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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

persons with and without β-amyloid. Similar analyses were carried out for *APOE* ε2 genotype. Additional analyses controlled for common comorbid brain pathology including neocortical LBs, macro and microscopic infarcts, and hippocampal sclerosis. To examine the possible confounding effect of the temporal spatial differences in β-amyloid and PHF-tau-tangle distributions on disease severity, we repeated the analyses controlling for the MMSE. Searching for possible differences in the association of *APOE* and PHF-tau tangles in participants with low β-amyloid burden, we also conducted stratified analyses regressing *APOE* ε4 status on PHF-tau tangles for each of 3 groups: without β-amyloid and without pathologic AD, presence of β-amyloid but without pathologic AD, and presence of β-amyloid and pathologic AD, adjusted for age and sex. We conducted the same analyses for *APOE* ε2 genotype. To investigate whether our results differ by brain regions, we repeated the analysis by examining mesial temporal and neocortical PHF-tau-tangle pathologies separately.

The measures of PHF-tau-tangle density were right skewed; we therefore applied square root transformation before the analyses. A nominal threshold of $p < 0.05$ was used to determine significance. Analyses were performed using SAS/STAT software, version 9.3 (SAS Institute Inc, Cary, NC, USA).

3. Results

Demographic and neuropathologic characteristics of participants by β-amyloid and *APOE* ε4 status are summarized in Table 1. *APOE* ε4 was present in 29.5% of persons with β-amyloid and only 7.2% of persons without β-amyloid ($X^2 = 33.4$, degrees of freedom = 1, $p = 7.7 \times 10^{-9}$). The density of both neocortical and mesial temporal tangles was considerably higher in those with β-amyloid, when compared to those without β-amyloid (*P*s < 0.001 for both neocortical tangles and mesial temporal tangles). Table 2 summarizes the characteristics of participants by β-amyloid and *APOE* ε2 status. *APOE* ε2 was present in 25.7% of persons without β-amyloid and only 13.7% of persons with β-amyloid ($X^2 = 14.2$, degrees of freedom = 1, $p = 0.0002$).

The association of *APOE* ε4 with PHF-tau tangle density was first examined in a linear regression model with an interaction term between *APOE* ε4 status and the presence of β-amyloid, adjusting for age and sex (Table 3). In this model, the term for *APOE* ε4 refers to the association of the ε4 allele with tangle density in persons with β-amyloid; and the interaction term compares the ε4 association among persons without β-amyloid relative to the reference group of persons with β-amyloid. The estimate was negative and the interaction was significant ($p = 0.013$), suggesting that the association of the ε4 genotype with tangle density was lower among persons without β-amyloid. We next performed separate regression analysis for persons with (N = 904) and without β-amyloid (N = 152). In persons with β-amyloid, *APOE* ε4 was strongly associated with a higher burden of PHF-tau tangles ($\beta = 0.757$, $p = 1.1 \times 10^{-15}$). By contrast, we did not find an association among persons without β-amyloid ($\beta = -0.201$, $p = 0.424$). Assuming the effect size of *APOE* ε4 was similar to the group with β-amyloid, our current sample size in the group without β-amyloid (N = 152) would be sufficient to detect such an effect with 80% power at alpha level of 0.05. The differences in burdens of tangle pathology by *APOE* and β-amyloid status are illustrated in Fig. 1. We repeated the analyses controlling for macroscopic and microscopic infarctions, cortical LBs, and hippocampal sclerosis, and the findings were essentially unchanged (data not shown). Additional stratified analyses controlled for MMSE proximate to death. The findings were essentially the same. *APOE* ε4 remained strongly associated with a higher burden of PHF-tau tangles in persons with β-amyloid ($\beta = 0.455$, $p = 7.6 \times 10^{-8}$), but not among persons without β-amyloid ($\beta = -0.198$, $p = 0.431$). Similarly, *APOE* ε2 was still associated with fewer PHF-tau tangles in persons with β-amyloid ($\beta = -0.300$, $p = 6.2 \times 10^{-3}$) but not in persons without β-amyloid ($\beta = -0.105$, $p = 0.495$).

The associations of *APOE* ε4 with both mesial temporal tangle density and neocortical tangle density were, in general, consistent with the finding for tangle density averaged across brain regions. The ε4 by β-amyloid interaction was attenuated and not significant for mesial temporal tangles ($p = 0.134$) and marginally significant for neocortical tangles ($p = 0.050$). However, the results from stratified analyses (Supplementary Tables 1 and 2) show that the association of *APOE* ε4 with mesial temporal tangle pathology was highly significant in persons with β-amyloid ($\beta = 0.874$, $p = 2.7 \times 10^{-11}$) but not significant in those without β-amyloid ($\beta = -0.026$, $p = 0.963$). Similarly, the neocortical tangle density was highly significant among ε4 carriers in persons with β-amyloid ($\beta = 0.771$, $p = 2.7 \times 10^{-15}$), and not significant in persons without β-amyloid ($\beta = 0.009$, $p = 0.926$).

Next, we examined the association of *APOE* ε2 with tangle pathology by β-amyloid status. Our first model included an interaction term between *APOE* ε2 and β-amyloid status (Table 4). In this model, the term for *APOE* ε2 refers to the association of the ε2 allele with tangle density in persons with β-amyloid; and the interaction term compares the ε2 association among persons without β-amyloid relative to the reference group of persons with β-amyloid. The ε2 by β-amyloid interaction was not significant ($p = 0.264$). However, the results from stratified models suggest that the magnitude of the ε2 association is likely to differ among persons with and without β-amyloid. In these models, *APOE* ε2 was associated with fewer PHF-tau tangles in persons with β-amyloid ($\beta = -0.425$, $p = 7.6 \times 10^{-4}$). By contrast, we did not find an association in persons without β-amyloid ($\beta = -0.102$, $p = 0.506$). Power calculation suggests that it

Table 1
Demographic and neuropathologic characteristics of participants by β-amyloid and *APOE* ε4 status

Mean (standard deviation) or N (%)	Without β-amyloid and without AD		With β-amyloid but without AD		With β-amyloid and with AD	
	APOE ε4+	*APOE* ε4−	*APOE* ε4+	*APOE* ε4−	*APOE* ε4+	*APOE* ε4−
N	11 (7.2)	141 (92.8)	42 (17.1)	203 (82.9)	225 (34.1)	434 (65.9)
Age at death	87.3 (6.3)	86.0 (7.3)	85.5 (7.2)	86.7 (7.3)	87.8 (5.9)	90.3 (5.8)
Male sex	5 (45.5%)	60 (42.5%)	22 (52.4%)	79 (38.9%)	76 (33.8%)	137 (31.6%)
Mini–Mental State Examination	24.2 (5.2)	23.9 (7.5)	22.6 (9.6)	25.4 (5.7)	16.9 (10.0	19.8 (9.3)
β-amyloid load	0	0	2.4 (2.5)	1.3 (1.8)	6.6 (4.2)	5.7 (4.2)
PHF-tau-tangle density	2.3 (3.9)	2.3 (2.5)	3.0 (3.5)	2.1 (2.2)	11.2 (11.2)	7.3 (6.8)
Neocortical PHF-tau	0.17 (0.37)	0.17 (0.47)	0.5 (1.8)	0.2 (0.6)	6.7 (10.7)	3.3 (5.5)
Mesial temporal PHF-tau	13.8 (21.8)	9.5 (9.5)	10.8 (11.2)	8.1 (8.1)	25.6 (18.0)	20.3 (14.2)
Cortical Lewy bodies	0	11 (7.8%)	4 (9.5%)	14 (6.9%)	37 (16.4%)	54 (12.4%)
Macroinfarcts	5 (45.5%)	44 (31.2%)	18 (42.9%)	69 (34.0%)	88 (39.1%)	149 (34.3%)
Microinfarcts	4 (36.4%)	37 (26.2%)	10 (23.8%)	55 (27.1%)	63 (28.0%)	134 (30.9%)
Hippocampal sclerosis	1 (10%)	5 (3.6%)	2 (5.0%)	10 (5.0%)	29 (12.9%)	36 (8.4%)

Key: AD, Alzheimer's disease; APOE, apolipoprotein E; PHF-tau, paired helical filament tau.

Table 2
Demographic and neuropathologic characteristics of participants by β-amyloid and *APOE* ε2 status

Mean (standard deviation) or N (%)	Without β-amyloid and without AD		With β-amyloid but without AD		With β-amyloid and with AD	
	APOE ε2+	*APOE* ε2−	*APOE* ε2+	*APOE* ε2−	*APOE* ε2+	*APOE* ε2−
N	39 (25.7)	113 (74.3)	43 (17.6)	202 (82.4)	81 (12.3)	578 (87.7)
Age at death	88.4 (8.3)	85.3 (6.7)	87.1 (7.0)	86.3 (7.3)	90.7 (5.9)	89.2 (5.9)
Male sex	11 (28.2%)	54 (47.8%)	17 (39.5%)	84 (41.6%)	25 (30.9%)	188 (32.5%)
Mini–Mental State Examination	23.0 (8.9)	24.3 (6.9)	23.5 (7.9)	25.3 (6.2)	20.7 (8.7)	18.6 (9.7)
B-amyloid load	0	0	1.0 (1.5)	1.6 (2.1)	5.2 (3.8)	6.1 (4.2)
PHF-tau tangle density	2.4 (3.0)	2.3 (2.5)	2.7 (2.5)	2.2 (2.5)	5.9 (5.8)	9.0 (9.0)
Neocortical PHF-tau	0.12 (0.25)	0.19 (0.51)	0.3 (1.3)	0.3 (0.8)	2.0 (4.3)	4.8 (8.1)
Mesial temporal PHF-tau	10.7 (12.0)	9.5 (10.1)	10.0 (8.3)	8.3 (8.9)	17.8 (14.7)	22.7 (15.8)
Cortical Lewy bodies	1 (2.6%)	10 (8.9%)	8 (18.6%)	10 (5.0%)	4 (4.9%)	87 (15.1%)
Macroinfarcts	14 (35.9%)	35 (31.0%)	12 (27.9%)	75 (37.1%)	39 (48.2%)	198 (34.3%)
Microinfarcts	14 (35.9%)	27 (23.9%)	10 (23.3%)	55 (27.2%)	28 (34.6%)	169 (29.2%)
Hippocampal sclerosis	3 (8.1%)	3 (2.7%)	0	12 (6.1%)	8 (9.9%)	57 (9.9%)

Key: AD, Alzheimer's disease; APOE, apolipoprotein E; PHF-tau, paired helical filament tau.

would require a sample size ~4400 to detect ε2 by β-amyloid interaction at a nominal α level of 0.05. Further adjustment for macroscopic and microscopic infarctions, cortical LBs, and hippocampal sclerosis did not change the results (data not shown).

Similar results were found in separate analyses of ε2 on mesial temporal tangles and neocortical tangles. Briefly, we did not find significant ε2 by β-amyloid interactions on either tangle pathology. However, stratified regression models show that in persons with β-amyloid, the ε2 carriers had lower mesial temporal ($\beta = -0.479$, $p = 0.005$) and neocortical tangle densities ($\beta = -0.551$, $p = 2.3 \times 10^{-5}$). By contrast, both associations were greatly attenuated and were not significant in persons without β-amyloid (Supplementary Tables 1 and 2).

Finally, we conducted stratified analyses regressing *APOE* ε4 status on PHF-tau tangles in 3 groups: without β-amyloid and without pathologic AD (N = 152), presence of β-amyloid but without pathologic AD (N = 245), and presence of β-amyloid and pathologic AD (N = 659) (Table 5). We observe that *APOE* ε4 was strongly associated with a higher burden of PHF-tau tangles in the group with pathologic AD ($\beta = 0.592$, $p = 5.8 \times 10^{-8}$). The association was significant but much weaker in the group with β-amyloid but without pathologic AD ($\beta = 0.266$, $p = 0.032$). Finally, as before, we did not find the association among persons without β-amyloid ($\beta = -0.201$, $p = 0.424$). We repeated the analysis for *APOE* ε2 allele (Table 5) and observe significant association of *APOE* ε2 and PHF-tau tangles only in the group with pathologic AD ($\beta = -0.536$, $p = 5.9 \times 10^{-4}$). Note the point estimate for the group with β-amyloid but without pathologic AD is about 70% of that for *APOE* ε4; thus, the lack of association could be the result of a reduction of power among those with this allele.

4. Discussion

In this study, we identified marked differences in the association between *APOE* and PHF-tau tangles among persons with and without β-amyloid deposits. Specifically, we found that *APOE* ε4 is strongly associated with a higher density of PHF-tau tangles and *APOE* ε2 is associated with fewer PHF-tau tangles in brains with β-amyloid but not in brains without β-amyloid. The association of *APOE* ε4 and PHF-tau tangles was also observed in the participants with low β-amyloid burden insufficient for a pathologic diagnosis of AD but not in the absence of β-amyloid suggesting that the association of *APOE* with PHF-tau tangles depends on β-amyloid presence. Results were similar when examining mesial temporal and neocortical tangle separately. Furthermore, they were unchanged after controlling for cognitive performance and other common neuropathologies. We are unaware of prior studies examining whether the association of *APOE* and PHF-tau tangles differs by β-amyloid deposition.

Many previous studies reported associations between *APOE* and β-amyloid deposition and NFT (Nagy et al., 1995; Ohm et al., 1995). Postmortem studies showed that β-amyloid levels in the brain, the load of β-amyloid, and NFTs vary according to *APOE* genotype (ε4 > ε3 > ε2) (Bennett et al., 2009; Nicoll et al., 2011; Sabbagh et al., 2013). These studies reveal a concurrent and symmetric association of *APOE* with both β-amyloid and NFT, suggesting that the association of *APOE* with NFT could be mediated by β-amyloid. Only 1 neuropathologic study found an association between *APOE* ε2

Table 3
Association of *APOE* ε4 status with PHF-tau-tangles density

Parameters	β (SE), *p*	β (SE), *p*
β-amyloid (absent)	−0.715 (0.109), <0.001	−0.633 (0.114), <0.001
APOE ε4+	0.702 (0.086), <0.001	0.752 (0.089), <0.001
β-amyloid (absent) × *APOE* ε4+	-	−0.968 (0.388), 0.013

β coefficient, standard error (SE), and *p* value from the models without and with interaction term between ε4 status and the presence of β-amyloid. Both models were adjusted for age and sex.
Key: APOE, apolipoprotein E; PHF-tau, paired helical filament tau.

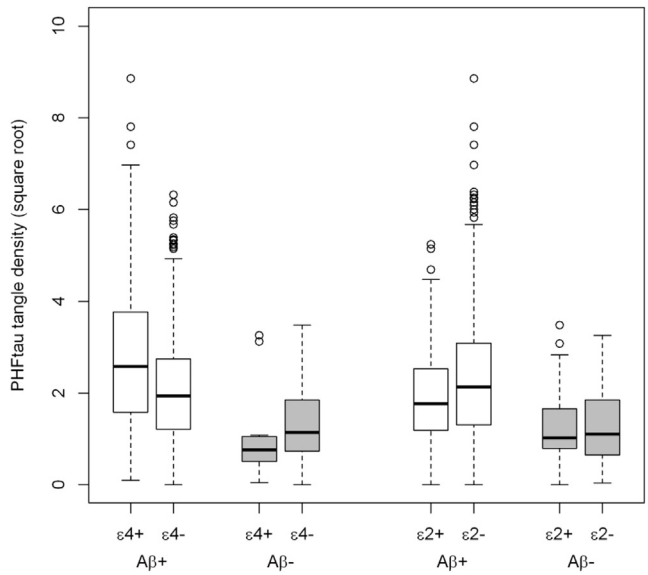

Fig. 1. PHF-tau-tangle density by *APOE* status and the presence or absence of β-amyloid. Abbreviations: APOE, apolipoprotein E; PHF-tau, paired helical filament tau.

Table 4
Association of *APOE* ε2 status with PHF-tau tangles density

Parameters	β (SE), *p*	β (SE), *p*
β-amyloid (absent)	−0.837 (0.107), <0.001	−0.905 (0.126), <0.001
APOE ε2+	−0.362 (0.107), <0.001	−0.424 (0.120), <0.001
β-amyloid (absent) × *APOE* ε2+	-	0.291 (0.260), 0.264

β coefficient, standard error (SE), and *p* value from the models without and with interaction term between ε2 status and the presence of β-amyloid. Both models were adjusted for age and sex.
Key: APOE, apolipoprotein E; PHF-tau, paired helical filament tau.

carriers and increased β-amyloid pathology. However, this study was performed in a population much older than the sample included in this and other studies suggesting that the relation between *APOE* and neuropathology may vary according to age (Berlau et al., 2009).

Robust experimental studies in transgenic mice link *APOE* ε4 and ε2 to distinct mechanisms of β-amyloid processing and metabolism. APOE-ε4-lipoproteins reportedly have lower binding affinity to β-amyloid and as result may be less efficient in mediating β-amyloid clearance (Castellano et al., 2011; LaDu et al., 1994). *APOE* ε4 isoform may also be less efficient in mediating degradation of β-amyloid (Jiang et al., 2008). *APOE* ε2 has been shown to be most effective in clearing β-amyloid from the brain and bloodstream and avoiding β-amyloid deposition by preventing the conversion of sustained forms of β-amyloid into pathologic oligomers (Fagan et al., 2002; Ma et al., 1994; Sharman et al., 2010). By contrast, the direct effect of both *APOE* ε4 and ε2 to PHF-tau pathology is less clear with few experimental studies showing that APOE-4 isoforms may induce fibrillary tangle-like intracellular inclusions in neurons containing phosphorylated tau (Brecht et al., 2004; Huang, 2010).

The results of this study support the hypothesis of a 2-process model underlying PHF-tau tangles in mesial temporal lobes (Crary et al., 2014; Jellinger et al., 2015; Mungas et al., 2014; Nelson et al., 2009; Royall and Palmer, 2014). The first one is the AD process characterized by the presence of β-amyloid deposits and recognized by the most recent guidelines for diagnosis of neuropathologic AD. Previous studies advocate that β-amyloid deposits are the initial AD event that triggers a cascade of events that will result in PHF-tau-tangles formation (Hardy and Selkoe, 2002; Jack et al., 2011). In our study, the density of PHF-tau tangles was much higher in brains with β-amyloid, and in the group with β-amyloid, *APOE* was very strongly associated with PHF-tau tangles. Even in the subjects with low β-amyloid burden, it was possible to find an association between *APOE* ε4 and PHF-tau tangles demonstrating that the presence of a low β-amyloid load is sufficient to find an association of *APOE* with PHF-tau tangles. These are all evidence in favor of the β-amyloid cascade hypothesis. The second process is the presence of PHF-tau tangles, especially in mesial temporal lobes in the absence of β-amyloid. In previous studies, PHF-tau tangles alone had been associated with age and cognitive impairment (Jellinger and Attems, 2007; Nelson et al., 2009; Yamada, 2003). Furthermore, PHF-tau tangles can characterize a number of conditions other than AD including chronic traumatic encephalopathy, progressive supranuclear palsy, corticobasal degeneration, and tau mutations (Klein et al., 2004; Nelson et al., 2009; Yoshiyama et al., 2005). In our study, this second process is characterized by a lower density of PHF-tau tangles in mesial temporal lobes, concentrates *APOE* ε2 carriers, and in these subjects, *APOE* is not associated with PHF-tau tangles when compared to the first process.

Table 5
Association of *APOE* status with PHF-tau tangles density, stratified by β-amyloid status

Parameters	*APOE* ε4+	*APOE* ε2+
	β (SE), *p*	β (SE), *p*
Without β-amyloid and without AD (N = 152)	−0.201 (0.205), 0.423	−0.102 (0.153), 0.505
With β-amyloid but without AD (N = 659)	0.266 (0.124), 0.03	−0.185 (0.122), 0.132
With β-amyloid and with AD (N = 245)	0.592 (0.107), <0.001	−0.536 (0.155), <0.001

β coefficient, standard error (SE), and *p* value from 4 models. All the models were adjusted for age and sex.
Key: AD, Alzheimer's disease; APOE, apolipoprotein E; PHF-tau, paired helical filament tau.

An alternative hypothesis conflicting with the β-amyloid cascade hypothesis is that AD begins with PHF-tau in mesial temporal lobe and spreads through the brain following a uniform and progressive pattern (Braak and Del Tredici, 2011). Recent animal models with transgenic mice suggest that tau pathology may be transmitted from neuron to neuron through a trans-synaptic mechanism from mesial temporal lobe to neocortical regions (Liu et al., 2012). What might be considered a hybrid hypothesis is one in which AD begins with mesial temporal lobe tangles and the spread of tangles through the brain is augmented by β-amyloid (Jack, 2014). Furthermore, experimental studies suggest that intraneuronal β-amyloid oligomers are associated to synaptic dysfunction, a process that may contribute to trans-synaptic tau spread and is enhanced in *APOE* ε4 carriers (Koffie et al., 2012; Sen et al., 2012). Thus, the presence of β-amyloid could augment the spreading of PHF-tau tangles. Our findings of a greater density of PHF-tau tangles in persons with β-amyloid are also consistent with this model of AD.

This study has limitations. As few, *APOE* ε4 carriers are without β-amyloid, a much larger sample might have identified an association between *APOE* and PHF-tau tangles in persons without β-amyloid. However, if there is any association between *APOE* and PHF-tau tangles in persons without β-amyloid, it remains much weaker than the association found in those with β-amyloid. Power calculations suggested that the sample size included in the group without β-amyloid would be sufficient to detect an association of *APOE* ε4 and PHF-tau tangles if the effect size was similar to the group with β-amyloid. Second, we did not determine specific associations of *APOE* to the different specific species of β-amyloid such as 42 or 40. Similarly, different tau antibodies identify slightly different subpopulations of tangles. Third, the convenience sample used in this study may limit the generalizability of our findings. Finally, the participants were much older than many other studies, and it is possible that the associations vary by age.

The study also had a number of strengths. The major strengths are the large sample size from 2 prospective community-based studies of older persons. Both enjoyed very high clinical follow-up and autopsy rates. Furthermore, they used identical uniform quantitative postmortem procedures by examiners blinded to *APOE* status.

Disclosure statement

The authors have no conflicts of interest to disclosure in relation to this study.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found at the online version at http://dx.doi.org/10.1016/j.neurobiolaging.2015.09.011.

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