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# Proteomic profiling of brain cortex tissues in a Tau transgenic mouse model of Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) involves regionalized neuronal death, synaptic loss, and an accumulation of intracellular neurofibrillary tangles and extracellular senile plaques. Although there have been numerous studies on tau proteins and AD in various stages of neurodegenerative disease pathology, the relationship between tau and AD is not yet fully understood. A transgenic mouse model expressing neuron-specific enolase (NSE)-controlled human wild-type tau (NSE-htau23), which displays some of the typical Alzheimer-associated pathological features, was used to analyze the brain proteome associated with tau tangle deposition. Two-dimensional electrophoresis was performed to compare the cortex proteins of transgenic mice (6- and 12-month-old) with those of control mice. Differentially expressed spots in different stages of AD were identified with ESI-Q-TOF (electrospray ionization quadruple time-of-flight) mass spectrometry and liquid chromatography/tandem mass spectrometry. Among the identified proteins, glutathione S-transferase P 1 (GSTP1) and carbonic anhydrase II (CAII) were down-regulated with the progression of AD, and secerin-1 (SCRN1) and V-type proton ATPase subunit E 1 (ATP6VE1) were up-regulated only in the early stages, and down-regulated in the later stages of AD. The proteins, which were further confirmed by RT-PCR at the mRNA level and with western blotting at the protein level, are expected to be good candidates as drug targets for AD. The study of up- and down-regulation of proteins during the progression of AD helps to explain the mechanisms associated with neuronal degeneration in AD.



## 1. Introduction

Alzheimer's disease (AD) is a progressive, degenerative neurological disorder, meaning that the disorder gets worse over time. AD is the most common form of dementia in the elderly, affecting more than 20% of those aged over 80 years.

Neuropathologically, AD is defined by loss of synaptic connections, regionalized neuronal death, extracellular deposition of ß-amyloid (Aß) protein (forming senile plaques), and intracellular precipitation of hyperphosphorylated tau protein (forming neurofibrillary tangles [NFTs]) within selective brain regions [1–3]. These tangles and plaques together act to disrupt brain function and are known to increase in quantity with the progression of AD.

The exact biochemical mechanism of the pathogenesis of AD is still unknown, but several hypotheses have been proposed to explain AD pathogenesis, including amyloid cascade, excitotoxicity, oxidative stress, and inflammation [4,5]. Several lines of evidence suggest an important role for oxidative stress in the pathogenesis and/or progression of AD. Protein oxidation, lipid peroxidation, and nucleic acid and carbohydrate oxidation products have been found to be elevated in the brain in AD. In addition, levels of antioxidant enzymes were found to be diminished in brains with AD, which strongly supports the role of oxidative stress in AD; the products of oxidative stress have been found in Aß-rich regions such as the cortex and hippocampus.

Previous studies of brain dysfunction in AD have only included a small number of samples from patients. In addition, studies that failed to find an enhancement effect in AD used stimuli lacking semantic coherence (e.g. lists of unrelated words, some that were emotional and others that were neutral). To circumvent these limitations, the present study examined a large number of transgenic (Tg) animal models over-expressing tau protein, followed by paired helical filament (PHF) formation, and investigated the modified protein expression levels in the cerebral cortex. The cortex is a

*Abbreviations:* 2-DE, 2-dimensional gel electrophoresis; AD, Alzheimer's disease; ANOVA, analysis of variance; CSF, cerebrospinal fluid; ESI-Q-TOF MS/MS, electrospray ionization quadruple time-of-flight mass spectrometry; IEF, isoelectric focusing; KFDA, Korea Food & Drug Administration; LC-MS/MS, liquid chromatography/tandem mass spectrometry; NDPK, nucleoside diphosphate kinase; NFT, neurofibrillary tangle; PHF, paired helical filament.

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region that plays an important role in memory and cognitive function. Signs of AD are first noticed in the entorhinal cortex, and then proceed to the hippocampus and other cortical regions. The level of proteins in the cortex may provide a better understanding of the pathological changes that occur.

Recently, neuroproteomics surveys provide a framework for large-scale protein research in AD, including the discovery of AD biomarkers, and a far-reaching systems biology understanding of how the brain responds to AD. The proteomic alterations in brain homogenates of two different ages of tau-Tg mice displaying PHFs, which were obtained from KFDA (Korea Food & Drug Administration), were examined.

In this study, 2-dimensional gel electrophoresis (2-DE) was used to evaluate the brain proteomes of these mice. Differentially expressed proteins in AD compared with healthy control subjects were identified using ESI-Q-TOF MS/MS and LC-MS/MS analysis.

## 2. Materials and methods

### 2.1. Animals

A Tau transgenic mouse expressing neuron-specific enolase (NSE)-controlled human wild-type tau (NSE/htau23) was obtained from the Division of Laboratory Animal Resources, Korea FDA (Food and Drug Administration), National Institute of Toxicological Research. According to their procedures [6], the pNSE/htau23 gene was constructed by inserting the human wild type tau cDNA (htau23) (GenBank accession No. J03778) linked to the NSE promoter and microinjecting the gene into the male pronucleus of a fertilized mouse egg. The egg was obtained by mating a female BDF1 mouse with a male BDF1 mouse. The injected eggs were then transferred into the oviducts of the pseudopregnant ICR recipient females. Then, the transgenic founder mice were crossed with the parental strain of the BDF1 background to establish the transgenic lines. 9-week-old transgenic and non-transgenic littermate mice were brought and acclimatized to the new laboratory conditions for 3 weeks following their arrival. The animals were fed a standard laboratory chow and water was freely available. The mice were maintained under the standard condition of a 12-h light–dark cycle, temperature at 23 ± 1 °C and humidity at 50 ± 10%. All animal experiments were approved by the Committee for the Care and Use of Laboratory Animals of Korea University.

### 2.2. Sample preparation

Mice were sacrificed through euthanasia using carbon dioxide gas at 6 and 12 months, respectively. The cortex samples were taken from the brain of the mice at 6 and 12 months. We use PlusOne Sample Grinding Kit (GE Healthcare) designed for the grinding of small tissue or cell sample for protein extraction. Extraction solution consisted of 8 M urea, 2% CHAPS, 40 mM DTT, 0.5% IPF buffer, and 2% protease inhibitor. After vortexing to resuspend the grinding resin, add up to 100 mg of the cerebral cortex to the tube. A disposable grinding pestle is used to grind the sample for 1 min. Cellular debris and grinding resin are by centrifugation for 10 min at maximum speed, carefully removed transfer the clear supernatant to another tube. The protein concentration of the homogenates was measured by the modified Bradford method with BSA as standard and then stored at 70 °C until further use [7].

### 2.3. PCR analaysis

The transgenes were identified by DNA-PCR analysis of the genomic DNA isolated from the tails of 4-week-old mice [8]. The quantity of isolated genomic DNA was measured by NanoDrop (Dea Myung Science Co. Ltd.) at 260 nm. After 25 cycles of amplification, the levels of the htau23 products (1112-bp) were quantified on 1% agarose gels. The total RNA from the brain cortex was extracted by Trizol (Life Technologies). In order to eliminate any contamination with genomic DNA, DNase I (Invitrogen) was utilized in accordance with the manufacturer's protocols. *Gstp1*, *CaII*, *Atp6v1e*, and *Scrn1* primers used in this study were obtained from COSMO (Korea). Initial denaturation at 94 °C for 2 min, followed by denaturation at 94 °C for 1 min, annealing at primer specific temperatures for 45 s (Table 1), and extension at 72 °C for 1 min; followed by a final extension of 72 °C for 1 min. PCR products were separated via electrophoresis in 1% agarose gel.

### 2.4. Isoelectric focusing

Ready-to-use Immobiline DryStrips (24 cm, pH 3–10 NL) were used for IEF. The DryStrips were rehydrated for 5 h in 450 mL of solubilization solution containing 8 M urea, 2% CHAPS, 40 mM DTT, 0.5% IPG buffer, a trace of bromphenol blue, together with the sample (100 μg). IEF was conducted using the IPGphor IEF system system (GE Healthcare) at 200 V for 30 min by step-n-hold, 500 V for 1 min by gradient, 8000 V for 1 h by gradient and constant of 8000 V until approximately 146,000 Vh were reached [9].

### 2.5. SDS–PAGE

The gel strips were equilibrated twice for 10 min with gentle shaking. The first equilibration solution was 50 mM Tris–HCl buffer (pH 8.8) containing 6 M urea, 20% glycerol, 2% SDS and 1% DTT in order to diminish electroendosmotic effects which result in a reduced protein transfer from the first to the second dimension. In the second equilibration buffer, DTT was replaced with 2.5% iodoacetamide (IAA). After equilibration, the IPG strips were slightly rinsed, then applied to 12.5% homogeneous SDS–PAGE gels ($26 \times 20$ cm$^2$) and overlaid with a solution of 0.5% agarose with a trace of bromophenol blue. Second-dimensional SDS–PAGE was conducted using the Ettan DALT II system (GE Healthcare) at 55 V for 1 h, 160 V for 1 h and 330 V for 4 h.

### 2.6. Silver staining

Proteins were visualized using the silver staining method as described previously [9,10] with some modifications.

### 2.7. Image analysis

The spot detection, volume calculation and comparasion analysis were performed using Image Master 2D Platinum Software Ver. 6.0 (Amersham Pharmacia Biotech). Expression levels of the spots were determined by the percentage volume of each spot. For each spot, the relative volume intensity was averaged and expressed as a mean ± standard error of the mean (SEM). The spots differently expressed were selected by one-way analysis of variance (ANOVA) followed by Bonferroni's test ($P < 0.05$).

### 2.8. LC–MS/MS

Proteins were subjected to in-gel trypsin digestion [11], and excised gel spots were destained with 100 μL of destaining solution (1:1 = 30 mM potassium ferricyanide: 100 mM sodium thiosulfate, v/v) with shaking for 10 min. After removal of the solution, gel spots were incubated with 200 mM ammonium bicarbonate for 20 min. The gel pieces were dehydrated with 100 μL of acetonitrile and dried in a vacuum centrifuge for 20 min. The dried gel pieces were kept in 20 μL of 50 mM ammonium bicarbonate containing 0.2 μg modified trypsin (Promega Corp.) overnight at 37 °C for

**Table 1**
Identification of differently expressed proteins in brain cortex of Tau transgenic mouse using LC–MS/MS.

| Spot no. | Protein | Classification | Change in expression level | MW (kDa) | Accession no. | pI |
|---|---|---|---|---|---|---|
| 670 | Dihydropyrimidinase-related protein 2 (DPYSL2) | Down-regulated | 6Tg → t12Tg ↓ | 62.2 | IPI00114375.2 | 5.95 |
| 724 | Secernin-1 (SCRN1) | Up-regulated | 12Ntg → t12Tg ↑ | 46.2 | IPI00112536.1 | 4.67 |
| 746 | Eno1; Alpha-enolase | Down-regulated | 6Ntg → t12NTg ↓ | 47.1 | IPI00462072.3 | 6.37 |
| 912 | F-actin-capping protein subunit alpha-2 (Capza2) | Down-regulated | 6Ntg → t6Tg ↓ | 32.9 | IPI00111265.3 | 5.57 |
| 935 | Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) isoform 1 | Down-regulated | 6Tg → 12Tg ↓ | 35.8 | IPI00135284.1 | 8.44 |
| 964 | Tubulin beta-2B chain (TUBB2b) | Up-regulated | 12Ntg → 12Tg ↑ | 49.9 | IPI00109061.1 | 4.78 |
| 986 | V-type proton ATPase subunit E 1 (ATP6VE1) | Down-regulated | 6Ntg → 12Tg ↓ | 26.1 | IPI00119115.2 | 8.44 |
| 1043 | Proteasome subunit alpha type-4 | Down-regulated | 12Ntg → 12Tg ↓ | 29.4 | IPI00277001.4 | 7.58 |
| 1072 | Creatine kinase B-type (CKB) | Up-regulated | 12Ntg → 12Tg ↑ | 42.6 | IPI00136703.1 | 5.40 |
| 1088 | Tubulin beta-2B chain (TUBB2B) | Up-regulated | 12Ntg → 12Tg ↑ | 49.9 | IPI00109061.1 | 4.78 |
| 1109 | Lactoylglutathione lyase (Glo1) | Up-regulated | 6Tg → 12Tg ↑ | 20.7 | IPI00321734.7 | 5.24 |
| 1134 | Isoform 1 of Astrocytic phosphoprotein (PEA-15a) | Down-regulated | 6Tg → 12Tg ↓ | 15.0 | IPI00121013.1 | 4.94 |
| 1140 | Glutathione S-transferase P 1 (GSTP1) | Down-regulated | 6Ntg → 12-Tg ↓ | 23.5 | IPI00555023.2 | 7.69 |
| 1200 | Nucleoside diphosphate kinase B (NME2) | Down-regulated | 12Ntg → 12Tg ↓ | 17.3 | IPI00127417.1 | 6.97 |
| 1214 | L-lactate dehydrogenase B chain (LDHB) | Down-regulated | 6Ntg → 6Tg ↓ | 36.5 | IPI00229510.5 | 5.70 |
| 1218 | Ubiquitin-conjugating enzyme E2 N (UBE2 N) | Up-regulated | 6Ntg → 12 Tg ↑ | 17.10 | IPI00165854.3 | 6.13 |
| 1218 | Ubiquitin-conjugating enzyme E2 N (UBE2 N) | Up-regulated | 6Tg → 12 Tg ↑ | 17.10 | IPI00165854.3 | 6.13 |
| 1218 | Ubiquitin-conjugating enzyme E2 N (UBE2 N) | Up-regulated | 6Ntg → 12NTg ↑ | 17.10 | IPI00165854.3 | 6.13 |
| 1325 | Voltage-dependent anion-selective channel protein 1 (Pl-VDAC1) | Down-regulated | 6Ntg → 12Tg ↓ | 32.3 | – | – |

Score is $-10 \times \log(P)$, where $P$ is the probability that the observed match is a random event, it is based on NCBInr database using MASCOT searching program as MS/MS data.

digestion. Peptides were extracted from the trypsin-digested gel pieces by applying 40 μL of 50% acetonitrile and 5% trifluotoacetic acid at 37 °C for 1 h. The peptide mixtures were desalted and concentrated prior to mass spectrometric analyses and LC–MS/MS was performed to identify proteins.

To identify the protein, all MS/MS spectra recorded on tryptic peptides derived from spots were searched against NCBI protein sequence databases using the MASCOT search program (www.matrixscience.com).

### 2.9. Western blotting

For immunoblotting, equal 10 μg protein per lane were separated by 8% (for ATP6V1E) and 12% (for CAII, GSTP1 and SCRN1) (w/v) SDS–PAGE, transferred to nitrocellulose (for CAII, GSTP1 and SCRN1) and PVDF (for ATP6V1E) membranes and then immunoblotted with anti-CAII antibody (Abcam), anti-GSTP1, anti-SCRN1 (Proteintech), and anti-ATP6V1E (Abcam), respectively. Values are mean ± standard error of the mean (SEM). The statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Bonferroni's test ($P < 0.05$).

### 2.10. Statistical analysis

All results are expressed as mean ± standard error of the mean (SEM). For each protein spot and band intensity from Western blotting, ANOVA was performed via Bonferroni's test to identify significant differences between four groups using the SPSS 12.1 for Windows computer software package (SPSS). *P*-Values less than 0.05 were considered statistically significant.

## 3. Results

### 3.1. RT-PCR analysis for the cerebral cortex in Tau-transgenic mouse

RT-PCR analysis was performed using mRNA from the cerebral cortex to examine the transcriptional levels of AD-specific markers (*Tau*, *Gsk3ß*, *Tpi*, and *Ckm*), which revealed specific differences in transcription level of these genes, as shown in Fig. 1. GSK3ß contributes to tau pathology in brains with AD. TPI and creatine kinase are enzymes related to glucose metabolism, which is the main source of energy for the brain under normal conditions.

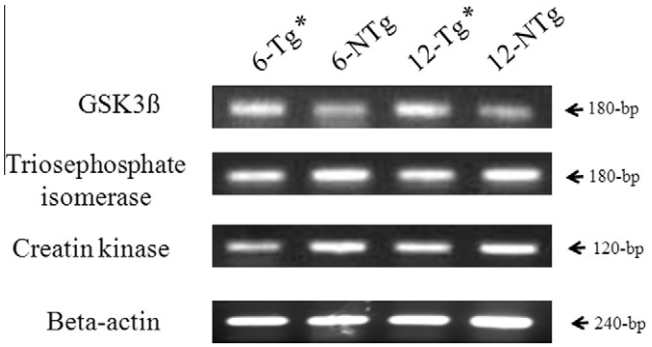


**Fig. 1.** Analysis of mRNA levels of *Tau*, *Gsk3ß*, *Tpi*, and creatine kinase (*Ckm*) transcripts in AD mouse.

### 3.2. Comparative analysis of protein expression in cortex of tau transgenic mice using 2-DE

Protein profiles of cortex samples were analyzed by 2-DE and stained with silver, as shown in Fig. 2. In the Tg group at 6 months of age, two spots (Spot Nos. 912 and 1214) were found to be significantly down-regulated compared with the normal group of the same age. In the Tg group at 12 months of age, five spots (Spot Nos. 965, 986, 1039, 1140 and 1325) were found to be down-regulated and one spot (Spot No. 1218) was up-regulated, compared with the normal group of the same age. In the Tg group at 12 months of age, 4 spots (Spot Nos. 670, 935, 1039 and 1134) were down-regulated and two spots (Spot Nos. 1109 and 1218) were up-regulated, compared with the Tg group at 6 months of age. In the Tg group at 12 months of age, two spots (Spot Nos. 1043 and 1200) were found to be down-regulated and five spots (Spot Nos. 724, 964, 1072, 1088 and 1100) were up-regulated compared with the non-Tg group at 12 months of age. Finally, the non-Tg group at 6 months of age was compared with the non-Tg group at 12 months of age to analyze the proteins affected by aging. Among the identified proteins, two spots (Spot Nos. 746 and 1164) were down-regulated and four spots (Spot Nos. 1181, 1211, 1218 and 1314) were up-regulated at 12 months of age. Overall, spots identified in each group were generally found to be significantly up-regulated by less than 50% and down-regulated by more than 200%.

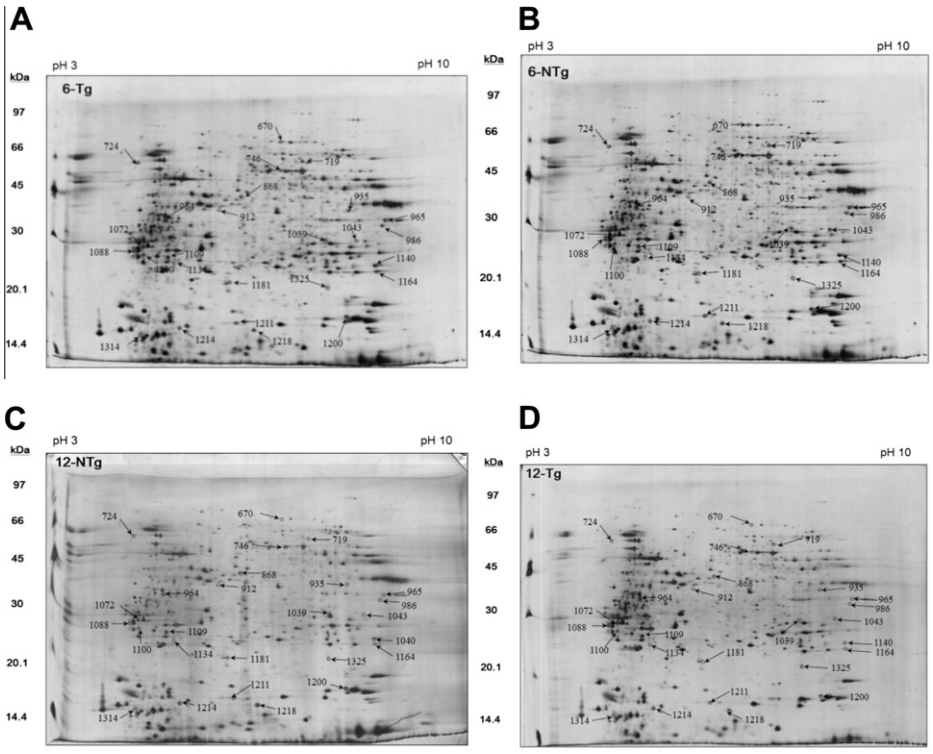


**Fig. 2.** 2-DE profile of (A) non-Tg group at 6 months of age, (B) Tg group at 6 months of age, (C) non-Tg group at 12 months of age, and (D) Tg group at 12 months of age.

### *3.3. Protein identification of differentially expressed proteins using LC–MS/MS*

As shown in Table 1, in the identified proteome, proteins associated with the progress of AD were analyzed. Thus, the proteins were divided into three groups by comparing the tendency of relative volume intensity among the four groups as non-Tg and Tg at 6 and 12 months (Fig. 3).

To identify proteins expressed differentially, the protein spots were excised from gels and subsequently identified by LC–MS/MS and database search. Table 1 shows proteins identified that are down-regulated or up-regulated in Tg-mice at 6 and 12 months of age, respectively. Up-regulated spots in Tg-mice at both 6 and 12 months, compared with controls, were identified as phosphoglycerate kinase and beta-tubulin. Down-regulated spots in Tg-mice at both 6 and 12 months, compared with controls, were identified as PI-VDAC1, nm23 protein, CAII, and GSTP1. Spots that are up-regulated at 6 months of age but down-regulated at 12 months of age were identified as secernin 1, ATP6VE1, GAPDH, dihydropyrimidinase-related protein 2, proteasome subunit alpha type-4, and NADH dehydrogenase [ubiquinone] iron-sulfur protein 8 (Table 1).

### *3.4. Confirmation by RT-PCR and western blotting*

In order to compare the mRNA expression level of identified proteins (GSTP1, ATP6VE1, CAII, and SCRN1) in each group, RT-PCR was performed. As a result, mRNA levels in each group were similar to the protein levels. However, SCRN1 expression did not show a great difference at the mRNA level (Fig. 4). Expression of GSTP1 (23 kDa), CAII (30 kDa), ATP6V1E1 (80 kDa), and SCRN1 (50 kDa) were confirmed by Western blotting to validate the results of 2-DE analyses (Fig. 4). Western blot analysis for each pro-

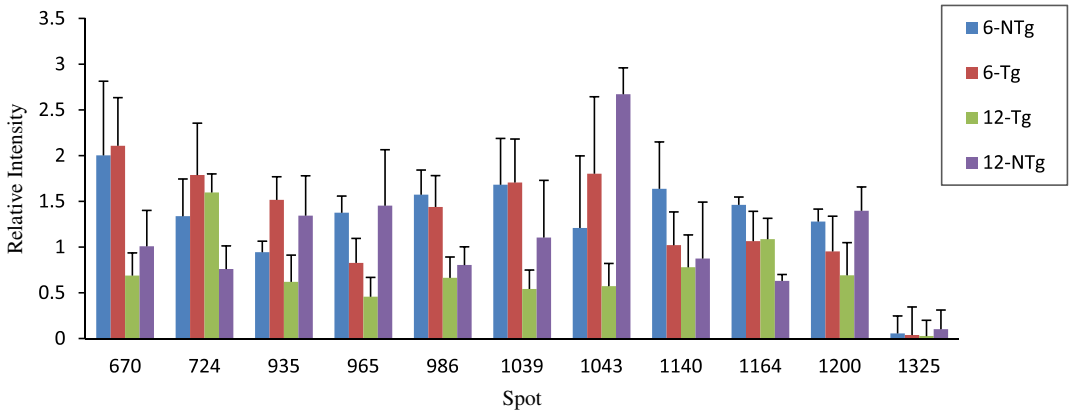


**Fig. 3.** The relative volume intensity of identified spots that were up-regulated and down-regulated in brain regions with significant change. Each bar represents the mean ± SEM of each spot. Statistical significance was evaluated by one-way ANOVA followed by Bonferroni's test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

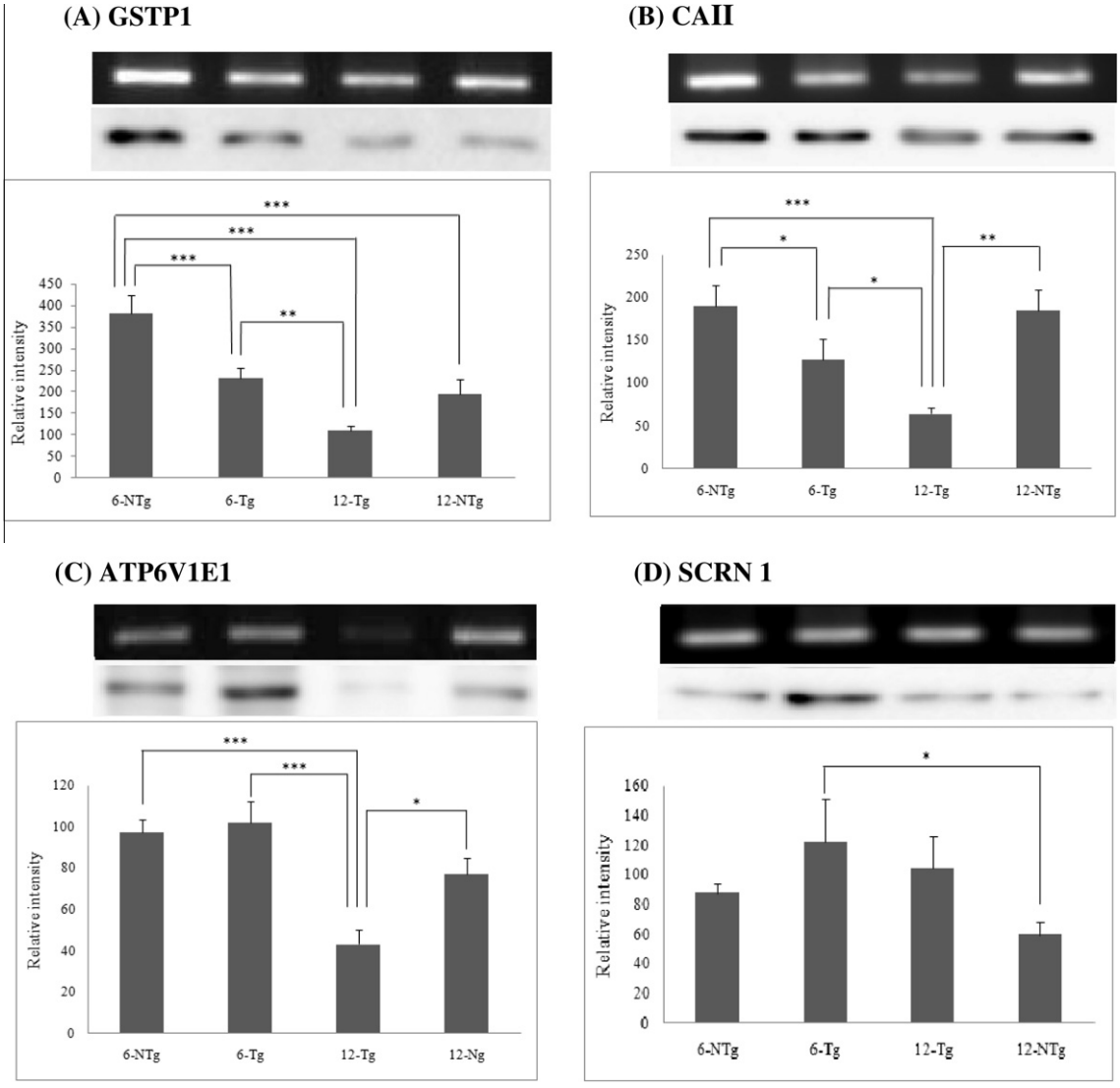


**Fig. 4.** Analysis of mRNA level and protein expression levels of GSTP1 (A), CAII (B), ATP6V1E1 (C), SCRN1 (D) confirmed by Western blot intensity analysis and RT-PCR. Tg group at 6 months of age; $n = 16$, Tg group at 12 months of age; $n = 9$, non-Tg group at 6 months of age; $n = 9$, and non-Tg group at 12 months of age; $n = 5$.

tein was conducted with 9 cortex samples from 6-month-old non-Tg-mice, 16 cortex samples from 6-month-old Tg-mice, 9 cortex samples from 12-month-old Tg-mice, and 5 cortex samples from 12-month-old non-Tg-mice.

The expression of the GSTP1 and CAII was progressively decreased in Tg-mice than in the control group. The expression of ATP6V1E1 was significantly decreased in 12-month-old Tg-mice ($P < 0.001$). However, a significant increase in the protein expression level was detected in 6-month-old Tg-mice compared with non-Tg-mice.

A difference in the expression level of SCRN1 was significant between the non-Tg and Tg groups. Protein expression in 6-month-old Tg-mice was higher than at 12 months. These proteins could be associated with protective mechanisms in early-stage AD.

## 4. Discussion

To identify the potential drug candidates to halt the cascade of disease-associated neurological damage, cerebral cortical proteomes of AD were analyzed by using 2-DE with ESI-Q-TOF MS/MS and LC–MS/MS. In this study, 14 proteins exhibiting changes in expression levels in the cortex of AD model mice were compared with levels in control tissue. These proteins are grouped together based on the expression levels with the progress of AD.

Voltage-dependent anion-selective channel protein 1 (PI-VDAC1), carbonic anhydrase II (CAII), glutathione *S*-transferase P 1 (GSTP1), and Nm23 protein are down-regulated with the progress of AD.

Secernin-1 (SCRN1), ATP6V1E1 and dihydropyrimidinase-related protein 2 (DRP2) were up-regulated in Tg-mice at 6 months of age but significantly down-regulated in Tg-mice at 12 months of age.

GSTP1 is a family of multifunctional detoxification enzymes that are mainly cytosolic, and which detoxify natural and exogenous toxic compounds by conjugation with glutathione; it is thus used as a marker of oxidative stress. It may play a role in the survival of neuronal cells in some neurodegenerative diseases. Reduced GST activity has been reported in multiple brain regions and in ventricular cerebrospinal fluid in AD patients. Proteins that are only up-regulated in the early stages of the disease process are likely related to neuroprotection, such as inflammation, cellular pH regulation, and glucose metabolism.

CAII has a wide tissue distribution and is found in bone, kidney, erythrocytes, glial cells, and osteoclasts. It plays an important role in regulating cellular pH, $CO_2$, and $HCO_3^-$ transport, and maintain-

ing $H_2O$ and electrolyte balance [12]. CAII is also involved in the production of cerebrospinal fluid (CSF) and the synthesis of glucose and lipids [13] [14]. The activity of CAII is decreased in the brain in AD. Initially, a deficiency in CAII could lead to an imbalance in pH, which could favor the formation of protein aggregates and may eventually lead to cognitive defects varying from disabilities to severe mental retardation [3]. CAII deficiency is an extremely rare autosomal recessive disorder, characterized by a triad of osteopetrosis [15]. Calcification results from excessive calcium or phosphorus in the blood, and is associated with the exceptionally high calcium concentration present in neurons before degeneration.

ATP6V1E1 is a large, multi-subunit complex organized into a peripheral domain (V1), responsible for ATP hydrolysis, and an integral domain (V0) that carries out transport for protons across the membrane. It regulates the pH of intracellular compartments, extracellular space, and the cytoplasm at the expense of ATP [16] [17]; it mediates the acidification of various intracellular organelles, including endosomes, lysosomes, the trans-Golgi network, and synaptic vesicles [18]. In the brain in AD, a deficiency in V-ATPase could lead to an imbalance in pH and disturb the acidification of various intracellular organelles.

SCRN1 is a novel 50 kDa cytosolic protein that appears to be involved in the regulation of exocytosis from peritoneal mast cells. SCRN1 increases both the extent of secretion and the sensitivity of mast cells to stimulation by calcium [19]. Mast cells are located in close proximity to neurons in the peripheral and central nervous system, suggesting a functional role in normal and aberrant neurodegenerative states [20]. It is able to secrete an array of potent mediators that may orchestrate neuroinflammation and affect the integrity of the blood–brain barrier. A functional interaction between mast cells and neurons may be an important neurobiological process. Various stimuli (such as neuroinflammatory conditions) induce mast cells to release these mediators. The possibility that an up-regulation of immune and inflammatory mediators in early-stage AD may be protective against the development and progression of AD has been gaining recognition [21]. Inflammation triggers the formation and progressive accumulation of NFTs and senile plaques, which in turn activate inflammatory reactions.

In early-stage AD, an up-regulation of SCRN1 leads to the release of immune and inflammatory mediators. In addition, ATP6V1E1 regulates the pH of intracellular compartments, extracellular space, and cytoplasm at the expense of ATP. It assists in the acidification of lysosomes, which is necessary for the activity of degradative enzymes. As a result, both these proteins may be neuroprotective, protecting neurons from degenerative processes in the early stage of the disease process.

This study suggests that the differential expression of proteins with the progress of AD might provide clues to establish potential targets to halt the cascade of disease-associated neurological damage.

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