

Research Article

Dipeptidyl Peptidase 10 (DPP10₇₈₉): A Voltage Gated Potassium Channel Associated Protein Is Abnormally Expressed in Alzheimer's and Other Neurodegenerative Diseases

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Received 13 January 2014; Accepted 23 April 2014; Published 16 June 2014

Academic Editor: Hanna Rosenmann

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The neuropathological features associated with Alzheimer's disease (AD) include the presence of extracellular amyloid- β peptide-containing plaques and intracellular tau positive neurofibrillary tangles and the loss of synapses and neurons in defined regions of the brain. Dipeptidyl peptidase 10 (DPP10) is a protein that facilitates Kv4 channel surface expression and neuronal excitability. This study aims to explore DPP10₇₈₉ protein distribution in human brains and its contribution to the neurofibrillary pathology of AD and other tauopathies. Immunohistochemical analysis revealed predominant neuronal staining of DPP10₇₈₉ in control brains, and the CA1 region of the hippocampus contained strong reactivity in the distal dendrites of the pyramidal cells. In AD brains, robust DPP10₇₈₉ reactivity was detected in neurofibrillary tangles and plaque-associated dystrophic neurites, most of which colocalized with the doubly phosphorylated Ser-202/Thr-205 tau epitope. DPP10₇₈₉ positive neurofibrillary tangles and plaque-associated dystrophic neurites also appeared in other neurodegenerative diseases such as frontotemporal lobar degeneration, diffuse Lewy body disease, and progressive supranuclear palsy. Occasional DPP10₇₈₉ positive neurofibrillary tangles and neurites were seen in some aged control brains. Western blot analysis showed both full length and truncated DPP10₇₈₉ fragments with the later increasing significantly in AD brains compared to control brains. Our results suggest that DPP10₇₈₉ is involved in the pathology of AD and other neurodegenerative diseases.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized pathologically by the presence of extracellular amyloid plaques containing the amyloid- β peptide (A β) and intracellular neurofibrillary tangles (NFTs) containing hyperphosphorylated microtubule-associated protein tau and loss of synapses and neurons in select brain regions [1–3]. The aetiology of AD is complex, with a majority of cases being sporadic and approximately 10% being inherited. Mutations have been identified in genes encoding amyloid precursor protein (APP) [4, 5], presenilin 1 (PS1), and PS2 in a small subset of familial AD cases. In sporadic AD cases, polymorphisms of apolipoprotein E4 and other genes have been associated with an increased risk of developing the disease [6].

Oxidative stress, impaired energy metabolism, and disruption of neuronal Ca²⁺ regulation have all been implicated in the cellular pathophysiology. Although experimental approaches indicate that A β deposition plays an important role in the neurodegenerative process in AD [7, 8], the mechanism underlying the relentless progression of neurofibrillary pathology and neuronal death remains to be fully elucidated. Over the last decade there have been a number of studies demonstrating that potassium channel dysfunction may be involved in the pathogenesis of AD [9–13].

Neuronal excitability is finely controlled by various membranous channels and associated proteins. In the central nervous system, the rapidly inactivating voltage-gated potassium channels (Kv channels) are the major determinants of dendritic excitability [14]. In particular, Kv4 channels, a subfamily of Kv channels, account for a large portion of the

TABLE 1: Primary antibodies used in this study.

Antibody/antiserum	Species	Epitope	Source	Dilution
DPP10 ₇₈₉ (polyclonal)	Rabbit	DPP10 ₇₈₉ N-terminal intracellular domain	This lab	1 : 100
Anti-GFP (polyclonal)	Rabbit	GFP	Invitrogen, USA	1 : 3000
AT8	Mouse	Specific for tau phosphorylation at Ser 202 and/or Thr 205	Innogenetics, Belgium	1 : 1000
Tau2	Mouse	Phosphorylated and nonphosphorylated tau	Novocastra, UK	1 : 1000
A4	Mouse	β -Amyloid	Professor Colin Masters, Melbourne University	1 : 250
Anti- β III-tubulin monoclonal Ab	Mouse	C-Terminus of β III-tubulin	Promega, USA	1 : 10000

somatodendritic inactivating current in neurons in regulating firing frequency and signal processing in dendrites. The Kv4 channels are composed of the pore-forming subunits, various auxiliary subunits, and other interacting proteins such as Kv channel interacting proteins and dipeptidyl peptidase-like proteins DPP6 and DPP10 [15–17].

Indeed, potassium channel dysfunction has been demonstrated in fibroblasts and platelets of AD patients [9, 10]. Post-mortem studies have also showed alterations of potassium channel expression in AD brains [18]. Several publications have demonstrated that $A\beta$ is involved in Kv channel function and may even play a physiological role in controlling neuronal excitability [19, 20]. In addition, Kv channel interacting protein 3, also known as calsenilin, is a presenilin binding protein [21] that preferentially interacts with the familial AD associated C-terminal fragment of PS2 [22]. Moreover, the application of $A\beta$ peptide to cultured cells increases both Kv channel interacting protein 3 mRNA and protein expression as well as cell death. The $A\beta$ toxicity can be prevented by blocking expression of Kv channel interacting protein 3 [23]. Furthermore, in a study using a transgenic model of Alzheimer’s disease, some potassium channels in hippocampal neurons were found to be absent [24]. Liu et al. reported that a potassium channel activator diazoxide ameliorated $A\beta$ and tau pathologies and improves memory in the 3xTg AD mouse model [25]. Thus there is a body of research that suggests that potassium channels or their associated proteins might be involved in steps leading to the neurodegeneration observed in AD.

DPP10 belongs to the dipeptidyl peptidase 4 (DPP4) gene family sharing 59% amino acid sequence similarity with DPP4, an atypical serine protease [26, 27]. However DPP10 is missing the nucleophilic Ser residue in the catalytic motif and lacks enzyme activity. So far, four splice variants have been reported for DPP10. As a result of alternate splicing of the first exon, each variant has a short divergent cytoplasmic N-terminus. The remaining protein of each variant is identical and consists of a highly conserved juxtamembrane, transmembrane, and large extracellular C-terminal domain. Kv4 channels containing different DPP10 variants display distinct inactivation kinetics and voltage dependence at depolarized potentials, indicating that the cytoplasmic portion of the DPP10 protein influences the inactivation properties [28]. We

have previously cloned the short form of DPP10 (DPP10₇₈₉) from human brain [27]. To further characterise its function, we generated an antibody specific to DPP10₇₈₉ to examine the expression of DPP10₇₈₉ in human brains. Here we report that DPP10₇₈₉ is predominantly localized to neuronal soma and dendrites in the neocortex and subcortical grey matter in control brains and abnormally accumulated in Alzheimer’s disease and other diseases characterised by tau positive pathologies.

2. Materials and Methods

2.1. Primary Antibodies. The peptide H-MRKVESRGGEGRE-OH containing the N-terminus of DPP10₇₈₉ was ordered from MIMOTOPES (VIC, Australia). This N-terminal sequence shares no sequence identity with the two other forms of DPP10 or the N-termini of the four DPP6 isoforms and therefore will only detect the DPP10₇₈₉ isoform of DPP10 [29]. Rabbits were immunised by the peptide mixed with Freund’s complete adjuvant. DPP10₇₈₉ antiserum was purified using an affinity column prepared from the respective immunising peptide (ABGENT, Inc., San Diego, CA, USA) conjugated to 6% cross-linked beaded agarose. A sequence homology search was performed using BlastP into the GenBank database and the peptide sequence only had significant homology with DPP10₇₈₉. All other primary antibodies, their source, and the dilution used in the present studies are listed in Table 1.

2.2. Human Cases. Samples from the hippocampus, the frontal and temporal cerebral cortex, the entorhinal and cingulate cortex, and the cerebellum were obtained from individuals listed in Table 2. Brain tissue was collected by the National Health Medical Research Council (NHMRC) Brain Bank of South Australia following NHMRC ethical guidelines. All diseased cases had a clinical diagnosis while control cases showed no clinical signs of dementia.

2.3. Cell Culture and Sample Preparation. The human kidney epithelial cell line 293T was cultured as described previously [30]. Transfection with GFP-DPP10₇₈₉ construct was carried out by methods previously described using Fugene 6 (Roche, USA) [27, 30]. After 48 hrs, cell extracts were resuspended in

TABLE 2: Human cases analysed in this study.

Brain Bank number	Gender	Age	Postmortem delay (h)	Neuropathological diagnosis
SA0123	M	80	21	AD
SA0126	F	79	31	AD
SA0134	F	69	23	AD
SA0143	M	84	15	AD
SA0148	M	81	14	AD
SA0129	F	88	9	Early AD
SA0087	M	71	15.5	FTLD
SA0069	M	69	31	DLBD
SA0079	F	82	31	DLBD
SA0063	F	81	7	DLBD
SA0094	M	74	24	DLBD
SA0035	F	72	28	PSP
SA0043	F	69	10	PSP
SA0106	M	66	29	PSP
SA0136	F	80	20	PSP
SA0010	M	58	14	Control
SA0013	M	64	23	Control
SA0021	F	72	7	Control
SA0030	M	69	48	Control
SA0031	F	61	8	Control

AD: Alzheimer's disease, DLBD: diffuse Lewy's body disease, FTLD: frontotemporal lobar degeneration, PSP: progressive supranuclear palsy, F: female, and M: male.

sonication buffer (20 mM Tris pH 7.5, 5 mM EGTA, 2 mM EDTA and 0.25 M sucrose) and sonicated for 3×10 sec. Following sonication cellular debris was removed by a brief 10,000 g centrifugation and then the supernatant underwent a 30 min 100,000 g centrifugation at 4°C to pellet membrane proteins. The membrane pellet was solubilized in sonication buffer containing 0.5% Triton X-100, mixed with 4x sample buffer (6% SDS, 40% sucrose, 150 mM Tris pH 6.8, and 0.03% Bromophenol Blue), and incubated at 37°C for 10 mins and run on 10% SDS-PAGE. The recombinant GFP-tagged DPP10₇₈₉ protein was detected using either an anti-GFP antibody (Cell Signaling Technology, USA) or the affinity purified anti-DPP10₇₈₉ antibody described above.

2.4. Tissue Preparation. Brain tissues were fixed routinely by immersion in a mixture of 4% paraformaldehyde and picric acid. Series of each frozen section (450 μm thick) were cut on a chilled microtome. Paraffin sections were cut (5–20 μm), mounted on gelatine coated glass slides, and dried in an oven at 37°C overnight.

2.5. Immunohistochemistry. A green fluorescent protein GFP-DPP10₇₈₉ construct with GFP fused to the N-terminus of DPP10₇₈₉ was transiently transfected into 293T cells cultured on a chamber slide as described previously [27]. After 48 h, cells were washed with PBS and then fixed with 2% paraformaldehyde made in PBS. The affinity purified anti-DPP10₇₈₉ Ab was used to detect recombinant DPP10₇₈₉ protein. Mouse

anti-rabbit Alexa 596 (Molecular Probes, Eugene, OR) was used as the secondary antibody for immunofluorescence studies.

Free-floating sections of human brain (50 μm) were incubated with primary antibody for 48 h at 4°C. Incubation with the secondary biotinylated anti-rabbit or anti-mouse IgG was performed for 1 hr at room temperature. Immunoreactions were visualised with the avidin-biotin-peroxidase complex (VECTASTAIN ABC system, Vector Labs, UK) and 3-3'-diaminobenzidine-4 HCl/H₂O₂ (DAB, D5637 Sigma, USA). Paraffin-embedded sections were treated similarly after dewaxing, except that they were heat-treated (105°C for 10 min 1 mM EDTA, pH 8.0 at 25°C) before immunostaining.

Double-labelled immunohistochemistry was performed on sections from AD cases. Selected free-floating sections were incubated with both anti-DPP10₇₈₉ and AT8 antibody (anti-phosphorylated tau protein), Tau2 antibody (detects both phosphorylated and nonphosphorylated tau), or A4 antibody (anti-amyloid-β). Anti-DPP10₇₈₉ antibody was then visualized by anti-rabbit IgG conjugated with Cy2 (Jackson ImmunoResearch Laboratories Inc., USA) while anti-tau antibody or anti-amyloid antibody was detected by anti-mouse IgG conjugated with Cy3 (Jackson ImmunoResearch Laboratories).

Whenever the A4 antibody was used, antigen retrieval was performed before the blocking step by incubating the sections in preheated sodium citrate buffer (10 mM, pH 8.5) at 80°C for 30 min.

2.6. Quantification of Lesions. Adjacent free-floating sections of hippocampus or cortex were immunostained with DPP10₇₈₉, AT8, or A4 antibody separately using the DAB reaction as described above. The number of lesions was quantified at 200 times of magnification under an Olympus BH-2 light microscope (Tokyo, Japan) coupled to a MicroPublisher 3.3 RTV digital camera (QIMAGING, Canada). In the hippocampus, three fields (0.01 mm²/field) from each region of Ammon's horn (CA1-4) were counted. In neocortical sections, three fields per case were counted in each of layers III to V. After each field was captured, the stage was moved manually to a new field using fiducial landmarks to ensure a completely nonredundant evaluation.

2.7. Tissue Homogenates. Hippocampuses of five AD cases and four control cases were dissected from fresh-frozen brain. Samples were homogenized in 10 volumes of ice-cold sucrose buffer (0.32 M sucrose, 1 mM EDTA, 5 mM Tris-HCl, and pH 7.4) containing 0.2% Triton X-100 and a protease inhibitor cocktail (Sigma, USA). The homogenate was centrifuged at 10,000 ×g for 20 min. The supernatant was saved and the pellet was then resuspended in ice-cold sucrose buffer and homogenized again. The homogenate was solubilised for 1 hr at 4°C. Insoluble material was removed by centrifugation at 10,000 ×g for 30 min. The second supernatant was combined with the first as hippocampal solubilised fraction. After the estimation of protein concentration, aliquots of homogenates were immediately subjected to incubation with 4 × SDS-PAGE sample buffer (250 mM Tris-HCl, 8% SDS, 40% glycerol, 0.4 M DTT, 0.04% Bromophenol Blue, and pH 6.8) at 37°C for 20 min. Protein (50 μg) from each sample was loaded and run on a 10% SDS-PAGE gel.

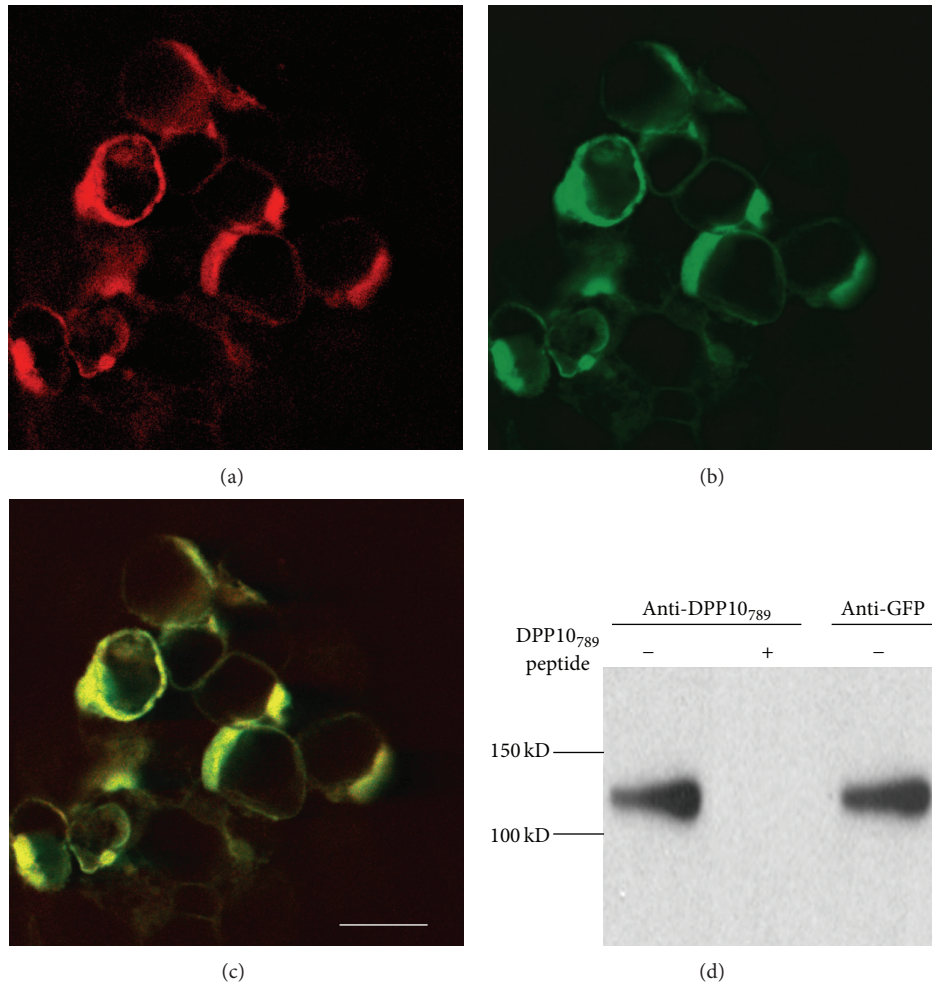


FIGURE 1: Anti-DPP10₇₈₉ antibody characterization. The GFP-DPP10₇₈₉ construct was transiently transfected into 293T cells. DPP10₇₈₉ protein was stained with anti-DPP10₇₈₉ antibody followed by Cy3 goat anti-rabbit IgG (a). The green GFP fluorescence (b) and the overlay of the Cy3 and the GFP images (c). Scale bar is 20 μ m. Membrane fractions were prepared and run on a 10% SDS-PAGE gel. The transferred membrane was immunoblotted with DPP10₇₈₉ antibody. The DPP10₇₈₉ antibody recognized a 120 kDa band corresponding to the full-length GFP-tagged DPP10₇₈₉ ((d) far left lane). This 120 kDa band can be totally blocked with addition of the antigenic peptide for DPP10₇₈₉ antibody in the blocking solution ((d) middle lane). The anti-GFP antibody detected the same band after the membrane was stripped and reprobed ((d) right lane).

2.8. Immunoblot. Immunoblots were performed as described previously [27] and incubated with the affinity purified DPP10₇₈₉ antibody (final concentration at 1.25 μ g/mL) or anti- β III-tubulin monoclonal antibody (final concentration 0.1 μ g/mL) at 4°C overnight. Bound antibodies were detected using the SuperSignal West Pico Chemiluminescent Substrate (Pierce, IL, USA). The membrane was exposed to X-OMAT diagnostic film (Kodak Scientific Light Systems, NY, USA) and developed on a Kodak X-OMAT 1000 Processor (Kodak Australasia, Pty. Ltd.).

2.9. Analytical Methods. The protein concentrations of brain homogenates were measured using the Bradford assay (BioRad, USA) according to the manufacturer's instructions. Levels of DPP10₇₈₉ in brain homogenates were estimated by immunoblot using the Quantity One (BioRad, USA) image analysis software package and were adjusted by the level of neuron specific protein β -tubulin. An independent *t*-test was

performed to compare the expression of DPP10₇₈₉ between control and AD brains.

3. Results

3.1. Specificity of the DPP10₇₈₉ Antibody. To confirm the specificity of the anti-DPP10₇₈₉ antibody, we used immunofluorescence and immunoblots to examine the 293T cells transfected with the GFP-DPP10₇₈₉ construct (Figures 1(a)–1(d)). With immunofluorescence, all cells that expressed GFP fluorescence were detected by the anti-DPP10₇₈₉ antibody (Figures 1(a), 1(b), and 1(c)), indicating the antibody specifically bound to recombinant GFP-DPP10₇₈₉ chimeric protein. Our DPP10₇₈₉ antibody was designed to detect the *N*-terminal sequence of DPP10₇₈₉, while the anti-GFP antibody recognises the GFP protein. On immunoblot (Figure 1(d)), the DPP10₇₈₉ antibody recognised a band around 120 kDa (Figure 1(d), far left lane), corresponding to the expected

molecular mass of GFP-tagged DPP10₇₈₉ and being consistent with our previous studies [27]. The 120 kDa band was also detected by the anti-GFP antibody (Figure 1(d), right lane) confirming that the band was the GFP-DPP10₇₈₉ chimeric protein. Because 293T cells are derived from human kidney epithelial cells that do not express the brain derived DPP10₇₈₉, no endogenous DPP10₇₈₉ was detected by immunoblot. Thus these results confirmed that the DPP10₇₈₉ antibody was specific to DPP10₇₈₉.

3.2. DPP10₇₈₉ in Control and AD Brains. To document the cellular localisation of DPP10₇₈₉ protein in human brains, we first immunostained free-floating brain sections from a selection of control cases and cases with various neurodegenerative diseases (Table 2). In control brains, positive DPP10₇₈₉ staining was predominantly associated with neurons in the CA1 region of the hippocampus (Figures 2(a), 3(a), and 3(b)). The pyramidal cells were particularly well stained, with DPP10₇₈₉ reactivity extending well into the distal portion of dendrites (Figures 3(a) and 3(b)).

In contrast, very strong punctate DPP10₇₈₉ immunoreactivity was observed in AD brains especially in the hippocampal regions. Close examination revealed that neurofibrillary tangles and dystrophic neurites were robustly stained with the DPP10₇₈₉ antibody. These positive structures were most abundant in the hippocampal region, where staining was most intense in the CA1 and subiculum areas (Figures 2(b)–2(e) and 2(h)). This immunoreactivity was completely abolished by the DPP10₇₈₉ antigenic peptide (Figure 2(i)), and sections incubated with preimmunization serum omitting the DPP10₇₈₉ antibody did not show any positive staining (data not shown), further indicating that the observed immunostaining was specific.

At high magnifications, three types of DPP10₇₈₉ positive neurofibrillary tangle-like structures were observed: (1) granular staining in the neuronal soma (Figure 2(j)), resembling pretangle phosphotau aggregates; (2) condensed intraneuronal staining (Figures 2(k), 2(l), and 2(o)), resembling intraneuronal tau NFTs; (3) less condensed extracellular staining (Figures 2(m), 2(n), and 5(h)), resembling extraneuronal tau NFTs and ghost tangles. In addition, DPP10₇₈₉ immunoreactivity was also detected significantly in dystrophic neurites surrounding amyloid plaques (Figures 2(d), 2(e), arrows, and 2(p)) and in neuropil threads (Figure 2(q)).

The DPP10₇₈₉ positive tangles and dystrophic neurites were also observed in the frontal, temporal, entorhinal, and cingulate regions as summarized in Table 3. No DPP10₇₈₉ positive staining was observed in the cerebellum.

Similar results were also observed in paraffin-embedded sections. DPP10₇₈₉ immunostaining was detected on the cell body and surface of dendrites of the hippocampal region in a control case (Figures 3(a), arrows, and 3(b)). DPP10₇₈₉ positive staining was also observed in NFTs and plaque-associated dystrophic neurites in AD brain (Figures 3(c)–3(h)). Thus the above results suggested that the increased DPP10₇₈₉ staining was primarily accumulated in neurofibrillary tangles and dystrophic neurites.

3.3. DPP10₇₈₉ in Other Tauopathies. Because neurofibrillary tangle and dystrophic neurite pathologies also occur in neurodegenerative diseases other than AD, including PSP and FTL and DLBD, these later cases were further examined. Some DPP10₇₈₉ positive tangles and plaques were also detected in these cases (Figure 4). Table 3 summarizes the presence of DPP10₇₈₉ positive tangles and dystrophic neurites. In the four DLBD cases examined, DPP10₇₈₉ positive tangles and dystrophic neurites were also observed in temporal, hippocampal, and entorhinal cortex regions (Figures 4(b) and 4(e)). A few positive DPP10₇₈₉ tangles and plaques in the hippocampal and entorhinal regions were observed in PSP cases (Figure 4(c)). Occasionally weak DPP10₇₈₉ positive tangles and plaques were detected in some aged control brains.

3.4. DPP10₇₈₉ Colocalizes with Tau Protein but Not with Amyloid- β in AD Brain. Because tau protein is a major component of neurofibrillary tangles and dystrophic neurites, we examined if DPP10₇₈₉ protein expression is associated with tau protein expression. We used the DPP10₇₈₉ antibody and anti-tau antibody (AT8) specific for phospho-Ser202/Thr205 to localise DPP10₇₈₉ and phosphorylated tau (p-tau) by double immunofluorescence staining in the brains of AD. We observed robust colocalization of DPP10₇₈₉ with p-tau in most tangles and dystrophic neurites (Figure 5). In neurofibrillary tangles, DPP10₇₈₉ and p-tau were fully colocalized (Figures 5(b), 5(e), and 5(g)). In plaques, DPP10₇₈₉ and p-tau were found in the larger sized dystrophic neurites (Figure 5(d)). DPP10₇₈₉ was also present in intracellular vesicle-like structures (Figure 5(f)) where only weak p-tau staining was present. In the entorhinal region of the temporal cortex, some DPP10₇₈₉ positive tangle-like structures were devoid of p-tau staining (Figure 5(h)), indicating that the DPP10₇₈₉ antibody might be able to detect either early or late tangle stages—ghost tangles which are not detected by AT8. Double staining using the DPP10₇₈₉ antibody and Tau2 antibody (detects both phosphorylated and nonphosphorylated tau) also showed colocalization of DPP10₇₈₉ and tau in neurofibrillary tangles and dystrophic neurites (Figures 5(a) and 5(c)).

The other major pathological hallmark of AD is the neuritic amyloid plaques located in the extracellular space of the brain, comprised of an aggregated A β peptide core surrounded by dystrophic neurites. As the DPP10₇₈₉ antibody showed strong immunoreactivity in plaque-associated dystrophic neurites, it was also examined if DPP10₇₈₉ colocalized with A β peptide. Double immunofluorescence revealed that DPP10₇₈₉ positive dystrophic neurites were mostly present in the A β core of senile plaques. While both DPP10₇₈₉ and A β positive staining were observed within the same plaque region, they were not colocalized; the red A β staining was distributed as amyloid aggregation, with the green DPP10₇₈₉ positive dystrophic neurites mingling randomly (Figure 5(i)).

The intrahippocampal distribution of DPP10₇₈₉ positive neurofibrillary tangles (NFTs) followed the pattern established for p-tau positive ones, with the greatest number of involved neurons lying within the CA1 region of Ammon's

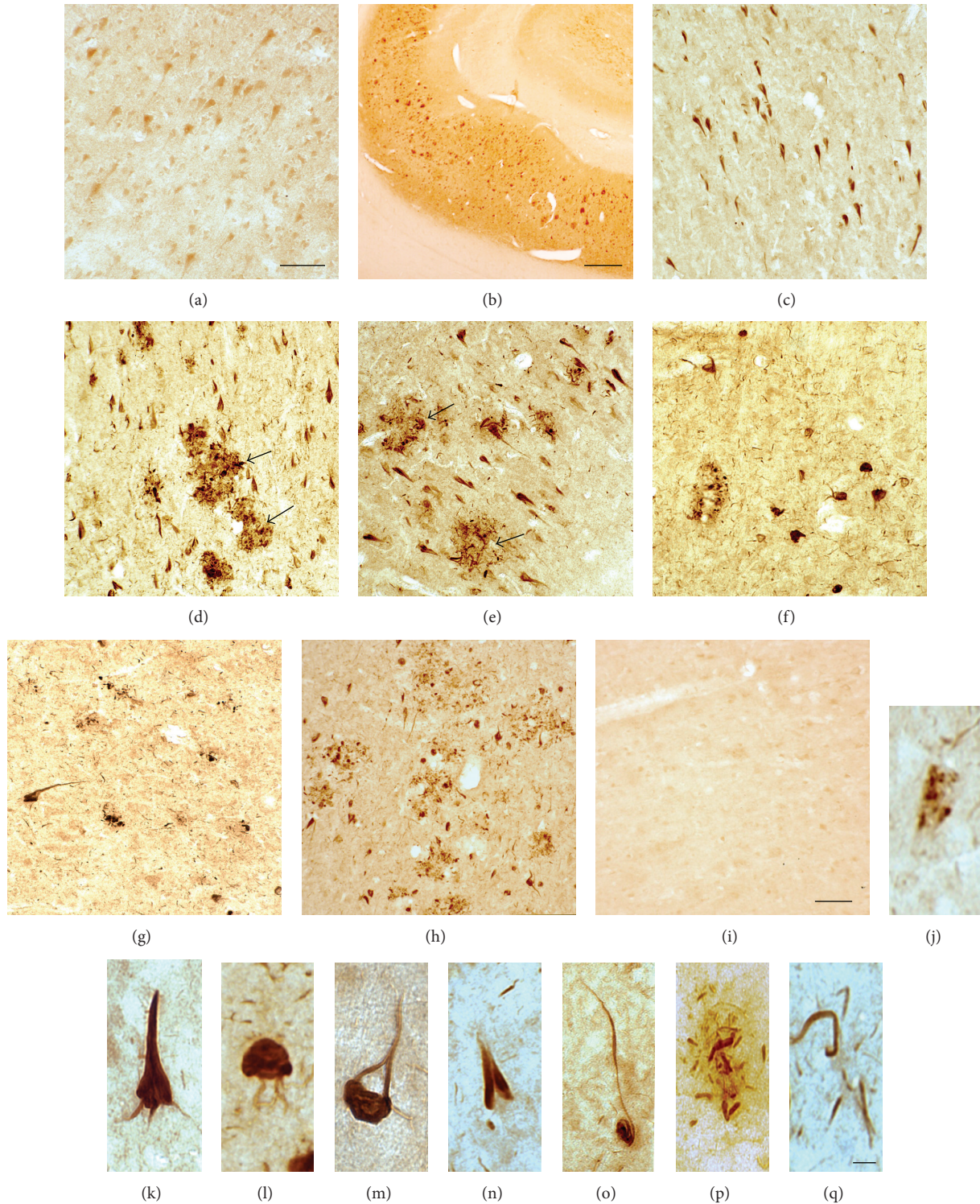


FIGURE 2: Immunostaining of DPP10₇₈₉ in free-floating sections of control and AD brains. DPP10₇₈₉ immunostaining in the CA1 region of a control brain (a), CA1 and subiculum region (b, c, and d), CA2 (e), inferior frontal cortex (f), cingulate cortex (g), inferior temporal cortex (h), and inferior temporal cortex section blocked with antigenic peptide (i) in AD brains. DPP10₇₈₉ staining was found in granular structures in the neuronal soma (j), NFT with extensions into dendrites of pyramidal neurons (k), globose NFT (l), extracellular ghost tangle (m), flame shaped NFT (n), slender tangle of the subiculum with long extension into apical dendrite (o), plaque-associated dystrophic neurites (p), and neuropil threads (q). Scale bar is 200 μ m (b), 50 μ m (a, c-i), and 10 μ m (j-q). Arrows in (d) and (e) indicate staining in plaque-associated dystrophic neurites in AD brain.

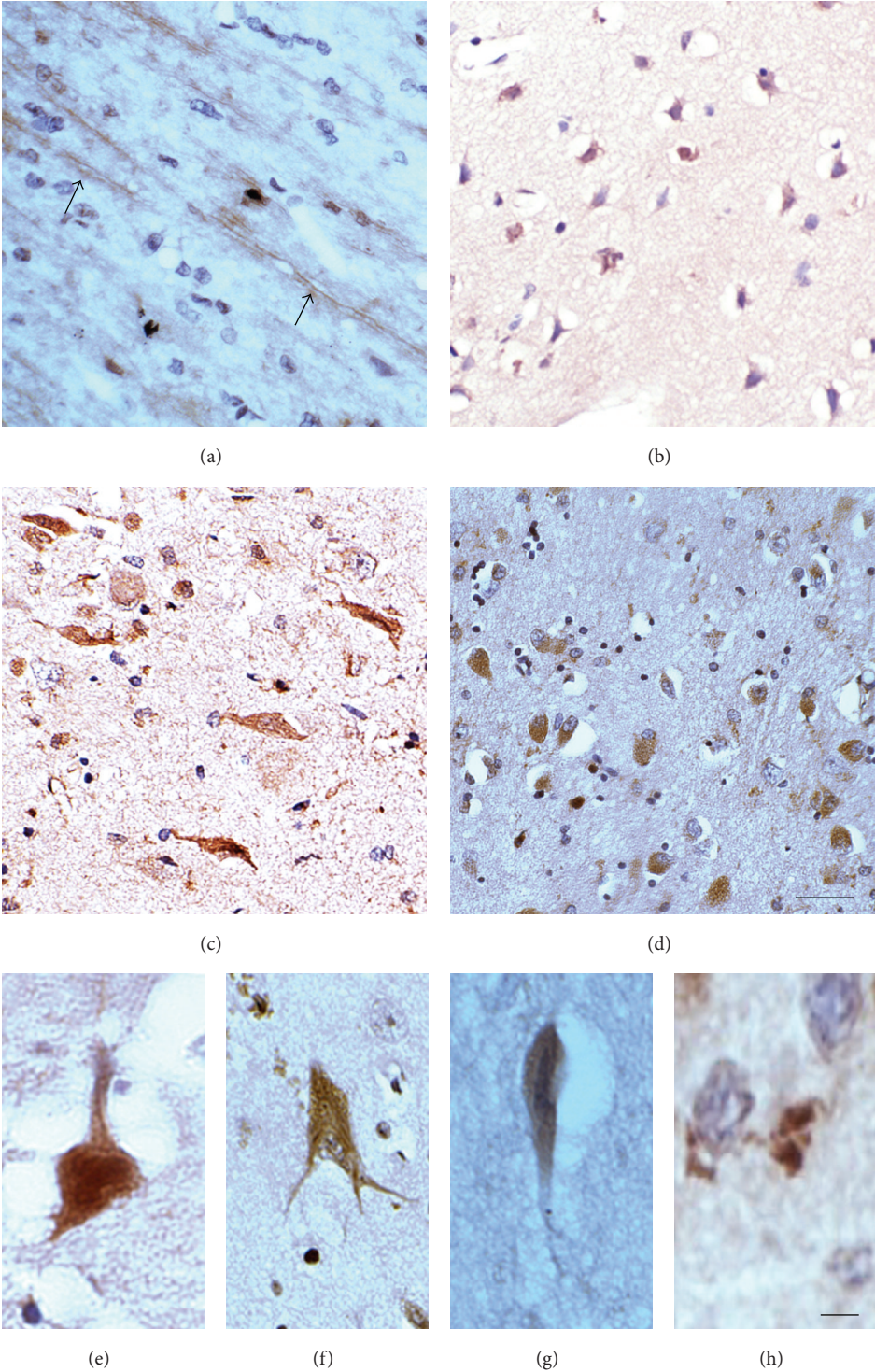


FIGURE 3: Immunostaining of DPP10₇₈₉ in paraffin sections from control and AD brains. DPP10₇₈₉ reactivity was observed in the frontal cortex of a control individual (a, b), and arrows indicate the surface staining of DPP10₇₈₉ in dendrites. DPP10₇₈₉ positive staining was increased in AD brain (c, d) and in NFTs (e-h). Scale bar is 50 μm (a-d) and 10 μm (e-h).

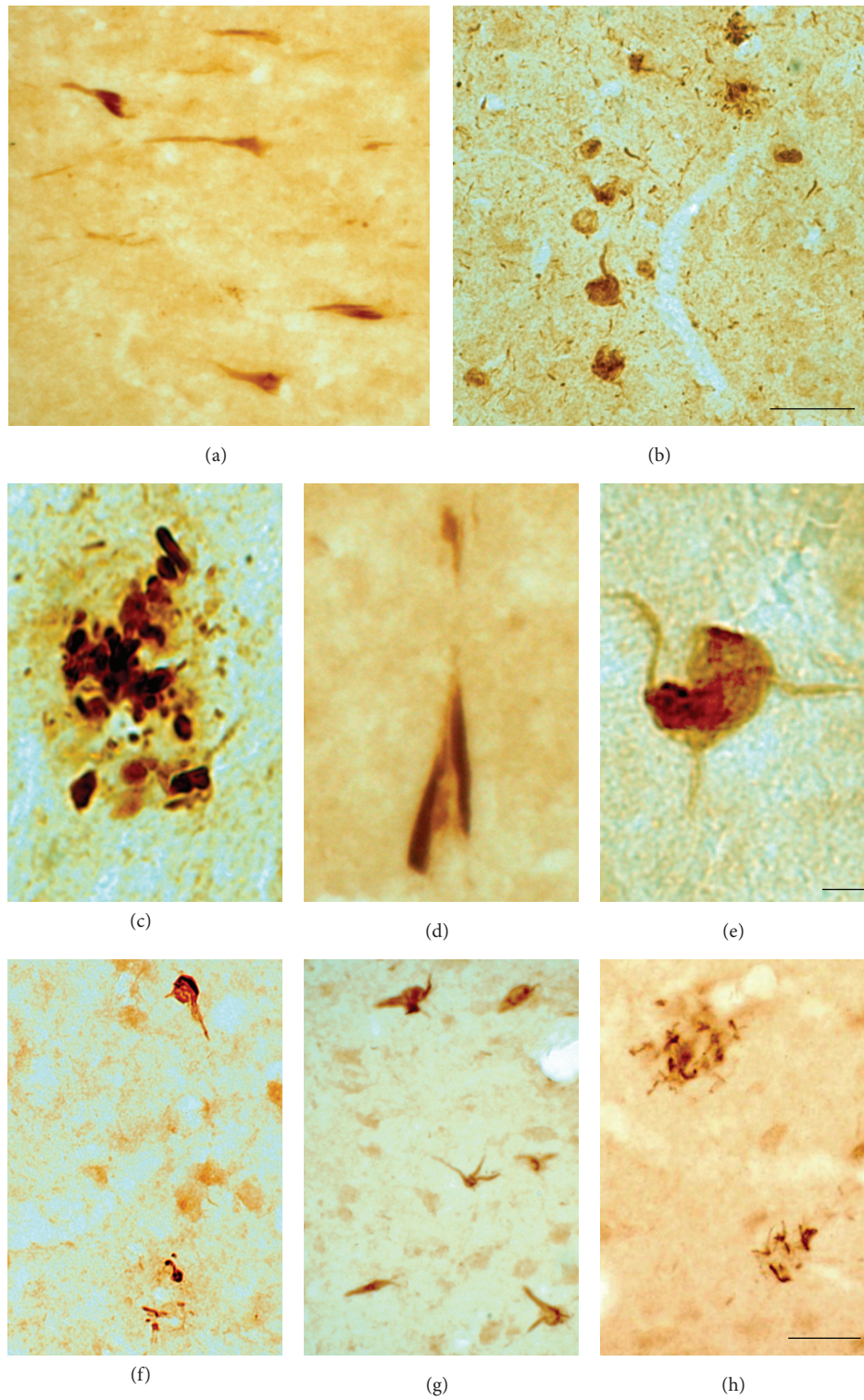


FIGURE 4: DPP10₇₈₉ immunostaining in free-floating brain sections of other tauopathies. DPP10₇₈₉ positive NFTs were observed in the hippocampus of FTLD (a, d and g) and PSP (f) and superior frontal brains sections from patients with DLBD (b, e). DPP10₇₈₉ staining in plaque-associated dystrophic neurites in DLBD and PSP brains (c and h, resp.). Scale bar is 50 μm (a, b, and f-h) and 10 μm (c-e).

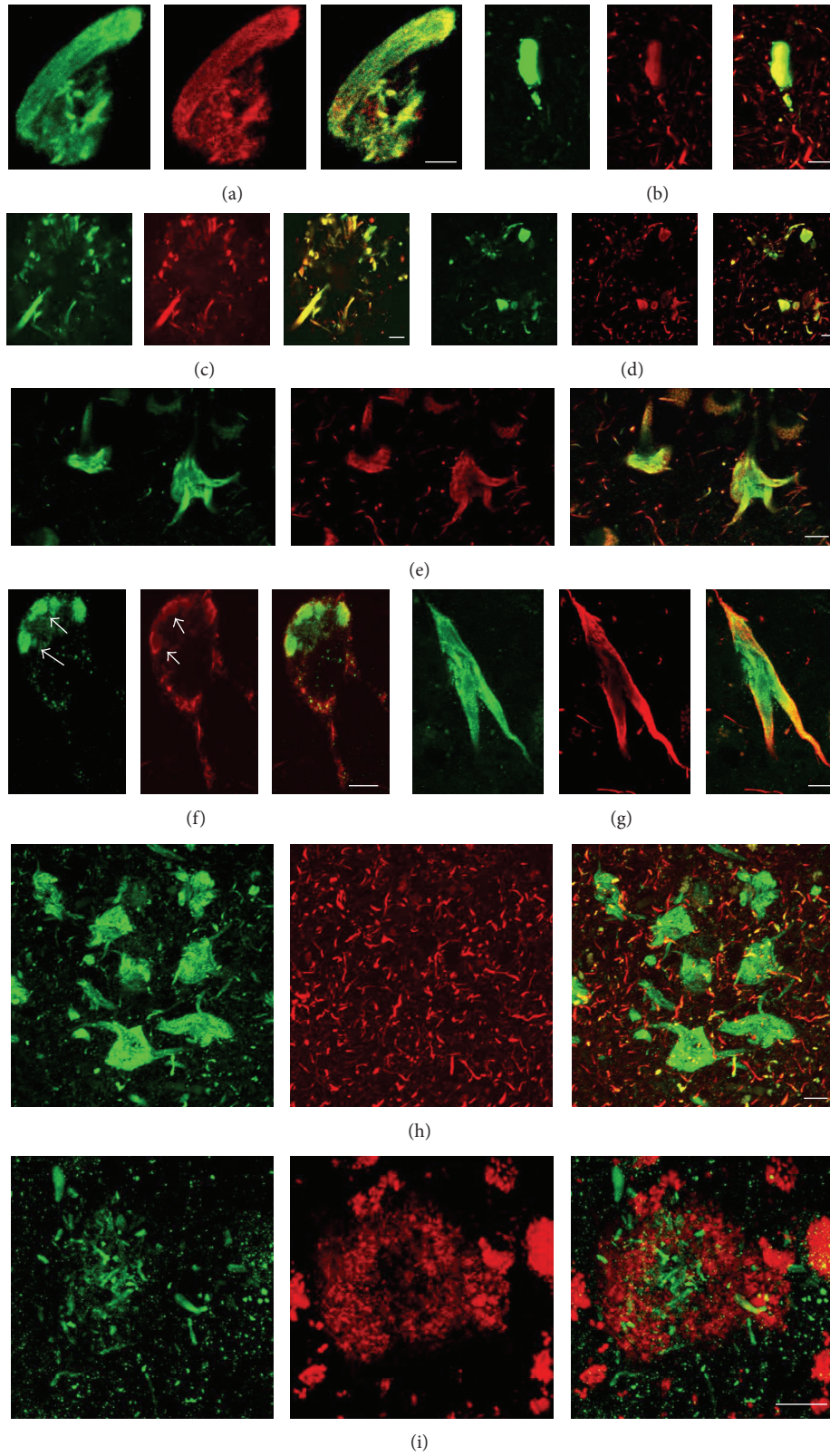


FIGURE 5: DPP10₇₈₉ and tau colocalization in AD. Immunohistochemical colocalization of DPP10₇₈₉ with phosphorylated tau (p-tau) in tangles and plaque-associated dystrophic neurites. DPP10₇₈₉ (green) partially or fully colocalized with p-tau (red) in tangles (a, b, e, and g) and in plaque-associated dystrophic neurites (c, d). DPP10₇₈₉ was also present in intracellular vesicle-like structures (f) where very weak staining for p-tau was present. DPP10₇₈₉ immunoreactivity was also found in some tangles in the entorhinal region which were devoid of p-tau staining (h). DPP10₇₈₉ (green) did not colocalize with amyloid- β plaques (red) in AD brain (i). Scale bar is 10 μ m. (a) and (c) used Tau2 ab and the remaining images are with AT8 ab.

TABLE 3: Summary of the DPP10₇₈₉ positive staining in NFT and plaque-associated dystrophic neurites observed in control and neurodegenerative disease cases.

Case number	Neuropathological diagnosis	Frontal cortex		Temporal cortex		Hippocampus		Entorhinal cortex		Cingulate cortex		
		Tangle	Plaque	Tangle	Plaque	Tangle	Plaque	Tangle	Plaque	Tangle	Plaque	
1	SA0123	AD	-	+++	+++	+++	+++	+++	+++	+++	++	+++
2	SA0126	AD	+++	++	+++	+++	++	+++	+++	++	++	++
3	SA0134	AD	+	+++	-	+++	+++	++	N/A	N/A	+	+++
4	SA0143	AD	+	+	+++	++	+++	+++	+++	+++	+	+
5	SA0148	AD	+++	+++	-	+++	+++	+++	+++	+++	+++	+++
6	SA0129	Early AD	+	+	+	+	+++	++	+++	+	-	-
7	SA0069	DLBD	++	++	-	+++	++	++	+++	+++	+	++
8	SA0079	DLBD	-	-	-	-	-	++	-	-	-	-
9	SA0094	DLBD	-	-	+++	+++	+	++	++	++	+	+
10	SA0063	DLBD	-	-	+	-	+	+	-	-	-	-
11	SA0087	FTLD	-	-	-	-	+++	+	++	-	-	-
12	SA0035	PSP	-	-	+	-	+	-	N/A	N/A	-	-
13	SA0043	PSP	+	+	+	+	++	+	++	++	+	-
14	SA0106	PSP	-	-	-	-	-	-	++	N/A	-	-
15	SA0136	PSP	-	-	-	+	-	++	+++	+	-	-
16	SA0010	Control	+	-	-	+	-	-	-	-	-	+
17	SA0013	Control	-	-	-	-	+	-	-	-	-	-
18	SA0021	Control	-	-	-	-	+	-	+	-	-	-
19	SA0030	Control	-	-	+	-	-	-	N/A	N/A	+	+
20	SA0031	Control	-	-	-	-	N/A	N/A	N/A	N/A	-	-

The number of lessons was counted at $\times 200$ magnification under an Olympus BH-2 light microscope (Tokyo, Japan).

N/A: sample not available; -: negative; +: 1-5 lesions/field; ++: 5-10 lesions/field; +++: >10 lesions/field.

horn, followed by CA2, CA3, and CA4 (Figure 6(a)). In the neocortical region, staining was detected mainly in layers III-V. Approximately 67% of p-tau positive NTFs and 40% A β positive neuritic plaques (NPs) contained anti-DPP10₇₈₉ immunoreactivity in these regions (Figure 6(b)).

3.5. Truncated DPP10₇₈₉ Is Elevated in AD Tissue. DPP10₇₈₉ protein levels were analyzed in the hippocampus as it is a rich source of both NFTs and NPs. The DPP10₇₈₉ Ab detected three major mobilities: 100 kDa, 50 kDa, and 37 kDa (Figure 7(a)) in immunoblots. The 100 kDa band represents the full-length DPP10₇₈₉ protein [27]. Samples from both AD and control brains showed similar density of this band. The 50 kDa and 37 kDa bands observed are probably truncated forms of DPP10₇₈₉ which are less than half the size of intact DPP10₇₈₉ and have retained the N-terminus, suggesting that the DPP10₇₈₉ has been proteolytically processed. Noticeably, AD brain samples had higher density bands of these two truncated forms compared to control samples. Quantitation of the immunoblot normalised to tubulin immunoreactivity confirmed that there was no significant difference in the 100 kDa intensity band between AD and control brains. However, the immunoreactive intensity of 50 kDa, 37 kDa, and 50 + 37 kDa was all significantly elevated in AD brain compared to control brain ($P \leq 0.05\%$) (Figure 7(b)), indicating the possibility that C-terminal truncated DPP10₇₈₉ may be aggregating and

thus may be directly involved in the formation of neurofibrillary tangles and dystrophic neurites in AD brain.

4. Discussion

DPP10₇₈₉ is a dipeptidyl peptidase-like protein that together with Kv-channel interacting proteins (KChIPs) forms multiprotein complexes that underlie subthreshold A-type currents (I_{SA}) in neuronal somatodendritic compartments [28]. This is the first study to assess the expression of DPP10₇₈₉ in postmortem brain samples in AD and other major tauopathies. Using immunohistochemistry we reveal predominant neuronal staining of DPP10₇₈₉ throughout the neocortex and subcortical grey matters with high expression in the pyramidal cells in control brain tissue. In contrast, in AD brains, robust DPP10₇₈₉ reactivity was also detected in neurofibrillary tangles and plaque-associated dystrophic neurites, most of which colocalized with the hyperphosphorylated Ser-202/Thr-205 tau epitope. DPP10₇₈₉ positive neurofibrillary tangles and plaque-associated dystrophic neurites also appeared in other neurodegenerative diseases such as FTL, DLB, and PSP. Occasional DPP10₇₈₉ positive neurofibrillary tangles and neurites were seen in some aged control brains. Furthermore using a quantitative analysis we showed that truncated DPP10₇₈₉ fragments increased significantly in AD brains compared to control brains. These results provide the

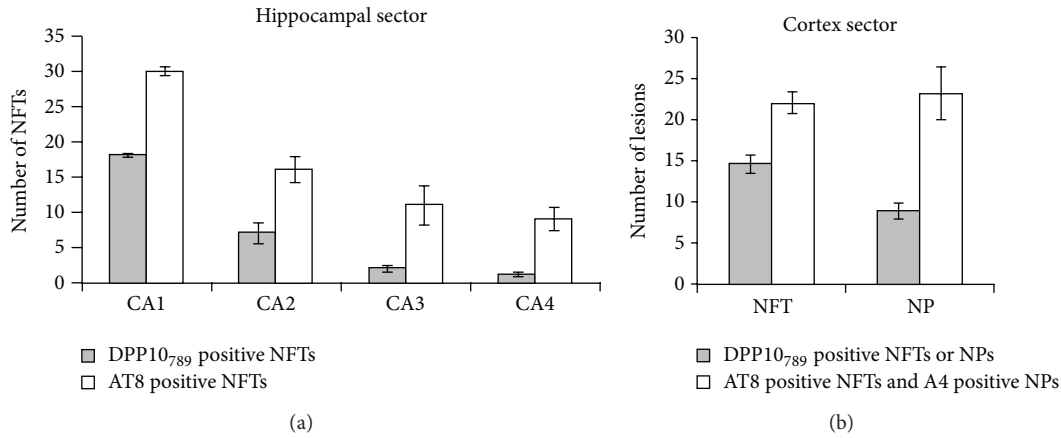


FIGURE 6: The regional distribution of DPP10₇₈₉ positive NFTs and NPs compared with phosphotau positive NFTs and amyloid- β positive plaques. The adjacent free-floating sections of hippocampus or cortex were immunostained with DPP10₇₈₉, AT8, or A4 antibody separately using the DAB reaction. (a) The distribution patterns of DPP10₇₈₉ positive NFTs and tau positive NFTs in AD hippocampal sector. Three fields (0.01 mm²/field) per case from each region of Ammon’s horn (CA1-4) were counted. (b) In the cortex sector of AD brain, the number of DPP10₇₈₉ positive NFTs and NPs was compared with tau positive NFTs and amyloid- β positive NPs. Three fields (0.01 mm²/field) per case were counted in each of layers III to V. Bars reflect mean \pm SE ($n = 9$; observations from 3 fields/case \times 3 cases).

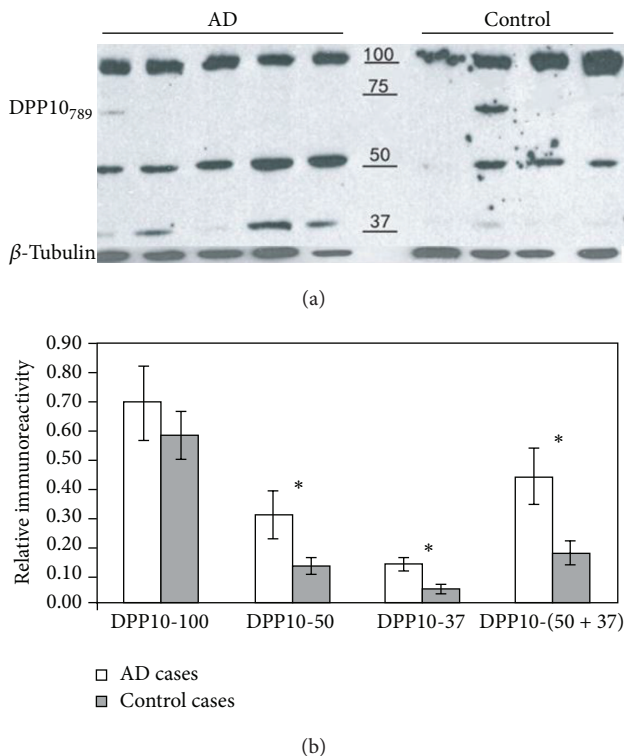


FIGURE 7: Truncated *N*-terminal DPP10₇₈₉ levels were elevated in AD hippocampus relative to control hippocampus. 50 μ g of solubilised fractions prepared from fresh-frozen AD and control hippocampus was subjected to immunoblot analysis with DPP10₇₈₉ antibody and class III β -tubulin antibody (a). Quantification of immunoblot data (b). The value of y -axis represents the relative immunoreactivity normalised to tubulin expression level. Bars represented mean \pm SE of duplicate determination from five AD and four control hippocampal homogenates. “*” represented one-tailed t -test $P \leq 0.05\%$.

first evidence that DPP10₇₈₉ may be involved in the pathology of AD and other neurodegenerative diseases.

Our immunohistochemistry studies revealed that in control brains DPP10₇₈₉ immunoreactivity is located in the cell body of neurons throughout the grey matter of cerebral cortex and axonal fibre tracts of white matter (Figures 2(a) and 3(a)), suggesting that DPP10₇₈₉ protein is widely distributed in the neuronal cells which is consistent with the expression of DPP10 mRNA reported in rat brain [28]. In human there are potentially four DPP10 mRNA isoforms which all have unique amino acid sequences in the *N*-terminal intracellular domain but identical C-terminal extracellular domains [31]. Multiple DPP10 isoforms have been observed by both Northern blot and reverse transcriptase PCR in both human brain and pancreas; however it is not known which transcript on the Northern blot represents DPP10₇₈₉ or whether any of these mRNA isoforms are translated into different forms of DPP10 protein *in vivo* [27, 31–33]. Takimoto et al. demonstrated the presence of four DPP10 mRNA isoforms in human brain. These isoforms are different only at the *N*-terminal amino acid sequence. When the different *N*-terminal forms of DPP10 were co-expressed with the Kv4.3 channel there were no differences in the gating ability of the channel [31]. In their study DPPYd is equivalent to DPP10₇₈₉ and this form was also detected in rat brain. Interestingly Takimoto et al. were unable to detect DPP10₇₉₆ in the adrenal gland and pancreas of humans or rats. In our laboratory we have found it extremely difficult to use reverse transcriptase PCR to amplify the other DPP10 isoforms (data not shown) from either postmortem brain tissue or neuronal cell lines; therefore these mRNA transcripts and thus protein isoforms appear to be present in very low amounts in human tissues.

Throughout the cortex of AD cases, although the background distribution pattern of DPP10₇₈₉ is similar to controls, it was the neurofibrillary tangles and plaque-associated

dystrophic neurites that, by virtue of their intense DPP10₇₈₉ staining, stand out compared to controls (some aged controls also had a few DPP10₇₈₉ tangles). Our data also revealed that the DPP10₇₈₉ positive tangles and plaque-associated dystrophic neurites are distributed mainly in layers III–V pyramidal cells of the neocortex and CA1 and CA2 pyramidal cells of the hippocampus in AD brains, consistent with the distribution of Kv4.2 and Kv4.3 subunits in brain [34]. DPP10 interacts with both Kv4.2 and Kv4.3 subunits [17, 35]. Additionally, Kv4 channels play important roles particularly in regulating neuronal membrane excitability; therefore these results indicate that deposition of DPP10₇₈₉ protein in these neurons may contribute to loss of channel function and lead to neuronal dysfunction and cell death in AD. Double immunofluorescence staining revealed that most of the DPP10₇₈₉ positive tangles were colocalized with the hyperphosphorylated phospho-Ser-202/Thr-205 tau epitope. In addition, the intrahippocampal distribution of DPP10₇₈₉ positive NFTs followed the pattern established for phospho-Ser-202/Thr-205 tau positive NFTs; however the amount of DPP10₇₈₉ positive NFTs was always less than that of phospho-Ser-202/Thr-205 tau (Figure 6(a)). This implies that, while AT8 can detect all six isoforms of human tau protein in brain [36], DPP10₇₈₉ may only be present in tangles containing a subset of the six isoforms. On the other hand, in the entorhinal cortex region, some DPP10₇₈₉ positive tangles were devoid of either AT8 (Figure 5(h)) or Tau2 (data not shown) antibody staining, indicating that DPP10₇₈₉ did not always cooccur with tau in tangles and it may on its own be involved with tangle formation without tau involvement. It is also possible that our DPP10₇₈₉ antibody can detect the late stage of tangles—the ghost tangles which have lost the phospho-Ser-202/Thr-205 tau epitope recognised by the AT8 antibody [37, 38]. Recently Lace et al. reported that the RD3 antibody that specifically recognises the 3-repeat tau shows an increased immunoreactivity in late-state ghost tangles [39]. Do RD3 positive tangles colocalize with DPP10₇₈₉ positive staining? Which tau isoform may be involved in DPP10₇₈₉ positive tangle formation? To answer these questions, further study needs to be done by using different tau antibodies specific to different tau isoforms.

Tau is a microtubule-associated protein and predominantly found in axons [40–42]. The best known functions of tau are the stabilization of microtubules and the regulation of axonal transport [43, 44]. Recently it was reported that tau has also been localized to dendrites under physiological conditions and at much lower levels [45]. In addition to its interaction with tubulin, tau binds to other partners such as the tyrosine protein kinase Fyn, dynactin, and postsynaptic density protein 95 (PSD95) [45–47]. Both tau-Fyn and tau-PSD95 interactions are involved in the complex formation of NMDA receptors with PSD95, which is required for excitotoxic downstream signalling, suggesting that the distribution of tau in dendrites is pivotal to healthy neurons [45, 47]. Noticeably, the intracellular distributions of Kv4 channels are also predominant in dendrites [34]. Besides, it has been reported that PSD95 has physical interactions with Kv4.2 [48, 49], suggesting that PSD95 may be involved in forming complexes between Kv4.2 and signalling molecules. Because

the dendritic A-type potassium current strongly influences the induction of long-term potentiation also by NMDA receptors [50, 51], it is possible that there exist some signalling connections between tau, Fyn, PSD95, and Kv4. As a Kv4 channel associated protein, DPP10 also has the potential to be involved in these connections. However, so far there is no direct evidence showing that tau interacts with Kv4, and one possible explanation is that these protein interactions may be transient or unstable; thus it is difficult to detect the interactions using immunoprecipitation, immunostaining techniques, or other proteomic based methods.

DPP10₇₈₉ positive immunoreactivity was also observed in dystrophic neurites in A β aggregated senile plaques. It was found that DPP10₇₈₉ positive dystrophic neurites did not colocalize but mingled with the A β deposition core of the senile plaques. This is consistent with the fact that plaques are composed of extracellular aggregated A β , while DPP10₇₈₉, according to our studies, is distributed intracellularly or in the membrane [26, 27]. In addition, we have observed that the number of DPP10₇₈₉ positive neurofibrillary tangles and plaque-associated dystrophic neurites present in AD is much more than that observed in other tauopathies (Table 3). As the accumulation of A β is a primary event driving AD pathogenesis, our finding indicates that DPP10₇₈₉ may be involved some way in the pathogenic process. So far, the mechanisms by which A β mediates neurotoxicity and initiates the degenerative processes of AD are still not clear. Recent studies revealed that A β inhibits the dendritic A-type K⁺ current in outside-out patches excised from distal dendrites of hippocampal CA1 pyramidal neurons, causing increases in back-propagating dendritic action-potential amplitude and associated Ca²⁺ influx. These results suggest that the sustained increased dendritic Ca²⁺ influx, which results from A β deposition in dendritic arborisation that induces persistent inhibition of the A-type K⁺ current, may cause a loss of Ca²⁺ homeostasis in dendrites of hippocampal neurons, an initiating event of synaptic failure and neurodegeneration [52]. Whether DPP10₇₈₉ is involved in this procedure needs further study.

The quantitative immunoblot on human brain samples showed that naturally *in vivo* two truncated forms of DPP10₇₈₉ are observed and the levels of these increase in AD brains compared to control brains, which has led us to hypothesise that the truncated forms may form aggregates in NFTs and dystrophic neurites. As there was no change in the full-length form of DPP10₇₈₉ between control and AD patients, it is assumed that if there is any increase in protein production in AD, the newly formed protein is likely to be rapidly degraded. DPP10₇₈₉ is a glycosylated transmembrane protein with a large extracellular C-terminal domain and a short intracellular N-terminal domain. The presence of DPP10₇₈₉ in NFTs and dystrophic neurites but not amyloid protein aggregated plaques suggests that DPP10₇₈₉ is confined to the neuronal cytoplasm. These observations suggest the possible aberrant trafficking of DPP10₇₈₉ in degenerating neurons.

DPP10 belongs to DPP4 gene family, some members of which have been reported to have multiple functions. Previous *in vitro* studies showed that DPP4 and FAP have roles in the cell-extracellular matrix interactions and apoptosis, and

these functions are independent of catalytic activity [53]. It is likely that most protein-protein interactions would occur on the β -propeller domains of these proteins [54]. With 47% amino acid similarity to DPP4, it is possible that DPP10, apart from associating with Kv4 channel complex through its single transmembrane domain [55], may also interact with other proteins and ligands via either its extracellular β -propeller domain or its intracellular domain and these interactions may control its biological function in normal and pathological conditions. Recently Lin et al. elegantly demonstrated that the highly related DPP6 interacts with filopodia-associated myosin and fibronectin within the extracellular matrix to play a role in hippocampal synaptic development [56]. In addition, DPP6 has been shown to interact with prion protein to mediate potassium channels and neuronal excitability [57].

In summary this paper reports the first evidence that DPP10₇₈₉ protein is present in NFTs and dystrophic neurites in AD. Interestingly, DPP10₇₈₉ protein colocalizes with hyperphosphorylated tau protein but not with A β . Moreover in neurodegenerative cases other than AD where neuronal tau pathology occurred, the same regions showed intense staining for DPP10₇₈₉. In addition this paper provides initial evidence that, as with other proteins involved in neurodegenerative diseases, DPP10₇₈₉ C-terminal truncation may lead to mistrafficking and aggregation of DPP10₇₈₉ protein but at this point it is unclear whether this occurrence contributes to AD pathology or is a result of AD pathology. Together this data suggests that DPP10 may play a significant role in the neurodegenerative process observed in AD and further investigation into this role and its interaction with tau is warranted.

Abbreviations

AD:	Alzheimer's disease
A β :	Amyloid- β peptide
DAB:	3-3-Diaminobenzidine-4 HCl/H ₂ O ₂
DLBD:	Diffuse Lewy's body disease
DP:	Dipeptidyl peptidase
FTLD:	Frontotemporal lobar degeneration
GFP:	Green fluorescent protein
Kv channel:	Voltage-gated potassium channel
KChIP:	Kv channel interacting protein
NFTs:	Neurofibrillary tangles
NPs:	Neuritic plaques
PSP:	Progressive supranuclear palsy.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Wei-Ping Gai and Catherine A. Abbott contributed equally to the paper. Tong Chen performed all experiments, data collection and analysis, and manuscript writing. Wei-Ping Gai and Catherine A. Abbott conceived the study, data interpretation, and manuscript writing.

Acknowledgments

Dr. Wei-Ping Gai holds an Australian National Health and Medical Research Council (NHMRC) Fellowship, and this work was supported by the NHMRC Brain Bank of South Australia and Flinders Medical Foundation. Dr. Tong Chen has been supported by an Australian Postgraduate Award and more recently by an Australian Alzheimer's Research Dementia Fellowship. The authors would like to acknowledge the technical assistance of Xiao-Feng Shen and Fariba Chegini with the immunostaining, the help of Kylie Lange with the statistical analysis, and the helpful paper comments of Associate Professor Mark Gorrell and Professor Zhuo-Hua Zhang. This work was supported by the NHMRC Brain Bank of South Australia managed by Robyn Flook and Flinders Medical Foundation.

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