

# p-tau/A $\beta$ 42 Ratio Associates with Cognitive Decline in Alzheimer's disease, Mild Cognitive Impairment, and Cognitively Unimpaired Older Adults

Ruchika Shaurya Prakash, PhD<sup>1\*</sup>; Michael R. McKenna, BA<sup>1</sup>; Oyetunde Gbadeyan, PhD<sup>1</sup>; Rebecca Andridge, PhD<sup>2</sup>; Douglas W. Scharre, MD<sup>3</sup>; for the Alzheimer's Disease Neuroimaging Initiative<sup>±</sup>

<sup>1</sup> Department of Psychology, The Ohio State University, OH, USA

<sup>2</sup> Division of Biostatistics, The Ohio State University, OH, USA

<sup>3</sup> Department of Neurology, Cognitive Neurology Division, The Ohio State University Wexner Medical Center, OH, USA

<sup>±</sup>Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database ([adni.loni.usc.edu](http://adni.loni.usc.edu)). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: [http://adni.loni.usc.edu/wp-content/uploads/how\\_to\\_apply/ADNI\\_Acknowledgement\\_List.pdf](http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf)

\*Correspondence concerning this article should be addressed to Ruchika Shaurya Prakash, Department of Psychology, The Ohio State University, 139 Psychology Building, 1835 Neil Avenue, Columbus, OH 43210.

Email: [prakash.30@osu.edu](mailto:prakash.30@osu.edu)

**Abstract Count: 248**

**Word Count (not including abstract): 3190**

## Study Funding

The project described in this publication was supported by Award Number UL1TR002733 from the National Center For Advancing Translational Sciences and Award Number R01AG054427 from the National Institute On Aging of the National Institutes of Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center For Advancing Translational Sciences, the National Institute on Aging or the National Institutes of Health.

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through contributions from the following groups: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health ([www.fnih.org](http://www.fnih.org)). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

## Disclosures

R Prakash reports no disclosures.

M McKenna reports no disclosures.

O. Gbadeyan reports no disclosures.

R. Andridge reports no disclosures.

D. Scharre reports no disclosures.

## Abstract

**INTRODUCTION:** The most well-studied biomarkers in AD are CSF amyloid beta-42 ( $A\beta_{42}$ ), tau, p-tau, and the ratio p-tau/ $A\beta_{42}$ . The ratiometric measure of p-tau/ $A\beta_{42}$  shows the best diagnostic accuracy, and correlates reliably with metrics of cognition in unimpaired participants. However, no study has examined the impact of the CSF p-tau/ $A\beta_{42}$  ratio in predicting cognitive decline in both healthy and AD individuals in one sample. The goal of this study was to examine whether CSF-based p-tau/ $A\beta_{42}$  predicts changes in global cognitive functioning, episodic memory, and executive functioning over a two-year period in cognitively impaired older adults (CU), and in individuals with Mild Cognitive Impairment (MCI) and Alzheimer's disease (AD).

**METHODS:** This study involves secondary analysis of data from 1215 older adults available in the Alzheimer's Disease Neuroimaging Initiative (ADNI). Neuropsychological variables, collected at baseline, 6-month, 12-month, and 24-month follow-ups, included the Preclinical Alzheimer's Cognitive Composite (PACC) to assess global cognitive functioning, ADNI-MEM to assess episodic memory functioning, and ADNI-EF to assess executive functioning. Linear mixed models were constructed to examine the effect of CSF p-tau/ $A\beta_{42}$ , diagnostic group, and change over time (baseline, 6-month, 12-month, and 24-month) on cognitive scores.

**RESULTS:** CSF p-tau/ $A\beta_{42}$  ratios predicted worsening cognitive impairment, both on global cognition and episodic memory in individuals with MCI and AD, but not in CU older adults and predicted decline in executive functioning for all three diagnostic groups.

**DISCUSSION:** Our study, including CU, MCI, and AD individuals, provides evidence for differential cognitive consequences of accumulated AD pathology based on diagnostic groups.

# Introduction

There has been an increased global focus in understanding the etiology and treatment possibilities for Alzheimer's disease (AD). AD has been traditionally conceptualized as a clinical pathologic syndrome, with multi-domain cognitive symptoms, considered the defining feature of the disease<sup>1-3</sup>. However, accumulating evidence from imaging and autopsy studies evince support for the onset of pathophysiological processes well before the onset of AD symptoms<sup>4,5</sup>, and in some instances, without the presence of known cognitive symptoms<sup>6-9</sup>, thus reinforcing a revised conceptualization of the disease away from a syndromal manifestation to a biological definition<sup>10,11</sup>. This redefinition of AD as a neuropathological disease prioritizes the investigation of misfolded and aggregated amyloid beta (A $\beta$ ) peptides and hyperphosphorylated tau proteins, detected in-vivo using either CSF- or PET-based examination, for the understanding of AD and its clinical manifestation.

Cerebral A $\beta$  accumulation can be estimated through a reduction in A $\beta$ <sub>42</sub> concentration, one of the most reliable diagnostic isoforms of A $\beta$ , in CSF<sup>12</sup> derived from a lumbar puncture. Importantly, reduction in CSF A $\beta$ <sub>42</sub> has been observed in cognitively unimpaired (CU) older adults, with clinicopathological studies evincing support for amyloid pathology as one of the earliest detectable markers of AD pathology in living persons<sup>13-15</sup>. However,  $\beta$ -amyloidosis does not perfectly predict the clinical expression of AD, with tauopathy essential for the manifestation of cognitive deterioration observed in AD<sup>11,16,17</sup>. Two of the most thoroughly examined CSF-based tau biomarkers are phosphorylated tau (p-tau) and total tau (t-tau) known to be made up of various protein isomers<sup>18</sup>. P-tau concentration is thought to be more specific to AD pathology, reflecting the hyperphosphorylated tau found in neurofibrillary tangles in the brain and correlating with the severity of paired helical filament tau aggregation at autopsy<sup>19,20</sup>, whereas t-tau concentration is thought to reflect general neurodegeneration and can be elevated in other neurological disorders like traumatic brain injury and stroke<sup>21,22</sup>.

Of these CSF-based markers, the ratio CSF tau/A $\beta$ <sub>42</sub>, combining variance across the two critically implicated proteinopathies of A $\beta$  and tau, provides one of the best diagnostic accuracies among fluid- and PET imaging-based biomarkers<sup>23-26</sup>. For example, Hansson et al. compared the accuracy rates for

Elecsys-based immunoassays of  $A\beta_{42}$ , t-tau/ $A\beta_{42}$ , and p-tau/ $A\beta_{42}$ , and showed that the two ratio measures have stronger concordance with amyloid PET than CSF  $A\beta_{42}$  alone<sup>27</sup>. This ratio allows us to capture a biologic measure of the two most prominent neuropathological features of AD creating high specificity for diagnostic classification of the disease.

Despite there being a shift from the traditional clinical-pathological conceptualization of AD emphasizing clinical consequences of the disease to a more biological definition highlighting underlying pathological processes, the two are inextricably linked, with studies systematically investigating associations between molecular and clinical changes. Although there have been studies reporting significant associations between accumulation of amyloid pathology and metrics of global cognition, episodic memory, and executive functioning<sup>28,29</sup>, there have also been studies reporting weaker correlations between CSF- $A\beta_{42}$  and cognitive functioning<sup>30,31</sup>. A handful of studies, notably, have examined the combined contribution of amyloidosis and tauopathy, and evince support for these models to best predict variance in baseline cognitive functioning and trajectory of decline in cognitive functioning<sup>32,33</sup>. Given the reconceptualization of AD as a biological disease, with accumulation of misfolded proteins occurring decades before the onset of cognitive sequelae, this study examined the interaction between diagnostic status, as defined in the Alzheimer's Disease Neuroimaging Initiative (ADNI) and the ratio of CSF p-tau/ $A\beta_{42}$ , in predicting changes in cognitive functioning over a two-year period.

## Methods

Data were obtained from the ADNIMERGE R package on March 27, 2020. ADNI is an ongoing, multi-site, longitudinal study designed to examine the role of fluid-based and imaging-based biomarkers in healthy and pathological aging. Ongoing since 2003, the study has had four waves of data collection (ADNI-1, ADNI-GO, ADNI-2, and ADNI-3), with baseline data from 2250 participants publicly available. In this study, anyone with available baseline CSF biomarker data from the ADNI1, ADNI-GO and ADNI-2 were included in the analysis, resulting in a total of 1215 participants.

*Participants:* We included ADNI CU older adults, ADNI MCI, and ADNI AD subjects in this study.

General inclusion criteria for the ADNI study included: ages between 55-90 years, English or Spanish speaking, Hachinski Ischemic Score of less than or equal to 4, adequate visual and auditory acuity, good general health, at least 6 years of education or equivalent work history, and a Geriatric Depression Scale score of less than 6.

Diagnostic criteria for classifying participants at baseline involved a combination of subjective reports, neuropsychological assessment, and physician assessment<sup>34</sup>. Specifically, CU participants had to meet the following criteria: 1) Mini-Mental Status Examination (MMSE) score  $\geq 24$ , 2) scoring above education-adjusted cut-offs on the delayed free recall of the Logical Memory II subscale ( $\geq 9$  for 16+ years of education,  $\geq 5$  for 8-15 years of education,  $\geq 3$  for 0-7 years of education), 3) a Clinical Dementia Rating score of 0, and 4) absence of AD dementia or any other neurological condition. Individuals with subjective memory concerns (SMC) had the same diagnostic criteria as CU individuals, except reported a significant subjective memory concern as indexed by the Cognitive Change Index; a self-report measure where participants are asked to compare present cognitive functioning with the last five years. The first 12 questions focus on memory concerns, and older adults who scored  $\geq 16$  on the first 12 questions were classified as SMC. As the only difference between CU and SMC was self-reported assessment of declines in memory functioning, we combined these two groups for this study. For MCI participants, the following criteria were employed: 1) MMSE score  $\geq 24$ , 2) scoring within education-adjusted range for the Logical Memory II subscale (EMCI: 9-11 for 16+ years of education, 5-9 for 8-15 years of education, 3-6 for 0-7 years of education; LMCI:  $\leq 8$  for 16+ years of education,  $\leq 4$  for 8-15 years of education,  $\leq 2$  for 0-7 years of education), 3) a Clinical Dementia Rating score of 0.5, 4) self or partner reported memory complaint(s), and 5) absence of AD dementia or any other neurological condition. Given that the only difference between EMCI and LMCI participants was performance on the Logical Memory II subscale, these two groups were combined for the purpose of the current study. AD participants were required to meet the following criteria: 1) MMSE score between 20-26, 2) scoring below education-adjusted cut-offs for the

Logical Memory Scale II (8 for 16+ of education,  $\leq 4$  for 8-15 years of education,  $\leq 2$  for 0-7 years of education), 3) a Clinical Dementia Rating score of 0.5 or 1.0, and 4) self or partner reported memory complaints.

Of the 1215 participants included in the study, 367 were classified as CU, 619 were classified as MCI, and 229 were classified as AD. Table 1 presents the demographic and relevant clinical characteristics for each of the three groups. Due to the low presence of double  $\epsilon 4$  alleles in the CU sample, participants with single and double  $\epsilon 4$  alleles were collapsed into one group “*APOE*  $\epsilon 4$  present.”

*Neuropsychological Measures and Composites:* Validated neuropsychological composites of global cognition, episodic memory, and executive functioning were employed to examine the impact of AD pathology on two-year (baseline, 6-month, 12-month, and 24-month) changes in key cognitive domains impacted in AD. For global cognition, we employed the Preclinical Alzheimer’s Cognitive Composite (PACC)<sup>35</sup>, assessing domains of episodic memory, executive functioning, and overall cognition. PACC includes the following measures: MMSE total score, Trails-Making Test B, delayed recall score from the Logical Memory II subscale, and the delayed word recall from the Alzheimer’s Disease Assessment Scale–Cognitive Subscale (ADAS-COG).

ADNI-MEM was employed to index changes in memory, and includes performance on the Logical Memory I and II tasks, several item scores on the Rey Auditory Verbal Learning Test, the cognitive subscale of the Alzheimer’s Disease Assessment Scale, and the three word recall items from the MMSE<sup>36</sup>. Similarly, to measure executive functioning, we employed the ADNI-EF made up of scores on the Digit Symbol Substitution test from the Weschler Adult Intelligence Scale-Revised, Digit Span Backwards Test, Trails-Making A and B, Category Fluency, and Clock Drawing<sup>37</sup>.

Table 1 presents the data available at each time point for the three diagnostic groups. Although ADNI has longer-term follow-up data, there is significant attrition as a function of the diagnostic group,

such that at 36 months, only 23 participants with AD, 441 with MC, and 132 with CU and baseline CSF data had the composite measure of EF. As such, we restricted our analysis to a two-year follow-up.

*CSF Biomarker Assessment and Classification:* CSF biomarker concentrations were pulled from the upennbiomk9.rdata file nested in the ADNIMERGE R package. P-tau and  $A\beta_{42}$  concentrations were measured in picograms per milliliter (pg/mL) by ADNI researchers using the highly automated Roche Elecsys immunoassays on the Cobas e601 automated system. Of the 1215 participants, 191 participants met the upper limit for the  $A\beta_{42}$  concentration (1700 pg/ml for the Roche-based assaying), and we employed the extrapolated values provided by the ADNI group. For all participants, we computed the ratio of p-tau/ $A\beta_{42}$ , with higher values representing higher pathological state of the two proteinopathies. Additionally, to quantify the distribution of clinically significant AD pathology in the three diagnostic groups, we employed the cut-off of .028 provided by Hansson et al.<sup>27</sup> for the ADNI dataset showing concordance of 91.8% with standardized uptake value ratio for amyloid- $\beta$  PET and 90.3% with visual read within the ADNI sample.

*Statistical Analysis:* Comparisons across diagnostic groups on baseline measures were made using ANOVA and chi-square tests as appropriate. Changes in outcomes (PACC, ADNI-MEM, ADNI-EF) over time were modeled using linear mixed effects models. An advantage of this method is that subjects who may be missing measurements at some time points can still contribute to the analysis. Initial exploratory analysis revealed that the trajectories of all three outcomes were linear, and thus time was included in the models as a linear effect. In the first set of models, fixed effects included time (months; linear), diagnostic group (CU, AD, MCI), and their interaction. Additional covariates included to guard against confounding were age (at baseline), years of education, sex, and *APOE* status ( $\epsilon 4$  allele present vs. absent). In the second set of models, baseline p-tau/ $A\beta_{42}$  ratio was added, including all two- and three-way interactions of tau/ $A\beta_{42}$  with time and diagnosis group, in order to test whether changes in cognitive measures over time were moderated by pathology. To capture the within-subject correlation arising due to repeated



measurements over time, an unstructured residual error covariance was used for random subject-level error. The Kenward-Roger adjustment to the degrees of freedom was used to control Type 1 error. Pairwise comparisons between groups were made using contrasts within these models.

*Data Availability:* All data used in the current study have been downloaded from the publicly available Alzheimer's Disease Neuroimaging Initiative database. We will also provide the curated data analyzed in the current study upon request by qualified investigators.

## Results

*Demographics Across Diagnostic Groups:* As shown in **Table 1**, there were statistically significant differences for all demographic characteristics across diagnostic groups ( $p < .05$  for all characteristics). Participants in the MCI and AD were more likely to be male and slightly more likely to be White. There were also small, but statistically significant differences in mean age and years of education across groups, with the MCI group having the youngest average age and the AD group having the lowest average number of years of education. There was also a large difference in *APOE* status across groups, with the  $\epsilon 4$  allele(s) present in 68% of the AD group, 50% of the MCI group, and 28% of the CU group.

*Distribution of Pathology Across Diagnostic Groups:*  $A\beta_{42}$ , p-tau, and the p-tau/ $A\beta_{42}$  ratio differed significantly across the diagnostic groups (**Table 2**). Going from CU to MCI to AD, mean  $A\beta_{42}$  decreased, p-tau increased, and the p-tau/ $A\beta_{42}$  ratio increased. p-tau/ $A\beta_{42}$  ratio explained a larger amount of between-group variability ( $R^2 = 0.20$ ) compared to  $A\beta_{42}$  ( $R^2 = 0.13$ ) or p-tau ( $R^2 = 0.12$ ). **Figure 1** shows the distribution of the three AD biomarkers by group.

*Change in Cognitive Functioning Across Diagnostic Groups:* At baseline there were large differences between groups on all three cognitive composites, with the AD group having lowest mean PACC, ADNI-MEM, and ADNI-EF and the CU group having the highest means (**Table 2; Figure 2a**). There were

significant differences in the rate of change over time across groups for PACC ( $F(2,1015)=120.6$ ,  $p<.0001$ ), ADNI-MEM ( $F(2,1104)=55.4$ ,  $p<.0001$ ), and ADNI-EF ( $F(2,1081)=51.23$ ,  $p<.0001$ ).

Estimated rates of change (slopes) for each cognitive measure are shown in **Table 3** and **Figure 2b**.

Across all three outcome measures, the AD group experienced the steepest decline, and the MCI group also declined but less steeply. The CU group did not experience a decline in either PACC ( $p=0.70$ ) or ADNI-EF ( $p=0.22$ ), but experienced a statistically significant, albeit small in magnitude, increase in ADNI-MEM ( $p=0.01$ ).

*Effects of AD Pathology Biomarkers and Diagnostic Group on Metrics of Cognitive Functioning:* When the p-tau/ $A\beta_{42}$  ratio was added into the mixed effects models, the three-way interaction of diagnosis group by p-tau/ $A\beta_{42}$  by time was significant for PACC ( $p = .02$ ) and ADNI-MEM ( $p = .004$ ) (**Table 4; Figure 3**). Contrasts in the models revealed that higher p-tau/ $A\beta_{42}$  was associated with steeper declines in PACC over time for the AD group ( $p = .001$ ) and the MCI group ( $p < .0001$ ), but for the CU group there was no effect of p-tau/ $A\beta_{42}$  on change over time ( $p = .51$ ). Similarly, higher p-tau/ $A\beta_{42}$  was associated with steeper declines in ADNI-MEM for the AD group ( $p = .001$ ) and the MCI group ( $p < .0001$ ) but not the CU group ( $p = .27$ ). The three-way interaction of p-tau/ $A\beta_{42}$  by diagnosis group by time was not significant for ADNI-EF ( $p = .31$ ), but the two-way interaction p-tau/ $A\beta_{42}$  by time was significant ( $p < .0001$ ) indicating that there was an effect of pathology on the rate of change in ADNI-EF over time, but that this effect did not significantly differ by diagnostic group. Pooled across groups, higher ratio of p-tau/ $A\beta_{42}$  was associated with a steeper decline in ADNI-EF.

## Discussion

The conceptualization of AD on the basis of AD neuropathology includes the presence of both  $\beta$ -amyloid pathology and phosphorylated tau as necessary evidence for AD<sup>11,38</sup>. In support of this, there is growing evidence for a strong concordance between combined  $A\beta$  and tau CSF-based measures and uptake of amyloid tracers in PET imaging<sup>26,27,29</sup>. For example, Roe et al. examining the relationships between CSF-

based and PET-based markers of pathology found that PiB uptake values were predicted by an interaction between CSF-A $\beta_{42}$  and CSF tau<sup>29</sup>. In our study, as would be expected, the ratio of p-tau/A $\beta_{42}$  was the highest in AD individuals, followed by MCI, and then CU older adults. Interestingly, the effect sizes for the CSF ratio was much larger compared to the effect size for either A $\beta_{42}$  or p-tau, suggesting that this combined metric of CSF-based biomarkers may be best able to distinguish the three diagnostic groups employed in the study.

Besides having AD pathology, the clinical diagnosis of Alzheimer-related MCI and dementia requires clinical symptomatology, with an emphasis on multi-domain cognitive deficits including memory impairment. Importantly, AD-specific pathology, quantified here as the ratio of CSF p-tau/A $\beta_{42}$ , predicted decline in global cognition (PACC) and episodic memory (ADNI-MEM) only for individuals with MCI and AD. Decrements in memory abilities are considered to be one of the earliest signs of cognitive dysfunction related to AD<sup>39-41</sup>, with declines in global functioning following the memory decline, and in the later stages of the disease serving as the leading predictor of long-term cognitive changes<sup>39,42,43</sup>. Executive ability impairments, a broad umbrella term encompassing a multitude of top-down, prefrontal-reliant operations<sup>44</sup>, and known to be sensitive to age-related decline<sup>45,46</sup>, are also seen early on in those with AD. This study showed that a composite measure of executive functioning (ADNI-EF) was associated with CSF p-tau/A $\beta_{42}$  across all diagnostic groups. Although the decline in cognitive functioning, and notably decrements in sub-domains of executive functioning, have been well documented with advancing age, there is significant individual variability in the temporal trajectory and scope of such deficits<sup>46</sup>. This study, by examining linkages between biomarkers of brain pathology and cognitive decline, provides support that the accumulation of amyloid and tau pathology worsens executive abilities not only in MCI and AD groups but also in CU older adults. This suggests that executive skills may be as early or perhaps an even earlier indicator of AD cognitive decline as memory impairments.

Our results are in agreement with the growing number of studies investigating the synergistic contribution of the two hallmark proteinopathies in predicting cognitive decline in preclinical AD. Individuals with lower levels of CSF A $\beta_{42}$  and elevated levels of CSF p-tau have an increased risk of

conversion to MCI<sup>47</sup>. Additionally, combined measures of amyloid beta and tau correlates with both cognitive and functional abilities. Increasing AD pathology biomarkers predicts progression on the Clinical Dementia Rating Scale<sup>48</sup>, cross-sectionally associates with driving performance<sup>49</sup>, and longitudinally predicts decline in global cognition,<sup>32</sup> memory, executive functioning, and semantic fluency<sup>33</sup>.

Strengths of this study includes the use of composite measures to quantify cognitive domains. By employing validated composite measures that included metrics across multiple measures and reducing variability and measurement error associated with individual tasks, our measures are likely to be more sensitive at detecting hypothesized effects. Another strength is the use of continuous measures of brain pathology as predictors of cognitive decline. Prior evidence suggests sizeable heterogeneity in classification of participants based on the differences in employed biomarkers. For example, in a recent investigation from the BioFINDER study, cut-off thresholds based on CSF p-tau resulted in a larger proportion of CU participants being classified as tau-positive compared with those classified using tau PET<sup>50</sup>. Additionally, in the same study, sensitivities of the various biomarkers in predicting cognitive decline was substantially reduced when dichotomized values were employed compared with the use of continuous measures, especially for CU individuals where pathology accumulation is still growing.

There are a number of limitations of this study that are important to note. We included a relatively short follow-up period of two years despite availability of additional longitudinal data in the ADNI dataset. However, this a priori decision was driven based on the significant attrition in this dataset by diagnostic groups, such that there were relatively fewer number of AD participants with available follow-up data. Additionally, we limited our analyses to CSF-based biomarkers of A $\beta$ <sub>42</sub> and p-tau. It is possible that PET-based biomarkers of beta amyloid and tau provide a different pattern of results, and inclusion of other synaptic and neuronal biomarkers of AD pathophysiology could provide additional valuable insights for prediction of the clinical progression of the disease.

In conclusion, the ratio of CSF-based biomarkers of amyloid and tau pathology, show larger between-group effect sizes than the individual biomarkers when comparing CU participants with MCI

and AD individuals. Worsening neuropathological changes seen in participants predicted declines in global metrics of cognition and episodic memory in those with MCI and AD and predicted declines in executive abilities in those with MCI, AD, and cognitively unimpaired participants suggesting executive impairments may occur very early in AD individuals. Amyloid and tau pathology are potential sources of heterogeneity explaining some of the variability in AD-related decline in global cognition, episodic memory, and executive functioning.

## APPENDIX 1: Authors

Name	Location	Contribution
Ruchika Prakash	The Ohio State University, OH	Designed and conceptualized the study; wrote the first draft of the manuscript; and incorporated feedback from all co-authors
Michael McKenna	The Ohio State University, OH	Downloaded and analyzed the data; revised manuscript for intellectual content
Oyetunde Gbadeyan	The Ohio State University, OH	Created figures; revised manuscript for intellectual content
Rebecca Andridge	The Ohio State University, OH	Analyzed the data; revised manuscript for intellectual content
Douglas Scharre	The Ohio State University, OH	Interpreted data; revised manuscript for intellectual content

# References

1. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. 1984;34(7):939-944. doi:10.1212/wnl.34.7.939
2. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 2011;7(3):263-269. doi:10.1016/j.jalz.2011.03.005
3. Petersen RC. *Mild Cognitive Impairment: Aging to Alzheimer's Disease*. Oxford University Press; 2003.
4. Price JL, McKeel DW, Buckles VD, et al. Neuropathology of nondemented aging: Presumptive evidence for preclinical Alzheimer disease. *Neurobiol Aging*. 2009;30(7):1026-1036. doi:10.1016/j.neurobiolaging.2009.04.002
5. Villemagne VL, Ong K, Mulligan RS, et al. Amyloid Imaging with 18F-Florbetaben in Alzheimer Disease and Other Dementias. *J Nucl Med*. 2011;52(8):1210-1217. doi:10.2967/jnumed.111.089730
6. Hulette CM, Welsh-Bohmer KA, Murray MG, Saunders AM, Mash DC, McIntyre LM. Neuropathological and Neuropsychological Changes in "Normal" Aging: Evidence for Preclinical Alzheimer Disease in Cognitively Normal Individuals. *J Neuropathol Exp Neurol*. 1998;57(12):1168-1174. doi:10.1097/00005072-199812000-00009

7. Price JL, Morris JC. Tangles and plaques in nondemented aging and “preclinical” Alzheimer’s disease. *Ann Neurol*. 1999;45(3):358-368. doi:10.1002/1531-8249(199903)45:3<358::AID-ANA12>3.0.CO;2-X
8. Bennett DA, Schneider JA, Aggarwal NT, et al. Decision Rules Guiding the Clinical Diagnosis of Alzheimer’s Disease in Two Community-Based Cohort Studies Compared to Standard Practice in a Clinic-Based Cohort Study. *Neuroepidemiology*. 2006;27(3):169-176. doi:10.1159/000096129
9. Rowe CC, Ellis KA, Rimajova M, et al. Amyloid imaging results from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging. *Neurobiol Aging*. 2010;31(8):1275-1283. doi:10.1016/j.neurobiolaging.2010.04.007
10. Jack CR, Barnes J, Bernstein MA, et al. Magnetic resonance imaging in Alzheimer’s Disease Neuroimaging Initiative 2. *Alzheimers Dement*. 2015;11(7):740-756. doi:10.1016/j.jalz.2015.05.002
11. Jack CR, Bennett DA, Blennow K, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer’s disease. *Alzheimers Dement J Alzheimers Assoc*. 2018;14(4):535-562. doi:10.1016/j.jalz.2018.02.018
12. Lee I-M, Shiroma EJ, Kamada M, Bassett DR, Matthews CE, Buring JE. Association of Step Volume and Intensity With All-Cause Mortality in Older Women. *JAMA Intern Med*. 2019;179(8):1105. doi:10.1001/jamainternmed.2019.0899
13. Hardy J, Allsop D. Amyloid deposition as the central event in the aetiology of Alzheimer’s disease. *Trends Pharmacol Sci*. 1991;12(10):383-388. doi:10.1016/0165-6147(91)90609-v
14. Bennett DA, Schneider JA, Wilson RS, Bienias JL, Arnold SE. Neurofibrillary Tangles Mediate the Association of Amyloid Load With Clinical Alzheimer Disease and Level of Cognitive Function. *Arch Neurol*. 2004;61(3):378-384. doi:10.1001/archneur.61.3.378



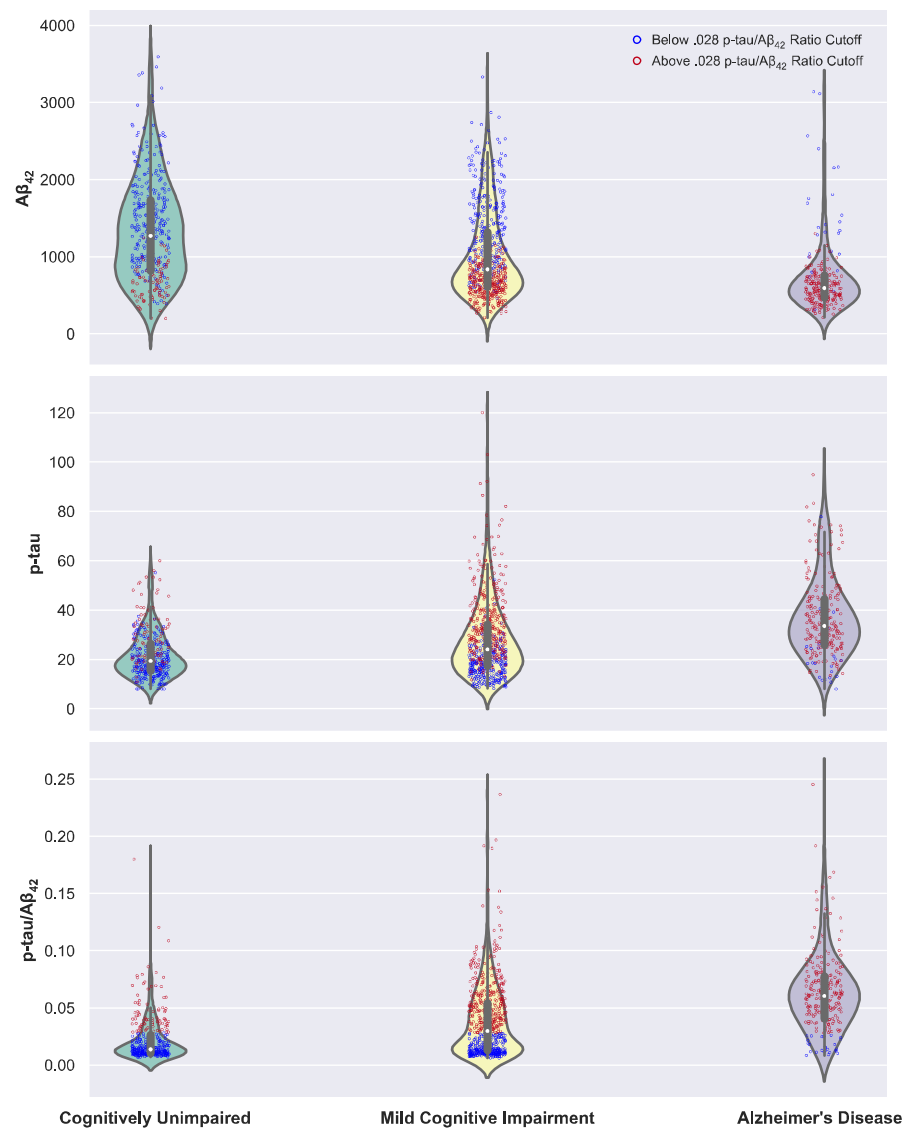
15. Bateman RJ, Xiong C, Benzinger TLS, et al. Clinical and Biomarker Changes in Dominantly Inherited Alzheimer's Disease. *N Engl J Med*. 2012;367(9):795-804. doi:10.1056/NEJMoa1202753
16. Montine TJ, Phelps CH, Beach TG, et al. National Institute on Aging–Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. *Acta Neuropathol (Berl)*. 2012;123(1):1-11. doi:10.1007/s00401-011-0910-3
17. Hyman BT, Phelps CH, Beach TG, et al. National Institute on Aging–Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimers Dement*. 2012;8(1):1-13. doi:10.1016/j.jalz.2011.10.007
18. Lee JC, Kim SJ, Hong S, Kim Y. Diagnosis of Alzheimer's disease utilizing amyloid and tau as fluid biomarkers. *Exp Mol Med*. 2019;51(5):1-10. doi:10.1038/s12276-019-0250-2
19. Buerger K, Ewers M, Pirttilä T, et al. CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease. *Brain J Neurol*. 2006;129(Pt 11):3035-3041. doi:10.1093/brain/awl269
20. Tapiola T, Alafuzoff I, Herukka S-K, et al. Cerebrospinal fluid {beta}-amyloid 42 and tau proteins as biomarkers of Alzheimer-type pathologic changes in the brain. *Arch Neurol*. 2009;66(3):382-389. doi:10.1001/archneurol.2008.596
21. Hesse C, Rosengren L, Andreasen N, et al. Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. *Neurosci Lett*. 2001;297(3):187-190. doi:10.1016/S0304-3940(00)01697-9
22. Ost M, Nylén K, Csajbok L, et al. Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury. *Neurology*. 2006;67(9):1600-1604. doi:10.1212/01.wnl.0000242732.06714.0f

23. Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. *Lancet Neurol.* 2006;5(3):228-234. doi:10.1016/S1474-4422(06)70355-6
24. Mattsson N, Zetterberg H, Hansson O, et al. CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. *JAMA.* 2009;302(4):385-393. doi:10.1001/jama.2009.1064
25. Palmqvist S, Zetterberg H, Mattsson N, et al. Detailed comparison of amyloid PET and CSF biomarkers for identifying early Alzheimer disease. *Neurology.* 2015;85(14):1240-1249. doi:10.1212/WNL.0000000000001991
26. Schindler SE, Gray JD, Gordon BA, et al. Cerebrospinal fluid biomarkers measured by Elecsys assays compared to amyloid imaging. *Alzheimers Dement.* 2018;14(11):1460-1469. doi:10.1016/j.jalz.2018.01.013
27. Hansson O, Seibyl J, Stomrud E, et al. CSF biomarkers of Alzheimer's disease concord with amyloid- $\beta$  PET and predict clinical progression: A study of fully automated immunoassays in BioFINDER and ADNI cohorts. *Alzheimers Dement.* 2018;14(11):1470-1481. doi:10.1016/j.jalz.2018.01.010
28. Gustafson DR, Skoog I, Rosengren L, Zetterberg H, Blennow K. Cerebrospinal fluid  $\beta$ -amyloid 1–42 concentration may predict cognitive decline in older women. *J Neurol Neurosurg Psychiatry.* 2007;78(5):461-464. doi:10.1136/jnnp.2006.100529
29. Roe CM, Fagan AM, Grant EA, Holtzman DM, Morris JC. CSF biomarkers of Alzheimer disease. *Neurology.* 2013;81(23):2028-2031. doi:10.1212/01.wnl.0000436940.78152.05

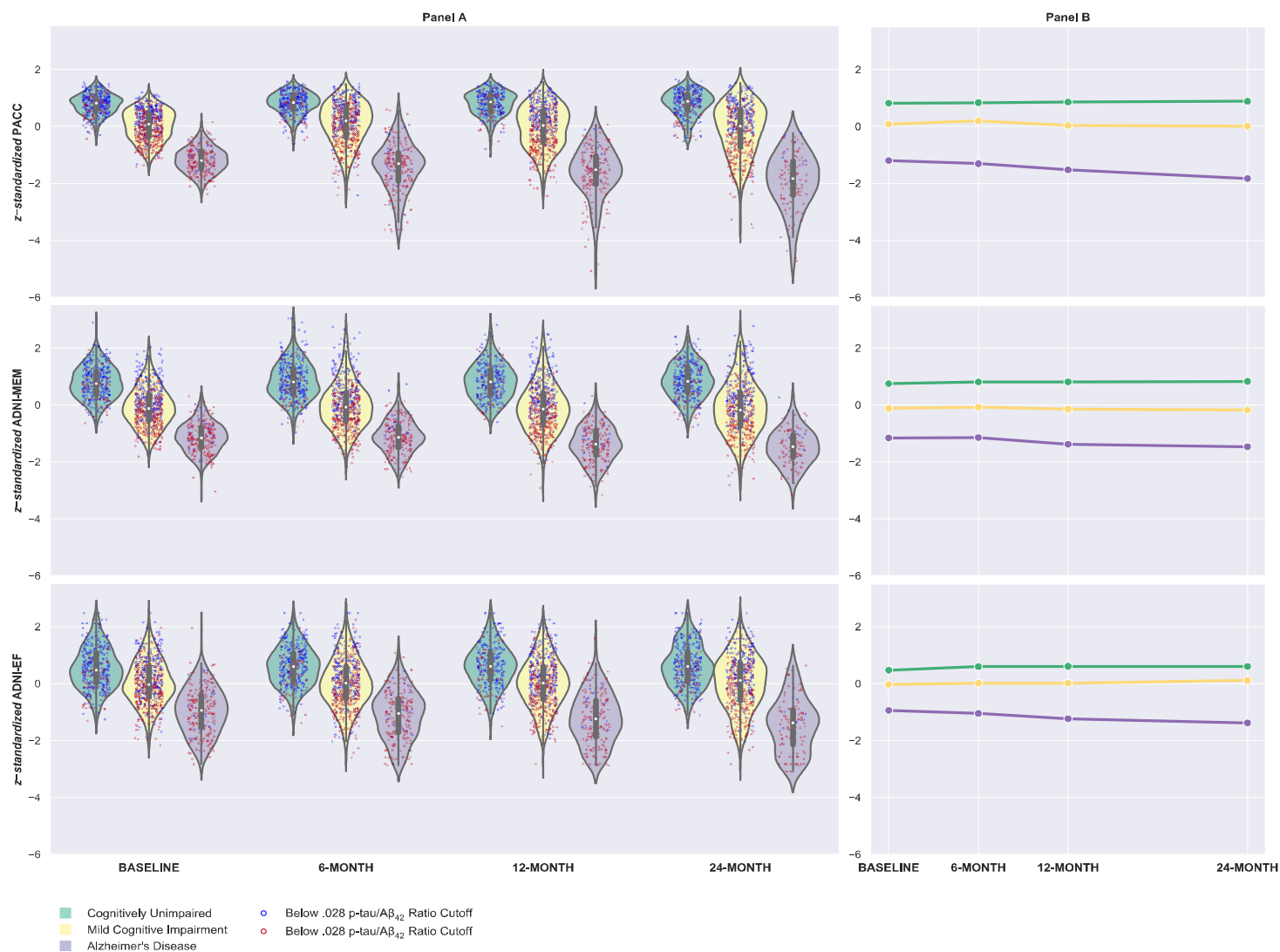
30. Glodzik L, de Santi S, Tsui WH, et al. Phosphorylated tau 231, memory decline and medial temporal atrophy in normal elders. *Neurobiol Aging*. 2011;32(12):2131-2141.  
doi:10.1016/j.neurobiolaging.2009.12.026
31. Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM. Cerebrospinal Fluid tau/ $\beta$ -Amyloid42 Ratio as a Prediction of Cognitive Decline in Nondemented Older Adults. *Arch Neurol*. 2007;64(3):343-349. doi:10.1001/archneur.64.3.noc60123
32. Soldan A, Pettigrew C, Cai Q, et al. Hypothetical Preclinical Alzheimer Disease Groups and Longitudinal Cognitive Change. *JAMA Neurol*. 2016;73(6):698-705.  
doi:10.1001/jamaneurol.2016.0194
33. Ho JK, Nation DA, for the ADNI Initiative. Neuropsychological Profiles and Trajectories in Preclinical Alzheimer's Disease. *J Int Neuropsychol Soc*. 2018;24(7):693-702.  
doi:10.1017/S135561771800022X
34. Petersen RC, Roberts RO, Knopman DS, et al. Prevalence of mild cognitive impairment is higher in men. *Neurology*. 2010;75(10):889-897. doi:10.1212/WNL.0b013e3181f11d85
35. Donohue MC, Sperling RA, Salmon DP, et al. The Preclinical Alzheimer Cognitive Composite: Measuring Amyloid-Related Decline. *JAMA Neurol*. 2014;71(8):961-970.  
doi:10.1001/jamaneurol.2014.803
36. Crane PK, Carle A, Gibbons LE, et al. Development and assessment of a composite score for memory in the Alzheimer's Disease Neuroimaging Initiative (ADNI). *Brain Imaging Behav*. 2012;6(4):502-516. doi:10.1007/s11682-012-9186-z

37. Gibbons LE, Carle AC, Mackin RS, et al. A composite score for executive functioning, validated in Alzheimer's Disease Neuroimaging Initiative (ADNI) participants with baseline mild cognitive impairment. *Brain Imaging Behav.* 2012;6(4):517-527. doi:10.1007/s11682-012-9176-1
38. Pimplikar SW. Reassessing the amyloid cascade hypothesis of Alzheimer's disease. *Int J Biochem Cell Biol.* 2009;41(6):1261-1268. doi:10.1016/j.biocel.2008.12.015
39. Schindler SE, Jasielec MS, Weng H, et al. Neuropsychological measures that detect early impairment and decline in preclinical Alzheimer disease. *Neurobiol Aging.* 2017;56:25-32. doi:10.1016/j.neurobiolaging.2017.04.004
40. Albert MS, Moss MB, Tanzi R, Jones K. Preclinical prediction of AD using neuropsychological tests. *J Int Neuropsychol Soc.* 2001;7(5):631-639. doi:10.1017/S1355617701755105
41. Bondi MW, Jak AJ, Delano-Wood L, Jacobson MW, Delis DC, Salmon DP. Neuropsychological Contributions to the Early Identification of Alzheimer's Disease. *Neuropsychol Rev.* 2008;18(1):73-90. doi:10.1007/s11065-008-9054-1
42. Insel PS, Palmqvist S, Mackin RS, et al. Assessing risk for preclinical  $\beta$ -amyloid pathology with APOE, cognitive, and demographic information. *Alzheimers Dement Diagn Assess Dis Monit.* 2016;4(1):76-84. doi:10.1016/j.dadm.2016.07.002
43. Amieva H, Le Goff M, Millet X, et al. Prodromal Alzheimer's disease: successive emergence of the clinical symptoms. *Ann Neurol.* 2008;64(5):492-498. doi:10.1002/ana.21509
44. Miyake A, Friedman NP, Emerson MJ, Witzki AH, Howerter A, Wager TD. The unity and diversity of executive functions and their contributions to complex "Frontal Lobe" tasks: a latent variable analysis. *Cognit Psychol.* 2000;41(1):49-100. doi:10.1006/cogp.1999.0734

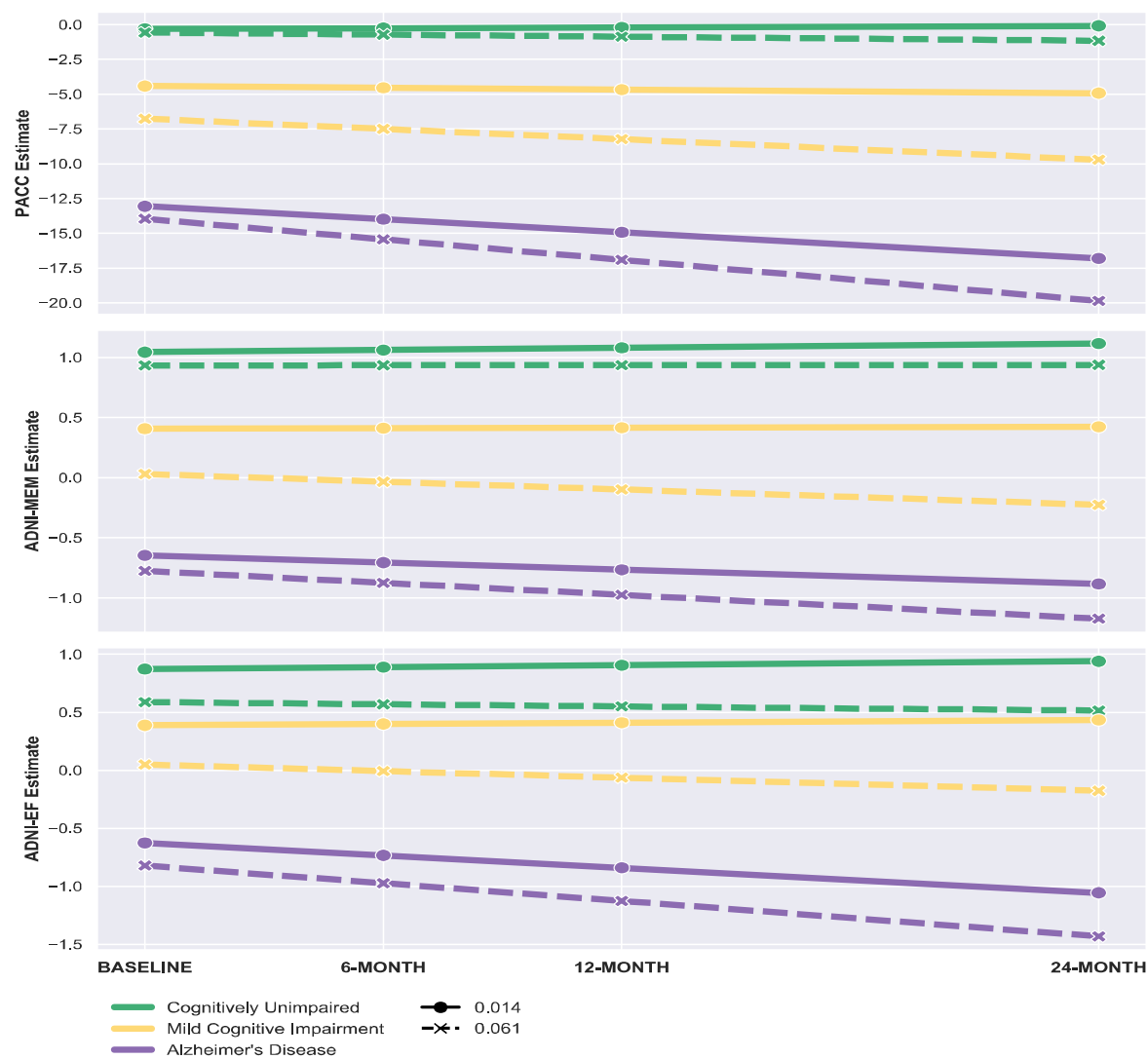
45. Park DC, Reuter-Lorenz P. The Adaptive Brain: Aging and Neurocognitive Scaffolding. *Annu Rev Psychol.* 2009;60(1):173-196. doi:10.1146/annurev.psych.59.103006.093656
46. Goh JO, An Y, Resnick SM. Differential trajectories of age-related changes in components of executive and memory processes. *Psychol Aging.* 2012;27(3):707-719. doi:10.1037/a0026715
47. Li G, Sokal I, Quinn JF, et al. CSF tau/Abeta42 ratio for increased risk of mild cognitive impairment: a follow-up study. *Neurology.* 2007;69(7):631-639. doi:10.1212/01.wnl.0000267428.62582.aa
48. Dumurgier J, Hanseeuw BJ, Hatling FB, et al. Alzheimer's Disease Biomarkers and Future Decline in Cognitive Normal Older Adults. *J Alzheimers Dis JAD.* 2017;60(4):1451-1459. doi:10.3233/JAD-170511
49. Roe CM, Barco PP, Head DM, et al. Amyloid Imaging, Cerebrospinal Fluid Biomarkers Predict Driving Performance Among Cognitively Normal Individuals. *Alzheimer Dis Assoc Disord.* 2017;31(1):69-72. doi:10.1097/WAD.0000000000000154
50. Mattsson-Carlgrén N, Leuzy A, Janelidze S, et al. The implications of different approaches to define AT(N) in Alzheimer disease. *Neurology.* Published online May 12, 2020:10.1212/WNL.00000000000009485. doi:10.1212/WNL.00000000000009485



**Figure 1:** Presents the distribution of  $A\beta_{42}$ , p-tau, and the ratio of p-tau/ $A\beta_{42}$  across the three diagnostic groups. The individual data points are color coded with red dots for participants having a p-tau/ $A\beta_{42}$  value above the cut-off, and blue dots representing participants below the cut-off.



**Figure 2:** Panel A presents the distribution of z-standardized global cognition (PACC scores), episodic memory (ADNI-MEM scores), and executive functioning (ADNI-EF scores) across the three diagnostic groups, and Panel B shows the decline in the three cognitive domains across the two-year period.



**Figure 3:** Predicted trajectories of each outcome by diagnosis group at two levels of p-tau/Aβ<sub>42</sub> ratio (solid lines: ratio = 0.014 = mean of values below established cutoff; dotted lines: ratio = 0.061 = mean of values above established cutoff)



**Table 1:** Baseline characteristics of participants by diagnostic group

Characteristic	CU (n=367)		MCI (n=619)		AD (n=229)		P-value
	Mean (SD) or N (%)	Range	Mean (SD) or N (%)	Range	Mean (SD) or N (%)	Range	
Sex							0.001
Female	193 (53%)		255 (41%)		95 (41%)		
Male	174 (47%)		364 (59%)		134 (59%)		
Race*							0.03
White	333 (91%)		583 (94%)		219 (96%)		
American Indian / Alaskan	1 (0.3%)		1 (0.2%)		0 (0%)		
Asian	4 (1%)		9 (1%)		4 (2%)		
Black	24 (7%)		15 (2%)		5 (2%)		
Hawaiian / Pacific Islander	0 (0%)		2 (0.3%)		0 (0%)		
More than One Race	5 (1%)		7 (1%)		1 (0.5%)		
Age (years)	73.8 (5.9)	56.2 to 89.6	72.3 (7.5)	54.4 to 91.4	74.6 (8.2)	55.6 to 90.3	<.0001
Years of education	16.4 (2.6)	6 to 20	16.1 (2.8)	6 to 20	15.4 (2.9)	4 to 20	0.0004
<i>APOE</i>							<.0001
ε4 allele absent	264 (72%)		314 (51%)		73 (32%)		
1 ε4 allele	94 (26%)		239 (39%)		107 (47%)		
2 ε4 alleles	9 (2%)		66 (11%)		49 (21%)		
<i>APOE</i> , collapsed							<.0001
ε4 allele absent	264 (72%)		314 (51%)		73 (32%)		
ε4 allele(s) present	103 (28%)		305 (49%)		156 (68%)		

CU = Cognitively unimpaired; MCI = Mild cognitive impairment; AD = Alzheimer's disease

*APOE* = apolipoprotein E genotype

\*Race missing for 2 subjects in the MCI group

**Table 2:** Summary of pathology markers and cognitive measures by diagnostic group

Measure	Diagnostic Group	Mean (SD) or N (%)	Range	P-value	R <sup>2</sup>
A $\beta$ <sub>42</sub>	CU	1336 (650)	200 to 3592	<.0001	0.13
	MCI	1018 (555)	210.9 to 3331		
	AD	693 (417)	212.3 to 3139		
p-tau	CU	21.8 (9.3)	8 to 60	<.0001	0.12
	MCI	27.8 (15.0)	8.2 to 120		
	AD	36.6 (15.8)	8 to 95		
p-tau/A $\beta$ <sub>42</sub>	CU	0.022 (0.019)	0.007 to 0.18	<.0001	0.20
	MCI	0.038 (0.031)	0.006 to 0.24		
	AD	0.063 (0.034)	0.008 to 0.25		
p-tau/A $\beta$ <sub>42</sub> > 0.028	CU	86 (23%)		<.0001	--
	MCI	319 (52%)			
	AD	203 (89%)			
Baseline PACC	CU	-0.26 (2.6)	-9.0 to 5.3	<.0001	0.67
	MCI	-5.6 (3.9)	-16.0 to 2.7		
	AD	-14.4 (3.1)	-23.0 to -4.7		
Baseline ADNI-MEM	CU	1.05 (0.57)	-0.37 to 3.1	<.0001	0.52
	MCI	0.20 (0.69)	-1.5 to 2.3		
	AD	-0.87 (0.52)	-2.8 to 0.57		
Baseline ADNI-EF	CU	0.80 (0.82)	-1.3 to 3.0	<.0001	0.31
	MCI	0.23 (0.89)	-2.3 to 3.0		
	AD	-0.90 (0.92)	-3.0 to 2.4		

CU = Cognitively unimpaired; MCI = Mild cognitive impairment; AD = Alzheimer's diseases

**Table 3** – Estimated slopes (change in outcome for a 1 month increase in time) for cognitive outcomes from linear mixed effects models

Outcome	Diagnostic Group	Slope (SE)	p-value
PACC	CU	0.0034 (0.0090)	0.70
	MCI	-0.074 (0.0071)	<.0001
	AD	-0.26 (0.014)	<.0001
ADNI-MEM	CU	0.0025 (0.0010)	0.01
	MCI	-0.0050 (0.00081)	<.0001
	AD	-0.017 (0.0016)	<.0001
ADNI-EF	CU	0.0018 (0.0015)	0.22
	MCI	-0.0038 (0.0012)	0.001
	AD	-0.026 (0.0024)	<.0001

Adjusted for age, years of education, sex, *APOE* status (ε4 allele present vs absent)

**Table 4** – Test statistics for the linear mixed models evaluating the effect of p-tau/A $\beta_{42}$  on cognitive outcomes.

Effect	PACC		ADNI-MEM		ADNI-EF	
	Test Statistic	P-value	Test Statistic	P-value	Test Statistic	P-value
time	F(1,1145) = 9.2	<b>0.003</b>	F(1,1224) = 0	0.99	F(1,1218) = 1.04	0.31
dx	F(2,1209) = 324.98	<b>&lt;.0001</b>	F(2,1205) = 172.61	<b>&lt;.0001</b>	F(2,1207) = 70.11	<b>&lt;.0001</b>
time*dx	F(2,1041) = 11.54	<b>&lt;.0001</b>	F(2,1131) = 6.12	<b>0.002</b>	F(2,1117) = 7.94	<b>0.0004</b>
ratio	F(1,1214) = 42.31	<b>&lt;.0001</b>	F(1,1205) = 37.44	<b>&lt;.0001</b>	F(1,1207) = 33.37	<b>&lt;.0001</b>
time*ratio	F(1,1023) = 54.67	<b>&lt;.0001</b>	F(1,1100) = 35.95	<b>&lt;.0001</b>	F(1,1095) = 22.34	<b>&lt;.0001</b>
ratio*dx	F(2,1207) = 17.09	<b>&lt;.0001</b>	F(2,1205) = 10.94	<b>&lt;.0001</b>	F(2,1204) = 1.31	0.27
time*ratio*dx	F(2,1026) = 3.96	<b>0.02</b>	F(2,1107) = 5.62	<b>0.004</b>	F(2,1098) = 1.17	0.31
age	F(1,1200) = 52.42	<b>&lt;.0001</b>	F(1,1205) = 45.08	<b>&lt;.0001</b>	F(1,1203) = 82.65	<b>&lt;.0001</b>
years of education	F(1,1200) = 63.65	<b>&lt;.0001</b>	F(1,1205) = 33.11	<b>&lt;.0001</b>	F(1,1201) = 35.09	<b>&lt;.0001</b>
sex	F(1,1196) = 11.37	<b>0.0008</b>	F(1,1205) = 62.27	<b>&lt;.0001</b>	F(1,1200) = 1.28	0.26
<i>APOE</i> ( $\epsilon 4$ present vs absent)	F(1,1202) = 2.63	0.11	F(1,1205) = 3.98	<b>0.046</b>	F(1,1203) = 0.44	0.51

dx = diagnostic group; ratio = p-tau/A $\beta_{42}$