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Association of *APOE* with tau-tangle pathology with and without β-amyloid

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ABSTRACT

This study tested the hypothesis that the association of apolipoprotein E (*APOE*) with paired helical filament tau (PHF-tau) tangle pathology differs in brains with and without β-amyloid. Participants were 1056 autopsied individuals from 2 clinical-pathologic cohort studies of aging and Alzheimer's disease (AD), the Religious Orders Study, and the Rush Memory and Aging Project. Neuropathologic measures were obtained using immunohistochemistry targeting β-amyloid and PHF-tau tangles in 8 brain regions. Linear regression was used to compare the relation of *APOE* ε4 and ε2 to PHF-tau-tangle density in persons with β-amyloid relative to persons without β-amyloid. We found an interaction between *APOE* ε4 carriers and presence of β-amyloid ($\beta = -0.968$, $p = 0.013$) such that the association of *APOE* ε4 with PHF-tau tangles was much stronger in brains with β-amyloid. Stratified analysis shows that the association of *APOE* ε4 with PHF-tau tangles was considerably stronger among those with β-amyloid ($\beta = 0.757$, $p = 1.1 \times 10^{-15}$) compared to those without β-amyloid which was not significant ($\beta = -0.201$, $p = 0.424$). Separately, *APOE* ε2 was associated with fewer tangles in brains with β-amyloid ($\beta = -0.425$, $p = 7.6 \times 10^{-4}$) compared to those without β-amyloid which was not significant ($\beta = -0.102$, $p = 0.506$). Thus, the presence of *APOE* ε4 and ε2 alleles was not associated with PHF-tau tangles in the absence of β-amyloid. The data provide additional evidence that PHF-tau tangles in the absence of β-amyloid may reflect a pathologic process distinct from Alzheimer's disease.

1. Introduction

The criteria for the neuropathologic diagnosis of Alzheimer's disease (AD) recently published by the National Institute on Aging-Alzheimer's Association differs from previous consensus statements (Hyman et al., 2012). A major change is the requirement of β-amyloid deposits for the neuropathologic diagnosis of AD, in addition to neocortical neuritic plaques (NPs) and neurofibrillary tangles (NFTs). The requirement for β-amyloid deposits also differs from another model of pathologic AD which suggests that the appearance of NFT in the entorhinal cortex and hippocampus is the earliest neuropathologic manifestation of AD regardless of the presence of β-amyloid (Braak and Braak, 1991, 1995). Recent articles referred to the condition of tangles in the absence of amyloid as "Primary age-related tauopathy" (PART) (Crary et al., 2014; Jellinger et al., 2015). Thus, the extent to which tangle pathology in the absence of β-amyloid represents AD or a separate process remains controversial.

The apolipoprotein E (*APOE*) polymorphism is the most robust genetic risk factor for AD dementia (Corder et al., 1993; Farrer et al., 1997). Substantial evidence suggests that *APOE* affects clinical AD in large part through β-amyloid metabolism and triggers a cascade of events that ultimately results in NFT formation or propagation and

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cognitive impairment (Polvikoski et al., 1995; Royall et al., 2012; Schmechel et al., 1993). The β-amyloid cascade hypothesis provides a biological background that can explain in part the relationship between *APOE*, β-amyloid, and NFT (Jack et al., 2010). This raises the possibility that NFT in the absence of β-amyloid represents a pathologic process distinct from AD. In prior studies, we found that β-amyloid mediated the association of *APOE* with paired helical filaments tau (PHFs-tau) and subsequent cognitive decline and clinical AD (Yu et al., 2014). We also reported separate effects of age on mesial temporal lobe tangles consistent with a 2-process model of mesial temporal lobe tangles, the site of most tangles in the absence of β-amyloid (Mungas et al., 2014). In this article, we extend our prior work by explicitly examining the association of *APOE* ε4 and ε2 with tangles in persons with and without β-amyloid. Finding robust associations of *APOE* with tangles in persons with β-amyloid but not in those without β-amyloid would provide additional evidence that tangles in the absence of β-amyloid represent a process distinct from AD.

2. Methods

2.1. Participants

Participants came from the Religious Orders Study (ROS) and the Rush Memory and Aging Project (MAP), both are community-based, clinical-pathologic cohort studies investigating AD and other chronic conditions of aging. Participants signed consent for annual clinical evaluations and an Anatomic Gift Act agreeing to brain donation at the time of death. Both studies enroll individuals free of known dementia. They were approved by the institutional review board of Rush University Medical Center. ROS, started in 1994, enrolls older Catholic religious clergy across the United States. MAP, started in 1997, enrolls older residents from retirement facilities and senior and subsidized housing in the Chicago metropolitan area. The follow-up rate exceeds 90%, and the autopsy rate exceeds 85%. More detailed information on study design and pathologic data collection of both ROS and MAP can be found in previous publications (Bennett et al., 2012a, 2012b).

At time of this analysis, 1385 of 3043 participants enrolled had died. Of the deceased, 1373 remained in the study, and 1198 (87.3%) were autopsied. Of these, the postmortem assessment was complete for 1092 persons, and *APOE* genotype was available on 1056 (96.7%). The average age at death was 88.2 years (standard deviation of 6.6 years, range 65.9–108.3 years); and 677 (64.1%) were female. ROS and MAP cohorts are convenience samples, and death is a form of informative censoring. We and others have reported that dementia is strongly associated with risk of death, and thus, the deceased group is enriched with persons with dementia and more neuropathologic burden. (Aguero-Torres et al., 1999; James et al., 2014; Rait et al., 2010; Tschanz et al., 2004). To investigate a possible bias resulting from an association between PART and mortality, we explored the distribution of PART in a subset of persons enrolled early in the study and compared to the distribution of PART in the entire cohort. Among the 201 persons enrolled in the Religious Orders Study from 1994 to 1997 aged 80 years and older at enrollment, 196 (98%) are deceased, and 181 (90%) autopsied. Among this subset, the distribution of PART (13.2%) is similar to what is seen in the entire cohort (14.4%) suggesting that PART is not likely to be associated with selective mortality relative to other pathologies.

2.2. APOE *genotyping*

DNA was extracted from white blood cells collected from participants or frozen brain tissue after death. *APOE* genotyping was performed by sequencing the codon 112 and codon 158 of exon 4 of the *APOE* gene (Boyle et al., 2010). Individuals with at least 1 copy of the ε4 were considered as ε4 carriers. Individuals with at least 1 copy of the ε2 were considered as ε2 carriers. Thus, in these analyses, ε2 or ε4 was used in both sets of models.

2.3. Neuropathology procedures

Brain removal and processing followed a standard protocol. One hemisphere was cut coronally into 1-cm slabs and fixed in 4% paraformaldehyde. β-amyloid deposits and PHF-tau tangles were assessed in 20 μm sections. Immunohistochemistry and computer-assisted image analysis were used for β-amyloid (10D5, 1:600; Elan Pharmaceuticals, San Francisco, CA, USA; 6F/3D, 1:50; DAKO North America Inc, Carpinteria, CA, USA, or 4G8, 1:9000 Covance Labs, Madison, WI, USA), and stereology was used for PHF-tau tangles (AT8, 1:2000; Thermoscientific, Waltham, MA, USA) across 8 different brain regions including the entorhinal cortex, the hippocampus at CA1, superior frontal cortex (Broadmann area [BA] 6/8), midfrontal cortex (BA 46/9), inferior temporal cortex (BA 20), angular gyrus cortex (BA 39/40), cingulate gyrus (BA 32/33), and calcarine cortex (BA 17). β-amyloid load and the density of PHF-tau-tangle density was obtained by averaging the mean percentage area per region, across all regions as previously reported (Bennett et al., 2004). The absence of β-amyloid was characterized by a β-amyloid load of 0 in all the 8 immunostained sections. Neocortical type Lewy bodies (LBs) were identifyied by alpha-synuclein positive LB (LB509, 1:100; Invitrogen/Zymed, Carlsbad, CA, USA or pSyn, 1:20,000; Wako Chemicals, Richmond, VA, USA) in 1 or more neocortical regions (midfrontal, middle temporal, and inferior parietal) (Schneider et al., 2012). NPs and NFTs were identified with modified Bielschowsky stain in 6 μm sections in 5 different regions (entorhinal cortex, hippocampus at CA1, midfrontal cortex, middle temporal gyrus, and inferior parietal cortex). CERAD (Consortium to Establish a Registry for Alzheimer's Disease) criteria assessed NP burden and a Braak staging assessed the distribution and severity of NFT pathology (Braak and Braak, 1991; Mirra et al., 1991) The diagnosis of pathologic AD was based on the National Institute on Aging (NIA)–Reagan criteria, 1997. Macroscopic infarcts were recorded from fixed slabs and confirmed by histology. Microscopic infarcts were documented in at least 9 different sections stained with hematoxylin and eosin as previously reported (Schneider et al., 2005). Severe neuronal cell loss and gliosis in the hippocampus on H&E was identified as hippocampal sclerosis (Nag et al., 2015).

2.4. Other variables

Age was calculated from birth data and date of death. Sex was self-reported. We used the Mini–Mental State Examination (MMSE) score proximate to death (Bennett et al., 2012a, 2012b; Folstein et al., 1975).

2.5. Statistical analysis

First, we examined the frequencies of *APOE* ε4 and ε2 in 3 groups: those without β-amyloid and without pathologic diagnosis of AD based on NIA-Reagan criteria, presence of β-amyloid but without pathologic AD, and a group with β-amyloid deposits and with pathologic AD. Next, we examined differences in the association of *APOE* ε4 with PHF-tau-tangle density in persons with and without β-amyloid. To do so, we fit a linear regression model with an interaction term between *APOE* ε4 and β-amyloid status, adjusted for age and sex. Because the variances of PHF-tau-tangle distribution differed between those with and without β-amyloid, and this might influence the model estimation, we also conducted stratified analyses regressing *APOE* ε4 status on PHF-tau tangles in