



Positive association of a *Sirt1* variant and parameters of oxidative stress on Alzheimer's disease

Daniela Camporez^{1,2} · Luciano Belcavello³ · Jucimara Ferreira Figueiredo Almeida^{1,2} ·
Geralda Gillian Silva-Sena⁴ · Lúcia Helena Sagrillo Pimassoni⁵ · Renato Lírio Morelato^{5,6} · Maria do Carmo
Pimentel Batitucci^{2,7} · Flavia de Paula^{1,2}

Received: 8 March 2020 / Accepted: 29 August 2020 / Published online: 14 September 2020

© Fondazione Società Italiana di Neurologia 2020

Abstract

Alzheimer's disease (AD) is a complex neurodegenerative disorder and the most common type of dementia in the elderly. Although its cause is not completely known, several studies suggest that oxidative stress plays an important role in the etiology of this disease. The SIRT1 and SOD2 proteins are linked to pathways that may impair oxidative stress. In this study, we analyzed the association between polymorphisms in these genes and in the *APOE* gene, through RT-PCR, as well as between environmental factors and the risk of AD. Additionally, the thiobarbituric acid reactive substance assay was performed to estimate the plasma level of malondialdehyde (MDA), a biomarker of lipid peroxidation. Furthermore, some cytogenetic studies indicate that cells of AD patients show increased chromosomal damage; thus, we performed the micronucleus cytome assay to assess cytogenetic damage in AD patients. As expected, the *APOE* polymorphisms were found to be highly associated with AD. Additionally, the CT genotype of the *SIRT1* gene showed a positive association with the disease. The frequencies of genomic damage (micronucleus, buds, nucleoplasmic bridges and binucleated cells), the presence of cell death biomarkers (condensed chromatin, karyorrhexis and pyknosis), and the plasma level of MDA were significantly greater in AD patients than in controls. Our results support the hypothesis that AD is a condition with increased oxidative stress and genomic instability, which may contribute to the neurodegeneration in AD.

Keywords Oxidative stress · SIRT1 · SOD2 · APOE · Genomic instability · Malondialdehyde

✉ Jucimara Ferreira Figueiredo Almeida
jucimarabiology@gmail.com

Daniela Camporez
danielacamporez@gmail.com

Luciano Belcavello
belcavello@gmail.com

Geralda Gillian Silva-Sena
ggsmais@gmail.com

Lúcia Helena Sagrillo Pimassoni
luciasagrillo@hotmail.com

Renato Lírio Morelato
renato.morelato@hotmail.com

Maria do Carmo Pimentel Batitucci
doCarmo_batitucci@yahoo.com.br

Flavia de Paula
flapvit@yahoo.com.br

¹ Programa de Pós-Graduação em Biotecnologia, Renorbio, Universidade Federal do Espírito Santo, CEP, Vitória, ES 29043-900, Brazil

² Departamento de Ciências Biológicas, Centro de Ciências Humanas e Naturais, Universidade Federal do Espírito Santo, Av. Fernando Ferrari, 514, Goiabeiras, Prédio Lydia Behar, sl. 102, Vitória, ES CEP 29075-910, Brazil

³ Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Sul, Viamão, RS, Brazil

⁴ Departamento de Educação Integrada em Saúde, Centro de Ciências da Saúde, Universidade Federal do Espírito Santo, Vitória, ES CEP 29043-900, Brazil

⁵ Escola Superior de Ciências da Santa Casa de Misericórdia de Vitória, Vitória, ES CEP 29027-502, Brazil

⁶ Hospital da Santa Casa de Misericórdia de Vitória, Vitória, ES CEP 29025-023, Brazil

⁷ Programa de Pós-Graduação em Biologia Vegetal, Centro de Ciências Humanas e Naturais, Universidade Federal do Espírito Santo, Vitória, ES CEP 29075-910, Brazil

Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly, affecting nearly 35 million people worldwide [1]. This devastating disease is characterized by progressive neurodegeneration with multifactorial etiology. AD dysfunctions include calcium dysregulation, proteolysis failure, altered cell signaling, mitochondrial dysfunction, and oxidative stress, which lead to synaptic dysfunction, nerve cell death, and neurodegeneration [2, 3].

Oxidative stress is accepted to play a key role in the pathology of AD. This process results from an imbalance between elevated free radical production and the decrease in either free radical scavenging or the mechanisms used to repair oxidized macromolecules. The overproduction of free radicals is capable of damaging proteins, lipids, and DNA, leading to cellular instability events, such as chromosomal damage and cell death [4–7]. These events can be easily measured by the micronucleus assay, considered a reliable biomarker of genetic instability in numerous applications [8–11].

Genes involved in cellular mechanisms of oxidative damage repair are strong candidates for AD [12]. These genes include *SIRT1* (*Silent Information Regulator Type I*), located on chromosome 10q21.3, and *SOD2* (*Superoxide dismutase 2*), on chromosome 6q25.3 [13]. Since the SIRT1 protein can increase life span through the regulation of cellular metabolism, it is a possible protective factor for AD [14]. The gene *SOD2* encodes a protein involved in the repair of oxidative damage and is located in a region that shows evidence of a relationship with AD in genome-wide association studies (GWAS) [15]. Despite the large number of genes supposedly related to AD, to date, the $\epsilon 4$ allele of the *APOE* gene on 19q13.2 is considered the major risk factor for the disease in several populations [16]. The study of genetic variants associated with the risk for AD is useful to understand the mechanisms of the disease, aiding in complementary diagnosis.

Additionally, lipid peroxidation is one of the most important manifestations of oxidative stress and results in the production of toxic aldehydes, such as malondialdehyde (MDA), a mutagenic compound measured by the chemical determination of thiobarbituric acid reactive substances—TBARs [17–20]. The search for biomarkers of genomic instability is fundamental to improving the implementation of diagnosis and treatment. In this context, the main objectives of this study were to validate the association of single-nucleotide polymorphisms (SNPs) in the *SIRT1* and *SOD2* genes with late-onset Alzheimer's disease and to estimate the level of cellular and genomic damage and lipid peroxidation in AD patients.

Materials and methods

Subjects

All patients fulfilled the clinical criteria for probable AD according to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) and the National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA). The complete evaluation of dementia were performed using imaging exams (computed tomography or magnetic resonance imaging); standard laboratory tests (complete blood count, serum electrolytes, serum glucose, blood urea nitrogen, vitamin B12, folate, thyroid function, and syphilis serology); the Clinical Dementia Rating (CDR) scale, which categorize functional impairment of dementia in values as 0 = normal; 0.5 = borderline; 1 = mild; 2 = moderate; and 3 = severe; and the Mini-Mental State Examination (MMSE) [21–25].

For controls samples, the geriatrician meticulously selected only participants with no family history of Alzheimer's and the non-dementia elders using the Mini-Mental State Examination (MMSE) (mean in controls samples = 21 ± 5). MMSE evaluates that cognitive status and several factors as sociocultural variables, age, and education levels could affect individuals scores [26, 27].

For genotyping, a total of 332 non-consanguineous subjects, comprising 109 late-onset AD patients (mean age: 82.3 ± 7.6) and 223 no demented healthy controls (mean age: 81.2 ± 9.9) from a single geographical location in Vitória, ES, Southeast of Brazil, were enrolled in the study. Peripheral blood (10 ml) was collected in 5% ethylenediaminetetraacetic acid (EDTA) tubes (Vacuette, Greiner Labortechnik, Germany).

In this study, we used the cytokinesis-block micronucleus cytome (CBMNcyt) assay in peripheral blood lymphocytes collected with heparin and the buccal micronucleus cytome (BMNcyt) assay in exfoliated buccal cells. For these two assays, cells were collected from 20 subjects. Both the AD and the control groups consisted of 7 females and 3 males (mean age: 81.0 ± 7.3 and 82.0 ± 8.7 , respectively, $p = 0.909$), with patients presenting the disease for a mean duration of 3.8 ± 3.0 years (range: 1–10 years). All patients were on cholinergic therapy at the time of enrollment in the study. During a face-to-face interview, volunteers or their caregivers were asked to answer an adapted questionnaire from the International Commission for Protection against Environmental Mutagens and Carcinogens [28] to minimize confounding factors; all subjects in these protocols were purposely selected to be non-smokers, non-alcohol drinkers with no recent X-ray exposure. AD patients and controls were matched for sex, age, and ethnic background.

For the TBARs study, blood samples were collected from 50 subjects by venipuncture with EDTA. Both the AD and the

control groups consisted of 20 females and 5 males (mean age: 80.0 ± 5.5 and 80.5 ± 10.7 , respectively, $p = 0.884$), with patients presenting the disease for a mean duration of 4.3 ± 3.0 years (range: 1–10 years).

This study was approved by the Research Ethics Committee of Emescam (School of Health Sciences of Santa Casa of Vitória), and written informed consent was obtained from each subject or from his/her surrogate prior to his/her inclusion into the study. All experiments were conducted in accordance with the Declaration of Helsinki.

Genotyping

For genotyping, the samples were stored at $4\text{ }^{\circ}\text{C}$ prior to analyses. The concentration and purity of genomic DNA were measured using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, DE, USA). SNPs in the *APOE* (rs429358: T > C, RefSeq NG_007084.2 and rs7412: C > T, RefSeq NG_007084.2), *SIRT1* (rs2273773: C > T, RefSeq NG_050664.1) and *SOD2* (rs4880: A > G, RefSeq NM_000636.3) genes were genotyped using real-time quantitative polymerase chain reaction (qPCR).

For each SNP, three standard genotypes identified through Sanger sequencing were used in all reactions of qPCR. The sequencing analysis was conducted using BioEdit software v.7.2.5 for Windows (Ibis Biosciences, Carlsbad, CA, USA) in ABI PRISM 3130XL Genetic Analyzer/HITACHI (Applied Biosystems, Carlsbad, California, USA).

Genomic DNA ($30\text{ ng}/\mu\text{l}$) was employed for qPCR on a 7500 Fast Real-Time PCR System according to the manufacturer's instructions (TaqMan SNP Genotyping Assay – Applied Biosystems, Carlsbad, CA, USA). Genotypes were analyzed using SDS v.2.0.5 software.

CBMNcyt assay

The lymphocytes were cultured according to the methodology developed by Fenech and Morley (1985) [29]. For each subject, 2000 binucleated cells with well-preserved cytoplasm (1000 cells from each of two replicate cultures) were scored for the presence of micronuclei (MN), nucleoplasmic bridges, and buds, following the criteria described by Bolognesi and Fenech (2013) [30]. Frequencies of cells carrying these abnormalities were expressed per 1000 binucleated cells scored. The cell cycle alteration was expressed by the variations of the Nuclear Division Index (NDI), which was calculated by counting 1000 cells per subject according to Eastmond and Tucker (1989). The analyses were performed using an optical microscope ($\times 1000$).

BMNcyt assay

Subjects were asked to rinse their mouths with water, and a premoistened cytobrush was used to sample cells from both sides of the interior cheek. The swab was immersed in 5 ml cold saline (0.9% w/v, aqueous NaCl) in a conical tube. The samples were centrifuged at 1500 rpm for 10 min, and the resulting pellets were washed three times more with 2 ml of saline under the same centrifugation conditions. The cell suspension was dropped onto a slide, air-dried, and fixed in 80% methanol. Slides were stained with pure Leishman for 2 min followed by 15 min in 10% Leishman aqueous solution, rinsed in distilled water, and air-dried. We used the criteria of scoring described by Thomas et al. (2009). The BMNcyt assay measures biomarkers of DNA damage (MN and buds), cell death (condensed chromatin, pyknosis, karyorrhexis and karyolysis), and cytokinetic defects (binucleated cells). Two thousand cells per subject (1000 from each duplicate slide) were scored blindly by the same person, using optical microscopy ($\times 1000$).

TBAR assay

In this work, we adopted a modified method from Buege and Aust (1978) [31]. Blood samples were kept in an ice bath until the time of centrifugation, which was performed at 1500 rpm for 10 min. Plasma samples were stored at $-80\text{ }^{\circ}\text{C}$ until analysis. To a volume of 1 ml of plasma, 2 ml of aqueous acid solution (15% trichloroacetic acid, 0.375% TBA, 0.25 N HCl, and 2.5 mM butylated hydroxytoluene—BHT), diluted in ethanol, was added. BHT, an antioxidant, was added to prevent MDA formation, which could result in falsely elevated TBA reactivity, during the assay. Samples were heated for 30 min at $95\text{ }^{\circ}\text{C}$, cooled at room temperature, and centrifuged at 3000 rpm for 10 min. Absorbance values were read in a spectrophotometer at 532 and 572 nm. Absorbance at 572 nm was subtracted from absorbance at 532 nm. MDA values were estimated with the extinction coefficient of MDA-TBA complex at 532 nm = $1.56 \times 10^5\text{ cm}^{-1}\text{ M}^{-1}$ [32].

Statistical analysis

To test the association between AD and the SNPs, Pearson's chi-square and Fisher's exact tests and logistic regression were performed. Additionally, the odds ratio (OR), confidence interval (CI, 95%), and Hardy-Weinberg equilibrium (H-WE) were calculated. Statistical analysis was performed using SPSS software v23.0 for Windows (IBM Corporation, Armonk, New York, USA). For the CBMNcyt, BMNcyt, and TBARs assays, the normality of the data was checked using the D'Agostino-Pearson test. Comparisons of mean values between groups were examined by parametric (unpaired Student's *t* test) or non-parametric (Mann-Whitney *U*)

tests, when applicable. Data were expressed as the mean \pm standard deviation, and $p < 0.05$, for a two-tailed test, was considered statistically significant.

Results

Genotyping

The characteristics of each sample group, such as ethnic background, sex, age, *APOE* status, and level of education, are shown in Table 1. The education of almost 90% of the literate of the total sample had less than 4 years of study. No significant differences between the samples were observed in relation to ethnic background, sex, or age. However, a low education level appeared to affect the predisposition for the disease.

The genotypic frequencies of the SNPs and results of logistic regression analysis are shown in Tables 2 and 3, respectively. As expected, the *APOE* $\epsilon 4\epsilon 4$ genotype frequency was significantly increased in AD patients. We also found a significant value for the *APOE* $\epsilon 3\epsilon 3$ genotype. A positive

association with AD was detected for a *SIRT1* variant but not for *SOD2*. All of the polymorphisms were in H-WE equilibrium in both the controls and AD patients.

Genomic instability and oxidative stress

The results of the CBMNcyt assay in blood lymphocytes and the BMNcyt assay in epithelial buccal cells are summarized in Table 4. We detected higher frequencies of MN, nucleoplasmic bridges, and buds in lymphocytes from AD patients, compared with controls. Moreover, the evaluation of epithelial buccal cells also revealed elevated frequencies of MN, buds, binucleated cells, condensed chromatin, karyorrhexis, and pyknosis in AD patients, in relation to controls.

We performed a TBAR assay to estimate the level of MDA, a biomarker of lipid peroxidation. The mean level of MDA in plasma was significantly higher in the AD patients than in the controls (Fig. 1).

Discussion

The current study allowed the identification of positive associations of *SIRT1* (rs2273773) and *APOE* (rs429358 and rs7412) polymorphisms with AD. Significant results were also found in AD patients for chromosomal damage and a higher level of plasma malondialdehyde. Our results support the hypothesis that oxidative stress may play an important role in the etiology of AD.

Several lines of evidence suggest that oxidative damage is involved in the aging process and, particularly, in neurodegenerative diseases such as AD. The brain metabolism of AD individuals appears to be more affected by reactive oxygen species damage than that of healthy older adults [17, 33]. This may partially explain the increase in oxidative damage to nucleic acids and a mitochondrial dysfunction in the brain affected by AD [34–36].

Our results show that individuals carrying the CT genotype of the *SIRT1* rs2273773 variant have a greater probability of developing AD than those who carry the TT genotype. Data provide evidence for implication of the TT genotype as a protective factor for AD. We did not find a positive result for the CC genotype, probably because of the sample size. *SIRT1* is one of the sirtuins involved in the control of apoptosis, cell survival, the modulation of reactive oxygen species (ROS) levels, and mitochondrial biogenesis and function [37]. It constitutes a molecular link between aging and human degenerative disorders [38].

The *SIRT1* rs2273773 polymorphism has been analyzed in several studies for a role in neuroprotection against the formation of ROS in AD and other neurotoxic conditions [39, 40]. As *SIRT1* can regulate the aging and metabolic processes involved in the pathogenesis of AD, it may represent a potential

Table 1 Characteristics of the sample

| Variable | AD patients <i>n</i> = 109 (100%) | Controls <i>n</i> = 223 (100%) | <i>p</i> value |
|--------------------|--------------------------------------|-----------------------------------|----------------------|
| Sex | | | |
| Women | 75 (68.8%) | 164 (73.5%) | 0.366 |
| Men | 34 (31.2%) | 59 (26.5%) | |
| Ethnic background | | | |
| Caucasians | 65 (59.6%) | 124 (55.6%) | |
| Afro-Brazilians | 39 (35.8%) | 96 (43.0%) | 0.113 |
| No identification | 5 (4.6%) | 3 (1.4%) | |
| Schooling | | | |
| Literate | 53 (48.6%) | 140 (62.8%) | |
| Illiterate | 37 (34.0%) | 65 (29.1%) | 0.012* |
| No identification | 19 (17.4%) | 18 (8.1%) | |
| <i>APOE</i> status | | | |
| $\epsilon 4 -$ | 49 (45.0%) | 146 (65.5%) | < 0.001 ^a |
| $\epsilon 4 +$ | 58 (53.2%) | 71 (31.8%) | |
| No identification | 2 (1.8%) | 6 (2.7%) | |
| Age (mean and SD) | 82.3 \pm 7.6 | 81.2 \pm 9.9 | 0.144 |
| MMSE (mean and SD) | 13 \pm 6 | 21 \pm 5 | < 0.001 |
| CDR | 2 | – | |

AD patients = Alzheimer's disease patients; $\epsilon 4 + = \epsilon 4$ carriers; $\epsilon 4 - = \epsilon 4$ non-carriers; SD = standard deviation; ^a = AD patient versus control group by chi-square test; ^b = *p* value of AD patient versus control group by Mann-Whitney *U* test; *p* value ≤ 0.05 considered significant. MMSE = Mini-Mental State Examination (value considering the schooling level and the average evolution time of the disease in AD patients); CDR = Dementia Rating Scale

therapeutic target. Our results support the hypothesis that the *SIRT1* rs2273773 polymorphism is associated with AD and reinforces the supposition that oxidative stress can be involved in its pathogenesis.

Some genetic variants increase the risk of developing AD, particularly the $\epsilon 4$ allele of the *Apolipoprotein E* (ApoE) gene, whose product is involved in the transport of cholesterol [41, 42]. In our work, an AD patient who carries two copies of this allele is 8 times more likely to develop the disease than those who develops the disease but carries only one or no copies of the *ApoE- $\epsilon 4$* allele (data not shown). Carrying the *APOE- $\epsilon 4$* allele is an established risk factor for sporadic AD [43, 44]. Our results showed that *APOE- $\epsilon 4\epsilon 4$* AD patients present an increased risk of developing AD, as reported worldwide, including in populations from North America, Europe, and Asia [44–46]. Regarding the *APOE- $\epsilon 3\epsilon 3$* genotype, our findings indicated that this SNP acts as a protective factor, which is in accordance with the previous reports in an Italian population [47]. The $\epsilon 3$ allele may act similarly to an antioxidant agent [48], protecting against AD [49]. However, there is no consensus regarding the effective role of the *APOE- $\epsilon 3$* allele in the pathology of AD in different populations [49, 50].

SODs are crucial to the endogenous signaling system that protects cells and tissues from oxidative stress [51]. Although functional studies have shown a role for SOD2 in the disease, in the present study, the *SOD2* polymorphism (rs4880) did not exhibit an association with AD. Early studies employing the CBMNcyt assay to assess chromosomal damage in cells of

AD patients reported a higher frequency of MN in peripheral lymphocytes of these patients than in those of controls [52, 53]. In our experiments, we additionally reported the frequencies of nucleoplasmic bridges and buds in the lymphocytes of AD patients. The inclusion of these markers of chromosomal damage allows the assessment of complementary events of genomic instability. The BMNcyt assay in MN-exfoliated buccal cells is a potential biomarker of genome instability. This assay has been used to measure increased risk for accelerated aging, cancer, and neurodegenerative diseases [54] as well as frailty syndrome and mild cognitive impairment [55, 56]. In our study, we adopted the cytome approach to score not only MN but also other biomarkers of nuclear abnormalities (buds), cytokinetic defects (binucleated cells), and cell death (condensed chromatin, karyorrhectic, karyolytic and pyknotic cells).

Using the buccal micronucleus cytome assay, Thomas and Fenech (2007) [57] detected a slight increase in MN frequency in their AD cohort, but this increase failed to reach significance. They also reported significantly lower frequencies of condensed chromatin cells and karyorrhectic cells in AD patients than in controls. Their results are in the opposite direction of the results obtained in our study; we found significantly increased frequencies of these markers in AD patients compared with controls. Additionally, we detected significantly enhanced frequencies of buds, binucleated cells, and pyknosis in AD patients. Such different results may be partially explained by the features of the cohorts: our AD cohort

Table 2 Genotype frequencies of polymorphisms in the study

| Polymorphisms | AD patients | Controls | OR (95% CI) | <i>p</i> value |
|--------------------------------------|-------------|-------------|----------------------|----------------|
| <i>SIRT1</i> (rs2273773) | | | | |
| TT | 78 (71.6%) | 194 (88.2%) | 0.376 (0.213–0.665) | 0.001* |
| CT | 29 (26.6%) | 22 (10.0%) | 3.312 (1.796–6.106) | < 0.001* |
| CC | 2 (1.8%) | 4 (1.8%) | 1.023 (0.185–5.675) | 0.641 |
| Total | 109 (100%) | 220 (100%) | | |
| <i>SOD2</i> (rs4880) | | | | |
| CC | 29 (26.6%) | 58 (26.9%) | 0.537 (0.587–1.662) | 0.988 |
| CT | 54 (49.5%) | 107 (49.5%) | 1.000 (0.631–1.585) | 0.546 |
| TT | 26 (23.9%) | 51 (23.6%) | 1.013 (0.590–1.741) | 0.533 |
| Total | 109 (100%) | 216 (100%) | | |
| <i>APOE</i> (rs429358 and rs7412) | | | | |
| $\epsilon 3\epsilon 4$ | 40 (37.4%) | 61 (28.1%) | 1.540 (0.945–2.509) | 0.082 |
| $\epsilon 2\epsilon 2$ | 0 (0.0%) | 1 (0.5%) | 0.996 (0.987–1.004) | 0.482 |
| $\epsilon 2\epsilon 3$ | 9 (8.4%) | 18 (8.3%) | 1.025 (0.445–2.263) | 0.954 |
| $\epsilon 2\epsilon 4$ | 3 (2.8%) | 6 (2.8%) | 1.024 (0.251–4.173) | 0.974 |
| $\epsilon 3\epsilon 3$ | 40 (37.4%) | 127 (58.5%) | 0.438 (0.274–0.702) | 0.001* |
| $\epsilon 4\epsilon 4$ | 15 (14.0%) | 4 (1.8%) | 8.737 (2.825–27.022) | < 0.001* |
| Total | 107 (100%) | 217 (100%) | | |

AD patients = Alzheimer's disease patients; OR = odds ratio; CI = confidence interval; *p* value ≤ 0.05 considered significant

Table 3 Logistic regression analysis of polymorphisms in the study

| Polymorphism | AD patients | Controls | OR (95% CI) | ^a <i>p</i> value | OR (95% CI) | ^b <i>p</i> value |
|-----------------------------------|-------------|-------------|----------------------|-----------------------------|----------------------|-----------------------------|
| <i>SIRT1</i> (rs2273773) | | | | | | |
| TT | 78 (71.6%) | 194 (88.2%) | 1 (Reference) | 1 (Reference) | 1 (Reference) | – |
| CT | 29 (26.6%) | 22 (10.0%) | 3.279 (1.776–6.054) | 0.000 | 4.067 (2.099–7.872) | < 0.001* |
| CC | 2 (1.8%) | 4 (1.8%) | 1.244 (0.223–6.928) | 0.804 | 1.253 (0.204–7.693) | 0.808 |
| Total | 109 (100%) | 220 (100%) | | | | |
| <i>SOD2</i> (rs4880) | | | | | | |
| CC | 29 (26.6%) | 58 (26.9%) | 1 (Reference) | 1 (Reference) | 1 (Reference) | – |
| CT | 54 (49.5%) | 107 (49.5%) | 1.009 (0.581–1.755) | 0.974 | 0.938 (0.522–1.688) | 0.832 |
| TT | 26 (23.9%) | 51 (23.6%) | 1.020 (0.533–1.952) | 0.953 | 0.872 (0.434–1.748) | 0.669 |
| Total | 109 (100%) | 216 (100%) | | | | |
| <i>APOE</i> (rs429358 and rs7412) | | | | | | |
| ε3ε4 | 40 (37.4%) | 61 (28.1%) | 1.540 (0.945–2.509) | 0.082 | 1 (Reference) | – |
| ε2ε2 | 0 (0.0%) | 1(0.5%) | 0.000 (0.000) | 1 | 0.000 (0.000) | 1 |
| ε2ε3 | 9 (8.4%) | 18 (8.3%) | 0.762 (0.212–1.864) | 0.552 | 0.491 (0.162–1.488) | 0.209 |
| ε2ε4 | 3 (2.8%) | 6 (2.8%) | 0.762 (0.180–3.225) | 0.712 | 0.796 (0.134–4.738) | 0.802 |
| ε3ε3 | 40 (37.4%) | 127 (58.5%) | 0.480 (0.282–0.819) | 0.007* | 0.450 (0.251–0.806) | 0.007* |
| ε4ε4 | 15 (14.0%) | 4 (1.8%) | 5.719 (1.770–18.478) | 0.004* | 5.197 (1.551–17.415) | 0.008* |
| Total | 107 (100%) | 217 (100%) | | | | |

AD patients = Alzheimer's disease patients; OR = odds ratio; CI = confidence interval; *p* value ≤ 0.05 considered significant; ^a = crude *p* value; ^b = *p* value adjusted by age, gender, educational attainment, ethnic background, and *APOE* ε4 status

comprised patients who had been diagnosed with the disease for a mean period of 3.8 ± 3.0 years and were undergoing treatment with cholinesterase inhibitors at the time of enrollment in the study. In contrast, the study by Thomas and Fenech (2007) [57] comprised patients newly diagnosed with AD prior to the commencement of any treatment. We suggest

that DNA damage increases with the progression of the disease, due to the elevated oxidative stress, a key factor in the degenerative neuronal death and progression of Alzheimer's disease [7, 58]. Nevertheless, these changes indicate that the buccal cells of AD patients show significant alterations in cellular kinetics and may be useful as predictive biomarkers

Table 4 The CBMNcyt assay in peripheral blood lymphocytes and the BMNcyt assay from AD patients and healthy controls.

| Parameter | AD patients (<i>n</i> = 10) Mean ± standard deviation | Controls (<i>n</i> = 10) Mean ± standard deviation | <i>p</i> value |
|-----------------------|---|--|--------------------|
| CBMNcyt assay | | | |
| MN | 11.5 ± 3.3 | 7.7 ± 2.7 | 0.015 ^a |
| Nucleoplasmic bridges | 1.4 ± 0.6 | 0.6 ± 0.2 | 0.002 ^a |
| Nuclear buds | 4.0 ± 1.9 | 1.9 ± 1.0 | 0.009 ^a |
| NDI | 1.9 ± 0.9 | 2.0 ± 0.2 | 0.150 |
| BMNcyt assay | | | |
| MN | 5.0 ± 1.6 | 3.1 ± 1.6 | 0.016 ^b |
| Nuclear buds | 2.6 ± 1.4 | 0.7 ± 0.6 | 0.001 ^b |
| Binucleated cells | 27.4 ± 15.4 | 12.0 ± 3.5 | 0.006 ^b |
| Condensed chromatin | 66.4 ± 25.4 | 33.6 ± 21.7 | 0.006 ^b |
| Karyorrhexis | 32.5 ± 17.9 | 16.9 ± 15.4 | 0.040 ^b |
| Karyolysis | 168.0 ± 129.8 | 283.7 ± 141.7 | 0.073 |
| Pyknosis | 4.2 ± 1.4 | 2.6 ± 1.2 | 0.018 ^b |

MN = micronucleus, NDI = nuclear index division in 1000 cells per subject, *n* = number of subjects. ^a Two thousand binucleated cells (one thousand from each of the duplicate cultures) were scored per subject in the CBMNcyt assay. ^b Two thousand epithelial buccal cells (one thousand from each of the duplicate slides) were scored per subject in the BMNcyt assay

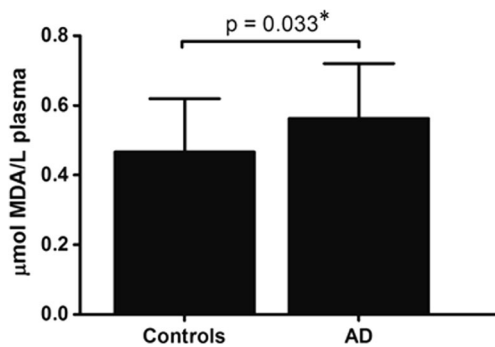


Fig. 1 Comparison of MDA levels in plasma of AD patients ($n = 25$) and healthy controls ($n = 25$)

in identifying individuals with elevated risk for developing or having AD.

We used the TBAR assay to estimate the level of MDA, the most abundant aldehyde resulting from lipid peroxidation [20]. Measurement of MDA by TBAR assay is the most widely used method to estimate the overall lipid peroxidation level [59]. Similar to the previous studies, we detected a significantly higher plasma level of MDA in AD patients than in controls, supporting the hypothesis that oxidative stress may be related to the etiology of AD [60, 61]. Since MDA is a toxic and mutagenic product and can damage genomic material and the membranes of cells [18, 19], we suggest that the higher frequencies of DNA damage observed in the CBM_Ncyt and BM_Ncyt in AD patients may be a consequence of elevated oxidative damage.

Therefore, our results suggest that AD patients show increased oxidative stress and genomic instability as compared with controls and the CT polymorphism of *SIRT1* is associated with increase the risk of the AD.

Conclusion

In conclusion, our results show that *SIRT1* and *APOE* variants are associated with AD, a condition with increased oxidative stress and genomic instability characterized by elevated lipoperoxidation and genomic and cell damage. Part of the discrepancy observed in our results and previous publications could be explained due to the presence of confounders factors in the samples not evaluated in this study such diet, antioxidant intake and inflammation. Although further research is required to achieve an overall understanding of the disease, we believe that our findings are important for the characterization of genes and other factors related to AD that could be useful in the future to improve strategies of treatment and diagnosis of the disease.

Funding This study was supported by Fundo de Apoio à Ciência e Tecnologia do Município de Vitória (grant number 5928/2011), Fundação de Amparo à Pesquisa do Estado do Espírito Santo,

Programa de Pesquisa para o Sistema Único de Saúde (grant number 65849124), Ministério da Ciência, Tecnologia e Inovação, Conselho Nacional de Desenvolvimento Científico e Tecnológico, Ministério da Educação, and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (grant number 552672/2011–4).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval None.

References

- Prince M, Comas-Herrera A, Knapp M et al (2016) World Alzheimer Report 2016 Improving healthcare for people living with dementia. Coverage, Quality and costs now and in the future. In: Alzheimer's Disease International (ADI). Alzheimer's Disease International (ADI), London, pp 1–140
- Pimplikar SW, Nixon RA, Robakis NK et al (2010) Amyloid-independent mechanisms in Alzheimer's disease pathogenesis. *J Neurosci* 30:14946–14954. <https://doi.org/10.1523/JNEUROSCI.4305-10.2010>
- Karch CM, Goate AM (2014) Alzheimer's disease risk genes and mechanisms of disease pathogenesis. *Biol Psychiatry* 1–9. <https://doi.org/10.1016/j.biopsych.2014.05.006>
- Hardas SS, Sultana R, Clark AM et al (2013) Oxidative modification of lipoic acid by HNE in Alzheimer disease brain. *Redox Biol* 1:80–85
- Jomova K, Vondrakova D, Lawson M, Valko M (2010) Metals, oxidative stress and neurodegenerative disorders. *Mol Cell Biochem* 345:91–104
- Lovell MA, Ehmman WD, Butler SM, Markesbery WR (1995) Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in Alzheimer's disease. *Neurology* 45: 1594–1601
- Lovell MA, Markesbery WR (2007) Oxidative damage in mild cognitive impairment and early Alzheimer's disease. *J Neurosci Res* 85:3036–3040
- Benedetti D, Nunes E, Sarmento M et al (2013) Genetic damage in soybean workers exposed to pesticides: evaluation with the comet and buccal micronucleus cytome assays. *Mutat Res Genet Toxicol Environ Mutagen* 752:28–33
- Celik A, Diler SB, Eke D (2010) Assessment of genetic damage in buccal epithelium cells of painters: micronucleus, nuclear changes, and repair index. *DNA Cell Biol* 29:277–284
- Holland N, Bolognesi C, Kirsch-Volders M et al (2008) The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: the HUMN project perspective on current status and knowledge gaps. *Mutat Res Rev Mutat Res* 659:93–108
- Migliore L, Coppèdè F, Fenech M, Thomas P (2011) Association of micronucleus frequency with neurodegenerative diseases. *Mutagenesis* 26:85–92
- Ortiz GG, Moisés FPP, Mireles-Ramírez M et al (2017) Chapter One-Oxidative stress: love and hate history in central nervous system. *Adv Protein Chem Struct Biol* 108:1–31
- Rizzi L, Roriz-Cruz M (2018) Sirtuin 1 and Alzheimer's disease: an up-to-date review. *Neuropeptides* 71:54–60. <https://doi.org/10.1016/j.npep.2018.07.001>
- Lee HJ, Yang SJ (2017) Aging-related correlation between serum sirtuin 1 activities and basal metabolic rate in women, but not in men. *Clin Nutr Res* 6:18–26

15. Wiener HW, Perry RT, Chen Z et al (2007) A polymorphism in SOD2 is associated with development of Alzheimer's disease. *Genes Brain Behav* 6:770–776. <https://doi.org/10.1111/j.1601-183X.2007.00308.x>
16. Zhao N, Liu C-C, Qiao W, Bu G (2018) Apolipoprotein E, receptors, and modulation of Alzheimer's disease. *Biol Psychiatry* 83:347–357. <https://doi.org/10.1016/j.biopsych.2017.03.003>
17. Islam MT (2017) Oxidative stress and mitochondrial dysfunction-linked neurodegenerative disorders. *Neurol Res* 39:73–82. <https://doi.org/10.1080/01616412.2016.1251711>
18. Marnett LJ (1999) Lipid peroxidation—DNA damage by malondialdehyde. *Mutat Res Fundam Mol Mech Mutagen* 424:83–95
19. Pizzimenti S, Ciamporcerio ES, Daga M et al (2013) Interaction of aldehydes derived from lipid peroxidation and membrane proteins. *Front Physiol* 4:242
20. Uchida K (2013) Redox-derived damage-associated molecular patterns: ligand function of lipid peroxidation adducts. *Redox Biol* 1:94–96
21. Morris JC (1993) The Clinical Dementia Rating (CDR): Current version and scoring rules. *Neurology* 43:2412–2412. <https://doi.org/10.1212/WNL.43.11.2412-a>
22. McKhann GM, Knopman DS, Chertkow H et al (2011) The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 7:263–269
23. McKhann G, Drachman D, Folstein M et al (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group* under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 34:939–939. <https://doi.org/10.1212/WNL.34.7.939>
24. Fagundes Chaves ML, Camozzato AL, Godinho C et al (2007) Validity of the clinical dementia rating scale for the detection and staging of dementia in Brazilian patients. *Alzheimer Dis Assoc Disord* 21:210–217. <https://doi.org/10.1097/WAD.0b013e31811ff2b4>
25. Macedo Montaña MBM, Ramos LR (2005) Validade da versão em português da Clinical Dementia Rating. *Rev Saude Publica* 39:912–917. <https://doi.org/10.1590/S0034-89102005000600007>
26. Arevalo-Rodríguez I, Smailagic N, Roqué Figuls M et al (2015) Mini-Mental State Examination (MMSE) for the detection of Alzheimer's disease and other dementias in people with mild cognitive impairment (MCI). *Cochrane Database Syst Rev*
27. Menegardo CS, Friggi FA, Scardini JB et al (2019) Sundown syndrome in patients with Alzheimer's disease dementia. *Dement Neuropsychol* 13:469–474. <https://doi.org/10.1590/1980-57642018dn13-040015>
28. Carrano AV, Natarajan AT (1988) Considerations for population monitoring using cytogenetic techniques. *Mutat Res Genet Toxicol* 204:379–406
29. Fenech M, Morley A (1985) Solutions to the kinetic problem in the micronucleus assay. *Cytobios* 43:233–246
30. Bolognesi C, Fenech M (2013) Micronucleus assay in human cells: lymphocytes and buccal cells. In: *Genotoxicity Assessment*. Springer, pp 191–207
31. Buege JA, Aust SD (1978) Microsomal lipid peroxidation. *Methods Enzymol* 52:302–310. [https://doi.org/10.1016/S0076-6879\(78\)52032-6](https://doi.org/10.1016/S0076-6879(78)52032-6)
32. Valenzuela A (1991) The biological significance of malondialdehyde determination in the assessment of tissue oxidative stress. *Life Sci* 48:301–309
33. Christen Y (2000) Oxidative stress and Alzheimer disease. *Am J Clin Nutr* 71:621 s–629 s
34. Coskun PE, Beal MF, Wallace DC (2004) Alzheimer's brains harbor somatic mtDNA control-region mutations that suppress mitochondrial transcription and replication. *Proc Natl Acad Sci U S A* 101:10726–10731
35. Mecocci P, MacGarvey U, Beal MF (1994) Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. *Ann Neurol* 36:747–751
36. Pei L, Wallace DC (2018) Mitochondrial Etiology of Neuropsychiatric Disorders. *Biol Psychiatry* 83:722–730
37. Treviño-Saldaña N, García-Rivas G (2017) Regulation of sirtuin-mediated protein deacetylation by cardioprotective phytochemicals. *Oxidative Med Cell Longev* 2017:1–16. <https://doi.org/10.1155/2017/1750306>
38. Hadar A, Milanese E, Walczak M et al (2018) SIRT1, miR-132 and miR-212 link human longevity to Alzheimer's disease. *Sci Rep* 8:8465. <https://doi.org/10.1038/s41598-018-26547-6>
39. Pasinetti GM, Zhao Z, Qin W et al (2007) Caloric intake and Alzheimer's disease. In: *Mechanisms of dietary restriction in aging and disease*. Karger Publishers, pp 159–175
40. Julien C, Tremblay C, Émond V et al (2009) Sirtuin 1 reduction parallels the accumulation of tau in Alzheimer disease. *J Neuropathol Exp Neurol* 68:48–58
41. Muñoz SS, Gamer B, Ooi L (2019) Understanding the role of ApoE fragments in Alzheimer's disease. *Neurochem Res* 44:1297–1305. <https://doi.org/10.1007/s11064-018-2629-1>
42. Uddin MS, Kabir MT, Al Mamun A et al (2019) APOE and Alzheimer's disease: evidence mounts that targeting APOE4 may combat Alzheimer's pathogenesis. *Mol Neurobiol* 56:2450–2465. <https://doi.org/10.1007/s12035-018-1237-z>
43. Corder E, Saunders A, Strittmatter W et al (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 261:921–923. <https://doi.org/10.1126/science.8346443>
44. Farrer LA, Cupples LA, Haines JL et al (1997) Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease: a meta-analysis. *Jama* 278:1349–1356
45. Ewbank DC (2004) The APOE gene and differences in life expectancy in Europe. *J Gerontol Ser A Biol Med Sci* 59:B16–B20
46. Ji Y, Liu M, Huo YR et al (2013) Apolipoprotein E ϵ 4 frequency is increased among Chinese patients with frontotemporal dementia and Alzheimer's disease. *Dement Geriatr Cogn Disord* 36:163–170
47. Bosco P, Guéant-Rodríguez RM, Anello G et al (2005) Allele ϵ 4 of APOE is a stronger predictor of Alzheimer risk in Sicily than in continental South Italy. *Neurosci Lett* 388:168–172. <https://doi.org/10.1016/j.neulet.2005.06.056>
48. Miyata M, Smith JD (1996) Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and β -amyloid peptides. *Nat Genet* 14:55–61
49. Rebeck GW, Kindy M, LaDu MJ (2002) Apolipoprotein E and Alzheimer's disease: the protective effects of ApoE2 and E3. *J Alzheimers Dis* 4:145–154
50. Nielsen HM, Chen K, Lee W et al (2017) Peripheral apoE isoform levels in cognitively normal APOE ϵ 3/ ϵ 4 individuals are associated with regional gray matter volume and cerebral glucose metabolism. *Alzheimers Res Ther* 9:5. <https://doi.org/10.1186/s13195-016-0231-9>
51. Nozik-Grayck E, Suliman HB, Piantadosi CA (2005) Extracellular superoxide dismutase. *Int J Biochem Cell Biol* 37:2466–2471
52. Migliore L, Testa A, Scarpato R et al (1997) Spontaneous and induced aneuploidy in peripheral blood lymphocytes of patients with Alzheimer's disease. *Hum Genet* 101:299–305
53. Petrozzi L, Lucetti C, Scarpato R et al (2002) Cytogenetic alterations in lymphocytes of Alzheimer's disease and Parkinson's disease patients. *Neurol Sci* 23:s97–s98
54. Fenech M, Holland N, Zeiger E et al (2011) The HUMN and HUMNxL international collaboration projects on human micronucleus assays in lymphocytes and buccal cells—past, present and future. *Mutagenesis* 26:239–245

55. Lee SL, Thomas P, Hecker J et al (2015) Chromosomal DNA damage measured using the cytokinesis-block micronucleus cytome assay is significantly associated with cognitive impairment in South Australians. *Environ Mol Mutagen* 56:32–40. <https://doi.org/10.1002/em.21890>
56. Sánchez-Flores M, Marcos-Pérez D, Lorenzo-López L et al (2018) Frailty syndrome and genomic instability in older adults: suitability of the cytochrome micronucleus assay as a diagnostic tool. *J Gerontol Ser A* 73:864–872. <https://doi.org/10.1093/gerona/glx258>
57. Thomas P, Fenech M (2007) A review of genome mutation and Alzheimer's disease. *Mutagenesis* 22:15–33. <https://doi.org/10.1093/mutage/gel055>
58. Migliore L, Fontana I, Trippi F et al (2005) Oxidative DNA damage in peripheral leukocytes of mild cognitive impairment and AD patients. *Neurobiol Aging* 26:567–573. <https://doi.org/10.1016/j.neurobiolaging.2004.07.016>
59. Nielsen F, Mikkelsen BB, Nielsen JB et al (1997) Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. *Clin Chem* 43:1209–1214
60. Bourdel-Marchasson I, Delmas-Beauviex MC, Peuchant E et al (2001) Antioxidant defences and oxidative stress markers in erythrocytes and plasma from normally nourished elderly Alzheimer patients. *Age Ageing* 30:235–241. <https://doi.org/10.1093/ageing/30.3.235>
61. Torres LL, Quaglio NB, De Souza GT et al (2011) Peripheral oxidative stress biomarkers in mild cognitive impairment and Alzheimer's disease. *J Alzheimers Dis*. <https://doi.org/10.3233/JAD-2011-110284>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.