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Brain Injury Biomarkers Are Not Dependent on β -amyloid in Normal Elderly

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Disclosures

Dr. Knopman serves as Deputy Editor for *Neurology*[®]; served on a Data Safety Monitoring Board for Lilly Pharmaceuticals; served as a consultant to TauRx, was an investigator in clinical trials sponsored by Baxter, Elan Pharmaceuticals, and Forest Pharmaceuticals in the past 2 years; and receives research support from the NIH.

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Author Contributions:

Dr. Knopman took part in data collection, supervised analyses, generated the first and final drafts and takes overall responsibility for the data and the manuscript.

Dr. Jack took part in data collection, supervised analyses, and critically reviewed the manuscript.

Ms. Wiste performed analyses and critically reviewed the manuscript.

Mr. Weigand performed analyses and critically reviewed the manuscript.

Dr. Vemuri performed analyses of imaging data and critically reviewed the manuscript.

Dr. Lowe supervised collection of PET imaging, and critically reviewed the manuscript.

Dr. Dr. Kantarci performed analyses of imaging data and critically reviewed the manuscript.

Dr. Gunter performed analyses of imaging data.

Mr. Senjem performed analyses of imaging data.

Dr. Roberts critically reviewed the manuscript.

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Dr. Boeve took part in data collection and critically reviewed the manuscript.

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Abstract

Background—The new criteria for preclinical Alzheimer’s Disease (AD) proposed 3 stages: abnormal levels of β -amyloid (stage 1); stage 1 plus evidence of brain injury (stage 2); and stage 2 plus subtle cognitive changes (stage 3). However, a large group of subjects with normal β -amyloid biomarkers have evidence of brain injury; we labeled them as “suspected non-Alzheimer pathway” (sNAP) group. The characteristics of the sNAP group are poorly understood.

Methods—Using the preclinical AD classification, 430 cognitively normal subjects from the Mayo Clinic Study of Aging who underwent brain MR, ¹⁸fluorodeoxyglucose (FDG) and Pittsburgh compound B (PiB) positron emission tomography (PET) were evaluated with FDG PET regional volumetrics, MR regional brain volumetrics, white matter hyperintensity (WMH) volume and number of infarcts. We examined cross-sectional associations across AD preclinical stages, those with all biomarkers normal, and the sNAP group.

Results—The sNAP group had a lower proportion (14%) with APOE ϵ 4 genotype than the preclinical AD stages 2 + 3. The sNAP group did not show any group differences compared to stages 2 + 3 of the preclinical AD group on measures of FDG PET regional hypometabolism, MR regional brain volume loss, cerebrovascular imaging lesions, vascular risk factors, imaging changes associated with α -synucleinopathy or physical findings of parkinsonism.

Conclusions—Cognitively normal persons with brain injury biomarker abnormalities, with or without abnormal levels of β -amyloid, were indistinguishable on a variety of imaging markers, clinical features and risk factors. The initial appearance of brain injury biomarkers that occurs in cognitively normal persons with preclinical AD may not depend on β -amyloidosis.

Keywords

Alzheimer’s disease; PET imaging; MR imaging; Epidemiology

Introduction

The development of β -amyloid and brain injury biomarkers has made it possible to study Alzheimer disease (AD) pathophysiology in asymptomatic persons. Relationships between β -amyloidosis and brain injury biomarkers in asymptomatic individuals may be different than in persons with cognitive impairment. In 2011, a workgroup of the National Institute on Aging and Alzheimer’s Association (NIA-AA) proposed a framework for preclinical AD [1]. The framework was based on a model that posited abnormal β -amyloid biomarkers as the first stage, which would then be accompanied by biomarkers of brain injury (stage 2), then later subtle cognitive decline (stage 3), and then symptomatic illness (mild cognitive impairment and dementia). Stratifying participants by both β -amyloidosis and brain injury biomarkers is a unique aspect of the NIA-AA preclinical framework.

We had the opportunity to study this preclinical framework in a large cohort of participants in the Mayo Clinic Study of Aging, a population-based epidemiological study [2, 3]. We used Pittsburgh compound B (PiB) positron emission tomography (PET) imaging as the biomarker for β -amyloid, and structural MR measures of hippocampal atrophy and ¹⁸fluorodeoxyglucose (FDG) PET measures of glucose metabolism as biomarkers of brain injury, ie neurodegeneration of the AD type. We defined abnormal biomarkers based on levels seen in 90% of clinically diagnosed AD dementia patients. While we found the

predicted graduated risk compared to stage 0 (no biomarkers abnormal) to stage 1 < stage 2 < stage 3, we also found something not envisioned by the underlying model. Twenty-three percent of our elderly cognitively normal subjects had abnormal brain injury biomarkers with levels β -amyloidosis measured by PiB PET below the diagnostic threshold for AD [2]. This group, dubbed “suspected non-AD pathophysiology” (sNAP), had a risk for cognitive decline after 1 year that was about the same as preclinical AD stage 1 and less than half the risk of stages 2 or 3 [3].

The current work took as its starting point our intention to characterize the sNAP group. Our working hypothesis was that the preclinical AD group possessed unique characteristics that would differ from the sNAP group, and that the sNAP group would show an excess of features observed in cerebrovascular disease or α -synucleinopathy, the two other major pathophysiologies that cause dementia in the elderly. That is not what we found. Instead, the sNAP group and the preclinical AD stages 2 and 3 were very similar in all respects except for APOE ϵ 4 genotype and β -amyloidosis. Our observations suggest that the initial appearance of brain injury biomarkers that occurs in cognitively normal persons with preclinical AD may not be dependent on β -amyloidosis.

Methods

Subjects

The 430 cognitively normal subjects were a subset of participants from the Mayo Clinic Study of Aging who had undergone imaging with MR and PET beginning in 2006 and continuing to the present [2, 3]. There were 450 in the baseline biomarker cohort; in the present analysis, 4 of those subjects were excluded because they lacked vascular imaging data, and 1 was excluded because volumetric MR data could not be calculated. An additional 15 participants were not classifiable by the NIA-AA scheme and were excluded from the analysis. The details of recruitment for the parent study have been described previously [4] as has the prevalence [5] and incidence [6] of mild cognitive impairment in this study.

Human Subjects Protections

All study protocols were approved by the Mayo and Olmsted Medical Center Institutional Review Boards, and all subjects provided signed informed consent to participate in the study and in the imaging protocols.

Assessments

The participants in this study were diagnosed as being cognitively normal through a consensus process that used information from three sources: a mental status examination performed by a study physician, a Clinical Dementia Rating that included interview of an informant as well as the participant completed by a trained study coordinator, and a psychometric battery containing 9 well-established instruments, previously described [4–6].

The neurological examination of the Unified Parkinson’s Disease Rating scale [7] was used to quantitate extrapyramidal features. We summed the following 15 exam items each rated on a scale from 0 to 4 (facial expression, tremor at rest (4 limbs), rigidity (neck and 4 limbs), arising from a chair, postural stability, posture, body bradykinesia/hypokinesia and gait) to give a total score that could range from 0 to 60.

All subjects in the MCSA underwent interviews in which a medical history was obtained. We inquired about the presence of diabetes, hypertension, smoking history, stroke history,

myocardial infarction, coronary artery disease interventions (surgery or angioplasty), congestive heart failure, atrial fibrillation and angina.

Imaging methods

Imaging methods for structural MR, FDG PET and amyloid PET were identical to those that have been described previously [2, 3, 8]. We used these imaging modalities to operationalize the preclinical AD groupings: amyloid PET imaging for defining abnormal brain β -amyloidosis, structural MR measurement of hippocampal atrophy or FDG PET for glucose hypometabolism for defining brain injury.

Amyloid PET images were acquired using a GE Discovery RX PET/CT scanner. Subjects are injected with 292–729 MBq ^{11}C Pittsburgh compound B (PiB). The PiB scan consisting of four 5-minute dynamic frames and was acquired from 40–60 minutes after injection [9, 10]. ^{18}F FDG-PET images were obtained on the same day one hour after the PiB scan. A CT image was obtained for attenuation correction. Subjects were injected with 366–399 MBq of fluorodeoxyglucose (^{18}F -FDG), and imaged after 30–38 minutes, for an 8-minute image acquisition consisting of four 2-minute dynamic frames.

Quantitative image analysis for both amyloid PET and FDG PET were performed using our in-house fully automated image processing pipeline [8, 11]. Statistics on image voxel values were extracted from automatically labeled cortical regions of interest (ROIs) using an atlas [12] modified in-house. A cortical amyloid PET standardized uptake value ratio (SUVR) was formed by combining the prefrontal, orbitofrontal, parietal, temporal, anterior cingulate, and posterior cingulate/precuneus ROI values normalized by the cerebellar gray matter ROI of the atlas. FDG PET scans were analyzed in a similar manner using angular gyrus, posterior cingulate, and inferior temporal cortical ROIs to define an “Alzheimer signature composite” [13], normalized to pons and vermis. In addition to this AD composite ROI, we also looked at individual ROIs defined by the atlas. Two α -synucleinopathy -related ROI composites were constructed based on changes seen in patients with Lewy Body Dementia [14], consisting of the occipital ROI and the ratio of posterior cingulate/(precuneus+occipital) ROIs.

All subjects underwent MR scanning at 3T with a standardized protocol that included a 3D-MPRAGE sequence [8]. MPRAGE images were corrected for image distortion and bias field [15] as previously described [8]. The primary MR measure used for group assignment was hippocampal volume measured with FreeSurfer software (version 4.5) [16]. Each subject's raw hippocampal volume was adjusted by their total intracranial volume [17]. We calculated an adjusted hippocampal volume as the residual from a linear regression of hippocampal volume on the y-axis versus total intracranial volume on the x-axis. Dorsal midbrain gray matter volume associated with α -synucleinopathy was calculated from an established ROI on a common template on SPM5 and scaled by the total intracranial volume [18]. The MR protocol included a FLAIR scan from which we assessed features of cerebrovascular disease. White matter hyperintensity burden was measured quantitatively using an algorithm developed in-house [19]. Supratentorial subcortical and cortical infarctions greater than 1 cm were ascertained visually by highly experienced image analysts and confirmed by a radiologist (KK).

We performed statistical parametric mapping (SPM) with SPM5 [20, 21] using our previously described methods [8] comparing the sNAP and the stage 2+3 groups on both MR grey matter density and FDG metabolism, both using false discovery rate correction and threshold at $p < 0.05$.

Definitions of preclinAD stages and sNAP group [2, 3]

As previously described, we chose the cutpoints for each imaging biomarker that corresponded to 90% sensitivity in clinically diagnosed subjects with AD dementia from the Mayo Alzheimer's Disease Research Center. For abnormal brain β -amyloidosis, a requirement for all stages of the preclinical criteria, we used the cutpoint for the PiB PET global cortical ratio of 1.5. For the markers of brain injury required for stages 2 and 3, subjects were classified as having brain injury if they had abnormal hippocampal atrophy or abnormal FDG PET hypometabolism. The 90% sensitivity cutpoint for hippocampal volume adjusted for total intracranial volume was -0.70 cm^3 , which is interpreted as 0.7 cm^3 below the normative average after accounting for head size. For the FDG PET hypometabolism ratio of the "AD signature" composite, the cutpoint value was 1.31.

For the subtle cognitive change required for stage 3 [1], we defined the cognitive cutpoint based on the 10th percentile on the global neuropsychological composite z-score from the baseline assessments of the 450 CN subjects who were part of the cross-sectional group with imaging biomarker assessments [2].

Subjects were divided into 5 groups based on the biomarker cutpoints described above: all biomarkers normal (stage 0), abnormal brain β -amyloidosis only (preclinAD stage 1), abnormal brain β -amyloidosis and brain injury without cognitive symptoms (preclinAD stage 2), abnormal brain β -amyloidosis and brain injury with cognitive symptoms (preclinAD stage 3), and normal brain β -amyloidosis with brain injury (sNAP).

Statistical methods

We used Wilcoxon rank sum tests and chi-square tests to test for pairwise differences in demographics, cerebrovascular features, cardiovascular risk factors, and α -synucleinopathy features between the sNAP group and stage 0 and preclinAD stage 1–3. Additionally, we combined preclinAD stages 2 and 3 to compare subjects with abnormal brain β -amyloidosis and brain injury (with or without cognitive symptoms) to subjects with normal brain β -amyloidosis and brain injury (sNAP). Because of the large number of tests, a p-value <0.01 was considered significant.

Results

The features of the 430 cognitively normal participants in this study (median age 78 yrs; 45% women) are shown in Table 1 along with the features of the entire group of cognitively normal subjects in the MCSA. The participants in the imaging studies are slightly healthier than the group as a whole. The features of the sNAP group compared to participants in stage 0 and preclinAD stages 1–3 are shown in Table 2.

One hundred thirty-seven (32%) participants had PiB SUVR ≥ 1.5 and 69 (50%) of them had biomarkers that met our operational definition for brain injury. Among the 293 (68%) participants with PiB SUVR < 1.5 , 102 (35%) had biomarkers indicative of brain injury (difference across amyloid groups, $p=0.002$, chi-square test). Among the entire group of our cognitively normal participants, 171 (40%) had abnormal brain injury biomarkers, and only 69 (40%) of them also had PiB SUVR ≥ 1.5 .

There were 102 subjects in the sNAP group. Focussing on the features used to define group membership, 20% ($n=20$) of the sNAP group had both low hippocampal volume and reduced glucose metabolism in the AD signature composite, 52% ($n=53$) had low glucose metabolism in the AD signature composite only, and 28% ($n=29$) had hippocampal atrophy only. Participants in preclinAD stages 2+3, the group which will be the principal comparison group to sNAP in these analyses, had similar proportions of participants with hippocampal

atrophy (25%), FDG PET hypometabolism (48%) in the AD signature regions or both biomarker abnormalities (28%). There were no significant differences in age, sex, or education between the sNAP group and the preclinAD stage groups. Like the preclinAD groups, the sNAP group was older than the stage 0 group (median age 80 vs. 76). The sNAP group had the fewest subjects who were APOE $\epsilon 4+$ (14%). The sNAP group had about the same proportion of participants with low cognition (17%) as the combined preclinAD stages 2+3 (19%).

Cortical volumetrics and regional glucose metabolism across groups

In addition to hippocampal volume and glucose hypometabolism in the AD signature composite used in our grouping definition, we examined all cortical regions and found no group-wise differences on SPM maps comparing the sNAP and the stage 2+3 groups on grey matter density or FDG metabolism (data not shown).

Associations with cerebrovascular disease

We found no consistent pattern of differences between structural imaging markers of cerebrovascular disease and group membership (Table 3) or in the presence of vascular risk factors (Table 4). White matter hyperintensity fractional volume was lower in stage 0 and preclinAD stage 1 compared to preclinAD stages 2+3 and sNAP. Some vascular risk factors were lower in stage 0 compared to sNAP. There were no differences between the sNAP group and the combined preclinAD stages 2+3.

Associations with α -synucleinopathy-related features

Similarly, there were no differences in clinical features (see Table 5) associated with α -synucleinopathy between the sNAP group and preclinAD stages 1–3. There were also no differences in midbrain volume across groups. Using the occipital to pons and posterior cingulate to (precuneus+occipital) hypometabolism on FDG PET as proxies for the pattern of regional hypometabolism seen in Dementia with Lewy Bodies, we found no differences between the sNAP group and the combined preclinAD stages 2+3 (Table 5).

Discussion

We performed analyses of imaging biomarkers, neurological findings and risk factors in cognitively normal persons grouped according to levels of fibrillar β -amyloidosis as indexed by PiB PET SUVR and biomarkers of brain injury, FDG PET hypometabolism in the AD signature region and hippocampal atrophy. The sNAP group and the preclinAD stages 2 + 3 group did not differ in any of the imaging biomarkers, vascular risk factors or clinical features that we examined, with the exception of carriage of APOE $\epsilon 4$ genotype. The latter was under-represented in the sNAP group, which was consistent with the lower levels of PiB retention and brain β -amyloidosis in the sNAP group [22, 23]. The qualitative and quantitative similarities across preclinAD stage 2 + 3 and sNAP on the battery of various non-AD related features suggests that changes in brain injury biomarkers that occur in cognitively normal persons may not be dependent on β -amyloidosis initially.

There are important caveats to our claim that the sNAP and preclinAD stages 2 + 3 groups do not differ in any features except β -amyloidosis and carriage of the APOE $\epsilon 4$ genotype. First, our subjects are elderly, and in younger persons, the relationships might be different. Second, we lacked power to rule out modest differences with many features. Small significant differences between sNAP and preclinical AD stages 2 + 3 may not diminish the claim of similarity, however. Third, it is possible that some as-yet-unknown brain injury biomarker would reveal large differences between the sNAP group and the combined preclinAD stages 2 + 3 that our current battery of features failed to detect. This is a more

significant concern, and one whose likelihood we cannot predict. Fourth, our use of amyloid imaging as the proxy for β -amyloid-related molecular changes might be less sensitive than cerebrospinal fluid β -amyloid₄₂ [24] and thus, the absence of imaging evidence does not imply an absence of pathological β -amyloidosis.

Prior to these analyses, we had hypothesized that the brain injury biomarker abnormalities in preclinAD stages 2 + 3 and sNAP arose from different pathophysiologies, and as a corollary that in the preclinAD pathway that the presence of β -amyloidosis had a unique impact on the initiation and development of brain injury. However, that hypothesis was not supported by observation. Our observations suggest that a modification to our model of AD pathophysiology [25] is needed, one that acknowledges that non- β -amyloid mechanisms may play a role in the development of brain injury that might be relevant to the development of AD dementia in cognitively normal persons. Our claims here represent conjecture based on cross-sectional data; ultimately, decades of longitudinal observation may be needed to clarify the true sequence of events.

The hypothesis that brain injury changes occur that are independent of β -amyloidosis but still relevant to the pathogenesis of AD is not without precedent. Tauopathy that has been linked by some authors to AD in brainstem nuclei and the medial temporal lobe [26, 27], appears to precede β -amyloidosis. Other non- β -amyloid related tauopathies [28–30], cerebrovascular disease [31, 32], α -synucleinopathy, or TDP43 proteinopathy [33, 34] are also possible bases for β -amyloid-independent brain injury. Several of these processes may become symptomatic during life and some might not [35]. Only some of these alternative pathophysiologies might be relevant for AD pathogenesis.

A key observation is that the joint presence of brain injury and β -amyloidosis appears to be necessary for the development of overt cognitive impairment in what becomes AD dementia, consistent with our own observations [3] and those of others [36–41]. Furthermore, because AD dementia is associated with more rapid brain atrophy [42, 43] and glucose hypometabolism [13, 44, 45] than MCI due to AD or preclinical AD in cognitively normal persons, it is possible that once certain levels of brain injury and β -amyloidosis are reached, possibly independently initially, an interaction of the two occurs and acceleration of brain injury results. That would account for the excess of brain injury biomarker changes in participants with PiB SUVR ≥ 1.5 .

The strengths of our study include the population-based origin of our participants, their well-characterized clinical and imaging features, as well as the large sample size of 102 in the sNAP group. There are also a number of caveats that we have previously described about how we operationalized the cutpoints in the analyses [2, 3]. We used a cutpoint for SUVR for PiB PET of 1.5. Some ($n=22$, 22%) of the participants in the sNAP group had PiB SUVR values between 1.4 and 1.5, some of whom may actually be in the AD pathophysiological pathway due to β -amyloidosis. We may have misclassified them because our cutpoint for “abnormal” was too stringent [46]. Although we grouped participants according to degree of hippocampal atrophy and glucose hypometabolism in AD signature regions, this manner of grouping did not necessarily constrain the range of the investigational features listed in Tables 3 to 5 to compare preclin AD stages and the sNAP group. Different choices for operationalizing neurodegeneration biomarkers could alter the composition of participants assigned to the sNAP group and preclinAD stages 2–3. Finally, levels of biomarkers are dynamic. Longitudinal observations may change relationships in unexpected ways.

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Abbreviations

APOE	apolipoprotein E
AD	Alzheimer's disease
FDG PET	¹⁸ fluorodeoxyglucose positron emission tomography
GM	grey matter
IQR	interquartile range
MR	magnetic resonance imaging
NIA-AA	National Institute on Aging/Alzheimer's Association
PiB PET	Pittsburgh compound B positron emission tomography
preclinAD	preclinical AD as defined by NIA-AA criteria
sNAP	suspected non-AD pathway
ROI	region of interest
SUVr	standardized uptake value ratio
TIV	total intracranial volume
UPDRS	Unified Parkinson's Disease Rating Scale
WMH	white matter hyperintensities

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Table 1

Comparison of Imaging Study Participants to all MCSA Participants

Characteristic	All MCSA CN Participants (n = 2399)	Study Participants (n = 430)
Age, years, median (IQR)	78 (74, 83)	78 (75, 83)
Female gender, no. (%)	1195 (50)	194 (45)
Education, years, median (IQR)	13 (12, 16)	14 (12, 16)
APOE ε4 positive no. (%)	586 (25)	119 (28)
Mini-Mental State Examination score, median (IQR)	28 (27, 29)	28 (27, 29)
Cognitive Z-scores, median (IQR)		
Global	0.12 (−0.54, 0.81)	0.51 (−0.12, 1.17)
Memory	0.03 (−0.71, 0.77)	0.51 (−0.27, 1.26)
Language	0.13 (−0.53, 0.72)	0.34 (−0.31, 0.96)
Executive	0.21 (−0.49, 0.77)	0.46 (−0.27, 1.05)
Visuospatial	0.12 (−0.60, 0.73)	0.50 (−0.15, 1.10)

APOE = apolipoprotein E; IQR = interquartile range

Table 2

Descriptive Characteristics of Participants according to Stage or Group

Characteristic	Stage 0 (n = 191)	Stage 1 (n = 68)	Stage 2 (n = 56)	Stage 3 (n = 13)	sNAP (n = 102)
Age, years, median (IQR)	76 (73, 79) [*] †	80 (75, 82)	80 (78, 82)	82 (80, 84)	80 (76, 84)
Women, no. (%)	94 (49)	34 (50)	19 (34)	6 (46)	41 (40)
Education, years, median (IQR)	13 (12, 16)	14 (12, 16)	15 (12, 18)	13 (12, 15)	14 (12, 16)
APOE ε4 carrier, no. (%)	43 (23) [†]	30 (44) [*]	25 (45) [*]	7 (54) [*]	14 (14) [†]
MMSE, median (IQR)	29 (28, 29) [†]	28 (27, 29)	28 (27, 29)	27 (27, 28) [*]	28 (27, 29)
Cognitive Z-scores, median (IQR)					
Global	0.84 (0.22, 1.40) [*] †	0.38 (−0.12, 1.29) [*] †	0.35 (−0.26, 0.80)	−1.22 (−1.32, −0.99) [*]	0.13 (−0.52, 0.96)
Memory	0.78 (0.01, 1.42) [*] †	0.63 (−0.37, 1.31) [†]	0.37 (−0.48, 0.93)	−1.23 (−1.36, −0.75) [*]	0.26 (−0.39, 1.02)
Language	0.58 (0.01, 1.11) [*] †	0.36 (−0.25, 0.79)	0.16 (−0.35, 0.72)	−0.90 (−1.12, −0.15) [*]	0.09 (−0.50, 0.85)
Executive	0.69 (0.13, 1.18) [*] †	0.42 (−0.17, 1.26) [†]	0.15 (−0.38, 0.79)	−0.64 (−0.98, −0.29) [*]	0.04 (−0.60, 0.93)
Visuospatial	0.73 (0.10, 1.27) [*] †	0.56 (0.00, 1.25) [*]	0.53 (−0.16, 0.96)	−1.43 (−1.71, −0.92) [*]	0.20 (−0.51, 0.74)
PIB global cortical ratio	1.33 (1.29, 1.38) [†]	1.86 (1.66, 2.07) [*]	1.93 (1.67, 2.26) [*]	2.35 (1.91, 2.81) [*]	1.35 (1.31, 1.39) [†]
AD composite FDG ratio	1.46 (1.40, 1.53) [*] †	1.42 (1.38, 1.48) [*] †	1.28 (1.21, 1.32)	1.23 (1.15, 1.27)	1.28 (1.24, 1.33)
Hippocampal volume, adjusted for TIV	0.47 (−0.01, 0.92) [*] †	0.28 (−0.12, 0.75) [*] †	−0.68 (−1.02, 0.10)	−0.87 (−1.17, −0.52)	−0.62 (−1.06, −0.14)

sNAP= suspected non-Alzheimer pathway; IQR = interquartile range; MMSE = Mini-mental State examination; PIB = Pittsburgh compound B; FDG = fluorodeoxyglucose; TIV = total intracranial volume

^{*} differs from sNAP group, p<.01;[†] differs from predinAD stages 2+3 combined, p<.01

Table 3

Cerebrovascular features of Participants according to Stage or Group

Characteristic	Stage 0 (n = 191)	Stage 1 (n = 68)	Stage 2 (n = 56)	Stage 3 (n = 13)	SNAP (n = 102)
WMH Fractional Volume, median (IQR)	0.017 (0.012, 0.026) ^{*,†}	0.019 (0.011, 0.028) ^{*,†}	0.030 (0.014, 0.047)	0.029 (0.017, 0.043)	0.022 (0.016, 0.035)
Cortical Infarctions					
Present, no. (%)	6 (3)	3 (4)	4 (7)	1 (8)	10 (10)
1	3 (2)	2 (3)	4 (7)	1 (8)	6 (6)
2	2 (1)	0 (0)	0 (0)	0 (0)	2 (2)
3+	1 (1)	1 (1)	0 (0)	0 (0)	2 (2)
Supratentorial subcortical Infarctions					
Present, no. (%)	32 (17)	9 (13)	11 (20)	3 (23)	18 (18)
1	25 (13)	7 (10)	6 (11)	1 (8)	8 (8)
2	5 (3)	1 (1)	4 (7)	1 (8)	6 (6)
3+	2 (1)	1 (1)	1 (2)	1 (8)	4 (4)

IQR = interquartile range; WMH = white matter hyperintensities

^{*} differs from sNAP group, p<.01;[†] differs from preclinAD stages 2+3 combined, p<.01

Table 4

Cardiovascular Risk Factors of Participants according to Stage or Group

Characteristic	Stage 0 (n = 191)	Stage 1 (n = 68)	Stage 2 (n = 56)	Stage 3 (n = 13)	SNAP (n = 102)
Self-reported cardiovascular risk factors *					
Diabetes	24 (13) ^{*†}	13 (19)	14 (25)	4 (31)	33 (32)
Hypertension	109 (57) *	46 (68)	38 (68)	10 (77)	77 (75)
Smoking	91 (48)	26 (38)	24 (43)	6 (46)	52 (51)
Stroke	10 (5)	4 (6)	7 (12)	1 (8)	10 (10)
Myocardial infarction	19 (10)	10 (15)	8 (14)	2 (15)	16 (16)
Coronary Bypass Surgery	6 (3) *	9 (13)	3 (5)	3 (23)	11 (11)
Angioplasty	23 (12)	12 (18)	7 (12)	2 (17)	13 (13)
Congestive heart failure	4 (2)	2 (3)	1 (2)	0 (0)	5 (5)
Atrial fibrillation	15 (8) *	9 (13)	10 (18)	2 (15)	21 (21)
Angina	26 (14)	10 (15)	6 (11)	1 (8)	22 (22)
Coronary artery disease	38 (20)	21 (31)	14 (25)	6 (46)	30 (29)

* differs from SNAP group, $p < .01$;[†] differs from preclinAD stages 2+3 combined, $p < .01$

Table 5Features associated with α -synucleinopathy of Participants according to Stage or Group

Characteristic	Stage 0 (n = 191)	Stage 1 (n = 68)	Stage 2 (n = 56)	Stage 3 (n = 13)	SNAP (n = 102)
UPDRS Parkinsonism Total, median (IQR)	0.0 (0.0, 0.0) *	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	1.0 (0.0, 2.5)	0.0 (0.0, 2.0)
UPDRS Parkinsonism $\geq$ 1, no. (%)	34 (18) *	16 (24)	13 (24)	6 (55)	36 (37)
Gait speed (seconds to walk 25ft), median (IQR)	7 (6, 8)	7 (6, 8)	7 (6, 8)	8 (7, 9)	7 (6, 8)
Midbrain GM Volume (% of TIV), median (IQR)	0.0043 (0.0033, 0.0056)	0.0042 (0.0034, 0.0051)	0.0044 (0.0032, 0.0056)	0.0055 (0.0037, 0.0079)	0.0047 (0.0037, 0.0059)
Occipital FDG PET Ratio, median (IQR)	1.58 (1.51, 1.65) [†]	1.57 (1.47, 1.63) [†]	1.46 (1.39, 1.55)	1.51 (1.44, 1.54)	1.47 (1.40, 1.56)
Posterior Cingulate/(Precuneus+Occipital) FDG PET Ratio, median (IQR)	1.09 (1.03, 1.13)	1.07 (1.02, 1.13)	1.09 (1.02, 1.13)	1.07 (1.01, 1.10)	1.07 (1.03, 1.13)

IQR = interquartile range; UPDRS = Unified Parkinson's Disease Rating Scale; GM = grey matter; TIV = total intracranial volume; FDG PET = ¹⁸fluorodeoxyglucose positron emission tomography* differs from sNAP group, $p < .01$;[†] differs from preclinAD stages 2+3 combined, $p < .01$