using a special drill bit and battery-powered drill with the slices being kept on a bed of ice during retrieval. The removed cores were placed in a small test tube and stored at −70°C until transported.

Two hundred milligrams of brain tissue was weighed (Sartorius Scales, type 1475) inside a biological safety cabinet (Gelman Sciences, Ann Arbor, MI, USA, BH series) and...
placed on a slide inside a petri dish and moistened with a few drops of phosphate-buffered saline (PBS; 120 mM sodium chloride, 2.7 mM potassium phosphate monobasic and 10 mM sodium phosphate dibasic, pH 7.4). Tissue was minced and chopped thoroughly using a scalpel blade (Blade No 11, Swann-Morton Ltd, Sheffield, UK) and transferred to TV-10 tube. PBS washings (2 ml) were taken of the slide within the inclined petri dish and transferred to the TV-10 tube. Three washings per slide were made. A glass pipette was used to draw up and release the cell suspension to encourage the separation of single cells. Care was taken to avoid the production of aerosols while pipetting. The tube was balanced and centrifuged at 2500 r.p.m. for 10 min. The supernatant was removed and replaced with 6 ml of PBS. The cell pellet was drawn up and released several times using a glass pipette to further encourage the production of single cells. Cells were drawn up into a 10-ml syringe and filtered into a TV-10 tube through a 100-µm nylon filter (Millipore, code MILNYH02500) held in a swinnex filter (Millipore, code MILSX0002500) to remove large aggregates of unseparated cells that hinder slide preparation and cell analysis. One millilitre of Carnoys fix consisting of ethanol: glacial acetic acid (3:1) was added and the suspension mixed. Tubes were spun for 2500 r.p.m. for 10 min. Supernatant was aspirated and fresh fix added to a volume of 6 ml and centrifuged at 2500 r.p.m. for 10 min. Fixation was repeated and cells were re-suspended in 4 ml of fix to produce a cloudy suspension. Cells were dropped onto clean slides, air-dried for 10 min and placed in a slide box at −20°C with desiccant ready for FISH analysis.

Preparation of chromosome 17 DNA probe

Centromeric chromosome 17-specific DNA probe was generated by PCR labelling with centromere-specific primers for the chromosome (α17A1: 5'-AATTCTGTGTGAAACGGGATAA TTTCAGCTG-3' and α17B2: 5'-CTTCTCTAGGTACCTTG TGTCTAGATGTC-3' (Geneworks, Thebarton, Adelaide, Australia) (23,24). The length of the amplification product is 227 bp (25). Briefly, PCR was carried out with 0.2 µM of each primer, 60 µM digoxigenin-11-dUTP (Roche, Basel, Switzerland, No: 1093088), 140 µM dTTP and 200 µM dATP, dCTP and dGTP (Roche, Basel, Switzerland, No: 1969064). The PCR was started with pre-denaturation at 94°C for 2 min, followed by 25 cycles of 92°C for 1 min, 61°C for 2 min, 72°C for 2 min and terminal extension at 72°C for 10 min. Chromosome 17 centromeric probe was validated by hybridizing to human metaphases to determine exact position and ensure no cross-hybridizing had occurred.
Hybridization and detection of the probe was performed according to the manufacturer’s instructions following a pre-treatment. Prior to in situ hybridization, the buccal slides were pre-treated with pepsin (Sigma) (300 μg/ml in 0.01 N HCl) for 10 min while lymphoblastoid control slides were treated with (5 μg/ml pepsin in 0.01 N HCl) at 37°C for 10 min and then washed twice in PBS (pH 7.0) for 5 min at room temperature. Afterwards, the slides were dehydrated in a cold ethanol series (70, 70, 80, 90 and 100%, 2 min each) and air-dried. Slides were denatured at 79°C for 5 min in pre-warmed 70% formamide/2× SSC and then dehydrated through an ethanol series (70, 70, 80, 90 and 100%, 2 min each) at 4°C and then placed in a 37°C oven to reach hybridization temperature. Ten microlitres of probe mixture per slide (1 μl LSI 21 probe, 2 μl distilled water and 6 μl LSI/WCP hybridization buffer) was prepared and denatured in a 79°C water bath for 5 min. Five microlitres of the probe mixture was added to each buccal cytospin spot, coveredslipped and sealed with rubber cement before incubating at 37°C overnight (~16 h) in a humidified box.

Coverslips were removed with forceps before slides were washed in three coplin jars of 50% formamide/2× SSC at 45°C for 10 min. Slides were further washed in coplin jars of 2× SSC and 2× SSC/0.1% Nonidet P40 (Roche, No: 1754599) at 45°C for 10 min. The nuclei were counterstained with DAPI (0.05 μg/ml in 4× SSC with 0.06% Tween 20) for 1 min 20 sec at room temperature and coveredslipped in anti-fade solution. The coverslips were sealed with nail varnish and kept at −20°C in dark.

**Scoring method**

A minimum of 1000 BCs or hippocampal brain tissue cells were scored for the presence of one, two or three signals (Figures 1 and 2). Only nuclei with at least one FISH signal were scored. In the cases of nuclei with more than one signal, only those nuclei in which the signals were of similar size and intensities and were separated by a distance of more than half the diameter of one FISH signal were scored (27). A Nikon E600 fluorescence microscope was used with a triple-band filter (for DAPI, FITC and rhodamine) allowing simultaneous visualization of the signals from both the fluorochrome and the nuclear counterstain.

**Statistical analyses**

One-way analysis of variance (ANOVA) was used to determine the significance of difference between groups for aneuploidy incidence, whereas pair wise comparison of significance was determined using Tukey’s test. Gender ratios were tested using the chi-square test. ANOVA values and cross-correlation analysis was calculated using Graphpad PRISM (Graphpad Inc., San Diego, CA, USA). Significance was accepted at $P < 0.05$.

**Results**

The results for the incidence of aneuploidy (monosomy and trisomy) for chromosomes 17 and 21 in BCs for all groups are shown in Figures 1, 3 and 4. The results showing the incidence of aneuploidy (monosomy and trisomy) for chromosomes 17 and 21 in hippocampal brain tissue from Alzheimer’s and control groups are shown in Figures 2, 5 and 6. Percentages of aneuploid cells scored in both BCs and brain tissue are outlined in Table II.

**Normal ageing—an euploidy in BCs**

Monosomy of chromosomes 17 and 21 was increased by 222.2% ($P < 0.001$) and 91.4% ($P < 0.001$) and trisomy of chromosomes 17 and 21 was increased by 89.0% ($P < 0.001$) and 97.4% ($P < 0.001$), respectively, in the old controls relative to the young controls. The total aneuploidy rate was 13.8% for chromosome 17 and 9.6% for chromosome 21 in old controls compared to 5.0 and 5.0%, respectively, in young controls.

**DS—an euploidy in BCs**

Monosomy and trisomy of chromosome 17 was increased by 292.7% ($P < 0.001$) and 79.7% ($P < 0.001$), respectively, in DS relative to young controls and were at a level similar to that observed in old controls. Total aneuploidy rate for chromosome 17 in DS was 16.0% compared to 5.0% in young controls.

![Fig. 1. FISH results showing non-disjunction events in BCs.](image-url)
AD—aneuploidy in BCs

Both chromosome 17 monosomy and trisomy were increased in the younger AD group by 19.4% ($P < 0.05$) and 16.1% ($P < 0.001$), respectively, relative to the old control group, but there were no differences between the younger and older AD groups. The total chromosome 17 aneuploidy rate in younger and older AD groups was 15.7 and 16.4% compared to 13.8% in the old control group. Chromosome 21 trisomy was increased by 183.3% ($P < 0.001$) in the AD groups relative to the old controls, but there was no difference in chromosome 21 monosomy. MMSE scores were not significantly correlated with chromosome 17 or chromosome 21 aneuploidy.

AD—aneuploidy in brain hippocampus

Chromosome 17 or chromosome 21 aneuploidy did not differ significantly in hippocampus tissue of Alzheimer’s cases and controls. Chromosome 17 and 21 aneuploidy rate in hippocampus was 18–18.2% and 11.8–12.8% compared to 13.8–16.4% and 9.6–11.6% in BCs of old controls and AD patients, respectively, suggesting a slightly higher aneuploidy rate in brain tissue relative to buccal mucosa.

Discussion

The aim of the study was to test the hypotheses that aneuploidy of chromosomes 17 and 21 in BCs (i) is a risk factor for neurodegenerative diseases such as AD and DS and (ii) increases with ageing.

We report a significant 1.2-fold increase in aneuploidy for chromosome 17 and a 1.5-fold increase in aneuploidy 21 within the buccal mucosa of AD patients compared to age- and gender-matched controls (Figures 1, 3 and 4). We also report a 1.8-fold increase in the incidence of chromosome 17 in DS BCs compared to a young control group ($P < 0.001$). The DS values were not significantly different from the old control, even though the old control group is 50 years senior to the DS group. DS aneuploidy rates are beyond those expected for normal ageing.
confirming its status as a premature ageing syndrome. Although a significant increase in aneuploidy rate for chromosome 17 ($P < 0.001$) and chromosome 21 ($P < 0.001$) occurred in BCs with normal ageing, our data show that the incidence of chromosome malsegregation in both the AD and DS groups are significantly increased and beyond the levels expected for healthy subjects in corresponding age groups. This confirms the findings from earlier studies that have shown elevated rates of aneuploidy in AD patients for a number of different chromosomes, such as 13, 18 and 21 indicating higher rates of genomic instability in this premature ageing syndrome (18,19,28). We found that the total aneuploidy rate for chromosome 21 was 92.1% in DS compared to 5.0% in young controls and 9.6% in old controls. Most DS individuals are cytogenetically determined as having full trisomy 21. Incomplete hybridization of the LSI 21 probe to BCs may be responsible for a lower rate of trisomy 21 with cells appearing disomic and therefore being scored as false negatives. However, it has been shown that DS individuals tend to exhibit increased disomy 21 with ageing, resulting in various frequencies of mosaicism for aneuploidy of chromosome 21, which are reflected in our findings (29,30). Given the strong association of trisomy 21 with premature ageing in DS, it is plausible that elevated trisomy 21 observed in AD may contribute to accelerated ageing in this condition.

![Fig. 4](image_url) Frequency of (a) chromosome 17 trisomy and (b) chromosome 21 trisomy following FISH in 1000 BCs of young controls ($n = 30$), DS ($n = 21$), old controls ($n = 26$), younger AD ($n = 23$) and older AD ($n = 31$). Groups sharing the same letters are not significant. Error bars indicate SEM.

![Fig. 5](image_url) Frequency of (a) chromosome 17 monosomy and (b) chromosome 21 monosomy from 1000 hippocampal brain tissue cells of Alzheimer’s patients ($n = 13$) and control hippocampal brain tissue ($n = 9$). Groups sharing the same letters are not significant. Error bars indicate SE of the mean.

![Fig. 6](image_url) Frequency of (a) chromosome 17 trisomy and (b) chromosome 21 trisomy from 1000 hippocampal brain tissue cells of Alzheimer’s patients ($n = 13$) and control hippocampal brain tissue ($n = 9$). Groups sharing the same letters are not significant. Error bars indicate SEM ($n = 9$).
Increased aneuploidy for chromosomes 17 and 21, which could lead to altered gene dosage and expression for tau and App, may be an important contributing factor leading to the early stages of the development of AD, if critical regions of the brain are enriched in aneuploid cells during early development due to mosaicism. The APP gene present on chromosome 21 may be over-expressed in the small population of cells that are trisomic and could result in aberrant proteolysis and the accumulation of the Aβ42 which is the core component of the senile plaques found in the brains of both DS and AD patients (31–34). This amyloidogenic pathway has traditionally held the view that the neurotoxic effects of these peptides contribute to the aetiology of the disease. Recently, this view has been challenged suggesting that APP itself may play an important neuronal role leading to cognitive impairment (35–37). The precise role of these peptides and proteins and their interaction and potential relationship to the development of AD will need to be conclusively determined through future studies. APP processing is also altered in terms of the ratio of (Aβ42) compared to the normal 40 amino acid β amyloid peptide, resulting in an increase of the more neurotoxic and amyloidogenic Aβ42. Similarly, an individual who is mosaic for trisomy 17 cells could potentially over-express the microtubule-associated protein tau which may lead to an increased aggregation of the protein; this could in turn result in increased paired helical filaments known to make up the neurofibrillary tangles that are the other cerebral hallmark of the disease. Abnormal accumulation or deficiency of tau could lead to a breakdown of the microtubule system causing chromosomal malsegregation as a result of microtubule disruption. Similarly, a breakdown in the neuronal transport system due to microtubule dysfunction may result in an apparent micro-nutrient deficiency affecting certain susceptible areas of the brain known to be affected in AD.

However, it is possible that genes other than APP contained on chromosome 21 and in particular in the DS critical region such as SOD1 (copper/zinc superoxide) may influence pathologies such as oxidative stress and the neurodegeneration associated with both AD and DS (38–40). Knockout mouse models for DS which exclude APP and SOD1 genes have shown that within the brain there are increased levels of oxidative stress, mitochondrial dysfunction and hyperphosphorylation of tau (41). This would suggest that over-expression of other genes within this region contribute to pathologies common to both conditions. Recently, cultured trophoblasts from trisomy 21 placentas were analysed for genome-wide expression and compared to trophoblasts from normal first trimester pregnancies. About 750 genes were significantly over-expressed in the trisomic placenta, with a 4.5-fold gene abundance being found to map to chromosome 21 compared to that expected from the control microarray. It was also found that genes implicated in AD were also over-expressed, such as a 2-fold increase in APP and a 3-fold increase in LOX as well as over-expression of the APP-binding protein. Other relevant genes that were identified were associated with ubiquitination and proteosomal degradation (42). This may be relevant as an alternative epigenetic mechanism involving post-translational modification of proteins common to both AD and DS. It has recently been shown that neuroblastoma cells treated with Aβ showed an increase in the DYRK1A transcript, a gene found within the DS critical region, resulting in elevated rates of tau hyperphosphorylation (43). This is significant as it provides a mechanism that links chromosome 21 aneuploidy and possible gene over-expression with histopathological cerebral hallmarks that are common to both AD and DS. Alterations in the DYRK1A and presenilin genes could lead to defects in anterograde fast axonal transport leading to neurodegeneration affecting brain function (44), while deficits in cellular transport leading to oxidative stress may indirectly influence spindle formation and chromosome segregation in all somatic and dividing cells.

It is plausible that trisomy 21 mosaicism in brain tissue, particularly within the hippocampus, may have originated from chromosome malsegregation events early in foetal development and may be a possible explanation for some sporadic forms of AD (19,45). Low level in utero mosaicism may be the result of nondisjunction caused by folate deficiency, which has been shown to increase the rate of chromosome 17 and 21 aneuploidy in lymphocytes (46). Recent studies have shown an increased predisposition to chromosome malsegregation in the young mothers of DS individuals (MDS) (47). Schumpf et al. (47) showed a 5-fold increase in the risk of AD within this cohort of young mothers and suggested that these individuals are biologically prematurely aged increasing their susceptibility to aneuploidy events. Chromosome 21 aneuploidy would predispose these individuals to DS pregnancies and potentially contribute to an increased risk for developing AD later in life (47).

The risk of aneuploidy due to chromosome malsegregation is likely to be increased in cerebral tissues undergoing post-maturation neurogenesis such as the dentate gyrus of the hippocampus involved in short-term memory and learning and the glial cells (48). Both populations of cells have been shown to continue cell division in the adult brain and so could be potential candidates to accumulate aneuploid cells throughout an individual’s lifetime (49). Our data shows that mosaicism for chromosomes 17 and 21 occurred within the hippocampus of histopathologically confirmed Alzheimer’s patients. However, the aneuploidy rate for these two chromosomes was not

<table>
<thead>
<tr>
<th>Chromosome 17</th>
<th>Young controls</th>
<th>DS</th>
<th>Old controls</th>
<th>Younger AD</th>
<th>Older AD</th>
<th>Alzheimer’s brain tissue</th>
<th>Control brain tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monosomy</td>
<td>3.1</td>
<td>12.4</td>
<td>10.1</td>
<td>12.1</td>
<td>11.3</td>
<td>14.6</td>
<td>14.3</td>
</tr>
<tr>
<td>Trisomy</td>
<td>1.9</td>
<td>3.6</td>
<td>3.7</td>
<td>4.3</td>
<td>4.4</td>
<td>3.6</td>
<td>3.7</td>
</tr>
<tr>
<td>Total aneuploidy</td>
<td>5.0</td>
<td>16.0</td>
<td>13.8</td>
<td>16.4</td>
<td>15.7</td>
<td>18.2</td>
<td>18.0</td>
</tr>
<tr>
<td>Chromosome 21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monosomy</td>
<td>3.1</td>
<td>2.9</td>
<td>5.9</td>
<td>5.5</td>
<td>5.8</td>
<td>8.9</td>
<td>7.9</td>
</tr>
<tr>
<td>Trisomy</td>
<td>1.9</td>
<td>89.2</td>
<td>3.7</td>
<td>5.4</td>
<td>5.8</td>
<td>3.9</td>
<td>3.9</td>
</tr>
<tr>
<td>Total aneuploidy</td>
<td>5.0</td>
<td>92.1</td>
<td>9.6</td>
<td>10.9</td>
<td>11.6</td>
<td>12.8</td>
<td>11.8</td>
</tr>
</tbody>
</table>

Table II. Percentages of monosomy and trisomic cells for chromosomes 17 and 21 in at least 1000 BCs of young controls (n = 30), DS (n = 21), old controls (n = 26), younger AD (n = 23) and older AD (n = 31) and hippocampal brain tissue cells of Alzheimer’s patients (n = 13) and control hippocampal brain tissue (n = 9).
controls, (iii) DS is associated with increased aneuploidy of chromosome 17 and 21 aneuploidy in BCs compared to segregation (61–63). In addition folate deficiency has been shown that abnormal folate metabolism, possibly as a result of polymorphisms within genes of the folate/methionine pathway, could modify gene expression (59,60). There is evidence to DNA metabolism and the maintenance of methylation patterns shown that AD patients tend to be deficient in vitamins such as 17 and 21 aneuploidy are needed to address this question. Polymorphisms of presenilins and ApoE genes that are known risk factors for both AD and DS are likely to play a pivotal role in chromosome malsegregation. Mutated presenilin genes that contribute to early onset familial AD have been shown to produce proteins localized within the nuclear membrane, centrosomes and kinetochores indicating a role in chromosome segregation and organization (50). An association has also been shown between polymorphisms within intron 8 of the presenilin 1 gene and errors within maternal meiosis II that led to trisomy 21 (51). This same polymorphism has also been identified as conferring an increased risk for late onset AD (19,52,53). It has been shown that certain alleles of ApoE influence the age of onset of dementia in both AD and DS. The ApoE4 allele has been shown to influence the development of sporadic AD and DS. DS individuals with two copies of this allele develop more severe forms of AD neuropathology compared to DS individuals lacking the e4 allele (18,54,55). The ApoE4 allele has also been shown to be significantly more common among young mothers of DS children, suggesting that this allele may predispose these individuals to chromosome 21 non-disjunction and a potential influential role for this allele on chromosome malsegregation (56).

Micronutrient deficiency may also have an influence upon chromosome malsegregation in both AD and DS. It has been shown that AD patients tend to be deficient in vitamins such as folate and B12 (57,58). Folate and B12 play an important role in DNA metabolism and the maintenance of methylation patterns that could modify gene expression (59,60). There is evidence to show that abnormal folate metabolism, possibly as a result of polymorphisms within genes of the folate/methionine pathway, may result in a state of DNA hypomethylation in the centromeric regions of chromosomes leading to abnormal chromosome segregation (61–63). In addition folate deficiency has been show to increase aneuploidy of chromosomes 17 and 21 (46).

In conclusion, the results of this study show that (i) aneuploidy of chromosomes 17 and 21 increases with ageing in BCs, (ii) AD patients exhibit an abnormally high rate of chromosome 17 and 21 aneuploidy in BCs compared to controls, (iii) DS is associated with increased aneuploidy of chromosome 17 in BCs and (iv) despite these differences in BCs, aneuploidy of chromosomes 17 and 21 in hippocampus tissue did not differ between AD cases and controls. These results suggest that aneuploidy of chromosomes 17 and 21 may be caused by genetic factors that predispose to AD but are unlikely to be a primary cause of brain pathology in AD.

Funding

CSIRO, Division of Human Nutrition, Adelaide, South Australia.

Acknowledgements

The authors would like to thank Associate Professor Michael Roberts for his valuable contribution and comments during the compilation of the manuscript. The authors gratefully acknowledge Jane Hecker and Jeffrey Faunt for clinically diagnosing the Alzheimer patients and for providing the authors with MMSE scores. Lastly, the authors greatly appreciate the efforts made by all the individuals who consented to participate in this study.

Conflict of interest statement: None declared.

References
