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Abstract

Chronic neuroinflammation is thought to potentiate medial temporal lobe (MTL) atrophy and memory decline in Alzheimer's disease (AD). It has become increasingly important to find novel immunological biomarkers of neuroinflammation or other processes that can track AD development and progression. Our study explored which pro- or anti-inflammatory cerebrospinal fluid (CSF) biomarkers best predicted AD neuropathology over 24 months. Using Alzheimer's Disease Neuroimaging Initiative data (N=285), CSF inflammatory biomarkers from mass spectrometry and multiplex panels were screened using stepwise regression, followed up with 50%/50% model retests for validation. Neuronal Pentraxin 2 (NPTX2) and Chitinase-3-like-protein-1 (C3LP1), biomarkers of glutamatergic synaptic plasticity and microglial activation respectively, were the only consistently significant biomarkers selected. Once these biomarkers were selected, linear mixed models were used to analyze their baseline and longitudinal associations with bilateral MTL volume, memory decline, global cognition, and established AD biomarkers including CSF amyloid and tau. Higher baseline NPTX2 levels corresponded to less MTL atrophy [R^2= .287, p<.001] and substantially less memory decline [R^2=.560, p<.001] by month 24. Conversely, higher C3LP1 modestly predicted more MTL atrophy [R^2=.083, p<.001], yet did not significantly track memory decline over time. In conclusion, NPTX2 is a novel pro-inflammatory cytokine that predicts AD-related outcomes better than any immunological biomarker to date, substantially accounting for brain atrophy and especially memory decline. C3LP1 as the microglial biomarker, by contrast, performed modestly and did not predict longitudinal memory decline. This research may advance the current understanding of AD etiopathogenesis, while expanding early diagnostic techniques through the use of novel pro-inflammatory biomarkers, such as NPTX2. Future studies should also see if NPTX2 causally affects MTL morphometry and memory performance.

Keywords: Alzheimer's disease; medial temporal lobe; inflammation; immunology; amyloid; tau; memory; biomarkers; NPTX2; C3LP1

1. Introduction

Alzheimer's disease (AD) is typified by progressive medial temporal lobe (MTL) atrophy and memory decline (Weintraub et al., 2012). It has become increasingly important to find novel, immunological biomarkers that can track AD development and progression. When exploring biomarkers, neuroinflammation may be a useful process to examine, as it is an early and continuous feature of AD (Hensley, 2010) that underlies neurodegeneration and cognitive deficits (Dursun et al., 2015).

It is now known that neurotoxic inflammatory mechanisms may initiate AD pathogenesis (Akiyama et al., 2000). Neuroinflammation, on the cellular subunit level, occurs through the release of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), Interleukin-1β (IL-1β), and Interleukin-6 (IL-6), primarily from microglia as well as astrocytes, brain endothelial cells (BECs), and neurons themselves (Akiyama et al., 2000; Guillot-Sestier and Town, 2013; Lee et al., 2008; Tai et al., 2015), all of which are potential immunological AD biomarkers. Levels of these cytokines and downstream effectors are higher in the AD brain (Griffin et al., 1989) and may mediate neural atrophy over time (Lee et al., 2008).

However, while the classic pro-inflammatory cytokines do potentiate brain atrophy, they are not necessarily ideal AD biomarkers, because they exhibit pleiotropic and concentration-dependent roles within the innate immune system. For example, pro-inflammatory cytokines at lower concentrations exert beneficial effects by inducing and maintaining hippocampal long-term potentiation (LTP) and neural plasticity, brain homeostasis, plaque clearance via activated microglia, and tissue repair (Ben Menachem-Zidon et al., 2011; Goshen and Yirmiya, 2009;

Hensley, 2010; Weiss, 2009), where these effects are impaired at higher concentrations (Yirmiya and Goshen, 2011). Therefore, there is no easily labeled "detrimental" phenotype based on the expression of pro-inflammatory cytokines by activated microglia in the brain (Weitz and Town, 2012), due to their context-dependent pleiotropic effects that vary considerably within and across individuals (Wyss-Coray, 2006). These paradigms highlight the difficulty in selecting effective pro- or anti- inflammatory biomarkers to detect and track AD.

It is important to consider other pro-inflammatory mechanisms that do not induce chronic neuroinflammation, yet are indicative of efficacious biomarkers. For example, synaptic plasticity in medial temporal lobe is integral to memory function and is in part regulated by the pentraxin superfamily, such as the pro-inflammatory protein neuronal pentraxin 2 or NPTX2 (Elbaz et al., 2015). Specifically, NPTX2, also known as neuronal-activity regulated protein (NARP), facilitates excitatory synapse formation, learning, and memory by clearing extracellular debris to anchor α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) channels (Elbaz et al., 2015; Hsu and Perin, 1995; Reti et al., 2011). As microglial activation is important for potentiating specific aspects of AD pathogenesis (Bales et al., 2000), related biomarkers have been investigated such as Chitinase 3-like Protein 1 (C3LP1). C3LP1, a derivative of chitin protein, is a marker of macrophage/microglial activation (Canto et al., 2015; Kester et al., 2015; Kzhyshkowska et al., 2007; Lautner et al., 2011; Lee et al., 2011; Sutphen et al., 2015). Serum and CSF C3LP1 levels are increased in preclinical and early AD (Canto et al., 2015; Craig-Schapiro et al., 2010), further suggesting its potential utility.

Thus, peptidomics and multiplex techniques may reveal novel immunological biomarkers of processes such as chronic neuroinflammation relevant to AD. Utilizing Alzheimer's Disease Neuroimaging Initiative (ADNI) data in 285 aged adults, we explored which CSF pro- or anti-inflammatory biomarkers were associated with baseline and longitudinal AD neuropathology and memory performance at baseline and across months 6, 12, and 24. Relationships with global cognition and CSF Aβ and tau species were also examined.

2. Methods

2.1. Participants

Baseline mass spectrometry and multiplex data from ADNI were available for 86 cognitively normal (CN), 135 Mild Cognitively Impairment (MCI), and 64 AD (adni.loni.usc.edu). The following ADNI data were also available for this cohort:

1. Demographics including age, sex and education;
2. Clinical diagnosis at baseline and month 24, as well as MCI conversion;
3. MRI scans;
4. CSF Aβ1-42, total tau, and phosphorylated tau (p-tau181);
5. Apolipoprotein E (APOE) ε4 genotype;
6. Global cognitive measures and factor scores.

By month 24, MCI participants were classified as either remaining stable (MCI-S, n =82) or progressing to AD (MCI-P, n =47), with the remainder diagnosed as CN. Details of the consensus procedure by the ADNI Conversion Committee are described elsewhere (Willette et

al., 2015). We chose to focus on month 24 as an endpoint for comparison to our previous work (Willette et al., 2015), and because there is much less MRI data available after month 24.

2.2. Standard Protocol Approvals, Registrations, and Patient Consents

Written informed consent was obtained from all ADNI participants at their respective ADNI sites. Site-specific institutional review boards approved the ADNI protocol.

2.3 Clinical and Cognitive Assessments

Global cognition and assessment scores for the Mini-Mental State Examination (MMSE), clinical dementia rating-sum of boxes (CDR-sob) and AD assessment scale-cognitive subscale 11 (ADAS-cog11) were examined at baseline and at 6, 12, and 24 months. Diagnoses were made by ADNI based on criteria described in the ADNI1 procedure manual (http://adni.loni.usc.edu/). A memory factor score (Crane et al., 2012) was also examined at baseline and longitudinally. Memory decline was defined as difference scores between baseline and either 6, 12, or 24 months after. Memory factor data from baseline to months 12 and 24 was missing for 0 and 27 participants respectively.

2.4. CSF Amyloid and Tau

CSF sample collection, processing, and quality control of p-tau181, total tau, and Aβ1-42 are described in the ADNI1 protocol manual (www.adni.loni.usc.edu) and elsewhere (Shaw et al., 2011). Total tau and Aβ1-42 values were not available for 3 and 1 participants respectively.

2.5. Mass Spectrometry and Multiplex Biomarkers in CSF

Data was downloaded from the Biomarkers Consortium CSF Proteomics liquid chromatography/ multiple reaction monitoring mass spectrometry (LC/MRM-MS) dataset. As described previously (Spellman et al., 2015), the ADNI Biomarkers Consortium Project investigated the extent to which selected peptides, measured with LC/MRM-MS, could discriminate among disease states. Briefly, 567 peptides representing 221 proteins were targeted in a single run (Caprion Proteome Inc., Montreal, QC, Canada). Raw intensities were derived and extensive quality control used to derive log intensities. The ADNI Biomarker core used the natural log to transform analyte values to normalize variance in the sample. Nine neuroinflammatory biomarkers were present, represented by 21 CSF peptides. A larger CSF multiplex array, containing 27 additional pro-inflammatory biomarkers (**Supplemental Table 1**), was also utilized for comparison to LC/MRM-MS (see below).

For the LC/MRM-MS panel, the nine biomarkers of interest were: Alpha-1-antitrypsin; Complement 3; CD14; IL-18; C3LP1/YKL-40; Osteopontin; C-Reactive Protein (CRP); Neuronal Pentraxin 1 (NPTX1); and NPTX2. **Supplemental Text 1** describes all nine derived peptides and protein functions specific to inflammation. Different peptides from a single protein were selected as candidate biomarkers based on peptides that best predicted diagnostic status (Spellman et al., 2015), or using stepwise regression analyses and follow up validation tests (see below). Due to the relatively small number of pro-inflammatory indices in the LC/MRM-MS peptide biomarker panel, we also explored if mass spectrometry analytes selected from that panel were again selected when simultaneously testing protein biomarkers from the larger CSF multiplex assay. Briefly, a Luminex xMAP immunoassay panel (Rules Based Medicine, Austin, TX) was used to measure 159 CSF analytes, including several pro- and anti-inflammatory

proteins. As shown in **Supplemental Table 1**, 27 CSF proteins were selected based on the literature linking them to one or more inflammatory processes.

2.6. MRI and Tensor Based Morphometry

T1-weighted volumes at baseline and months 6, 12, and 24 were downloaded. Bilateral MTL gray matter (GM) volume was derived, as it shows reliable atrophy over the AD spectrum and is susceptible to neuroinflammation (Risacher and Saykin, 2013). Baseline images were processed using FreeSurfer 4.3 as described previously (see "UCSF FreeSurfer Methods" at www.adni.loni.usc.edu). Fifty-five baseline scans were rejected for analysis based on failed QC checks. Tensor Based Morphometry (TBM) was used to gauge atrophy over time. Jacobian maps were generated between baseline and either month 6, 12, or 24 volumetric scans (Hua et al., 2013). Degree of contraction was expressed as a percentage decrease relative to baseline, reflecting progressive brain atrophy. T1-weighted scans at months 6, 12 and 24 were missing for 13, 18 and 58 participants respectively.

2.7. APOE Genotype

The ADNI Biomarker core at the University of Pennsylvania conducted APOE ε4 genotyping. We characterized participants as being "non-APOE4" (i.e., zero APOE ε4 alleles) or "APOE4" (i.e., one to two APOE ε4 alleles).

2.8. Statistical Analyses

All statistical mixed model analyses were conducted using SPSS 23.0 software (IBM Corp., Armonk, NY). All variables had homoscedastic variance and were normally distributed or log transformed.

2.8.1. Stepwise Regression: Biomarker Selection

The nine peptide biomarkers of interest (See Section 2.5) were screened using stepwise regression which, when used correctly, is useful for variable selection and model building (Astin and Denson, 2009; Knüppel et al., 2013; Smith et al., 2013). The first goal was to determine which inflammation-related LC/MRM-MS biomarkers were significant predictors of MTL volume and memory performance at 24 months. A subsequent goal was take selected mass spectrometry biomarkers and use stepwise regression while incorporating multiplex proteins, to see if MRM peptides and/or multiplex proteins were selected for model building. Covariates were entered into a given model as the first step. The nine peptide analytes representing nine candidate proteins were added in a stepwise step. In models with multiplex biomarkers, they were added in a subsequent stepwise step. The default threshold of $P<.05$ for inclusion and $P>.10$ for exclusion of variables were used. Based on these regression analyses (see Section 3.2 in Results), NPTX2 and C3LP1 were the only consistently significant biomarkers, and thus became the main predictor variables for the focus of our study. Stepwise regression iterates through each potential biomarker and removes it from the model if $P>.10$, minimizing the need for type 1 error correction.

2.8.2. Linear Mixed Models: Biomarker Testing on Outcomes

The two selected peptide biomarkers, NPTX2 and C3LP1, were subsequently analyzed with linear mixed models, to determine their baseline and longitudinal associations with GM atrophy in bilateral MTL or a memory factor. We used a single model to examine the main effects of NPTX2 and C3LP1 at baseline, or their interaction with Time longitudinally, on global cognition, memory, and bilateral MTL volume. Time was defined as change relative to baseline at months 6, 12, and 24. Similar analyses were conducted for global cognition, Clinical Dementia Rating (CDR), and CSF amyloid and tau. Longitudinal analyses of CSF tau, p-tau181 and Aβ1-42 were not performed due to lack of longitudinal ptau-181 data in ADNI. Again, the CSF samples of NPTX2 and C3LP1 used for our statistical analyses were derived from the LC/MRM-MS (see Section 2.5).

Linear mixed models, followed by least significant differences (LSD) follow-up tests, also gauged if CSF NPTX2 or C3LP1 levels differed by baseline diagnosis (CN, MCI, or AD) or MCI conversion (MCI-S or MCI-P). All subsequent models except for cognitive outcomes included the following covariates: age at baseline, education, sex, APOE ε4 genotype, and either baseline diagnosis or MCI conversion. Mixed models also covaried the random effect of subject. Models gauging global cognition, the CDR assessment, and the memory factor did not covary baseline diagnosis or MCI conversion, because these measures are directly used to diagnose participants as CN, MCI, or AD or are direct outcomes of disease diagnosis.

Finally, on an exploratory basis, interactions were examined between both NPTX2 and C3LP1 and covariates that were statistically related to them, including APOE ε4 genotype, age, and education.

3. RESULTS

3.1. Demographics and Inflammation Biomarkers

Table 1 lists demographics, APOE ε4 genotype data, and other baseline sample characteristics. Based on subsequent analyses, log-transformed CSF analyte levels of NPTX2 (TESTLNALLQR) and C3LP1 (ILGQQVPYATK) are noted.

3.2. CSF Inflammatory Biomarker Selection (Stepwise Regression)

As a first step, stepwise regression was used to select inflammatory biomarkers that best predicted memory decline and atrophy by 24 months. As described in **Supplemental Text 1**, 9 peptides representing 9 proteins were chosen as candidate inflammatory biomarkers. All peptides were log-transformed by the ADNI Biomarker Core to achieve normality (Spellman et al., 2015). Results were similar when considering the outcomes at 12 months, or when all 21 peptide analytes were entered into the stepwise step for month 24.

For memory performance by 24 months, covariates accounted for a moderate proportion of variance [Adjusted R^2=.164, F=11.10, P<.001]. Stepwise selection of NPTX2 [Adjusted R^2=.202, F-change=12.91, P<.001] and then C3LP1 [Adjusted R^2=.215, F-change=5.17, P<.001] significantly improved the model. Using 10 random samples of 50% of the cohort or Lasso regression to validate model selection (**Supplemental Text 2**), NPTX2 and C3LP1 were consistently selected as the only significant predictors.

For MTL volume by 24 months, a similar pattern emerged. Covariates initially explained nearly half of the variance [Adjusted R^2=.477, F=42.27, *P*<.001]. NPTX2 [Adjusted R^2=.514, F=17.62, *P*<.001] and subsequently C3LP1 [Adjusted R^2 = .546, F-change=16.79, *P*<.001] were again selected as the only significant predictors. Using stepwise regression with random sampling or Lasso regression to validate the model (**Supplemental Text 2**), NPTX2 and C3LP1 were again selected as the only significant predictors.

Finally, as described in **Supplemental Text 3**, NPTX2 and C3LP1 were selected in the stepwise step when they were iteratively added into a model with 27 CSF proteins related to inflammation from the multiplex immunoassay (**Supplemental Table 1**). These results suggest that NPTX2 and C3LP1, respectively biomarkers of inflammation-mediated excitatory synaptic plasticity (Elbaz et al., 2015) and macrophage/microglia (Craig-Schapiro et al., 2010) activity, may be useful for tracking AD neuropathology and cognitive decline and should be investigated further.

3.3. Effects of Diagnosis and Covariates on NPTX2 and C3LP1 (Mixed Models)

Having selected NPTX2 and C3LP1, their associations with clinical diagnosis and covariates were then ascertained with linear mixed models. There was a main effect of baseline diagnosis on NPTX2 [F=4.120, *P*=.017]. **Table 1** indicates a modest step-wise decrease in log-transformed NPTX2 levels from CN to AD [*P*=.005] and MCI to AD [*P*=.034], but not CN to MCI [*P*=.242]. MCI-P had lower NPTX2 levels than MCI-S [F=4.04, *P*=.047]. A main effect of baseline diagnosis on C3LP1 was also significant [F=3.32, *P*=.037]. **Table 1** indicates a modest step-wise increase in log-transformed C3LP1 levels from CN to AD [*P*<.001], MCI to AD [*P*=.045] and CN to MCI [*P*=.002]. MCI-S and MCI-P did not differ for C3LP1 values [F=0.358, *P*=.551].

For covariates, on an exploratory basis, APOE4 carriers had higher C3LP1 [F=7.81, *P*=.006], but similar NPTX2 values [F=0.15, *P*=.696]. Older age at baseline was related to higher C3LP1 [R^2=.391, F=63.91, P<.001], but not NPTX2 [F=0.38, *P*=.539]. There was a trend for more years of education predicting higher NPTX2 [F=1.77, *P*=.053], but not C3LP1 [F=1.00, *P*=.317]. Sex was not a significant predictor for NPTX2 [F=0.01, *P*=.973] or C3LP1 [F=1.32, *P*=.251].

3.4. Neuropsychological Testing: Baseline and Over 24 Months (Mixed Models)

Next, the associations of NPTX2 and C3LP1 were investigated with baseline and longitudinal indices of global cognition and function, as well as memory with linear mixed models. As shown in **Figure 1**, higher baseline NPTX2 and C3LP1 levels were, respectively, related to better and worse baseline global cognitive and assessment outcomes. Specifically, higher NPTX2 levels were correlated with higher MMSE [β±SE=1.24±0.22, F=32.85, *P*<.001], lower CDR-sob [β±SE=-0.81±0.15, F=28.22, *P*<.001] and lower ADAScog-11 [β±SE=-3.34±0.54, F=38.40, *P*<.001] (**Figure 1A,C,E**). Higher C3LP1, conversely, was associated with lower MMSE [β±SE=-1.43±0.37, F=15.26, *P*<.001], higher CDR-sob [β±SE=1.18±0.26, F=21.13, *P*<.001], and higher ADAS cog-11 scores [β±SE=4.45±0.92, F=23.55, *P*<.001] (**Figure 1B,D,F**). Similar patterns were seen across time (see **Supplemental Text 4**).

For the memory factor, higher NPTX2 and C3LP1 at baseline respectively corresponded to better [R^2=.051, F=11.76, *P*<.001] or worse [R^2= .072, F=9.67, *P*=.002] baseline performance. A NPTX2 x Time [F=8.88, *P*<.001] interaction revealed that higher baseline NPTX2 strongly corresponded to less memory decline over time relative to baseline, particularly by month 24

where NPTX2 explained 56% of the variance (**Figure 2**). By contrast, a C3LP1 main effect [F=8.851, *P*=.003], with a non-significant C3LP1 x Time interaction [F=1.73, *P*=.180], indicated that higher baseline C3LP1 showed a weak association (R^2=.04) with memory decline regardless of time. See **Supplemental Figure 1** for a trajectory curve showing predicted change in memory decline over time for NPTX2. As a confirmation analysis using 10 randomized iterations of 50% of the sample (**Supplemental Text 5**), relative effect sizes and P values for NPTX2 and C3LP1 were comparable. Exploratory interactions with covariates revealed no significant effects.

3.5. Brain: Baseline MTL volume and Atrophy over 24 Months (Mixed Models)

Next, the associations of NPTX2 and C3LP1 were investigated with baseline MTL volume and longitudinal, cumulative MTL atrophy relative to baseline with linear mixed models. Higher baseline NPTX2 [R^2=.050, F=6.91, *P*=.009] was correlated with more basal MTL volume. A NPTX2 x Time interaction [F=16.61, *P*<.001] showed that higher NPTX2 corresponded to less MTL atrophy over time, particularly by month 24 (**Figure 3A-C**). By contrast, C3LP1 showed no association with MTL volume at baseline [R^2=.008, F=0.05, *P*=.817]. A C3LP1 x Time interaction [F=12.09, *P*<.001] indicated that while baseline C3LP1 was slightly associated with atrophy over time, it was relatively modest compared to NPTX2 (**Figure 3D-F**). **Supplemental Figure 2** shows trajectory curves for predicted change in MTL atrophy over time for NPTX2 and C3LP1. These results were confirmed (**Supplemental Text 5**) when testing models with randomly selected 50% sub-samples of the cohort. Exploratory interactions with covariates revealed no significant effects.

3.6. CSF Biomarkers: Baseline Amyloid and Tau (Mixed Models)

Finally, it was important to gauge how NPTX2 and C3LP1 were related to amyloid and tau, which are hallmarks of AD, with linear mixed models. Higher NPTX2 and C3LP1 were respectively related to a less or more AD-like CSF amyloid and tau profile. Specifically, higher NPTX2 was associated with higher CSF Aβ1-42 [β±SE=9.09±4.44 F=4.20, *P*=.041], lower total tau [β±SE=-23.89±4.07, F=34.43, *P*<.001], and lower p-tau181 [β±SE=-4.34±1.45, F=8.93, *P*=.003]. By contrast, higher C3LP1 was not significantly associated with CSF Aβ1-42 [β±SE=-9.52±7.48, F=1.61, *P*=.204], but corresponded to higher total tau [β±SE=25.67±6.87, F=13.96, *P*<.001] and higher p-tau181 (β±SE= 7.71±2.46, F=9.83, *P*=.002].

Finally, we explored interactions between C3LP1 or NPTX2 and age, education, and APOE ε4 genotype, given that the covariates predicted variation in C3LP1 and NPTX2. **Supplemental Figure 3** shows that higher levels of NPTX2 were related to less amyloid pathology for non-APOE4 carriers [β±SE= 22.15±7.93, F=7.79, *P*=.006], but not for APOE4 carriers. No other interactions were significant.

4. Discussion

The aim of our study was to explore which established or novel pro- and anti-inflammatory CSF biomarkers from ADNI Biomarker Core panels best predicted MTL atrophy and memory decline, as well as other AD indices affected by neuroinflammation. NPTX2 and C3LP1 consistently loaded as the only significant predictors of both MTL volume and memory performance by 24 months. They also predicted other AD aspects including global cognition and function, as well as CSF measures of amyloid and tau. Links with APOE4 status and age were found, where age has also been linked to chronic neuroinflammation over time due to age-related

pro-inflammatory effects on the brain (Godbout and Johnson, 2006). Along the AD spectrum in our study, there was a modest step-wise increase in C3LP1 and decrease in NPTX2.

These juxtaposed patterns are underscored by the global neuropsychological findings. As shown in **Figure 1** and supplemental data, higher NPTX2 reflected a significantly less AD-like pattern of global function at baseline and relative decline through month 24, while higher C3LP1 modestly corresponded to a slightly more AD-like pattern. Curiously by month 24, C3LP1 was a poor predictor for memory across time, while NPTX2 accounted for more than half of the variance among all participants. This could reflect NPTX2's role in synaptic plasticity and long-term potentiation (Dong et al., 2015; Elbaz et al., 2015; Tsui et al., 1996). However, NPTX2 could merely reflect the AD process, while synaptic loss reliably accompanies dementia onset (Crews and Masliah, 2010; Serrano-Pozo et al., 2011; Shankar and Walsh, 2009). Higher NPTX2 similarly correlated with less MTL atrophy over time and AD neuropathology at baseline, further highlighting its potential use to track etiopathogenesis and progression. Non-APOE4 carriers showed a relationship between Aβ and NPTX2, while APOE4 carriers did not, suggesting that the APOE risk factor may modulate the effect of NPTX2 or an upstream mechanism. It is unclear if NPTX2 exercises a causal or correlational effect on one or more neurological and cognitive aspects of AD.

The lack of association of C3LP1 with memory scores may be due to its modest relationship with MTL atrophy over two years. Chronic neuroinflammation in AD arises from Aβ-dependent and independent activation of microglia and astrocytes (Heneka et al., 2015). The release of pro-inflammatory cytokines is thought to potentiate AD pathogenesis. It is clear in this report that

higher baseline C3LP1 has some modest association with AD progression, as it is significantly corresponded to global cognition, tau, and AD risk factors such as age and APOE4 status. However, baseline levels of C3LP1 were not significantly related to CSF amyloid levels. Sutphen and colleagues (Sutphen et al., 2015) similarly found in middle-aged, cognitively normal participants that YKL-40 (i.e., C3LP1) levels increased with age and APOE4 status, where longitudinal but not baseline associations were seen with amyloid positivity. Kester and colleagues (Kester et al., 2015) found that YKL-40 levels at baseline and longitudinally were higher in patients with MCI and AD.

Our report is particularly novel because we investigated the degree to which NPTX2 and C3LP1 track neuropathology and memory decline over time along the AD spectrum. Several limitations and strengths should be noted. Protein expression of the NPTX2 and C3LP1 peptides cannot be validated in the current dataset, as ADNI CSF samples are not readily accessible. The ADNI Biomarker Core has only assessed peptides at baseline, where longitudinal collection is needed for future work. Thus, no causal inferences can be made, and results should be considered exploratory for driving hypothesis generation. To contain type 1 error, we chose to focus structural analyses on MTL and consequent memory performance. It could be that C3LP1 is a better predictor for global atrophy or regions other than MTL. Finally, we only analyzed subjects in ADNI, where there are to our knowledge no other readily accessible AD datasets with mass spectrometry, MRI, and neuropsychological data. For strengths, this large sample size study used an unbiased stepwise selection process and follow up stepwise validation test to select candidate biomarkers in CSF. We also highlight that NPTX2 was an excellent predictor of AD neuropathology and especially cognitive decline over time.

In conclusion, NPTX2 is a novel immunological cytokine that accounts for several neurobiological and cognitive aspects of AD, particularly cognitive decline across the AD spectrum. The microglial biomarker C3LP1, by contrast, performed modestly or did not account for AD-related indices. This research may advance the current understanding of AD etiopathogenesis, while expanding early diagnostic techniques by using novel pro-inflammatory biomarkers such as NPTX2.

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Figure Legends.

Figure 1. Mass Spectrometry Biomarkers and Baseline Global Cognition

Associations between baseline global cognitive and assessment outcomes with baseline CSF NPTX2 or C3LP1. Blue and red circles respectively correspond to NPTX2 and C3LP1 CSF values in predicting MMSE (A,B), CDR-sob (C,D), and ADAS-cog11 (E,F). The R^2 value reflects the proportion of variance in cognitive scores explained by each biomarker. Covariates included age at baseline, sex, Apolipoprotein ε4 genotype, and education. ADAS-cog11, Alzheimer's Disease Assessment Scale-cognitive subscale 11; C3LP1, chitinase-3-like-protein 1; CDR-sob, Clinical Dementia Rating sum of boxes; MMSE, Mini-Mental State Examination; NPTX2, neuronal pentraxin 2.

Figure 2. NPTX2 and Memory Performance across Time

Associations between baseline NPTX2 and change over time for the memory factor score relative to baseline at months 6 (A), 12 (B), and 24 (C) thereafter. Blue circles correspond to NPTX2 values. The R^2 reflects the proportion of variance in the memory factor as explained by NPTX2. Covariates included the fixed effects of age at baseline, sex, APOE ε4 genotype, and education, as well as the random effect of subject. NPTX2, neuronal pentraxin 2.

Figure 3. Mass Spectrometry Biomarkers and Medial Temporal Atrophy across Time

Associations between baseline NPTX2 (A,B,C) or C3LP1 (D,E,F) and cumulative change in medial temporal lobe (MTL) gray matter (GM) volume, expressed as a percentage relative to baseline at months 6, 12, and 24 thereafter. The blue and red circles correspond to NPTX2 and C3LP1 values respectively. The R^2 reflects the proportion of variance in MTL GM volume as explained by a given biomarker. Covariates included the fixed effects of age at baseline, sex,

APOE ε4 genotype, and education, as well as the random effect of subject. C3LP1, chitinase-3-like-protein 1; NPTX2, neuronal pentraxin 2.

Table 1. Demographics and Summary Indices

	CN (n=86)	MCI (n=135)	AD (n=66)	MCI-S (n=82)	MCI-P (n=47)
Age	75.70 ± 5.54	74.69 ± 7.35	74.98 ± 7.57	74.77 ± 7.37	74.64 ± 7.40
Education	15.64 ± 2.97	16.00 ± 2.96	15.11 ± 2.96	15.78 ± 3.19	16.32 ± 2.58
Sex (F,M)	42, 44	44, 91	29, 37	22, 60	20, 27
APOE4 (-/+)	65, 21	64, 71	19, 47	41, 41	20, 27
CDR-sob	0.02 ± 0.11	1.56 ± 0.88	4.34 ± 1.56	1.52 ± 0.87	1.65 ± 0.94
MMSE	29.05 ± 1.02	26.91 ± 1.74	23.52 ± 1.85	26.98 ± 1.68	26.85 ± 1.81
ADAS-cog11	6.05 ± 2.90	11.72 ± 4.33	18.88 ± 6.71	11.52 ± 4.33	12.33 ± 4.34
Memory Factor	0.98 ± 0.50	-0.15 ± 0.57	-0.91 ± 0.55	-0.10 ± 0.56	-0.26 ± 0.57
C3LP1	23.03 ± 0.03	23.13 ± 0.02	23.20 ± 0.03	23.14 ± 0.03	23.10 ± 0.03
NPTX2	10.70 ± 0.08	10.62 ± 0.06	10.31 ± 0.09	10.71 ± 0.09	10.43 ± 0.11

Abbreviations: AD, Alzheimer's disease; ADAS-cog11, AD Assessment Scale-Cognitive Subscale; APOE4, apolipoprotein ε4 allele status; C3LP1, Chitinase 3-like Protein 1; CDR-sob, Clinical Dementia-Rating Sum of Boxes; MMSE, Mini-Mental State Examination; NPTX2, Neuronal Pentraxin 2.

Note: Variables are shown as mean ± standard error or frequency count.