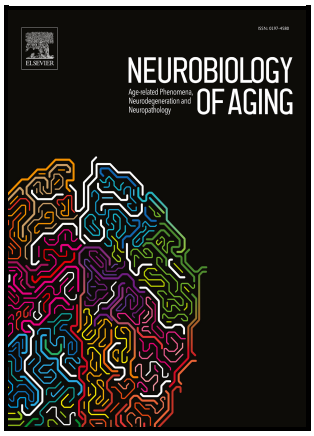

Annualized changes in rate of amyloid deposition and neurodegeneration are greater in participants who become amyloid positive than those who remain amyloid negative

Renée C. Groechel, Yorghos Tripodis, Michael L. Alosco, Jesse Mez, Wei Qiao Qiu, Gustavo Mercier, Lee Goldstein, Andrew E. Budson, Neil Kowall, Ronald J. Killiany

PII: S0197-4580(23)00051-9

DOI: https://doi.org/10.1016/j.neurobiolaging.2023.03.005

Reference: NBA11515

To appear in: *Neurobiology of Aging*

Received date: 6 July 2022
Revised date: 1 March 2023
Accepted date: 6 March 2023

Please cite this article as: Renée C. Groechel, Yorghos Tripodis, Michael L. Alosco, Jesse Mez, Wei Qiao Qiu, Gustavo Mercier, Lee Goldstein, Andrew E. Budson, Neil Kowall and Ronald J. Killiany, Annualized changes in rate of amyloid deposition and neurodegeneration are greater in participants who become amyloid positive than those who remain amyloid negative, *Neurobiology of Aging,* (2022) doi:https://doi.org/10.1016/j.neurobiolaging.2023.03.005

Annualized changes in rate of amyloid deposition and neurodegeneration are greater in participants who become amyloid positive than those who remain amyloid negative

Author(s):

Renée C. Groechel, PhD[1*]; Yorghos Tripodis, PhD[2,3]; Michael L. Alosco, PhD[3,4]; Jesse Mez, MD[3,4]; Wei Qiao Qiu MD/PhD[3,5]; Gustavo Mercier, MD/PhD[3,6]; Lee Goldstein, MD/PhD[3,4]; Andrew E. Budson, MD [3,4]; Neil Kowall, MD[3,4]; Ronald J. Killiany[1,3,4,6] for the Alzheimer's Disease Neuroimaging Initiative*

*Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (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

Corresponding Author:

Renée C. Groechel, PhD

groechelrc@nih.gov
National Institute of Neurological Disorders & Stroke (NINDS) Intramural Research Program, National Institute of Health (NIH)
10 Center Drive
Building 10 Room: 4D37
Bethesda, MD 20814

Affiliation Information for All Authors:

[1]Department of Anatomy & Neurobiology, Boston University School of Medicine, Boson MA
[2]Department of Biostatistics, Boston University School of Public Health, Boston, MA
[3]Boston University Alzheimer's Disease Research Center, Boston MA
[4]Department of Neurology, Boston University School of Medicine, Boson MA
[5]Department of Psychiatry, Boston University School of Medicine, Boson MA
[6]Department of Radiology, Boston University School of Medicine, Boson MA

[*] Disclaimer: This article was prepared while first author, Dr. Renee C. Groechel, was at Boston University School of Medicine. Dr. Groechel is now employed at the National Institute of Neurological Disorders & Stroke (NINDS) Intramural Research Program, National Institute of Health (NIH). Dr. Groechel's present address and email are stated above. The opinions expressed in this article are the author's own and do not reflect the view of the National Institutes of Health, the Department of Health and Human Services, or the United States Government.

Abstract

This study longitudinally examined participants in the Alzheimer's Disease Neuroimaging Initiative (ADNI) who underwent a conversion in amyloid-beta (Aβ) status in comparison to a group of ADNI participants who did not show a change in amyloid status over the same follow-up period. Participants included 136 non-demented ADNI participants with 2 florbetapir positron emission tomography (PET) scans. Of these participants, 68 showed amyloid conversion as measured on florbetapir PET, and the other 68 did not. Amyloid converters and non-converters were chosen to have representative demographic data (age, education, sex, diagnostic status, and race). The amyloid converter group showed increased prevalence of *APOE* ε4 ($p < 0.001$), greater annualized percent volume loss in selected magnetic resonance imaging (MRI) regions ($p < 0.05$), lower cerebrospinal fluid (CSF) $A\beta_{1\text{-}42}$ ($p < 0.001$), and greater amyloid retention (as measured by standard uptake value ratios) on florbetapir PET scans ($p < 0.001$) in comparison to the non-converter group. These results provide compelling evidence that important neuropathological changes are occurring alongside amyloid conversion.

Keywords: Alzheimer's disease; amyloid; cognition; dementia; magnetic resonance imaging; positron emission tomography

Abbreviations: A- = amyloid negative; A+ = amyloid positive; Aβ = amyloid-beta; $A\beta_{1\text{-}42}$ = amyloid-beta 1-42 peptide; AD = Alzheimer's disease; ADNI = Alzheimer's Disease Neuroimaging Initiative; ANOVA = analysis of variance; ANCOVA = analysis of covariance; APOE = apolipoprotein E; APP = amyloid precursor protein; AT(N) = amyloid, tau, neurodegeneration; CI = confidence interval; CN = cognitively normal; CSF = cerebrospinal fluid; DICOM = digital imaging and communications in medicine; eTIV = estimated total intracranial volume; MCI = mild cognitive impairment; MRI = magnetic resonance imaging; (N)- = neurodegeneration negative; (N)+ = neurodegeneration positive; PET = positron emission tomography; p-tau = tau phosphorylated at threonine 181; ROI = region of interest; SUVR = standard uptake value ratio; T- = tau negative, T+ = tau positive; t-tau = total tau

Funding: This work was supported in part by NIH/NIA P30-AG072978.

Disclosure Statement: None of the authors part of this work have any conflicts of interests.

Introduction

Cleavage of the amyloid precursor protein, an essential element of the cell membrane, results in amyloid-beta (Aβ) peptides leaving the cell membrane. Some peptides are cleared by the cerebrospinal fluid (CSF), and others by the vascular system (Brothers et al., 2018). Other

peptides are more difficult to clear and have the capacity to change shape and interact with similar molecules prior to self-aggregating into long fibrils that form β-sheets (Brothers et al., 2018; Chen & Mobley, 2019; Hensley et al., 1994). The progression of insoluble fibrillar Aβ plaque deposition in the brain has been linked to aging and increasing severity of Alzheimer's disease (AD) (Braak & Braak, 1991). Thus, detection of amyloid in aging adults has become a paramount focus of AD research and amyloid is the target of many drug therapeutics (Brothers et al., 2018).

Soluble and insoluble Aβ burden can be measured *in vivo* in CSF. Insoluble Aβ can also be measured using radioactive ligands, such as the [^{18}F]-labeled florbetapir ligand, with positron emission tomography (PET) (Landau et al., 2013; Mattsson et al., 2015). Using established florbetapir PET standardized uptake values (SUVR) thresholds, the presence of Aβ can be categorized as amyloid negative (A-) or amyloid positive (A+) (Jack et al., 2018).

Studies have begun investigating how AD biomarkers and cognition are modified in people with changing Aβ burden (Dubois et al., 2018; Harrison et al. 2021; Landau et al., 2018; Sperling et al., 2020; van der Kall et al., 2021). In the present study, we sought to longitudinally examine participants in the Alzheimer's Disease Neuroimaging Initiative (ADNI) who underwent a conversion in amyloid status from negative (A-) to positive (A+) in comparison to a group of participants who remained A- over the same follow-up period. Conversion from A- to A+ was based upon established florbetapir PET SUVR thresholds (Jagust et al., 2015; Landau et al., 2013;). Our hypothesis was that the amyloid converter group would show an increased frequency of apolipoprotein E *(APOE)* ε4 alleles, greater annualized percent volume loss in selected magnetic resonance imaging (MRI) regions, and lower CSF concentration of the amyloid beta 1-42 peptide ($A\beta_{1-42}$), in comparison to the non-converter group. Based on previous

studies in the literature, we did not expect to see differences in CSF measures of p-tau or cognitive performance (Gordon et al., 2018; Harrison et al., 2021; Ossenkoppele et al., 2019).

2. Material and Methods

2.1 Study Design and Participants

Data used in the preparation of this article were obtained from the ADNI database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial MRI, PET, other biological markers, and clinical/neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD.

This study utilized data from 136 ADNI participants. Selection criteria was at least two florbetapir amyloid PET scans. All florbetapir PET data used in this study were downloaded in July 2021. All 136 participants were A- at the first scan and 68 participants were classified as amyloid converters because they later became A+. Amyloid positivity on florbetapir PET was defined as retention above the established SUVR threshold of 1.11 (Landau et al., 2013; see Jagust et al., 2015 for additional details on ADNI PET protocols). The global SUVR used for this study was calculated by combining binding measures from the frontal, angular/posterior cingulate, lateral parietal, and temporal cortices then dividing them by the mean uptake value of the cerebellum. To identify participants who fit conversion criterion, we collected SUVR data from the first PET visit global SUVR became > 1.11 and the PET visit prior. The average time between PET visits in amyloid converters was 26.65 months.

Using this same florbetapir SUVR threshold, we identified 68 ADNI participants who were non-converters. Non-converting participants were selected from the ADNI dataset based upon 2 criteria: (1) that they maintained A- on *all* existing ADNI PET scans (through time of

data collection) and (2) that they had representative (comparable) demographic data of the 68 amyloid converters. For instance, if we identified PET data from a male participant with MCI and 18 years of education who converted from A- to A+ at age 70, we looked for PET data from a 70-year-old male participant with MCI and 18 years of education who remained A- on existing ADNI PET scans. PET data for non-converters were downloaded from two visits which best corresponded to the timeframe and ages of the amyloid converter group. The average time between visits in non-converters was 30.88 months.

In addition to having at least two florbetapir PET scans, all participants had to be diagnosed by the ADNI Clinical Core as cognitively normal (CN) or MCI (Petersen et al., 1999; Petersen et al., 2014). Other variables of interest downloaded from the ADNI repository included demographics (age, years of education, sex, race), medical history such as cardiovascular health and smoking status, and *APOE* ε4. Available T1 MRI, CSF, and cognitive data were also downloaded from the time of both PET visits. Figure 1 outlines the data collection process. Written informed consent or assent was obtained from all participants, and study procedures were approved by the institutional review board at each of the ADNI participating sites.

2.2 Imaging Assessments

The neuroimaging methods and parameters utilized by ADNI for T1 scans have been described previously (Jack et al., 2008). Visual inspection for artifact and unexpected neuropathology by the ADNI MRI Core was completed at the time of image upload to the ADNI.

Two T1 scans from 129 participants were downloaded in their native DICOM format. T1 scans from seven participants were not used because the timing of scans did not correspond to the time of amyloid conversion and/or the time of the PET, CSF, or cognitive data used in this study. The first T1 scan for amyloid converters was selected to be from the ADNI visit (or within

six months after) the participant became A+. The second T1 scan for amyloid converters was chosen to come from a visit prior to conversion that best coincided with PET, CSF, and cognitive data. T1 scans from non-converters were selected to be from the same visits that PET data were collected. After downloading and throughout image processing, images were visually inspected for artifacts that could have impaired image processing.

All 258 T1 MRI scans were processed on a Mac Pro 2013 running OS version 10.14.5 using Freesurfer version 7.2 (Desikan et al., 2006; Iglesias et al., 2015). Scans were processed cross-sectionally followed by the longitudinal stream to extract reliable volume and thickness estimates in FreeSurfer (Reuter et al., 2012). An unbiased within-subject template space and image was created using robust, inverse consistent registration (Reuter et al., 2010; Reuter & Fischl, 2011). Several processing steps, such as skull stripping, Talairach transforms, atlas registration, spherical surface maps and parcellations were then initialized with common information from the within-subject template. This processing stream significantly increases reliability and statistical power (Reuter et al., 2012).

Volumes of regions of interest (ROI) from the Desikan-Killiany atlas were extracted and estimated total intracranial volume (eTIV) was generated by Freesurfer version 7.2 (Desikan et al., 2006; Iglesias et al., 2015). Nineteen cortical parcellations were selected as ROI based upon previous florbetapir PET studies providing evidence that amyloid accumulation frequently occurs in these regions in early phases of AD (Guo et al., 2017; Palmquist et al., 2017; Villemange et al., 2017). These cortical ROI included the following regions: banks of superior temporal sulcus, caudal anterior division of cingulate cortex, inferior parietal cortex, inferior temporal gyrus, isthmus division of cingulate cortex, lateral occipital cortex, lateral division of orbitofrontal cortex, medial division of the orbitofrontal cortex, middle temporal gyrus,

paracentral lobule, pars orbitalis, pars opercularis, pars triangularis, posterior division of cingulate cortex, precuneus, rostral anterior cingulate, superior parietal cortex, superior temporal gyrus, and frontal pole. Additionally, seven subcortical ROI, including the thalamus, hippocampus, caudate, putamen, pallidum, accumbens, and amygdala, were examined. Total volume (right and left hemispheres added together) was calculated for both cortical and subcortical ROI. For each ROI, rate of volume change was annualized then converted to percent change for analyses.

2.3 CSF Sampling and Analysis

One hundred and twenty-six participants had CSF measures generated by the ADNI Biomarker Core. CSF data was used if acquired within ~24 months of the PET data of interest (n = 100; 36 amyloid converters and 64 non-converters). Standard practice of the ADNI Biomarker Core is to measure concentrations of $A\beta_{1-42}$, total tau (t-tau), and tau phosphorylated at threonine 181 (p-tau) in collected CSF samples. Samples were obtained at the various ADNI sites via lumbar puncture as described in ADNI procedures and previous studies (Shaw et al., 2009).

2.4 AT(N) Classification

Ninety-four participants (31 amyloid converters and 63 non-converters) had the necessary data available to create full amyloid, tau, neurodegeneration [AT(N)] biomarker profiles (Jack et al., 2018). Criteria for A+ on florbetapir PET is described previously (see Section 2.1). Threshold values for tau and neurodegeneration were chosen based upon data presented in other studies that have assessed the AT(N) framework. Tau positivity (T+) was defined as CSF p-tau $\geq$ 22 pg/mL (Guo et al., 2020). Neurodegeneration positivity (N+) was defined as total hippocampal volume (as measured on structural MRI) being less than 1.5 standard deviations below that of the sample's mean total hippocampal volume (Ingala et al., 2021).

2.5 Cognitive Evaluation

All participants in the study completed cognitive evaluations in English at the ADNI sites. Details pertaining to ADNI cognitive testing have been described previously (Aisen et al., 2010; Aisen et al., 2015). Cognitive data downloaded from the ADNI included performance on Logical Memory Delayed Recall (modified from the Wechsler D. Wechsler Memory Scale-Revised, San Antonio, Texas: Psychological Corporation; 1987), the Rey Auditory Verbal Learning Test (RAVLT; immediate recall), the Clock Drawing Test (both Drawing Administration and Copying Administration), and Part B of the Trailmaking Test. These five measures were chosen because none were used previously by the ADNI Clinical Core in determining clinical syndrome groups and they represent a multitude of cognitive domains (e.g. memory, executive function, and visuospatial functioning).

Data from amyloid converters were collected from the visit prior to and at the time of PET conversion. Data from non-converters were matched to that of the amyloid converter group so that comparisons were based on a similar length follow-up period. Cognitive scores were standardized using z-scores and the rate of decline was annualized between timepoint 1 and 2. Rates of annualized change were calculated so that negative numbers denoted worsening, 0 indicated no change, and positive numbers indicated improvement.

2.6 Statistical Analysis

All analyses were performed in JMP Pro V15.2 on a MacBook Pro 2015 running OS version 10.15.7. One-way analyses of variance (ANOVAs) were used to assess differences in age and years of education between amyloid converters and non-converters. Age was that recorded at the time of the second MRI (closest to time of conversion). For the seven participants who did not have usable MRI data, age at time of PET conversion and/or cognitive testing was used. Chi-

square testing was used to assess differences between groups in categorical variables such as sex, diagnostic status (CN/MCI), race, history of cardiovascular health, smoking status, and *APOE* ε4. Statistical significance was set at $p < 0.05$ without correction.

One-way ANOVA models were used to assess the effect of group on SUVR from the two florbetapir PET scans, annualized percent change in total volume of cortical and subcortical MRI ROI, CSF measures, and rate of annualized change on the five cognitive measures. The effect of group on annualized change in PET SUVR as well as the effect of group on MRI and cognitive data from both timepoints 1 and 2 was also assessed. For measures showing significant differences, 95% confidence intervals (CI) for the mean of the outcome measure are reported. Prior to creating models, linear regressions were conducted to determine whether covariates (e.g. age, sex, years of education, and diagnostic status) influenced these measures. For MRI data, linear regressions were used to assess the influence of eTIV. Multiple comparisons were corrected for by use of the Benjamini-Hochberg method (Benjamini & Hochberg, 1995). As a sensitivity analysis, all analyses were performed separately in participants classified as CN (n = 83) or MCI (n = 53) by the ADNI Clinical Core.

3. Results

3.1 Demographics and APOE ε4

One-way ANOVAs showed no significant differences in age nor years of education between groups (amyloid converter vs. non-converter). Chi-square testing showed no differences between groups in sex, diagnostic status, race, or history of cardiovascular health/smoking. Chi-square testing revealed a significant difference between groups in proportion of *APOE* ε4 carriers ($p < 0.001$) and in frequency of ε4 alleles ($p < 0.001$). The amyloid converter group had more carriers

and greater frequency of ε4 alleles than the non-converter group. Demographic and APOE ε4 data are reported in Table 1.

3.2 SUVR Uptake on Florbetapir PET Scans

Linear regressions showed that sex, but not age, years of education or diagnosis, had a significant effect on PET SUVR and was used as a covariate in subsequent models. Analysis of covariance (ANCOVA) models assessing the main effect of group (amyloid converter vs. non-converter) with sex as a covariate showed a significant effect of group on the SUVR for both florbetapir PET scans. On the first florbetapir PET scan when both groups were A-, the ANCOVA showed a significant effect of group ($F(2,133) = 58.60$, $p < 0.001$; 95% CI: 1.04 – 1.05) (Table 1). On the second scan when the amyloid converter group had become A+, the ANCOVA also showed a significant effect of group ($F(2,133) = 307.60$, $p < 0.001$; 95% CI: 1.08 – 1.09) (Table 1). Figure 2 shows longitudinal change in SUVR between timepoints. The effect of sex was significant on both scans ($p < 0.021$). The ANCOVA assessing the main effect of group with sex as a covariate on annualized change in SUVR on florbetapir scans also showed a significant effect of group ($F(2, 133) = 75.39$, $p < 0.001$; 95% CI: 0.016 – 0.022), but the effect of sex was not significant ($p = 0.13$). Two-way ANCOVAs exploring the interaction of sex with age and sex with diagnostic status were not significant.

3.3 Volumetric Analyses

Linear regressions showed covariates including age, sex, years of education, diagnosis, and eTIV had no effect on annualized percent volume change. One-way ANOVAs showed a significant effect of group (amyloid converter vs non-converter) on annualized percent change in total volume of 8 of the 19 cortical ROI assessed. These regions included: frontal pole ($F(1,127) = 7.65$, $p = 0.007$; 95% CI: 1.06 – 5.06), caudal anterior cingulate ($F(1,127) = 7.54$, $p = 0.007$;

95% CI: 0.56 – 2.44), lateral occipital cortex (F(1,127) = 6.19, p = 0.014; 95% CI: 0.61 – 3.02), medial orbitofrontal gyrus (F(1,127) = 5.21, p = 0.024; 95% CI: 0.44 – 3.00), pars orbitalis (F(1,127) = 4.88, p = 0.029; 95% CI: 0.53 – 3.57), superior temporal gyrus (F(1,127) = 4.85, p = 0.029; 95% CI: 0.51 – 2.25), posterior cingulate cortex (F(1,127) = 4.76, p = 0.031; 95% CI: 0.33 – 2.51), and the middle temporal gyrus (F(1,127) = 4.65, p = 0.033; 95% CI: 0.56 – 2.39). These ROI are illustrated on the pial surface of the right hemisphere in Figure 3. One-way ANOVAs showed a significant effect of group on annualized percent change in total volume of three of the seven subcortical ROI. These regions included: pallidum (F(1,127) = 5.73, p = 0.018; 95% CI: 0.97 – 6.61), amygdala (F(1,127) = 5.12, p = 0.025; 95% CI: 1.61 – 7.40), and hippocampus (F(1,127) = 4.35, p = 0.039; 95% CI: 0.96 – 3.79). The main effect of group remained significant for the reported cortical and subcortical regions after correction for multiple comparisons using the Benjamini-Hochberg method. One-way ANOVAs used to assess the main effect of group on total volume at each MRI scan (timepoints 1 and 2) showed no effect of group on total volume in 18 of the 19 cortical ROI and all 7 subcortical ROI on either scan. The difference initially shown between groups in total volume of the superior parietal gyrus did not survive corrections for multiple comparisons.

3.4 CSF Sampling

Linear regressions showed that age, sex, years of education, and diagnosis had no effect on CSF measures. One-way ANOVAs revealed a significant effect of group on CSF $A\beta_{1\text{-}42}$ (F (1, 98) = 42.46, p < 0.001; 95% CI: 1166.38 – 1309.20) (Table 1). There was no effect of group on CSF t-tau or CSF p-tau.

3.5 AT(N) Classification

We found 0 out of 31 amyloid converters were A+T+(N)+. Eight amyloid converters were A+T+(N)- and two were A+T-(N)+. The remaining 21 amyloid converters were A+T-(N)-. All 63 non-converters were A- and none were found to be A-T+(N)+. Of the 63 non-converters, 18 were A-T+(N)- and 4 were A-T-(N)+. Data, including diagnostic status, is reported in Table 2.

3.6 Cognition

Linear regressions showed that age, sex, years of education, and diagnosis had no effect on annualized cognitive change. One-way ANOVAs showed no significant effect of group on annualized change in performance on any of the five measures ($p > 0.05$) or on performance at either cognitive testing visit (timepoint 1 or 2). Figure 4 displays how (minimally) performance on Logical Memory Delayed Recall differed between groups.

3.7 Sensitivity Analyses

Consistent with findings observed in the entire sample, sensitivity analyses conducted separately in the CN and MCI diagnostic groups showed the same significant effect of group (amyloid converter vs. non-converter) from assessment of SUVR of both PET scans and when annualized change in SUVR was evaluated. Likewise, the same significant between-group difference in CSF $A\beta_{1-42}$ (lower CSF $A\beta_{1-42}$ in amyloid-converters) was shown in separate analyses of CN ($F(1,54) = 10.98$, $p = 0.002$) and MCI participants ($F(1,42) = 31.53$, $p < 0.001$) and no group differences were shown in CSF t-tau or CSF p-tau. From evaluation of MRI data in the two diagnostic groups, the only regions to show a significant group difference (in either annualized percent change or total volume at timepoint 1 or 2) following Benjamini-Hochberg correction for multiple comparisons was annualized percent volume change in the pallidum, hippocampus, and accumbens in the CN group. Assessment of cognitive measures in both groups revealed a significant group difference in the CN group in annualized change in performance on the Copy

Administration of the Clock Drawing Test. Data pertaining to SUVR, CSF measures, and other significant findings are shown in Supplemental Table 1.

4. Discussion

In this study examining changes that occur while participants convert from A- to A+, we observed an increased prevalence of *APOE* ε4, greater annualized percent volume loss in 8 cortical and 3 subcortical brain regions, and lower CSF $A\beta_{1-42}$ relative to participants who maintained A-. Many existing studies that have examined participants with varying Aβ status have analyzed participants who are *already* A+ in comparison to participants who are A- and found mixed results (Dubois et al., 2018; Harrison et al., 2021; Sperling et al., 2020; Tosun et al., 2021). Therefore, goals of the present study were to 1) expand upon previous literature by examining biomarker changes that occur while participants convert from A- to A+ and 2) investigate the less-explored relationship between changes in Aβ with neurodegeneration.

Many of our findings were supportive of previous observations of amyloid deposition in aging individuals (Chen & Mobley, 2019; Selkoe & Hardy, 2016). We saw expected differences between the amyloid converter and non-converter groups in *APOE* ε4 and CSF $A\beta_{1-42,}$ but no differences in CSF t-tau or p-tau between the two groups. An increased prevalence of *APOE* ε4 carriers in the amyloid converter group is expected because the presence of the ε4 allele is believed to modify how Aβ is cleared from the brain (Fouqet et al., 2014; Selkoe & Hardy, 2016). Similarly, lower CSF $A\beta_{1-42}$ is expected in participants who are A+ on florbetapir PET because less Aβ can be in the CSF if plaques (reflected by PET) have already formed (Chen & Mobley, 2019; He et al., 2021; Selkoe & Hardy, 2016). Importantly, our findings showed that the groups in this study did not differ in demographic factors or medical history such as cardiovascular health or smoking status. This finding provides support that the differences found

between the amyloid converter and non-converter groups were not driven by these potentially confounding factors.

Arguably our most striking findings were within PET and MRI data which provided evidence of greater annualized change in amyloid converters compared to non-converters. Since global cortex SUVR > 1.11 was the positivity threshold, it was inherent to our study design that amyloid converters would have significantly greater SUVR values at the second florbetapir PET scans. We also observed higher SUVR in females which at least one other study has shown (Gottesman et al., 2016) but is inconsistent across cohorts (Jack et al., 2015; Jansen et al., 2022). Moreover, we saw that amyloid converters had significantly greater SUVR at the first PET scan which occurred over two years prior to the second PET scan. In the non-converter group, we saw essentially no change between the first and second PET scans indicating a slower rate of amyloid accumulation. These findings demonstrate that increased amyloid retention may be a gradual process that begins some time (years) before conversion takes place. Previous studies have begun to emphasize that the magnitude of amyloid change (as indicated by SUVR), rather than baseline Aβ burden, may be an equally or more effective measure for assessing progression of AD (Farrell et al., 2018; Landau et al., 2018; van der Kall et al., 2022). Meanwhile, others have shown that measuring SUVR in specific ROI rather than a larger composite region may be more sensitive to initial changes in amyloid in addition to displaying greater correspondence with cognitive changes (Guo et al., 2020).

The importance of assessing magnitude and annualized change was further reflected in our cortical volume findings. From the 19 ROI assessed, greater annualized percent volume loss was shown in 8 ROI in the amyloid converter group relative to the non-converter group.

Interestingly, when isolated total volume was assessed at either the first or second MRI scan, there were no differences between the groups. These findings contrast previous studies which have shown increased cortical thickness or volume in participants who are A+ individuals compared to those who are A- when measured cross-sectionally (Fortea et al., 2011; Harrison et al., 2021; Johnson et al., 2014; Montal et al., 2018). This discordance in findings could be explained by the two-phase phenomenon; characterized by an initial phase of cortical thickening followed by cortical atrophy, which is believed to take place during amyloidosis (Fortea et al., 2014; Harrison et al., 2021; Montal et al., 2018). It is possible amyloid converters in the present study had already progressed to cortical atrophy and that is why we saw greater annualized percent volume loss in this group.

In the subcortical ROIs assessed, greater annualized percent volume loss was observed in the hippocampus, amygdala, and pallidum in the amyloid converter group. Many previous studies have not explored subcortical regions outside of the hippocampus. Moreover, those that have examined hippocampal volume in participants with different Aβ status have only examined participants at one timepoint and not shown any group differences (Chen et al., 2021; Dubois et al., 2018). From assessing hippocampal volume and volume of other subcortical ROI at the first or second MRI scan alone, there were no differences between groups in isolated total volume. The difference between groups was only detectable when annualized percent volume loss was measured. When we separated the diagnostic groups, we saw the difference in annualized change persisted in the pallidum and hippocampus in the CN group with greater annualized percent volume loss in the amyloid converter group. We also saw greater annualized percent volume loss in the accumbens in the CN non-converter group. Seeing changes in the CN group but not the MCI group was unexpected since one might anticipate that participants with cognitive

impairment might be more sensitive to changes in biomarkers. Nonetheless these findings are meaningful to our interpretation of the MRI results because they provide evidence that the greater atrophy rate in the amyloid converters in the full sample is not driven by findings in the MCI group alone.

Since the nature of this study already categorized participants as A+/A-, we further categorized full AT(N) biomarker profiles in participants with the necessary biomarker data. Per the AT(N) framework, the presence of the Aβ biomarker alone is enough to categorize whether someone is on the AD continuum, but the additional presence of tau is required to make a neuropathologic diagnosis of AD (Jack et al., 2018). This differentiation continues to be heavily debated by experts ranging from neuroradiologists to clinicians as the field strives to implement biomarkers to identify which individuals are at the greatest (lifetime) risk to develop AD (Strikwerda-Brown et al., 2022). Our sample consisted of eight amyloid converters who were A+T+(N)-, which means their neuropathologic profile could be interpreted as consistent with AD despite ADNI Clinical Core guidelines which led to these participants being diagnosed by the ADNI as MCI (n = 4) or CN (n = 4) (Jack et al., 2018). Meanwhile, two other amyloid converters with biomarker profiles of A+T-(N)+ were diagnosed as CN despite their AT(N) profiles indicating changes in two of three biomarkers. The presence of this profile in addition to our overall findings of changes in amyloid and neurodegeneration without changes in tau is somewhat unexpected given widespread belief that the progression of AD is characterized by changes in A followed by T and lastly, N. However, other studies have found evidence of the A+T-(N)+ biomarker group and interpret this to mean that non-AD pathology may be driving neurodegeneration and potential cognitive impairment in the absence of tau (McCollum et al., 2021). Similarly, we observed other profiles that indicate non-AD related pathologic changes as

we saw that 22 of 63 non-converters were A-T+(N)- or A-T-(N)+. Per the AT(N) framework, this can be interpreted to mean that while these 22 non-converters have neuropathology, they are not on the AD continuum (Jack et al., 2018). Further work characterizing biomarkers in addition to cognitive changes in participants without dementia may strengthen our understanding as to what changes are characteristic of AD versus what biomarker changes may accompany normal aging without the development of clinical symptoms.

Lastly, we found no difference between the two groups (amyloid converters and non-converters) in annualized rate of cognitive change or in cognitive performance at either cognitive testing visit. Other studies have shown disparate findings when comparing cognition between participants who are A- and A+ (Dubois et al., 2018; Johnson et al., 2014; Rentz et al., 2021). There was a greater rate of annualized change (worsening) in the CN non-converter group on the Copying Administration of the Clock Drawing Test whereas the CN amyloid converter group showed virtually no change. It was unclear why this difference existed in CN participants. Altogether, the lack of cognitive differences in the full analytic sample may seem unexpected given the difference observed between the two groups in neurodegenerative changes. Still, we often observe that cognitive change is closely tied to tau aggregation (Guo et al., 2021; Jack et al., 2013; Pichet Binette et al., 2022). Interestingly in this sample, a relatively small proportion of amyloid converters with full neuropathologic profiles (8 of 31 participants) had an above threshold concentration of both Aβ and tau. This leads us to wonder if having more A+T+ participants in the sample would have resulted in more notable cognitive differences between the two groups. Furthermore, the lack of cognitive differences may reflect our choice to only include CN and MCI participants in the analytic sample. Per inclusion criteria, no one who progressed to AD by the second timepoint was included in this study. Lastly, these findings may reflect

cognitive reserve and how older adults are able to maintain cognitive abilities despite pathological diseases burden (Stern, 2012).

4.1 Limitations

Although we had access to 68 participants who showed conversion on florbetapir PET from A- to A+, the sample size of this study remains modest. While some participants had tau PET scans as part of the ADNI, we elected to focus our tau findings on CSF p-tau measures. Use of CSF p-tau may further be a limitation as we did not have data from all 136 participants, nor did we look at longitudinal change in p-tau. Once consensus is reached as to how researchers can best interpret tau PET positivity, it would be interesting to see what including longitudinal tau PET data adds to our findings and knowledge about biomarker changes in the trajectory of AD.

Using the ADNI population allows us to examine data longitudinally and in participants across North America, but certain limitations exist when using a clinical trials population which has selection criteria that restricts many in the general population from study entry. Furthermore, assessing biomarker changes that accompany a specific timeframe, such as *when* amyloid conversion occurs, is limited when we only have data from scheduled study visits.

4.2 Conclusions

The results of this study confirmed our hypothesis that the amyloid converter group would show an increased prevalence of *APOE* ε4, greater annualized percent volume loss in selected MRI regions, and lower CSF $A\beta_{1\text{-}42}$ in comparison to the non-converter group in this ADNI sample. These results provide compelling evidence that important neuropathological changes are occurring prior to amyloid conversion and that studying rates of change may be most sensitive to establishing meaningful differences in biomarkers rather than static measures.

Acknowledgements

Data collection and sharing for this project was funded by the by the Boston University-Alzheimer's Disease Research Center (NIH- NIA P30-AG072978), 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 generous contributions from the following: 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). 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.

References

Aisen, P. S., Petersen, R. C., Donohue, M. C., Gamst, A., Raman, R., Thomas, R. G., Walter, S., Trojanowski, J. Q., Shaw, L. M., Beckett, L. A., Jack Jr., C. R., Jagust, W., Toga, A. W., Saykin, A. J., Morris, J. C., Green, R. C., Weiner, M. W., & Initiative, A. D. N. Clinical core of the Alzheimer's disease neuroimaging initiative: Progress and plans. *Alzheimers Dement*. 2010; 6(3), 239–46. https://doi.org/10.1016/j.jalz.2010.03.006

Aisen, P. S., Petersen, R. C., Donohue, M., & Weiner, M. W. Alzheimer's Disease Neuroimaging Initiative 2 Clinical Core: Progress and plans. *Alzheimers Dement.* 2015; 11(7): 734–9. https://doi.org/10.1016/j.jalz.2015.05.005

Aschenbrenner, A. J., Gordon, B. A., Benzinger, T. L. S., Morris, J. C., & Hassenstab, J. J. Influence of tau PET, amyloid PET, and hippocampal volume on cognition in Alzheimer disease. *Neurology*. 2018; 91(9), e859–66. https://doi.org/10.1212/WNL.0000000000006075

Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol.* 1995; 57:289–300

Braak, H. & Braak, E. Demonstration of Amyloid Deposits and Neurofibrillary Changes in Whole Brain Sections. *Brain Pathol*. 1991;1(3):213-6. doi:10.1111/j.1750-3639.1991.tb00661.x

Brothers, H. M., Gosztyla, M. L., & Robinson, S. R. The Physiological Roles of Amyloid-β Peptide Hint at New Ways to Treat Alzheimer's Disease. *Front Aging Neurosci.* 2018; 10, 118. https://doi.org/10.3389/fnagi.2018.00118

Chen, X., Cassady, K. E., Adams, J. N., Harrison, T. M., Baker, S. L., & Jagust, W. J. Regional Tau Effects on Prospective Cognitive Change in Cognitively Normal Older Adults. *J Neurosci.* 2021;41(2), 366–75. https://doi.org/10.1523/JNEUROSCI.2111-20.2020

Chen, X.-Q., & Mobley, W. C. Alzheimer Disease Pathogenesis: Insights From Molecular and Cellular Biology Studies of Oligomeric Aβ and Tau Species. *Front Neurosci*. 2019; 13, 659. https://doi.org/10.3389/fnins.2019.00659

Desikan, R. S., Ségonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., Buckner, R. L., Dale, A. M., Maguire, R. P., Hyman, B. T., Albert, M. S., & Killiany, R. J. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage*. 2006; *31*(3), 968–80. https://doi.org/10.1016/j.neuroimage.2006.01.021

Dubois, B., Epelbaum, S., Nyasse, F., Bakardjian, H., Gagliardi, G., Uspenskaya, O., Houot, M., Lista, S., Cacciamani, F., Potier, M.-C., Bertrand, A., Lamari, F., Benali, H., Mangin, J.-F., Colliot, O., Genthon, R., Habert, M.-O., & Hampel, H. N. Cognitive and neuroimaging features and brain β-amyloidosis in individuals at risk of Alzheimer's disease (INSIGHT-preAD): A longitudinal observational study. *Lancet Neurol*. 2018; *17*(4), 335–46. https://doi.org/10.1016/S1474-4422(18)30029-2

Farrell, M. E., Chen, X., Rundle, M. M., Chan, M. Y., Wig, G. S., & Park, D. C. Regional amyloid accumulation and cognitive decline in initially amyloid-negative adults. *Neurology*. 2018; 91(19), e1809–21. https://doi.org/10.1212/WNL.0000000000006469

Fortea, J., Sala-Llonch, R., Bartrés-Faz, D., Lladó, A., Solé-Padullés, C., Bosch, B., Antonell, A., Olives, J., Sanchez-Valle, R., Molinuevo, J.L & Rami, L. Cognitively preserved subjects with transitional cerebrospinal fluid ss-amyloid 1-42 values have thicker cortex in Alzheimer's disease vulnerable areas. *Biol Psychiatry*. 2011; *70*(2), 183-190.

Fortea, J., Vilaplana, E., Alcolea, D., Carmona-Iragui, M., Sánchez-Saudinos, M. B., Sala, I., Antón-Aguirre, S., González, S., Medrano, S., Pegueroles, J., Morena,s E., Clarimón, J., Blesa, R., Lleó, A. & Alzheimer's Disease Neuroimaging Initiative. Cerebrospinal fluid β-amyloid and phospho-tau biomarker interactions affecting brain structure in preclinical Alzheimer disease. *Ann Neurol.* 2014; *76*(2), 223-230.

Fouquet, M., Besson, F. L., Gonneaud, J., La Joie, R., & Chételat, G. Imaging Brain Effects of APOE4 in Cognitively Normal Individuals Across the Lifespan. *Neuropsyhol Rev.* 2014; 24(3), 290–9. https://doi.org/10.1007/s11065-014-9263-8

Gordon, B. A., McCullough, A., Mishra, S., Blazey, T. M., Su, Y., Christensen, J., Dincer, A., Jackson, K., Hornbeck, R. C., Morris, J. C., Ances, B. M., & Benzinger, T. L. S. Cross-sectional and longitudinal atrophy is preferentially associated with tau rather than amyloid β positron emission tomography pathology. *Alzheimer's Dement Diagnosis Assess Dis Monit*. 2018; *10*(1), 245–52. https://doi.org/10.1016/j.dadm.2018.02.003

Gottesman RF, Schneider ALC, Zhou Y, et al. The ARIC-PET amyloid imaging study: Brain amyloid differences by age, race, sex, and APOE. *Neurology*. 2016;87(5):473-80.

Guo, T., Brendel, M., Grimmer, T., Rominger, A., & Yakushev, I. Predicting Regional Pattern of Longitudinal β-Amyloid Accumulation by Baseline PET. *J Nucl Med.* 2017; *58*(4), 639–45. https://doi.org/10.2967/jnumed.116.176115

Guo, Y., Huang, Y.-Y., Shen, X.-N., Chen, S.-D., Hu, H., Wang, Z.-T., Tan, L., & Yu, J.-T. Characterization of Alzheimer's tau biomarker discordance using plasma, CSF, and PET. *Alzheimers Res Ther.* 2021; 13(1), 93. https://doi.org/10.1186/s13195-021-00834-3

Guo, T., Korman, D., La Joie, R., Shaw, L. M., Trojanowski, J. Q., Jagust, W. J., Landau, S. M., & for the Alzheimer's Disease Neuroimaging Initiative. Normalization of CSF pTau measurement by Aβ40 improves its performance as a biomarker of Alzheimer's disease. *Alzheimers Res Ther*. 2020; *12*(1), 97. https://doi.org/10.1186/s13195-020-00665-8

Guo, T., Landau, S., & Jagust, W.J. Detecting earlier stages of amyloid deposition using PET in cognitively normal elderly adults. *Neurology*. 2020; 94:e1512-e1524. doi:10.1212/WNL.0000000000009216

Harrison, T. M., Du, R., Klencklen, G., Baker, S. L., & Jagust, W. J. Distinct effects of beta-amyloid and tau on cortical thickness in cognitively healthy older adults. *Alzheimers Dement.* 2021; 17(7), 1085–96. https://doi.org/10.1002/alz.12249.

He, B., Wang, L., Xu, B., & Zhang, Y. Association between CSF Aβ42 and amyloid negativity in patients with different stage mild cognitive impairment. *Neurosci Lett*. 2021; *754*, 135765. https://doi.org/10.1016/j.neulet.2021.135765

Hensley, K., Carney, J. M., Mattson, M. P., Aksenova, M., Harris, M., Wu, J. F., Floyd, R. A., & Butterfield, D. A. A model for beta-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: Relevance to Alzheimer disease. *Proc Natl Acad Sci U S A*. 1994; *91*(8), 3270–4.

Iglesias, J. E., Augustinack, J. C., Nguyen, K., Player, C. M., Player, A., Wright, M., Roy, N., Frosch, M. P., McKee, A. C., Wald, L. L., Fischl, B., Van Leemput, K., & Alzheimer's Disease Neuroimaging Initiative. A computational atlas of the hippocampal formation using ex vivo, ultra-high resolution MRI: Application to adaptive segmentation of in vivo MRI. *Neuroimage*. 2015; *115*, 117–37. https://doi.org/10.1016/j.neuroimage.2015.04.042

Ingala, S., De Boer, C., Masselink, L. A., Vergari, I., Lorenzini, L., Blennow, K., Chételat, G., Di Perri, C., Ewers, M., Flier, W. M., Fox, N. C., Gispert, J. D., Haller, S., Molinuevo, J. L., Muniz-Terrera, G., Mutsaerts, H. J., Ritchie, C. W., Ritchie, K., Schmidt, M., Schwarz, A.J., Vermunt, L., Waldman, A.D., Wardlaw, J., Wink, A.M., Wolz, R., Wottschel, V., Scheltens, P. Visser, P.J., Barkhof, F. & the EPAD consortium. Application of the ATN classification scheme in a population without dementia: Findings from the EPAD cohort. *Alzheimers Dement*. 2021; *17*(7), 1189–1204. https://doi.org/10.1002/alz.12292

Insel, P. S., Donohue, M. C., Sperling, R., Hansson, O., & Mattsson-Carlgren, N. The A4 study: β -amyloid and cognition in 4432 cognitively unimpaired adults. *Ann Clin Transl Neurol.* 2020; 7(5), 776–85. https://doi.org/10.1002/acn3.51048

Jack Jr, C.R., Bennett, D.A., Blennow, K., Carrilo, M.C., Dunn, B., Haeberlein, S.B., Holtzman, D.M., Jagust, W., Jessen, F., Karlawish, J., Liu ,E., Molinuevo, J.L., Montine, T., Phelp, C., Rankin, K.P., Rowe, C.C., Scheltens, P., Siemers, E., Synder, H.M., & Sperling, R. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement.* 2018;14(4):535-62.10.1016/j.jalz.2018.02.018.

Jack Jr, C.R. Bernstein, M.A. Fox, N.C., Thompson, P., Alexander, G., Harvey, D., Borowski, B., Britson, P.J., Whitwell, J.L., Ward, C., Dale, A.M., Felmlee, J.P., Gunter, J.L., Hill, D.L.G., Killiany, R., Schuff, N., Fox-Bosetti, S., Lin, C., Studholme, C., DeCarli, C.S., Krueger, G., Ward, H.A., Metzger, G.J., Scott, K.T., Mallozzi, R., Blezek, D., Levy, J., Debbin, J.P., Fleisher, A.S., Albert, M., Green, R., Bartzokis, G., Glover, G., Mugler, J., & Weiner, M.W. The Alzheimer's Disease Neuroimaging Initiative (ADNI): MRI methods. *J Magn Reson Imaging*. 2008; 27(4):685–91.10.1002/jmri.21049.

Jack, C. R., Knopman, D. S., Jagust, W. J., Petersen, R. C., Weiner, M. W., Aisen, P. S., Shaw, L. M., Vemuri, P., Wiste, H. J., Weigand, S. D., Lesnick, T. G., Pankratz, V. S., Donohue, M. C., & Trojanowski, J. Q. Tracking pathophysiological processes in Alzheimer's disease: An updated hypothetical model of dynamic biomarkers. *Lancet Neurol*. 2013; *12*(2), 207–16. https://doi.org/10.1016/S1474-4422(12)70291-0

Jagust, W. J., Landau, S. M., Koeppe, R. A., Reiman, E. M., Chen, K., Mathis, C. A., Price, J. C., Foster, N. L., & Wang, A. Y. The Alzheimer's Disease Neuroimaging Initiative 2 PET Core: 2015. *Alzheimers Dement.* 2015; *11*(7), 757–71. https://doi.org/10.1016/j.jalz.2015.05.001

Jansen, W.J., Janssen, O., Tijms. B.M., Vos, S.J.B., Ossenkoppele, R., Visser, P.J., & the Amyloid Biomarker Study Group. Prevalence Estimates of Amyloid Abnormality Across the Alzheimer Disease Clinical Spectrum. *JAMA Neurol*. 2022;79(3):228. doi:10.1001/jamaneurol.2021.5216

Johnson, S. C., Christian, B. T., Okonkwo, O. C., Oh, J. M., Harding, S., Xu, G., Hillmer, A.T., Wooten, D.W., Murali, D., Barnhart, T.E., Hall, L.T., Racine, A.M., Kluck, W.E., Mathis, C,A., Bendlin, B.B., Gallagher, C.L., Carlsson, C.M., Rowley, H.A., Hermann, B.P., Maritza Dolwing, N., Asthana, S. & Sager, M. A. Amyloid burden and neural function in people at risk for Alzheimer's Disease. *Neurobiol Aging.* 2014; *35*(3), 576-584.

Landau, S. M., Horng, A., Jagust, W. J., & For the Alzheimer's Disease Neuroimaging Initiative. Memory decline accompanies subthreshold amyloid accumulation. *Neurology*. 2018; *90*(17), e1452–60. https://doi.org/10.1212/WNL.0000000000005354

Landau, S. M., Lu, M., Joshi, A. D., Pontecorvo, M., Mintun, M. A., Trojanowski, J. Q., Shaw, L. M., Jagust, W. J., & For the Alzheimer's Disease Neuroimaging Initiative. Comparing positron emission tomography imaging and cerebrospinal fluid measurements of β-amyloid. *Ann Neurol*. 2013; *74*(6), 826–36. https://doi.org/10.1002/ana.23908

Mattsson, N., Insel, P. S., Donohue, M., Landau, S., Jagust, W. J., Shaw, L. M., Trojanowski, J. Q., Zetterberg, H., Blennow, K., Weiner, M. W., & for the Alzheimer's Disease Neuroimaging Initiative*. Independent information from cerebrospinal fluid amyloid-β and florbetapir imaging in Alzheimer's disease. *Brain*. 2015; *138*(3), 772–83. https://doi.org/10.1093/brain/awu367

McCollum, L.E., Das, S.R., Xie, L., de Flores, R., Wang, J., Xie, S.X., Wisse, L.E.M., Yushkevich, P.A., Wolk, D.A., and Alzheimer's Disease Neuroimaging Initiative. "Oh brother, where art tau? Amyloid, neurodegeneration, and cognitive decline without elevated tau." *NeuroImage:Clinical*. 2021; 31: 102717.

Montal, V., Vilaplana, E., Alcolea, D., Pegueroles, J., Pasternak, O., González-Ortiz, S., Clarimón, J., Carmona-Iragui, M., Illán-Gala, I., Morenas-Rodríguez, E., Ribosa-Nogué, R., Sala, I., Sánchez-Saudinós, M-B., García-Sebastian, M., Villanúa, J., Izagirre, A., Estanga, A., Ecay-Torres, M., Iriondo, A., Clerigue, M., Tainta, M., Pozueta, A., González, A., Martínez-Heras, E., Llufriu, S., Blesa, R., Sanchez-Juan, P., Martínez-Lage, P., Lleó, A., & Fortea, J.

Cortical microstructural changes along the Alzheimer's disease continuum. *Alzheimers Dement*. 2018; *14*(3), 340-351.

Ossenkoppele, R., Smith, R., Ohlsson, T., Strandberg, O., Mattsson, N., Insel, P. S., Palmqvist, S., & Hansson, O. Associations between tau, Aβ, and cortical thickness with cognition in Alzheimer disease. *Neurology*. 2019; *92*(6), e601–12. https://doi.org/10.1212/WNL.0000000000006875

Palmqvist, S., Schöll, M., Strandberg, O., Mattsson, N., Stomrud, E., Zetterberg, H., Blennow, K., Landau, S., Jagust, W., & Hansson, O. Earliest accumulation of β-amyloid occurs within the default-mode network and concurrently affects brain connectivity. *Nature Commun.* 2017; *8*(1), 1214. https://doi.org/10.1038/s41467-017-01150-x

Petersen, R., Caracciolo, B., Brayne, C., Gauthier, S., Jelic, V., & Fratiglioni, L. Mild cognitive impairment: a concept in evolution. *J Intern Med.* 2014; 275:214–28. 10.1111/joim.12190

Petersen R.C., Smith, G.E., Waring, S.C., Invik, R.J., Tangalos, E.G., & Kokmen, E. Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol.*1999;56(6)

Pichet Binnette, A., Franzmeire, N., Spotorno, N., Ewers, M., Brendel, M, Biel, D., et al. Amyloid-associated increased in soluble tau relate to tau aggregation rates and cognitive decline in early Alzheimer's disease. *Nature Commun.* 2022;13: 6634. https://doi.org/10.1038/s41467-022-34129-4

Rentz, D. M., Papp, K. V., Mayblyum, D. V., Sanchez, J. S., Klein, H., Souillard-Mandar, W., Sperling, R. A., & Johnson, K. A. Association of Digital Clock Drawing With PET Amyloid and Tau Pathology in Normal Older Adults. *Neurology*. 2021; *96*(14), e1844–54. https://doi.org/10.1212/WNL.0000000000011697

Reuter, M. & Fischl. B. Avoiding Asymmetry-Induced Bias in Longitudinal Image Processing. *Neuroimage.* 2011; 57(1): 19-21

Reuter, M., Rosas, H.D., & Fischl, B. Highly Accurate Inverse Consistent Registration: A Robust Approach. *Neuroimage*. 2010; 53(4): 1181-96,

Reuter, M., Schmansky, N.J., Rosas, H.D., & Fischl, B. Within-Subject Template Estimation for Unbiased Longitudinal Image Analysis. *Neuroimage.* 2012; 61(4): 1402-18

Selkoe, D. J., & Hardy, J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med*. 2016; *8*(6), 595–608. https://doi.org/10.15252/emmm.201606210

Shaw, L. M., Vanderstichele, H., Knapik-Czajka, M., Clark, C.M., Aisen, P.S., Petersen, R.C., Blennow, K., Soares, H., Simon, A., Lewczuk, P., Dean, R., Seimers, E., Potter, W., Lee, V., Trojanowski, J.Q. & for the Alzheimer's Disease Neuroimaging Initiative. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Ann Neurol*. 2009;65(4),403-13.

Sperling, R. A., Donohue, M. C., Raman, R., Sun, C.-K., Yaari, R., Holdridge, K., Siemers, E., Johnson, K. A., Aisen, P. S., & for the A4 Study Team. Association of Factors With Elevated Amyloid Burden in Clinically Normal Older Individuals. *JAMA Neurology*. 2020; *77*(6), 735. https://doi.org/10.1001/jamaneurol.2020.0387

Stern Y. Cognitive reserve in ageing and Alzheimer's disease. *Lancet Neurol.* 2012; 11(11):1006–1012

Strikwerda-Brown, C., Hobbs, D. A., Gonneaud, J., St-Onge, F., Binette, A. P., Ozlen, H., Provost, K., Soucy, J-P., Buckley, R.F., Benzinger, T.L.S., Morris, J.C., Villemagne, V.L., Doré, V., Sperling, R.A., Johnson, K.A., Rowe, C.C., Gordon, B.A., Poirer, J., Breitner, J.C.S., Villeneuve, S. Association of elevated amyloid and tau positron emission tomography signal with near-term development of Alzheimer disease symptoms in older adults without cognitive impairment. *JAMA neurology*. 2022. *79*(10), 975-985.

Tosun, D., Veitch, D., Aisen, P., Jack, C. R., Jagust, W. J., Petersen, R. C., Saykin, A. J., Bollinger, J., Ovod, V., Mawuenyega, K. G., Bateman, R. J., Shaw, L. M., Trojanowski, J. Q., Blennow, K., Zetterberg, H., & Weiner, M. W. Detection of β-amyloid positivity in Alzheimer's Disease Neuroimaging Initiative participants with demographics, cognition, MRI and plasma biomarkers. *Brain Commun*. 2021; *3*(2), fcab008. https://doi.org/10.1093/braincomms/fcab008

van der Kall, L. M., Truong, T., Burnham, S. C., Doré, V., Mulligan, R. S., Bozinovski, S., Bozinovski, S., Lamb, F., Bourgeat, P., Fripp, J., Schultz, S., Lim, Y.Y., Laws, S.M., Ames, D., Fowler, C., Rainey-Smith, S.R., Martins, R.N., Salvado, O., Robertson, J., Maruff, P., Masters, C.L., Villemagne, V.L & Rowe, C. C. Association of β-amyloid level, clinical progression, and longitudinal cognitive change in normal older individuals. *Neurology*. 2021;*96*(5), e662-e670.

Villemagne, V. L., Doré, V., Bourgeat, P., Burnham, S. C., Laws, S., Salvado, O., Masters, C. L., & Rowe, C. C. Aβ-amyloid and Tau Imaging in Dementia. *Semin Nucl Med*. 2017; *47*(1), 75–88. https://doi.org/10.1053/j.semnuclmed.2016.09.006

Tables

Table 1: Demographic, APOE ε4, SUVR, and CSF Data

	Amyloid Converters (n = 68)	**Non-Converters (n = 68)**	***p* value**
Age mean (SD), y	76.41 (7.24)	76.01 (7.38)	0.75
Education mean (SD), y	16.56 (2.40)	16.84 (2.80)	0.53
Sex (M/F)	40 M 28 F	42 M 26 F	0.73
Diagnostic Status (CN/MCI)	41 CN 27 MCI	42 CN 26 MCI	0.86

Race	65 White 1 Black 2 More than One	67 White 1 Black	0.36
History of Cardiovascular Health	40 with history 27 without	42 with history 26 without	0.81
Smoking Status	25 Smokers 42 Non-Smokers	32 Smokers 36 Non-Smokers	0.25
APOE ε4 carrier n (%)	28 (41.18)	8 (11.76)	<0.001
0 ε4 alleles	40 (58.82)	60 (88.24)	
1 ε4 alleles	22 (32.35)	8 (11.76)	<0.001
2 ε4 alleles	6 (6.82)	0 (0)	
PET SUVR Scan #1	1.07 (0.03)	1.01 (0.05)	<0.001
PET SUVR Scan #2	1.16 (0.04)	1.01 (0.04)	<0.001
CSF $A\beta_{1-42}$ (n = 100) mean (SD), pg/mL	1003.31 (415.06)	1472.26 (299.85)	<0.001
CSF t-tau (n = 100) mean (SD), pg/mL	242.91 (90.86)	226.26 (64.74)	0.29
CSF p-tau (n = 100) mean (SD), pg/mL	21.29 (7.88)	19.62 (5.64)	0.22

One-way ANOVAs used for continuous data, chi-square testing used for categorical data. Response for medical history recorded as "yes" or "no," (n = 135). APOE ε4 carrier indicating participants with presence of one or more ε4 alleles. Sex was added as a covariate to models for SUVR on PET scans.

Key: $A\beta_{1-42}$ = amyloid beta 1-42 peptide; ANOVA = analysis of variance; APOE = Apolipoprotein E; CN = cognitively normal; CSF = cerebrospinal fluid; F = Female; M = Male; MCI = mild cognitive impairment; n = number, PET = positron emission tomography; p-tau = phosphorylated tau; t-tau = total tau; SUVR = standard uptake value ratio; y = years

Table 2: AT(N) Profiles of Participants

		ADNI Clinical Core Diagnosis: CN	**ADNI Clinical Core Diagnosis: MCI**	**Total ADNI Conversion Participants** (n = 94)
	A+T+(N)+	-	-	-
Amyloid	**A+T+(N)-**	4	4	8 (25.81)
Converters	**A+T-(N)+**	2	-	2 (6.45)
(n = 31)	**A+T-(N)-**	8	13	21 (67.74)
	Total	14	17	31 (100)
	A-T+(N)-	14	4	18 (28.57)
Non-	**A-T-(N)+**	1	3	4 (6.35)

Converters	**A-T+(N)+**	-	-	-
(n = 63)	**A-T-(N)-**	24	17	41 (65.08)
	Total	39	24	63 (100)

Values reported as number or number (percent). Participants without complete AT(N) biomarker data excluded. Key: A- = amyloid negative; A+ = amyloid positive; ADNI = Alzheimer's Disease Neuroimaging Initiative; CN = cognitively normal; MCI = mild cognitive impairment; n = number; T- = tau negative; T+ = tau positive; (N)- = neurodegeneration negative; (N)+ = neurodegeneration positive

Figure Captions

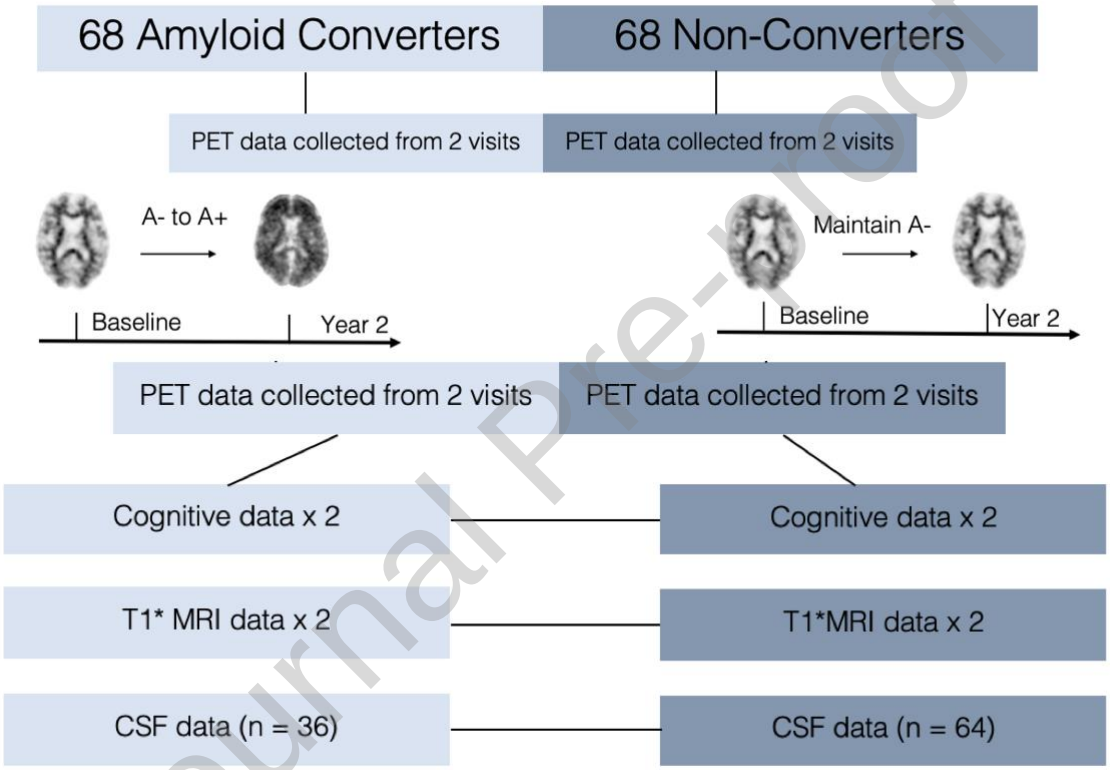

Figure 1: Schematic displaying data collection from both amyloid converters and non-converters. PET data from ADNI amyloid converters was collected from the first visit a participant became A+ and the visit prior. PET data from ADNI non-converters was chosen from participants who remained A- over the same time frame. From both groups, cognitive and MRI data were collected from the same timepoints as PET data (when possible). CSF data for both groups were only collected from 1 visit. Only CSF data acquired within ~24 months of the PET data of interest were included in analyses. *Note: "Year 2" (as written above) does not describe the exact timeframe between PET visits but is used to reflect that many ADNI participants in this study had PET scans taken at their baseline and "year 2" ADNI visits.* Abbreviations: A- = amyloid negative; A+ = amyloid positive; ADNI = Alzheimer's Disease Neuroimaging Initiative; CSF = cerebrospinal fluid; MRI = magnetic resonance imaging; PET = positron emission tomography.

Figure 2

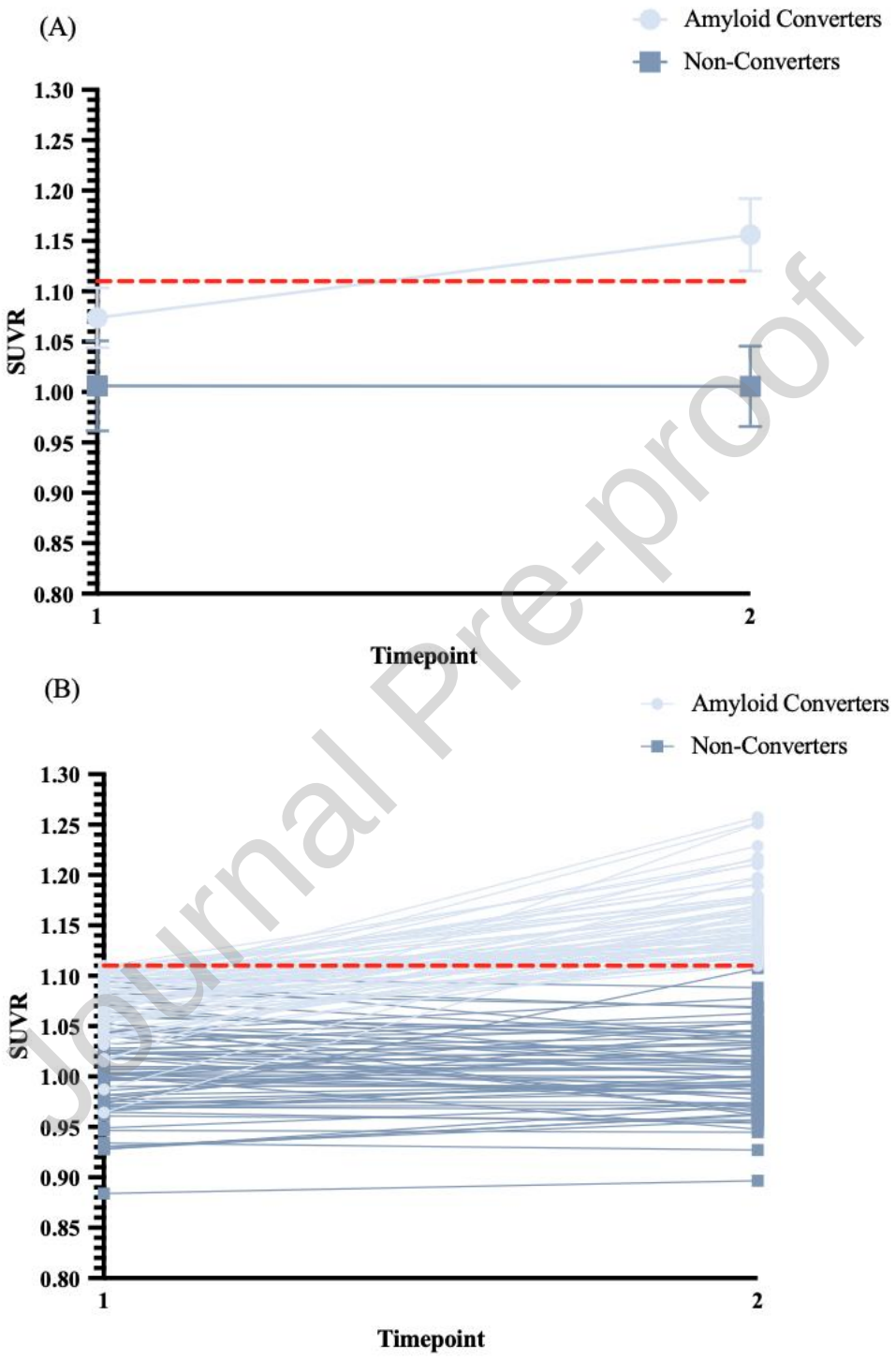

Figure 2: (A) Higher global florbetapir SUVR shown in amyloid converters at timepoint 1 and timepoint 2. Difference in non-converters is relatively unchanged between timepoints. B) Spaghetti plot illustrates longitudinal change of all participants. Threshold florbetapir SUVR(= 1.11) denoted in red. Abbreviations: SUVR = standardized uptake value ratio

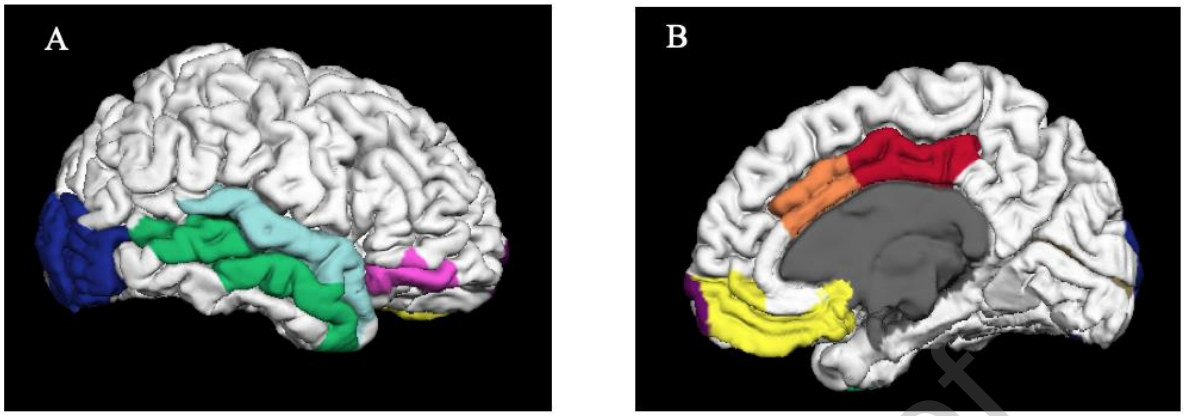

Figure 3: Cortical ROI that showed greater annualized percent volume loss in amyloid converters compared to non-converters shown ($p < 0.05$ following Benjamini-Hochberg method). Lateral surface (A) illustrates lateral occipital cortex, superior temporal gyrus, middle temporal gyrus, and pars orbitalis. Medial surface (B) illustrates caudal anterior division of cingulate cortex, posterior division of cingulate cortex, medial division of orbitofrontal cortex, and frontal pole. Abbreviations: ROI = regions of interest

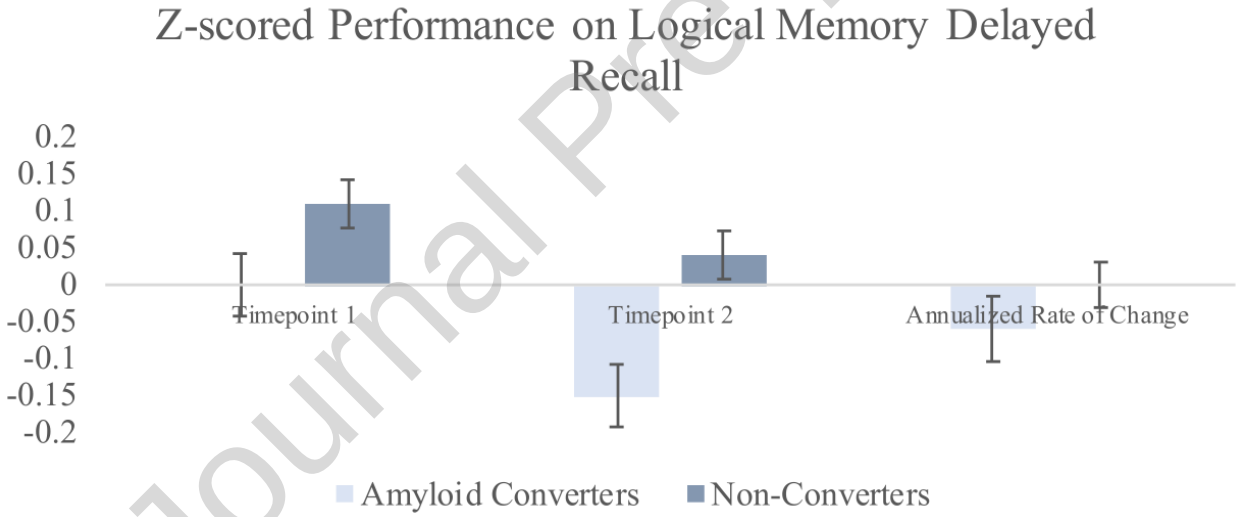

Figure 4: Z-scored performance of both amyloid converters and non-converters on Logical Memory Delayed Recall. Scores calculated so that negative numbers denote worsening, 0 indicate no change, and positive numbers indicate improvement. Group differences between amyloid converters and non-converters at both timepoints and annualized rate of change (in performance) were not significant.

Verifications

This work has not been published previously or submitted for publication elsewhere.

Disclosure Statement: None of the authors part of this work have any conflicts of interests.

CRediT authorship contribution statement

Renee C. Groechel: Conceptualization; Investigation; Formal analysis; Writing-Original Draft; Visualization. **Yorghos Tripodis:** Methodology; Formal analysis; Writing – Review & Editing. **Michael Alosco:** Conceptualization; Resources; Writing – Review & Editing. **Jesse Mez:** Conceptualization; Resources; Writing – Review & Editing. **Wei Qiao Qiu:** Conceptualization; Resources; Writing – Review & Editing. **Gustavo Mercier**: Writing – Review & Editing; Visualization. **Lee Goldstein:** Resources; Writing – Review & Editing; **Andrew E. Budson:** Resources; Writing – Review & Editing; **Neil Kowall:** Resources; Supervision; Funding Acquisition **Ronald J. Killiany:** Conceptualization; Methodology; Resources; Writing – Review & Editing; Supervision

Highlights

- Studying changes in amyloid burden is important to Alzheimer's disease research
- Examination of participants who converted in amyloid status vs those who did not
- Amyloid converters showed greater annualized percent volume loss in MRI regions
- Amyloid converters showed greater rates of amyloid accumulation on florbetapir PET
- Important neuropathological changes may be occurring alongside amyloid conversion