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Cyclophilin D deficiency improves mitochondrial function and learning/memory in aging Alzheimer disease mouse model

Heng Du^a, Lan Guo^a, Wensheng Zhang^{a,b}, Monika Rydzewska^a, and Shidu Yan^{a,c,*}

^aDepartment of Surgery, and Taub Institute for Research on Alzheimer's Disease and the Aging Brain, College of Physicians & Surgeons of Columbia University, 650 West 168th Street, New York, NY 10032, USA

^bInstitute of Natural Medicine and Chinese Medicine Resources, Beijing Normal University, Beijing 100875, PR China

^cDepartment of Pathology, and Taub Institute for Research on Alzheimer's Disease and the Aging Brain, College of Physicians & Surgeons of Columbia University, 650 West 168th Street, New York, NY 10032, USA

Abstract

Mitochondrial stress is one of the early features of Alzheimer disease (AD). Mitochondrial A β has been linked to mitochondrial toxicity. Our recent study demonstrated that cyclophilin D (CypD) mediated mitochondrial permeability transition pore (mPTP) is an important mechanism for neuronal and synaptic stress induced by both A β and oxidative stress. In transgenic AD-type mice overexpressing mutant amyloid precursor protein (APP) and A β (mAPP), CypD deficiency improves mitochondrial and synaptic function and learning/memory up to 12 months old. Here we provide evidence of the protective effects of CypD deficiency in aged AD mice (22–24 months). CypD deficient mAPP mice demonstrate less calcium-induced mitochondrial swelling, increased mitochondrial calcium uptake capacity, preserved mitochondrial respiratory function and improved spatial learning/memory even in old age (known to be the age for late stage AD pathology and synaptic dysfunction). These data demonstrate that abrogation of CypD results in persistent life-long protection against A β toxicity in an Alzheimer's disease mouse model, thereby suggesting that blockade of CypD may be of benefit for Alzheimer disease treatment.

Keywords

Alzheimer's disease; Amyloid beta; Mitochondrial permeability transition pore; Cyclophilin D

1. Introduction

Alzheimer disease (AD) is the most common form of dementia affecting the aging population. Patients suffering from AD exhibit progressive deterioration in cognitive function and may present with mental symptoms such as delusions. Late stage AD patients manifest even more severe clinical and neuropathological changes including massive amyloid beta (A β) deposition in parenchyma and cerebrovascular walls (McGowan et al., 2005; Rensink et al., 2003; Selkoe, 2000) as well as neuronal loss (Eucher et al., 2007) and

neurofibrillary tangles (Crowther and Goedert, 2000). A large body of evidence *in vitro* and *in vivo* indicates that A β exerts neurotoxicity via mediation of apoptosis pathways (Kadowaki et al., 2005; Mattson et al., 1998; Paradis et al., 1996), oxidative stress (Tanaka et al., 2002), and intracellular calcium perturbation (Brzyska and Elbaum, 2003). Importantly, mitochondria play a central role in mediating apoptosis, generating reactive oxygen species (ROS), and maintaining intracellular calcium homeostasis; thus, mitochondrial abnormalities may be linked to A β -induced neurotoxicity and the pathogenesis of AD.

Recent studies provide substantial evidence that the prevalent mitochondrial damage in AD is linked to A β toxicity (Lustbader et al., 2004; Reddy and Beal, 2008; Rui et al., 2006; Takuma et al., 2005). Decreased mitochondrial respiration chain function (Bosetti et al., 2002; Takuma et al., 2005), increased mitochondrial ROS generation (Ohta and Ohsawa, 2006; Takuma et al., 2005) and changes in mitochondrial structure (Aleardi et al., 2005; Parks et al., 2001) have been observed in AD brains, AD mouse models and cellular experiments *in vitro*. A β species are found in the mitochondria and progressively accumulate within mitochondria, contributing to neuronal and synaptic stress. Furthermore, interaction of mitochondrial A β with certain mitochondrial molecules, such as amyloid binding alcohol dehydrogenase (ABAD) (Lustbader et al., 2004; Takuma et al., 2005) and cyclophilin D (CypD) (Du et al., 2008), exaggerates aberrant mitochondrial function through generation of reactive oxygen species (ROS) or dysregulation of mitochondrial permeability transition pore (mPTP), lending strong support for the pathogenic role of mitochondrial A β in AD pathogenesis.

CypD is generally considered to be a crucial component for mitochondrial permeability transition pore (mPTP) formation (Baines et al., 2005; Basso et al., 2005). Further, mPTP is involved in the processes of cellular necrosis and apoptosis (Baines et al., 2005; Eliseev et al., 2007; Schinzel et al., 2005) by way of collapsing mitochondrial membrane potential, repressing mitochondrial respiratory function, releasing mitochondrial calcium and cytochrome c, and enhancing ROS generation (Baines et al., 2005; Connern and Halestrap, 1994; Jordan et al., 2003). Our recent study demonstrated that A β decreases the threshold of mPTP formation by interacting with CypD and upregulating CypD expression levels in an AD mouse model overexpressing a mutant human form of amyloid precursor protein (mAPP, Line J-20). We found that the mitochondrial function and learning/memory were significantly improved in CypD-deficient mAPP up to 12 months old (Du et al., 2008). However, whether CypD deficiency has a persistent protective effect on A β -mediated mitochondrial and cognitive dysfunction in the late stage of the AD mouse model remains unclear. To address this question, we evaluated the effects of CypD deficiency on mitochondrial function and learning/memory at the late stage of Alzheimer's disease mice up to 22–24 months old.

2. Materials and methods

2.1. Transgenic mice

Animal studies were approved by the Animal Care and Use Committee of Columbia University in accordance with the National Institutes of Health guidelines for animal care. CypD homozygous null mice (Ppif^{-/-}) were kind gifts from Dr. Jeffery D. Molkentin (Baines et al., 2005). These animals were backcrossed 10 times into the C57BL6 background. Transgenic mice overexpressing a mutant human form of amyloid precursor protein (APP) that encodes hAPP695, hAPP751, and hAPP770 bearing mutations linked to familial AD (V717F, K670M, N671L, J-20 line), were crossed with Ppif^{-/-} mice to generate four genotypes of mice: double transgenic (Tg) mice overexpressing mutant APP and CypD deficiency (APP/Ppif^{-/-}); single Tg mice overexpressing mutant APP (mAPP); CypD-

deficient mice (Ppif^{-/-}); and, nonTg littermate controls. Offsprings of Tg mice were identified by PCR using primers for each specific transgene as previously described (Du et al., 2008). All mice used for the described experiments were 22–24 months old.

2.2. Mitochondria isolation

Mitochondria were isolated from mice of 22–24 months old. Brain cortices were homogenized five times the volume of ice-cold isolation buffer (225 mM mannitol, 75 mM sucrose, 2 mM K₂PO₄, 0.1% BSA, 5 mM HEPES, and 1mM EGTA, 0.25 mM DTT, pH 7.2). Mitochondria were isolated as previously described (Caspersen et al., 2005). Briefly, the homogenate was centrifuged at 1500 × g for 5 min and the resultant supernatant was incubated in 10% Percol with 0.01% digitonin on ice for 5 min and then subjected to centrifugation at 12,000 × g for 10 min. The pellet was collected and resuspended in EGTA-free ice-cold isolation buffer and centrifuged for 10 additional min at 8000 × g. Resultant pellets were resuspended in EGTA-free ice-cold isolation buffer and recentrifuged at 8000 × g.

2.3. Western blot and antibodies

We have performed Western blot to determine cyclophilin D content in mitochondrial fraction. The mitochondria fraction were lysed in RIPA buffer (25 mM Tris–HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) containing protease inhibitor cocktail (Calbiochem, set V, EDTA free). Equal amount of proteins were loaded and separated by SDS–PAGE (12% Bis–tris gel, Invitrogen), and then transferred to nitrocellulose membrane (Amersham). Membrane was blocked in TBST buffer (20 mM Tris–HCl, 150 mM NaCl, 0.1% Tween-20) containing 5% nonfat dry milk (Santa Cruz) for 1 h at room temperature, and then incubated and gently shaken overnight (at 4 °C) with anti CypD Ig G (1:2000, generated in our laboratory) in TBST containing 5% nonfat dry milk; this was followed by incubation with corresponding secondary antibody for 1 h at room temperature. Chemiluminescence was detected by using ECL (GE). The same membrane was stripped and reprobed with antibody to cytochrome c oxidase (1:4000, Abcam) as the protein loading control.

For Aβ detection, mice cortices were homogenized in RIPA buffer containing protease inhibitor as described above. Equal amounts of protein were loaded and separated by 12% SDS–PAGE and transferred to nitrocellulose membrane. The membrane was dried and then boiled in PBS for 5 min. After boiling, membrane was blocked in TBST containing 1% non-fat dry milk for 1 h at room temperature, and incubated with anti Aβ Ig G (6E10) over night at 4 °C followed by incubation of secondary antibody for 1 h. After chemiluminescence analysis, the membrane was stripped and reprobed with anti β actin (1:10,000, Sigma). The intensity of blots was analyzed and compared using the NIH Image J program (Du et al., 2008).

2.4. Mitochondrial swelling assay

The calcium-triggered mitochondrial swelling assay was performed as previously described (Du et al., 2008). Briefly, mitochondria from mice cortices were suspended in 1 ml swelling assay buffer (100 μg protein, 150 mM KCl, 5 mM HEPES, 2 mM K₂HPO₄, 5 mM glutamate, 5 mM malate, pH 7.2). Mitochondrial swelling was triggered by the addition of calcium (500 nmol/mg protein). For cyclosporin A (CSA) (Sigma) treatment, CSA was added at a concentration of 1 μM for 5 min on ice before the addition of calcium. Swelling was observed by immediately recording changes in optical density (OD) values at 540 nm on an Amersham Biosciences Ultrospect 3100 pro spectrophotometer continuously for 10 min. Data were analyzed and are presented as the percentage of the initial OD value.

2.5. Mitochondria calcium retention capacity (CRC) assay

Mitochondrial calcium retention capacity (CRC) was measured using fluorescent indicator Calcium Green 5N (Invitrogen); 100 μ g mitochondria were suspended at room temperature in 1 ml potassium buffer (150 mM KCl, 5 mM HEPES, 2 mM K_2HPO_4 , pH 7.2) containing 1 μ M Calcium Green 5N with pulsate injection of calcium into the cuvette. To observe the effect of CSA on mPTP formation, 1 μ M CSA was used to treat mitochondria for 5 min on ice before the addition of calcium. Fluorescence changes at an excitation/emission of 506/531 nm were monitored with a FluoroMax-2 spectrophotometer (Jobin Yvon-Spex instruments S.A. Inc.).

2.6. CypD translocation

Analysis of CypD translocation to the inner membrane was performed as described by Connern and Halestrap (1994). In brief, mitochondria isolated from mice cortices were resuspended in buffer (150 mM KCl, 5 mM HEPES, 2 mM K_2HPO_4 , 5 mM glutamate, 5 mM malate, 150 mM potassium thiocyanate, pH 7.2) and subjected to freezing–thawing treatment five times. Inner membrane was extracted by a centrifugation at $150,000 \times g$ for 60 min and the resultant supernatant (mitochondrial matrix content) and pellet (mitochondrial membrane) were subjected to immunoblotting with CypD antibody.

2.7. Mitochondrial respiration control ratio

Mitochondrial respiration rate was measured using a Clark oxygen electrode (Oxytherm, Hansatech) at 30 °C as previously described (Du et al., 2008). 300 μ M mitochondria were added into 1 ml potassium buffer. State 3 respiration was triggered by the addition of 150 μ M ADP in the presence of 5 mM glutamate and 5 mM malate, and state 4 respiration is defined as oxygen consumption after ADP has been consumed. The respiratory control ratio (RCR) of mitochondria is calculated as state 3/state 4.

2.8. Cytochrome c oxidase activity assay

The cytochrome c oxidase activities of mitochondrial fractions were measured with a cytochrome c oxidase kit (Sigma). Briefly, a suitable volume of mitochondria fraction and enzyme solution was added to 950 μ l assay buffer. The reaction was initiated by the addition of 50 μ l freshly prepared ferrocytochrome c substrate solution into the cuvette. Changes in OD values at 550 nm were recorded immediately using a kinetic program with 5 s delay, 10 s interval; a total of six readings were obtained utilizing an Amersham Biosciences Ultrospect 3100 pro spectrophotometer.

2.9. Behavioral analysis

Mouse (age- and gender-matched) spatial learning and memory were assessed in a 6-radial arm water maze as previously described (Arancio et al., 2004; Du et al., 2008; Lustbader et al., 2004). Mice were allowed 60 s to find the hidden platform and errors were counted. Mice that failed to find the platform within 60 s were guided to the hidden platform and stayed on the platform for 10 s. Retention memory was assessed 30 min after the initial water maze experiment. Investigators were blinded to mouse genotypes until all tests were completed.

2.10. Statistical analysis

Statistical analysis was performed using Statview statistics software. Differences between means were assessed by Student's *t*-test or one-way analysis of variance (ANOVA). *P* < 0.05 was considered significant.

3. Results

3.1. Expression of CypD in mAPP cortical mitochondria

CypD is a necessary component of mPTP formation, triggering the opening of mPTP by translocation of CypD to the inner membrane. Previously, we observed upregulation of CypD in mAPP mice as compared to nonTg mice at the age of 12 months (Du et al., 2008). For the current study, we compared CypD levels of elderly mAPP mice to nonTg mice. According to Western blot analysis, CypD levels in mAPP cortical mitochondria were significantly increased (32%) as compared to nonTg cortical mitochondria (Fig. 1A and B, $P < 0.001$). CypD was not present in the cortical mitochondria from either the Ppif^{-/-} or mAPP/Ppif^{-/-} mice (Fig. 1C).

3.2. A β levels in Tg mouse brain

To accurately evaluate the effect of CypD deficiency on A β toxicity, we compared A β levels in mAPP brains to those in mAPP/Ppif^{-/-} brains to exclude the possibility of deviation due to the effect of A β in brains of Tg mice. There were no significant differences in A β levels between mAPP and mAPP/Ppif^{-/-} mice at the age of 22–24 months (Fig. 2, $P > 0.05$), consistent with our previous findings that cerebral A β levels were comparable between these two genotypes of mice at the age of 12 months, and suggesting that CypD deficiency does not interfere with A β process or generation.

3.3. Effect of CypD deficiency on mitochondrial permeability transition pore (mPTP) in mAPP mice

Since increased expression of CypD is associated with a decreased threshold for mPTP opening; (Naga et al., 2007), we first examined mPTP formation by comparing calcium-induced mitochondrial swelling and calcium retention capacity among the four indicated Tg mice. Cortical mitochondrial from mAPP mice demonstrated a greater magnitude of swelling compared to nonTg mitochondria in response to the same amount of calcium insult (37.4% in mAPP mitochondria vs. 29.8% in nonTg mitochondria, Fig. 3A1, $*P < 0.05$). In contrast, cortical mitochondria from mAPP/Ppif^{-/-} mice are resistant to calcium-induced changes in mitochondrial volume as demonstrated by a significantly smaller magnitude of swelling (Fig. 3A1, $\#P < 0.001$ vs. either nonTg or mAPP mitochondria).

The addition of cyclosporin A (CSA), an inhibitor that blocks translocation of CypD to mitochondrial inner membrane, to mAPP cortical mitochondria significantly protected mitochondria from Ca²⁺-induced swelling. Notably, CypD deficiency in mAPP cortical mitochondria from mAPP/Ppif^{-/-} mice also exhibited significant attenuation of swelling in response to Ca²⁺ (Fig. 3A2, $*P < 0.01$ compared to mAPP mice). The suppression of mAPP mitochondrial swelling in the presence of CSA was comparable to that of mAPP/Ppif^{-/-} mitochondria. These results indicate that CypD is critical for PTP opening and that blockade of CypD in an A β enriched environment leads to improving mPTP function.

Mitochondrial calcium retention capacity (CRC) is another important parameter for evaluation of the mPTP formation threshold in that reduced CRC manifests a decreased mPTP formation threshold. mAPP mitochondria revealed a significantly reduced CRC (65.9%) as compared to nonTg mitochondria (112 ± 10.13 nmol/mg protein in mAPP mitochondria vs. 170 ± 8.56 nmol/mg protein in nonTg mitochondria, $P < 0.001$, Fig. 3B), whereas mAPP/Ppif^{-/-} mitochondria demonstrated normal CRC (285 ± 17.07 nmol/mg protein, $P < 0.001$ vs. mAPP mitochondria Fig. 3B). The Ppif^{-/-} mice had a greater CRC (840 ± 18.59 nmol/mg protein, $P < 0.001$ vs. nonTg) (data not shown). Similarly, inhibition of CypD after the addition of CSA to mAPP mitochondria significantly increased CRC to 272 ± 13.48 nmol/mg protein (Fig. 3B, $*P < 0.001$ vs. mAPP without CSA treatment).

These results demonstrate that deleting or blocking CypD in mAPP mitochondria significantly improves calcium uptake capacity.

As the formation of mPTP is achieved by the translocation of CypD to mitochondrial inner membrane, it is important to study CypD translocation to the inner membrane in static conditions. We therefore compared CypD translocation in the mitochondrial inner membrane between mAPP and nonTg mitochondria. As expected, CypD levels in the inner mitochondrial membrane from mAPP mice were significantly higher than in nonTg inner mitochondrial membrane; in parallel, CypD levels in mitochondrial matrix of mAPP mice were lower than in nonTg mitochondrial matrix (Fig. 4A, $P < 0.05$), suggesting that more CypD is translocated to the inner membrane in mAPP mitochondria than in nonTg mitochondria. This scenario promotes mPTP opening in response to stress conditions such as A β or oxidant insults. A β -rich mitochondria lacking or blocking CypD are still capable of protecting mitochondria from calcium-mediated impaired membrane permeability transition pore and calcium loading in aging AD mice, although they have only limited effects on mitochondrial properties.

3.4. Improved mitochondrial respiratory function and cytochrome c oxidase activity in mAPP/Ppif^{-/-} mitochondria

Mitochondrial respiration is critical for energy metabolism, ATP production and cell survival. Decreased mitochondrial respiratory function manifests severe damage to mitochondria. We analyzed mitochondrial respiration by measuring ADP-stimulated respiration control ratios (RCR). During the consumption of 150 μ M ADP, mAPP cortical mitochondria showed compromised RCR as compared to RCR in nonTg mitochondria, whereas mAPP/Ppif^{-/-} mitochondria revealed significantly improved RCR. There were no significant differences in RCR between nonTg and Ppif^{-/-} mitochondria (Fig. 5A1 and A2).

Cytochrome c oxidase is the key enzyme in the terminal step of mitochondrial electron transport chain. It involves in the establishment of the transmembrane difference of proton by receiving electron from cytochrome c. The activity of cytochrome c oxidase was significantly decreased by 30–40% in mAPP cortical mitochondria as compared with those in nonTg mitochondria. In contrast, mAPP/Ppif^{-/-} mitochondria showed a higher activity of cytochrome c oxidase activity than mAPP mitochondria. The activity of cytochrome c oxidase was comparable between nonTg mitochondria and Ppif^{-/-} mitochondria (Fig. 5B1 and B2).

Taken together with our previous observations that mAPP/Ppif^{-/-} mice have more highly preserved mitochondrial respiratory function and cytochrome c oxidase activity than mAPP mice at middle age, it appears that similar to other effects of aging, the absence of CypD has a persistent protective effect on mitochondrial respiratory function and cytochrome c oxidase activity even in the late stage AD mouse model exhibiting full brown amyloid pathology and neuronal perturbation.

3.5. Improvement of spatial learning and memory in mAPP/Ppif^{-/-} mice

Behavioral change is one of the outward manifestations of neuronal dysfunction. Our previous studies have shown that mAPP/Ppif^{-/-} mice have substantially improved spatial learning and memory capacities than their mAPP littermates up to 12 months of age (Du et al., 2008). To assess the effect of CypD deficiency on learning and memory in aged mAPP mice, we evaluated spatial learning and memory in mice at age of 22–24 months using a radial arm water maze as previously described (Arancio et al., 2004; Du et al., 2008; Lustbader et al., 2004). Both nonTg and Ppif^{-/-} mice had good learning and memory for finding hidden platforms during trials. Consistent with our previous results (Du et al., 2008),

mAPP mice displayed impaired spatial learning memory (5–6 errors during trials 3 and 4 and retention test) as compared to nonTg mice (1–1.5 errors during trials 3 and 4 and retention test). Notably, mAPP/Ppif^{-/-} mice made significantly fewer errors in finding the platform (2.5–3 errors in trials 3 and 4 and average 3.3 errors in retention test) than mAPP mice (Fig. 6), although learning memory was still impaired in mAPP/Ppif^{-/-} mice as compared to nonTg and Ppif^{-/-} mice. The four groups of mice did not show any significant difference in their speed of swimming or in the time required to reach the platform in the visible platform test (data not shown).

3.6. Discussion

In the present study, we extend our previous findings on the effects of CypD blockade in an AD mouse model (Du et al., 2008) by investigating whether CypD deficiency in aged mAPP mice is able to protect mitochondrial and cognitive function. We found that that 22–24 month old mAPP/Ppif^{-/-} mice have a higher mPTP formation threshold than mAPP mice. Further, mitochondrial respiratory function was significantly improved in CypD - deficient mAPP mice versus mAPP mice. Consistent with these results, CypD deficiency yielded significantly improved spatial learning and memory in aged mAPP/Ppif^{-/-} mice similar to results found in young and middle aged mice of the same genotype mice. Therefore, the present results, together with the previous findings, suggest that ablation of CypD gives life-long persistent protection against A β toxicity on mitochondrial and behavioral function.

Increasing evidence indicate that mitochondria serve as an A β targeted organelle. The presence of mitochondrial A β decreases mitochondrial key enzyme activities and mediates oxidative injury in neurons (Cardoso et al., 2004; Caspersen et al., 2005; Chen et al., 2007; Chen and Yan, 2006; Jhoo et al., 2004; Lustbader et al., 2004; Takuma et al., 2005). Specific selection of ion transport is critical for maintenance of the mitochondrial matrix pH gradient and mitochondrial membrane potential for sustained oxidative phosphorylation. It has been reported that A β causes intracellular calcium perturbation (Abramov et al., 2004; Begley et al., 1999), and that overload of mitochondrial calcium will trigger mPTP formation in the mitochondrial membrane to violate mitochondrial integrity (Gunter et al., 2000; Gunter et al., 1998). As CypD is considered to be a crucial component of mPTP, genetic deletion of CypD greatly increases mitochondrial capacity to buffer calcium and limits mPTP formation under conditions of stress (Baines et al., 2005; Basso et al., 2005; Nakagawa et al., 2005). Further, CypD deficiency failed to fully impede mitochondrial calcium efflux in the presence of intensive insults (Baines et al., 2005), implying a limitation of the protection of CypD deficiency on mitochondrial calcium modulation, especially under certain pathological conditions.

We previously demonstrated that mPTP formation is enhanced by A β in an AD mouse model from youth to middle age. Since extensive ROS generation and calcium perturbation are commonly seen during aging and can be aggravated by A β , it was important to determine the stage of aging at which CypD deficiency reverses mitochondrial abnormalities and neuronal function in AD mice that present with full blown amyloid pathology and increased A β toxicity. Indeed, the current studies in aged mice demonstrate that CypD depletion has persistent protective effects on mitochondrial and neuronal function in an A β -rich environment, such as in CypD-deficient mAPP mice up to the age of 22–24 months. Similarly, blockade of CypD in mAPP mitochondria by CypD inhibitor, cyclosporine A(CSA), rescues mitochondrial function as evidenced by attenuating calcium-induced swelling.

Mitochondrial respiration is the most important function of mitochondria, and healthy respiratory function is the manifestation of normal mitochondrial function. The rescued mitochondrial respiration control rate and cytochrome c oxidase activity in cortical

mitochondria from aged mAPP/Ppif^{-/-} mice suggest that CypD deficiency increases mitochondrial resistance to A β -induced insults, which is consistent with our observation of the effect of such a deficiency on mPTP formation. Although the respiration control ratio (RCR) of mitochondria from the aged mAPP/Ppif^{-/-} mice was still lower than that of mitochondria from the nonTg mice, cytochrome c oxidase activities in CypD-deficient mAPP mice completely recovered even at the late age of 22–24 months. Since the respiratory chain reflects electron flux through complexes I, III, and IV, inactivation of enzyme activities associated with any one of these complexes in the respiratory chain could disturb the respiratory chain function causing decreased oxygen consumption. Thus, the reduction in the activity of cytochrome c oxidase, a component of complex IV of the mitochondrial electron transport chain, could be one of the mechanisms for perturbing mitochondrial respiratory chain function observed in mAPP mice.

Considering the potential for damage to the mitochondria, especially, for A β -containing mitochondria (increase in the vulnerability of mitochondria to mechanical damage), during the isolation of mitochondria, we paid special attention to balancing mitochondrial yield against retention of a potentially fragile subpopulation of mitochondria. The mitochondria were prepared by the method described previously (Caspersen et al., 2005) with careful controlling of the mitochondria yielding and minimum mechanical damage to mitochondria integrity. By ultrastructural studies using electron microscopy, preparations of brain mitochondria appeared homogeneous, >95% enrichment and intact. There were no significant differences in enzymatic activities representative of complexes I and II from mitochondria harvested from mAPP mice or nonTg littermate controls (Caspersen et al., 2005). The activity of citrate synthase, mitochondrial matrix enzyme, was comparable between mAPP mitochondria and nonTg mitochondria (data not shown). Furthermore, levels of A β in mAPP mice was comparable to those in cyclophilin D-deficient mAPP mice (mAPP/Ppif^{-/-} mice), suggesting that the mitochondria from the two types of transgenic mice are exposed to similar A β -induced insults, whereas CypD-deficient mAPP mitochondria preserved respiratory function and cytochrome c oxidase activity. Therefore, it is unlikely that impaired mitochondrial function observed in mAPP mice, as evidenced by decreases in the oxygen consumption and cytochrome c oxidase, was due to the potential damage to mitochondria during the isolation procedure. Our results indicate that the absence of CypD makes mitochondria and neurons more resistant to injury induced by A β and oxidative stress.

Consistent with these changes in mitochondrial respiratory function, aged mAPP/Ppif^{-/-} mice demonstrate better performance on working and retention memory abilities in behavioral tests as compared to mAPP mice, although the aged mAPP/Ppif^{-/-} mice still showed impairment in both working and retention memory abilities compared to nonTg or Ppif^{-/-} mice. CypD therefore may also be critical, at least in part, for A β -mediated deficits in learning and memory through the late stages of AD.

Compromised mitochondrial respiration function and learning/memory ability in mAPP/Ppif^{-/-} mice is consistent with the decreased mPTP threshold. We attribute such compromised protection to increased A β toxicity at late stages of the AD mouse model. Mitochondria from aged mAPP mice showed sustained A β insults during aging. Long-lasting intensive A β toxicity induces diverse irreversible mitochondrial damage via several pathways other than triggered mPTP formation, such as increased oxidative injury (Kaminsky and Kosenko, 2008; Lustbader et al., 2004; Tamagno et al., 2003). Importantly, blockade of CypD in A β -containing mitochondria, either by deleting the CypD gene or by specific inhibition of CypD, has protective effects on mitochondrial and neuronal function through the late stages of AD mouse model.

In summary, these studies significantly increase the body of evidence that CypD-mediated mitochondrial membrane permeability transition pore formation contributes to mitochondrial and neuronal failure in an A β -rich environment. Blockade of CypD protects mitochondria from A β toxicity even in the aged AD mouse model, although the protective effects of inhibiting CypD might be compromised at more advanced stages. Thus, CypD may be a potential therapeutic target for halting AD progress.

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Fig. 1.

CypD expression in aged transgenic mice. Panel (A) densitometry of immunoreactive bands using NIH imageJ program ($n = 8$ mice per group; $P < 0.001$). Panels (B and C). Representative images of Western blots for CypD. Western blot for cytochrome c oxidase was used as a protein loading control showing an equal amount of protein added to each lane.

Fig. 2. Cerebral Aβ levels in transgenic mice at age of 22–24 months. Panel (A) densitometry of Aβ immunoreactive bands by using NIH imageJ program. Panel (B) Representative images of Western blot for Aβ and β-actin. β-Actin was used as a protein loading control ($n = 4-5$ per group).

Fig. 3.

Effect of CypD deficiency on mitochondrial swelling and calcium uptake capacity. (A) Comparison of mitochondrial swelling among Tg mice cortical mitochondria ($n = 4-5$ mice per group; * $P < 0.05$ vs. nonTg mitochondria; # $P < 0.001$ vs. mAPP or nonTg mitochondria panel A1). Mitochondrial swelling was induced by the addition of 500 nmol calcium per mg mitochondrial protein. The addition of CSA completely rescued mAPP mitochondria from calcium-induced swelling (* $P < 0.001$ vs. mAPP/Ppif^{-/-} mitochondria or mAPP mitochondria incubated with CSA panel A2). The calcium buffering capacity of mAPP mitochondria is much lower than that of nonTg, mAPP/Ppif^{-/-} mitochondria or mAPP mitochondria with CSA (* $P < 0.01$; $n = 4-6$ mice per group panel B).

* $P = 0.044$ vs nonTg mitochondrial membrane, # $P = 0.023$ vs nonTg mitochondrial matrix

Fig. 4. CypD translocation. Mitochondrial membrane and matrix were isolated from cortices of the indicated Tg mice and then subjected to Western blotting for CypD ($n = 4$ mice per group). Panel (A) indicates densitometry of CypD immunoreactive bands and panel (B) shows representative images of Western blotting for CypD and cytochrome c oxidase. Cytochrome c oxidase was used as a protein loading control indicating an equal amount of mitochondrial protein employed for each of the experiments.

(A2)

	nonTg	mAPP	Ppif ^{-/-}	mAPP/ Ppif ^{-/-}
RCR	5.68 ± 0.17	3.5 ± 0.24 *	5.75 ± 0.23	4.73 ± 0.12 #

* $P < 0.005$ vs. nonTg, Ppif^{-/-} and mAPP/Ppif^{-/-}

$P < 0.01$ vs. nonTg and Ppif^{-/-}

(B2)

	nonTg	mAPP	Ppif ^{-/-}	mAPP/ Ppif ^{-/-}
nmol /min/mg protein	86.46 ± 7.21	53.1 ± 6.1 *	86.96 ± 3.54	79.8 ± 4.19

* $P < 0.05$ vs. nonTg, Ppif^{-/-} and mAPP/Ppif^{-/-}

Fig. 5.

Effect of CypD deficiency on mitochondrial respiratory function and cytochrome c oxidase activity in mAPP mice. Respiration control rate (RCR) in the cortical mitochondria from the indicated Tg mice at 22–24 months old (panel (A1)). RCR values are shown in panel (A2) ($n = 4–5$ mice per group, * $P < 0.005$ vs. nonTg, mAPP/Ppif^{-/-} or Ppif^{-/-} mitochondria and # $P < 0.01$ vs. nonTg or Ppif^{-/-} mitochondria). Cytochrome c oxidase activity in the indicated Tg mice at 22–24 months old (panel (B1)). Value for cytochrome c oxidase activity (panel (B2)) ($n = 4–5$ mice per group, * $P < 0.05$ vs. other groups of mice).

* $P < 0.01$ vs. nonTg, Ppif^{-/-} and mAPP/Ppif^{-/-}

$P < 0.01$ vs. nonTg and Ppif^{-/-}

Fig. 6.

Effect of CypD deficiency on spatial learning and memory. Mice were tested in a 6-arm radialwater maze. mAPP mice made more errors in locating the hidden platform than other groups of mice (* $P < 0.01$). mAPP/Ppif^{-/-} mice demonstrated better performance than mAPP mice but worse than nonTg and Ppif^{-/-} mice (# $P < 0.01$; $n = 6-7$ mice per group).