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Microsomal Prostaglandin E Synthase-2: Cellular Distribution and Expression in Alzheimer's Disease

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Abstract

Nonsteroidal anti-inflammatory drugs, such as cyclooxygenase (COX)-2 inhibitors, have been unsuccessful in slowing or reversing Alzheimer's disease (AD). Thus, understanding the expression patterns of the downstream effectors for the regulation of prostaglandin synthesis may be important for understanding the pathological processes involved in AD and formulating more effective pharmacotherapeutics for this disease. In this study, we used immunofluorescence, immunohistochemistry, and Western blot analysis to compare patterns of microsomal prostaglandin E synthase (mPGES)-2 expression in the middle frontal gyrus (MFG) of AD patients and age-matched controls. In control human brain sections, mPGES-2 immunoreactivity was observed in neurons, activated microglia, and endothelium, but not in resting microglia, astrocytes, or smooth muscle cells. Microsomal PGES-2 immunoreactivity was particularly elevated in the pyramidal neurons of brains from three of five sporadic and four of five familial AD patients compared with four of five age-matched control brains that showed minimal immunoreactivity. In contrast, Western blot analysis revealed no difference in mPGES-2 levels between end-stage AD brain tissue and control brain tissue. These results suggest that in human cortex, mPGES-2 is constitutive in neurons and endothelium and induced in activated microglia. Furthermore, the high immunoreactivity of mPGES-2 in pyramidal neurons of AD brains indicates that it might have a potential role in the functional replacement of cytosolic PGES or inactive mPGES-1 in later stages of AD.

Keywords

amyloid β -peptide; COX; eicosanoid; neuroinflammation; NSAIDs; prostaglandin E₂

Introduction

Nonsteroidal anti-inflammatory drugs have been shown to reduce the risk of developing Alzheimer's disease (AD) (Breitner et al., 1995; McGeer et al., 1996; Stewart et al., 1997; in t' Veld et al., 2001; Szekely et al., 2004). Nevertheless, clinical trials have shown

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cyclooxygenase (COX)-2 inhibitors to be ineffective at alleviating AD symptoms (Pasinetti, 2002; Aisen et al., 2003; Reines et al., 2004), making their use in AD controversial. However, nonselective COX inhibitors are showing some promise (McGeer and McGeer, 2007). Such findings imply that the problem may lie more in COX-1 and certain coupled prostaglandin (PG) synthases than in COX-2. COX-1 and COX-2 catalyze the rate-limiting steps for the production of prostaglandins (PGE₂, PGD₂, PGF_{2α}, and PGI₂) and thromboxane A₂ (TXA₂) from arachidonic acid. COX-2 inhibitors may be beneficial in preventing β-amyloid (Aβ) deposition (Xiang et al., 2002), and they decrease PGE₂ levels, thus reducing the activity of the neurotoxic PGE₂ receptors EP1 and EP3 (Lim et al., 2000; Carlson, 2003; Ahmad et al., 2008; Saleem et al., 2009). However, reducing PGE₂ levels also reduces the activity of the neuroprotective EP2 and EP4 receptors (Echeverria et al., 2005; Doré, 2006). Interestingly, PGE₂ levels have been found to correlate positively with patient survival and decrease in advanced stages of AD, indicating that suppression of PGE₂ may not always be medically optimal (Combrinck et al., 2006). Therefore, because COX inhibitors can have mixed effects in AD, identification of the respective contribution of the various terminal PG synthases might promote the development of more selective therapeutic targets to reverse or slow the progression of AD.

PGE₂ stimulation of the EP2 receptor has been found to provide neuroprotection in rat neuronal cultures during Aβ₁₋₄₂-induced neurotoxicity (Echeverria et al., 2005) and in a mouse model of excitotoxicity and cerebral ischemia (McCullough et al., 2004; Ahmad et al., 2006). In contrast, a study that used EP2 knockout APP_{Swe}-PS1ΔE9 mice suggested that EP2 receptors are proamyloidogenic and proinflammatory (Liang et al., 2005). Furthermore, Takadera et al. (2006) reported that in the presence of N-methyl-D-aspartate (NMDA), PGE₂-stimulated EP2 receptor activation becomes neurotoxic in rats, whereas others showed PGE₂ to be neuroprotective in the presence of NMDA (McCullough et al., 2004; Ahmad et al., 2005; Liu et al., 2005). Our previous studies suggest that constitutive PGE₂ synthase (cPGES) is markedly diminished in end-stage AD, while microsomal (or membrane-bound) PGES (mPGES)-1 is elevated, most likely as a compensation mechanism (Chaudhry et al., 2008; Chaudhry and Doré, 2009). The evidence to date suggests that constitutive levels of PGE₂ in end-stage AD may be improperly regulated despite upregulation of mPGES-1 and mPGES-2. It is likely that the effect of PGE₂ depends on its concentration and to which receptor it binds.

Microsomal PGES-2 is derived from the PTGES2 gene on chromosome 9, according to the Human Genome Organization (HUGO Gene Nomenclature Committee at the European Bioinformatics Institute). Constitutively expressed mPGES-2 mRNA is most abundant in the brain, heart, kidney, and small intestine (Yang et al., 2006). According to the Allen Brain Atlas, mPGES-2 in normal brain is distributed primarily in the cortex, hippocampus, hippocampal formation, retrohippocampal region, and olfactory areas, indicating some involvement in learning and memory. Of all PGES, mPGES-2 is the least dense and has the lowest levels. It has maximal cortical levels of 22%, compared to 64% for cPGES and 100% for mPGES-1 (see Table 1 for regional distribution of mPGES-2 in mouse brain). It is synthesized as a Golgi membrane-associated protein and becomes a mature cytosolic enzyme after proteolytic removal of the hydrophobic N-terminal domain (Tanikawa et al., 2002). It forms a dimer that attaches to lipid membranes via the N-terminal section (Yamada et al., 2005). Unlike mPGES-1, mPGES-2 has been found to be glutathione (GSH)-independent and is activated by a wide variety of sulfhydryl-reducing reagents, such as GSH, dithiothreitol, β-mercaptoethanol, and dihydrolipoic acid (Jakobsson et al., 1999). The sulfhydryl group of Cys110 in the glutaredoxin/thioredoxin-like domain is most likely its catalytic site (Watanabe et al., 2003). Previous studies have shown that mPGES-2 is generally not elevated in inflammation, except, for example, in human colorectal cancer (Murakami et al., 2003).

Studies regarding mPGES-2 have only recently begun, and none has yet elucidated the localization and expression patterns of mPGES-2 in normal human brain or in AD. The purpose of this study was to characterize the cellular location of mPGES-2 in human middle frontal gyrus (MFG) by immunofluorescence and to use immunohistochemistry and Western blot analysis to determine the expression patterns of mPGES-2 in the brains of patients with AD.

Materials and methods

Specimen Processing

Tissue from the brains of nine control, 10 sporadic AD, and five familial AD cadavers was obtained from the Johns Hopkins Brain Resource Center within 4.0 to 19.0 h of a patient's death (Table 1). Informed consent was obtained from the patient before death. At autopsy, the brain was removed, weighed, and examined for lesions. Both halves of the brain were cut coronally. Sections from the right half were frozen in methylbutane; snap-frozen sections were fixed at 4°C in buffered paraformaldehyde, cryoprotected, and frozen at -70°C. Sections from the left half of the brain were fixed in buffered formalin for 2 weeks and embedded in paraffin.

Neuropathologic Evaluation and Criteria for Disease Stage

Control brain tissue was taken from normal, healthy human cadavers. Definite or probable cases of AD were established via the Consortium to Establish a Registry of Alzheimer's Disease (CERAD) criteria (Mirra et al., 1991). Braak scores (which measure neurofibrillary tangle progression) of greater than II of VI with CERAD C were considered as definite AD (Braak and Braak, 1991). All other cases of CERAD 0 and any Braak score were considered to be controls. Immunostaining for A β and tau and the modified Bielschowski silver-staining technique confirmed diagnosis. Case details for the groups are summarized in Table 2. All AD patients included in the study had Braak scores of V or VI; therefore, we examined the MFG because it is known to be highly affected in these latter stages of AD.

Immunohistochemistry

Paraffin sections of the MFG were cut into 10- μ m sections with a rotary microtome and mounted onto Superfrost/Plus microscope slides (Fisher, Pittsburgh, PA). The samples were deparaffinized at 60°C for 30 min and then hydrated by immersion in four changes of xylene (Fisher), three changes of 100% ethanol, three changes of 95% ethanol, and a final rinse in distilled H₂O. For antigen retrieval, 1 M citric acid (pH 6) was placed in a microwave oven on high for 6 min and added to the slides. The samples were then placed in a microwave oven for 6 more min before being cooled in distilled H₂O. Liquid Blocker was applied with a Super Pap Pen (Daido Sangyo Company, Tokyo, Japan) to create a waterproof barrier that prevented applied reagents from leaking. Sections were then washed with Tris-buffered saline (TBS, pH 7.3) and exposed to 0.3% H₂O₂ (Sigma, St. Louis, MO) and 1% methanol in TBS for 30 min. To reduce non-specific antigen binding, sections were incubated in 10% normal goat serum (NGS; Cayman Chemical, Ann Arbor, MI) and 3% Triton X-100 in TBS for 4 h at room temperature. Sections were then incubated in rabbit anti-mPGES-2 (1:7000, Cayman Chemical) overnight at 4°C and subsequently in secondary antibody (biotinylated goat anti-rabbit IgG, Vector Laboratories, Burlingame, CA) for 1 h. Corresponding negative control sections were incubated with 2% NGS in TBS without primary antibody. Next, sections were incubated in Avidin D-horseradish peroxidase (HRP, ABC Elite; Vector Laboratories) for 1 h, followed by incubation in diaminobenzadine tetrahydrochloride (DAB, Vector Laboratories) for 5 min. To stain non-specific nuclei, sections were immersed in distilled water for 5 min, counterstained with Mayer's hematoxylin for 20 min, and washed in running tap water for 15 min. The slides were then dehydrated in three 3-min rinses of 95% ethanol, 100% ethanol, and xylene. Cover slips were adhered with Permount mounting medium (Fisher). Sections were imaged and photographed with a Nikon Eclipse TE-2000-E motorized focus microscope

(Nikon, Nanjing, China), Nikon digital camera DXM 1200F (Nikon, Japan), and the software program Nikon ACT-1 Version 2.62 (Surrey, UK). Background staining was removed via Brilliantphoto Version 1.2.0.87 (Tioga, FL) software to analyze results because specimens were not stained simultaneously.

Double immunofluorescence

Frozen sections of MFG from two patients (C2 and C5) were cut into 10- μ m sections with a cryostat, mounted on Superfrost/Plus microscope slides, and stained in separate batches. Sections were fixed in 4% paraformaldehyde (Sigma) for 30 min and incubated in 10% NGS for 4 h at room temperature. Sections were then incubated overnight at 4°C with rabbit polyclonal antibody against mPGES-2 (mPGES-2, 1:7000; Cayman Chemical) and an antibody against one of each of the following cell markers: rat anti-glial fibrillary acid protein (GFAP, 1:500; Zymed, San Francisco, CA) for astrocytes; rat anti-CD11b (1:1000; Serotec, Kidlington, Oxford, UK) for microglia; mouse anti-nonphosphorylated neurofilament (SMI-32, 1:1000; Sternberger Monoclonals Lutherville, MD) for neurons; mouse anti-smooth muscle actin (SMC, 1:200; Zymed) for smooth muscle cells; and mouse anti-von Willebrand factor (vWB, 1:200; Vector Laboratories) for endothelial cells. Negative control sections were incubated in 2% NGS in TBS. All sections were then incubated at room temperature for 4 h in the dark in corresponding fluorescent markers: red Cy3-conjugated Affinipure goat anti-mouse IgG (0.5 mg, 1:200; Jackson IR Laboratories) or goat anti-rat IgG (0.5 mg, 1:200; Jackson IR Laboratories) (respective to the host of the corresponding primary antibody) and green Alexa Fluor 488-conjugated goat anti-rabbit IgG (2 mg/mL, 1:500; Molecular Probes). A set of control and AD sections was subsequently double stained with mouse monoclonal antibodies against neuronal nuclear marker (neu-N, 1:500; Chemicon, Temecula, CA) and rabbit polyclonal antibodies against mPGES-2 (1:7000, Cayman) and immersed in phosphate-buffered saline (PBS) for 5 min, 70% ethanol for 5 min, Autofluorescence Eliminator Reagent (Chemicon) for 5 min, and finally three 1-min changes of 70% ethanol. Cover slips were applied to sections with Vectashield dry mount media hard set (Vector Laboratories). Sections were stored at 4°C away from light. Stained sections were viewed in the dark with a Nikon microscope (as described above), SPOT RT monochrome camera (Diagnostic Instruments, Inc. Sterling Heights, MI), and Metavue imaging software (Molecular Devices, Sunnyvale, CA).

Western blot analysis

Western blot was used to analyze the expression of mPGES-2 in the MFG of control (n = 9) and sporadic AD (n = 10) brains. Tissue blocks were melted and sonicated in lysis buffer (Cell Signaling Technology, Beverly, MA) that contained complete protease inhibitor (Roche, Indianapolis, IN), 1 nM dimethyl sulfoxide (Sigma), and 10 mM sodium fluoride (Sigma). The BCA assay (Pierce, Rockford, IL) was used to quantify proteins, which were subsequently subjected to polyacrylamide gel electrophoresis on 12% gels (Invitrogen, Carlsbad, CA). The resolved proteins were transferred to nitrocellulose membranes (BioRad, Hercules, CA), which were stained with Ponceau S Solution (Sigma) to verify that equal amounts of protein were loaded in each lane. The membranes were then blocked with 5% skim milk in PBS with 0.1% Tween 20 overnight at 4°C. Blots were washed and incubated with rabbit polyclonal antibodies against mPGES-2 (1:70,000; Cayman) or actin (1:500; Sigma) and goat anti-rabbit secondary antibody for 1 h each at room temperature. Blots were developed with enhanced chemiluminescence (ECL; Amersham Biosciences, Piscataway, NJ). The immunoreactivity of mPGES-2 and actin was analyzed by densitometry and the ratio of mPGES-2/actin calculated. Comparisons between the control and AD groups were made by the Student's t test, and the statistical significance was set at $p < 0.05$.

Results

Microsomal PGES-2 colocalizes to activated microglia, neurons, and endothelial cells in control brain tissue

In control brain tissue, mPGES-2 colocalized with microglia in activated (amoeboid-shaped) cells (Fig. 1A – C); resting (ramified-shaped) microglia were not stained by mPGES-2 (data not shown). Microsomal PGES-2 also colocalized with neurons (Fig. 1D – F), and endothelial cells (Fig. 1G – I), but not with astrocytes (Fig. 1J – L) or smooth muscle cells (Fig. 1M – O).

Immunohistochemical analysis reveals that mPGES-2 is immunopositive in pyramidal neurons in AD

In four of five control patients, staining for mPGES-2 in pyramidal neurons was not evident (Fig. 2A). However, pyramidal neurons from three of five sporadic and four of five familial AD patients were positively stained for mPGES-2 (Fig. 2C, E). As expected, in the absence of the primary antibody, tissues were negatively stained (Fig. 2B, D, F). Staining was generally diffuse and negligible throughout the brain parenchyma in mPGES-2 immunostained controls (Fig. 2G); however, in AD tissue, dark immunostaining was apparent in two neuronal layers in the grey matter of the MFG (Fig. 2H). Staining of the glia, subcortical white matter, and axons was absent in both control and AD tissues. One control brain sample of a 91-year-old woman showed darkly stained neurons, similar to those of AD, but the neurons were diffusely scattered as in the other control samples.

mPGES-2 protein expression in control and AD cases

Western blot analysis of mPGES-2 protein expression in the MFG of control (n = 9) and sporadic AD (n = 10) brains (Fig. 3) revealed no significant differences.

Discussion

Previous investigators have studied mPGES-2 in animal brain tissue (Murakami et al., 2003; Bosetti et al., 2004; Sang et al., 2005; Schroder et al., 2006; Zhang et al., 2006), but to our knowledge, we are the first to examine mPGES-2 localization in human control and AD brains. Similar descriptive immunohistochemical studies have been conducted on COX isoforms (Yermakova and O'Banion, 2001; McGeer and McGeer, 2007), and we have patterned our study to complement them. Using immunofluorescence, we observed constitutive cytoplasmic expression of mPGES-2 in neurons, activated microglia, and endothelium in normal human MFG, but not in resting microglia, astrocytes, or smooth muscle cells. Also, mPGES-2 immunohistochemical staining was stronger in pyramidal neurons in AD than in control brain tissue, which suggests a potential upregulation of mPGES-2 in end-stage AD. We were able to see such a pattern mainly in pyramidal neurons. Western blot analysis of MFG homogenate revealed no overall difference in mPGES-2 expression between control and AD samples; it is possible that Western blot might not be sensitive enough to reveal slight increases in expression within restricted brain sub-regions. Although using immunohistochemical approaches with post-mortem tissue has intrinsic limitations, similar studies in human brain tissue have revealed increases in neuronal levels of mPGES-1 and COX-2 (Hoozemans et al., 2001; Chaudhry et al., 2008).

Glia and mPGES-2

Whereas Ikeda-Matsuo et al. (2005) found mPGES-2 to be constitutively expressed in microglia, others have reported that mPGES-2, but not mPGES-1, can be induced and upregulated in microglia (De Simone et al., 2003; Zhang et al., 2006), results that support our current findings. Furthermore, COX-1, which has been suggested to be more functionally

coupled to mPGES-2 than to mPGES-1 in rat microglial cell cultures (Zhang et al., 2006), was more immunoreactive in microglia of human AD tissue (Hoozemans et al., 2001) and in inflamed human post-injury brain tissue than in control tissue (Schwab et al., 2000). Although some have questioned the presence of COX isoforms in human astrocytes (Hoozemans et al., 2001), others have shown human astrocytes to contain COX-1 and COX-2 mRNA (Hull et al., 2006). Another study showed that COX-2 is induced in primary human midbrain astrocytes via β -amyloid peptides and causes PGE₂ release (Hull et al., 2006), indicating that PGES may be present in astrocytes. Here, immunoreactive mPGES-2 was absent in control and AD brain astrocytes.

Cerebral endothelial cells, smooth muscle cells, and mPGES-2

Investigators have shown through Western blotting that COX-1 and COX-2 are expressed constitutively but at variable levels in human neonatal cerebral endothelial cells (Parfenova et al., 2002). Here, we did not detect significant mPGES-2 immunostaining in endothelium in AD specimens. Interestingly, studies have shown that β -amyloid dose-dependently enhances COX-2-dependent-PGE₂ production in rat brain-derived smooth muscle cell/pericyte cultures but not in brain endothelial cells (Paris et al., 2002). In contrast, a study of post-brain-injury inflammation showed positive immunostaining of COX-1 in adult human brain endothelium and smooth muscle for several months after the initial insult (Schwab et al., 2002), indicating that COX-1-coupled mPGES-2 or cPGES may also be induced or made active in this process; however, we did not observe obvious immunostaining for mPGES-2, mPGES-1, or cPGES in smooth muscle cells (data not shown).

Pyramidal neurons and mPGES-2

Microsomal PGES-2 has been found to be coupled to COX-2 and/or COX-1 in pyramidal neurons in AD (Murakami et al., 2003). As shown here, mPGES-2 appears to be located cytoplasmically, perinuclearly, and in dendritic processes, particularly in pyramidal neurons, as is COX-2, and both are localized in the cerebral cortex and hippocampus (Kaufmann et al., 1996; Tai et al., 1998; Tanikawa et al., 2002; Kim and Jang, 2006). These findings are consistent with those of other investigators, who have reported mPGES-1, mPGES-2, and COX-2 to be present in rat dendritic spines (Sang et al., 2005) and have shown that mPGES-2 and COX-2 were elevated in apoptotic-like subsets of rat pyramidal neuron layers after kainic acid-induced seizures (Tocco et al., 1997). Furthermore, mPGES-2 has been shown to be diffusely expressed in pyramidal neurons and elevated slightly in brains from AD patients (Yermakova et al., 1999). Conflicting data have shown COX-2 to be either upregulated (Hoozemans et al., 2001) or downregulated (Yermakova and O'Banion, 2001) in end-stage AD. Interestingly, higher PGE₂ levels are found during early stages of AD, whereas lower levels are present in end-stage AD, when neurons are dying (Combrinck et al., 2006). Thus, an ideal range and/or tight regulation of PGE₂ may be necessary for viability.

Together, our data suggest that mPGES-2 is localized in activated microglia, neurons, and endothelial cells of normal MFG tissue and that it is elevated in neurons of the pyramidal layers in the MFG of AD patients. These observations provide a framework for further investigation of mPGES-2 in normal brain and in neuroinflammatory states.

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Fig. 1.

Double immunofluorescent labeling of mPGES-2 and markers for various cell types. MFG sections from normal brain tissue were double labeled with antibodies against mPGES-2 (green) and cell markers (red); merged signals appear yellow. mPGES-2 colocalized with CD11b in activated (amoeboid-shaped) microglia (A–C), with SMI-32 in neurons (D–F), and with vWB in endothelial cells (G–I). No clear colocalization was observed with GFAP in astrocytes (J–L) or with SMC in smooth muscle cells (M–O). Green scale bar (A–L) = 50 μ m; White scale bar (M–O) = 25 μ m.

Fig. 2.

Patterns of mPGES-2 immunohistochemical labeling of neuronal cells in MFG from patients with AD and age-matched controls. Brain slices were immunostained with antibody against mPGES-2, reacted with DAB, and counterstained with Mayer's hematoxylin. (A) Control tissue shows negligible staining for mPGES-2. (B) Unstained control is shown for comparison. (C) Sporadic AD tissue reveals intense mPGES-2 immunolabeling of pyramidal neurons, including apical dendrites and cell bodies. No other cells or white matter are visibly labeled. (D) Unstained sporadic AD tissue is shown for comparison. (E) Familial AD tissue shows intense mPGES-2 immunolabeling. (F) Unstained familial AD tissue is shown for comparison. (G) Age-matched control tissue slice at 4x magnification, with inset at 10x, immunolabeled

with anti-mPGES-2 shows negligible neuronal staining. (H) Familial AD tissue slice at a magnification of 4x, with inset at 10x, immunolabeled with anti-mPGES-2 shows intense neuronal staining of pyramidal neurons in two neocortical layers (arrows). Such staining was evident in both sporadic and familial AD cases. Scale bars: A–H: 100 μm ; insets of G and H: 500 μm .

Fig. 3. Protein expression of mPGES-2 and actin in control and sporadic AD brain tissue. Case numbers are indicated above each lane; see Table 1 for patient demographics. The graph depicts the densitometric analysis of the ratio of mPGES-2/actin immunoreactivity. Values shown are means $\pm$ standard deviation; ns, not significant.

Table 1
Percent Regional Expression Levels and Density Values for PTGES2 (Allen Brain Atlas)

Area	CB	CTX	HIP	HPF	HY	LSX	MB	MY	OLF	P	PAL	sAMY	STR	STRd	STRv	TH	RHP
Level (%)	9	22	17	19	1	1	2	2	16	4	3	4	2	1	2	4	21
Densit (%)	6	26	15	17	1	1	1	1	15	2	3	5	2	2	2	4	20

CB = cerebellum, CTX = cerebral cortex, HIP = hippocampus, HPF = hippocampal formation, HY = hypothalamus, LSX = lateral septal complex, MB = midbrain, MY = medulla, OLF = olfactory areas, P = pons, PAL = pallidum, sAMY = striatum-like amygdalar nuclei, STR = striatum, STRd = striatum dorsal region, STRv = striatum ventral region, TH = thalamus, RHP = retrohippocampal region.

TABLE 2

Description of the Subjects

Diagnosis	Case ID	Age (years)	Sex (M/F)	PMT (hours)	CERAD *	Braak stage
C	C1	74	M	4.0	Negative	II
C	C2	71	F	16.0	Negative	0
C	C3	87	M	8.0	Negative	II
C	C4	80	F	8.0	Negative	0
C	C5	68	M	10.0	Negative	II
C	C6	94	M	16.0	Negative	III
C	C7	80	M	22.0	Negative	IV
C	C8	nd	nd	Nd	Negative	nd
C	C9	91	F	8.0	Negative	I
SAD	AD1	79	M	10.5	C	VI
SAD	AD2	82	F	6.0	C	VI
SAD	AD3	85	M	3.5	C	VI
SAD	AD4	89	M	9.5	C	VI
SAD	AD5	80	F	12.0	C	V
SAD	AD6	72	F	10.0	C	VI
SAD	AD7	54	F	14.5	C	VI
SAD	AD8	62	F	11.0	C	VI
SAD	AD9	84	F	5.0	C	V
SAD	AD10	72	F	19.0	C	VI
FAD	AD11	63	M	9.0	C	VI
FAD	AD12	74	F	5.5	C	VI
FAD	AD13	78	M	4.0	C	VI
FAD	AD14	92	F	8.0	C	VI
FAD	AD15	94	F	4.0	C	VI

* CERAD = Consortium to Establish a Registry for Alzheimer Disease. Scoring is as follows: control, CERAD negative and Braak 0-IV; (Senile dementia, CERAD negative and Braak IV); probable AD, CERAD B and Braak IV-V; definite, CERAD C and Braak V-VI. M, male; F, female; PMT, post-mortem time; C, Control; SAD, Sporadic AD; FAD, Familial AD; nd, not determined.