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Cerebrospinal Fluid proNGF: A Putative Biomarker for Early Alzheimer's Disease

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Abstract

The discovery of biomarkers for the onset of Alzheimer's disease (AD) is essential for disease modification strategies. To date, AD biomarker studies have focused on brain imaging and cerebrospinal fluid (CSF) changes in amyloid- β (A β) peptide and tau proteins. While reliable to an extent, this panel could be improved by the inclusion of novel biomarkers that optimize sensitivity and specificity. In this study, we determined whether CSF levels of the nerve growth factor (NGF) precursor protein, proNGF, increased during the progression of AD, mirroring its up regulation in postmortem brain samples of people who died with a clinical diagnosis of mild cognitive impairment (MCI) or AD. Immunoblot analysis was performed on ventricular CSF harvested from participants in the Rush Religious Orders Study with an antemortem clinical diagnosis of no cognitive impairment (NCI), amnesic MCI (aMCI, a putative prodromal AD stage), or mild/moderate AD. ProNGF levels were increased 55% in aMCI and 70% in AD compared to NCI. Increasing CSF proNGF levels correlated with impairment on cognitive test scores. In a complementary study, we found that proNGF was significantly increased by 30% in lumbar CSF samples derived from patients with a clinical dementia rating (CDR) of 0.5 or 1 compared to those with a CDR = 0. Notably, proNGF/A β ₁₋₄₂ levels were 50% higher in CDR 0.5 and CDR 1 compared to CDR 0 controls. By contrast, ELISA measurements of CSF brain-derived neurotrophic factor (BDNF) did not distinguish aMCI from NCI. Taken together, these results

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

suggest that proNGF protein levels may augment the diagnostic accuracy of currently used CSF biomarker panels.

Keywords

Alzheimer's disease; amyloid; biomarker; cerebrospinal fluid; mild cognitive impairment; proNGF; tau

INTRODUCTION

Alzheimer's disease (AD) is believed to have an extensive preclinical stage [1]. Neuropathological examinations of older people who died with a clinical diagnosis of no cognitive impairment (NCI) or mild cognitive impairment (MCI) often reveal similar pathological signatures to those with frank AD [2–6]. Hence, the identification of individuals in the preclinical stages of AD is essential for the timely administration of disease modifying therapies. Several initiatives have been undertaken to define a panel of diagnostic and prognostic cerebrospinal fluid (CSF) biomarkers that identifies people at the earliest stages of the disease, particularly those with amnesic MCI (aMCI) who preferentially transition to AD [1, 7]. Current multi-center efforts such as the AD Neuroimaging Initiative (ADNI) have focused on detecting biomarkers associated with changes in amyloid- β ($A\beta$) peptide and tau protein levels in the CSF of people at varying stages of AD, since these markers may reflect underlying cerebral pathology [8]. In particular, changes in the ratio of total tau or tau phosphorylated at residue T181 (phospho-tau) to $A\beta_{42}$ are predictive for longitudinal changes in cognitive measures [9–12]. However, the inherent variability of AD neuropathology in the aged NCI, MCI and diseased brain is also reflected by variation of these CSF markers, resulting in equivocal specificity and sensitivity for identifying at-risk individuals. This highlights the need to augment a CSF biomarker panel with novel proteins to improve diagnostic accuracy in longitudinal studies [13–15].

During our ongoing studies of the involvement of nerve growth factor (NGF) in AD pathogenesis, we discovered that protein levels of the NGF precursor, proNGF, were selectively increased in postmortem neocortex [16] and hippocampus [17] of subjects who died with a clinical diagnosis of MCI or mild AD compared to NCI, respectively. Moreover, increased proNGF protein levels in these brain regions correlated with poorer performance on antemortem cognitive tests [16, 17]. Biomechanistic studies indicate that proNGF binds with low affinity to TrkA and with high affinity to the panneurotrophin receptor p75^{NTR} [18, 19]. It has been suggested that reduced TrkA in AD [20] results in a shift from proNGF-mediated survival to pro-apoptotic signaling [21, 22]. Moreover, the loss of TrkA is associated with an increase in proNGF during disease progression, further exacerbating the pathophysiological shift from cell survival to neurodegeneration [21, 23, 24]. Based on these findings, we tested whether CSF proNGF levels are an early marker of cognitive impairment using two sample sources: 1) ventricular CSF obtained postmortem from Rush Religious Orders Study (RROS) participants who died with an antemortem clinical diagnosis of NCI, aMCI, or mild/moderate AD, and 2) antemortem lumbar CSF collected from Washington

University Knight AD Research Center subjects clinically diagnosed by the Clinical Dementia Rating (CDR) scale as CDR 0 (no dementia), CDR 0.5 (MCI or very mild AD), or CDR 1 (mild AD). In complementary CSF studies, we also measured levels of brain-derived neurotrophic factor (BDNF) immunoreactivity, which we have previously shown to be decreased in the brains of MCI and AD subjects [25, 26].

MATERIALS AND METHODS

Subjects and CSF Sampling

Postmortem ventricular CSF was extracted from the posterior ventricle using a 50 ml syringe attached to a 30 gauge needle from 66 participants in the RROS [27] who were clinically diagnosed within a year of death with NCI (n = 26), aMCI (n=20) or mild/moderate AD (n = 20; see Table 1 for demographics). Details of clinical evaluations and diagnostic criteria have been previously published [6, 27–29]. In addition to an annual clinical evaluation, subjects were administered the Mini Mental State Exam (MMSE) and a battery of 19 neuropsychological tests referable to multiple cognitive domains (*e.g.*, episodic memory, perceptual speed) [6]. A Global Cognitive Score (GCS), consisting of a composite z-score, was calculated from this test battery for each participant [27]. The aMCI population was defined as subjects who exhibited cognitive impairment on neuropsychological testing of episodic memory alone, but did not meet the clinical criteria for AD recommended by the joint working group of the National Institute of Neurologic and Communicative Disorders and Stroke/AD and Related Disorders Association (NINCDS/ADRDA) [27, 30]. We also tested CSF collected by lumbar puncture from age-matched subjects (age > 60 years) who were clinically examined using the CDR scale and classified as CDR 0, CDR 0.5, or CDR 1 (Table 2) [3]. In addition to age and clinical diagnosis, data was obtained for gender, years of education, medication history, and apoE genotype. Finally, we also tested for proNGF levels in 13 samples of lumbar CSF obtained from early stage Parkinson's disease (PD) patients (60% male, mean age = 60.1 ± 10.3, mean Hoehn and Yahr scale = 1.45 ± 0). All samples from each cohort were frozen at –80°C until processed for analysis.

Frozen CSF samples were thawed on ice, spiked with a protease inhibitor cocktail (Sigma, St. Louis, MO), and screened for blood contamination using Hemastix reagent strips (Bayer, Elkhart, IN) to detect hemoglobin (Hb). Using serial dilutions of a control blood sample with a known Hb concentration, we estimated that the cut-off value for detectable Hb contamination was ~0.6 mg/dL or ~200 erythrocytes/μL. CSF samples were then microaliquotted and stored at –80° C. Samples were analyzed only if Hb concentration fell below the cut-off value. Lumbar CSF levels of Aβ₁₋₄₂, total tau, and phospho-tau (pT181 tau), were provided by the Washington University Knight AD Research Center [9].

Neuropathological Analysis

Neuropathological evaluation was available only for the RROS cases, including visualization and quantitation of amyloid plaques and neurofibrillary tangles (NFTs) in multiple cortical and hippocampal regions using antibodies directed against amyloid-β peptide (Aβ; 4G8, Covance, AZ), thioflavin-S, modified Bielschowsky silver stain, and pathological tau (PHF1, Gift from Dr. Peter Davies) [6, 27], respectively. Additional sections were stained for Lewy

bodies using antibodies directed against ubiquitin and α -synuclein. Exclusion criteria included argyrophilic grain disease, frontotemporal dementia, Lewy body disease, mixed dementias, Parkinson's disease, and large strokes. A board certified neuropathologist blinded to the clinical diagnosis performed the neuropathological evaluation. Neuropathological designations were based on NIA-Reagan, CERAD and Braak staging criteria [31–33]. The new guidelines for the neuropathologic evaluation of AD require data related to transactive response DNA-binding protein of 43 kDa (TDP-43) inclusions [34]. The Rush neuropathology core is in the processes of evaluating all RROS cases for TDP-43 inclusions. Amyloid burden and apolipoprotein E (ApoE) genotype were determined for each case as described previously [6, 27].

CSF proNGF Immunoblot Analysis

To ensure specificity of proNGF measurements among other potential NGF moieties in the CSF, we performed western blotting of CSF samples. Proteins from 100 μ l CSF were precipitated with methanol/chloroform and resuspended in Laemmli sample buffer. Volume equivalents of 25 μ l CSF were separated by SDS-PAGE (10% acrylamide) and electroblotted to polyvinylidene difluoride membranes (Immobilon P, Millipore, Billerica, MA). Membranes were cut at ~50 kDa to remove high-molecular-weight proteins and then blocked with Tris-buffered saline (TBS)/0.05% Tween-20/5% nonfat milk powder for one hour at ambient room temperature (RT) and probed overnight at 4°C with affinity-purified polyclonal rabbit antiserum raised against human NGF [H-20, Santa Cruz (Santa Cruz, CA), 1:100 in blocking buffer] [16–18]. Immunoreactive proteins were visualized by enhanced chemiluminescence (Pierce, ThermoFisher, Waltham, MA) on a Kodak Image Station 440CF (Perkin-Elmer, Waltham, MA). Three independent samples from each case were analyzed in separate assays. An operator blinded to sample characteristics performed the quantitative analysis using Kodak 1D image analysis software.

NGF and BDNF ELISA

NGF-immunoreactive protein levels were measured by two-site, sandwich ELISA using an affinity-purified rabbit polyclonal antibody MC-51, following protocols from Boehringer Mannheim, with fluorescent substrate 4-methylumbelliferyl β -D-galactoside. Fluorescence was read on a Safire plate reader (Tecan, Männedorf, Switzerland) with background subtracted. Samples were measured in duplicate on separate plates.

BDNF levels in ventricular CSF were measured in duplicate using the human BDNF DuoSet ELISA (R&D Systems, Minneapolis, MN) according to the manufacturer's protocols, but using Thermo Scientific QuantaBlu Fluorogenic Peroxidase substrate (Life Technologies, Burlington, Canada) in place of the colorimetric substrate tetramethylbenzidine- H_2O_2 to increase sensitivity. Three samples were removed from analysis based on high total protein levels (>3.5 mg/ml), and one outlier in the MCI group was removed because BDNF levels were more than 2 standard deviations higher than the mean.

Statistical Analysis

Demographic variables were compared by one-way ANOVA with Bonferroni correction (age, education, postmortem interval, cognitive test scores), Fisher's Exact test (% males, %

apoE ϵ 4 status) or Kruskal-Wallis testing (neuropathological diagnosis). Ventricular and lumbar CSF proNGF levels as well as previously recorded lumbar CSF A β ₄₂ and tau levels were compared by one way ANOVA with Bonferroni correction. Spearman rank correlation analysis was used to test for associations between ventricular CSF proNGF levels and clinical and neuropathological variables. Linear regression was performed to test for interactions between lumbar CSF proNGF levels and gender, apoE ϵ 4 status, and use of cholinesterase inhibitors or antidepressants. Statistical significance was set at $\alpha = 0.05$.

RESULTS

Case Demographics

Table 1 shows characteristics of the RROS subjects by clinical diagnostic group. Groups did not differ by age, education, gender, postmortem interval, or presence of an apoE4 allele. Post hoc analyses revealed that AD cases had lower MMSE scores compared to both aMCI and NCI cases ($p < 0.002$), while the latter two groups did not differ statistically (Table 1). Global Cognitive Scores were also significantly lower in the AD compared to NCI and aMCI groups ($p < 0.001$). The subjects displayed considerable heterogeneity with respect to neuropathological criteria. Neuropathological evaluation revealed that 92% of NCI and 100% of the aMCI cases were classified as Braak stages I-IV, whereas 80% of the AD cases were classified as Braak stages III-VI (Table 1). Using NIA-Reagan criteria, 96% of NCI and 95% of MCI cases were classified as low or intermediate likelihood of AD, whereas 85% of AD cases were classified as intermediate to high likelihood of AD. The AD cases had more advanced CERAD diagnoses compared to aMCI ($p < 0.005$), but differences between AD and NCI did not reach statistical significance (Table 1).

Table 2 shows characteristics of the Washington University Knight AD Research Center subjects by clinical diagnostic group. The subjects did not differ by age (mean age $\pm$ SD). However, education in years was significantly lower in the CDR 1 mild AD group (14 ± 3) compared to the CDR 0 (17 ± 3) ($p < 0.01$). The CDR 1 group contained a higher percentage of male subjects ($p < 0.001$). The CDR 0.5 and CDR 1 also differed from CDR 0 with respect to the number of subjects carrying at least one apoE ϵ 4 status ($p < 0.001$, Table 2).

Ventricular CSF proNGF Protein Levels in NCI, aMCI, and Mild/Moderate AD

Western blotting of ventricular CSF from RROS cases revealed a consistent increase in the ~32 kDa proNGF-immunoreactive band in aMCI and AD samples (Fig. 1A) [16–18]. Quantitative analysis revealed a significant 55% increase in proNGF levels in CSF samples obtained from aMCI compared to NCI subjects ($p < 0.001$, Fig. 1B). Although proNGF protein levels were increased 70% in AD compared to NCI ($p < 0.001$, Fig. 1B), they did not differ significantly from aMCI subjects. Mature NGF was variably detectable in ventricular CSF but could not be reliably quantified.

Ventricular CSF proNGF Associations with Clinical and Neuropathological Data

Spearman rank correlations revealed a strong, significant inverse association between increasing ventricular CSF proNGF levels and cognitive deterioration measured by MMSE ($r = -0.62$, $p < 0.0001$; Fig. 2A) and GCS ($r = -0.7$, $p < 0.0001$; Fig. 2B) for the RROS

cohort. By contrast, proNGF levels were not associated with postmortem interval (Fig. 2C) or AD neuropathological diagnostic criteria as measured by Braak NFT staging, CERAD diagnosis, or NIA-Reagan likelihood of AD (Table 3).

Lumbar CSF proNGF Levels in CDR-Scored and PD Cases

Western blotting of lumbar CSF samples also revealed a proNGF-immunoreactive band migrating at ~32 kDa (Fig. 3A). Quantitative analysis revealed a significant 30% increase in proNGF levels in subjects clinically diagnosed as CDR 0.5 and CDR 1 compared to CDR 0 controls ($p = 0.008$; Fig. 3B). By contrast, there were no differences in levels of A β_{1-42} , total tau, phospho-tau, or phospho-tau/A β_{1-42} among the groups, whereas the ratio of total tau/A β_{1-42} levels was 50% higher in CDR 1 subjects compared to CDR 0 ($p < 0.01$; Fig. 3B). To gauge whether the inclusion of proNGF might improve the reliability of these current biomarkers, we calculated proNGF/A β_{1-42} , proNGF/total tau, and proNGF/phospho-tau in the same cases. Notably, proNGF/A β_{1-42} levels were 50% higher in CDR 0.5 and CDR 1 compared to CDR 0 controls ($p = 0.001$; Fig. 3B), whereas proNGF/total tau and proNGF/phospho-tau were not different between the groups. Moreover, lumbar CSF proNGF levels were not influenced by gender ($r^2 = 0.03$, $p = 0.33$), the presence of an apoE $\epsilon 4$ allele ($r^2 = 0.03$, $p = 0.36$), use of cholinesterase inhibitors ($r^2 = 0.0003$, $p = 0.93$), or use of antidepressant medication ($r^2 = 0.003$, $p = 0.77$). We also evaluated proNGF levels in lumbar CSF samples from PD cases. However, we were unable to consistently detect the proNGF band on the western blots (data not shown) suggesting possible technical caveats associated with these samples.

NGF AND BDNF ELISA LEVELS

Although lumbar CSF NGF-immunoreactivity was undetectable, very low levels were detected in ventricular CSF. The amount of NGF-immunoreactivity detected in NCI subjects varied from 8-33 pg/ml, which was at the lower limits of the assay. This variability and low sensitivity of the NGF ELISA precluded a systematic statistical analysis of NGF immunoreactivity (data not shown).

BDNF-immunoreactive levels were assayed in RROS ventricular CSF samples. Although no statistically significant differences were found among NCI, MCI and AD subjects (one-way ANOVA, $p = 0.26$), BDNF levels showed a trend towards lower levels in AD compared to NCI subjects (unpaired t-test, NCI vs AD, $p = 0.048$) (Fig. 4A). BDNF levels were 20-25% higher in female than in male subjects (Fig. 4B), in agreement with a previous report [35].

DISCUSSION

The disappointment of Phase III clinical trials for AD to date suggest that the mild/moderate AD patients selected for these studies are already on an inexorable disease trajectory. Therefore, AD therapeutics may have disease-modifying properties only if administered during the preclinical or prodromal stages of the disease. Reliable biomarkers to identify people in these incipient stages of disease will be essential for therapeutic efficacy. Current AD CSF biomarkers utilizing A β and tau measurements are good at separating control from AD but equivocal for identifying subjects with aMCI [36]. Here, we show that protein levels

of proNGF are significantly elevated in ventricular CSF from aMCI subjects and in lumbar CSF of CDR 0.5 subjects relative to aged controls. Moreover, CSF proNGF levels were highly correlated with MMSE and GCS across subjects clinically diagnosed with NCI, aMCI, or mild/moderate AD. Hence, the inclusion of proNGF protein levels may improve the reliability of current biomarkers to detect early stages of AD. In support of this hypothesis, proNGF/A β_{1-42} levels were significantly greater than proNGF alone in CDR 0.5 and CDR 1 compared to CDR 0 subjects. By contrast, A β_{1-42} levels alone were not different across the groups and total tau/A β_{1-42} levels only distinguished the CDR 0 and CDR 1 groups.

The present findings report an increase in proNGF levels in lumbar CSF from AD patients relative to controls, lending support to a previous study [37]. In this earlier report, the NGF moiety measured migrated at ~30-32 kDa on western blots, suggesting that the authors were measuring proNGF and not mature NGF. This result was in contrast to previous ELISA studies reporting increased mature NGF in the CSF of AD subjects [38, 39]. Herein, we were only able to detect mature NGF in a small number of CSF samples, in line with data showing that proNGF is the predominant NGF protein species found in postmortem human brain [18].

The present study supports a growing list of candidate biomarkers for AD derived from CSF, including apolipoprotein isoforms [40, 41], prostaglandin D2 synthase: transthyretin dimers [42], synuclein isoforms [43], ubiquitin [44, 45], SNAP-25 [46], and neurogranin [47]. Although CSF BDNF ELISA distinguishes NCI from AD in this and other studies [35], it does not distinguish NCI from aMCI groups. This may be, in part, due to the plasticity of BDNF levels in response to external and internal factors such as exercise and depression, respectively [48]. In addition, plasma phospholipid profiles may complement the current CSF panel along with imaging and clinical variables [49]. These fluid biomarkers represent several putative mechanistic pathways underlying AD progression. Notably, increased CSF proNGF levels mirror the proNGF increase seen in the neocortex and hippocampus in MCI and AD subjects [16, 18]. The functional consequences of proNGF accumulation during the prodromal stages of AD are unclear. *In vitro* studies suggest that recombinant proNGF binds the high-affinity NGF receptor TrkA and promotes neuronal survival and neuritic outgrowth similar to mature NGF, but is approximately five-fold less active than the mature NGF peptide [50, 51]. On the other hand, proNGF binding to p75^{NTR} has been shown to promote apoptotic signaling [19]. We have previously shown that TrkA protein levels are reduced in the cortex in early stage AD whereas p75^{NTR} levels remain stable [20]. Given that both reduced TrkA levels and increased proNGF levels in the cortex were associated with poorer antemortem cognitive performance [16, 20], the accumulation of cortical proNGF may reflect a shift from pro-survival to pro-apoptotic signaling [21, 23, 24]. Hence, we posit that the increase in CSF proNGF levels in prodromal AD clinically diagnosed as aMCI or CDR 0.5 is an early pathobiological marker for disease onset. A caveat to our findings is that the sample sizes were limited and the study was cross-sectional by design. Future efforts will involve improving the sensitivity of a proNGF ELISA for high throughput screening of antemortem CSF on a larger scale and a longitudinal study with adequate power for gauging the utility of including proNGF in a CSF biomarker panel for the onset of AD.

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Biographies

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Fig. 1. CSF proNGF levels are increased in aMCI and AD relative to NCI

A) Representative immunoblot showing proNGF immunoreactivity in ventricular CSF derived postmortem from NCI, aMCI, and AD subjects. B) Plot shows individual CSF proNGF levels in the subjects examined. Quantitative analysis revealed a significant 50-60% increase in proNGF levels in CSF samples obtained from aMCI compared to NCI subjects and a 70% increase in AD compared to NCI. CSF proNGF levels did not differ between aMCI and AD. Units on the Y-axis are arbitrary based on relative densitometric measurements. ***, $p < 0.001$ via one way ANOVA with Bonferroni correction.

Fig. 2. Ventricular CSF proNGF levels correlate with cognitive performance

A) Scattergram shows a significant association between increasing ventricular CSF proNGF levels and poorer performance on the MMSE ($r = -0.62$, $p < 0.0001$). Tests were administered within a year of death. B) Scattergram shows a significant association between increasing CSF proNGF levels and global cognitive deterioration as measured by global composite z-score (GCS) consisting of 19 neuropsychological tests administered to Religious Orders Study participants ($r = -0.74$, $p < 0.0001$). C) Scattergram shows the lack of association between CSF proNGF levels and PMI ($r = 0.1$, $p = 0.5$), indicating that ventricular CSF proNGF levels were not influenced by autolysis time. Units on the Y-axis are arbitrary based on relative densitometric measurements.

Fig. 3. CSF proNGF levels are increased in lumbar samples from CDR 0.5 and CDR 1 subjects relative to CDR 0

A) Representative immunoblot showing proNGF immunoreactivity in lumbar CSF derived postmortem from subjects diagnosed as CDR 0, CDR 0.5, or CDR 1. Mature NGF was not detected. B) Plots show proNGF levels compared to Aβ₁₋₄₂ and tau levels in lumbar CSF samples. Quantitative analysis revealed a significant 30-40% increase in proNGF levels in CSF samples obtained from CDR 0.5 and CDR 1 subjects compared to CDR 0 subjects. CSF proNGF levels did not differ between the CDR 0.5 and CDR 1 groups. By contrast, Aβ₁₋₄₂ and tau levels differentiated CDR 0 and CDR 1 subjects only when total tau/Aβ₁₋₄₂ levels were compared. Quantitative analysis revealed a significant 50% increase in proNGF/Aβ₁₋₄₂ levels in CSF samples obtained from CDR 0.5 and CDR 1 subjects compared to CDR 0 subjects. Units on the Y-axis are arbitrary based on relative densitometric measurements. **, $p < 0.01$ via one way ANOVA with Bonferroni correction.

Fig. 4. BDNF levels in ventricular CSF samples as determined by ELISA

A) BDNF levels are not different between NCI, MCI and AD (one-way ANOVA, $p=0.26$).

BDNF levels showed a trend towards lower levels in AD compared to NCI subjects (unpaired t test, NCI vs AD, $p<0.05$). B) BDNF levels are 20-25% higher in females than in males (Student's t test, $*p<0.05$).

Table 1

Clinical, demographic, and neuropathological characteristics of Rush Religious Orders Study subjects.

	NCI	aMCI	AD	<i>p</i> value	Pair-wise comparisons
N	26	20	20		
Age, years (mean $\pm$ SD) (range)	84.5 $\pm$ 4.6 (79 – 96)	85.5 $\pm$ 6.3 (74 – 100)	86.4 $\pm$ 5.0 (80 – 98)	0.64 ^a	
Education, years (mean $\pm$ SD) (range)	19.3 $\pm$ 2.6 (14 – 23)	18.9 $\pm$ 4.6 (12 – 30)	18.0 $\pm$ 2.9 (12 – 25)	0.36 ^a	
Number (%) males	13 (54%)	12 (60%)	9 (45%)	0.8 ^b	
Number (%) apoE4 ϵ 4	7 (30%)	8 (40%)	8 (40%)	0.1 ^b	
PMI in hours (mean $\pm$ SD) (range)	6.0 $\pm$ 4.7 (1.0 – 22.0)	5.7 $\pm$ 3.2 (1.5 – 10.0)	4.7 $\pm$ 1.0 (2.5 – 6.0)	0.5 ^a	
MMSE score (mean $\pm$ SD) (range)	28.1 $\pm$ 1.5 (26 – 30)	26.4 $\pm$ 2.4 (22 – 29)	18.7 $\pm$ 6.1 (0 – 27)	0.002 ^a	(NCI, MCI) > AD
GCS z-score (mean $\pm$ SD) (range)	-0.2 $\pm$ 0.3 (0.5 – -0.7)	-0.3 $\pm$ 0.5 (1.3 – -1.2)	-1.7 $\pm$ 0.9 (-0.7 – -3.9)	0.001 ^a	(NCI, MCI) > AD
Braak Stage 0 I/II III/IV V/VI	0 12 12 2	0 6 14 0	0 4 8 8	0.01 ^a	NCI < AD
CERAD Diagnosis NO POSSIBLE PROBABLE DEFINITE	8 4 7 6	12 2 3 2	3 1 5 11	0.01 ^a	MCI < AD
Reagan Diagnosis NO LOW INTERMEDIATE HIGH	0 11 14 1	0 13 6 1	0 3 9 8	0.05 ^a	(NCI, MCI) < AD

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Kruskal-Wallis test, with Bonferroni correction for multiple comparisons.
 q
Fisher's exact test, with Bonferroni correction for multiple comparisons.

Clinical, demographic, and neuropathological characteristics of Washington University Knight AD Center subjects.

Table 2

N	CDR 0		CDR 0.5		CDR 1		p value	Pair-wise comparisons
	20		20		20			
Age, years (mean ± SD) (range)	70.5 ± 4.9 (63 – 79)		74.3 ± 6.7 (64 – 85)		86.4 ± 5.0 (80 – 98)		0.09 ^a	
Education, years (mean ± SD) (range)	17 ± 3.1 (12 – 20)		15 ± 3.3 (8 – 20)		14 ± 3.4 (8 – 20)		0.01 ^a	CDR 0 > CDR 1
Number (%) males	4 (54%)		14 (70%)		12 (60%)		0.001 ^b	(CDR 0.5, 1 > CDR 0)
Number (%) apoE4 ε4	5 (20%)		12 (60%)		11 (55%)		0.001 ^b	(CDR 0.5, 1 > CDR 0)

^aKruskal-Wallis test, with Bonferroni correction for multiple comparisons.

^bFisher's exact test, with Bonferroni correction for multiple comparisons.

Table 3

Associations between CSF proNGF levels and AD neuropathological diagnostic criteria in RROS subjects

AD neuropathology criteria	<i>r</i> value ^a	<i>p</i> value
Braak	0.06	0.64
CERAD	−0.03	0.82
Reagan	−0.28	0.08

^aSpearman rank correlation.