ORIGINAL ARTICLE

Astroglial Activation of Extracellular-Regulated Kinase in Early Stages of Alzheimer Disease

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Abstract

Characterization of the earliest neuropathologic features of Alzheimer disease (AD) indicates that synaptic degeneration accompanied by tau hyperphosphorylation and amyloid deposition might be an important feature. The mechanisms involved are unclear; however, dysregulation of signaling cascades such as the extracellular signal-regulated kinase (ERK) pathway might play a role. In this context, the main objective of this study was to determine whether ERK hyperactivation occurs in early stages of AD. We compared the patterns of total and phosphorylated ERK (pERK) expression in the midfrontal cortex of patients clinically and neuropathologically characterized with early, intermediate, or advanced AD. Immunocytochemical and Western blot analysis showed that in early AD, there was extensive activation of ERK in astroglial cells in the white matter accompanied by intense astrogliosis. In contrast, in patients with more advanced AD, pERK immunoreactivity was associated with neuronal cell bodies and dystrophic neurites around plaques. Levels of astroglial pERK immunoreactivity in the white matter were strongly correlated with scores of cognitive performance (Blessed, Mini-Mental Status Examination, and Clinical Dementia Rating) and with the severity of AD neuropathology (Braak stage). These findings suggest that astroglial ERK activation may be an important early response to the onset of AD pathology. Identification of cell signaling events unique to early AD may provide therapeutic targets for the prevention or delay of dementia.

Key Words: Amyloid β, Astrogliosis, Dementia, Extracellular signal-regulated kinase, GFAP, Mini-Mental Status Examination, Preclinical Alzheimer disease.

INTRODUCTION

Recent studies in Alzheimer disease (AD) have been focused at characterizing the earliest clinical and neuropathologic features of this condition. Neurologic studies indicate that mild cognitive impairment (MCI) is a clinically diagnos-
able condition that is coming to be recognized as an important means of predicting future progression to AD (1, 2). Patients with MCI show measurable impairments predominantly in episodic memory, often with some of the neuropsychiatric features common to patients with AD, including dysphoria, apathy, irritability and anxiety (1, 3, 4). The presence of impairments and delayed recall in patients with MCI has been found to have the greatest predictive value for progression to AD in two separate studies (3, 5). In one study, 77% of patients meeting the criteria for MCI beyond pure memory loss progressed to AD within 7 years of follow up compared with 24% of patients not meeting these criteria (6). Probably accounting for these memory alterations, patients with MCI display degeneration of the perforant pathway manifested with loss of neurons in the entorhinal cortex and synapses in the molecular layer of the hippocampal dentate gyrus and frontal cortex (7-10). In addition, increased phosphorylation of tau in pyramidal neurons and amyloid deposition has been detected in such cases (11). In experimental animal models of AD, similar early neuropathologic alterations have been described, including loss of synapses in the hippocampus and frontal cortex accompanied by astrogliosis (12, 13). The mechanisms involved are not completely clear; however, some studies suggest that dysregulation of signaling pathways such as the extracellular-regulated kinase (ERK) cascade might play a significant role (14).

The ERK proteins (ERK1 and ERK2) belong to the mitogen-activated protein kinase (MAPK) pathway and include p38 and Jun kinase (JNK) (15). The ERK cascade (like in other MAPK cascades) contains a central core of three protein kinases: 1) the ERKs themselves; 2) kinases that phosphorylate and activate ERKs (the MAPK-kinase [MAPKK] or MEK); and 3) kinases that phosphorylate and activate MEKs (MAPK-kinase-kinase [MAPKK] or MAPK/ERK-kinase-kinase [MEKK]) (16, 17). Activation of this cascade results in transcriptional regulation through several downstream activators such as cAMP-response-element-binding protein (CREB), which is necessary for cell proliferation, differentiation, and survival (17). Several lines of evidence support a role for the ERK signaling pathway in the pathogenesis of AD, including studies that have shown ERK activation in degenerating neurons in AD (18–20) and amyloid precursor protein (APP) transgenic (tg) mice (21). Furthermore, ERK is activated by amyloid β protein (Aβ) in vitro and plays a role in APP processing and secretion and can phosphorylate tau protein in a manner similar to paired helical filament (PHF)-tau (22, 23).
Activation of the ERK pathway in astrocytes is known to occur after a variety of neurologic insults, including hypoxia–ischemia, kainate excitotoxicity, and traumatic brain injury (24–26). The consequences of astroglial ERK activation after these insults are not well understood, but are thought to contribute to a proliferative inflammatory response around the site of injury. In vitro studies of cultured cortical rat astrocytes have shown that Aβ promotes the phosphorylation and nuclear translocation of ERK, suggesting that astroglial ERK activation may potentially occur in AD (27). The ERK pathway controls the synthesis and release of apolipoprotein E, a protein that has been implicated in the pathogenesis of AD, by astrocytes (28); however, it is unknown whether this occurs and contributes to AD progression in vivo. Despite a number of studies that have investigated the role of ERK activation in neuronal cells in AD, none has explored the role of this signaling pathway in astrocytes or in the early stages of AD. In this context, the purpose of the present study was to characterize the patterns of ERK-signaling abnormalities in the earliest stages of AD. Remarkably, we found that in patients with early AD, there was extensive ERK activation in the white matter that was correlated with the severity of the dementia as well as the neuropathologic stage.

MATERIALS AND METHODS

Specimen Processing, Neuropathologic Evaluation, and Criteria for Disease Stage

A total of 24 nondemented (n = 5) control subjects and AD (n = 19) cases were included for the present study. Autopsy material was obtained from patients (Table 1) studied neurologically and psychometrically at the Alzheimer Disease Research Center/University of California, San Diego (ADRC/UCSD). The last neurobehavioral evaluation was performed within 12 months before death and included Blessed score, Mini-Mental Status Examination (MMSE) and dementia rating scale (DRS) (29, 30). Brains were processed and evaluated according to standard methods (31). At autopsy, brains were divided sagittally, the left hemibrain was fixed in formalin of 4% paraformaldehyde for subsequent neuropathologic analysis, and the right was frozen at –70°C for neurochemical analysis. Paraffin sections from 10% buffered formalin-fixed neocortical, limbic system and subcortical material stained with hematoxylin and eosin (H&E) and thioflavine-S were used for routine neuropathologic analysis that included assessment of plaque and tangle density in the neocortex and hippocampus (32). An additional group of five non-AD demented patients were included as control subjects for immunocytochemical studies (Huntington disease, n = 2; frontotemporal dementia, n = 3).

Based on previously published clinical and pathologic findings (9, 33), cases were divided into the following groups: 1) nondemented age-matched control subjects (n = 5); 2) early AD (n = 7); 3) intermediate AD (n = 5); and 4) advanced AD (n = 7). The nondemented control group had a Clinical Dementia Rating (CDR) score of zero, an MMSE score of 25 or greater, and a Blessed score of zero to 2. For neuropathologic assessment, plaque counts were divided into total and neuritic groups and expressed as numbers per square millimeter. The neuropathologic criteria for the control group required a Braak score of zero, total plaque count of 3 or less, and a neuritic plaque count of zero. The criteria for the early AD group included a CDR score of 0.5, MMSE score of 25 or greater, a Blessed score of 1 to 4, Braak score of 1 to 2, total plaque count of 25 or greater, and a neuritic plaque count of 10 or less. The criteria for the intermediate-stage group included a CDR score of 1, MMSE score of 20 or less, a Blessed score of 5 to 20, Braak score of III to V, total plaque count of 25 or greater, and a neuritic plaque count of 15 to 25 or less. For the advanced AD group, the criteria included a CDR score of 2, MMSE score of 10 or less, a Braak stage of VI, a total plaque count of 50 (which is the maximal count and means ±50), and a neuritic plaque count of 25 or greater.

Immunohistochemistry, Image Analysis, and Laser Scanning Confocal Microscopy

Briefly, as previously described (33), free-floating 40-μm-thick vibratome sections from the midfrontal cortex were washed with Tris-buffered saline (TBS, pH 7.4) and 0.5% Triton X-100 (Sigma, St. Louis, MO) followed by pretreatment in 3% H2O2, 10% methanol in TBS, and blocking with 10% normal goat serum (Vector), 3% bovine serum albumin (Sigma), and 0.2% gelatin in TBS-Tx. Sections were then incubated at 40°C overnight with the mouse monoclonal antibodies against phosphorylated ERK (pERK, Catalog 9106, Cell Signaling Technology, Beverly, MA) or total ERK (tERK, Catalog 554095, 10 μg/mL; BD Pharmingen, San Diego, CA) in 3% normal goat serum and 0.2% gelatin in TBS-Tx or mouse monoclonal antibodies against glial fibrillary acidic protein (GFAP, Catalog MAB3402, 2 μg/mL; Chemicon, Temecula, CA) and Aβ protein (4G8, Catalog 9220, 0.5 μg/mL; Signet Laboratories, Dedham, MA). To further confirm the specificity of the patterns of pERK immunostaining in AD, an additional set of vibratome sections were

**TABLE 1.** Summary of Clinicopathologic Features

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Age (Range)</th>
<th>Sex (F/M)</th>
<th>PMT</th>
<th>DRS</th>
<th>MMSE</th>
<th>CDR</th>
<th>Braak</th>
<th>NP</th>
<th>Percent Amyloid</th>
<th>NFT</th>
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<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>84 ± 4</td>
<td>2/3</td>
<td>&lt;3 hours</td>
<td>2 ± 1</td>
<td>135 ± 3</td>
<td>28 ± 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Early Alzheimer disease</td>
<td>7</td>
<td>86 ± 3</td>
<td>5/2</td>
<td>&lt;3 hours</td>
<td>3 ± 2</td>
<td>134 ± 3</td>
<td>28 ± 1</td>
<td>0.5</td>
<td>1</td>
<td>8.4 ± 3.5</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>Intermediate</td>
<td>5</td>
<td>82 ± 3</td>
<td>4/1</td>
<td>&lt;3 hours</td>
<td>21 ± 2</td>
<td>104 ± 9</td>
<td>18 ± 2</td>
<td>1 IV–V</td>
<td>23 ± 8</td>
<td>18 ± 1</td>
<td>1.25 ± 1</td>
</tr>
<tr>
<td>Advanced</td>
<td>7</td>
<td>79 ± 3</td>
<td>2/5</td>
<td>&lt;3 hours</td>
<td>30 ± 1</td>
<td>67 ± 13</td>
<td>3 ± 1.5</td>
<td>2 VI</td>
<td>37 ± 5</td>
<td>38 ± 5</td>
<td>6.5 ± 2</td>
</tr>
</tbody>
</table>

PMT, postmortem time; BI, Blessed score; DRS, dementia rating scale; MMSE, Mini-Mental Status Examination; CDR, Clinical Dementia Rating; NP, neuritic plaques; NFT, neurofibrillar tangles.
immunolabeled with a rabbit polyclonal anti-pERK antibody from a different source (Catalog AF1018, 1.0 μg/mL; R&D Systems, Minneapolis, MN). Sections were then incubated in biotinylated horse antimouse secondary antibody (1:75; Vector Laboratories, Burlingame, CA), followed by Avidin D-horseradish peroxidase (HRP, ABC Elite; Vector) and reacted with diaminobenzidine (DAB, 0.2 mg/mL) in 50 mM Tris (pH 7.4) with 0.001% H2O2. Control experiments consisted of incubation with preimmune rabbit serum. To investigate the effects of postmortem delay and fixation on the levels of pERK immunoreactivity, preliminary studies were performed in a subset of cases (n = 5) with postmortem delay ranging from 4 to 48 hours. In addition, tests were performed in paraffin sections and in tissues overfixed in formalin for more than 2 months.

Immunostained sections were imaged with a digital Olympus microscope and assessment of levels of pERK, tERK, and GFAP immunoreactivity was performed using the Image-Pro Plus program (Media Cybernetics, Silver Spring, MD). For each case, a total of three sections (10 images per section) were analyzed to estimate the average number of immunolabeled cells per unit area (mm2) and the average intensity of the immunostaining (corrected optical density). Additional analysis of amyloid burden in the frontal cortex was performed in vibratome sections immunostained with the 4G8 antibody. For this purpose, a total of 3 sections (4 images per section) were analyzed with the Image-Pro Plus program by setting a threshold parameter that only detects the area of the neuropil covered by Aβ-immunoreactive material. The area occupied by amyloid was divided by the total area of the image and results were averaged as percent area of the neuropil covered by Aβ-immunoreactive material. To confirm the identity of the labeled cells, double immunocytochemical analyses were performed using the Tyramide Signal Amplification-Direct (Red) system (NEN Life Sciences, Boston, MA). Specificity of this system was tested by deleting each primary antibody. For this purpose, sections were double-labeled with the monoclonal antibodies against pERK (1:20,000; Cell Signaling) detected with Tyramide Red and either the astroglial marker, GFAP (5 μg/mL; Chemicon), or the oligodendroglial marker galactocerebroside (GC). Immunostained sections were imaged with a digital Zeiss 63X objective on an Axiovert 35 microscope (Zeiss, Germany) with an attached MRC1024 laser scanning confocal microscope (LSCM) system (BioRad, Watford, U.K.).

**Western Blot Analysis**

Immunoblot analysis was performed as previously described (34). Briefly, frontal cortex brain homogenates from 24 cases were solubilized in lysis buffer (1% Triton X-100, 10% glycerol, 50 mM HEPEs, pH 7.4, 140 mM NaCl, 1 mM EDTA, 1 mM Na2VO4, 20 mM β-glycerophosphate, and proteinase inhibitor cocktails) and separated into cytosolic and particulate fractions by centrifugation. Twenty micrograms of the particulate fractions were then resolved by SDS-PAGE and electroblotted onto Immobilon membranes (Millipore, Billerica, MA). The membranes were blocked with phosphate-buffered saline (PBS) with 0.2% Tween-20 (PBST) containing 3% skim milk or bovine serum albumin (BSA) followed by incubation with primary mouse monoclonal antibodies against pERK (1:2500; Cell Signaling), tERK (1 μg/mL; BD Pharmingen), pMEK (Catalog 9121, 1:1000; Cell Signaling), tMEK (Catalog 4694, 1:1000; Cell Signaling), or GFAP (1 μg/mL; Chemicon) in PBST containing 5% BSA. After washing with PBS, the membranes were incubated with rabbit antimouse secondary antibodies (1:5000) and analyzed with enhanced chemiluminescence (ECL; PerkinElmer, Wellesley, MA) on the VersaDoc gel imaging system (BioRad, Hercules, CA).

**Statistical Analysis**

All experiments were conducted in triplicate on blind-coded samples. After the results were obtained, the code was broken and data were analyzed with the StarView program (SAS Institute, Inc., Cary, NC). Comparisons of pERK, tERK, pMEK, tMEK, and GFAP levels among groups were performed by one-way analysis of variance with post hoc Dunnett’s or Tukey-Kramer. Linear regression analysis was performed to determine the correlation between levels of pERK immunoreactivity and clinical and neuropathologic markers. Correction for multiple comparisons was applied by using the Bonferroni test. All results were expressed as mean ± standard error of mean.

**RESULTS**

**Increased Extracellular-Regulated Kinase Activation in White Matter Glial Cells Occurs in Early Alzheimer Disease**

Cases were grouped according to clinical and neuropathologic criteria into nondemented control subjects and early, intermediate, or advanced AD (Table 1). Immunohistochemical analysis of the frontal cortex revealed a unique pattern of pERK immunoreactivity in the early stages of AD. Compared with nondemented control subjects (Figs. 1A, 2A), cases with early AD displayed intense pERK immunolabeling predominantly in glial cells in the white matter (Figs. 1B, 2A). Cases with intermediate and advanced AD (Figs. 1C, 2A) showed lower levels of pERK immunoreactivity in glial cells in the white matter. Similar results were observed with an anti-pERK antibody from a different source (Fig. 11–L). In contrast to the intense astroglial pERK immunolabeling in the white
matter of early AD cases, only mild pERK immunolabeling was observed in the gray matter of control and AD cases at all stages (Fig. 1E–H) and in the white or gray matter of non-AD control subjects (Fig. 1D, H, L). Analysis of levels of pERK immunoreactivity revealed a significant increase in the number of pERK-immunoreactive glia in early AD cases and lower numbers in pERK-immunoreactive glial cells around the plaques (Fig. 3A). In contrast, intermediate and advanced AD cases showed pERK immunoreactivity in cortical pyramidal neurons (Fig. 3B) and in dystrophic neurites within plaques (Fig. 3C, D). Early AD cases did not show significant neuronal or plaque labeling for pERK (not shown), and only mild or no pERK immunoreactivity was detected in glial cells around the plaques (not shown). Immunohistochemical analysis with an antibody against tERK showed moderate levels of immunoreactivity in neurons and mild labeling of glial cells (Fig. 1M–P) with no significant differences among the groups (Fig. 2C). Overall pERK immunoreactivity was detected in vibratome sections and

FIGURE 1. Comparison of the patterns of extracellular-regulated kinase immunoreactivity during the progression of Alzheimer disease (AD). Sections from the midfrontal cortex were immunostained with antibodies against pERK and tERK and reacted with DAB. (A) In the control white matter, pERK immunolabeled some glial cells. (B) In early AD, glial cells in the white matter displayed intense pERK immunoreactivity (arrows). (C) In advanced AD, glial cells in the white matter showed mild pERK immunoreactivity. (D) In non-AD dementia (Huntington disease), there is mild labeling of neuronal cell bodies. (E) In control sections, pyramidal neurons in layer 5 of the neocortex showed mild pERK immunostaining. (F) In early AD, glial cells in the neocortex displayed moderate levels of pERK immunoreactivity. (G) In the neocortex of advanced AD cases, some pyramidal cells displayed intense pERK immunoreactivity. (H) In non-AD demented cases, there was mild immunostaining of pyramidal cell neurons. (I–L) Levels of pERK immunolabeling with a second antibody showed that compared with control brain (I), intense reactivity was observed in early AD (J), advanced AD (K) and a non-AD control (L). (M–P) Similar levels of tERK immunoreactivity were observed among control (M), early (N), advanced (O), and advanced Huntington disease (P) groups. *, Antibody obtained from Cell Signaling Technology; **, antibody obtained from R&D Systems. Scale bar = 25 μm.
also in paraffin sections when antigen retrieval was used (not shown). The antibody immunostaining was unaffected when tissues were obtained within 24 hours of death and fixed in paraformaldehyde. pERK immunoreactivity was not detected in brain tissue fixed in formalin for a long period of time.

Consistent with the immunohistochemical studies, Western blot analysis of all 24 cases showed that in early AD, there was an increase in pERK immunoreactivity compared with intermediate and advanced AD (Fig. 4A, B). Furthermore, the levels of pMEK upstream of ERK were similarly upregulated (Fig. 4A, C), and levels of GFAP in the white matter were increased in early AD cases compared with controls and later disease stages (Fig. 4A).

Extracellular-Regulated Kinase Activation in Early Alzheimer Disease Colocalizes With Astroglial Cell Markers and Is Accompanied by Intense White Matter Astrogliosis

To determine which glial cell population exhibits pERK expression, double immunohistochemical experiments with antibodies against astroglial (GFAP), oligodendroglial (galactocerebroside), and microglial (CD45) cell markers were performed. Analysis of the sections with the LSCM showed that in the control (Fig. 5A–C) and AD cases (Fig. 5D–L), the pERK-immunoreactive glial cells in the white matter were positive for GFAP immunoreactivity but negative for galactocerebroside and CD45 immunoreactivity (not shown). Consistent with the single-labeling data (Fig. 1), double-labeling studies showed that the pERK immunoreactivity in astroglial cells was more intense in early AD (Fig. 5D–I) compared with advanced AD (Fig. 5J–L). In control and early AD cases, approximately 45% of the GFAP-immunolabeled astroglial cells were pERK immunoreactive (Fig. 2D). In contrast, in the intermediate and advanced AD groups, 25% or less of the astroglial cells in the white matter were pERK immunoreactive (Fig. 2D).

To determine the relationship between pERK activation and astrogliosis, immunohistochemical analysis for GFAP was performed on adjacent brain sections from the frontal cortex. This study showed that compared to control cases (Fig. 6A), in early AD cases, there was intense astrogliosis in the white matter.

**FIGURE 2.** Image analysis of levels extracellular-regulated kinase and glial fibrillary acid protein (GFAP) immunoreactivity during the progression of Alzheimer disease (AD). (A) Estimation of the numbers of pERK-immunolabeled glial cells in the white matter; (B) average levels of pERK immunostaining (expressed as optical density) in the glial cells in the white matter; (C) average levels of tERK immunostaining (expressed as optical density) in the glial cells in the white matter; (D) proportion of GFAP immunolabeled astroglial cells in the white matter that displayed pERK immunostaining. *, p < 0.05 compared with control by one-way analysis of variance with post hoc Dunnett’s; **, p < 0.05 compared with intermediate and advanced AD by one-way analysis of variance with post hoc Tukey-Kramer.

**FIGURE 3.** Patterns of pERK immunolabeling in astroglial and neuronal cells in Alzheimer disease (AD). Images are from the midfrontal cortex of sections immunostained with an antibody against pERK, reacted with DAB, and counterstained with hematoxylin. (A) Intense astroglial labeling in the white matter in an early AD case; (B) pERK immunostaining of granular structures in the cytoplasm of pyramidal neurons in the neocortex; (C, D) in advanced AD cases the pERK antibody recognized axons in the vicinity and dystrophic neurites associated with the plaques (arrows). Scale bars = (A, B) 20 μm; (C, D) 30 μm.
matter, where astroglial cells were diffusely distributed along the myelinated axons and around blood vessels (Fig. 6B). In cases with intermediate and advanced AD, only mild levels of GFAP immunoreactivity were detected in the white matter (Fig. 6C). The distribution of GFAP immunoreactivity in the gray matter differed in each of the groups. In normal control cases, there was a relatively even distribution of astroglia in cortical layers I through III (Fig. 6D). In early AD cases, there was moderate astroglial reaction diffusely distributed in layers IV to VI (Fig. 6E). In contrast, in the intermediate and advanced AD cases, abundant GFAP-immunoreactive astroglial cells were detected around the plaques (Fig. 6F).

**Relationship Between Clinical and Neuropathologic Markers of Alzheimer Disease and pERK Immunoreactivity in Astroglial Cells**

To further investigate the relationship between clinical and neuropathologic markers of the progression of AD and pERK expression in astroglial cells, linear regression analysis was performed (Table 2). This study showed that the numbers of pERK-positive astroglial cells in the white matter were strongly correlated with the scores of cognitive performance (Table 2; Fig. 7A), the neuropathologic stage of AD as evaluated by the Braak system (Table 2; Fig. 7B), and the intensity of the astrogliosis in the white matter (Table 2; Fig. 7C). In contrast, pERK activation and astrogliosis in the white matter were correlated to a lesser extent with amyloid plaque formation (Table 2).

**DISCUSSION**

Experiments conducted in this study have uncovered a previously unreported event unique to early AD, namely intense astroglial ERK activation in the white matter that is inversely correlated with the severity of the dementia and the neuropathology. Previous studies in cases with early AD have
FIGURE 5. Laser scanning confocal microscopy of pERK and glial fibrillary acid protein (GFAP) immunoreactivity in Alzheimer disease (AD). Midfrontal cortex sections were double-labeled with antibodies against pERK (red) and GFAP (green); merged signals are in yellow. (A–C) Nondemented control case; (D–I) colocalization of pERK and GFAP in astroglial cells in early AD; (J–L) advanced AD case. Scale bar = 10 μm.
been focused at characterizing the relationship between amyloid plaques (35), neurofibrillary tangles (NFTs) (36), and psychometric testing, but not with astrogliosis or signal transduction alterations. Thus, this is the first study to report cell signaling and neuropathologic events that correspond to the onset of AD pathology. The white matter astrogliosis pathology observed in early AD cases is consistent with previous studies in the aged macaque (37) and APP tg models of AD pathology (38), in which this process is accompanied by synaptic loss. Interestingly, the rise in astrogial pERK immunoreactivity was transient and more characteristic of the early stages of AD. Similarly, in studies of excitotoxicity mediated by kainic acid (26) and of SIV in the central nervous system (39), ERK activation occurs for a restricted period of time and has been linked to neuroprotective pathways. This suggests that in early stages of AD, astrogliosis and ERK activation in the white matter might represent a response to the ongoing axonal damage and presynaptic terminal loss characteristic of the disease (9).

The mechanisms involved in triggering ERK activation in astroglial cells in the white matter in early AD are unclear. One possibility is that damaged axons and neurons might release molecular signals that in turn activate astrocytes. Interestingly, previous studies have shown that neurotrophic factors such as fibroblast growth factor-2 (FGF2), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF) stimulate this signaling pathway leading to proliferation, differentiation, and migration of astroglial cells (40–42). Furthermore, in vitro studies have shown that ERK activation in mature astroglial cells is not associated with proliferative responses after stimulation with conditioned media containing neurotrophic factors (40, 43). Therefore, it has been suggested that ERK activation in nonproliferating mature human astroglia might be linked to the promotion of cell survival (43) and functional activation (44). Moreover, recent studies have shown that neurotrophic factors promote the expression of glutamate transporters via ERK activation (44). In this regard, studies in primary glial cultures have

TABLE 2. Summary of Significant Correlations

<table>
<thead>
<tr>
<th></th>
<th>BL</th>
<th>DRS</th>
<th>MMSE</th>
<th>Braak Stage</th>
<th>NP</th>
<th>Percent Amyloid</th>
<th>NFT</th>
<th>GFAP GM</th>
<th>GFAP WM</th>
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<tr>
<td>pERK+ glial cells r</td>
<td>-0.92</td>
<td>+0.70</td>
<td>+0.85</td>
<td>-0.87</td>
<td>-0.70</td>
<td>-0.60</td>
<td>-0.51</td>
<td>+0.81</td>
<td>+0.73</td>
</tr>
<tr>
<td>WM p</td>
<td>0.0001</td>
<td>0.001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0008</td>
<td>0.006</td>
<td>0.02</td>
<td>0.0001</td>
<td>0.0003</td>
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</table>

Bl, Blessed score; DRS, dementia-rating scale; MMSE, Mini-Mental Status Examination; NP, neuritic plaques; NFT, neurofibrillary tangles; GM, gray matter; WM, white matter.
shown that FGF2, EGF, transforming growth factor-β1 (TGFβ1), and platelet-derived growth factor play a role in affecting the function and expression of glutamate transporters through early growth response signaling (44). Thus, after injury, reactive astrogliosis and ERK activation might limit secondary neuronal damage resulting from excitotoxicity by promoting increased activity of astroglial glutamate transporters. Furthermore, release by activated astrogliosis of trophic factors such as FGF2 might promote neuroprotective effects in vulnerable neuronal populations (45, 46).

Alternatively, astrogliosis and ERK activation might also represent a response to amyloid production and deposition in early stages of AD. In particular, several studies of later stages of AD have shown that fibrillar amyloid activates astrocytes to secrete cytokines and other neuroinflammatory factors (47, 48). However, it is worth noting that in the present study, astrogliosis and astrogial ERK activation was not associated with amyloid deposits, and it was also not correlated with amyloid density or plaque counts. Taken together, this suggests that either soluble amyloid aggregates or other molecules released by injured axons might signal astrogliosis and astroglial activation in the white matter in early AD.

Although these events may represent a reaction to pre-existing pathology, astrogliosis also has the potential to contribute to further neuronal and axonal damage in later stages of the disease progression. Previous studies have emphasized the role of astrogliosis in the pathogenesis of AD (49, 50). The ability of astrogliosis to secrete damaging toxins such as cytokines, complement, reactive oxygen species, nitrogen intermediates, and proteases suggests that the high densities of astrogliosis observed in preclinical AD may potentially contribute greatly to the early pathology of AD (49, 50). Although astrogliosis may represent the activation of an innate protective response, the activation of this cell type has been shown to be pathogenic in a variety of neurologic insults and neurodegenerative disorders (53), including Parkinson disease (54) and HIV dementia (55).

In conclusion, the present study showed that astrogliosis and ERK activation in the white matter occur early in the pathogenesis of AD and is a strong correlate to the severity of the dementia. Therefore, if the astrogliosis response is related to the early neurodegenerative process, it is possible that activation of ERK may be a part of the trophic response to the ongoing synaptic pathology in early AD.

FIGURE 7. Linear regression analysis between pERK expression in the white matter in Alzheimer disease (AD) and neuropsychologic and neuropathologic markers. (A) Correlation between Blessed score and pERK-immunoreactive cell counts in the white matter of AD cases. (B) Correlation between Braak stage and pERK cell counts in the white matter of AD cases. (C) Correlation between glial fibrillary acid protein and pERK cell counts in the white matter of AD cases.

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