Published in final edited form as:

Neurobiol Aging. 2013 October ; 34(10): 2399–2407. doi:10.1016/j.neurobiolaging.2013.04.014.

Mitochondrial DNA damage in a mouse model of Alzheimer's disease decreases amyloid beta plaque formation

Milena Pinto^{#a}, Alicia M. Pickrell^{#b,2}, Hirokazu Fukui^b, and Carlos T. Moraes^{a,b,c,*}

^aDepartment of Neurology, University of Miami Miller School of Medicine, Miami, FL, USA

^bNeuroscience Graduate Program, University of Miami Miller School of Medicine, Miami, FL, USA

^cDepartment of Cell Biology and Anatomy, University of Miami Miller School of Medicine, Miami, FL, USA

[#] These authors contributed equally to this work.

Abstract

Mitochondrial DNA (mtDNA) damage and the generation of reactive oxygen species have been associated with and implicated in the development and progression of Alzheimer's disease. To study how mtDNA damage affects reactive oxygen species and amyloid beta (A β) pathology in vivo, we generated an Alzheimer's disease mouse model expressing an inducible mitochondrial-targeted endonuclease (Mito-PstI) in the central nervous system. Mito-PstI cleaves mtDNA causing mostly an mtDNA depletion, which leads to a partial oxidative phosphorylation defect when expressed during a short period in adulthood. We found that a mild mitochondrial dysfunction in adult neurons did not exacerbate A β accumulation and decreased plaque pathology. Mito-PstI expression altered the cleavage pathway of amyloid precursor protein without increasing oxidative stress in the brain. These data suggest that mtDNA damage is not a primary cause of Ab accumulation.

Keywords

mtDNA damage; Alzheimer; Plaque formation; APP cleavage; ROS

© 2013 Elsevier Inc. All rights reserved.

*Corresponding author at: 1420 NW 9th Avenue, Room 229, Miami, FL 33136, USA. Tel.: +1 305 243 5858; fax: +1 305 243 6955. cmoraes@med.miami.edu (C.T. Moraes).

²Present address: Surgical Neurology Branch, NINDS/National Institutes of Health Bethesda, MD, USA.

Disclosure statement

The authors declare no conflicts of interest.

All experiments and animal husbandry were performed according to a protocol approved by the University of Miami Institutional Animal Care and Use Committee.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neurobiolaging.2013.04.014>.

1. Introduction

Alzheimer's disease (AD) is one of the most common age-related neurodegenerative diseases characterized by declines in memory and cognition. One pathological feature of AD is the presence of abnormal plaques composed of amyloid beta (A β) protein deposited in the cortex and hippocampus, regions that are affected in AD.

Mitochondrial dysfunction has been implicated in contributing to the development and progression of AD. Early studies reported defects in oxidative phosphorylation (OXPHOS), specifically cytochrome c oxidase, in postmortem AD brains (Chagnon et al., 1995; Long et al., 2012; Mutisya et al., 1994; Sheng et al., 2012). Mutations in mitochondrial DNA (mtDNA), which encodes proteins for several OXPHOS complexes, have also been found in affected patients (Coskun et al., 2004; Krishnan et al., 2012; Lin et al., 2002). Aging, the major risk factor in developing AD, is associated with declines in mitochondrial function in the central nervous system (Bowling et al., 1993; Hauptmann et al., 2009; Hong et al., 2008; Ross et al., 2010; Yao et al., 2009). Moreover, the cybrid model of AD, in which mtDNA from AD patients' platelets was transferred into cells lacking mtDNA, showed decreased mitochondrial mobility, increased oxidative stress, decreased cytochrome oxidase activity, and decreased mitochondrial membrane potential, suggesting that mitochondria and mtDNA abnormalities contribute to AD pathogenesis (Costa et al., 2012; Swerdlow, 2007).

In support of an alternative hypothesis, in which mitochondrial dysfunction is a consequence rather than a trigger of AD, A β negatively affects mitochondrial function. A β directly associates with the mitochondria inhibiting OXPHOS (Anandatheerthavarada et al., 2003; Calkins et al., 2011; Casley et al., 2002; Chen and Yan, 2010; Devi et al., 2006; Hansson Petersen et al., 2008; Manczak et al., 2006; Reddy and Beal, 2008). Mitochondrial dynamics are also affected in AD either by the downregulation of fission and/or fusion proteins, or by nitrosylation of dynamin-related protein 1 (DRP1), a mitochondrial fission protein (Manczak et al., 2011; Su et al., 2010; Wang et al., 2009). It is unknown to what extent OXPHOS defects affect and progress the pathophysiology of AD.

Oxidative stress and reactive oxygen species (ROS) damage also contribute to AD pathophysiology. Previous reports showed that ROS enhances β -secretase activity and exacerbates A β aggregation (Guglielmotto et al., 2009; Paola et al., 2000; Tamagno et al., 2002; Yao and Brinton, 2012). ROS also influence amyloid precursor protein (APP) processing, promoting A β through β - and β -secretase activation (Leuner et al., 2012a, 2012b; Shen et al., 2008). The electron transport chain is a known source of ROS that can damage proteins, lipids, and DNA. Moreover, some functional alterations in the respiratory chain have been reported to increase ROS production. However, it is unclear whether mitochondria are the sole contributor to the ROS damage seen in AD, because alternative sources of ROS have also been identified (Abramov et al., 2004; Cutler et al., 2004; Yao and Brinton, 2012).

We examined the effect of mtDNA damage on A β accumulation and plaque formation to determine how a mild mitochondrial dysfunction affects the pathophysiological changes that occur in a mouse model of AD.

2. Methods

2.1. Mice procedures

The generation of Mito-PstI transgenic mice has been previously described (Fukui and Moraes, 2009). The AD transgenic mice, carrying mutant APP and mutant presenilin 1 (The Jackson Laboratory, Bar Harbor, Maine.), were first crossed with CaMKII α -tTa mice (The Jackson Laboratory). Double-positive mice, AD/CaMKII α -tTa, were then crossed with Mito-PstI mice to obtain AD/ CaMKII α -tTa/*PstI* mice that we called “AD-mito-PstI mice”. We called “AD mice” double-positive mice, either AD/CaMKII α -tTa or AD/Mito-*PstI*.

Expression of PstI was suppressed starting in utero until 6 months of age with a doxycycline diet (200 mg/kg; BioServ, Frenchtown, NJ). After the suppression period, animals were fed with standard rodent diet. All animals regardless of genotype were given this feeding regimen and sacrificed at 8 months of age. Only male animals were used and compared in this study. Each individual strain was on a pure C57Bl/6J background crossed at least 10 generations. All experiments and animal husbandry were performed according to a protocol approved by the University of Miami Institutional Animal Care and Use Committee. Mice were housed in a virus- and antigen-free facility of the University of Miami Division of Veterinary Resources in a 12-hour light/dark cycle at room temperature and fed ad libitum.

2.2. Western blot analyses

Protein extracts were prepared from the cortical, hippocampal, striatal, and cerebellar neuroanatomical regions homogenized in phosphate-buffered saline (PBS) containing a protease inhibitor mixture (Roche life Sciences, Branford, Connecticut). Sodium dodecyl sulfate was added to the homogenate at the final concentration of 4%. Protein (30 mg) was run on a 4%e20% gradient Tris-HCl gel (BioRad, Hercules, CA) and transferred to a polyvinylidene difluoride (PVDF) or nitrocellulose membrane (BioRad).

Membranes were blocked in 1:1 Odyssey blocking solution (LICOR Biosciences, Lincoln, NE) for 1 hour at room temperature. Primary and secondary antibodies were diluted in 1:10 Odyssey blocking solution. A list of primary and secondary antibodies used, dilutions, and incubation times is provided in the Supplementary Methods.

2.3. mtDNA deletions and depletion quantitation

DNA was isolated from cortical, striatal, hippocampal, and cerebellar homogenates using phenol:chloroform extraction. As previously described (Pickrell et al., 2011b), we designed primers to amplify mtDNA in a region outside of the PstI sites to determine the relative quantity of mtDNA (wild type [wt] and recombined molecules) in each sample. We used the comparative Ct method (Schmittgen and Livak, 2008), normalizing the amplification of mtDNA copy number to primers that amplify a genomic DNA region. Relative quantity (DDCt) was corrected for relative polymerase chain reaction (PCR) amplification efficiency using BioRad CFX Manager Software (version 3.0).

To detect mtDNA deletion events, DNA was used in a qPCR to detect potential recombination events that involve mtDNA deletions around the PstI restriction endonuclease sites. Primers sequences are provided in the Supplementary Methods.

2.4. β -Secretase activity assay

β -secretase activity was measured in cortical and hippocampal lysates using a Fluorescence resonance energy transfer (FRET)-based substrate, H-RE(EDANS)EVNLDAEFK(DABCYL)R-OH (Calbiochem, Billerica, MA), which contains a β -secretase cleavage sequence with Swedish-type mutations. Homogenates lysed in PBS 1% Triton-X and protease inhibitor cocktail (Roche) were centrifuged at 10,000 g for 5 minutes at 4 °C, and the supernatant was used for the analysis. The activity assay was carried out on 4 μ g of protein in a 100- μ L volume reaction volume that contained 12.5 μ M substrate and 20 mM sodium acetate (pH 4.4). The fluorescence from the released EDANS fluorophore was measured at 37 °C with excitation and emission wavelengths of 355 and 460 nm, respectively, using a Synergy H1 hybrid reader (Biotek, Winooski, VT). Background fluorescence from the substrate alone was subtracted from the readings. Fluorescence (arbitrary units), recorded 30 minutes after the initiation of the reaction, was used for the comparison between the “wt,” “AD,” and “AD-mito-PstI” groups.

2.5. Quantification of the A β ₁₋₄₂ fragment

Cortices and hippocampi were homogenized in cold guanidine buffer (5 M guanidine HCl, 50 mM Tris-HCl, pH 8.0). Total A β ₄₂ content was quantified using a human A β ₄₂ colorimetric enzyme-linked immunosorbent assay kit (Invitrogen/Life Technologies, Grand Island, NY) according to the manufacturer's instruction with an incubation time of 16 hours at 4 °C.

2.6. Immunostaining and stereological quantification of amyloid plaques

Immunostained brain section (see the Supplementary Methods for details) images were captured using an Olympus BX51 microscope. As previously described (Fukui et al., 2007), the total number of amyloid plaques per tissue area (cerebral cortex and hippo-campus) were counted from 15 consecutive stained sections per animal (n = m 5-7 per group), scanning the whole area using a 40x objective.

Antibodies used for morphology analysis were: monoclonal anti-NeuN (1:500; Millipore, Billerica, MA), monoclonal anti-gial fibrillary acidic protein (GFAP) (1:500; Cell Signaling, Danvers, MA) and monoclonal Anti-Neuronal Class III b-Tubulin (Clone TUJ1) (1:500; Abcam).

For slides that were Nissl-stained, sections were prepared as already stated herein. Sections were deparaffinized in xylenes and rehydrated in gradient alcohol washes. Sections were stained for 3 minutes with Nissl stain at room temperature. Sections were then dehydrated with gradient alcohol washes and cleared with xylenes. Samples were mounted with SurgiPath Sub-X mounting media (Leica, Wetzlar, Germany). Images were captured using an Olympus BX51 microscope.

2.7. Measurement of hydrogen peroxide generation from isolated mitochondria

Mitochondria were isolated from the cerebral cortex as previously reported (Pickrell et al., 2011a). Final mitochondrial pellets were resuspended into ice-cold mitochondria incubation buffer containing 130 mM KCl, 2 mM KH_2PO_4 , 2 mM MgCl_2 , 10 mM Hepes, and 1 mM ethylenediaminetetraacetic acid or EDTA (pH 7.2). Protein content was determined using a Bradford protein assay (BioRad). Protein (15 μg) was incubated with 10 mM Amplex Red (Invitrogen-Molecular Probes, Grand Island, NY), 3 units per mL horseradish peroxidase, 10 mM glutamate, 2 mM malate, and 2 mM adenosine diphosphate (ADP). Fluorescence originating from resorufin produced after the reaction between Amplex Red and peroxides was measured using the Wallac Victor2 1420 Multilabel counter (PerkinElmer Life Sciences, Waltham, MA) with excitation and emission wavelengths of 535 and 590 nm, respectively. A standard curve was created for each assay using different concentrations of hydrogen peroxide (0.25-8 mM). Antimycin A (1 mM), a known inducer of ROS, was used as a positive control. Background fluorescence that originated from Amplex Red itself or nonspecific oxidation of Amplex Red was also monitored in the absence of mitochondria and subtracted from the values recorded in the presence of mitochondria.

2.8. Insulin-degrading enzyme activity

Cortices and hippocampi were homogenized in cold PBS containing a protease inhibitor mixture (Roche). Total insulin-degrading enzyme (IDE) activity was quantified using an Immozyme Insulysin/IDE Immunocapture Activity Assay Kit (Calbiochem) according to the manufacturer's instructions.

2.9. Statistical analysis

A 2-tailed, unpaired Student t test was used to determine the statistical significance between the different groups. If more than 2 groups were analyzed, significance of the differences was evaluated using a 1-way analysis of variance followed by a Bonferroni post-test. Double asterisks indicate $p < 0.01$, a single asterisk indicates $p < 0.05$. Error bars represent standard error of the mean.

3. Results

3.1. Generation of AD-mito-PstI mice

We previously described the generation and the characteristics of mito-PstI mice using the CaMKII α promoter (Fukui and Moraes, 2009). Briefly, these mice express a mammalianized version of the bacterial *PstI* targeted to mitochondria, inserted downstream of a tetracycline response element promoter. Another transgenic allele expresses a tetracycline trans-activator (tTA) gene using the neuronal specific CaMKII α promoter. When mice harbor both transgenic alleles (PstI⁺/tTA⁺), mito-*PstI* is expressed in CaMKII α -positive neurons only in the absence of doxycycline (Fig. 1A).

Mito-*PstI* is successfully controlled temporally and spatially, and when mito-PstI mice are continuously induced from birth; they develop a severe neuronal OXPHOS deficiency attributed mostly to an mtDNA depletion, and die at 3 months of age (Fukui and Moraes, 2009).

To study how mtDNA damage affects A β pathologies in vivo, we crossed our mito-*PstI* mice with a mouse model of AD (Jankowsky et al., 2004) that overexpresses mutant APP and mutant presenilin 1 under the control of a brain-specific mouse prion protein promoter, heretofore referred to as “AD-mito-*PstI*” (Fig. 1A). This AD model accumulates A β -containing plaques in cortex and hippocampus with age (Garcia-Alloza et al., 2006). Plaques become evident and numerous by 6 months of age. To analyze the effect of mtDNA damage when the plaque formation and accumulation processes had already started, we induced mito-*PstI* expression in CaMKII β -positive neurons in adulthood. Mito-*PstI* expression was suppressed with a doxycycline diet for 6 months and induced with standard rodent diet for 2 months before mice were sacrificed for analyses (Fig. 1B).

To verify the induction conditions, we performed a Western blot analysis on brain region-specific homogenates using an anti-*PstI* antibody. We detected the expression of mito-*PstI* in cortex, hippocampus, striatum, and to a much lower extent, in cerebellum in 8-month-old AD-mito-*PstI* animals, and expression was absent in control animals (Fig. 1C).

3.2. Induction of mito-*PstI* in vivo causes mtDNA damage and a decrease in the levels of mtDNA-encoded proteins

To test whether the induction of mito-*PstI* damaged mtDNA, we analyzed the presence of recombination events at the *PstI* cutting sites. Recombination events after mito-*PstI*-induced double-strand breaks are relatively rare, because most of the mtDNA is degraded. Using quantitative PCR primers flanking the *PstI* sites in the mtDNA (see section 2. Methods), we were able to detect recombination events in mtDNA from cortex, hippocampus, and striatum, but not in cerebellum of some of the AD-mito-*PstI* mice (Fig. 1D). We did not detect any recombination events in the brain of AD mice not expressing mito-*PstI*. We then analyzed the total mtDNA content using quantitative PCR (see section 2. Methods). We analyzed the same 4 brain regions in wt, AD, and AD-mito-*PstI* animals; and we observed mtDNA reduction of approximately 40% in cortex of AD-mito-*PstI* mice compared with wt and AD mice (Fig. 1E). These data confirmed that mito-*PstI* caused mtDNA double-strand breaks and subsequent elimination in forebrain neurons.

To analyze the consequences of the reduced mtDNA, we examined the levels of several OXPHOS proteins in the different regions of AD-mito-*PstI* brains. We found that, among the proteins analyzed (cytochrome oxidase subunit I [CoxI];

NADH-ubiquinone oxidoreductase subunits Ndufs3, Ndufa9; succinate dehydrogenase subunits SDHA and SDHB; and ATPase subunit AtpA), spanning all the complexes of the OXPHOS chain, only the mtDNA-encoded CoxI protein was reduced in cortex and hippocampus of AD-mito-*PstI* animals (Fig. 2A and B). The subunits of Complexes I, II, and V analyzed were encoded by the nuclear DNA and therefore less affected by mtDNA damage.

Cytochrome oxidase enzyme activity in mito-*PstI*-expressing mice showed a significant reduction (approximately 50%) only in hippocampal samples (Fig. 2C) (see Supplementary Methods). Considering that in addition to neurons, the homogenates also contain other cell types (such as astrocytes and oligodendrocytes) that do not express the CaMKII α tTA, it is

likely that the partial enzyme defect in cortical neurons was masked when homogenates were analyzed.

3.3. AD-mito-PstI mice brains do not show signs of neurodegeneration

AD mice do not show major neuroanatomical changes in cortex or hippocampus (Verret et al., 2007). Because we were analyzing the role of mtDNA damage in plaque formation, we wanted to ensure that our studies on A β pathology would not be confounded by a large number of degenerating neurons. To analyze whether the mtDNA damage in these regions after 2-month expression of mito-*PstI* led to neuroanatomical impairment, we analyzed the gross neuroanatomy of 8-month-old AD and AD-mito-*PstI* mice. Neither hematoxylin and eosin (data not shown) nor Nissl staining (Supplementary Fig. 1A), 2 different techniques to visualize the cytoarchitecture in the brain, showed changes in the anatomy of the cortex and hippocampus or in the density of the cell population in any of the mouse groups. We then performed immunostaining analysis using different markers: anti-NeuN (Supplementary Fig. 1B) and anti-TUJ1/ β -III tubulin, neuronal markers, to detect any possible neuronal loss and anti-GFAP to identify any sign of gliosis. We found no significant differences between AD and AD-mito-*PstI* brains nor signs of neurodegeneration in cortex and hippocampus at this time point (8 months). We also confirmed using western blot analysis that there were no changes in TUJ1 or GFAP content in the cortex from the 2 groups of animals at 8 months (Supplementary Fig. 1C and D).

3.4. AD-mito-PstI brains have fewer amyloid plaques and fewer A β ₁₋₄₂ fragments

AD mice show many cerebral plaque formations at 6 months of age. At 8 months of age, the plaque number increases to 1.5e2 plaques per mm² (Fukui et al., 2007; Jankowsky et al., 2004) and most of them are present in the cerebral cortex and the hippocampus. To evaluate the effect of mtDNA damage on plaque number, we performed immunohistochemistry on serial coronal sections from 8-month-old AD and AD-mito-*PstI* brains with an anti-human A β antibody (6E10). AD-mito-*PstI* brains showed significant reductions in the number of plaques (both dense and diffuse) compared with age-matched control AD brains, in cortex and hippocampus (Fig. 3AeC).

In mouse models of AD, it has been shown that plaque number is correlated with A β content and to secretase activity (Li et al., 2004b; Mohajeri et al., 2004; Vassar et al., 1999). In particular, the most fibrillogenic amyloid fragment derived from the cleavage of APP is A β ₁₋₄₂. To determine whether the diminished number of plaques in AD-mito-*PstI* mouse cortices and hippocampi correlated with the amount of produced A β peptides, we performed a sandwich enzyme-linked immunosorbent assay to estimate the amount of A β ₁₋₄₂ from protein homogenates of wt, AD, and AD-mito-*PstI* mice. Cortical lysates from AD-mito-*PstI* brains showed a 70% reduced amount of A β ₁₋₄₂ compared with the ones from AD brains. When we measured A β ₁₋₄₂ levels in the hippocampus, we observed a trend of AD-*PstI* mice to have fewer fragments, although these data did not reach significance. The A β ₁₋₄₂ amount was undetectable in brains from age-matched wt animals, which were used as negative controls, because they do not express the human mutant APP (Fig. 3D).

3.5. Mito-Pstl is associated with altered APP processing

Either a decrease in the formation or an increase in the degradation of A β ₁₋₄₂ fragments could explain its reduced content in mito-*PstI*-expressing brains. To analyze the steps involved in A β ₁₋₄₂ formation, we performed western blot analyses of the different fragments derived from the APP sequential cleavage.

The 6E10 A β antibody is specific for human APP and recognizes amino acid residues 1e16 of the A β ₁₋₄₂ fragment (represented by a hexagon in Fig. 4A). Using the 6E10 antibody on cortical lysates, we could detect that the total amount of mutant APP expressed by the transgenic mice was not changed between AD and AD-mito-*PstI* (Fig. 4B). We then used an antibody that recognizes the carboxy terminal portion of APP (amino acid residues 676-695; shown as the hexagon combined with the circle in Fig. 4A). This antibody recognizes the human and the murine form of the epitope, showing both the mutant and the endogenous protein. From this analysis we detected an increase in the ratio between the β carboxy terminal fragment (β -CTF) and the full length APP, suggesting that this processing event was upregulated (Fig. 4C). We obtained similar results when we analyzed the hippocampal samples (data not shown).

One of the crucial events triggering the formation of amyloid fragments from APP is the cleavage by β -secretase (Citron et al., 1995). We measured β -secretase activity with a FRET-based assay in cortical lysates from AD and AD-mito-*PstI* brains, but we did not detect any significant difference between the 2 groups (Fig. 4D), suggesting that an increase in β -secretase was not the explanation for elevated relative levels of β -CTF/APP. We obtained similar results in hippocampal samples (data not shown).

To investigate the possibility that the decrease in A β ₁₋₄₂ fragments was because of an increase in its degradation, we analyzed some of the major proteases involved in this process, including IDE, and metalloprotease (MMP) 1 and 2. We also analyzed 2 major proteins involved in general cellular pathways for protein degradation: 20S proteasome, for the ubiquitin proteasome system (UPS), and LC3B for the autophagy pathway. We did not detect significant changes in the protein level of MMP1, MMP2, and the 20S proteasome nor changes in LC3B lipidation (Fig. 4F-H). Contrary to what was expected; we found that IDE activity was actually decreased in cortical samples of AD-mito-*PstI* mice (Fig. 4E).

3.6. AD-mito-Pstl mice did not show changes in ROS production or oxidative stress

Plaque formation has been correlated with ROS levels, and increased levels of lipid peroxidation, of DNA strand breaks, and the presence of specific oxidized DNA bases and adducts have been described in AD patient brains (Colurso et al., 2003; Williams et al., 2006). Moreover, animal models of AD in which a mitochondrial antioxidant enzyme (superoxide dismutase 2 or SOD2) was knocked out showed increased amyloid deposition and enhanced Ab levels (Li et al., 2004a).

To detect if, in our model, the mtDNA damage and partial deficiency in mtDNA-coded proteins provoked by mito-*PstI* expression stimulated ROS production, we measured hydrogen peroxide production (generated from superoxide anions derived from Complexes I-III) in mitochondria isolated from cortical homogenates of AD and AD-mito-*PstI* animals.

We did not detect significant changes in the rate of hydrogen peroxide production between mitochondria purified from the 2 groups of animals (Fig. 5A).

To investigate for other signs of oxidative stress, we also measured 4-hydroxy-2-nonenal (HNE) adducts using Western blot analysis and analyzed 8-hydroxy-deoxy-guanosine (8-OHdG)/ 8-hydroxy-guanosine (8-OHG) staining using immunohisto-chemistry (see Supplementary Methods). HNE is a major product of endogenous lipid peroxidation, and the presence of HNE adducts is a sign of increased cellular free radicals; 8-OHdG and 8-OHG are respectively a product of DNA and RNA damage induced by ROS (Kasai and Nishimura, 1984). We did not detect changes between AD and AD-mito-*PstI* mice in either HNE content (Fig. 5C) or in 8-OHdG and 8-OHG staining (Fig. 5B), showing that mtDNA damage in brain does not necessarily exacerbate cellular oxidative stress.

4. Discussion

In this study, we caused mtDNA damage in adult cortical and hippocampal neurons during the period of b-amyloid plaque formation by inducing mito-*PstI* expression at 6-8 months of age. This is the period when AD mice show marked accumulation of A β fragments and amyloid plaques. A 2-month induction period was sufficient to produce a decrease in CoxI content, an mtDNA encoded protein, indicating an mtDNA depletion consequent to *PstI* induction. We showed that mtDNA damage, which was associated with a mild OXPHOS deficiency, had a negative effect on Ab₁₋₄₂ content and, consequently, on plaque burden. In addition, we found that this effect was associated with an alteration in APP processing, but not with an increased degradation of the A β fragments, and that this phenomenon was independent from oxidative stress.

One hypothesis is that oxidative stress in AD can be directly tied to the toxicity of A β , which negatively affects the mitochondrial function. A β binds to A β binding alcohol dehydrogenase (ABAD), which is a mitochondrial enzyme, negatively affecting its activity and causing free radical production (Lustbader et al., 2004). When ABAD is inhibited, mitochondrial dysfunction, ROS formation, and A β accumulations are reduced. Accordingly, behavioral phenotypes of AD mouse models are ameliorated (Yao et al., 2011). In contrast, the upregulation of ABAD caused by behavioral stress or transgenic overexpression exacerbates AD phenotypes in these mouse models (Lustbader et al., 2004; Seo et al., 2011). Also, studies in AD mouse models have reported mitochondrial dysfunctions in the central nervous system when compared with age-matched littermates suggesting that A β oligomerization impairs mitochondrial function (Fukui et al., 2007; Yao et al., 2009).

ROS generation and damage has been implicated in the development of AD (Subbarao et al., 1990). Complex I dysfunction and Complex III-derived ROS also influence APP processing leading to A β generation or increased Ab production (Leuner et al., 2012a). Recent studies have also reported that the treatment of different AD mouse models with mitochondrial-targeted antioxidants have shown protective effects such as reversing survival trends, behavioral deficits, A β production, and plaque accumulation (Mao et al., 2012; McManus et al., 2011). However, these mouse model studies began treatment either in utero or from 2

months of age long before A β formation and deposition begins (Mao et al., 2012; McManus et al., 2011). In addition, it is controversial whether antioxidants or ROS scavengers specific to the mitochondria reduce AD pathologies in humans (Galasko et al., 2012; Morris et al., 2002). Therefore, although ROS can exacerbate AD pathology, it is not clear if it has a primary role in the development of the disease.

We previously described (Fukui et al., 2007) another mouse model in which cytochrome oxidase 10, COX10 (a gene coding for a nuclear subunit of Complex IV) was knocked out from birth in forebrain neurons in an AD background. This model developed a severe Complex IV deficiency, showing a striking age-related neurodegeneration of forebrain neurons and reduced number of plaques that correlated with a decreased ROS damage. Oxidative stress increases expression and activity of β -secretase (Tamagno et al., 2002), and therefore, the significant reduction in the β -secretase activity in AD-COX10 knockout mice was ascribed to attenuated ROS (Fukui et al., 2007). The COX10 model, however, had 3 major limitations: (1) the COX10 ablation from fetal development caused massive neurodegeneration; (2) because of the severe loss of neurons, mice had to be studied early (4 months), when the levels of A β accumulated were very low; and (3) a severe Complex IV deficiency does not have the same potential that partial defects in other respiratory complexes have in producing ROS.

In the model described here, we did not observe major neuronal loss, allowing for a better analysis of A β accumulation. We detected a decrease in β -secretase activity in AD-mito-*PstI* mice compared with wt mice, but we did not observe changes in β -secretase activity when comparing AD-mito-*PstI* with AD mice. However, we did detect a significant reduction in the levels of the A β ₁₋₄₂ fragment in AD-mito-*PstI* mice compared with AD mice, and increased relative levels of b-CTF indicating an alteration in APP processing. Even though a significant reduction in β -secretase activity was not observed, it appears that a mild decrease in OXPHOS can lead to an alteration in A β production and accumulation. Because we did not observe a significant change in ROS levels, it is possible that the defect in OXPHOS function or mtDNA affect APP processing in a ROS-independent manner.

Another important process involved in plaque accumulation is A β degradation. Many proteases are involved in this process, including: neprilysin, endothelin-converting enzyme, IDE, angiotensin-converting enzyme, tissue plasminogen activator (tPA) and urokinase (uPA), cathepsin D, gelatinase A, gelatinase B, coagulation factor XIa, antibody light chain c23.5 and hk14, and α 2-macroglobulin complexes (Wang et al., 2006). Besides these proteases, MMPs like MMP2 and MMP9 are partially responsible for oligomer degradation (Backstrom et al., 1996; Roher et al., 1994; Yamada et al., 1995). Microarray studies performed on cell cultures showed that several genes involved in extracellular matrix remodeling like MMP1, tissue inhibitor of metalloproteinase (TIMP1) and TIMP2 are differentially expressed during an OXPHOS dysfunction (van Waveren et al., 2006). Therefore, we analyzed the possibility that the mtDNA damage led to an upregulation of specific MMPs leading to a protective degradation of oligomers thus resulting in fewer A β plaques.

We examined many of the markers of the protein degradation pathway responsible for removing A β and, even though the analysis of LC3 lipidation levels, alone, is not a clear indication of an impairment of the autophagy pathway, we found that none of the analyzed markers were significantly changed.

Unexpectedly, we observed a paradoxical decrease in IDE activity in AD-mito-PstII cortices. Mitochondrial dysfunction is implicated in AD and in the development of diabetes mellitus, and patients with diabetes are twice as likely to develop AD (Moreira et al., 2007; Ohara et al., 2011). IDE also has an alternative form that can localize to the mitochondria (Leissring et al., 2004).

Besides a direct role, mitochondrial function could influence A β accumulation indirectly, by affecting the endoplasmic reticulum (ER) function. These organelles are physiologically related (de Brito and Scorrano, 2008) and a great deal of cross-signaling between them has been reported. In particular, A β -induced ER stress activates a mitochondrial apoptotic cascade that involves loss of mitochondrial membrane potential and caspase 9-3 activation (Ferreiro et al., 2008), but this pathway seems to require fully functional mitochondria because it is inhibited in a cell model deprived of mtDNA (Costa et al., 2010). We did detect a marked increase in the levels of C/EBP homologous protein (CHOP) (Fig. 5D), a transcription factor associated with the expression of molecular chaperones, mostly in the ER (Ryan and Hoogenraad, 2007). It is possible that, in our model, the role that ER stress has in activating the mitochondrial apoptotic cascade is inhibited by the OXPHOS deficiency itself. In addition, a recent report pointed to ER-Mitochondrial connections as key sites for the development of APP pathology (Area-Gomez et al., 2012). The role of this potential crosstalk between organelles will require further exploration.

In summary, the observations described in this report showed that mtDNA damage in the adult mouse brain (in the form of double-strand breaks) does not cause an increase in A β accumulation, but rather a decrease. Although the molecular damage to mtDNA during normal aging is likely different in type or intensity, this model allowed us to answer a question that cannot be addressed in pharmacological models. Our results support a model in which mitochondrial dysfunction in AD patients and mouse models is not the cause of A β oligomer accumulation. Rather, our data are more compatible with a model in which OXPHOS function is decreased because of A β toxicity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by the National Institutes of Health Grants 1R01AG036871, 1R01NS079965, and 5R01EY010804 (CTM), 5T32NS007492, 5T32NS007459, American Heart Association Grant 11Pre7610007, and the Lois Pope LIFE Fellowship (AMP). The authors thank David Jackson (Neuroscience Program) for technical assistance, and Dr Beata Frydel and the Lois Pope LIFE Center Histology Core for the access and use of their microtome, reagents, and light microscopes.

References

- Abramov AY, Canevari L, Duchen MR. Beta-amyloid peptides induce mitochondrial dysfunction and oxidative stress in astrocytes and death of neurons through activation of NADPH oxidase. *J. Neurosci.* 2004; 24:565–575. [PubMed: 14724257]
- Anandatheerthavarada HK, Biswas G, Robin MA, Avadhani NG. Mitochondrial targeting and a novel transmembrane arrest of Alzheimer's amyloid precursor protein impairs mitochondrial function in neuronal cells. *J. Cell Biol.* 2003; 161:41–54. [PubMed: 12695498]
- Area-Gomez E, Del Carmen Lara Castillo M, Tambini MD, Guardia-Laguarta C, Groof de AJ, Madra M, Ikenouchi J, Umeda M, Bird TD, Sturley SL, Schon EA. Upregulated function of mitochondria-associated ER membranes in Alzheimer disease. *EMBO J.* 2012; 31:4106–4123. [PubMed: 22892566]
- Backstrom JR, Lim GP, Cullen MJ, Tokes ZA. Matrix metalloproteinase-9 (MMP-9) is synthesized in neurons of the human hippocampus and is capable of degrading the amyloid-beta peptide (1-40). *J. Neurosci.* 1996; 16:7910–7919. [PubMed: 8987819]
- Bowling AC, Mutisya EM, Walker LC, Price DL, Cork LC, Beal MF. Age-dependent impairment of mitochondrial function in primate brain. *J. Neurochem.* 1993; 60:1964–1967. [PubMed: 8473911]
- Calkins MJ, Manczak M, Mao P, Shirendeb U, Reddy PH. Impaired mitochondrial biogenesis, defective axonal transport of mitochondria, abnormal mitochondrial dynamics and synaptic degeneration in a mouse model of Alzheimer's disease. *Hum. Mol. Genet.* 2011; 20:4515–4529. [PubMed: 21873260]
- Casley CS, Canevari L, Land JM, Clark JB, Sharpe MA. Beta-amyloid inhibits integrated mitochondrial respiration and key enzyme activities. *J. Neurochem.* 2002; 80:91–100. [PubMed: 11796747]
- Chagnon P, Betard C, Robitaille Y, Cholette A, Gauvreau D. Distribution of brain cytochrome oxidase activity in various neurodegenerative diseases. *Neuroreport.* 1995; 6:711–715. [PubMed: 7605932]
- Chen JX, Yan SS. Role of mitochondrial amyloid-beta in Alzheimer's disease. *J. Alzheimers Dis.* 2010; 20(suppl 2):S569–S578. [PubMed: 20463403]
- Citron M, Teplow DB, Selkoe DJ. Generation of amyloid beta protein from its precursor is sequence specific. *Neuron.* 1995; 14:661–670. [PubMed: 7695913]
- Colurso GJ, Nilson JE, Vervoort LG. Quantitative assessment of DNA fragmentation and beta-amyloid deposition in insular cortex and midfrontal gyrus from patients with Alzheimer's disease. *Life Sci.* 2003; 73:1795–1803. [PubMed: 12888118]
- Coskun PE, Beal MF, Wallace DC. Alzheimer's brains harbor somatic mtDNA control-region mutations that suppress mitochondrial transcription and replication. *Proc. Natl. Acad. Sci. U.S.A.* 2004; 101:10726–10731. [PubMed: 15247418]
- Costa RO, Ferreira E, Cardoso SM, Oliveira CR, Pereira CM. ER stress-mediated apoptotic pathway induced by Aβ peptide requires the presence of functional mitochondria. *J. Alzheimers Dis.* 2010; 20:625–636. [PubMed: 20182029]
- Costa RO, Ferreira E, Martins I, Santana I, Cardoso SM, Oliveira CR, Pereira CM. Amyloid beta-induced ER stress is enhanced under mitochondrial dysfunction conditions. *Neurobiol. Aging.* 2012; 33:824.e5–824.e16. [PubMed: 21704433]
- Cutler RG, Kelly J, Storie K, Pedersen WA, Tammara A, Hatanpaa K, Troncoso JC, Mattson MP. Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* 2004; 101:2070–2075. [PubMed: 14970312]
- de Brito OM, Scorrano L. Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature.* 2008; 456:605–610. [PubMed: 19052620]
- Devi L, Prabhu BM, Galati DF, Avadhani NG, Anandatheerthavarada HK. Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction. *J. Neurosci.* 2006; 26:9057–9068. [PubMed: 16943564]

- Ferreiro E, Oliveira CR, Pereira CM. The release of calcium from the endoplasmic reticulum induced by amyloid-beta and prion peptides activates the mitochondrial apoptotic pathway. *Neurobiol. Dis.* 2008; 30:331–342. [PubMed: 18420416]
- Fukui H, Diaz F, Garcia S, Moraes CT. Cytochrome c oxidase deficiency in neurons decreases both oxidative stress and amyloid formation in a mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* 2007; 104:14163–14168. [PubMed: 17715058]
- Fukui H, Moraes CT. Mechanisms of formation and accumulation of mitochondrial DNA deletions in aging neurons. *Hum. Mol. Genet.* 2009; 18:1028–1036. [PubMed: 19095717]
- Galasko DR, Peskind E, Clark CM, Quinn JF, Ringman JM, Jicha GA, Cotman C, Cottrell B, Montine TJ, Thomas RG, Aisen P. Antioxidants for Alzheimer disease: a randomized clinical trial with cerebrospinal fluid biomarker measures. *Arch. Neurol.* 2012; 69:836–841. [PubMed: 22431837]
- Garcia-Alloza M, Robbins EM, Zhang-Nunes SX, Purcell SM, Betensky RA, Raju S, Prada C, Greenberg SM, Bacskai BJ, Frosch MP. Characterization of amyloid deposition in the APP^{swe}/PS1^{dE9} mouse model of Alzheimer disease. *Neurobiol. Dis.* 2006; 24:516–524. [PubMed: 17029828]
- Guglielmotto M, Tamagno E, Danni O. Oxidative stress and hypoxia contribute to Alzheimer's disease pathogenesis: two sides of the same coin. *ScientificWorldJournal.* 2009; 9:781–791. [PubMed: 19705038]
- Hansson Petersen CA, Alikhani N, Behbahani H, Wiehager B, Pavlov PF, Alafuzoff I, Leinonen V, Ito A, Winblad B, Glaser E, Ankarcrona M. The amyloid beta-peptide is imported into mitochondria via the TOM import machinery and localized to mitochondrial cristae. *Proc. Natl. Acad. Sci. U.S.A.* 2008; 105:13145–13150. [PubMed: 18757748]
- Hauptmann S, Scherping I, Droese S, Brandt U, Schulz KL, Jendrach M, Leuner K, Eckert A, Muller WE. Mitochondrial dysfunction: an early event in Alzheimer pathology accumulates with age in AD transgenic mice. *Neurobiol. Aging.* 2009; 30:1574–1586. [PubMed: 18295378]
- Hong MG, Myers AJ, Magnusson PK, Prince JA. Transcriptome-wide assessment of human brain and lymphocyte senescence. *PLoS One.* 2008; 3:e3024. [PubMed: 18714388]
- Jankowsky JL, Fadale DJ, Anderson J, Xu GM, Gonzales V, Jenkins NA, Copeland NG, Lee MK, Younkin LH, Wagner SL, Younkin SG, Borchelt DR. Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase. *Hum. Mol. Genet.* 2004; 13:159–170. [PubMed: 14645205]
- Kasai H, Nishimura S. Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. *Nucleic Acids Res.* 1984; 12:2137–2145. [PubMed: 6701097]
- Krishnan KJ, Ratnaike TE, De Gruyter HL, Jaros E, Turnbull DM. Mitochondrial DNA deletions cause the biochemical defect observed in Alzheimer's disease. *Neurobiol. Aging.* 2012; 33:2210–2214. [PubMed: 21925769]
- Leissring MA, Farris W, Wu X, Christodoulou DC, Haigis MC, Guarente L, Selkoe DJ. Alternative translation initiation generates a novel isoform of insulin-degrading enzyme targeted to mitochondria. *Biochem. J.* 2004; 383:439–446. [PubMed: 15285718]
- Leuner K, Muller WE, Reichert AS. From mitochondrial dysfunction to amyloid beta formation: novel insights into the pathogenesis of Alzheimer's disease. *Mol. Neurobiol.* 2012a; 46:186–193. [PubMed: 22833458]
- Leuner K, Schutt T, Kurz C, Eckert SH, Schiller C, Occhipinti A, Mai S, Jendrach M, Eckert GP, Kruse SE, Palmiter RD, Brandt U, Droese S, Wittig I, Willem M, Haass C, Reichert AS, Muller WE. Mitochondrion-derived reactive oxygen species lead to enhanced amyloid beta formation. *Antioxid. Redox Signal.* 2012b; 16:1421–1433. [PubMed: 22229260]
- Li F, Calingasan NY, Yu F, Mauck WM, Toidze M, Almeida CG, Takahashi RH, Carlson GA, Flint Beal M, Lin MT, Gouras GK. Increased plaque burden in brains of APP mutant MnSOD heterozygous knockout mice. *J. Neurochem.* 2004a; 89:1308–1312. [PubMed: 15147524]
- Li R, Lindholm K, Yang LB, Yue X, Citron M, Yan R, Beach T, Sue L, Sabbagh M, Cai H, Wong P, Price D, Shen Y. Amyloid beta peptide load is correlated with increased beta-secretase activity in sporadic Alzheimer's disease patients. *Proc. Natl. Acad. Sci. U.S.A.* 2004b; 101:3632–3637. [PubMed: 14978286]

- Lin MT, Simon DK, Ahn CH, Kim LM, Beal MF. High aggregate burden of somatic mtDNA point mutations in aging and Alzheimer's disease brain. *Hum. Mol. Genet.* 2002; 11:133–145. [PubMed: 11809722]
- Long J, He P, Shen Y, Li R. New evidence of mitochondria dysfunction in the female Alzheimer's disease brain: deficiency of estrogen receptor-beta. *J. Alzheimers Dis.* 2012; 30:545–558. [PubMed: 22451324]
- Lustbader JW, Cirilli M, Lin C, Xu HW, Takuma K, Wang N, Caspersen C, Chen X, Pollak S, Chaney M, Trinchese F, Liu S, Gunn-Moore F, Lue LF, Walker DG, Kuppusamy P, Zewier ZL, Arancio O, Stern D, Yan SS, Wu H. ABAD directly links Abeta to mitochondrial toxicity in Alzheimer's disease. *Science.* 2004; 304:448–452. [PubMed: 15087549]
- Manczak M, Anekonda TS, Henson E, Park BS, Quinn J, Reddy PH. Mitochondria are a direct site of A beta accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression. *Hum. Mol. Genet.* 2006; 15:1437–1449. [PubMed: 16551656]
- Manczak M, Calkins MJ, Reddy PH. Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: implications for neuronal damage. *Hum. Mol. Genet.* 2011; 20:2495–2509. [PubMed: 21459773]
- Mao P, Manczak M, Calkins MJ, Truong Q, Reddy TP, Reddy AP, Shirendeb U, Lo HH, Rabinovitch PS, Reddy PH. Mitochondria-targeted catalase reduces abnormal APP processing, amyloid beta production and BACE1 in a mouse model of Alzheimer's disease: implications for neuroprotection and lifespan extension. *Hum. Mol. Genet.* 2012; 21:2973–2990. [PubMed: 22492996]
- McManus MJ, Murphy MP, Franklin JL. The mitochondria-targeted antioxidant MitoQ prevents loss of spatial memory retention and early neuropathology in a transgenic mouse model of Alzheimer's disease. *J. Neurosci.* 2011; 31:15703–15715. [PubMed: 22049413]
- Mohajeri MH, Saini KD, Nitsch RM. Transgenic BACE expression in mouse neurons accelerates amyloid plaque pathology. *J. Neural Transm.* 2004; 111:413–425. [PubMed: 14991462]
- Moreira PI, Santos MS, Seica R, Oliveira CR. Brain mitochondrial dysfunction as a link between Alzheimer's disease and diabetes. *J. Neurol. Sci.* 2007; 257:206–214. [PubMed: 17316694]
- Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Aggarwal N, Wilson RS, Scherr PA. Dietary intake of antioxidant nutrients and the risk of incident Alzheimer disease in a biracial community study. *JAMA.* 2002; 287:3230–3237. [PubMed: 12076219]
- Mutisya EM, Bowling AC, Beal MF. Cortical cytochrome oxidase activity is reduced in Alzheimer's disease. *J. Neurochem.* 1994; 63:2179–2184. [PubMed: 7964738]
- Ohara T, Doi Y, Ninomiya T, Hirakawa Y, Hata J, Iwaki T, Kanba S, Kiyohara Y. Glucose tolerance status and risk of dementia in the community: the Hisayama study. *Neurology.* 2011; 77:1126–1134. [PubMed: 21931106]
- Paola D, Domenicotti C, Nitti M, Vitali A, Borghi R, Cottalasso D, Zaccheo D, Odetti P, Strocchi P, Marinari UM, Tabaton M, Pronzato MA. Oxidative stress induces increase in intracellular amyloid beta-protein production and selective activation of betaI and betaII PKCs in NT2 cells. *Biochem. Biophys. Res. Commun.* 2000; 268:642–646. [PubMed: 10679257]
- Pickrell AM, Fukui H, Wang X, Pinto M, Moraes CT. The striatum is highly susceptible to mitochondrial oxidative phosphorylation dysfunctions. *J. Neurosci.* 2011a; 31:9895–9904. [PubMed: 21734281]
- Pickrell AM, Pinto M, Hida A, Moraes CT. Striatal dysfunctions associated with mitochondrial DNA damage in dopaminergic neurons in a mouse model of Parkinson's disease. *J. Neurosci.* 2011b; 31:17649–17658. [PubMed: 22131425]
- Reddy PH, Beal MF. Amyloid beta, mitochondrial dysfunction and synaptic damage: implications for cognitive decline in aging and Alzheimer's disease. *Trends Mol. Med.* 2008; 14:45–53. [PubMed: 18218341]
- Roher AE, Kasunic TC, Woods AS, Cotter RJ, Ball MJ, Fridman R. Proteolysis of A beta peptide from Alzheimer disease brain by gelatinase A. *Biochem. Biophys. Res. Commun.* 1994; 205:1755–1761. [PubMed: 7811262]
- Ross JM, Oberg J, Brene S, Coppotelli G, Terzioglu M, Pernold K, Gojny M, Sitnikov R, Kehr J, Trifunovic A, Larsson NG, Hoffer BJ, Olson L. High brain lactate is a hallmark of aging and

- caused by a shift in the lactate dehydrogenase A/B ratio. *Proc. Natl. Acad. Sci. U.S.A.* 2010; 107:20087–20092. [PubMed: 21041631]
- Ryan MT, Hoogenraad NJ. Mitochondrial-nuclear communications. *Annu. Rev. Biochem.* 2007; 76:701–722. [PubMed: 17227225]
- Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. *Nature Protocols.* 2008; 3:1101–1108.
- Seo JS, Lee KW, Kim TK, Baek IS, Im JY, Han PL. Behavioral stress causes mitochondrial dysfunction via ABAD up-regulation and aggravates plaque pathology in the brain of a mouse model of Alzheimer disease. *Free Radic. Biol. Med.* 2011; 50:1526–1535. [PubMed: 21382475]
- Shen C, Chen Y, Liu H, Zhang K, Zhang T, Lin A, Jing N. Hydrogen peroxide promotes Abeta production through JNK-dependent activation of gamma-secretase. *J. Biol. Chem.* 2008; 283:17721–17730. [PubMed: 18436531]
- Sheng B, Wang X, Su B, Lee HG, Casadesus G, Perry G, Zhu X. Impaired mitochondrial biogenesis contributes to mitochondrial dysfunction in Alzheimer's disease. *J. Neurochem.* 2012; 120:419–429. [PubMed: 22077634]
- Su B, Wang X, Bonda D, Perry G, Smith M, Zhu X. Abnormal mitochondrial dynamics: a novel therapeutic target for Alzheimer's disease? *Mol. Neurobiol.* 2010; 41:87–96. [PubMed: 20101529]
- Subbarao KV, Richardson JS, Ang LC. Autopsy samples of Alzheimer's cortex show increased peroxidation in vitro. *J. Neurochem.* 1990; 55:342–345. [PubMed: 2355227]
- Swerdlow RH. Mitochondria in cybrids containing mtDNA from persons with mitochondrialriopathies. *J. Neurosci. Res.* 2007; 85:3416–3428. [PubMed: 17243174]
- Tamagno E, Bardini P, Obbili A, Vitali A, Borghi R, Zaccheo D, Pronzato MA, Danni O, Smith MA, Perry G, Tabaton M. Oxidative stress increases expression and activity of BACE in NT2 neurons. *Neurobiol. Dis.* 2002; 10:279–288. [PubMed: 12270690]
- van Waveren C, Sun Y, Cheung HS, Moraes CT. Oxidative phosphorylation dysfunction modulates expression of extracellular matrix remodeling genes and invasion. *Carcinogenesis.* 2006; 27:409–418. [PubMed: 16221732]
- Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J, Jarosinski MA, Biere AL, Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G, Citron M. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science.* 1999; 286:735–741. [PubMed: 10531052]
- Verret L, Jankowsky JL, Xu GM, Borchelt DR, Rampon C. Alzheimer's-type amyloidosis in transgenic mice impairs survival of newborn neurons derived from adult hippocampal neurogenesis. *J. Neurosci.* 2007; 27:6771–6780. [PubMed: 17581964]
- Wang DS, Dickson DW, Malter JS. beta-Amyloid degradation and Alzheimer's disease. *J. Biomed. Biotechnol.* 2006; 2006:58406. [PubMed: 17047308]
- Wang X, Su B, Lee HG, Li X, Perry G, Smith MA, Zhu X. Impaired balance of mitochondrial fission and fusion in Alzheimer's disease. *J. Neurosci.* 2009; 29:9090–9103. [PubMed: 19605646]
- Williams TI, Lynn BC, Markesbery WR, Lovell MA. Increased levels of 4-hydroxynonenal and acrolein, neurotoxic markers of lipid peroxidation, in the brain in mild cognitive impairment and early Alzheimer's disease. *Neurobiol. Aging.* 2006; 27:1094–1099. [PubMed: 15993986]
- Yamada T, Miyazaki K, Koshikawa N, Takahashi M, Akatsu H, Yamamoto T. Selective localization of gelatinase A, an enzyme degrading beta-amyloid protein, in white matter microglia and in Schwann cells. *Acta Neuropathol.* 1995; 89:199–203. [PubMed: 7538720]
- Yao J, Brinton RD. Estrogen regulation of mitochondrial bioenergetics: implications for prevention of Alzheimer's disease. *Adv. Pharmacol.* 2012; 64:327–371. [PubMed: 22840752]
- Yao J, Du H, Yan S, Fang F, Wang C, Lue LF, Guo L, Chen D, Stern DM, Gunn Moore FJ, Xi Chen J, Arancio O, Yan SS. Inhibition of amyloid-beta (Abeta) peptide-binding alcohol dehydrogenase-Abeta interaction reduces Abeta accumulation and improves mitochondrial function in a mouse model of Alzheimer's disease. *J. Neurosci.* 2011; 31:2313–2320. [PubMed: 21307267]
- Yao J, Irwin RW, Zhao L, Nilsen J, Hamilton RT, Brinton RD. Mitochondrial bioenergetic deficit precedes Alzheimer's pathology in female mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106:14670–14675. [PubMed: 19667196]

Fig. 1.

The generation of AD-mito-*PstI* mice to study neuronal mitochondrial DNA (mtDNA) damage in an Alzheimer's disease (AD) background. (A) Breeding scheme used to generate "AD-mito-*PstI*" mice that harbor 3 transgenic alleles. Final experimental animals are "AD +/tTA+/PstI+" and "AD" controls are "AD+/tTA/+" or "AD+/PstI/+". Wild type (wt) controls used for some experiments are age-matched pure C57Bl/6J. (B) Depiction of the suppression and induction scheme to induce mito-*PstI* expression in an AD background only in adult animals. (C) Representative Western blot analysis detecting the expression of mito-*PstI* protein in the cortex, hippocampus (hippo), striatum (str), and cerebellum (crbl) of AD-mito-*PstI* mice. Anti-*PstI* antibody shows an aspecific band that is indicated by an asterisk (*). Loading was normalized for α -tubulin. (D) Quantification and detection of recombined mtDNA molecules in AD-mito-*PstI* mice in the cortex, hippo, and str. No deleted mtDNA molecules were detected in AD or wt controls. Each square represents an individual animal

(n = 4-5 animals per group). (E) mtDNA copy number is reduced in the cortices of AD-mito-*PstI* mice compared with AD and wt control mice using qPCR (n = 4-5 animals per group). * $p < 0.05$. Abbreviations: Dox, doxycycline; E0, embryonic day 0; IRES, internal ribosome entry site; IVS, intervening sequence; nDNA, nuclear DNA.

Fig. 2.

Temporal mito-*PstI* expression leads to a decline in oxidative phosphorylation activity. (A) Representative Western blot analysis probing for different oxidative phosphorylation subunits in cortical, hippocampal, striatal, and cerebellar regions comparing expression between Alzheimer's disease (AD) and AD-mito-*PstI* mice. (B) Optical density (O.D.) of cytochrome oxidase (Cox) I in cortical homogenates from Western blot analyses comparing AD-mito-*PstI* and AD mice normalized to α -tubulin ($n = 4$ per group). (C) Spectrophotometric assay measuring cytochrome oxidase (COX) activity in cortical and hippocampal homogenates of AD and AD-mito-*PstI* mice ($n = 4-5$ per group). * $p < 0.05$. Abbreviations: Atp, adenosine triphosphate subunit; crbl, cerebellum; hippo, hippocampus; Nduf, NADH-ubiquinone oxidoreductase subunit; Sdh, succinate dehydrogenase subunit; str, striatum.

Fig. 3. AD-mito-*PstI* mice have a significant reduction in the number of amyloid beta (Ab) plaques. (A) Representative images of coronal Alzheimer's disease (AD) and AD-mito-*PstI* brains immunostained with an anti-Aβ antibody to detect dense and diffuse plaques. Bar = 0.5 mm. (B and C) Quantification of dense and diffuse Aβ plaques in AD and AD-mito-*PstI* mice in cortex (B) and hippocampus (C) (n = 5-7 per group). Squares represent the quantity of plaques per section from an individual animal. * p < 0.05. (D) Content of Aβ₁₋₄₂ fragments in cortical and hippocampal homogenates from AD and AD-mito-*PstI* mice (n = 4/group). Abbreviation: wt, wild type. * p < 0.05.

Fig. 4. Mito-*PstI* alters A β ₁₋₄₂ fragment production but not its degradation. (A) Diagram of the proteolytic processing of APP. The hexagon represents the epitope of the hAPP recognized by 6-10 Ab (only transgenic APPmut), the hexagon combined with the circle represents the epitope of APP recognized by APP-Ct Ab (transgenic and endogenous). (B) Western blot analysis detecting the presence of APPmut in cortical samples from Alzheimer's disease (AD) and AD-mito-*PstI* mice. (C) Western blot analysis detecting the presence of total APP and β carboxy terminal fragment (CTF) in cortical samples from AD and AD-mito-*PstI* mice; optical density of APP, β -CTF, and β -CTF/APP signal from Western blot analysis normalized to α -tubulin from cortical samples from AD and AD-mito-*PstI* mice. * $p < 0.05$. (D) Enzymatic activity of β -secretase in cortical homogenates of AD and AD-mito-*PstI* mice ($n = 4$ per group). (E) Enzymatic activity of IDE in cortical homogenates of AD and AD-mito-*PstI* mice ($n = 5$ per group) * $p < 0.05$. (F) Western blot detecting the presence of 20s proteasome, MMP1, and MMP2 in cortical samples from AD and AD-mito-*PstI* mice. (G) Western blot detecting the presence of LC3B (I and II) in cortical samples from AD and AD-mito-*PstI* mice. (H) Optical density (O.D.) of LC3bII/(LC3bI+II) signal from Western blot analysis normalized to α -tubulin from cortical samples from AD and AD-mito-*PstI* mice. For all the Western blot images: AD, $n = 4$; AD-mito-*PstI*, $n = 4$. Abbreviations: A β ,

antibody; APP, amyloid precursor protein; AICD, amyloid precursor intracellular domain; APPmut, mutant APP; A.U., arbitrary unit; Ct, control; hAPP, human APP; IDE, insulin-degrading enzyme; LC, microtubule-associated protein 1A/1B-light chain; MMP, metalloprotease; sAPP, soluble APP; wt, wild type.

Fig. 5.

AD-mito-*PstI* mice do not show increased reactive oxygen species damage or increased reactive oxygen species production in cortical regions. (A) Rate of hydrogen peroxide production from isolated mitochondria from the cortical regions of Alzheimer's disease (AD) and AD-mito-*PstI* mice ($n = 4$ per group). (B) Representative immunohistochemistry with antibody anti-8-hydroxy-guanosine (8-OHG) and 8-hydroxy-deoxy-guanosine (8-OHdG) on AD and AD-mito-*PstI* brains ($n = 3$ mice per group). Scale bar, 200 μ m. Negative controls were treated with DNase and RNase, positive controls were treated with 30% H₂O₂. (C) Western blot detecting the presence of 4-hydroxy-2-nonenal (HNE) and SOD2 in cortical samples from AD and AD-mito-*PstI* mice (AD: $n = 3$; AD-mito-*PstI*: $n = 4$) and relative optical densities (O.D.) from Western blot analyses normalized to α -tubulin. * $p < 0.05$. (D) Western blot analysis probing for CHOP protein expression in the cortical regions of AD and AD-mito-*PstI* mice (AD: $n = 4$, AD-mito-*PstI*: $n = 3$). Abbreviations: CHOP, C/EBP homologous protein; SOD, superoxide dismutase.