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Mild to Moderate Alzheimer Dementia with Insufficient Neuropathological Changes

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Abstract

Recently, ~16% of participants in an anti-A β passive immunotherapy trial for mild-to-moderate Alzheimer disease (AD) had a negative baseline amyloid positron emission tomography (PET) scan. Whether they have AD or are AD clinical phenocopies remains unknown. We examined the 2005-2013 National Alzheimer's Coordinating Center autopsy database and found that ~14% of autopsied subjects clinically diagnosed with mild-to-moderate probable AD have no or sparse neuritic plaques, which would expectedly yield a negative amyloid PET scan. More than half of these "A β -negative" subjects have low neurofibrillary tangle Braak stages. These findings support the implementation of a positive amyloid biomarker as an inclusion criterion in future anti-A β drug trials.

Amyloid positron emission tomography (PET) imaging is a valuable biomarker in clinical trials of anti-A β passive immunotherapy in mild-to-moderate Alzheimer disease (AD) patients,^{1,2} and to diagnose AD in the clinical setting.³ The results of the largest amyloid PET imaging substudy of a clinical trial were recently communicated.^{4,5} Remarkably, although all had a clinical diagnosis of mild-to-moderate probable AD, 30 of 184 (16.3%)

participants had a negative baseline amyloid PET scan. Interestingly, 22 of 61 (36.1%) *APOEε4* non-carriers were amyloid PET negative, whereas only 8 of 123 (6.5%) *APOEε4* carriers were amyloid PET negative, and 1 of these 8 was homozygous for the *APOEε4* allele.

Because of its potential adverse impact on the design of ongoing and future clinical trials for AD, it is imperative to recognize and characterize this subset of “amyloid PET-negative mild-to-moderate AD dementia subjects.” To explore the clinical and neuropathologic characteristics of subjects in this category, we examined the 2005-2013 autopsy cohort of the National Alzheimer's Coordinating Center (NACC) database, a large multicenter longitudinal cohort study of aging involving 35 past and present AD centers across the United States.⁶ We selected all subjects with a clinical diagnosis of probable AD (PRAD) and a last Mini-Mental State Examination (MMSE) score between 16 and 26 within 2 years of death, and excluded subjects with a non-AD primary neuropathological diagnosis. Based on published amyloid PET—postmortem correlations,^{7,10} we approximated the cutoffs for amyloid PET imaging sensitivity and classified as amyloid positive ($A\beta^{+}$) those subjects with moderate or frequent neuritic plaques, and as amyloid negative ($A\beta^{-}$) those with no or sparse neuritic plaques according to Consortium to Establish a Registry for Alzheimer's Disease (CERAD) score,¹¹ appreciating that the CERAD score is of neuritic plaques and therefore not identical to a determination of amyloid positivity. We investigated the demographic, clinical, and neuropathological characteristics of $A\beta^{+}$ and $A\beta^{-}$ subjects.

Subjects and Methods

Eligibility Criteria

To approximate the clinical characteristics of anti- $A\beta$ immunotherapy clinical trials, subjects were selected from the 2005–2013 NACC autopsy cohort if they met the following inclusion criteria: (1) age of death $\geq$ 50 years; (2) last clinical evaluation (including MMSE) within 2 years before death; (3) last MMSE score between 16 and 26, inclusive; (4) clinical diagnosis of PRAD; and (5) if any primary neuropathological diagnosis was present, this had to be AD, although meeting the neuropathological criteria for AD¹² was not required. To maximize the correlation between a clinical diagnosis of PRAD and AD neuropathological changes, the following conservative exclusion criteria were implemented: (1) a primary neuropathological diagnosis other than AD (eg, frontotemporal lobar degeneration, dementia with Lewy bodies, hippocampal sclerosis, vascular dementia, prion disease, Parkinson disease, Huntington disease, hypoxia, ischemia, hemorrhage); and (2) cognitive impairment attributable to alcohol use, depression, medication use, or medical illness.

Data Collection

Demographic characteristics included sex, age at death, education, and *APOE* genotype. Clinical variables included age of onset, disease duration, Unified Parkinson Disease Rating Scale, MMSE score, Clinical Dementia Rating Scale Sum of Boxes (CDR-SOB) score, and part B of the Trail Making Test. Neuropathological data included CERAD score of neuritic plaques,¹¹ Braak stage of neurofibrillary tangles,¹³ presence of any vascular pathology, presence and severity of cerebral amyloid angiopathy (none, mild, moderate, severe),

atherosclerosis (ie, circle of Willis, none, mild, moderate, severe), and arteriolosclerosis (none, mild, moderate, severe), and presence of 1 lacunar infarcts, 1 cortical microinfarcts, 1 large infarcts, subcortical arteriosclerotic leukoencephalopathy, cortical laminar necrosis, 1 brain hemorrhages, hippocampal sclerosis, and incidental Lewy bodies (in any brain region, brainstem, limbic system, neocortex, unspecified).

Statistical Analyses

Statistical analyses were performed with Prism version 5.0 for Mac (GraphPad, La Jolla, CA). Normality of continuous variables in the data set was evaluated with the D'Agostino–Pearson omnibus test. For continuous variables, pairwise comparisons between $A\beta^-$ and $A\beta^+$ subjects were performed with the Mann-Whitney U test or unpaired Student t test, as appropriate. For categorical variables, comparisons of proportions between $A\beta^-$ and $A\beta^+$ subjects were done using Fisher exact test. All the hypothesis tests were 2-sided, and a p value of <0.05 was considered to be statistically significant.

Results

F1 The flowchart in the Fig 1 depicts the selection process based on the eligibility criteria. A total of 161 subjects were eligible for this study. The Table shows the demographic, clinical, and neuropathological characteristics of the subjects. Groups were comparable regarding sex, education, age, and disease duration from symptom onset. Of note, compared to $A\beta^+$ subjects, $A\beta^-$ subjects had slightly but significantly better cognitive function, as indicated by antemortem MMSE and CDR-SOB scores. A trend was also observed with part B of the Trail Making Test, a measure of executive function.

Regarding the neuropathological findings, more than half of the $A\beta^-$ subjects (13 of 22, 59.1%) had a Braak stage 0/I/II, indicating that many $A\beta^-$ subjects with dementia do not have the neuropathological signs of AD. We reasoned that other concomitant pathologies may account for the cognitive decline of these individuals.^{14,15} However, $A\beta^-$ and $A\beta^+$ subjects did not differ in the frequency of concurrent moderate/severe cerebral amyloid angiopathy (CAA), arteriolosclerosis, or atherosclerosis, nor in the frequency of coexisting lacunar infarcts, large infarcts, cortical microinfarcts, subcortical arteriosclerotic leukoencephalopathy, cortical laminar necrosis, cerebral hemorrhages, or hippocampal sclerosis. If anything, there was a trend toward a significantly higher frequency of “any vascular pathology” in the $A\beta^+$ group. Last, Lewy body pathology was more frequent in $A\beta^+$ subjects than in $A\beta^-$ subjects.

The *APOE* genotype was available for 137 of the 161 eligible subjects. The *APOE* $\epsilon 4$ genotype was overrepresented within the $A\beta^+$ group compared to the $A\beta^-$ group (see Table). *APOE* $\epsilon 4$ noncarriers were twice as likely to be $A\beta^-$ than *APOE* $\epsilon 4$ carriers (12 of 70 [17.1%] vs 6 of 67 [8.9%], odds ratio = 2.1; 95% confidence interval = 0.7–6.0), although these differences did not reach statistical significance in this relatively small autopsy sample.

Discussion

The recent finding of a higher than expected proportion (16%) of amyloid PET-negative subjects who participated in a large multicenter phase 3 anti- $A\beta$ passive immunotherapy trial has raised concerns about the design of this and similar clinical trials.^{4,5} Do these individuals really have AD? If not, should participation in subsequent trials require demonstration of a positive amyloid biomarker?

We interrogated the 2005–2013 NACC autopsy database and selected a convenience sample of subjects with a clinical diagnosis of PRAD (MMSE = 16–26) excluding those with a primary clinical or neuropathological diagnosis of a disease other than AD and those with cognitive dysfunction attributed to medications, alcohol, medical conditions, or depression. Thus, these were individuals who otherwise would qualify for and may have been enrolled in clinical trials of potentially disease-modifying anti- $A\beta$ drugs. Surprisingly, we discovered that a not negligible proportion (22 of 161, $\approx 14\%$) of these subjects actually have no or only sparse neuritic plaques, which is not sufficient to meet the neuropathological diagnostic criteria for AD.^{12,16} Had we not excluded individuals with a clinical diagnosis of AD but a non-AD pathological diagnosis, the percentage of individuals without substantial $A\beta$ deposits would have been even higher, similar to an earlier report by Beach et al.¹⁴ Importantly, extrapolating the published studies correlating amyloid PET radiotracer uptake and postmortem plaque burden,^{7–10} these subjects would have had a negative amyloid PET scan in vivo, referred to in this study as $A\beta^-$. Although 2 of these 22 individuals had severe CAA and this could be associated with a positive amyloid PET scan,¹⁷ the proportion of $A\beta^-$ subjects observed in this study is strikingly similar to the 16% recently observed in clinical trials of anti- $A\beta$ passive immunotherapy^{4,5} and to that reported in the validation studies of some of the amyloid PET radiotracers.^{18–20}

We next asked whether this singular group of $A\beta^-$ subjects could be differentiated from $A\beta^+$ subjects on the basis of demographic, clinical, or neuropathological signatures. We found that: (1) both groups have similar gender, education level, and age of death and symptom onset; (2) *APOE ϵ 4* carriers were half as likely to be $A\beta^-$ as noncarriers; (3) although meeting criteria for mild-to-moderate dementia, the $A\beta^-$ subjects were slightly less impaired than $A\beta^+$ subjects; (4) more than half of the $A\beta^-$ subjects had a Braak stage 0/I/II of neurofibrillary tangles, which is clearly insufficient to account for their mild-to-moderate dementia; but (5) frequency and severity of concurrent incidental vascular and Lewy body pathologies did not differ between $A\beta^-$ and $A\beta^+$ subjects; therefore, these known pathologies—although acknowledging the limitations of NACC scoring—do not appear to account for cognitive loss among the $A\beta^-$ subjects.

In summary, our results document that a sizeable number of individuals clinically diagnosed with PRAD at a mild-to-moderate dementia stage in specialized AD centers have insufficient plaques and tangles to meet established neuropathological guidelines for AD. The pathological substrate of these clinical phenocopies of AD remains elusive, and further investigation in other cohorts may help elucidate their distinct clinical or neuropathological features. Our findings support the implementation of a baseline amyloid PET radiotracer

uptake above a prespecified cutoff and/or a positive cerebrospinal fluid AD biomarker as inclusion criteria in future clinical trials with anti- $A\beta$ therapies.

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Figure 1.

Flowchart of the selection algorithm of the study subjects from the original 2005-2013 National Alzheimer's Coordinating Center (NACC) autopsy cohort. The final sample size of this study is 161. The number of subjects excluded and the reasons for their exclusion are shown on the right side of the flowchart. bvFTD = behavioral variant frontotemporal dementia; CBD = corticobasal degeneration; DLB = dementia with Lewy bodies; HD = Huntington disease; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; NP Dx = neuropathological diagnosis; PD = Parkinson disease; POAD = possible Alzheimer disease; PosVaD = possible vascular dementia; PPA = primary progressive aphasia; PRAD = probable Alzheimer disease; Prob-VaD = probable vascular dementia; PSP = progressive supranuclear palsy; SCA = spinocerebellar ataxia.

Table 1
Comparison between A β ⁺ and A β ⁻ Subjects with Mild-to-Moderate (MMSE = 16-26)
Probable Alzheimer Disease

Characteristic	A β ⁺ , n = 139	A β ⁻ , n = 22	P
Demographic characteristics			
Sex, No. (%) female	80 (57.5)	15 (68.2)	0.485
Education, yr	15.3 $\pm$ 3.2	14.4 $\pm$ 2.9	0.272
Age at death, yr	84.4 $\pm$ 7.7	86.3 $\pm$ 6.7	0.267
Age at onset, yr	76.4 $\pm$ 8.6	78.7 $\pm$ 6.4	0.273
Disease duration, yr	8.0 $\pm$ 3.8	7.5 $\pm$ 3.9	0.708
Cognitive performance			
MMSE	20.0 [17.0–22.0] ^a	22.0 [19.7–23.2] ^a	0.021 ^a
CDR-SOB	9.0 [6.0–12.0] ^a	6.0 [5.0–9.25] ^a	0.023 ^a
TMT-B, s	245 $\pm$ 71 ^b	205 $\pm$ 86 ^b	0.074 ^b
Motor performance, UPDRS	11.0 $\pm$ 7.8	10.1 $\pm$ 9.7	0.411
<i>APOE</i> genotype			
<i>APOE</i> ϵ 4 carriers, No. (%)	61/119 (51.3)	6/18 (33.3)	0.208
<i>APOE</i> ϵ 4 alleles, No. (%)	69/238 (29.0)	7/36 (19.4)	0.318
Neuropathology, No. (%)			
Braak stage 0/I/II	5 (3.6) ^a	13 (59.1) ^a	<0.0001 ^a
Braak stage III/IV	49 (35.2) ^a	5 (22.7) ^a	
Braak stage V/VI	85 (61.1) ^a	4 (18.2) ^a	
Any vascular pathology present	113 (81.3) ^b	14 (63.6) ^b	0.088 ^b
Moderate/severe CAA	45/134 (33.6)	4/22 (18.2)	0.215
Moderate/severe arteriosclerosis	37/109 (33.9)	6/20 (33.3)	0.802
Moderate/severe atherosclerosis	61 (43.9)	8 (36.4)	0.644
Large artery infarct	8/130 (5.8)	2/22 (9.1)	0.629
Cortical microinfarcts	23/138 (16.7)	4/22 (18.2)	0.768
Lacunar infarcts	19/138 (13.8)	4/22 (18.2)	0.527
Subcortical arteriosclerotic leukoencephalopathy	15/137 (10.9)	2/22 (9.1)	1.000
Cortical laminar necrosis	1/136 (0.7)	0/21 (0.0)	1.000
Hippocampal sclerosis	13/136 (9.6)	4/21 (19.0)	0.249
Hemorrhage	7 (5.0)	0 (0.0)	0.595
Lewy bodies any region	34 (24.5) ^a	1 (4.5) ^a	0.048 ^a
Lewy bodies neocortex	8 (5.7)	0 (0.0)	0.103
Lewy bodies limbic	17 (12.2)	0 (0.0)	
Lewy bodies brainstem	4 (2.9)	1 (4.5)	
Lewy bodies unspecified	5 (3.6)	0 (0.0)	

Education, age of death, age of onset, disease duration, and TMT-B are presented as mean $\pm$ standard deviation. MMSE and CDR-SOB scores are presented as median [interquartile range]. A higher MMSE score and a lower CDR-SOB score indicate better cognitive performance. Denominators vary due to missing data.

^aStatistically significant difference ($p < 0.05$).

^bStatistical trend ($p < 0.1$).

$A\beta^+$ = CERAD score of moderate or frequent neuritic plaques; $A\beta^-$ = CERAD score of none or sparse neuritic plaques; MMSE = Mini-Mental State Examination; CDR-SOB = Clinical Dementia Rating Scale Sum of Boxes; CERAD = Consortium to Establish a Registry for Alzheimer's Disease; TMT-B = part B of Trail Making Test; UPDRS = Unified Parkinson Disease Rating Scale; CAA = cerebral amyloid angiopathy.