



## Original Contribution

## Increased oxidative stress and astrogliosis responses in conditional double-knockout mice of Alzheimer-like presenilin-1 and presenilin-2

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## ABSTRACT

Conditional presenilin 1 and presenilin 2 double knockout causes memory dysfunction and reproduces neurodegenerative phenotypes of Alzheimer disease (AD) in mice. Oxidative stress has been long implicated predominantly in amyloidosis-mediated AD pathologies; however, its role in response to the loss-of-function pathogenic mechanism of AD remains unclear. In this study, we examined the oxidative stress status in PS1 and PS2 double-knockout (PS cDKO) mice using F<sub>2</sub>-isoprostanes (iPF<sub>2α</sub>-III) as the marker of lipid peroxidation. Lipid peroxidation was enhanced in a gender- and age-related manner in the PS cDKO mice independent of brain Aβ deposition. Such oxidative abnormalities predominantly in cerebral cortex at 2–4 months of age preceded the onset of many pronounced AD neuropathologies, suggesting that increased lipid peroxidation is not only an early pathophysiological response to PS inactivation, but also a potential culprit responsible for the AD-like neurodegenerative pathologies in the PS cDKO mice. Western blot analysis of cortical glial fibrillary acidic protein demonstrated an increased astrogliosis response to PS inactivation, in particular in the PS cDKO mice at as young as 2 months of age, suggesting that lipid peroxidation and neuronal injury may be closely associated with the loss-of-function neuropathogenic mechanism of AD.

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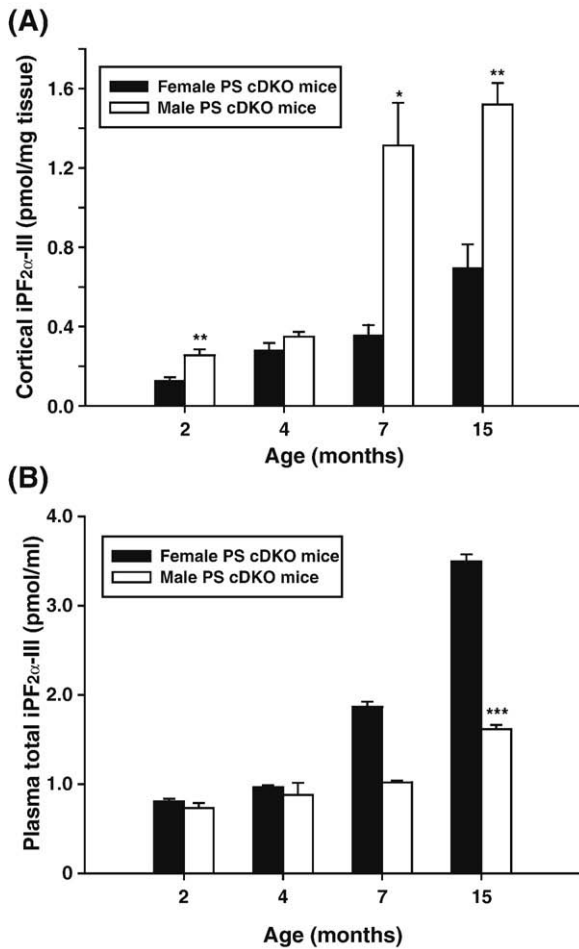
Alzheimer disease (AD)<sup>1</sup> is the most common late-life neurodegenerative disease and the main cause of dementia [1]. Previous research interests in AD were mainly focused on the amyloid-cascade hypothesis, which links amyloid β-peptide (Aβ) deposition in plaques in brain tissue with neuronal dysfunction and the induction of tangles and dementia [2]. Mutations in the presenilin 1 (PS1) and presenilin 2 (PS2) genes account for the majority of early onset familial AD [3,4]. Presenilins regulate the γ-secretase-mediated processing of amyloid precursor protein and are also involved in stimulation of the Notch signaling pathways [5]. PS1 knockout mice develop severe cerebral hemorrhage and widespread skeletal abnormalities [6,7]. PS2 knockout mice lack any detectable phenotype [8]. Whereas conventional PS1 and PS2 double-knockout mice often result in embryonic lethality, precluding the functional analysis of PS in adult [8,9], the forebrain-specific and conditional PS1 and PS2 double-knockout (PS cDKO) mice are indistinguishable from littermate controls during early adulthood and show age-dependent AD-like pathologies and phenotypes, including synaptic dysfunction, hyperphosphorylation of tau, severe neurodegeneration, and memory impairment [10,11]. Reduced Aβ production and increased inflammatory responses have been reported in the PS cDKO mice [12].

Free radical oxidative stress has been implicated in a variety of human diseases, including neurodegenerative disease and aging. The brain is particularly susceptible to oxidative stress because of its high oxygen consumption rate, it is rich in unsaturated lipids, and it has a relatively high abundance of redox-capable transition metal ions and a relatively low availability of antioxidant enzymes compared with other organs [13]. Indeed, increasing evidence has suggested the pivotal role of oxidative stress, predominantly manifested as lipid peroxidation, in the pathogenesis of AD [14–16]. Whereas oxidative damage to neuronal nucleic acids, lipids, and proteins in AD has been frequently linked to brain amyloidosis [17,18], it also has been demonstrated increasingly in human studies [19] and in AD-like transgenic animals [14] that oxidative stress is an early event preceding Aβ deposition in the formation of AD pathologies. Furthermore, previous investigations into oxidative imbalance in the underlying AD pathogenic mechanism have been largely based on transgenic animal models of AD-like brain amyloidosis. However, data derived from PS cDKO mice, which represent the loss-of-function pathogenic mechanism of AD with reduced Aβ production, are currently unavailable. The present study was conducted to investigate age- and gender-related oxidative imbalance in response to PS inactivation in the brain by measuring cerebral cortical and circulating levels of F<sub>2</sub>-isoprostanes (also referred to as iPF<sub>2α</sub>-III, 15-F<sub>2t</sub>-IsoP, or 8-iso-PGF<sub>2a</sub>), a specific and sensitive *in vivo* biomarker of lipid peroxidation, in PS cDKO mice. To assess further the oxidative stress status during the early development of AD pathologies, the levels of endogenous antioxidant enzymes, including superoxide

Abbreviations: AD, Alzheimer disease; PS1, presenilin-1; PS2, presenilin-2; PS cDKO, PS1 and PS2 double knockout; GFAP, glial fibrillary acidic protein.

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**Fig. 1.** Differential effects of gender on formation of iPF<sub>2α</sub>-III in (A) cerebral cortex and (B) plasma samples from PS cDKO mice at ages 2, 4, 7, and 15 months. \* $p < 0.05$ , \*\* $p \leq 0.01$ , and \*\*\* $p \leq 0.001$ .

dismutase (SOD), glutathione peroxidase (GSH-px), and catalase (CAT), were determined in blood samples from female and male PS cDKO mice at 2, 4 (for plasma SOD), and 7 months (for both GSH-px and CAT) of age and in gender- and age-matched wild-type (WT) mice. Meanwhile, the expression of glial fibrillary acidic protein (GFAP) in the cerebral cortex of the female and male PS cDKO mice was also analyzed as the astrocytosis marker to explore potential interactions between oxidative stress and astrocytic activation in the brain toward the loss-of-function pathogenic mechanism of AD-like presenilins.

## Materials and methods

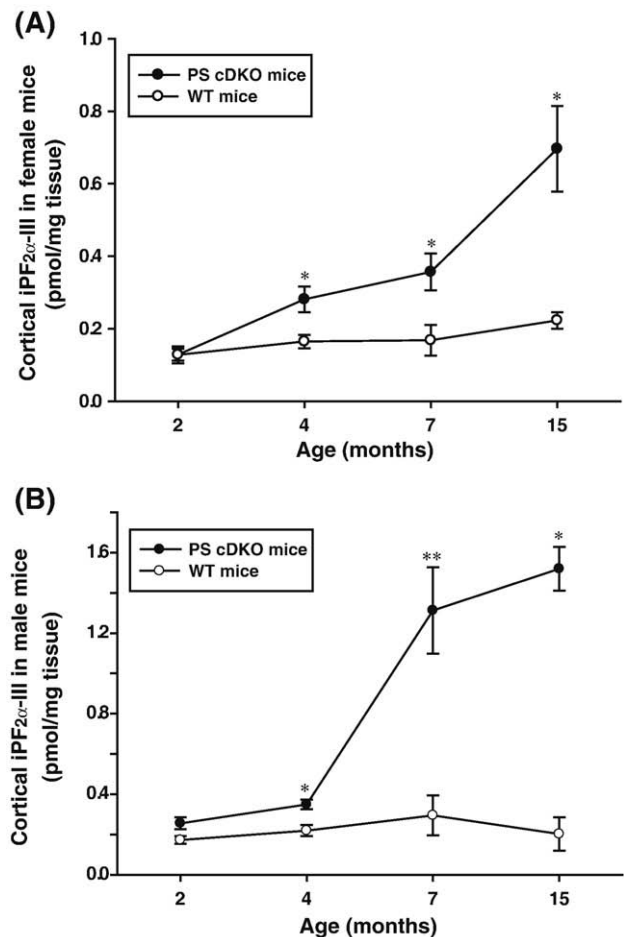
### Animals

The study was approved by the Animal Ethics Committee and all procedures complied with international standards of humane care in animal experimentation. The PS knockout mice were generated as previously published [8,10,11,20]. Briefly, PS cDKO mice were obtained by crossing forebrain-specific PS1 heterozygous knockout mice with conventional PS2 knockout mice on a B6CBA genetic background. Genotyping of the cDKO mice was performed as described previously [8,10,11,20]. A total of 12 male and 12 female PS cDKO mice ages 2, 4, 7, and 15 months (three mice per age and gender group) were used in the study. Age- and gender-matched mice with the same B6CBA genetic background served as WT controls (three mice per age and gender group). All mice were housed under constant temperature and humidity with 12-h light/dark cycles and fed ad libitum water and

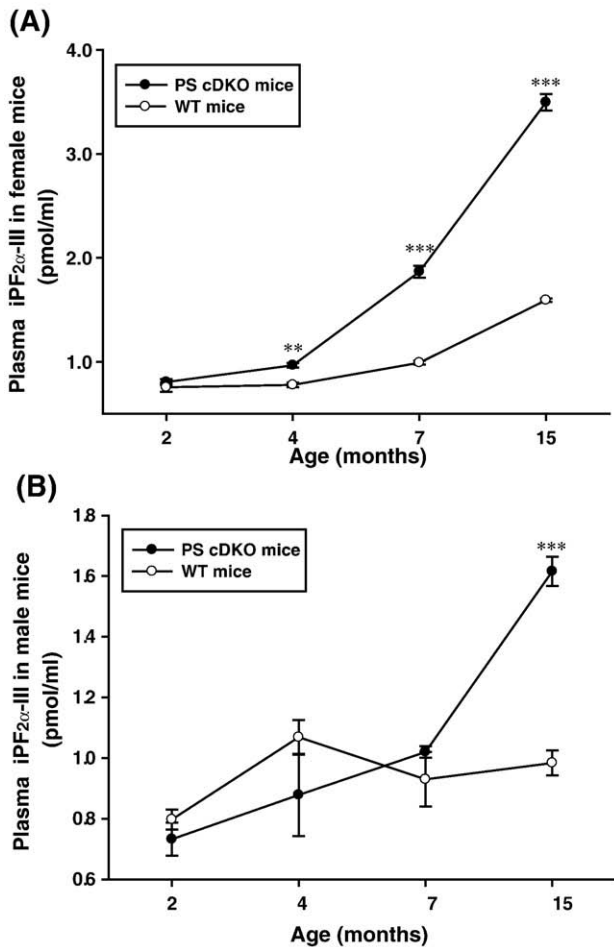
standard laboratory chow. Blood samples were collected from a cohort of these mice, and their fasting plasma samples were kept at  $-70^{\circ}\text{C}$ . The cerebral cortex samples were dissected and thoroughly washed with ice-cold 0.9% NaCl to avoid contamination with plasma proteins. The samples were frozen in liquid nitrogen immediately and then stored at  $-70^{\circ}\text{C}$ . The protein concentration of each sample was determined by the Bradford reagent method with BSA standard.

### Hydrolysis and solid-phase extraction of plasma and cortical iPF<sub>2α</sub>-III

The purification of iPF<sub>2α</sub>-III from the cerebral cortex and plasma was performed as described [21,22], with modifications. Briefly, the tissue sample (50–100 mg) was homogenized in 4 ml ice-cold chloroform:methanol (2:1, v/v). For plasma, aliquots (500  $\mu\text{l}$ ) were transferred to Eppendorf tubes. The internal standard iPF<sub>2α</sub>-III-d<sub>4</sub> (50 ng for cerebral cortex and 5 ng for plasma) was added. For the cerebral cortex sample, the homogenate was mixed at  $4^{\circ}\text{C}$  for 1 h, and 1.6 ml of 0.9% NaCl was added to the sample followed by centrifuging at 2000 g for 10 min. Precipitates were dried under nitrogen. KOH (1 M; 1 ml for the cerebral cortex residual and 500  $\mu\text{l}$  for the plasma) was added to the tubes containing the samples. After both cerebral cortex and plasma samples were hydrolyzed at  $40^{\circ}\text{C}$  for 45 min, 1 M HCl (1 ml for the cerebral cortex sample and 500  $\mu\text{l}$  for the plasma sample) and 1 ml of 10 mM formate buffer (pH 3.0) was added. Samples were centrifuged at 12,000 g for 20 min; the supernatants were removed and applied to an Oasis HLB extraction cartridge [21]. The extraction steps were programmed into an ASPEC XL SPE System (Gilson S.A.S., France) and run automatically.



**Fig. 2.** Age-related changes in cortical iPF<sub>2α</sub>-III levels in (A) female and (B) male PS cDKO mice at ages 2, 4, 7, and 15 months. \* $p < 0.05$  and \*\* $p \leq 0.001$ .



**Fig. 3.** Age-related changes in plasma iPF<sub>2α</sub>-III levels in (A) female and (B) male PS cDKO mice at ages 2, 4, 7, and 15 months. \*\* $p < 0.01$  and \*\*\* $p \leq 0.001$ .

#### Derivatization and GC-MS analysis of plasma and cortical iPF<sub>2α</sub>-III

The iPF<sub>2α</sub>-III extracts were analyzed using gas chromatography-negative ion chemical ionization-mass spectrometry as described [21]. After derivatization with pentafluorobenzyl bromide (PFB) and *N,O*-bis-(trimethylsilyl) trifluoroacetamide, the PFB-TMS derivatives of iPF<sub>2α</sub>-III were analyzed on a TRACE DSQ gas chromatograph-mass spectrometer (ThermoFisher Scientific, USA). Selected ion monitoring was performed to monitor the carboxylate anion (*M*-181) at *m/z* 569 for iPF<sub>2α</sub>-III and *m/z* 573 for iPF<sub>2α</sub>-III-d<sub>4</sub>.

#### Determination of blood antioxidant enzymes

Plasma activities of SOD and GSH-px were measured using the SOD Assay Kit-WST (Dojindo Laboratories, Japan) and a GSH assay kit (Jiancheng Company, Nanjing, China), respectively. Erythrocyte CAT activities and the protein levels of the samples were determined using the Catalase Assay Kit and the BCA Protein Assay Kit (Beyotime Institute of Biotechnology, Jiangsu, China), using the protocols provided by the manufacturer.

#### Measurement of cortical GFAP by Western blot

Cerebral cortex samples were homogenized with a Tefloomogenizer in lysis buffer (20 mM Tris, pH 7.8–8.2, 150 mM NaCl, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 10 mM EDTA, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1 mg/ml leupeptin, and 1 mM PMSF) on ice followed by centrifugation at 12,000 g at 4°C. Loading buffer was

added to each supernatant and heated at 100°C for 4 min. The samples were then resolved by SDS-PAGE and transferred to Hybond-P PVDF membrane (Amersham). After being blocked in TBST containing 5% nonfat milk at room temperature for 45 min, the membranes were washed with TBST and incubated with antibodies against GFAP (1:2000; Santa Cruz) overnight at 4°C. The membranes were washed with TBST and incubated with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG (1:2000; Chemicon) at room temperature for 45 min. The membranes were washed again with TBST and the proteins were visualized with an ECL chemiluminescence kit (Amersham) according to the manufacturer's protocol. The GFAP levels were quantified using BandsScan software and normalized to GAPDH.

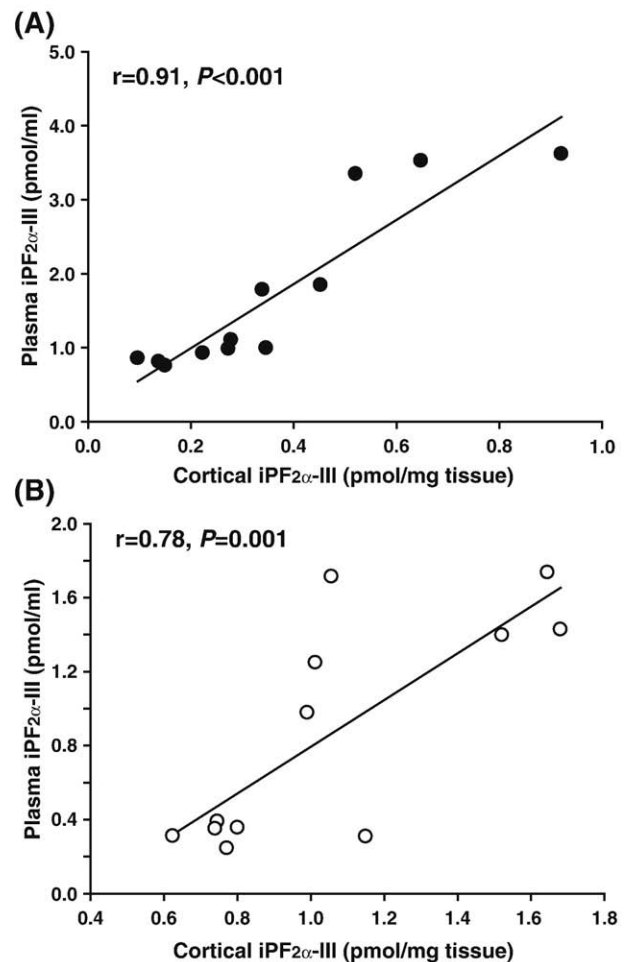
#### Statistical analysis

Data are expressed as the means ± SEM. The differences between the mean values of two groups were determined by Student's *t* test. Associations between the different variables were examined by Pearson correlation analysis. Statistical significance was set at  $p < 0.05$ .

## Results

### Differential effects of gender on iPF<sub>2α</sub>-III levels in cerebral cortex and plasma of PS cDKO mice

To study the effects of gender on both cortical and plasma iPF<sub>2α</sub>-III production in PS cDKO mice, the iPF<sub>2α</sub>-III data from mice of different ages (2, 4, 7, and 15 months) were analyzed based on gender status



**Fig. 4.** Positive correlation between age-related changes in plasma and cortical iPF<sub>2α</sub>-III levels in (A) female and (B) male PS cDKO mice.

( $n=3$  per age and gender group). The cortical  $iPF_{2\alpha}$ -III levels were significantly higher in the male mice than in the female mice over the age points examined, although the difference for cortical  $iPF_{2\alpha}$ -III levels in 4-month-old mice seemed not to reach statistical significance (Fig. 1A). Such gender-related differences in cortical  $iPF_{2\alpha}$ -III levels were not observed in the age-matched WT mice. By contrast, the mean levels of plasma total  $iPF_{2\alpha}$ -III in the males seemed to be lower than those in the females over the age points examined; however, the differences, except for that in 15-month mice, were not statistically significant (Fig. 1B). These results show that in PS cDKO mice there are differential effects of gender on the concentration of  $iPF_{2\alpha}$ -III in cerebral cortex and plasma.

#### Age-related changes in $iPF_{2\alpha}$ -III levels in cerebral cortex and plasma of PS cDKO mice

Because there were gender-related differences in  $iPF_{2\alpha}$ -III concentrations, especially in the cerebral cortex samples, of PS cDKO mice, the assessments of age-related changes in cortical and plasma total  $iPF_{2\alpha}$ -III levels upon the same set of  $iPF_{2\alpha}$ -III data were performed separately in female and male PS cDKO mice and their gender- and age-matched WT controls. Notably, the levels of  $iPF_{2\alpha}$ -III in the cerebral cortices from both female and male PS cDKO mice were not changed at 2 months of the age but were significantly elevated from 4 months onward compared with those of WT mice (Figs. 2A and 2B). A similarly changing pattern of plasma total  $iPF_{2\alpha}$ -III levels at the same ages was also found in the female but not the male PS cDKO mice (only 15-month male PS cDKO mice showed a significant difference) compared to WT mice (Figs. 3A and B). Furthermore, the  $iPF_{2\alpha}$ -III concentration increased in an age-dependent manner over the ages examined similarly for the cerebral cortex (Figs. 2A and B) and plasma (Figs. 3A and B) samples from both female and male PS cDKO mice. The data revealed that there was a significant increase in oxidative stress, particularly in the cerebral cortex, for PS

cDKO mice at and after the age of 4 months. Meanwhile, it was interesting to find in this study that the age-related production of  $iPF_{2\alpha}$ -III in the cerebral cortex samples was strongly correlated with that in the circulation in both female ( $r=0.92$ ,  $p<0.001$ ) and male ( $r=0.78$ ,  $p=0.001$ ) PS cDKO mice (Figs. 4A and B), suggesting that the increase in circulating  $iPF_{2\alpha}$ -III might reflect the increased oxidative damage in the PS cDKO brain.

#### Endogenous antioxidant activities in blood samples from PS cDKO mice

The enzymatic activities of three endogenous antioxidant enzymes (SOD, GSH-px, and CAT) were analyzed in the blood samples from PS cDKO and WT mice. The analysis of plasma SOD activities in the 2- and 4-month mice revealed that the enzyme activities were significantly higher only in 4-month-old (mean 60.30 vs 48.61 U/ml,  $p<0.05$ ), and not in 2-month-old, male PS cDKO mice compared with the age-matched male WT mice, and there were also no significant changes in the activities of this enzyme in female PS cDKO mice over the ages examined. No changes in plasma GSH-px activities were observed in either female or male PS cDKO mice at 2, 4, and 7 months compared to the gender- and age-matched WT mice. However, erythrocyte CAT activities exhibited increasing trends in the female and male PS cDKO mice, notably at 4 and 7 months, although only the elevation in 7-month female PS cDKO mice reached statistical significance (mean 9.33 vs 6.28 U/g protein,  $p=0.018$ ), compared to that in WT mice. The data suggest that during the early development of AD-like pathologies there would be a systemic compensatory mechanism attempting to redress the oxidative imbalance in response to the loss of PS function in the brain.

#### Age-related changes in cortical GFAP in PS cDKO mice

To investigate the possible source of the age-related increase in lipid peroxidation in the cerebral cortex of PS cDKO mice, we

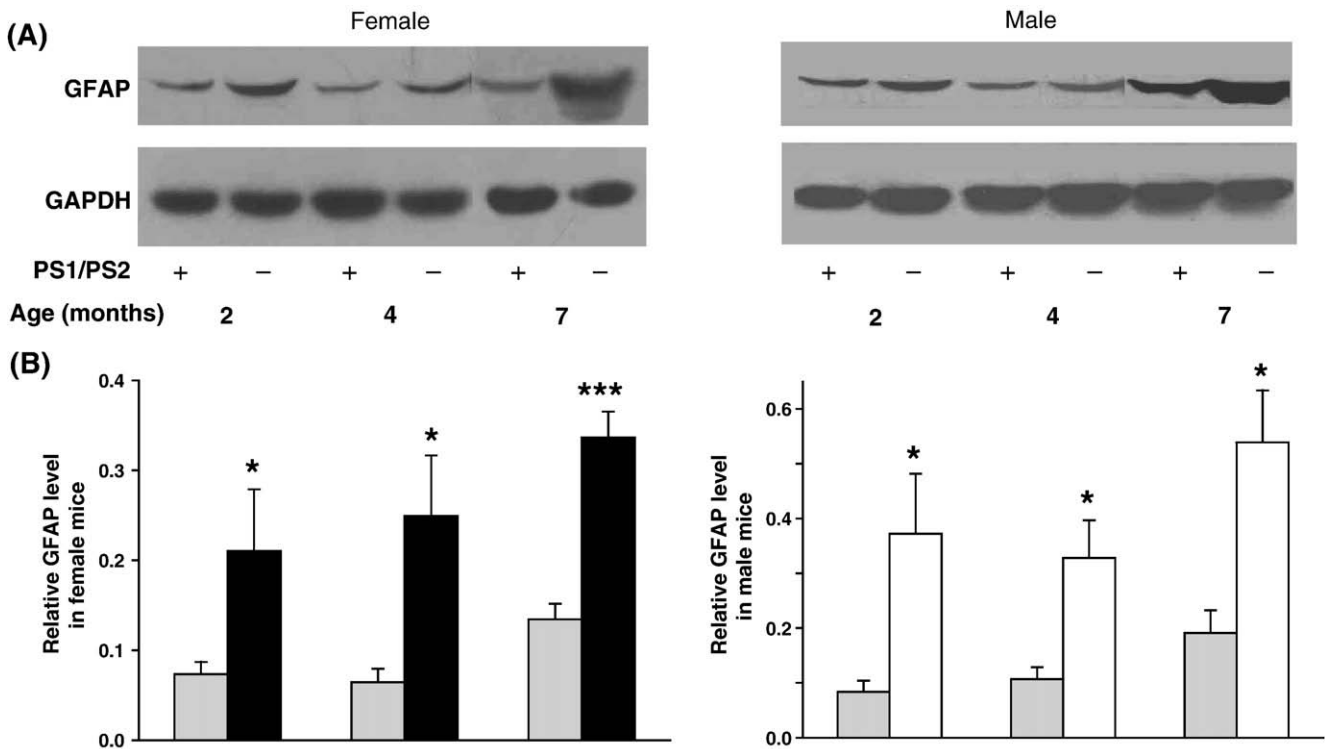
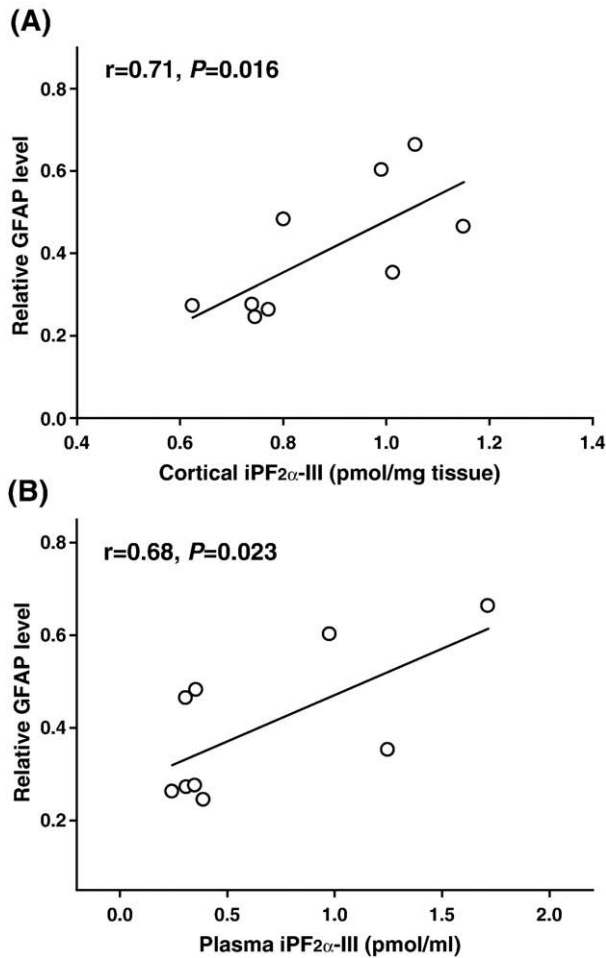


Fig. 5. Increased cortical GFAP expression in female and male PS cDKO mice at ages 2, 4, and 7 months. (A) Western analysis and (B) quantification show elevated levels of GFAP in PS cDKO cerebral cortices ( $n=3$ ) compared to the gender- and age-matched WT mice ( $n=3$ ). The results were quantified using Bandscan (Glyko). Values are normalized to GAPDH levels. \* $p<0.05$  and \*\*\* $p\leq 0.001$ .





**Fig. 6.** Significant correlations of cortical GFAP expression with age-related changes in (A) cortical and (B) plasma  $iPF_{2\alpha-III}$  levels in male PS cDKO mice.

measured the levels of cortical GFAP, a marker of astrocytosis, in female and male PS cDKO mice at 2, 4, and 7 months and in the gender- and age-matched WT mice. The results revealed that cortical GFAP levels were higher in both female and male PS cDKO mice, notably at as young as 2 months, in comparison with the WT mice and were elevated significantly in an age-related manner (Figs. 5A and B). The interrelations of the astrogliosis responses with oxidative stress status of both cerebral cortex and circulation were further evaluated. Pearson correlation analysis revealed strong correlations between GFAP levels and  $iPF_{2\alpha-III}$  concentrations in cerebral cortex ( $r=0.71$ ,  $p=0.016$ ) (Fig. 6A) and plasma ( $r=0.68$ ,  $p=0.023$ ) (Fig. 6B) in male (but not in female) PS cDKO mice.

## Discussion

PS mutations linked to early onset familial AD can cause alterations in  $A\beta$  production similar to those described in the amyloid-cascade hypothesis [23]. However, accumulated evidence indicates that the complete loss of PS function in the brain will also lead to neurodegeneration independent of brain  $A\beta$  deposition [10,11,24]. The cDKO mice are a well-characterized model of a loss-of-function pathogenic mechanism of AD [25], although the precise genetic relevance of the model to human familial AD is not yet fully understood. Previous morphological and behavioral studies have shown that PS cDKO mice maintain normal brain cytoarchitecture but exhibit mild memory impairment and synaptic plasticity deficit at 2 months and begin to display severe memory and synaptic dysfunc-

tions at 6 months accompanied by age-dependent neuronal degeneration [11]. Although oxidative stress has been long implicated predominantly in amyloidosis-mediated AD pathologies [26–28], its role in response to the loss-of-function pathogenic mechanism of AD remains unclear. This study provides compelling evidence that the enhanced oxidative stress and lipid peroxidation occurred independent of brain  $A\beta$  deposition in the cerebral cortex and plasma of PS cDKO mice compared with WT mice (Figs. 2 and 3). Importantly, such oxidative abnormalities predominantly in cerebral cortex at 2–4 months of age (Fig. 2) precede, rather than follow, the onset (i.e., at 6 months) of much of the pronounced AD neuropathology, such as progressive and striking loss of cortical gray and white matter, enlargement of the lateral ventricles, and significant reduction in neuronal number and neocortex volume [11]. As stated above, the oxidative damage mechanism suspected in AD has been largely attributed to  $A\beta$  deposits in the brain. Indeed, it has been reported that Tg2576, a well-characterized mouse model of AD amyloidosis, exhibits increased  $F_2$ -isoprostanes levels in the cortex and hippocampus at 7–8 months of age that precede significant brain  $A\beta$  deposition [14]. In human studies, increased oxidative damage was demonstrated in the brains of patients with mild cognitive impairment, a condition often being the earliest clinical manifestation of AD [29,30], and with early AD [30]. An inverse relationship between levels of neuronal oxidized RNA (8OHG) and amino acid (nitrotyrosine) and both  $A\beta$  deposits and duration of dementia was also observed in AD patients, which was even more significant in ApoE  $\epsilon 4$  carriers [19]. Collectively, these findings suggest that oxidative damage to critical biomolecules in the brain may be one of the earliest events in the onset and progression of AD. Our data further provide important and novel evidence supporting the role of oxidative stress and lipid peroxidation in the loss-of-function pathogenic mechanism of AD. Thus, proteins to relieve oxidative stress may serve as a potential therapeutic target for slowing the disease process.

A significant increase in  $iPF_{2\alpha-III}$  production in PS cDKO mice seemed to be gender and age associated (Figs. 1, 2, and 3). The gender seemed to have differential effects on  $iPF_{2\alpha-III}$  production in cerebral cortex and plasma of the transgenic mice over the age points examined (Fig. 1). In the cerebral cortex,  $iPF_{2\alpha-III}$  concentrations in the males were significantly higher (2.43-fold on average) than in the females, whereas the levels of plasma total  $iPF_{2\alpha-III}$  in the males tended to be lower (1.49-fold on average) than in the females. The underlying explanation for such a differential influence of gender on  $iPF_{2\alpha-III}$  formation is unknown. It possibly reflects a different oxidative response in the brains of the males and females to PS inactivation. Epidemiological data have suggested that aging women have a higher incidence of AD than men, due most likely to pleiotropic effects of estrogen on neurons and brain [31]. Aged male ApoE knockout and human ApoE  $\epsilon 4$  transgenic knockout mice had significantly higher brain  $F_2$ -isoprostanes levels than the age- and genotype-matched female mice, which closely paralleled the ApoE-genotype- and gender-dependent changes in brain  $A\beta$  [32]. The gender effect on brain oxidative stress status in PS cDKO mice may suggest that PS cDKO mice also recapitulate another feature of the human disease: the gender effect. Age-dependent alterations in oxidative stress and lipid peroxidation, as indicated by  $F_2$ -isoprostane levels, have also been evident in other AD-like transgenic animals such as the amyloidotic Tg2576 mice [14] and the ApoE knockout mice with or without transgenic human ApoE  $\epsilon 3$  or  $\epsilon 4$  alleles [32]. The present study also demonstrates an age-dependent feature of brain and systemic  $iPF_{2\alpha-III}$  production in response to the loss of PS function in PS cDKO mice at the age points examined (Figs. 2 and 3). This thus provides a new perspective for in-depth understanding of AD pathogenesis. Furthermore, the age-dependent increases in  $iPF_{2\alpha-III}$  levels in cerebral cortex and circulation of PS cDKO mice were significantly correlated (Fig. 4), suggesting that peripheral monitoring of this highly specific biomarker of lipid peroxidation may be useful

for assessing oxidative stress status and pathological changes related to loss of PS function in AD brain.

Endogenous antioxidant enzymes, such as SOD, GSH-px, and CAT, are critical components of cellular antioxidant defenses against oxygen free radical-mediated damages [18]. Whereas previous publications showed that the activities of some of these enzymes, for example CAT and/or SOD, are decreased in various brain regions of AD patients [33,34] and in the cortex of SAMP8 mice [35], it has been increasingly demonstrated that one or more of these enzymes is activated to counteract the enhanced oxidative stress related to AD pathologies. SOD activity was significantly elevated in the fibroblast cell lines derived from familial Alzheimer patients [36]. Marcus et al. observed elevated SOD expression in several hippocampal regions of AD brains compared to those of age-matched controls [37]. Cortical CAT and GSH-px activities were all higher in AD patients with at least one ApoE  $\epsilon$ 4 allele [38], whereas significant increases in blood SOD and CAT activities were also observed in patients with AD-like dementia compared to the age-matched control subjects [39]. These findings support the idea that the vulnerable neuronal cells mobilize antioxidant defenses under increased oxidative stress [40]. The potential influence of gender status on the measurements of these enzymes, however, was not clarified in these studies. Given the fact that the gender status may have a differential effect on iPF<sub>2 $\alpha$</sub> -III levels in PS cDKO mice (Fig. 1), this study thus attempted to assess antioxidant abilities in the circulation of both female and male animals. The results showed no significant reduction in the activities of any of the antioxidant enzymes measured in the blood samples from either female or male PS cDKO mice over the age points examined compared with the gender- and age-matched WT mice. Although generally remaining unchanged in the animals at other ages, the erythrocyte CAT activities in 7-month female and plasma GSH-px activities in 4-month male PS cDKO mice were significantly elevated compared to those of their matched WT controls. Collectively, our data provide further evidence suggesting a systemic oxidative resistance mechanism during the early development of AD neuropathologies in response to the loss of PS functions.

This study confirms previous observations by others that astrogliosis responses, as measured by GFAP expression, were significantly increased and responsible for the inactivation of PS in the mice at 6 months [12]. We further revealed that such astrocytic responses were initiated in both male and female PS cDKO mice at as young as 2 months of age and further changed age-dependently compared to the age- and gender-matched WT mice (Fig. 5). GFAP, an intermediate filament protein, is increasingly expressed in response to astrocytic activation. The protein was reported to be present at increased levels with A $\beta$  plaques in the brains of AD patients [41–43]. However, it was also observed that up-regulation of GFAP expression in response to astrogliosis in the AD brain was not dependent on A $\beta$  accumulation but was correlated with the duration of the disease, suggesting that the elevation of GFAP level was not a consequence of A $\beta$  accumulation but likely a reaction to neuronal and synaptic loss and tangle formation [43]. Chronic glial activation can cause oxidative stress in the brain [44]. In accordance with these observations, our data demonstrate that increased astrogliosis responses precede the oxidative impairments of critical cellular components, such as membrane lipids in the PS cDKO brain, and that both increased oxidative stress and astrogliosis may be contributors toward the underlying loss-of-function pathogenic mechanism of AD. To support this, we observed significant correlations of the cortical GFAP expressions with both cortical and plasma iPF<sub>2 $\alpha$</sub> -III concentrations, although only in male PS cDKO mice (Fig. 6). The reason such a relationship existed only in the male PS cDKO mice is unclear, but it might be explained, at least in part, by the differential effect of gender on iPF<sub>2 $\alpha$</sub> -III formation in the transgenic animals. Nevertheless, the connection between oxidative stress and astrogliosis in this AD-like model needs to be further studied.

## Summary

Despite the use of a relatively small sample size owing to the limitation of the transgenic resources, this study provides evidence from F<sub>2</sub>-isoprostanes that the gender- and age-related increase in both cerebral cortex and circulation of oxidative stress contributes to the loss-of-function pathogenic mechanism of AD in PS cDKO mice. A systemic antioxidant defense under enhanced oxidative stress responsible for the PS inactivation is also documented. The synergistic relationship between oxidative stress signaling and astrogliosis response toward the underlying amyloidosis-independent mechanism of AD is worth further investigation.

## Acknowledgments

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