Increased proNGF Levels in Subjects with Mild Cognitive Impairment and Mild Alzheimer Disease

SHIYONG PENG, JOANNE WU, ELLIOTT J. MUFSON, PHD, AND MARGARET FAHNESTOCK, PHD

Abstract. Nerve growth factor (NGF) is critical for the regulation, differentiation, and survival of basal forebrain cholinergic neurons that degenerate in the late stage of Alzheimer disease (AD). The precursor of NGF (proNGF) is the predominant form of NGF in brain and is increased in end stage AD. To determine whether this increase in proNGF is an early or late change during the progression of cognitive decline, we used Western blotting to measure the relative amounts of proNGF protein in the parietal cortex from subjects clinically classified with no cognitive impairment (NCI; n = 20), mild cognitive impairment (MCI; n = 20), or mild to moderate AD (n = 19). We found that proNGF increased during the prodromal stage of AD. The amount of proNGF protein was 1.4-fold greater in the MCI group as compared to NCI, and was 1.6-fold greater in mild-moderate AD as compared to NCI, similar to our previous findings of a 2-fold increase in end stage AD. There was a negative correlation between proNGF levels and Mini Mental Status Examination (MMSE) score, demonstrating that the accumulation of proNGF is correlated with loss of cognitive function. These findings demonstrate that proNGF levels increase during the preclinical stage of AD and may reflect an early biological marker for the onset of AD.

Key Words: Alzheimer disease; Cholinergic; Nerve growth factor (NGF); Neurodegeneration; Neurotrophic factor; Parietal cortex; Western blotting.

INTRODUCTION

Dysfunction and degeneration of cholinergic basal forebrain (CBF) neurons occur in end stage Alzheimer disease (AD), with a corresponding reduction in neocortical choline acetyltransferase (ChAT) activity (1). In the late stages of AD, this reduction of cortical ChAT activity correlates with the degree of dementia and thus is thought to be associated with cognitive impairment (2). Recently, several studies have shown that these cholinergic abnormalities are not found in people with mild cognitive impairment (MCI) and early AD (3–6). For example, nucleus basalis neurons containing ChAT and the vesicular acetylcholine transporter are preserved (3), and ChAT activity in the hippocampus and frontal cortex are increased in MCI (5, 6). ChAT levels in these areas decline to normal only during the transition from MCI to AD and fall below normal in late stage AD (5, 6).

The prototypic neurotrophin, nerve growth factor (NGF), maintains and regulates the morphological features and cholinergic phenotype of CBF neurons (7–10). NGF is synthesized in CBF target regions such as the hippocampus and cerebral cortex (11) and is retrogradely transported by CBF neurons (12–14). Blocking NGF availability to CBF target regions results in memory deficits (15, 16). This led to the concept that a deficit in NGF production underlies CBF neuronal degeneration in AD (17).

Although there is no difference in NGF mRNA expression between aged human controls and subjects with AD (18, 19), NGF protein has been reported to be either increased or stable in hippocampus and cortex in the late stages of AD (20–24). NGF protein is synthesized as a precursor, proNGF, which can be cleaved to the mature form of NGF. Most NGF studies have used ELISA or immunohistochemistry, which cannot distinguish between proNGF and mature NGF. These methods most properly detect NGF-like immunoreactivity, although based on studies in the mouse submandibular gland, it has generally been assumed that the species under study is mature NGF. Recently, however, Western blotting experiments revealed that it is proNGF, and not NGF, that is present in the human cortex and is increased in the later stage of this disease (25). Together with observations showing decreases in TrkA message and protein in the basocortical projection system in AD (26–31), the increase in proNGF is consistent with a defect in retrograde transport of proNGF in AD (32, 33). Neurofibrillary tangle density and phosphorylated tau immunoreactivity are increased in the entorhinal and perirhinal cortices in MCI, suggesting that an axonal transport defect occurs early in the disease process (34). Interestingly, impaired retrograde transport of NGF-like immunoreactivity has been implicated in CBF neuronal degeneration in a mouse model of Down syndrome (35). The number of TrkA- and p75NTR-immunoreactive nucleus basalis neurons is reduced in people diagnosed with MCI (29, 30, 36). However, it is unknown whether the accumulation of proNGF seen in late stage AD occurs early in the
Neuropathological Evaluation

For each case, a pathological diagnosis was made by a neuropathologist blinded to the clinical diagnosis. Designations of “normal” (with respect to AD or other dementing processes), “possible” or “probable AD,” and “definite AD” were based on the CERAD and NINCDS/ADRDA criteria (39, 40), which include semiquantitative estimation of neuritic plaque density, an age-adjusted plaque score, and presence or absence of dementia (CERAD, 40). All cases were also assigned neuropathological diagnosis based upon the Braak and Braak staging of neurofibrillary tangle pathology (41) and National Institute on Aging (NIA)-Reagan criteria (42; Table).

Brain Tissue

The procedure for brain tissue collection and processing was described previously (3, 5, 14, 28, 29, 36, 37). Cases were excluded if substantial brain abnormalities were found, including stroke, tumors, or other neurologic disorders. Brains were cut into 1-cm-thick slabs using a plexiglas cutting device and processed as previously reported (5, 24). Briefly, 1 hemisphere was immersion-fixed in 4% paraformaldehyde (in 0.1 M phosphate buffer, pH 7.2) for 48 hours at 4°C and cryoprotected. From the opposite hemisphere, slabs alternating around an anchor slab at the level of the crossing of the anterior commissure were either snap-frozen in liquid nitrogen or placed in 4% paraformaldehyde and paraffin-embedded. The frozen slabs were stored at −80°C. Cortical samples of gray matter were dissected based on fiducial landmarks. All frozen dissections were performed on dry ice to prevent thawing of the tissue.

MATERIALS AND METHODS

Grey matter from postmortem parietal cortex samples harvested at autopsy from participants enrolled in a longitudinal study of aging and AD of Catholic clergy, the Religious Orders Study (ROS) (3, 5), categorized with NCI, MCI, or mild AD were examined (see below). Each participant agreed to an annual detailed clinical evaluation and to brain donation at the time of death. The present investigation is based on 20 subjects meeting clinical criteria for no cognitive impairment (NCI), 20 for mild cognitive impairment (MCI), and 19 for mild to moderate AD (Table). The Human Investigations Committee of Rush Medical Center approved this study.

Clinical Evaluation

Details of the clinical evaluation in the ROS have been published elsewhere (3, 5, 13, 28, 36–38). The NCI group had no evidence of brain disease and was considered cognitively normal. The present MCI population is defined as those subjects rated as impaired on neuropsychological testing but not having dementia (3, 28, 37, 38). A summary of subject demographic data is shown in the Table.

<table>
<thead>
<tr>
<th>Clinical Diagnosis</th>
<th>NCI (n = 20)</th>
<th>MCI (n = 20)</th>
<th>AD (n = 19)</th>
<th>Total (n = 59)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) at death:</td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td>p value</td>
</tr>
<tr>
<td>Number (%) of males:</td>
<td>14 (70%)</td>
<td>7 (35%)</td>
<td>10 (53%)</td>
<td>31 (53%)</td>
<td>0.10*</td>
</tr>
<tr>
<td>Years of education:</td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td></td>
</tr>
<tr>
<td>Number (%) with ApoE ε4 allele:</td>
<td>4 (22%)*</td>
<td>3 (15%)</td>
<td>5 (26%)</td>
<td>12 (21%)*</td>
<td>0.6*</td>
</tr>
<tr>
<td>Mini mental state exam:</td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td></td>
</tr>
<tr>
<td>Postmortem interval (hours):</td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td></td>
</tr>
<tr>
<td>Yield of protein (µg/mg):</td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td></td>
</tr>
<tr>
<td>Distribution of Braak scores:</td>
<td>Low</td>
<td>Intermediate</td>
<td>High</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pathological Reagan Dx (likelihood of AD):</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* ApoE data unavailable for 2 NCI cases; PMI unavailable for 1 NCI case.
* Kruskal-Wallis test.
* Fisher exact test.
* ANOVA with Tukey’s studentized range test for multiple comparison.

Subject Characteristics

Grey matter from postmortem parietal cortex samples harvested at autopsy from subjects clinically categorized with no cognitive impairment (NCI), MCI, or mild to moderate AD.

For each case, a pathological diagnosis was made by a neuropathologist blinded to the clinical diagnosis. Designations of “normal” (with respect to AD or other dementing processes), “possible” or “probable AD,” and “definite AD” were based on the CERAD and NINCDS/ADRDA criteria (39, 40), which include semiquantitative estimation of neuritic plaque density, an age-adjusted plaque score, and presence or absence of dementia (CERAD, 40). All cases were also assigned neuropathological diagnosis based upon the Braak and Braak staging of neurofibrillary tangle pathology (41) and National Institute on Aging (NIA)-Reagan criteria (42; Table).

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Brain Tissue Preparation

Sixty mg of tissue was homogenized directly from the frozen state using a Polytron homogenizer (Brinkmann, Mississauga, Canada) in 0.6 ml of homogenization buffer [0.05 M Tris, pH 7.5, 10 mM EDTA, 0.5% Tween-20, 2 μg/ml aprotinin, 2 μg/ml pepstatin, and 100 μg/ml phenylmethane sulphonyl fluoride (PMSF)] on ice. The homogenates were incubated for 5 to 10 min on ice and then centrifuged at 12,000 g for 15 min at 4°C. Supernatants were collected and protein concentrations were determined using a DC protein assay kit (Bio-Rad Laboratories, Hercules, CA), which is a colorimetric assay for protein concentration following detergent solubilization. A single control sample (0.1–0.3g) used on each gel was homogenized in 5 ml homogenization buffer/g tissue and treated as above.

Antibodies and Chemicals

The primary antibodies were an affinity-purified rabbit polyclonal anti-NGF IgG from Dr. M. D. Coughlin (25, 43), monoclonal NGF antibody 27/21 (Roche Diagnostics, Laval, Canada), and monoclonal anti-β-actin (Sigma, St. Louis, MO). HRP-conjugated donkey anti-rabbit IgG and sheep anti-mouse (Amersham Biosciences, Baie d’Urfé, Canada) were used as secondary antibodies. Aprotinin, pepstatin, and PMSF were purchased from Sigma (Oakville, Canada), Boehringer-Mannheim (Laval, Canada), and Life Technologies Inc. (Burlington, Canada), respectively. The 2.5S form of NGF (mature NGF) was prepared from murine submandibular gland NGF (44) and further purified by reverse-phase high performance liquid chromatography (45). Polyvinylidene difluoride (PVDF) membrane was obtained from Amersham Biosciences. Precast acrylamide gels, prestained broad-range protein molecular weight markers, and DC protein assay reagents were purchased from Bio-Rad Laboratories Ltd. All other chemicals were reagent grade.

Western Blotting

Samples were separated on 17% SDS-polyacrylamide gels at 120V for 60 min as previously described (25). Briefly, proteins were transferred onto PVDF membranes in transfer buffer (25 mM Tris, pH 8.0, 133 mM NaCl, 0.2% (v/v) Tween-20) with 10% (w/v) Carnation nonfat milk powder. The blots were incubated with 1:1,000 primary antibody (affinity-purified rabbit polyclonal anti-NGF IgG raised against whole murine 2.5S NGF), which recognizes both mature NGF and proNGF (25, 43), overnight at 4°C in TBS-T. After washing in TBS-T, membranes were incubated in 1:5,000 HRP-conjugated donkey anti-rabbit secondary antibodies in TBS-T with 5% nonfat milk powder for 1 hour at room temperature. Finally, a chemiluminescence system (ECL®, Amersham Biosciences) was used to detect immunoreactive protein.

After NGF Western blotting, each blot was re-probed with 1:20,000 monoclonal anti-β-actin as the primary antibody and sheep anti-mouse as the second antibody for detecting the corresponding β-actin level of each sample. To avoid interference between the 32-kDa proNGF band and the 46-kDa β-actin band, HRP activity was allowed to decay for at least 4 hours before performing Western blotting for β-actin.

Densitometry and Statistical Analysis

Each Western blot contained a standard curve consisting of 30 to 120 μg protein per lane of a single human parietal cortex sample. The amount of protein from samples in the NCI, MCI, and mild AD groups (60 μg) was chosen so as to fall within the linear range of the standard curve for both proNGF and β-actin immunoreactivity. The volume of the immunoreactive bands was determined by densitometry of films using an MCID image analysis system (Brock University, St. Catherine’s, Canada) with a Microtek Scanner and Scion Image Beta 4.01 Acquisition and Analysis software (Scion Corporation, Frederick, MD). Local background was subtracted from each band and each sample was analyzed 3 times in separate experiments. The standard curves for both proNGF and β-actin immunoreactive bands were used to normalize pixel values between blots.

Subject characteristics and proNGF and β-actin levels were compared among the clinical diagnosis groups by the Kruskal-Wallis test, Fisher exact test, and, for pair-wise comparisons, the analysis of variance (ANOVA) with post hoc Tukey test. ProNGF and β-actin levels were log-transformed in the ANOVA model. Correlation between clinical variables and proNGF measurements were performed using the Spearman rank correlation or Wilcoxon rank-sum test. The level of statistical significance was set at p = 0.05 (2-sided).

Apolipoprotein E Genotyping

Apolipoprotein E (ApoE) genotyping was performed on all subjects according to a previously described procedure (14, 28, 30, 46–48). Briefly, ApoE restriction isotyping of genomic DNA isolated from plasma was performed by polymerase chain reaction (PCR) amplification using a GNOME® DNA Kit (Qiogene, Carlsbad, CA). After PCR amplification, 40 μl of each product was digested with 20 units of HhaI at 37°C overnight and then electrophoresed on an 8% polyacrylamide gel, stained with ethidium bromide, and photographed under ultraviolet light. PCR products were purified over a DNA-binding matrix with a GENECLEAN® kit (Qiogene). DNA sequencing using PCR was performed using a Stratagene Cyclist® kit (Stratagene, La Jolla, CA) with 32P labeled dATP incorporation. Samples were electrophoresed through an 8 M urea-6% polyacrylamide gel containing 25% formamide, dried, and autoradiographed.

RESULTS

Case Demographics

There was no difference in age, sex, educational level, prevalence of ApoE e4 allele, or yield of protein per gram tissue across the 3 clinical groups (Table). The MMSE scores of the AD group (15.6 ± 7.9) were significantly lower than those of the MCI (26.4 ± 2.8) and NCI (27.9 ± 1.6) groups (p < 0.0001). The NCI group had a significantly greater postmortem interval (PMI) than the other 2 groups (p = 0.0082) (Table). We have previously shown that human CNS proNGF protein levels are stable up to at least 20 hours postmortem (25).

Specificity of the Primary Antibody

Western blotting of human parietal cortical homogenates with a polyclonal anti-NGF antibody gives 2 bands...
Fig. 1. NGF-immunoreactive bands. Sixty µg of early AD sample homogenate (Lane 1) and 0.7 ng 2.5S NGF (Lane 2) were loaded on each blot. A: Western blotting carried out as described in Materials and Methods, using the polyclonal anti-NGF antibody. B: As in (A), but polyclonal anti-NGF antibodies were incubated overnight with a 60-fold molar excess of murine 2.5S NGF at 4°C before adding to the blot. C: As in (A), but using monoclonal antibody 27/21. D: As in (C), but with a longer exposure to equal the intensity of the 32-kDa band in (A).

at 81 kDa and 32 kDa (Fig. 1A). The 32-kDa band has been previously identified as proNGF (25, 49, 50). The higher molecular weight band at ~81 kDa is similar to a ~60 kDa glycosylated form of proNGF previously identified in both recombinant human preparations and rat tissues (49). This high molecular weight NGF is thought to be an intracellular form only (51) and will not be examined in this study. Mature NGF was not detected in any human samples, in agreement with our previous findings (25).

Western blotting of human cortical homogenates with monoclonal antibody 27/21 gives a strong band at 32 kDa and multiple weaker bands (Fig. 1D). The sensitivity of the 27/21 antibody is substantially weaker than for the polyclonal antibody (compare Fig. 1A and 1C), and therefore only the polyclonal antibody was used for quantification in this study.

To demonstrate the specificity of the immunoreactive bands, an excess (compared to antibody) of 2.5S NGF was added at the time of incubation with the primary antibody and incubated overnight at 4°C. The 81-kDa, 32-kDa proNGF, and 13.2-kDa mature NGF bands were all blocked by the addition of excess 2.5S NGF (Fig. 1B), demonstrating specificity. This antibody has also been shown not to cross-react with NGF-like proteins such as BDNF and NT-3 (25).

Relative levels of proNGF in the parietal cortex samples were determined using Western blotting. Each gel was loaded with 60 µg total protein for each NCI, MCI, or AD sample. Each gel also contained a standard curve consisting of 4 different total protein concentrations (i.e. 30, 60, 90, and 120 µg) of 1 parietal cortex NCI sample common to all blots. Figure 2 shows a representative blot of the 32-kDa proNGF band with the 46-kDa β-actin immunoreactive band in each sample shown below. β-actin was used as an internal control for each of the samples, since its mRNA and protein levels have been demonstrated to be unchanged between aged control and AD brain (52, 53).

ProNGF Levels

The intensity of the 32-kDa NGF-immunoreactive band (proNGF) was significantly increased in AD and MCI compared to NCI samples (Fig. 3; ANOVA, post hoc Tukey test, p < 0.0001 for AD compared to NCI, p = 0.0008 for MCI compared to NCI). There were no differences in β-actin levels between clinical groups (Fig. 3; ANOVA, p > 0.05). When normalized to the β-actin
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Fig. 2. Representative Western blot for proNGF and β-actin. Lane 1: 0.7 ng mature NGF. Lanes 2–5: a single human cortex sample with increasing total protein loaded of 30, 60, 90, and 120 μg. Lanes 6–7: 60 μg of 2 NCI samples; Lane 8: 60 μg of 1 MCI sample; Lane 9: 60 μg of 1 AD sample. Western blotting was carried out as described in Materials and Methods for proNGF and β-actin.

Fig. 3. ProNGF and β-actin levels in NCI, MCI and mild AD samples. Error bars represent SEM. *p < 0.001.

Our previous studies have shown that proNGF, the precursor to NGF, is the predominant form of NGF in the human brain and is increased 2-fold in parietal cortex between normal and late stage AD (25). To determine whether this increase in proNGF occurs in the prodromal stages of AD, we evaluated proNGF protein in postmortem parietal cortex from subjects clinically classified as NCI, MCI, or early AD. We found that the accumulation of proNGF occurs even in the earliest stages of cognitive decline. The accumulation of proNGF is independent of age, sex, educational level, yield of total protein, or β-actin levels, and is not correlated with PMI. The lack of a correlation between any ApoE e4 genotype and proNGF levels is interesting, since ApoE e3 and e4 alleles are associated with a greater reduction in cortical ChAT activity and CBF neuron loss in end stage AD (54–57).

One of the earliest correlates of AD was traditionally thought to be the loss of CBF neurons and the corresponding reduction in ChAT activity (1, 58). Recent investigations demonstrate that ChAT activity increases in hippocampus and superior frontal cortex in MCI, normalizes again in the early stages of AD, and then declines below normal only in late stage AD (3, 5, 6). Since mature NGF is known to regulate the cholinergic phenotype of CBF neurons (8) and since proNGF exhibits many of the neurotrophic properties attributed to mature NGF (59), one might expect proNGF levels to follow a similar pattern. Although proNGF levels increase in MCI similar to that reported for ChAT (5), it does not mirror the subsequent significant reduction in cortical ChAT levels seen in AD.
Fig. 4. Ratio of proNGF/β-actin in NCI, MCI, and mild AD samples. A: Mean ± SEM. *p < 0.0005. B: Box plot: The horizontal line in the middle of the box indicates the median, while the top and bottom borders of the box mark the seventy-fifth and twenty-fifth percentiles, respectively. The vertical lines above and below the box extend to the farthest observation (denoted by the bracket) that is within 1.5 times the distance between the quartiles (inter-quartile range). Circles denote outliers or observations falling outside of this range. NCI vs MCI vs mild AD, p < 0.0001, Kruskal-Wallis test.

Fig. 5. Relationship between proNGF levels and Mini mental state exam (MMSE) scores, by group. A: ProNGF vs MMSE score. B: ProNGF/β-actin ratio vs MMSE score. AD (○), MCI (▲), NCI (□).

in the late stage of AD (4, 5). A possible reason for the lack of correlation between proNGF and ChAT levels is the degeneration of entorhinal cortex layer 2 stellate neurons early in the disease (37, 60), which results in elevated levels of NGF-like immunoreactivity (61) and induces sprouting of septal cholinotrophic projections into the denervated hippocampus (62, 63). This early compensatory response to hippocampal denervation may then be followed by CBF neurodegeneration. Thus, although proNGF accumulation may provide some compensatory neurotrophic activity by upregulating ChAT activity in the prodromal stage of AD, it may be ineffective later in the disease progression.

ProNGF accumulation in CBF target sites, and subsequent atrophy of CBF cell bodies, may be due to failed retrograde transport following early loss of its receptors, TrkA and p75NTR, on CBF neurons (29, 30, 36). CBF dysfunction in AD may also be associated with a reduction or inactivation of TrkA-mediated signaling cascades that underlie CBF basocortical and/or septohippocampal activity (64), resulting in accumulation of proNGF in target tissues. TrkA expression is known to be regulated by mature NGF (64). ProNGF has been shown to bind and activate TrkA and its signaling cascade (59), although its ability to regulate TrkA levels has not been investigated. However, it is possible that a loss of proNGF-mediated...
signaling may further reduce TrkA receptor numbers within CBF neurons, affecting cell survival.

A recent study using an ELISA procedure to detect NGF-like immunoreactivity in several human cortical areas, including samples from many of the same parietal cortex cases used in the present experiments, failed to detect a difference in NGF-like immunoreactivity between NCI, MCI, and early and late stage AD subjects (24). Curiously, that investigation used the same NGF monoclonal antibody 27/21 (24) which, as demonstrated here, detects only proNGF in human brain homogenates by Western blotting. One possible reason for the discrepancy between these 2 studies is that the NGF monoclonal antibody 27/21 used for the ELISA is less sensitive and displays more non-specific binding in Western blots than the NGF polyclonal antibody used for the present study (Fig. 1; 25). In addition, the single epitope recognized by the monoclonal antibody is intact in the denatured proNGF present on the Western blot but not in mature NGF, whereas the polyclonal antibody recognizes multiple epitopes, some present in proNGF and some in mature NGF. An ELISA assay using a different antibody, such as the polyclonal antibody used here, might succeed in detecting differences in NGF-like immunoreactivity between clinical groups.

The biological consequences of the accumulated proNGF seen in this study have yet to be determined. Many investigators have shown that proNGF exhibits neurotrophic activity similar to mature NGF (59, 66–69), and our recent data demonstrates that it binds to and activates TrkA (59, 69). However, the retrograde transport of proNGF has not yet been demonstrated. In contrast, it has been reported that proNGF exhibits apoptotic activity (70). In that study, a modified, histidine-tagged proNGF was shown not to bind TrkA, but to bind p75NTR with high affinity. Binding of mature NGF to p75NTR in the absence of TrkA binding activates apoptotic pathways (71). Therefore, the accumulation of proNGF in the AD brain has been interpreted as a possible contributor to cell death in AD (72). This is despite the fact that significant amounts of proNGF, and little or no mature NGF, are found in normal brain as well (25). Nevertheless, whether proNGF in vivo binds p75NTR leading to apoptosis (70) or binds TrkA, leading to neurotrophic activity (59, 69), is unclear. Until this is determined, the effects of accumulated proNGF in AD remain controversial (69).

We demonstrate in this study a negative relationship between a diagnostic index for dementia, the MMSE, and proNGF levels; the accumulation of proNGF is correlated with loss of cognitive function. ProNGF levels increase during the preclinical stage of AD and may serve as an early biological marker for the onset of AD.

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