Published in final edited form as:

J Neuroimmunol. 2008 March ; 195(1-2): 81–87.

Expression and Localization of Prostaglandin Transporter in Alzheimer Disease Brains and Age-Matched Controls

Koyi Choi¹, Hean Zhuang, MD¹, Barbara Crain, MD PhD², and Sylvain Doré, PhD¹

¹Anesthesiology/Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland

²Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland

Abstract

Neuroinflammation, a major contributor to neurodegenerative diseases, involves the contribution of activated microglia, reactive astrocytes, and infiltrating inflammatory cells. Stress and various acute or chronic brain injuries stimulate the generation of free radicals and glutamate, triggering inflammatory pathways that lead to increases in chemokines, cytokines, and prostaglandins. Prostaglandins are lipid mediators of inflammation that are produced from arachidonic acid by cyclooxygenase enzymes. They are generally believed to be in all tissues and organs. Their transport through the lipid bilayers of the cell membranes/organelles is facilitated by the prostaglandin transporter (PGT). In this study, middle frontal gyrus brain tissue from patients diagnosed with Alzheimer disease (AD) and that of age-matched control brains were examined to determine the protein expression pattern of PGT and its possible role in modulating neuroinflammation associated with AD. Immunohistochemical and immunofluorescent studies showed that PGT protein was expressed in all the brain tissues examined and was localized in neurons, microglia, and astrocytes. Interestingly, Western blot analysis revealed that the PGT level was significantly less in AD than in age-matched control brain homogenates. Further work is warranted to address the possibility and implications that prostaglandins might not be cleared at a proper rate in AD brains.

Keywords

central nervous system; cyclooxygenases; neuroinflammation; PGE₂

1. Introduction

Alzheimer disease (AD), one of the most prominent neurodegenerative diseases, is characterized by progressive mental decline, including memory loss, behavioral abnormalities, and language impairment. The neuritic plaques and neurofibrillary tangles associated with AD, strongly imply a link with neuroinflammation (Halliday et al., 2000; McGeer and McGeer, 2003; Tuppo and Arias, 2005). When an area of the brain is injured, a high level of glutamate is generated that initiates the inflammatory pathway by activating microglia, astrocytes, and infiltrating leukocytes (Engelhardt et al., 1989; Engelhardt et al., 1993; Kawamata et al., 1992; Lampson et al., 1990; Schiffer et al., 1996; Troost et al., 1990). The inflammatory mediators produced include cytokines, cyclooxygenase (COX) 1 and 2, and prostaglandins. Thus, it has been reported that COX activity and prostaglandin levels are higher in the brains

of AD patients than in control brains (Consilvio et al., 2004; Donnelly and Hawkey, 1997; Montine et al., 1999a; Montine et al., 1999b; Pasinetti, 1998).

Prostaglandins are carried across the cell membrane by the prostaglandin transporter (PGT). PGT has been identified and shown to have a role in the release of newly synthesized prostaglandins from cells, the transepithelial transport of prostaglandins, and the clearance of prostaglandins from the circulation for the termination of signaling (Kanai et al., 1995). PGT protein is expressed by prostanoid-releasing cells (Bao et al., 2002), and Northern blot analysis has shown that PGT mRNA is expressed in amygdala, caudate nucleus, corpus callosum, hippocampus, substantia nigra, subthalamic nucleus, and thalamus (Lu et al., 1996). A recent study also reported that the highest expression was in the cortex, followed by cerebellum and hippocampus, with the lowest expression in the brainstem/diencephalon of mouse brain during development (Scafidi et al., 2007).

PGT has been studied extensively in peripheral organs (Arosh et al., 2004; Banu et al., 2003; Chan et al., 1998; Endo et al., 2002; Kashiwagi et al., 2002; Pucci et al., 2004; Reid et al., 2003; Topper et al., 1998), but to our knowledge, no one has investigated the expression pattern or function of PGT in the human brain. Moreover, the correlation between prostaglandins and neuroinflammation usually has been approached by the investigation of prostaglandins and prostaglandin receptors rather than PGT (Ahmad et al., 2005; Ahmad et al., 2006a; Ahmad et al., 2006b; Echeverria et al., 2005). Because prostaglandins are major components of the neuroinflammatory process, studying the expression pattern of PGT in normal and diseased human brains will help to clarify not only the localization of PGT but also the disease processes of AD. Therefore, in this study, we used immunohistochemistry, immunofluorescence, and Western blot analysis to examine the expression pattern of PGT in age-matched control human brain tissue and in tissue from the brains of patients with AD. This study is designed to help us understand the role of PGT in neuroinflammation and its potential contribution in AD.

2. Materials and methods

2.1 Specimens

Human brain tissue samples were acquired from autopsy patients at the Johns Hopkins Brain Resource Center within 19 hours postmortem. Patients gave informed consent before the time of death. Neuropathological diagnosis of AD was established by the Consortium to Establish a Registry of Alzheimer Disease (CERAD) standardized criteria. In general, cases with Braak scores of >II or VI were considered to be AD; however Braak score alone is never used for a final diagnosis. Three patients in our control group had Braak scores of III or IV, but were not considered to have AD because their CERAD ratings were normal. Samples comprised middle frontal gyrus (MFG) tissue from seven patients with familial AD, 10 with sporadic AD, and nine control patients (Table 1). Age, sex, and postmortem time for each patient were recorded (Table 1). The AD tissue also was examined with the Bielchowski silver-staining technique and A β and tau immunostaining to confirm diagnosis. Control brains had no substantial evidence of neuropathological changes at autopsy. In addition to the fresh tissue samples, frozen human brain tissue from one AD and one age-matched control patient was used to determine the cellular localization of PGT.

At autopsy, the dissection was conducted according to the following steps: 1) brain removal, 2) brain weighing, 3) external examination, and 4) sagittal bisection. The right hemisphere was cut coronally, frozen in methylbutane, and stored at -70°C . Snap-frozen sections were fixed in buffered paraformaldehyde at 4°C , cryoprotected, and frozen at -70°C . The left hemisphere was fixed in buffered formalin for 2 weeks and embedded in paraffin.

2.2 Immunohistochemistry

Paraffinized samples were cut into 10- μ m sections with the Leica Rotary Microtome (Meyer Instruments, Houston, TX), mounted on slides, and incubated at 37°C overnight. To deparaffinize the samples, sections were heated at 60°C for 30 minutes and placed in xylene (Fisher, Pittsburgh, PA), 100% ethanol (Harleco, Kansas City, MO), and 95% ethanol (Harleco) three times for 3 minutes each. For antigen unmasking, sections were boiled in citric acid for 5 minutes and then washed in double distilled water and tris-buffered saline (TBS). Endogenous peroxidase was blocked with hydrogen peroxide (H₂O₂; Sigma, St. Louis, MO) in methanol (Fisher) and TBS for 30 minutes. Sections were washed again in TBS, and nonspecific binding sites were blocked with 4% normal goat serum (NGS) and 3% Triton-X 100 in TBS for 1 hour. Sections were incubated with PGT anti-mouse polyclonal antibody (1:750; Cayman, Ann Arbor, MI) in TBS + 2% NGS overnight at 4°C. Negative controls were treated similarly, but without primary antibody. Sections then were washed in TBS + 2% NGS and incubated with biotinylated goat anti-rabbit secondary antibody (Vector Laboratories, Burlingame, CA) in TBS + 2% NGS for 1 hour at room temperature. After being washed in TBS + 2% NGS, sections were incubated with ABC kit (Vector Laboratories) for 1 hour for immunohistochemical localization. Finally, sections were washed in TBS and visualized with DAB solution (Vector Laboratories). Sections were washed in TBS and tap water, counterstained in Mayer's hematoxylin for 10 minutes and washed in running tap water for 10 minutes. After dehydration in 95% ethanol, 100% ethanol, and xylene, sections were mounted with Permount (Fisher) and cover-slipped (Corning Labware, No. 1, 24 × 44 mm, Corning, NY). Tissues were examined on the Bright Field Zeiss Axioskop 20 microscope (Zeiss, Thornwood, NY), and on the Nikon Eclipse TE 2000-E (Nikon Instruments Inc., Melville, NY). Images were acquired with the Nikon ACT-1 Version 2.62 (Nikon Corporation, Tokyo, Japan).

2.3 Immunofluorescence

Frozen tissue of control and AD brains was cut into 10- μ m sections with a Leica Cryostat (Meyer Instruments) and mounted on Superfrost/Plus microscope slides (Fisher). Sections were fixed in 4% formaldehyde for 30 minutes and washed in TBS. Nonspecific binding sites were blocked with 4% NGS and 3% triton-X 100 in TBS for 1 hour. Sections were stained with anti-PGT (1:750; Cayman), mouse anti-SMI32 (neuronal marker, 1:1000; Sternberger Monoclonals, Lutherville, MD), mouse anti-GFAP (astrocyte marker, 1:500; Zymed Laboratories, Carlsbad, CA), or rat anti-CD11b (microglial marker, 1:300; Serotec, Raleigh, NC) in TBS + 2% NGS at 4°C for 48 hours. Samples used as negative controls were treated the same way but without primary antibody. Then sections were washed in TBS and incubated with appropriate secondary antibodies, Alexa Fluor, 1:500 (rabbit, Molecular Probes, Carlsbad, CA), or Cy3, 1:200 (mouse or rat, Jackson IR Laboratories) in TBS overnight at 4°C. Sections then were washed in TBS in the dark, mounted with Vectashield hardset mounting media (Vector Laboratories), and cover-slipped. Sections were examined with the Nikon Eclipse TE 2000-E (Nikon). Photographs were obtained with Nikon ACT-1 Version 2.62 (Nikon).

2.4 Western blots

Frozen human cortical brain tissues were homogenized by sonication in tissue lysis buffer (Cell Signaling Technology, CST, Danvers, MA) containing complete protease inhibitor (Roche Molecular Biochemicals, Indianapolis, IN), 1 mM phenyl methyl sulphonyl fluoride (PMSF, Sigma), and 10 mM sodium fluoride (Sigma). After protein concentration was measured (Bio-Rad, Hercules, CA), equal amounts were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (BA83 0.2 μ m; Bio-Rad). After visualization with Ponceau S solution (Sigma), membranes were washed with TBS containing 0.2% Tween 20, and blocked with 10% non-fat milk for 45 minutes. Membranes were incubated with the

primary antibody overnight at 4°C and then with the secondary antibody for 1 hour at room temperature. Immunoreactive bands were visualized with enhanced chemiluminescence (ECL; Pharmacia Biotech, Piscataway, NJ).

3. Results

3.1 Immunohistochemical expression of PGT in control human brain

Immunohistochemistry revealed that PGT was expressed in the MFG of control brain tissues (Fig. 1). Neurons were intensely stained in the brain tissues of both younger patients (those below the mean patient age of 81.3 years; Fig. 1D) and of older patients. Immunoreactivity for PGT in microglia and astrocytes also was visible but not clearly demonstrable by immunohistochemical techniques. However there appeared to be greater numbers of those cell types in the brain tissues of patients older than 81.3 years with a Braak score greater than II (Fig. 1F). Negative controls showed no immunoreactivity to PGT (Compare Fig. 1B and its inset). These results confirm and extend previous evidence showing the expression of PGT in the human brain as assessed by Northern blot analysis (Lu et al., 1996).

3.2 Immunohistochemical expression of PGT in AD human brain

Similar to the findings in the age-matched control brains, immunohistochemistry also revealed PGT expression in the MFG of AD brain tissues (Fig. 1A, C, E, G). PGT was most evident in neurons (Fig. 1C, arrow heads), but less intense immunostaining was also visible in other cell types, including astrocytes (Fig. 1G, dashed arrows) and microglia (Fig. 1C, E, G, solid arrows), the main cells that synthesize prostaglandins. PGT expression also was observed in blood vessels (Fig. 1A, asterisk). Negative control samples showed no immunoreactivity (Compare Fig. 1A and its inset). These results were consistent regardless of whether the tissue was from a familial or sporadic AD brain. Although relative, the intensity of PGT staining in neurons and astrocytes appeared to be stronger in the control brain than in AD brain tissues, whereas the intensity of PGT staining in microglia appeared to be stronger in AD brain tissues. As reported in previous studies (McGeer et al., 1993; McGeer and McGeer, 2004), the number and size of astrocytes and microglia in AD brains appeared to be higher than those in control brains.

3.3 Double-label immunofluorescent localization of PGT in human brain tissues

Double-label immunofluorescence with specific antibodies for neurons (SMI32), microglia (GFAP), and astrocytes (CD11b) was used to identify the cell types that express PGT. We found that in control (not shown) and AD brains, PGT co-localized with microglia, astrocytes, and neurons, mostly in the cell bodies (Fig. 2).

3.4 Comparison of PGT expression levels in AD and age-matched controls

Western blot analysis of 10 AD brains and 9 controls revealed that PGT bands were weaker in AD than in control brain tissue (Fig. 3A). The ratio of PGT to actin (loading control) band intensity was approximately 9 for control tissue and 3 for AD tissue, indicating significantly less PGT protein in the AD tissue ($P < 0.001$; Fig. 3B).

4. Discussion

To find more effective treatments for neurodegenerative diseases, researchers have investigated the roles of prostaglandins and COX enzymes in neuroinflammation, but the prostaglandin transporter has not been as intensively studied. We have examined the pattern of PGT expression in adult control and diseased human brain tissue and found PGT to be most prominently expressed in the neurons, astrocytes, and microglia of the MFG. Of interest,

Western blot analysis suggested that PGT protein expression was lower in the AD brains than in the age-matched controls.

Neuroinflammation is associated with increased levels of reactive microglia and astrocytes (Kawamata et al., 1992). In AD, immunohistochemical examination has shown clusters of activated microglia and astrocytes surrounding the A β deposits (McGeer and McGeer, 2004). Knowing that PGs can act as either autocrine- or paracrine-signaling bioactive molecules, it is possible that in our study, the PGT staining in microglia was more intense in AD than in age-matched brain tissue because microglia are reactive in AD brains but mostly resting in control brains. Microglia, which are phagocytic, support and protect neuronal function (McGeer et al., 1993; Perlmutter et al., 1990; Sastre et al., 2006). For example, when the brain is injured, microglia become activated by A β deposits and recruit astrocytes by secreting acute-phase proteins such as complement factors and cytokines. Reactive microglia and astrocytes additionally generate proinflammatory mediators, including cytokines, chemokines, prostaglandins, neurotoxic secretory products, reactive oxygen species, and nitric oxide (Griffin et al., 1998; Tuppo and Arias, 2005). Cytokines and chemokines, in turn, stimulate the synthesis of other enzymes, such as COXs and prostaglandin synthases. In AD, the expression of COX-2, the inducible isoform, increases in response to inflammatory agents in neurons and glial cells (Pasinetti and Aisen, 1998; Sairanen et al., 1998). Because COX is the rate-limiting enzyme in the production of prostaglandins (O'Banion, 1999; Smith et al., 1991), the increase in COX activity leads to an increase in prostaglandin production (Consilvio et al., 2004). Although, there have been few *in vivo* studies to pinpoint the cellular location of the prostaglandin increase, Consilvio, et al (Consilvio et al., 2004) suggested that the ability of astrocytes to synthesize prostaglandin after an injury increases over a long period of time. They based their hypothesis on studies showing that COX-2 is present in astrocytes (Chang et al., 1996) and in infarcted human brains (Sairanen et al., 1998). The increase in prostaglandin synthesis could be either protective or injurious, depending on the specific prostaglandins produced and the receptors that they bind and activate (Doré, 2006). In addition, one prostaglandin can promote different effects, depending on the target cells (Consilvio et al., 2004).

Studies of the human PGT in peripheral organs such as the kidney, uterus, and endometrium have shown that this transporter plays two important roles: releasing newly synthesized prostaglandins and removing prostaglandins for intracellular clearance (Bao et al., 2002; Kanai et al., 1995; Lu et al., 1996; Scafidi et al., 2007). Of these two functions, it is unclear which is active in the brain, but both have the potential to play essential roles in neuroinflammation. During brain injury, the rapidity with which prostaglandins reach the affected area could substantially impact their function in a given subregion (such as a synaptic microenvironment) and the survival of surrounding neurons. However, our Western blot data showed that the protein expression of PGT was actually lower in AD brains than in control brains, suggesting that free prostaglandin levels might be altered, thus affecting cell function and neuronal death.

A second possibility is that cellular uptake of prostaglandins may be decreased in AD. It was reported that in Madin-Darby canine kidney cells, PGT mediates the influx of prostaglandins across the apical membrane but that prostaglandins are released from the cell by passive diffusion (Chi et al., 2006; Endo et al., 2002). If true in the brain, it is likely that the clearance of prostaglandins following the uptake of PGT also plays a key role in neuroinflammation. As the levels of proinflammatory prostaglandins increase, PGT must also increase to maintain clearance of the prostaglandins and prevent additional inflammation. Because our data indicate lower than normal levels of PGT protein in AD brains, it is possible that the prostaglandins might not be cleared fast enough to limit the inflammatory cascade, which then could lead to neuronal cell death; however more work addressing this specific question is warranted.

In summary, we have shown that PGT protein is expressed in the neurons, microglia, and astrocytes of human brain tissue, but is present at lower levels in the brains of AD patients. To our knowledge, this is the first report to characterize PGT expression in the human brain. At this point, it is still unclear why the level of PGT protein would be lower in AD or which of the mechanisms discussed above is active, but it is likely that PGT is associated with the neuroinflammatory process. Additional study of the role of PGT in AD could help clarify the mechanism.

Acknowledgments

This work was supported in part by NIH grants AG022971 (SD) and NS046400 (SD, BC). The authors would like to thank Claire Levine for her assistance in preparing this manuscript and all members of the lab team for their insightful comments.

References

- Ahmad AS, Ahmad M, de Brum-Fernandes AJ, Doré S. Prostaglandin EP4 receptor agonist protects against acute neurotoxicity. *Brain Res* 2005;1066:71–77. [PubMed: 16360658]
- Ahmad AS, Saleem S, Ahmad M, Doré S. Prostaglandin EP1 receptor contributes to excitotoxicity and focal ischemic brain damage. *Toxicol Sci* 2006a;89:265–270. [PubMed: 16237196]
- Ahmad M, Saleem S, Zhuang H, Ahmad AS, Echeverria V, Sapirstein A, Doré S. 1-HydroxyPGE₁ reduces infarction volume in mouse transient cerebral ischemia. *Eur J Neurosci* 2006b;23:35–42. [PubMed: 16420413]
- Arosh JA, Banu SK, Chapdelaine P, Madore E, Sirois J, Fortier MA. Prostaglandin Biosynthesis, Transport, and Signaling in Corpus Luteum: A Basis for Autoregulation of Luteal Function. *Endocrinology* 2004;145:2551–2560. [PubMed: 14736737]
- Banu SK, Arosh JA, Chapdelaine P, Fortier MA. Molecular cloning and spatio-temporal expression of the prostaglandin transporter: A basis for the action of prostaglandins in the bovine reproductive system. *Proc Natl Acad Sci U S A* 2003;100:11747–11752. [PubMed: 13130075]
- Bao Y, Pucci ML, Chan BS, Lu R, Ito S, Schuster VL. Prostaglandin transporter PGT is expressed in cell types that synthesize and release prostanoids. *Am J Physiol Renal Physiol* 2002;282:F1103–1110. [PubMed: 11997327]
- Chan BS, Satriano JA, Pucci M, Schuster VL. Mechanism of Prostaglandin E2 Transport across the Plasma Membrane of HeLa Cells and Xenopus Oocytes Expressing the Prostaglandin Transporter “PGT”. *J. Biol. Chem* 1998;273:6689–6697. [PubMed: 9506966]
- Chang J, Coleman P, O'Banion M. Prostaglandin G/H synthase-2 (cyclooxygenase-2) mRNA expression is decreased in Alzheimer's disease. *Neurobiol Aging* 1996;17:801–808. [PubMed: 8892355]
- Chi Y, Khersonsky SM, Chang Y-T, Schuster VL. Identification of a new class of prostaglandin transporter inhibitors and characterization of their biological effects on prostaglandin E2 transport. *J Pharmacol Exp Ther* 2006;316:1346–1350. [PubMed: 16269530]
- Consilvio C, Vincent AM, Feldman EL. Neuroinflammation, COX-2, and ALS--a dual role? *Exp Neurol* 2004;187:1–10. [PubMed: 15081582]
- Donnelly MT, Hawkey CJ. Review article: COX-II inhibitors--a new generation of safer NSAIDs? *Aliment Pharmacol Ther* 1997;11:227–235. [PubMed: 9146759]
- Doré S. GPCR antagonists as an alternative to COX-2 inhibitors: a case for the PGE₂ EP1 receptor. *Trends Pharmacol Sci* 2006;27:458–460. [PubMed: 16870271]
- Echeverria V, Clerman A, Doré S. Stimulation of PGE₂ receptors EP2 and EP4 protects cultured neurons against oxidative stress and cell death following β -amyloid exposure. *Eur J Neurosci* 2005;22:2199–2206. [PubMed: 16262658]
- Endo S, Nomura T, Chan BS, Lu R, Pucci ML, Bao Y, Schuster VL. Expression of PGT in MDCK cell monolayers: polarized apical localization and induction of active PG transport. *Am J Physiol Renal Physiol* 2002;282:F618–622. [PubMed: 11880322]
- Engelhardt JJ, Appel SH, Killian JM. Experimental autoimmune motoneuron disease. *Ann Neurol* 1989;26:368–376. [PubMed: 2802536]

- Engelhardt JJ, Tajti J, Appel SH. Lymphocytic infiltrates in the spinal cord in amyotrophic lateral sclerosis. *Arch Neurol* 1993;50:30–36. [PubMed: 8093428]
- Griffin WST, Sheng JG, Royston MC, Gentleman SM, McKenzie JE, Graham DI, Roberts GW, Mrak RE. Glial-neuronal interactions in Alzheimer's disease: the potential role of a 'cytokine cycle' in disease progression. *Brain Pathology* 1998;8:65–72. [PubMed: 9458167]
- Halliday G, Robinson SR, Shepherd C, Kril J. Alzheimer's disease and inflammation: a review of cellular and therapeutic mechanisms. *Clin Exp Pharmacol Physiol* 2000;27:1–8. [PubMed: 10696521]
- Kanai N, Lu R, Satriano JA, Bao Y, Wolkoff AW, Schuster VL. Identification and characterization of a prostaglandin transporter. *Science* 1995;268:866–869. [PubMed: 7754369]
- Kashiwagi K, Kanai N, Tsuchida T, Suzuki M, Iizuka Y, Tanaka Y, Tsukahara S. Comparison between Isopropyl Unoprostone and Latanoprost by Prostaglandin E2 Induction, Affinity to Prostaglandin Transporter, and Intraocular Metabolism. *Experimental Eye Research* 2002;74:41–49. [PubMed: 11878817]
- Kawamata T, Akiyama H, Yamada T, McGeer PL. Immunologic reactions in amyotrophic lateral sclerosis brain and spinal cord tissue. *Am J Pathol* 1992;140:691–707. [PubMed: 1347673]
- Lampson LA, Kushner PD, Sobel RA. Major histocompatibility complex antigen expression in the affected tissues in amyotrophic lateral sclerosis. *Ann Neurol* 1990;28:365–372. [PubMed: 2241118]
- Lu R, Kanai N, Bao Y, Schuster VL. Cloning, in vitro expression, and tissue distribution of a human prostaglandin transporter cDNA(hPGT). *J Clin Invest* 1996;98:1142–1149. [PubMed: 8787677]
- McGeer EG, McGeer PL. Inflammatory processes in Alzheimer's disease. *Prog Neuropsychopharmacol Biol Psychiatry* 2003;27:741–749. [PubMed: 12921904]
- McGeer PL, Kawamata T, Walker DG, Akiyama H, Tooyama I, McGeer EG. Microglia in degenerative neurological disease. *Glia* 1993;7:84–92. [PubMed: 8423066]
- McGeer PL, McGeer EG. Inflammation and the degenerative diseases of aging. *Ann N Y Acad Sci* 2004;1035:104–116. [PubMed: 15681803]
- Montine TJ, Markesbery WR, Zackert W, Sanchez SC, Roberts LJ, 2nd, Morrow JD. The magnitude of brain lipid peroxidation correlates with the extent of degeneration but not with density of neuritic plaques or neurofibrillary tangles or with APOE genotype in Alzheimer's disease patients. *Am J Pathol* 1999a;155:863–868. [PubMed: 10487843]
- Montine TJ, Montine KS, Olson SJ, Graham DG, Roberts LJ, 2nd, Morrow JD, Linton MF, Fazio S, Swift LL. Increased cerebral cortical lipid peroxidation and abnormal phospholipids in aged homozygous apoE-deficient C57BL/6J mice. *Exp Neurol* 1999b;158:234–241. [PubMed: 10448437]
- O'Banion MK. Cyclooxygenase-2: molecular biology, pharmacology, and neurobiology. *Crit Rev Neurobiol* 1999;13:45–82. [PubMed: 10223523]
- Pasinetti GM. Cyclooxygenase and inflammation in Alzheimer's disease: experimental approaches and clinical interventions. *J Neurosci Res* 1998;54:1–6. [PubMed: 9778144]
- Pasinetti GM, Aisen PS. Cyclooxygenase-2 expression is increased in frontal cortex of Alzheimer's disease brain. *Neuroscience* 1998;87:319–324. [PubMed: 9740394]
- Perlmuter LS, Barron E, Chui HC. Morphologic association between microglia and senile plaque amyloid in Alzheimer's disease. *Neuroscience Letters* 1990;119:32–36. [PubMed: 2097581]
- Pucci ML, Chakkalakal B, Liclican EL, Leedom AJ, Schuster VL, Abraham NG. Augmented heme oxygenase-1 induces prostaglandin uptake via the prostaglandin transporter in micro-vascular endothelial cells. *Biochemical and Biophysical Research Communications* 2004;323:1299–1305. [PubMed: 15451438]
- Reid G, Wielinga P, Zelcer N, van der Heijden I, Kuil A, de Haas M, Wijnholds J, Borst P. The human multidrug resistance protein MRP4 functions as a prostaglandin efflux transporter and is inhibited by nonsteroidal antiinflammatory drugs. *Proc Natl Acad Sci U S A* 2003;100:9244–9249. [PubMed: 12835412]
- Sairanen T, Ristimäki A, Karjalainen-Lindsberg ML, Paetau A, Kaste M, Lindsberg PJ. Cyclooxygenase-2 is induced globally in infarcted human brain. *Ann Neurol* 1998;43:738–747. [PubMed: 9629843]
- Sastre M, Klockgether T, Heneka MT. Contribution of inflammatory processes to Alzheimer's disease: molecular mechanisms. *Int J Dev Neurosci* 2006;24:167–176. [PubMed: 16472958]

- Scafidi S, Douglas RM, Farahani R, Banasiak KJ, Haddad GG. Prostaglandin transporter expression in mouse brain during development and in response to hypoxia. *Neuroscience* 2007;146:1150–1157. [PubMed: 17428614]
- Schiffer D, Cordera S, Cavalla P, Migheli A. Reactive astrogliosis of the spinal cord in amyotrophic lateral sclerosis. *J Neurol Sci* 1996;139:27–33. [PubMed: 8899654]
- Smith WL, Marnett LJ, DeWitt DL. Prostaglandin and thromboxane biosynthesis. *Pharmacol Ther* 1991;49:153–179. [PubMed: 1905023]
- Topper JN, Cai J, Stavrakis G, Anderson KR, Woolf EA, Sampson BA, Schoen FJ, Falb D, Gimbrone MA, Jr. Human prostaglandin transporter gene (hPGT) is regulated by fluid mechanical stimuli in cultured endothelial cells and expressed in vascular endothelium in vivo. *Circulation* 1998;98:2396–2403. [PubMed: 9832484]
- Troost D, Van den Oord JJ, Vianney de Jong JM. Immunohistochemical characterization of the inflammatory infiltrate in amyotrophic lateral sclerosis. *Neuropathol Appl Neurobiol* 1990;16:401–410. [PubMed: 2263315]
- Tuppo EE, Arias HR. The role of inflammation in Alzheimer's disease. *Int J Biochem Cell Bio* 2005;37:289–305. [PubMed: 15474976]

Fig. 1.

Expression of PGT in human AD and control brains. Immunohistochemical analysis shows that PGT is expressed in the middle frontal gyrus (MFG) of AD and control brains. Microglia are indicated with solid arrows, neurons with arrow heads, blood vessels with an asterisk, and astrocytes with dashed arrows. (A) MFG of 80-year-old female (AD6) and (B) age-matched control (C6). Negative control tissue that received no primary antibody did not show immunoreactivity (insets). (C) MFG of 72-year-old female (AD3) and (D) age-matched control (C3). (E) MFG of 95-year-old female (AD4) and (F) age-matched control (C4). (G) MFG of 72-year-old female (AD5) and (H) age-matched control (C5).

Fig. 2.

Localization of PGT in AD brain tissue. Double-label immunofluorescence shows that PGT is expressed in neurons, astrocytes, and microglia. (A) green immunofluorescent staining of PGT, (B) red staining of neurons with anti-SMI32, (C) merge of PGT and SMI32; (D) green immunofluorescent staining of PGT, (E) red staining of astrocytes with anti-GFAP, (F) merge of PGT and GFAP; (G) green immunofluorescent staining of PGT, (H) red staining of microglia with anti-CD11b, (I) merge of PGT and CD11b.

Fig. 3. Differential expression of PGT protein in human cortical brain tissues. (A) representative Western blot of PGT in tissue from control and AD brains. Actin was used as a loading control. (B) The ratio of PGT to actin (which was unchanged in AD) was significantly lower in AD brain tissue (n=10) than in control brain tissue (n=9). ** $P < 0.001$.

Table 1

Human brain samples used for expression of PGT

Diagnosis	Sample No.	Age	Sex	PMT (hours)	CERAD	BRAAK stage
AD (F)	AD1	92	F	8	C	VI
AD (F)	AD2	74	F	5.5	C	VI
AD (S)	AD3	72	F	19	C	VI
AD (F)	AD4	95	F	4	C	VI
AD (S)	AD5	72	F	10	C	VI
AD (F)	AD6	80	F	13	C	VI
AD (S)	AD7	80	F	12	C	V
AD (F)	AD8	63	M	9	C	VI
AD (F)	AD9	78	M	4	C	VI
AD (F)	AD10	94	F	4	C	VI
AD (S)	AD11	79	M	10.5	C	VI
AD (S)	AD12	82	F	6	C	VI
AD (S)	AD13	85	M	3.5	C	VI
AD (S)	AD14	89	M	9.5	C	VI
AD (S)	AD15	54	F	14.5	C	VI
AD (S)	AD16	62	F	11	C	VI
AD (S)	AD17	84	F	5	C	V
CTRL	C1	91	F	8		I
CTRL	C2	74	M	4		II
CTRL	C3	68	M	10		II
CTRL	C4	94	M	16		III
CTRL	C5	71	F	16		0
CTRL	C6	80	F	8		0
CTRL	C7	87	M	8		II
CTRL	C8	80	M	22		IV
CTRL	C9	87	M	17		IV

PMT, post mortem time; AD, Alzheimer disease; F, familial; S, sporadic; CTRL, control