INTRODUCTION

Alzheimer’s disease (AD), the most common cause of dementia, is defined by progressive and irreversible neurodegeneration of the central nervous system that eventually leads to the gradual decline of cognitive function (Czech, Tremp, & Pradier, 2000; Oddo et al., 2003). The main feature of this disease is the deposition of amyloid-β...
(Aβ) protein in the extracellular space of the cerebral cortex and the walls of cerebral blood vessels. It has traditionally been thought that the Aβ deposited in the brain originates from the brain itself. However, it has recently been speculated that it may also come from Aβ at the periphery circulating in the blood. It is known that peripheral exposure to Aβ-rich brain extracts can also induce cerebral Aβ deposits in transgenic mice (Meyer-Luehmann et al., 2006). In this regard, insoluble Aβ oligomers have been experimentally shown to cause memory dysfunction, inhibit LTP and prolong LTD (Ding et al., 2019).

In addition to these neuronal lesions, some biomarkers of genomic instability have been found in patients with AD, including micronuclei (markers of loss and breakage of chromosomes), aneuploidies of chromosome 21, shortening of telomeres in lymphocytes and fibroblasts (Thomas, O'Callaghan, & Fenech, 2008), and increased oxidative stress markers (Butterfield & Sultana, 2011).

Telomeres are essential non-coding deoxyribonucleic acid (DNA)-protein complexes that cap the ends of the linear chromosomal DNA protecting the genome from damage. In mitotic cells, telomeres can shorten with each division, unless this can be counteracted or reversed by the telomere-lengthening enzyme, telomerase (Blackburn, 1991; Wolkowitz et al., 2011), telomere length regulation in mammalian cells is complex, and telomere length differs between tissues (Prowse & Greider, 1995) or even cells (Friedrich et al., 2000).

An increase in tissue cell proliferation results in the progressive shortening of telomeres, which triggers a DNA damage response that if repaired, restores proliferation. On the contrary, if the deterioration is irreversible, the cell keeps the cell cycle at halt and can be directed to apoptosis or cellular senescence (Campisi, 2011; Meeker et al., 2004). Telomere length depends on several factors such as the speed of degradation and the speed and time of action of telomerase in each chromosome (Cawthon, Smith, O’Brien, Sivatchenko, & Kerber, 2003; Jaskelioff et al., 2011). Hence, telomere length decreases differentially with time in all mitotic tissues.

The brains of patients diagnosed with AD show a significant extent of oxidative damage associated with the abnormal marked accumulation of β-amyloid and the deposition of neurofibrillary tangles (Christen, 2000); stress contributes to a significant decrease in telomerase activity and consequently causes an increase in the telomere-shortening rate (Kurz et al., 2004). Biochemical studies show TTAGGG repeats are preferred sites for iron binding and iron-mediated Fenton reactions, which generate hydroxyl radicals that induce the 5’ cleavage of GGG (Oikawa, Tada-Oikawa, & Kawanishi, 2001). There is a minimal shortening of less than 20 bp per cell division in cells with high antioxidative capacity, and this rate increases in cells with lower antioxidative defence. Cultivating cells under enhanced oxidative stress like mild hyperoxia (40% normobaric oxygen) shortens the telomeres prematurely and reduces the replicative lifespan accordingly (Saretzki & Von Zglinicki, 2002).

Telomere length has been associated with lifespan as it reflects the number of times a cell has divided (Boonekamp, Simons, Hemerik, & Verhulst, 2013); during ageing, 50–150 bp of telomeric DNA is lost with each proliferation cycle (Hochstrasser, Marksteiner, & Humpel, 2012). It also has been associated with disease conditions such as mental stress, obesity, smoking, type 2 diabetes mellitus, ischaemic heart diseases, AD and Parkinson’s disease (Allsopp et al., 1992; Brouilette, Singh, Thompson, Goodall, & Samani, 2003; Harley, Futerch, & Greider, 1990; Zakian, 1995).

Specifically, it has been reported that patients with AD show shorter telomeres in peripheral blood mononuclear cells, monocytes, T and B cells (Panossian et al., 2003), buccal cells and leucocytes compared to controls (Thomas et al., 2008). Also, significant differences in leucocyte telomere length (LTL) between controls, amnestic mild cognitive impairment and AD patients have been recently reported (Scarabino, Broggio, Gambina, & Corbo, 2017). However, there are no reports, to our knowledge, of how and if progression of AD affects telomere length. Hence, we decided to evaluate changes in telomere length and oxidative stress due to the progression of AD in a murine model. As our model has a genetic disposition to develop AD, we expected to see an intrinsic effect of disease progression (changes in oxidative stress) on telomere length.

2 | MATERIALS AND METHODS

2.1 | Animals

Homozygous 3xTg-AD (3xTg-AD; B6; 129-Psen1 <tm1Mpm> Tg (APPswe, tauP301L) 1Lfa) male mice were used as an AD murine model, and B6129SF2/J WT male mice were used as controls because this is the genetic background originally described for the transgenic mice used. Mice were bred in our laboratory from parents purchased from the Jackson Laboratory, and housed in the Bioterio de la Coordinación de Investigación en Salud. Mice were housed in a 12:12 light/dark cycle at 20–22°C with water and food ad libitum. All animals were genotyped. Animals of three different ages were used for this project 5, 9 and 13 months. Groups for telomere length measurement were as follows: 5 months 3xTgAD n = 7, 5 months WT n = 12; 9 months 3xTgAD n = 9, 9 months WT n = 12; and 13 months 3xTgAD n = 8, and 13 months WT n = 12. Groups for oxidative stress measurements were as follows: 5 months 3xTgAD n = 6, 5 months WT n = 6; 9 months 3xTgAD n = 6, 9 months WT n = 6.
2.2 Biological samples and DNA extraction

Blood samples were obtained from the mandibular vein (~0.2 ml) and treated with an erythrocyte lysis buffer. After the last blood sample was obtained from each group of age, mice were sacrificed with an overdose of pentobarbital administered intraperitoneally. The animals were then decapitated with a guillotine, the brain was carefully removed, and tissue from the hippocampus was dissected and homogenized with lysis buffer. DNA was extracted according to the manufacturer's instructions (Thermo Scientific DNA extraction kit, K0512). Purified DNA samples were stored at −70°C until use.

2.3 Telomere length assessment

All measurements were performed on samples from 5-, 9- and 13-month-old mice from both strains. We followed the qPCR (StepOnePlus Real-Time PCR System, Applied Biosystems) method published by O’Callaghan and Fenech (O’Callaghan & Fenech, 2011), an absolute quantification method that introduced an oligomer standard. A standard curve was used to determine telomere length; the 36B4 housekeeping gene was used as an endogenous calibrator. The number of copies of telomeric repeats was determined by the standard curve of telomere standard, while the standard curve of 36B4 STD was used as control; oligomers was HPLC-purified synthetized.

The Ct values of each sample were extrapolated in their corresponding curves by a linear regression test. The Maxima SYBR Green/ROX qPCR Master Mix 2X (Thermo Scientific) was used. The cycling conditions for both genes were as follows: 10 min at 95°C, followed by 40 cycles of 95°C for 15 s, and 60°C for 1 min, followed by a melting curve. Results are expressed as absolute telomere length (aTL); Kb telomere/diploid genome and were obtained as suggested by O’Callaghan and Fenech (O’Callaghan & Fenech, 2011).

2.4 Oxidative Stress measurements

All measurements were performed on samples from 5- and 9-month-old mice from both strains. No 13-month-old mice were used for these measurements.

2.4.1 Reactive oxygen species

Reactive oxygen species (ROS) were measured by the oxidation of 2-7-dichlorofluorescein (DCFH) to the fluorescent oxidized compound 2-7- dichlorofluorescein (DCF) by the presence of hydrogen peroxide. Several reactive intermediates can oxidize DCFH, so it cannot be used to determine the presence of a specific reactive species.

To evaluate the formation of reactive oxygen species (ROS) by fluorometry, 60 µl of homogenates of the hippocampus and whole blood cells were used. A final volume of 200 µl was obtained with 1x PBS buffer, 10 µl of a DCFH diacetate at 75 µM. Samples were incubated in the dark for 30 min at 37°C, then centrifuged at 5,590 g for 10 min; the supernatants were read in a fluorometer at an excitation wavelength of 480 nm and emission of 532 nm. Results are expressed in nM of DCFH in mg of tissue samples or nM of DCFH in µl of serum.

2.4.2 Lipid peroxidation

Lipid peroxidation was measured by the production of malondialdehyde (MDA); 120 µl of the homogenate of the hippocampus and 60 µl of whole blood cells were mixed with 60 µl of 1x PBS and 120 µl of the TBA reagent (0.375 g of TBA + 15 g of trichloroacetic acid + 2.54 ml of concentrated HCl). Samples were placed in a boiling bath (94°C) for 20 min subsequently centrifuged at 6,163 g for 15 min. The optical density of the supernatant was determined (BioTek plate reader) at a wavelength of 532 nm. Results are expressed as µM of MDA in µl of serum and µM of MDA in mg of tissue sample.

2.4.3 Mitochondrial viability

Mitochondrial viability was measured by the reduction of 3-(4,5-dimethylthiazol-2-yl)bromide-2,5-diphenyltetrazole (MTT nM/µl); 100 µl of brain tissue homogenate was used, to which 10 µl of an MTT solution (5mg/ml) was added, and incubated for 30 min at 37°C in the dark. After incubation, samples were centrifuged at 8,050 g for 3 min. The supernatant was removed, and 500 µl of isopropanol acid was added to the pellet where it was suspended. Samples were read at a wavelength of 560 nm in a plate reader. Results are expressed as µM of MTT in mg of tissue.

2.5 Statistical analysis

The data analysis was carried out in Prism 6.0e and SPSS 21 commercial software. As a first approach to explore the molecular changes in the progression of Alzheimer’s disease in a murine transgenic model (3xTgAD), we measured absolute telomere length (aTL; kb) at 5, 9 and 13 months, in addition to reactive oxygen species (DCFH; nM/µl), lipid peroxidation (MDA; µM/µl) and mitochondrial functionality (MTT;
nM/µl) at 5 and 9 months. All measurements were carried out on the hippocampus homogenate and whole blood cells. Quantitative variables are presented as the arithmetic median and maximums and minimums per age group: 3, 5 and 13 months.

Scattergrams show the distributions of aTL among age groups, p-values were calculated by Kruskal–Wallis non-parametric test, and when statistical significance between groups was obtained, we performed a post hoc test (Dunn’s test) to discriminate the specific groups with significantly different medians that differ from the others.

Differences between ROS, MDA and MTT among age groups are represented as scattergrams. A Kruskal–Wallis (KW) non-parametric test was used to calculate p-values when statistical significance between groups was obtained, we performed a post hoc test (Dunn’s test) to discriminate the specific groups with significantly different medians that differ from the others; in some cases, a Mann–Whitney test was used to compare between two groups.

To determine the possible relationship and strength of the relationship between ROS, MDA and MTT with absolute telomere length among whole blood cells or hippocampus from either AD (3xTgAD) or Wt (B6129SF2/J) mice at 5 or 9 months, we performed a Spearman’s rank-order correlation (r_s) analysis and considered the statistical significance of the correlation according to p-values <.05.

### RESULTS

We measured telomere length and oxidative stress in the progression of AD in a murine model. Table 1 shows the mean values of telomere length, measured in whole blood cells and hippocampus at 5, 9 and 13 months, as well as ROS, lipid peroxidation and mitochondrial viability measured at 5 and 9 months, for both transgenic and wild-type strains.

#### 3.1 Telomere length

Telomere length in whole blood cells of the transgenic strain showed significant differences between age groups (KW; H(2) = 14.01, p < .001), the 13-month-old group was significantly different from the other two groups (Dunn’s post hoc; p = .014, p = .002). No significant differences were found between age groups from the wild-type strain (KW; H(2) = 0.70 p = .70). When compared between mice strains, significant differences were found between 13-month-old transgenic and wild-type mice (Dunn’s post hoc; p = .016) as can be seen in Figure 1a.

Telomere length was also measured in the hippocampus, one of the first structures affected by AD. We observed a telomere length decreasing tendency for HC of transgenic mice; nevertheless, we did not find significant differences between age groups within strains (KW; H(2) = 2.45 p = .31 for transgensics and KW; H(2) = 2.02 p = .37 for wild type).

| Strain | Age (months) | Sample      | aTL (Mb/diploid genome) | DCFH (nM) | MTT (µM) | MDA (µM) |
|--------|--------------|-------------|-------------------------|-----------|----------|----------|
| Wt     | 5            | Hippocampus | 172.1 ± 132.9           | 100.8 ± 67.6 | 0.08 ± 0.03 | 0.50 ± 0.17 |
|        |              | Whole blood cells | 131.3 ± 22.7          | 635.7 ± 158.9 | –        | 0.07 ± 0.05 |
|        | 9            | Hippocampus | 78.8 ± 28.7            | 215.2 ± 40.3 | 0.03 ± 0.01 | 0.94 ± 0.28 |
|        |              | Whole blood cells | 133.2 ± 124.2        | 1,295.0 ± 252.5 | –        | 0.09 ± 0.06 |
|        | 13           | Hippocampus | 134.0 ± 85.6           | –             | –        | –        |
|        |              | Whole blood cells | 96.7 ± 60.5          | –             | –        | –        |
| Tg     | 5            | Hippocampus | 106.9 ± 64.4           | 444.5 ± 197.3 | 0.10 ± 0.01 | 0.35 ± 0.14 |
|        |              | Whole blood cells | 149.0 ± 20.9         | 1,777.2 ± 339.5 | –        | 0.10 ± 0.04 |
|        | 9            | Hippocampus | 104.8 ± 62.3           | 1,442.3 ± 1,395.5 | 0.03 ± 0.01 | 1.25 ± 0.38 |
|        |              | Whole blood cells | 153.4 ± 64.7         | 3,796.1 ± 1,061.9 | –        | 2.81 ± 1.49 |
|        | 13           | Hippocampus | 52.2 ± 49.5            | –             | –        | –        |
|        |              | Whole blood cells | 15.4 ± 9.3           | –             | –        | –        |

Note: Mean ± SD

Abbreviations: aTL, absolute telomere length; Mb, megabase.
or between wild-type and transgenic mice (KW; H(6) = 5.58 \ p = .34) as can be seen in Figure 1b.

3.2 | Oxidative stress

In order to determine whether oxidative stress increased with the progression of AD, we measured ROS (by DCFH) and lipid peroxidation (by MDA) at 5 and 9 months of age. When measured in blood cells, both ROS and lipid peroxidation showed a significant increase between age groups within strains. As shown in Figure 2a, a Kruskal–Wallis test shows differences between groups (KW; H(3) = 18.79 \ p < .001). ROS in blood cells of 9-month-old transgenic mice is significantly higher than that of 5-month-old mice (Dunn’s post hoc; \ p < .001). ROS also increased between 5- and 9-month-old groups of the wild-type strain (Dunn’s post hoc; \ p = .002). ROS increases with age in both strains, but significantly more in the transgenic mice where the 9-month group also showed differences between strains (Dunn’s post hoc; \ p = .021).

Lipid peroxidation, measured by MDA (µM/µl), is shown in Figure 2b, where increases can only be seen in the 9-month-old transgenic mice group which is significantly different from every other group (KW; H(3) = 10.99 \ p = .01). No other significant differences in lipid peroxidation were found.

When measured in the hippocampus, ROS increased with age within both strains; a Mann–Whitney test showed differences between wild-type (Mdn = 83.30, Mdn = 210.3, \ U = 4 \ p = .026) and transgenic mice in both ages (Mdn = 427.5, Mdn = 905.3, \ U = 2 \ p = .032); moreover, we could observe that increase in ROS was more in transgenic mice at 9 months than wild-type mice at the same age (Mdn = 905.3, Mdn = 210.3, \ U = 0 \ p = .004) as can be seen in Figure 3a. In lipid peroxidation significant increase between 5- and 9-month-old mice for both strains, transgenic (Mann–Whitney; Mdn = 0.284, Mdn = 1.131, \ U = 0 \ p = .008) and wild type (Mann–Whitney;
Mdn = 0.495, Mdn = 0.874, \( U = 3 \ p = .015 \), however no differences were observed between strains (Figure 3b). Similarly, mitochondrial viability (measured by MTT) decreased with age in both strains, transgenic mice (Mann–Whitney; Mdn = 0.098, Mdn = 0.027, \( U = 0 \ p = .008 \)) and wild-type mice (Mann–Whitney; Mdn = 0.076, Mdn = 0.028, \( U = 0 \ p = .002 \)) but showed no differences between strains as shown in Figure 3c.

Finally, a correlation analysis showed a low/moderate positive correlation for 5-month-old wild-type mice between telomere length and ROS for the hippocampus (HC \( r_s = .3 \)) and whole blood cells (WB \( r_s = .5 \)) as well as for MDA (HC \( r_s = .3 \) and WB \( r_s = .7 \)), while a low negative correlation was found for MTT in the hippocampus (HC \( r_s = -.5 \)). Similar correlations were seen for the 9-month-old wild-type mice with the exception of a low negative correlation between HC telomere length and MDA (HC \( r_s = -.1 \)) and a high positive correlation between WB telomere length and MDA (HC \( r_s = .9 \), \( p = .04 \)) as can be seen in Figure 4.

For the transgenic strain, the hippocampus from 5-month-old mice showed low negative correlations between telomere length and ROS, MTT and MDA (HC \( r_s = -.2, -.1, \) and \(-.3, \) respectively) and high negative correlation for whole blood cells and MDA (HC \( r_s = -.8 \)). Finally, 9-month-old mice showed moderate and high positive correlations between HC telomere length and ROS (HC \( r_s = .9 \), \( p = .04 \)) and MTT (HC \( r_s = .7 \), respectively, while positive and negative moderate correlations for whole blood cells with ROS (HC \( r_s = .7 \)) and MDA (HC \( r_s = -.7 \)), respectively, as can be seen in Figure 4.

**FIGURE 2** Oxidative stress in blood cells of transgenic (●black) and wild-type (◇grey) mice of 5 and 9 months of age. (a) Measurement of ROS by DCFH. Blood cells ROS increases with age in both strains and is statistically significant at 9 months of age between strains. (b) Lipid peroxidation measured by MDA. Significant differences were found between 9-month-old transgenic mice and every other group. *\( p < .05 \)

### DISCUSSION

Telomere length and oxidative stress are affected by AD progression in the 3xTg-AD murine model. Shorter telomeres were found in blood cells of older transgenic mice compared to younger transgenic mice and same-age wild-type mice. This could be a reflection of a systemic effect due to the progression of AD in our murine model as it is not ageing but the disease itself that is causing the changes in telomere length.

On the matter, it has been reported that chronic inflammation promotes telomere attrition by increasing white blood cell replacement (Samani, Boulby, Butler, Thompson, & Goodall, 2001). An increase of glial fibrillary acidic protein...
**FIGURE 3** Oxidative stress in hippocampus homogenates of transgenic (●black) and wild-type (◽grey) mice of 5 and 9 months of age. (a) Measurement of ROS by DCFH. ROS increases with age in both strains and is statistically significant at 5 and 9 months of age between strains. (b) Lipid peroxidation measured by MDA. Significant differences were found between 5- and 9-month groups of both strains but not between strains. (c) Mitochondrial viability measured by MTT. Significant differences were found between 5- and 9-month groups of both strains but not between strains. *p < .05
immunoreactivity, indicative of astrogliosis, has been reported in the retinal ganglion cell layer in the late symptomatic stages of 3xTg-AD (Edwards et al., 2014). During disease progression in the 3xTg-AD mouse model, retinal microglia showed a pro-inflammatory phenotype, with less ramified morphology, and neurodegenerative associated markers (Grimaldi et al., 2018). Hence, inflammation could promote white blood cell replacement which would reflect in telomere shortening for this murine model.

Additionally, it has been shown that elevated levels of Aβ 1–40 and Aβ 1–42 are associated with increased oxidation products in peripheral blood cells from AD patients (Coppedé & Migliore, 2015). On the matter, plasma levels of Aβ 1–40 and Aβ 1–42 of 3xTg-AD mice have been found to increase progressively from 5 to 9 months of age (age at which this model does not yet show accumulation of Aβ in brain tissue), observing a significant decrease at 12 months (Cho et al., 2016).

Hence, the elevation of oxidative stress that we see with age, and that is more evident and prominent in the transgenic model, could be due to the presence of Aβ in blood, which appears sooner than in brain tissue. However, at the time we cannot statistically prove that the elevated oxidative stress causes telomere attrition, even when it seems a likely scenario.

We found increased oxidative stress, measured by ROS and lipid peroxidation, in blood cells of older compared to younger transgenic mice; however, we found no statistical association between the increase in oxidative stress and telomere length in our model. For that purpose, 8OHdG may have been a better biomarker to associate with telomere length as it is directly related to DNA damage due to oxidative stress. Accordingly, a previous paper reported that 8OHdG is elevated in lymphocytes of AD patients compared to a control group (Mecocci, MacGarvey, & Flint Beal, 1994). Unfortunately, this measurement was impossible to obtain at the time, and the latter study was made in humans.

Regarding our findings on telomere length in the hippocampus (HC), we only found a tendency, but no significant differences between the transgenic 13-month-old mice and the younger groups. On that matter, Franco and co-workers found no changes in telomere length of the subcortical and granular areas as well as the HC between transgenic mice for APP and their controls. They concluded that the accumulation of amyloid-beta has no relation to telomere shortening (Franco et al., 2006). Moreover, Lukens et al. found
no changes in telomere length in the cerebellum of patients with AD against controls despite a significant difference in leucocyte telomere length (Lukens, Van Deerlin, Clark, Xie, & Johnson, 2009). However, Thomas et al., 2008, found a significant increase in telomere length in the hippocampus of brain tissue of AD patients compared to controls as well as a significantly shorter telomere of the younger AD compared to older AD patients (Thomas et al., 2008). Finally, Cardillo et al., 2018, found no differences in the hippocampal telomere length of 3xTg-AD 11-month-old mice compared to WT controls (Cardillo et al., 2018).

Changes in telomere length in brain tissue are tricky to interpret as most neurons do not replicate, and specifically, the hippocampus shows reduced neurogenesis in AD (Dhaliwal et al., 2018). A recent stereological study in eleven-month-old 3xTg-AD mice indicated that, in spite of the occurrence of cerebral atrophy and reduced hippocampal volume, there was preservation of the total number of CA1 pyramidal neurons, suggesting a self-preservation mechanism that may be reflected in enhanced telomere maintenance (Manaye et al., 2013; Schaeffer, Catanoz, West, & Gattaz, 2017). Therefore, if shortening occurs, it is more likely to be due to environmental factors or to increased replication of glial cells (Cattan et al., 2008; Thomas et al., 2008), a distinction we did not make at the moment.

Here, we found that the progression of the disease does not affect telomere length in brain tissue as it does on blood cells. On the matter, it has been reported that telomere length in skeletal muscle (a minimally replicating somatic tissue), and leucocytes (a highly proliferative hematopoietic system) shorten with age, but only leucocytes show statistically significant attrition with time (Chahine et al., 2019). Telomeres in blood cells may shorten at a greater rate than other tissues because of their high turnover rate (Nakagawa, Gemmell, & Burke, 2004). Hence, inter-species, interindividual and intertissue differences are accounted for mainly by factors such as different rates of cell replication, levels of telomerase activity and levels of oxidative stress (Aviv, 2002).

On that matter, it is worthy to note that here we did not take into account the effects of interstitial telomeric sequences (ITSs). The dynamics for telomere estimation are complex because telomeric repeats exist both within true telomeres at the ends of chromosomes and as ITSs in the interior of chromosomes. Quantitative PCR (qPCR) detects both true telomere and ITSs (Foote, Vleck, & Vleck, 2013; Nakagawa et al., 2004). ITSs are likely to affect telomere estimates in any species that has substantial ITSs relative to true telomeres. This is problematic because it could increase the variance within a group of samples, as we can see in our measurements. Both of these effects reduce statistical power to detect significant differences in telomere length between groups (Foote et al., 2013).

Regarding ageing and telomere shortening, a negative correlation between age and telomere length (Lukens et al., 2009) has been reported in human tissue and peripheral blood cells, a feature we did not find in any of our mice, wild type or transgenic. Accordingly, a comparative study in mice established no correlation between telomere length and age in these animals; the replicative ageing mechanism controlling the number of cell divisions where telomere length is shortened, as has been reported in humans, appears to be different in mice (Gomes et al., 2011). Murine telomeres do not serve as a mitotic clock for replicative ageing, as primary cells constitutively express telomerase, in contrast to humans, in whom telomeres play a part in replicative senescence and telomerase expression is repressed (Calado & Dumitriu, 2013; Reichert & Stier, 2017). Hence, the data obtained here must be taken with caution when compared to humans.

One of the limitations of this study was the use of a control strain that did not belong to littermates from the transgenic strain. As has been discussed previously, using strains from other colonies means that mice also differ in epigenetic and environmental causes of selection (Holmdahl & Malissen, 2012). Nevertheless, all mice were born in our facilities and both transgenic and control strains were bought from the Jackson Laboratory, every used mouse was exposed to the same environmental conditions such as food, light/dark cycle or cage rotation. Moreover, our main findings relate to changes within the transgenic strain. However, we acknowledge that the use of an approximate control might affect the variability of our results.

Taking it all into account, human and mice studies have both described no changes in telomere length in brain tissue samples but a shortening in telomere in whole blood cells of AD patients. Although oxidative damage can cause telomere shortening through double-stranded breaks to DNA, most telomere loss due to oxidative stress occurs during DNA replication (Calado & Dumitriu, 2013; Reichert & Stier, 2017). Oxidation of biomolecules in the context of AD is mainly related to neuronal membrane biomolecules and to a disruption of membrane integrity (Cheignon et al., 2018), which is in accordance with our findings of a general negative correlation found between lipid peroxidation (measured by MDA) and telomere length for both brain tissue and whole blood cells of transgenic mice. This correlation is mainly evident and statistically significant for whole blood cells of transgenic 5-month-old mice.

Our results show that telomere attrition is due to the progression of the disease, supporting the newest discovery of AD being a disease that does not only affect the central nervous system but has a systemic effect.

Ageing, as well as AD, has an effect on oxidative stress (Panossian, 2003; de Souza-Pinto, Wilson, Stevnsner, & Bohr, 2008) which we were able to confirm here. Both mice
strains had increased markers of oxidative stress for blood and tissue samples when compared to a younger group, but transgenic mice showed even higher values suggesting an additive effect of age and Alzheimer’s disease. However, no statistical correlation between oxidative stress increase and telomere length was found. Independently of age, our results point to a distinct, not ROS-related, and yet to be determined, the global mechanism by which the Alzheimer phenotype promotes cell proliferation and consequently telomere shortening.

5 | CONCLUSION

Higher oxidative stress and shorter telomere length in peripheral blood were observed with the progression of the disease, but no statistical correlation between them was found. No changes were found in telomere length, but an increase in oxidative stress was found in the hippocampus of both strains. The presence of changes in peripheral blood cells of this mouse model suggests that AD affects the individual systemically due to its progression.

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CONFLICT OF INTEREST

We declare no conflict of interest.

AUTHOR CONTRIBUTIONS

K MG and A IH contributed to methodology, investigation and writing. JD ME contributed to data curation and writing. F BR contributed to resources, and review and editing. P GT contributed to conceptualization, funding acquisition, investigation, supervision and writing.

ETHICAL APPROVAL

All procedures were performed in accordance with the current rulings in Mexican law (NOM-062-ZOO-1999) and with the approval of the local Science and Ethics Committees (R-2012-785-049).

DATA AVAILABILITY STATEMENT

https://osf.io/pygce/?view_only=06b96ac7527a4385b765bf3b5036d77b

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