Transient OGG1, APE1, PARP1 and Polβ expression in an Alzheimer's disease mouse model

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ABSTRACT

Alzheimer's disease (AD) is a disease of major public health significance, whose pathogenesis is strongly linked to the presence of fibrillar aggregates of amyloid-beta (Aβ) in the aging human brain. We exploited the transgenic (Tg)-ArcSwe mouse model for human AD to explore whether oxidative stress and the capacity to repair oxidative DNA damage via base excision repair (BER) are related to Aβ pathology in AD. Tg-ArcSwe mice express variants of Aβ, accumulating senile plaques at 4–6 months of age, and develop AD-like neuropathology as adult animals. The relative mRNA levels of genes encoding BER enzymes, including 8-oxoguanine glycosylase (OGG1), AP endonuclease 1 (APE1), polymerase β (Polβ) and poly(ADP-ribose) polymerase 1 (PARP1), were quantified in various brain regions of 6 weeks, 4 months and 12 months old mice. The results show that OGG1 transcriptional expression was higher, and APE1 expression lower, in 4 months old Tg-ArcSwe than in wildtype (wt) mice. Furthermore, Polβ transcriptional expression was significantly lower in transgenic 12 months old mice than in wt. Transcriptional profiling also showed that BER repair capacity vary during the lifespan in Tg-ArcSwe and wt mice. The BER expression pattern in Tg-ArcSwe mice thus reflects responses to oxidative stress in vulnerable brain structures.

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1. Introduction

One theory of aging and neurodegenerative diseases proposes that damaged DNA and proteins accumulate in older cells and organisms, leading to phenotypical changes and genome instability (Harman, 1956; Hazra et al., 2007; Kirkwood, 2005; Tchou and Grollman, 1993). Oxidative stress induced by reactive oxygen species (ROS) may play a key role in this process, leading to cancer and neurodegenerative diseases, such as Alzheimer's disease (AD) (Lovell and Marquesbery, 2007; Patten et al., 2010).

AD, a multifactorial and progressive neurodegenerative disease which leads to impaired memory and cognition, is the most common form of dementia worldwide, accounting for 60–70% of all dementia cases (Ferri et al., 2005). The neurodegenerative process in AD is initially characterized by synaptic damage accompanied by neuronal loss, formation of extracellular amyloid-β (Aβ) plaques and intracellular neurofibrillary tangles (NFTs) (Querfurth and LaFerla, 2010). Age is the major risk factor for AD and onset of disease is insidious with an initial loss of short-term memory, followed by progressive impairment of multiple cognitive functions that affect the activities of daily living. The presence of Aβ plaques is a hallmark of AD neuropathology. Aβ peptides are derived from the proteolysis of amyloid-β precursor protein (AβPP), and the accumulation of aberrant Aβ peptides is a result of an imbalance between the level of Aβ production and clearance (Hardy and Selkoe, 2002; Hardy and Higgins, 1992). Oligomeric assemblies of Aβ may be central to the pathogenesis of AD, because the concentration of soluble Aβ in the human brain correlates better with the degree of cognitive dysfunction than senile plaque counts in AD patients (Hefti et al., 2013). Moreover, insoluble Aβ in plaques can structurally and functionally disrupt neuronal networks.

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Although genetic studies of AD provide insight into the etiology of familial AD, the factors that increase risk of sporadic AD, whose incidence is much higher and is trending upward, is poorly understood. Age is considered to be a main risk factor (Marques et al., 2010), but environmental factors may also play a significant role. There is overwhelming evidence that ROS-induced damage to cell membranes, proteins and DNA play a significant role in AD (Lovell and Markesbery, 2007; Patten et al., 2010). This could indicate an increased level of ROS-production or a decreased capacity to repair ROS-induced damage (Lovell and Markesbery, 2007; Sayre et al., 2008; Sultana et al., 2009; Wang et al., 2005). ROS are formed in the mitochondria during normal metabolism, during chronic inflammation and in response to exogenous chemicals and ultraviolet and ionizing radiation (Bjelland and Seeberg, 2003). Oxidative stress is generated when there is an imbalance between the generation, handling and manifestation of ROS.

Base excision repair (BER) is the major pathway for repairing oxidative DNA damage and involves the cooperative interaction of several proteins that work sequentially to excise the target damage and restore DNA to its original, unmodified form (Seeberg et al., 1995). Defects in DNA repair have been associated with progerias, cancer, dementia and other neurodegenerative diseases (Bohr et al., 2007; Jeppesen et al., 2011; Bass et al., 2007; Weissman et al., 2007; Wilson and Bohr, 2007). Analysis of BER enzyme expression in AD mice models in this context is warranted. In the Tg-ArcSwe mouse model of AD, there is neuronal expression of a mutant human AβPP gene carrying the Arctic (AβPP E693G) and Swedish (KM670/671NL) mutations (Lord et al., 2006, 2009). Intraneuronal aggregates of Aβ are observed already in approximately 1-month-old mice, and senile plaques accumulate in the brains of these mice at 5–6 months of age, partly simulating AD neuropathology. However, the brains of 4 months old or younger mice remain free of plaques. Amyloid deposits generate significant oxidative stress in the brain. Since the BER components under study contribute to the repair of oxidative damage of the macromolecule DNA, these genes and gene products were selected for analysis. Aβ is liberated following cleavage of AβPP by β-site AβPP-cleaving enzyme-1 (BACE-1) and the γ-secretase complex, in which presenilin contributes to the catalytic activity. The first AD-associated mutation identified was located outside the AβPP gene encoding the Aβ-domain that increased the expression of Aβ-42, while a point mutation in the APP gene (an amino-acid substitution Val/Ile close to the carboxy terminus of the Aβ peptide) suggested that some cases of AD could be caused by mutations in the APP gene itself (Goate et al., 1991). AβPP gene mutations either enhance the steady-state level of Aβ, like the Swedish AβPP mutation (K670N/M671L) (Citron et al., 1992), or selectively increase the level of Aβ-42 and/or alter the Aβ-42/Aβ-40 ratio, like the Presenilin (PS) and London-type AβPP mutations do (Price and Sisodia, 1998). The Arctic AβPP mutation (E693G) (Nilsberth et al., 2001) in humans is associated with clinical features of early-onset AD commencing at 52–62 years. In young mice, the Arctic mutation increased intraneuronal Aβ accumulation in an age-dependent manner (Knochlo et al., 2007; Lord et al., 2006). Previous analyses of the Tg-ArcSwe mice also depict perivascular amyloid angiopathy as well as plaques confined to the neuropil. Interestingly, those results suggest that the development of amyloid aggregates at an advanced stage is located extracellularly and are associated with a loss of astrocyte polarization (Yang et al., 2011). The possible perturbation of water and homeostasis could contribute to cognitive decline and seizure propensity in AD patients. Animal models with loss of astrocyte polarity reveal delayed potassium clearance and increased seizure intensity (Amiry-Moghaddam et al., 2003). Investigations in other AβPP-targeted mice models for AD including the triple-transgenic model (3 × Tg-AD) harbouring PS1 (M146V), AβPP (Swe), and tau (P301L) transgenes (Oddo et al., 2003) have also demonstrated evidence of increased oxidative stress in the early AD phase (Resende et al., 2008). These alterations are evident during the Aβ oligomerization period, before the appearance of Aβ plaques and neurofibillary tangles, supporting the view that oxidative stress occurs early in the development of the disease (Resende et al., 2008) and can be alleviated by the mitochondria-aimed antioxidant MitoQ to prevent loss of spatial memory retention and early neuropathology (McManus et al., 2011). Furthermore, Aβ deposition in vivo has been shown to be associated with increased lipid peroxidation and reduced levels of antioxidants such as glutathione and vitamin E in early AD also in other AβPP mice models (Pratico et al., 2001).

Here, we employed the Tg-ArcSwe mouse model to examine whether BER capacity or oxidative stress play a role in susceptibility to AD. The relative mRNA levels of potentially BER-rate-influencing genes, including 8-oxoguanine glycosylase (OGG1), AP endonuclease 1 (APE1), poly(ADP-ribose) polymerase 1 (PARP1) and polymerase β (Polβ) were quantified in the frontal cortex, hippocampus, cerebellum, and remaining brain regions of 6 weeks, 4 months, and 12 months old mice. Wildtype (wt) mice were used as controls. The results show that OGG1 transcriptional expression was higher and APE1 expression was lower, in 4 months old transgenics than in 4 months old wt mice and that Polβ transcriptional expression was significantly lower in transgenic 12 months old mice, and that hippocampal PARP1 expression was reduced in both wt and Tg-ArcSwe mice at 12 months. Transcriptional profiling also showed that overall DNA repair capacity may vary considerably during the lifespan in both Tg-ArcSwe and wt mice.

2. Materials and methods

2.1. Mouse model and tissue preparation

Inbred C57BL/6 non-transgenic wt and AβPP transgenic mice harbouring human AβPP with the Arctic (E693G) and Swedish (K670N, M671L) mutations (Tg-ArcSwe mice) (Lord et al., 2006) were employed in this study (Table 1). The Tg-ArcSwe mouse model was constructed by inserting the human AβPP containing the Arctic mutation (AβPP E693G) and the Swedish mutation (KM670/671NL) into a Thy-1 expression vector and microinjecting purified DNA into fertilized oocytes of C57BL/6-CBA-F1 mice. The Arctic mutation (AβPP E693G) is located within the sequence encoding Aβ which makes it quite unique compared to other AD-causing AβPP mutations that are typically used in transgenic models (Duyckaerts et al., 2008). The Tg-ArcSwe model has an early onset of senile plaque formation (4–6 months) and increased intraneuronal Aβ aggregation (1 month) prior to the extracellular Aβ deposition (Lord et al., 2006, 2009, 2011) The mice were age-matched and all experimental procedures were performed following institutionally approved protocols in accordance with strict international regulations for the care and use of laboratory animals. The experiment was approved by the section for comparative medicine at the University of Oslo and the Norwegian Animal Research Authority/ Biological Research Ethics Committee, and complied with national laws, institutional regulations and EU Directive 86/609/EEC governing the use of animals in research.

Table 1

| Age (weeks) | 6 | 4 | 12 |
|-----------|---|---|----|
| wt B16 female | 4 | 4 | 6 |
| non-transgenic | | | |
| wt B16 male | 2 | 4 | 5 |
| non-transgenic | | | |
| Tg-ArcSwe female | 4 | 4 | 6 |
| Tg-ArcSwe male | 4 | 4 | 6 |
| Total animals | | | |
| Frontal cortex | 14 | 16 | 23 |
| Hippocampus | 10 | 7 | 21 |
| Cerebellum | 10 | 7 | 23 |
| Rest of brain | 12 | 6 | 22 |
The animals used for gene expression and Western blot were humanely sacrificed, the cerebral cortex and other brain structures dissected out and quickly frozen on dry ice and stored at −80 °C until processed. For gene expression analysis and Western blot, the brain was divided into four main parts: the frontal cortical parts of the hemispheres front cortex (FC), hippocampus (HC), cerebellum (CB) and the rest of brain (RB) (Table 1). Each age-group studied contained between 6 and 12 pairs of animals (Tg-ArcSwe/wt) (Table 1). Mice were studied at 6 weeks, 4 months and 12 months of age. Altogether, 53 mice (25 wt and 28 Tg-ArcSwe) and 171 brain specimens were subjected to gene expression and Western blot analysis (Table 1). More samples and mice at additional ages would have provided useful information, however, this was beyond the range of accessibility in this study.

2.2. RNA isolation and cDNA synthesis

RNA was isolated manually from brain tissue of Tg-ArcSwe and wt mice. The samples were put into MagNA Lyser Green Beads (Roche Diagnostics, Mannheim, Germany) with TriZol Reagent (Invitrogen, CA, US) within few seconds after detaching for minimum RNA degradation, and then homogenized using FastPrep™ FPI 20 device (Bio 101 Systems, US). The supernatant was collected, and RNA was isolated using chloroform and ethanol combined with the RNeasy™ Mini kit (QIAGEN, Germany) spin column protocol. The RNA was eluted in RNAse-free water. An extra DNase treatment was applied to the RNA samples using Turbo DNA-free DNase treatment (Ambion, Texas, US) according to the manufacturer’s protocol. The samples were then quantified using Nanodrop 1000 (Thermo-Scientific, Montchanin, DE, US) for indication of purity and yield. RNA integrity was checked in all RNA samples using agarose gel electrophoresis with SYBR™ Safe DNA Gel Stain, and those samples which did not show the distinct 28S and 18S bands were further analyzed using Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, US). Samples with RIN value <7 were excluded (see Supplementary data). RNA samples were also monitored for the presence of genomic DNA that could disturb the gene expression analysis. RNA was stored in −80 °C until further processing.

Reverse transcription PCR (cDNA synthesis and quantitative RT-PCR) was performed with the two-step procedure High capacity RNA-to-cDNA kit (Invitrogen, US) according to the recommendations of the manufacturer using the same RNA concentration for all samples.

2.3. Determination of mRNA levels

 Primer design for all genes (Table 2) was performed based on general recommendations (see Supplementary data, Table S1). mRNA levels were determined by quantitative real-time PCR (qRT PCR) using the StepOnePlus™ system (Applied Biosystems Ltd., NC, US) 96 wells plate with SYBR™ green according to the recommendations of the manufacturer (see Supplementary data). Holding stage correspond to one initial cycle at 95 °C for 10 min, the cycling stage at 95 °C for 15 min and 58 °C for 1 h (55 cycles), and the melt curve stage at 95 °C for 15 min, 60 °C for 1 h and 95 °C for 15 min. PCR was performed in parallel for each sample for each gene.

Normalization of gene expression qPCR data was performed in several steps and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was selected as the endogenous reference gene (see Supplementary data Fig. S1). The relative standard curve method was used to calculate the relative gene expression of the target genes using the Ct difference between the target gene and the y-intercept, divided by the slope and normalized to the reference gene (GAPDH) and adjusted for minute efficiency (see Supplementary data).

2.4. Western blot analysis

Protein concentrations were determined with a DC protein assay (Bio-Rad, US). After diluting the samples in loading buffer (75% LDS Sample buffer (4×) NuPAGE (Invitrogen, US) and 25% 1 M DTT), 40 μg of protein was added per lane, separated by electrophoresis on 4–12% polyacrylamide gels (NuPAGE Bis-Tris Midi Gel, Invitrogen, US) and transferred onto PVDF membranes (iBlot™ Gel Transfer Stack, Invitrogen US). Membranes were blocked overnight in Tris-buffered saline (TBS (1 M Tris pH 7.5, 150 mM NaCl) plus 0.05% Tween (TBS-T) and 4% skim milk powder (Sigma–Aldrich, UK) followed by 1-h incubation in primary antibody diluted in TBS-T. Membranes were subsequently incubated for 1 h with the secondary antibody. All experiments were carried out at room temperature and after each step membranes were washed in TBS-T. Several antibodies against OGG1 and Polβ were also tested, however, none of them provided sufficient sensitivity and specificity. Antibody specifications are listed in the Supplementary data.

2.5. Statistical analysis

A linear mixed model rather than the ANOVA model was used to accommodate the specific nature of this study and the fact that the study was multifactorial and not completely balanced as the number of observations (gene expression measurements) was not equal for all brain regions for both types of mice (depending on scarce availability). To test whether any gene is differentially expressed between the Tg-ArcSwe and wt mice in each region of the brain, the linear mixed model was fitted to each gene. Each time point was considered separately. The model contains the fixed effects of mouse type (Tg-ArcSwe or wt), brain region (HC, CB, FC or RB) and the interaction between mouse type and brain region. A random mouse effect was included to account for the potential correlation between the measurements within each mouse. If the interaction effect was not significant, the main effect of mouse type could be interpreted directly and we can decide whether the gene is differentially expressed between Tg-ArcSwe and wt mice in general. However, if the interaction effect was significant, the effect of type of mouse depends on the brain region, and each brain region was considered separately.

We first tested whether there was an overall significant interaction effect, i.e. we tested whether all interaction terms in the model were equal to zero against the alternative that at least one was not, using an F-test (Table S2). The significance level selected was 5%. If the null hypothesis was not rejected, the final overall difference between Tg-ArcSwe and wt mice was tested using a t-test for the main effect of type in the model (Tables 3 and 4).

If the null hypothesis was rejected, there was evidence that the difference in expression between Tg-ArcSwe and wt mice differed between brain regions. We then proceeded to test for differential expression within each region. We fitted a reduced version of the model where the main effect of mouse was left out. The model then included the fixed effect of brain region and the interaction effect between mouse type and brain region, as well as the random effect of mouse type. For each region of the brain, a t-test was used to test whether the interaction effect of mouse type with that region of the brain was significantly different from zero

Table 2

| Gene | Amplicon length (nt) | Sequence accession no. | Chromosome | Number of introns |
|------|---------------------|------------------------|------------|------------------|
| APE1 | 546                 | NM_009687               | 14         | 4                |
| OGG1 | 1556                | NM_010957.4             | 16         | 4                |
| Polβ | 5658                | NM_011130               | 6          | 6                |
| PARP1| 3845                | NM_007415.2             | 8          | 6                |
| GAPDH| 1254                | NM_008084.2             | 13         | 6                |

Table 3

| Gene | Age | Interaction effect (brain region specific) | Main type of mouse effect (not brain region specific) |
|------|-----|-------------------------------------------|---------------------------------------------------|
|      |     | M.S. Lillenes et al. / Mechanisms of Ageing and Development 134 (2013) 467–477 | 469 |
| APE1 | 6 weeks | 1.50 | 0.23 | −1.59 | 0.12 |
|      | 4 months | 1.92 | 0.15 | 2.83 | 0.009* |
|      | 12 months | 1.12 | 0.34 | −1.15 | 0.26 |
| Polβ| 6 weeks | 0.80 | 0.50 | 0.34 | 0.74 |
|      | 4 months | 1.32 | 0.29 | 1.81 | 0.08 |
|      | 12 months | 2.88 | 0.04* | 0.03 | 0.98 |
| OGG1 | 6 weeks | 0.20 | 0.89 | 0.24 | 0.81 |
|      | 4 months | 13.89 | 0.00001* | −3.20 | 0.003 |
|      | 12 months | 0.98 | 0.14 | −0.69 | 0.49 |
| PARP1 | 6 weeks | 0.38 | 0.76 | 1.24 | 0.22 |
|      | 4 months | 0.48 | 0.70 | −0.06 | 0.96 |
|      | 12 months | 0.24 | 0.87 | 0.52 | 0.60 |
Table 4

Results of brain region specific transcriptional expression of OGG1 and Polβ when comparing Tg-ArcSwe mice and wildtype (wt) mice. OGG1 expression was significantly different between Tg-ArcSwe and wt mice in the hippocampus, frontal cortex and rest of brain, and Polβ expression was significantly different in the cerebellum and rest of brain. Significant results are marked with *.

| Gene | Age   | Brain part | Effect of type of mouse within brain part | t-value | p-Value |
|------|-------|------------|------------------------------------------|---------|---------|
| OGG1 | 4 months | Hippocampus | −3.20 | 0.003* |
|      |       | Cerebellum  | −1.63 | 0.11   |
|      |       | Frontal cortex | −2.53 | 0.02* |
|      |       | Rest of brain | −3.70 | 0.0009* |
| Polβ | 12 months | Hippocampus | 0.028 | 0.98   |
|      |       | Cerebellum  | 4.18  | 0.00007* |
|      |       | Frontal cortex | 1.58  | 0.12   |
|      |       | Rest of brain | 2.64  | 0.01*  |

(Table 4) To test whether the gene expression changes significantly between the different ages within the same mouse type, the same procedure was applied, except that the age effect was included instead of type of mouse. The two models are fitted for each age comparison (4 months versus 6 weeks and 12 months versus 4 months) and each type of mouse (Tg-ArcSwe and wt) separately (Tables S2 and S3). For APE1 in the Tg-ArcSwe mice, including another interaction term did not improve the model fit, and therefore only the main effect of the age comparison was tested.

3. Results

3.1. Transcription of BER genes in Tg-ArcSwe mice

OGG1, APE1, PARP1 and Polβ transcripts were quantified by qRT-PCR in the FC, HC, CB and RB regions of the brains of Tg-ArcSwe and wt mice 6 weeks, 4 months and 12 months old. In 6 weeks old mice, there was low basal expression of OGG1, APE1, PARP1 and Polβ in Tg-ArcSwe and wt mice (Table 3; Figs. 1–4). However, in 4 months old mice, the correlation of brain region and mouse type was significant on OGG1 mRNA levels where the OGG1 expression in the Tg-ArcSwe mice was significantly higher in HC (p < 0.01), FC (p < 0.05) and RB (p < 0.001) at 4 months (Table 4) than in wt mice. At the same time, APE1 expression at 4 months was generally and significantly lower (p < 0.01), but there was no difference between the various brain regions (Table 3). At 4 months, Polβ transcriptional expression was moderately lower in Tg-ArcSwe mice than in wt and significantly higher at 12 months in the CB (p < 0.001) and RB (p < 0.01) compared to wt (Table 4; Fig. 3). In 12 months old mice, OGG1 and APE1 were expressed at a similar level in Tg-ArcSwe and wt mice (Figs. 1 and 2). There were no significant differences in PARP1 expression between Tg-ArcSwe and wt mice at any age (Fig. 4).

3.2. BER gene expression by age of Tg-ArcSwe and wt mice

In Tg-ArcSwe mice, OGG1, APE1 and PARP1 expression varied in animals of different age (Figs. 1–4). OGG1 expression was significantly higher in 4-month-old mice than in 6 weeks old mice in all four brain regions: HC (p < 0.001), FC (p < 0.001) and RB (p < 0.0001), and was lower in 12 months old mice in all brain regions: HC (p < 0.0001), FC (p < 0.05), FC (p < 0.01) and RB (p < 0.0001) (Fig. 1). APE1 was expressed at a higher level (p < 0.05) in 12 months old mice than in 4-month mice (Fig. 2). APE1 expression tended to be lower in the 4 months old mice than in 6 weeks old mice, however, this finding was not significant (p < 0.052). The transcriptional expression of OGG1, APE1, PARP1 and Polβ also varied throughout the lifespan of wt mice (Figs. 1–4). OGG1 expression was higher (p < 0.01) in 4 months old mice than in 6 weeks old mice in general. OGG1 expression was lower in 12 months old wt mice, but this difference was not significant (p = 0.057). Thus, the OGG1 transcriptional profile pattern was similar to Tg-ArcSwe during the lifespan of wt mice, however, the total levels of OGG1 mRNA were significantly higher in the Tg-ArcSwe than in the wt mice (Fig. 1). APE1 expression was significantly higher in the cerebellum of 4 months old wt mice than in 6 weeks old wt mice (p < 0.001), opposite to the pattern in Tg-ArcSwe mice (Fig. 2), and Polβ expression was significantly higher in the cerebellum (p < 0.0001), frontal cortex (p < 0.05) and rest of brain (p < 0.01) in 12 month than in 4-month-old mice (Fig. 3). PARP1 expression was lower in the hippocampus (p < 0.0001) of both Tg-ArcSwe and wt mice at 12 months as compared to 4 months (Fig. 4). A complete transcriptional expression profile of all the BER genes analyzed in both mouse types in all age groups is presented in the Supplementary data (Fig. S2).

3.3. Protein expression in AD mice

Western blot analysis was performed using sections of the same brain samples in the transcriptional analysis, revealing that the level of APE1 protein expressed was significantly lower in 4 months old Tg-ArcSwe mice than in the wt controls (p < 0.01) (Fig. 5 and Fig. S3). GAPDH and beta-actin were used as endogenous controls, and the GAPDH level was significantly lower (p < 0.001) in Tg-ArcSwe mice than in wt, illustrating a change in GAPDH protein levels but not in the initial protein load, since the level of expressed beta-actin in the same samples was not changed. APE1 and GAPDH protein levels were not different between Tg-ArcSwe and wt mice at 6 weeks and 12 months (Figs. 5B and S3). Due to poor antibody reactivity/specificity, Western analysis for OGG1 and Polβ could not be completed.

3.4. Aβ deposits in Tg-ArcSwe mice

Corroborating previous data (Lord et al., 2009; Philipson et al., 2012), by the end of 4 months, the Tg-ArcSwe model had aberrant Aβ aggregate accumulation representative for AD (Fig. 6). The presence of intracellular Aβ at 3–4 months, corroborating the time point for BER gene expression fluctuation, was presented by light microscopy (LM) analysis with immunocytochemistry (Fig. 6A–D). LM with immunocytochemistry and an electron micrograph also show that at 12 months also extracellular Aβ aggregates are present (Fig. 6E and F), at a time when the genotoxic stress is alleviated.

4. Discussion

The goal of this study was to evaluate whether oxidative stress and/or accumulated oxidative DNA damage in the brain might promote and/or correlate with development of AD-like pathology in the life span of Tg-ArcSwe mice. Data collected here generally support this idea, as follows.

4.1. Comparison between the of transcription of BER genes in Tg-ArcSwe and wt mice

At 6 weeks, there were low baseline transcription expression of OGG1, APE1, PARP1 and Polβ and no difference in the expression of these genes between Tg-ArcSwe and wt mice. However, at 4 months there is significantly higher OGG1 expression in the Tg-ArcSwe mice as compared to wt mice (Fig. 1). This may indicate a response to oxidative stress in that age group, possibly reflecting increased level of oxidative stress occurring during pre-plaque Aβ deposition (Tabner et al., 2005) in these parts of the brain. At the same time, APE1 mRNA and protein levels at 4 months were
significantly reduced in general in the Tg-ArcSwe mice (Figs. 2 and 5), simultaneously with the peaked OGG1 response. The rate-limiting BER enzyme APE1 is generally highly expressed in the brain (Hegde et al., 2012) and its mRNA and protein are normally upregulated as a response to oxidative stress (Hill et al., 2001). The low APE1 expression in 4 months old Tg-ArcSwe mice could represent aberrant regulation of APE1 correlating with Aβ pathology. Further, because inactive APE1 stimulates OGG1 (Hill et al., 2001), if a feedback loop is in effect, reduced APE1 could in turn stimulate transcription of OGG1, as seen in the Tg-ArcSwe mice. Alternate interpretations are possible, including that OGG1 transcription increases in response to higher oxidative stress, correlated to Aβ accumulation, and that an increase in APE1 expression lags behind. Notably, at 12 months, the expression of Polβ was significantly lower in Tg-ArcSwe mice than in wt mice, with significant differences in the cerebellum and rest of brain, possibly representing a Polβ bottleneck function in multiple DNA repair pathways.
4.2. Reduced GAPDH protein levels implicate a role in oxidative stress

The notable finding of reduced amounts of GAPDH at the protein level in 4-month Tg-ArcSwe mice may indicate altered GAPDH expression or other mechanisms affecting protein levels. In fact, studies show that GAPDH expression in humans is age-dependent and may be related to oxidative stress-induced GAPDH modifications (Mazzola and Sirover, 2005), and polymorphism variation within GAPDH genes has been associated with late onset AD (Allen et al., 2012). However, we found no alteration in mRNA levels of GAPDH across both types of mice and age (Fig. S1), indicating that other mechanisms affect protein levels of GAPDH. Studies suggest that the protein GAPDH exhibits aggregating properties, and Aβ or oxidant exposure are suggested to lead to increased accumulation of disulfide-bonded isoforms of GAPDH that in turn act as seeds to facilitate the aggregation of misfolded GAPDH, leading to insoluble aggregates that may be cytotoxic or indirectly trigger apoptosis. During apoptosis, the insoluble
GAPDH is released and after cell death, the insoluble GAPDH aggregates may accumulate in extracellular plaques (Cumming and Schubert, 2005). Thus, GAPDH taking part in mitochondrial bioenergetics, glucose degradation and axonal transport may be influenced by both oxidative stress and Aβ, also indicating that GAPDH at the protein level is not an optimal endogenous control when studying Aβ or other factors influenced by oxidative stress.

4.3. BER gene expression during the lifespan of Tg-ArcSwe and wt mice

Transcriptional expression of OGG1, APE1, PARP1 and Polβ also differed over the lifespan of both the Tg-ArcSwe and wt mice (Figs. 1–4). The pattern of lifespan OGG1 expression profile appeared similar in both Tg-ArcSwe mice and wt mice, however, the magnitude of these changes were higher in the Tg-ArcSwe mice than in wt. The increase of OGG1 at 4 months in all four brain parts
may indicate a response to increased level of oxidative stress with age, particular at the time the animals attains adulthood. This is supported by other studies showing that accumulation of DNA damage increase with age, but increases more dramatically between young (7 days) and aged (6 months) rats than between aged and old rats (≥24 months) (Swain and Subba Rao, 2011), suggesting a possible time point of initiation of enhanced oxidative stress in the aging process, occurring in both mouse types, that is further accelerated in the Tg-ArcSwe mice possibly by the intraneuronal Aβ accumulation. Intraneuronal Aβ is shown to interact with membranes and subcellular organelles such as the mitochondria, and studies demonstrate the presence of Aβ in brain mitochondrial fractions of a transgenic AD mouse model (Gillardon et al., 2007). Aβ-42 is a potent inhibitor of mitochondrial enzymes (Crouch et al., 2005) causing dysregulation of mitochondrial proteins mainly related to oxidative phosphorylation (Rhein et al., 2009), possibly contributing to increasing the level of oxidative stress possibly contributing to the increased expression of OGG1 in the Tg-ArcSwe mice. APE1 expression was, however, inversely affected in the two mouse types at this age, expressed at lower levels
APE1 was generally stably expressed in the wt mice over time compared to the Tg-ArcSwe mice, and the elevated APE1 expression in the wt mice at 4 months may be a normal response to the increased DNA damage occurring at this age. The lower expression in the Tg-ArcSwe mice, however, may represent altered DNA damage response in these mice due to the accumulating intraneuronal Aβ interacting with, e.g., ER, endosomes, lysosomes, ribosomes, the
ubiquitin-proteasome system, mitochondria and the microtubuli system (Penke et al., 2012). Polβ expression was higher in the 12-month-old wt mice compared to 4-month wt mice in the CB, FC and RB, possibly representing a late response to general aging presented with accumulation of DNA damage, at a stage when Aβ is aggregating extracellularly. This delayed and differentiated response in Polβ expression, an enzyme which normally functions as a bottleneck in multiple DNA repair pathways, may represent a long-term consequence of the increased load of oxidative stress earlier in the life span. PARP1 expression was generally stable across the lifespan of both Tg-ArcSwe and wt mice, with the exception of a distinctly lower expression in the hippocampus of both mouse types at 12 months compared to the other brain regions. PARP1 activity and the poly(ADP)-ribosylation of proteins have been shown to be required for changes in synaptic plasticity related to memory stabilization in the mouse hippocampus (Fontan-Lozano et al., 2010), while PARP1 activation is also required for long-term neuronal plasticity in mice (Cohen-Armon et al., 2004; Goldberg et al., 2009). Cognitive processes require modification in gene expression to consolidate information (Abel and Kandel, 1998; Abel and Lattal, 2001; Milner et al., 1998), and histone modification has been demonstrated to be critical in regulating gene expression during learning and memory (Alarcon et al., 2004; Fontan-Lozano et al., 2008; Gupta et al., 2010; Korzus et al., 2004; Levenson et al., 2004; Vecsey et al., 2007; Woolf, 2005). Learning provokes an increase in the poly(ADP)-ribosylation of histone H1 in brain regions relevant for learning and memory such as the hippocampus (Fontan-Lozano et al., 2010). Changes and modulation of gene expression needed for consolidation of objective recognition memory require PARP1 activation in the hippocampus (Fontan-Lozano et al., 2010), and PARP1 is also involved in the changes of gene expression needed for long-lasting synaptic plasticity (Fontan-Lozano et al., 2010). Given these critical roles of PARP1 related to the hippocampus, the concurrent low expression of PARP1 in the hippocampus of both mouse types may explain some of the common memory disturbances acquired by age, apparently unaffected by the presence Aβ. PARP1 expression has also been found not to be significantly lower in older mice versus younger mice in other studies (Noren Hooten et al., 2012), corroborating the data presented here. However, PARP1 activity rather than expression has been found to be higher in centenarians than in the general population (Muiras et al., 1998). Thus, future work lies in determining the relationship between PARP1 expression and the activity in aging and longevity and neurodegenerative diseases such as AD.

In general, this study demonstrates that the expression of DNA repair genes varies in a tissue and age-specific manner in both Tg-ArcSwe and wt mouse brains, both linked to and independent of the presence of Aβ pathology. Although higher DNA repair capacity may reflect response to Aβ deposition, it cannot be definitely ascertained at present. It is logical to speculate that amyloid deposits are linked to incipient AD, higher oxidative stress, and higher concurrent DNA damage; however, whether or not this leads to induced transcription of OGG1, APE1, PARP1 and Polβ especially in vulnerable brain regions, remains to be determined. Taken together, the inverse longitudinal patterns of BER expression observed in Tg-ArcSwe mice may reflect the level of oxidative stress occurring during Aβ depositions (Tabner et al., 2005), more so in particularly vulnerable parts of the brain. However, the fact that the BER components do not vary coordinately during the lifespan in both mouse types nor with Aβ pathology may reflect the complex nature of DNA repair enzymes, particularly APE1, Polβ and PARP1 whose multiple roles may be affected through alternative pathways in addition to the DNA repair response that is influenced differently by oxidative stress and Aβ pathology. Additional studies are required to elucidate the role of these enzymes and their impact in and on aging and AD pathology. Nonetheless, the model presented here demonstrates how differential DNA repair capacity can occur, relevant for the DNA damage response to oxidative stress in AD and also in the brain in general.

However, without functional studies one cannot determine what is the antecedent cause or what is the consequence of Aβ-related oxidative stress and/or DNA damage, as BER expression could both be caused by or initiate elevated oxidative stress. In conclusion, the data presented here demonstrate different levels of BER gene expression in specific brain regions in mice with and without genetically engineered AD-like pathology. The Tg-ArcSwe mouse model may thus in the future prove to be a valuable experimental system to further explore and better understand the relationship between AD pathology, oxidative stress and BER capacity.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mad.2013.09.002.

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