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RESEARCH ARTICLE

Amyloid is associated with accelerated atrophy in cognitively unimpaired individuals

Henry Gilreath Stephenson[1,2,3] | **Tobey J. Betthauser**[1,2,3] | **Rebecca Langhough**[1,3,4] | **Erin Jonaitis**[1,3] | **Lianlian Du**[1,3] | **Carol Van Hulle**[1,3] | **Gwendlyn Kollmorgen**[5] | **Clara Quijano-Rubio**[5,6] | **Nathaniel A. Chin**[1,3] | **Ozioma C. Okonkwo**[1,2,3] | **Cynthia M. Carlsson**[1,3,7] | **Sanjay Asthana**[1,3,7] | **Sterling C. Johnson**[1,2,3,4] | **Kaj Blennow**[8,9,10,11] | **Henrik Zetterberg**[1,3,9,12,13,14] | **Barbara B. Bendlin**[1,2,3]

[1]School of Medicine and Public Health, University of Wisconsin-Madison, Health Sciences Learning Center, Madison, Wisconsin, USA

[2]Neuroscience Training Program, University of Wisconsin-Madison, Wisconsin Institute for Medical Research, Madison, Wisconsin, USA

[3]Wisconsin Alzheimer's Disease Research Center, University of Wisconsin-Madison, Madison, Wisconsin, USA

[4]Wisconsin Alzheimer's Institute, University of Wisconsin-Madison, Madison, Wisconsin, USA

[5]Roche Diagnostics GmbH, Penzberg, Germany

[6]Roche Diagnostics International Ltd, Rotkreuz, Switzerland

[7]Veterans Administration, Madison, Wisconsin, USA

[8]Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

[9]Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden

[10]Paris Brain Institute, ICM, Pitié-Salpêtrière Hospital, Sorbonne University, Paris, France

[11]Neurodegenerative Disorder Research Center, Division of Life Sciences and Medicine, and Department of Neurology, Institute on Aging and Brain Disorders, University of Science and Technology of China and First Affiliated Hospital of USTC, Hefei, P.R. China

[12]Department of Neurodegenerative Disease, University College London Institute of Neurology, Queen Square, London, UK

[13]UK Dementia Research Institute at University College London, London, UK

[14]Hong Kong Center for Neurodegenerative Diseases, Hong Kong, P.R. China

Correspondence
Henry Gilreath Stephenson, School of Medicine and Public Health, University of Wisconsin-Madison, Health Sciences Learning Center, 600 Highland Avenue MC 2420, Madison, WI 53792, USA.
Email: hstephenson2@wisc.edu

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Abstract

INTRODUCTION: This study examined the association of longitudinal atrophy with baseline cerebrospinal fluid (CSF) amyloid beta (Aβ, A) and phosphorylated tau (p-tau, T) biomarkers (Aβ42/40, p-tau181) in 406 cognitively unimpaired (CU) individuals (6.670 years of follow-up on average, up to 13 imaging visits) to assess whether A+ is associated with Alzheimer's disease–like atrophy and whether this depends on p-tau181 levels.

METHODS: An A-T- CU group free from abnormal neurodegeneration (N) was identified using a robust normative approach and used to model normal age-related atrophy

via *z*-scoring. Linear mixed-effects models tested differences in longitudinal atrophy between A+ and A-T-N- individuals and between A/T subgroups.

RESULTS: A+ was associated with worse atrophy within and beyond the medial temporal lobe, even at low levels of p-tau181.

DISCUSSION: Neurodegeneration likely begins soon after the onset of abnormal Aβ pathology. Clinical intervention at the earliest signs of Aβ pathology may be needed to mitigate further neurodegeneration.

KEYWORDS
AD, amyloid beta, atrophy, CSF biomarkers, MRI, phosphorylated tau, preclinical AD, volumetrics

Highlights

- An A-T-N- control group was identified using a robust normative approach
- A+ was associated with accelerated atrophy in cognitively unimpaired individuals
- Atrophy was observed even at low p-tau181 levels

1 | BACKGROUND

A considerable body of research examining in vivo biomarkers and *post mortem* neuropathology supports a long preclinical period of sporadic Alzheimer's disease (AD) during which amyloid beta (Aβ, A) pathology, which is present in 20%–30% of elderly individuals without cognitive impairment,[1] begins to accumulate 15–25 years prior to the onset of symptoms.[2,3] Cognitively unimpaired (CU) individuals harboring Aβ pathology are at increased risk of cognitive decline and dementia due to AD.[4–6] However, it is unclear whether all Aβ-positive (A+) individuals will develop dementia. Research has suggested that brain atrophy in CU A+ individuals, as measured via T1-weighted magnetic resonance imaging (MRI), is more temporally aligned with cognitive decline and dementia risk than Aβ, particularly in the hippocampus or AD-associated cortical regions.[7] Given the current interest in targeting preclinical populations with anti-Aβ immunotherapies,[8] understanding whether Aβ itself leads to atrophic change is key for clinical decision-making.

Prior research suggests that, at the group level, atrophy occurs after increases in phosphorylated tau (p-tau, T) biomarkers. Studies on individuals with dominantly-inherited AD (DIAD) have shown that significant reductions in volume and thickness occur after increases in cerebrospinal fluid (CSF) p-tau measures.[3,9] Likewise, both cross-sectional[10–12] and longitudinal studies[13–16] examining atrophy in the context of sporadic disease have found that T+ is a stronger predictor of atrophy in CU individuals than Aβ alone. Taken together, these results suggest that A+ in the absence of T+ is a largely benign finding. However, studies to date are limited by relatively small samples of A+ CU individuals, which reduces sensitivity to subtle atrophy that may occur in the earliest stages of AD.

Furthermore, little attention has been paid to the problem of defining normal aging in the context of brain atrophy. Typically, A- CU individuals are considered "healthy-aging" controls and used as a point of reference for A+ individuals.[10–16] However, AD is only one of many possible neurodegenerative processes observed in aging populations,[17] and A- CU samples are likely contaminated with individuals positive for neurodegenerative (N+) change, thereby reducing sensitivity to detect early Aβ-associated atrophy. Furthermore, early atrophy in AD may shadow normal aging,[18] making the disambiguation of AD-related from typical age-related atrophy critical. Research on cognitive decline has benefited from robust normative approaches to establish sensitive cut-points for the detection of subtle preclinical changes.[19,20] Researchers from our group have recently applied similar normative approaches to diffusion-weighted imaging measures of neurodegeneration[21] and volumetric measures[22] to define abnormality cross-sectionally. However, few if any studies on volumetric change have used these approaches longitudinally to facilitate the disambiguation of pathological and age-related atrophy occurring over time.

In this study, we used longitudinal volumetric data measured via T1-weighted MRI from CU individuals to assess the impact of core AD neuropathologies on longitudinal atrophy patterns. First, we developed covariate-adjusted *z*-scores for regional neurodegeneration using a robust normative approach to screen out A-T-N+ individuals and quantify abnormality in volumes relative to a well-characterized A-T-N- group. Then we assessed whether abnormal AD biomarkers were associated with accelerated atrophy relative to A-T-N- individuals. Temporo-parietal regions of interest (ROIs) were selected based on previously observed associations with AD dementia (see Figure 1 and Methods 2.4). Analyses explored the magnitude and spatial extent of longitudinal atrophy in (1) A+ versus A-T-N- individuals and (2) A+T- and A+T+ groups versus A-T-N- controls. It was hypothesized that even at low levels of p-tau, A+ would be associated with an emerging pattern of AD-like atrophy.

2 | METHODS

2.1 | Study design and sample selection

Data were drawn from the Wisconsin Registry of Alzheimer's Prevention (WRAP) and the Wisconsin Alzheimer's Disease Research Center Clinical Core Study (ADRC) cohorts. Informed written consent was provided by all participants according to the Declaration of Helsinki. Study procedures were approved by the University of Wisconsin-Madison Institutional Review Board. Data included in this analysis were collected between November 24, 2009 and March 29, 2023. Participants were included in this analysis if they had available (1) CSF data using the NeuroToolKit (NTK) research platform; (2) at least two T1-weighted volumetric imaging visits with the first "baseline" visit occurring within 2 years of CSF collection; and (3) available clinical and genetic data available including a summary diagnosis of CU, determined via a clinical consensus conference occurring within 2 years of baseline MRI, and apolipoprotein E (*APOE*) genotyping. Clinical diagnosis was determined according to National Institute on Aging (NIA)–Alzheimer's Association (AA) (NIA-AA) guidelines, and confirmed in a clinical consensus conference, blinded to biomarker data.[23,24] The mean (SD) time difference between CSF collection and baseline imaging visit was 0.310 (0.694) years, whereas the mean time difference between clinical consensus diagnosis and baseline imaging was 1.150 (0.870) years, suggesting that both CSF collection and diagnosis tended, on average, to occur after baseline imaging. Participants were classified as A+ or A- as well as T+ or T- based on in-house cutoffs for Aβ42/40 and p-tau181 (described below). A-T+ individuals were excluded to filter out individuals with potential non-AD neuropathological change. This resulted in a sample of 96 A+ individuals (67 A+T-, 29 A+T+) and 310 A-T- controls (1593 observations total, 6.670 years of longitudinal follow-up on average). The A-T- group was then used to identify a robust control sample free from neurodegenerative change (see Methods 2.5). See Figure 2 for an overview of sample selection.

RESEARCH IN CONTEXT

1. **Systematic review**: The lead author reviewed the literature using PubMed and Google Scholar to identify articles detailing the association between atrophic change and Alzheimer's disease (AD) biomarkers in cognitively unimpaired (CU) individuals. Although a number of such articles exist, most studies are limited by low sample size, a lack of specialized longitudinal neuroimaging methods, and insufficient characterization of healthy aging control samples. These citations, as well as general citations on AD, are appropriately referenced.
2. **Interpretation**: Findings point to early amyloid beta (Aβ) pathology as a key predictor of accelerated atrophic change in CU individuals, even at low levels of phosphorylated tau-181 (p-tau181). This indicates that Aβ may be associated with abnormal atrophy even prior to increases in p-tau, possibly due to mixed pathology.
3. **Future directions**: Future studies will incorporate Aβ and tau-PET (positron emission tomography) measures of AD pathology to study the exact timing of these changes more precisely using PET-derived A/T groupings as well as the impact of mixed pathology on Aβ-associated change.

2.2 | CSF collection/analysis

CSF biomarkers were measured using the NTK, a panel of exploratory robust prototype assays including markers of AD pathology: the Aβ42/40 ratio and pTau181 (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). Details of processing have been described elsewhere.[25]

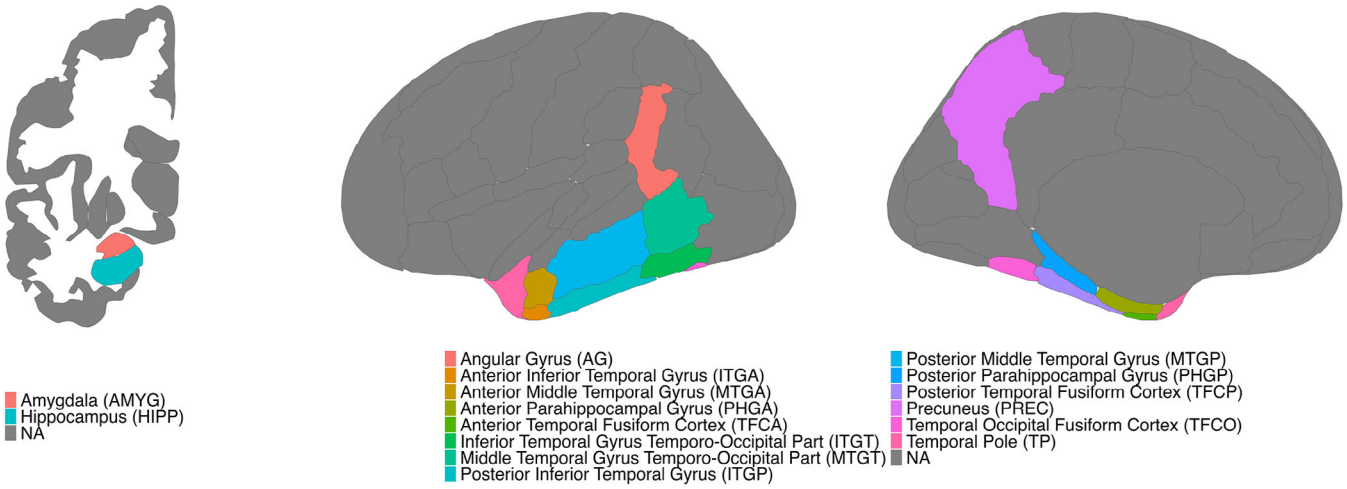

FIGURE 1 Harvard-Oxford Atlas subcortical (left) and cortical (right) ROIs selected for analysis based on sensitivity to AD-associated neurodegeneration. AD, Alzheimer's disease; ROI, region of interest.

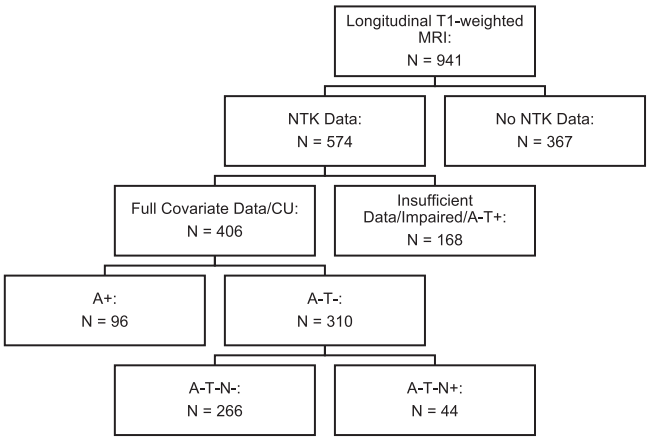

FIGURE 2 Overview of sample selection.

CSF assays using the NTK occurred over two waves. The first involved lumbar punctures collected using Fisher 1.5 mL tubes, whereas the second used Sarstedt 0.5 mL tubes. Our group has shown that tube type has strong effects on CSF measurements, especially for Aβ.[26] Therefore, cutoffs were derived separately for each wave. Participants were considered A+ if their Aβ42/40 ratio was <0.046 in the first wave or <0.055 in the second wave, as determined using an receiver-operating characteristic (ROC) analysis against Aβ positivity measured via the Aβ positron emission tomography (PET) tracer Pittsburgh compound B (PiB).[25,27] Participants were considered T+ if their p-tau181 level was >24.8 pg/mL in the first wave or >26.6 pg/mL in the second wave.[25] These values correspond with levels >2 SD above the mean of separate A-/CU subsamples (data not shown). Individuals were then grouped according to A+ status as well as A/T status.[28]

2.3 | Longitudinal volumetric MRI

Volumetric data were collected using T1-weighted imaging sequences acquired on one of two, 3-Tesla (3T) Discovery MR750 (GE Healthcare) MRI scanners with either an 8-channel Excite (GE Healthcare) or 32-channel (Nova Medical) head coil. A longitudinal segmentation pipeline was employed using Statistical Parametric Mapping 12 (SPM12) to initialize the processing of individual volumes using a within-person longitudinal template to increase within-person reliability of volumes and increase sensitivity to longitudinal change. See Supplemental Methods for longitudinal processing details. See Tables S1 and S2 for sequencing details as well as scanner and head coil numbers.

2.4 | ROI selection

ROIs were selected based on the work of Schwarz and colleagues.[29] Specifically, temporoparietal ROIs roughly corresponding to their AD signature meta-ROI were selected for analysis. Note that although Schwarz and colleagues recommended thickness measures due to their lower correlation with intracranial volume (ICV), volumetric measures were chosen for this analysis given the longitudinal design and the higher test–retest reliability of volume measures in that study. (See Figure 1 for ROIs analyzed.) Given that the current study's hypotheses were agnostic to hemisphere, left and right volumes for each ROI were averaged to reduce the number of multiple comparisons. Regional results were visualized using the ggseg package.[30]

2.5 | Robust normative *z*-scoring

A robust normative approach was used to identify an A-T- CU sample free from abnormal neurodegenerative change and quantify volumes according to this normal aging standard. Visits within 310 A-T- CU individuals were selected randomly from each individual's longitudinal data set. Random selection was done to ensure adequate sampling of scans from both head coils, given that most individuals switched from the 8-channel to the 32-channel over the course of the observation period. ROI volumes were entered into a linear regression predicting regional volumes with age, sex, ICV, and head coil as covariates. Parameter estimates from these models and their root mean squared errors (RMSEs) were then used to *z*-score regional volumes using the following formula:

$$Z\text{-Scored Volume} = (\text{Observed Volume} - \text{Predicted Volume}) / \text{RMSE}$$

In this scheme, an individual with an observed volume equal to their predicted volume would have a *z*-score of 0, suggesting normal volume given their age, controlling for sex, ICV, and head coil. If an individual had a lower volume than predicted, their score would be negative, suggesting abnormal volume given their age. Longitudinally, if an individual's atrophy did not exceed the age-related atrophy predicted by the model, their score would remain at 0. If their volume loss exceeded age-related volume loss predicted by the model, their score would become more negative over time. For the purposes of these analyses, if A+ is associated with volume loss above and beyond what can be expected from normal aging, we would expect their *z*-scored volumes to become more negative over time, while the A- group's *z*-scored volumes remain around 0.

To isolate a robust normative sample we applied methods used in developing robust cognitive norms that have been employed previously been in our group.[19–22] Specifically, we identified individuals in the A-T- control group as having abnormal neurodegeneration (N+) if their *z*-scored volume was <−2 SD in one or more ROI ($N = 44$). We excluded them to obtain a robust, healthy control sample and then obtained the linear regression model parameter estimates and RMSE using the same parameters on this reduced data set. The above model was applied once more to raw volumes from this final control sample, and the resultant parameter estimates and RMSEs were used to calculate *z*-score volumes for the entire sample.

We also investigated higher-order terms in the final control sample to account for possible non-linearity in age-related decline[18] and modification of age-related atrophy by sex.[31] Neither an interaction

between age and sex nor a quadratic effect of age was significant in any ROI (data not shown) before or after the removal of A-T-N+ individuals. Therefore, neither term was included.

2.6 | Statistical analysis

Data analysis and visualization were done in R.[32] Visualization was done using the tidyverse package.[33] Sample characteristics were compared between A+ and A- groups using unpaired *t*-tests, chi-square tests, or other tests appropriate for the distribution of the variable being compared. Age, sex, and ICV were added as covariates when comparing hippocampal volumes, whereas age, sex, and NTK batch were added as covariates when comparing p-tau181 levels.

Following *z*-scoring, all linear mixed-effects models were adjusted for *APOE* ε4 status (one or more copies of the *APOE* ε4 allele) and included random intercepts for participants as well as random slopes for time (years since baseline). Linear mixed-effects models were estimated using the lmerTest package.[34,35] *p*-Values from all analyses were corrected for multiple comparisons using the false discovery rate (FDR) correction.[36] Interaction effects were further characterized using simple slopes analysis from the emmeans package.[37] Effect sizes were assessed using partial η^2 estimates from the effect size package.[38] Partial η^2 estimates can be benchmarked according to the recommendations of Cohen (small: $0.02 \leq$ partial $\eta^2 < 0.13$, medium: $0.13 \leq$ partial $\eta^2 < 0.26$, large: partial $\eta^2 \geq 0.26$).[39]

2.7 | Longitudinal atrophy and Aβ pathology

To test whether longitudinal atrophy differed between CSF-based Aβ groups, linear mixed-effects models predicting regional *z*-scored volumes were estimated with A+, time, and an interaction between A+ and time.

2.8 | Longitudinal atrophy and A/T groupings

To examine whether longitudinal atrophy further differed between CSF-based A/T groupings, dummy-coded A+ group contrasts with the A-T-N- group as a reference were used to compare each A+ group to A-T-N- controls. Linear mixed-effects models predicting *z*-scored volumes were estimated with time, contrasts between the A+ groups and the A-T-N- group, and interactions between the A+ group contrasts with time.

2.9 | Sensitivity analyses

To assess whether results depended on the removal of A- CU individuals who were N+ in the robust normative step, primary analyses were repeated with the full A-T- CU sample, regardless of N status. To assess the extent to which results depended on longer periods of observation, the primary analyses were repeated with follow-up truncated to observation periods within 1, 3, and 5 years of baseline.

3 | RESULTS

3.1 | Sample characteristics

A+ and A-T- individuals did not differ in terms of sex. They did, however, differ significantly in terms of *APOE* ε4 status and age at baseline, such that the A+ group had significantly higher proportion of *APOE* ε4 carriers and was significantly older on average compared to A-T- individuals. The gap between baseline MRI and CSF collection differed significantly between the A+ and A-T- groups, such that CSF collection tended to occur earlier relative to MRI collection in A+ compared to A-T- individuals. The A+ group showed significantly higher p-tau181 levels. Groups did not differ in terms of self-reported ethnicity, length of follow-up, number of visits, baseline hippocampal volume (non-*z*-scored), or the gap between baseline MRI and clinical diagnosis. See Table 1 for details; see Table S3 for sample characteristics between A/T groupings.

3.2 | Robust normative *z*-scoring

After the initial *z*-scoring in the CU, A-T- control group, 44 A-T- individuals (≈14%) were considered neurodegeneration-positive (N+) in at least one ROI and were removed from the final control sample and excluded from all subsequent analyses. See Tables S4 and S5 for regional model outputs in the initial and final robust control samples, respectively. See Figure S1 for counts of N+ by ROI in the initial control sample. See Figures S2 and S3 for uncorrected longitudinal volumes and *z*-scored longitudinal volumes by ROI in the final control sample.

3.3 | Longitudinal atrophy and Aβ pathology

In the primary analysis, A+ individuals showed accelerated atrophy relative to A-T-N- controls as evidenced by significant, negative interactions between A+ and time in all ROIs examined. Partial η^2 estimates ranged from small (partial $\eta^2 = 0.032$ for temporal occipital fusiform) to medium (partial $\eta^2 = 0.161$ for amygdala). The magnitude of the interaction effect was larger in the MTL and relatively weaker in lateral temporal and parietal areas. The top four ROIs by magnitude of the interaction were the amygdala, hippocampus, and the anterior and posterior parahippocampal gyri. Simple slopes analyses corroborated these findings, showing considerably more negative effects of time in MTL ROIs in A+ compared to A-T-N- individuals and relatively more subtle differences in the remaining ROIs. Partial η^2 estimates show that the top four ROIs by size of the interaction effect were the amygdala, hippocampus, anterior inferior temporal gyrus, and temporal occipital inferior temporal gyrus. Notably, simple slopes for time were positive

TABLE 1 Sample characteristics.

	Whole sample	A-T-	A+	*p*-value
N	406	310	96	NA
% Female	65.025%	67.419%	57.292%	0.900
% White	94.335%	93.548%	96.875%	0.327
% *APOE ε4+*	37.192%	28.065%	66.667%	< 0.001
Age at baseline MRI, years, mean (SD)	61.227 (7.187)	59.854 (6.806)	65.658 (6.597)	< 0.001
Length of follow-up, years, mean (SD)	6.672 (3.110)	6.793 (3.050)	6.284 (3.284)	0.162
Number of visits, mean (SD)	3.924 (1.837)	3.994 (1.871)	3.698 (1.711)	0.169
Aβ42/40, mean (SD)	0.064 (0.017)	0.072 (0.010)	0.038 (0.007)	NA
p-tau181, pg/mL, mean (SD)	17.156 (6.134)	15.345 (4.373)	23.001 (7.280)	< 0.001
Baseline hippocampal volume, mL, mean (SD)	3.721 (0.443)	3.744 (0.431)	3.645 (0.474)	0.225
MRI/CSF age difference, years, mean (SD)	−0.310 (0.694)	−0.242 (0.643)	−0.530 (0.802)	< 0.001
MRI/diagnosis age difference (years), mean(SD)	−1.150 (0.869)	−1.182 (0.853)	−1.045 (0.916)	0.177

Note: *p*-values taken from unpaired samples *t*-tests or chi-square tests comparing Aβ42/40 groupings, as appropriate.
Abbreviations: Aβ, amyloid beta; CSF, cerebrospinal fluid; mL, milliliters; MRI, magnetic resonance imaging; pg, picograms; p-tau181, phosphorylated tau-181; SD, standard deviation.

within the A-T-N- group in a few ROIs, suggesting slower declines over time than the age trend would predict in this group. Neither A+ contrast nor *APOE ε4+* showed the main effects. See Figure 3 for visualization of the results. See Table S6 for slopes, confidence intervals, uncorrected and FDR-corrected *p*-values, and effect size estimates.

3.4 | Longitudinal atrophy and A/T groupings

In the secondary analysis, it was shown that both A+ groups showed significantly accelerated atrophy relative to controls in a majority of ROIs. For the A+T- group, the magnitude of the interaction effect was larger in the MTL and relatively weaker elsewhere, similar to the primary analysis. The top four ROIs by magnitude of the interaction were the amygdala, hippocampus, and posterior and anterior parahippocampal gyri. The interaction was not significant in the anterior middle temporal, temporal occipital middle temporal, or anterior temporal fusiform gyri. For the A+T+ group, the magnitude of the interaction effect was larger in both medial and lateral aspects of the temporal lobe. The top four ROIs by magnitude of the interaction were the anterior parahippocampal, posterior temporal fusiform, anterior inferior temporal, and posterior middle temporal gyri. The interaction was not significant in the anterior middle temporal gyrus. Simple slopes analysis complemented these findings, showing greater effects of time in MTL ROIs in both A+ groups compared to A-T-N- individuals, with a number of ROIs showing worse effects of time in the A+T+ group compared to A+T- individuals. Similar to the prior analysis, simple slopes for time were positive within the A-T-N- group in a few ROIs, suggesting slower declines over time than the age trend would predict. Neither A+ contrast nor *APOE ε4+* showed the main effects. See Figure 4 and Table S7.

3.5 | Sensitivity analyses

For the primary analysis, inclusion of the entire sample without removing A-T-N+ individuals from the A-T- CU group yielded a largely identical pattern of results with the exception of the anterior middle temporal gyrus, which was no longer significant. Considering A+ vs A-T-N- individuals with longitudinal follow-up restricted to 1, 3, and then 5 years, there were no significant interactions between A+ and time with 1 year of follow-up. With follow-up durations extended to 3 years or less, there were significant interactions in the amygdala and hippocampus, suggesting worse atrophy in the A+ group. At 5 years or less, this pattern extended to the anterior inferior temporal gyrus and temporal pole. In addition, the 5-year window showed significant interactions in the temporal occipital inferior temporal, posterior middle temporal, and the anterior and posterior parahippocampal gyri, but these did not survive FDR correction. See Figures S4 and S5 and Tables S8 and S9. See Figures S6 and S7 and Tables S10, S11, and S12 for secondary sensitivity analyses examining A/T groupings. An additional sensitivity analysis comparing A/T groupings with A-T+ individuals included can be found in Figure S8 and Table S13.

4 | DISCUSSION

This study examined the association between Aβ and p-tau pathology and longitudinal atrophy in CU individuals. Using a robust normative approach, we identified a group free from abnormal neurodegeneration and *z*-scored longitudinal volumes in our full sample in relation to this group. Results of the first analysis comparing atrophy in A+ vs A-T-N- individuals indicated that A+ individuals showed more rapid volume loss than A-T-N- individuals, not just in the MTL, but in all ROIs examined. Analyses using A/T groupings refined these results,

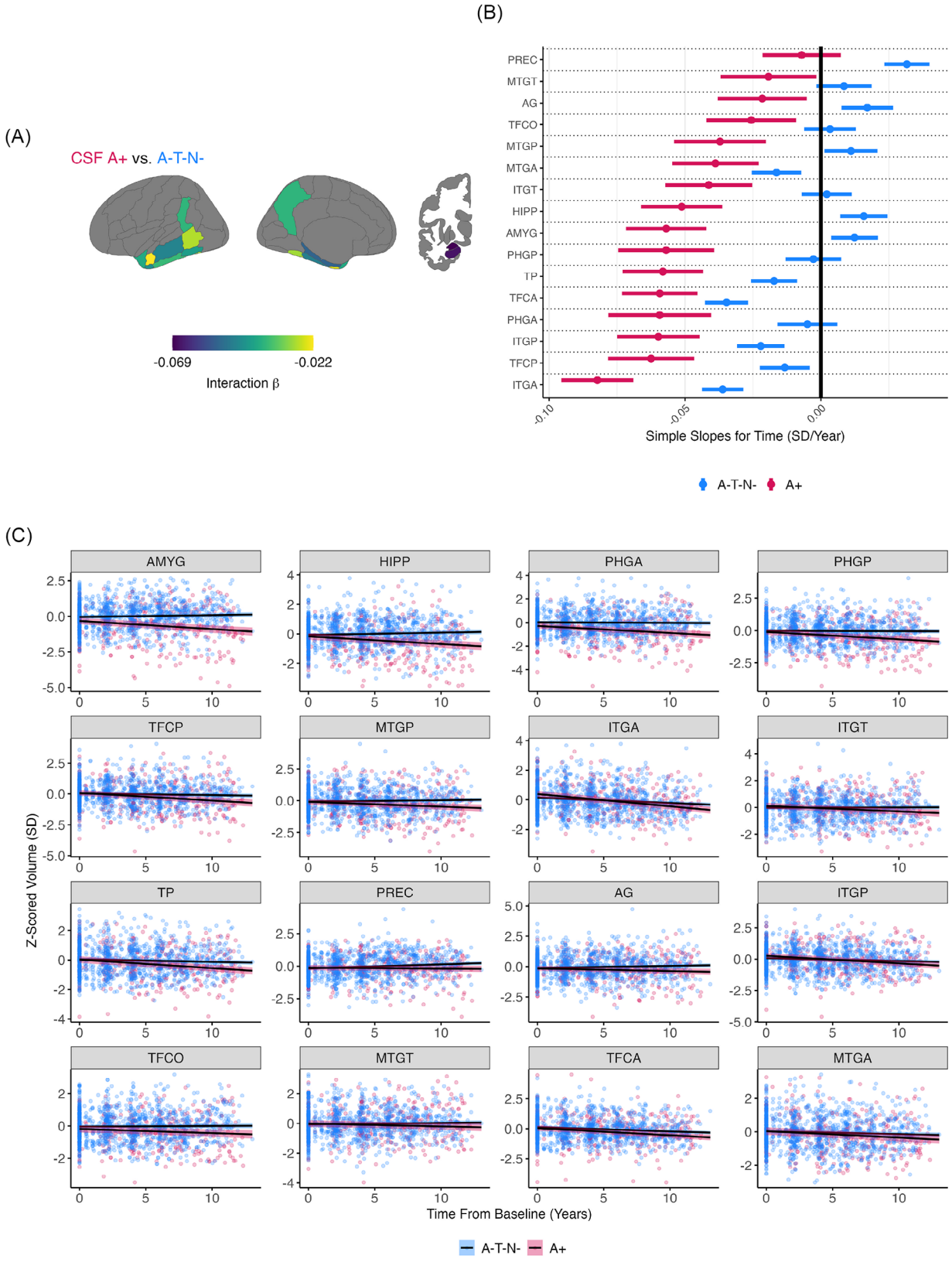

FIGURE 3 Regional effects of A+ from linear mixed-effects models using the robust ROI *z*-scores. (A) β estimates of interactions between A+ and time that survived FDR correction. (B) Forest plot of simple slopes for time by Aβ group arranged by observed magnitude in the A+ group. More negative numbers indicate worse effects of time. Points show simple slopes, while lines show the lower and upper bounds of 95% confidence intervals. (C) Interaction plots depicting the effects of time for A+ versus A-T-N- groups. Aβ, amyloid beta; FDR, false discovery rate; ROI, region of interest.

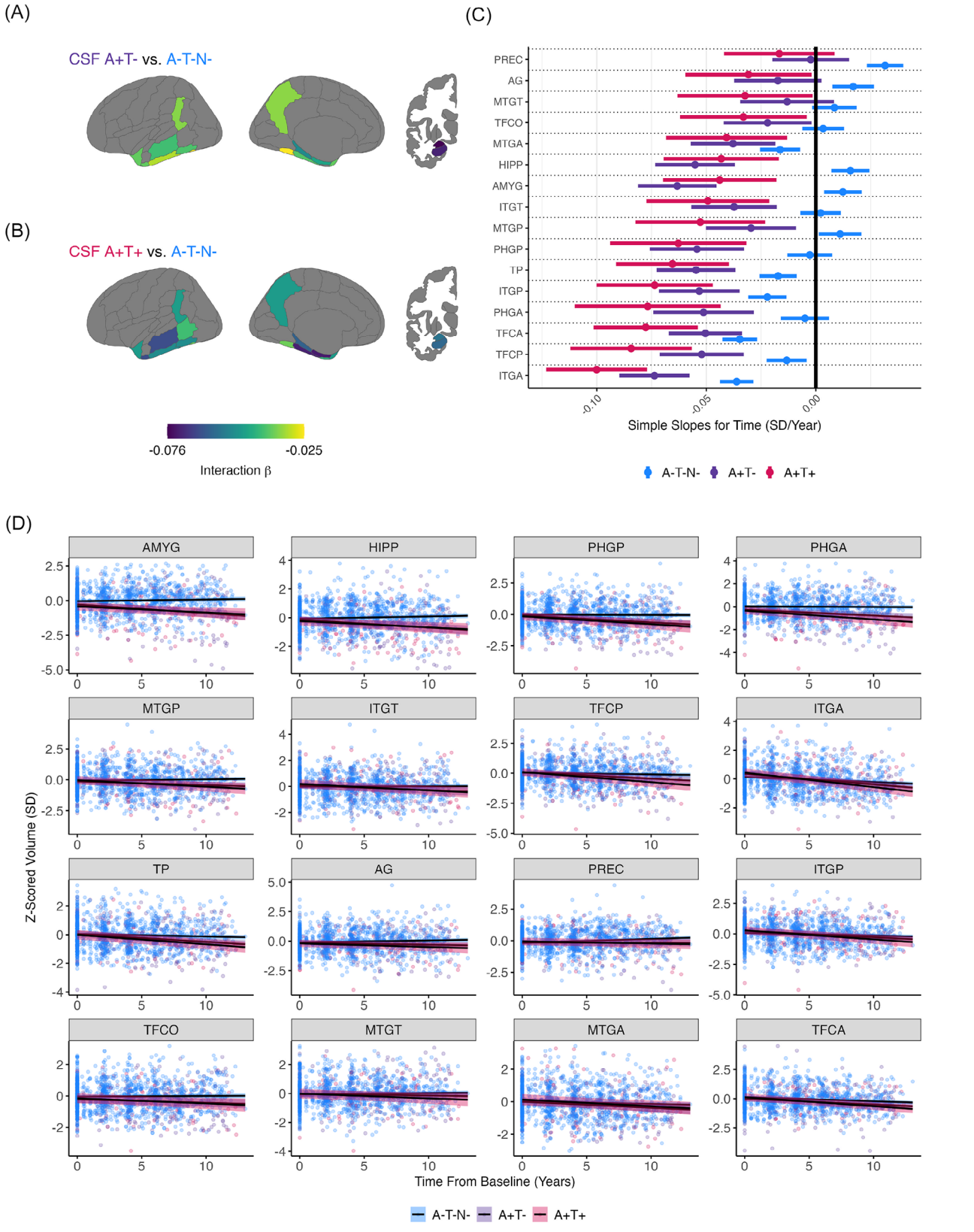

FIGURE 4 Regional effects of A+ groups from linear mixed-effects models using the robust ROI *z*-scores. (A, B) β estimates of interactions between those that survived FDR correction in the A+T- and A+T+ groups, respectively. (C) Forest plot of simple slopes for time arranged by observed magnitude in the A+T+ group. More negative numbers indicate worse effects of time. Points show simple slopes, whereas lines show the lower and upper bounds of 95% confidence intervals. (D) Interaction plots depicting the effects of time within A/T groups. FDR, false discovery rate; ROI, region of interest.

showing that A+T- and A+T+ individuals showed largely concordant patterns of atrophy. Sensitivity analyses for the primary results showed that these results did not depend on the removal of A-T-N+ individuals, demonstrating their robustness. Notably, restriction of longitudinal follow-up to shorter durations revealed accelerated amygdalar and hippocampal atrophy in A+ individuals by 3 years that extended to the anterior inferior temporal gyrus and temporal pole within 5 years. These results suggest that among CU individuals, A+, even at low levels of p-tau181, is associated with abnormal atrophy in the MTL, which gradually extends to a broad swath of ROIs consistent with an AD-like pattern of atrophy.

Results affirm models of AD pathophysiological progression whereby neurodegeneration in the MTL begins after the onset of Aβ pathology and increases in magnitude and extent thereafter. However, these results contradict existing literature in regard to the timing of neurodegeneration in relation to CSF AD biomarkers. Prior research indicates that significant neurodegeneration is observed only in individuals with a concomitant A+ and T+ profile in the CSF.[10–16] In this study, A+ individuals, regardless of T+, showed accelerated atrophy in a broad set of temporoparietal ROIs. This would place the onset of neurodegeneration considerably earlier than previously thought. These observations are not without precedent. Insel and colleagues showed that accelerated atrophy in the hippocampus, amygdala, precuneus, and insula occurred before their threshold for A+,[40] similar to what was observed here in the A+T- group. Mattsson and colleagues similarly showed accelerated neurodegeneration in multiple neocortical ROIs within individuals who converted from CSF A- to A+, suggesting an association between emerging Aβ pathology and widespread neurodegeneration.[41] Results from this study agree with both in suggesting that neurodegeneration in and beyond the medial temporal lobe accompanies Aβ pathology in its early stages. This might help explain why cognitive decline has been observed in CU A+ individuals, even without T+.[42] Prior studies may not have detected atrophy among A+ CU individuals due to a number of limitations. Notably, many prior studies had a low CU A+ sample size relative to this study,[10,11,13–16] thereby lowering statistical power to detect subtle, preclinical effects. Similarly, past studies lacked a robust normative approach and may have had control samples contaminated with N+ individuals. Finally, several longitudinal studies have lacked specialized longitudinal segmentation methods.[10,12–15] This is especially important, as cross-sectionally–derived volumes are inherently noisy, which may have obfuscated longitudinal trends in neurodegeneration.[43]

The pathophysiological mechanism underlying the observed results may be Aβ-mediated neuronal loss. At autopsy in later or end-stage AD, neurodegeneration appears to show the greatest spatial overlap with regions of neurofibrillary tangle (NFT) pathology,[44] contributing to the conclusion that tau, not Aβ, is the key predictor of atrophy. However, the aforementioned studies demonstrating accelerated atrophy alongside early Aβ pathology may contradict this view.[40,41] The possibility of pre-NFT atrophy in humans would agree with observations of neurodegenerative change in some AD model mice that overproduce Aβ but do not develop NFTs.[45] Atrophic changes in this study did not necessarily correspond to areas of early plaque deposition. They were observed in both relatively early- (e.g., precuneus) and late-plaque–bearing (e.g., hippocampus) regions.[46] However, discrepancies in the spatial distribution of Aβ plaques and neurodegeneration do not preclude a possible link between the two. Indeed, Aβ pathology has been suggested to promote pathological changes in spatially distant, but anatomically connected ROIs such as neuronal hyperexcitability[47] or glial hyperactivation,[48] which can produce neurodegenerative change. Neurodegeneration in A+ individuals at low levels of p-tau may then be a product of Aβ itself or Aβ-mediated changes occurring through one or more of these mechanisms.

Early, Aβ-related elevations in tau may still have played a role in this study, albeit below the supra-threshold levels that prior research suggests are necessary for neurodegenerative change. Although the A+T- group did not have p-tau181 levels above our T+ threshold, they did have significantly elevated levels relative to controls (See Table S3). Aβ has been shown to promote the prefilamentous hyperphosphorylation of tau in non-plaque–bearing regions,[49] which can be neurotoxic, even in the absence of NFT formation.[50] Neurodegeneration in A+ individuals may have resulted from this process, suggesting that even low levels of p-tau are sufficient to produce neurodegeneration. If this is the case, tau and neurodegeneration may be more contemporaneous processes than previously thought. Some individuals in this study may have had mature NFTs, especially in the entorhinal cortex. It has been shown that many phospho-tau species are poor markers for early NFT burden and could reflect Aβ-associated tau hyperphosphorylation in addition to mature tangles.[9] Tau-PET was not available for this study, as most of the CSF and imaging data were collected prior to the development and implementation of the tau-PET tracer MK-6240 in WRAP and ADRC. Future studies incorporating tau-PET will be needed to characterize atrophy according to NFT pathology.

Another contributor to the observed patterns of atrophy in A+ CU individuals could be mixed neuropathology. Measures of neurodegeneration are not specific to AD. Although our robust normative approach renders the presence of abnormal non–AD-related neurodegeneration less likely in the control group, it cannot disambiguate AD versus non–AD-related neurodegeneration in the A+ group. Autopsy studies have shown that Aβ is often co-morbid with other neuropathological entities including TAR DNA-binding protein 43 (TDP-43), Lewy body disease, and vascular disease, which can exacerbate the rate of decline.[17] This may have accounted for some of the observed atrophy in the A+ group, such that those showing more rapid atrophy may have had a greater number of co-pathologies. Few biomarkers yet exist for non-AD neuropathologies. However, once they become available, these biomarkers will enable more rigorous characterization of both A+ and A- individuals.

This study has several limitations worth noting. First, the present sample consists predominantly of non-Hispanic, White individuals. As such, the current results may not generalize to the broader population. Second, changes to MRI acquisition occurred over the course of this longitudinal study; however, this is unlikely to account for the current results, as all individuals, regardless of AD pathological status, underwent the same changes in image acquisition over time, and these

changes were accounted for statistically as part of the robust *z*-scoring approach. Nevertheless, caution is warranted in interpreting longitudinal changes in any large-scale neuroimaging studies, where changes in acquisition parameters are common. Finally, caution is warranted in regard to our A/T analyses. First, we used an SD approach to define abnormality in pTau181 levels because there is no ground truth to define abnormality in p-tau isoforms themselves, except in regard to other pathological changes (Aβ or tau PET abnormality, clinical diagnosis, and so on). As a result, our delineation of T+ may have been imprecise with regard to an underlying pathological state. In addition, our A+T+ sample was small with only 29 individuals. Larger A+T+ sample sizes will be necessary in future studies to more reliably assess atrophy in this group.

In conclusion, we have shown that among CU individuals, elevated measures of Aβ and p-tau pathology are associated with neurodegeneration within and beyond the MTL. These results clarify and expand existing models of neurodegeneration in AD and further suggest that abnormal Aβ deposition in CU individuals may not be a benign finding. Anti-Aβ immunotherapies may be necessary even at preclinical stages of disease to spare gray matter and forestall future cognitive decline.

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CONFLICT OF INTEREST STATEMENT

G.K. is a full-time employee of Roche Diagnostics GmbH, Penzberg, Germany. C.Q-R. is a full-time employee of Roche Diagnostics International Ltd, Rotkreuz, Switzerland. S.C.J. has served as an advisor to Enigma, Roche Diagnostics, Merck, and ALZPath. He has also received research funding from Cerveau Technologies for unrelated work. K.B. has served as a consultant and on advisory boards for Abbvie, AC Immune, ALZPath, AriBio, BioArctic, Biogen, Eisai, Lilly, Moleac Pte. Ltd, Neurimmune, Novartis, Ono Pharma, Prothena, Roche Diagnostics, and Siemens Healthineers; has served on data monitoring committees for Julius Clinical and Novartis; has given lectures, produced educational materials, and participated in educational programs for AC Immune, Biogen, Celdara Medical, Eisai, and Roche Diagnostics; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this article. H.Z. has served on scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. B.B.B. has consulted for New Amsterdam Pharma, Cognito Therapeutics, and Merry Life Biomedical, and is the co-founder of Cognovance, LLC. None of these competing interests are related to the content of the manuscript. No other disclosures were reported. Author disclosures are available in the Supporting Information.

CONSENT STATEMENT

Written informed consent was given by all participants and their study partners.

DISCLAIMER

The NeuroToolKit (NKT) is a panel of exploratory prototype assays designed to robustly evaluate biomarkers associated with key pathologic events characteristic of AD and other neurological disorders, used for research purposes only and not approved for clinical use (Roche Diagnostics International Ltd, Rotkreuz, Switzerland).

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SUPPORTING INFORMATION

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