APOE ϵ 4 is associated with severity of Lewy body pathology independent of Alzheimer pathology

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Abstract

Objective

To evaluate whether *APOE* ϵ 4 is associated with severity of Lewy body (LB) pathology, independently of Alzheimer disease (AD) pathology.

Methods

Six hundred fifty-two autopsy-confirmed LB disease (LBD) cases and 660 clinical controls were genotyped for *APOE*. In case-control analysis, LBD cases were classified into 9 different groups according to severity of both LB pathology (brainstem, transitional, diffuse) and AD pathology (low, moderate, high) to assess associations between *APOE* ϵ 4 and risk of different neuropathologically defined LBD subgroups in comparison to controls. In LBD cases only, we also measured LB counts from 5 cortical regions and evaluated associations with ϵ 4 according to severity of AD pathology.

Results

As expected, *APOE* ϵ 4 was associated with an increased risk of transitional and diffuse LBD in cases with moderate or high AD pathology (all odds ratios ≥ 3.42 , all $p \leq 0.004$). Of note, ϵ 4 was also associated with an increased risk of diffuse LBD with low AD pathology (odds ratio = 3.46, $p = 0.001$). In the low AD pathology LBD subgroup, ϵ 4 was associated with significantly more LB counts in the 5 cortical regions, independently of Braak stage and Thal phase (all $p \leq 0.002$).

Conclusions

Our results indicate that *APOE* ϵ 4 is independently associated with a greater severity of LB pathology. These findings increase our understanding of the mechanism behind reported associations of ϵ 4 with risk of dementia with Lewy bodies and Parkinson disease with dementia, and suggest that ϵ 4 may function as a modifier of processes that favor LB spread rather than acting directly to initiate LB pathology.

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Glossary

AD = Alzheimer disease; **DLB** = dementia with Lewy bodies; **LB** = Lewy body; **LBD** = Lewy body disease; **NFT** = neurofibrillary tangle; **OR** = odds ratio; **PD** = Parkinson disease; **PDD** = Parkinson disease with dementia; **SP** = senile plaque.

Disorders associated with α -synuclein deposits within neuronal cell bodies (Lewy bodies [LBs]¹) and their processes (Lewy neurites²) are classified as LB disease (LBD). Diseases in which LB pathology is considered to have a key role in pathogenesis and clinical presentation are Parkinson disease (PD),³ PD with dementia (PDD),⁴ and dementia with Lewy bodies (DLB).⁵ DLB and PDD often have some degree of concomitant Alzheimer-type pathology.^{5–8}

PD genetics has greatly advanced over recent years.⁹ Although less is known about the genetics of PDD and DLB, the most replicated genetic risk factor for these diseases is the *APOE* $\epsilon 4$ allele.^{10–17} Conversely, most studies have failed to show an association between $\epsilon 4$ and PD.^{18,19} Despite these reports of an association of *APOE* $\epsilon 4$ with increased risk of DLB and PDD, the causal mechanism remains unclear. Given the strong association between *APOE* $\epsilon 4$ and Alzheimer disease (AD)^{20–23} and the presence of AD pathology in many patients with DLB and PDD, one possibility is that $\epsilon 4$ contributes to β -amyloid deposition and LB pathology through an amyloid cascade hypothesis comparable to that proposed for neurofibrillary pathology in AD.^{24–26} It is also possible that at least some of the increased risk of DLB and PDD attributable to *APOE* $\epsilon 4$ is due to a direct association between $\epsilon 4$ and LB pathology.

To evaluate the latter hypothesis, in the current study, we assessed associations between *APOE* $\epsilon 4$ and risk of different neuropathologically defined LBD subgroups in a case-control analysis and subsequently examined associations between $\epsilon 4$ and cortical LB counts in our LBD series.

Methods

Standard protocol approvals, registrations, and patient consents

This study was approved by the Mayo Clinic institutional review board.

Study patients

A total of 652 autopsy-confirmed LBD cases and 660 living controls were included. Controls were free of neurologic disease and seen at Mayo Clinic in Jacksonville, FL. All controls provided informed consent. LBD cases were obtained from the brain bank for neurodegenerative disorders at Mayo Clinic in Jacksonville, FL, and were evaluated by a single neuropathologist (D.W.D.). Cases of AD with amygdala-predominant LBs²⁷ and cases with incidental LBs²⁸ were excluded, as were cases in which LBs were concomitant with significant coexisting pathology (e.g., progressive supranuclear palsy, corticobasal

degeneration, Pick disease, or multiple system atrophy). Autopsies were performed after informed consent from the legal next of kin or a person with legal power of attorney. All patients were unrelated non-Hispanic Caucasians. Exploratory retrospective clinical diagnoses that were based on review of available medical records (rather than on uniform guidelines) revealed that 282 LBD cases (43%) had dementia without parkinsonism, 171 (26%) had parkinsonism without dementia, and 199 (31%) had both parkinsonism and dementia. Median age at death of LBD cases was 79 years (range: 50–99 years) and 381 patients (58%) were men. In controls, median age was 69 years (range: 45–88 years) and 286 (43%) were men.

Neuropathologic assessment

Neuropathologic methods have been described previously,^{29,30} but, briefly, neuroanatomical sampling and thioflavin S fluorescence microscopy was performed using the protocol of Terry et al.³¹ with some modifications. Formalin-fixed, paraffin-embedded tissue samples from neocortical and limbic regions were cut at 5- μ m thickness and mounted on glass slides. Senile plaques (SPs) and neurofibrillary tangles (NFTs) were evaluated with thioflavin S fluorescent microscopy. SP counts were truncated at 50 (twice the amount required to meet Khachaturian³² AD criteria). LB pathology was assessed using an α -synuclein antibody (NACP, 1:3,000 rabbit polyclonal, Mayo Clinic antibody³³) following formic acid pretreatment of the slides and processed using the DAKO Autostainer (DAKO Auto Machine Corporation, Carpinteria, CA) with the DAKO EnVision+ HRP System, which is a sensitive method for detecting a range of Lewy-related pathologies.³⁴ LB counts were measured in 5 cortical regions (middle frontal, superior temporal, inferior parietal, cingulate, and entorhinal gyri; table 1); of the 652 LBD cases, 638 had LB counts measured in at least one cortical region. In addition, NFT counts were recorded in 13 different brain regions, and SP counts were recorded in 12 different regions (table 1).

Pathologic subgroups

To assess associations between *APOE* $\epsilon 4$ and risk of different neuropathologically defined LBD subgroups that are based on severity of both LB and AD pathology, LBD cases were divided into 9 different categories for comparison with controls as follows. First, the staging scheme of Kosaka et al.³⁵ was utilized to classify distribution of LBD as brainstem ($n = 75$), transitional ($n = 219$), and diffuse ($n = 358$) subtypes.

Second, the severity of AD pathology was classified as low, moderate, or high when considering both the distribution of NFTs with Braak stage³⁶ and distribution of SPs with Thal amyloid phase.³⁷ Specifically, patients with both Braak stage 0–III and Thal phase 0–2 were categorized as “low AD

Table 1 Summary of Lewy body, NFT, and SP counts according to LBD subtype (brainstem, transitional, diffuse) and severity of AD pathology (low, moderate, high)

Pathologic measure	Low AD pathology			Moderate AD pathology			High AD pathology		
	Brainstem LBD (n = 46)	Transitional LBD (n = 80)	Diffuse LBD (n = 33)	Brainstem LBD (n = 10)	Transitional LBD (n = 27)	Diffuse LBD (n = 115)	Brainstem LBD (n = 19)	Transitional LBD (n = 111)	Diffuse LBD (n = 209)
Lewy body counts									
Middle frontal	0 (0, 2)	1 (0, 5)	3 (1, 10)	1 (0, 1)	2 (0, 4)	6 (0, 30)	0 (0, 2)	1 (0, 21)	6 (0, 35)
Superior temporal	1 (0, 5)	4 (1, 9)	7 (3, 51)	1 (0, 2)	5 (0, 8)	12 (2, 40)	1 (0, 6)	4 (0, 16)	15 (2, 50)
Inferior parietal	0 (0, 1)	1 (0, 3)	2 (1, 8)	0 (0, 1)	1 (0, 3)	5 (0, 27)	0 (0, 1)	0 (0, 3)	5 (0, 30)
Cingulate	2 (0, 7)	6 (1, 14)	9 (3, 30)	2 (0, 6)	6 (2, 12)	14 (3, 45)	2 (0, 10)	3 (0, 15)	15 (1, 35)
Entorhinal	2 (0, 6)	7 (1, 20)	14 (3, 27)	2 (1, 5)	8 (3, 16)	18 (4, 40)	3 (0, 10)	8 (1, 35)	22 (4, 45)
NFT counts									
Middle frontal	0 (0, 0)	0 (0, 2)	0 (0, 0)	0 (0, 0)	0 (0, 1)	0 (0, 3)	3 (0, 15)	5 (0, 30)	2 (0, 34)
Superior temporal	0 (0, 1)	0 (0, 2)	0 (0, 1)	0 (0, 1)	0 (0, 9)	0 (0, 3)	5 (1, 23)	11 (1, 45)	4 (0, 30)
Inferior parietal	0 (0, 0)	0 (0, 1)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 1)	5 (0, 28)	7 (0, 40)	3 (0, 25)
Motor cortex	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 3)	1 (0, 3)	1 (0, 12)	0 (0, 10)
Visual cortex	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	2 (0, 7)	2 (0, 12)	0 (0, 18)
Hippocampus									
CA4	0 (0, 2)	0 (0, 4)	0 (0, 4)	0 (0, 2)	0 (0, 3)	1 (0, 6)	3 (1, 8)	3 (0, 15)	2 (0, 22)
CA2/3	0 (0, 15)	0 (0, 28)	0 (0, 35)	1 (0, 14)	0 (0, 24)	1 (0, 30)	6 (0, 16)	5 (0, 40)	3 (0, 50)
CA1	1 (0, 30)	1 (0, 10)	1 (0, 30)	2 (0, 26)	1 (0, 20)	2 (0, 20)	7 (1, 31)	15 (1, 60)	8 (0, 55)
Subiculum	1 (0, 12)	1 (0, 22)	2 (0, 16)	1 (0, 17)	2 (0, 8)	2 (0, 15)	14 (1, 60)	25 (0, 98)	11 (1, 68)
Entorhinal	4 (0, 25)	5 (0, 50)	5 (0, 25)	2 (0, 26)	8 (0, 20)	8 (0, 30)	12 (3, 35)	21 (3, 45)	12 (1, 40)
Amygdala									
Basolateral	0 (0, 2)	0 (0, 6)	0 (0, 2)	0 (0, 3)	1 (0, 3)	1 (0, 21)	4 (0, 16)	10 (0, 35)	3 (0, 20)
Corticomedial	1 (0, 3)	1 (0, 25)	0 (0, 3)	0 (0, 5)	1 (0, 10)	2 (0, 25)	9 (3, 50)	24 (0, 80)	6 (0, 65)
Basal nucleus	1 (0, 3)	1 (0, 6)	1 (0, 5)	1 (0, 3)	1 (0, 4)	1 (0, 12)	12 (2, 22)	10 (0, 45)	3 (0, 24)

Continued

Table 1 Summary of Lewy body, NFT, and SP counts according to LBD subtype (brainstem, transitional, diffuse) and severity of AD pathology (low, moderate, high) *(continued)*

Pathologic measure	Low AD pathology			Moderate AD pathology			High AD pathology		
	Brainstem LBD (n = 46)	Transitional LBD (n = 80)	Diffuse LBD (n = 33)	Brainstem LBD (n = 10)	Transitional LBD (n = 27)	Diffuse LBD (n = 115)	Brainstem LBD (n = 19)	Transitional LBD (n = 111)	Diffuse LBD (n = 209)
SP counts									
Middle frontal	0 (0, 40)	0 (0, 50)	29 (0, 50)	45 (8, 50)	50 (10, 50)	50 (0, 50)	50 (20, 50)	50 (20, 50)	50 (0, 50)
Superior temporal	0 (0, 40)	0 (0, 50)	14 (0, 50)	23 (5, 50)	50 (15, 50)	50 (0, 50)	50 (16, 50)	50 (7, 50)	50 (10, 50)
Inferior parietal	0 (0, 40)	0 (0, 50)	18 (0, 50)	48 (3, 50)	50 (10, 50)	50 (0, 50)	50 (32, 50)	50 (4, 50)	50 (0, 50)
Motor cortex	0 (0, 8)	0 (0, 10)	0 (0, 25)	6 (0, 12)	10 (0, 45)	10 (0, 40)	19 (1, 50)	25 (2, 50)	18 (0, 50)
Visual cortex	0 (0, 35)	0 (0, 26)	1 (0, 26)	9 (1, 24)	10 (0, 50)	16 (0, 50)	43 (3, 50)	40 (0, 50)	29 (0, 50)
Entorhinal	0 (0, 50)	2 (0, 50)	8 (0, 50)	26 (5, 50)	45 (12, 50)	50 (0, 50)	50 (12, 50)	50 (15, 50)	50 (0, 50)
Hippocampus									
CA4	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 1)	0 (0, 10)	0 (0, 10)	4 (0, 12)	4 (0, 25)	2 (0, 22)
CA2/3	0 (0, 0)	0 (0, 1)	0 (0, 0)	0 (0, 5)	0 (0, 7)	0 (0, 10)	1 (0, 6)	2 (0, 12)	2 (0, 16)
CA1	0 (0, 1)	0 (0, 3)	0 (0, 4)	1 (0, 15)	4 (0, 46)	3 (0, 30)	5.5 (0, 25)	8 (0, 36)	8 (0, 45)
Subiculum	0 (0, 3)	0 (0, 20)	0 (0, 28)	4 (0, 36)	8 (0, 30)	10 (0, 40)	10 (0, 36)	20 (0, 50)	20 (0, 50)
Entorhinal	0 (0, 50)	2 (0, 50)	8 (0, 50)	26 (5, 50)	45 (12, 50)	50 (0, 50)	50 (12, 50)	50 (15, 50)	50 (0, 50)
Amygdala									
Basolateral	0 (0, 20)	0 (0, 45)	4 (0, 20)	9 (2, 30)	15 (3, 50)	16 (1, 50)	15 (0, 50)	18 (0, 50)	20 (0, 50)
Corticomedial	0 (0, 20)	0 (0, 50)	4 (0, 50)	11 (2, 36)	20 (4, 50)	20 (0, 50)	50 (2, 50)	50 (2, 50)	40 (0, 50)

Abbreviations: AD = Alzheimer disease; LBD = Lewy body disease; NFT = neurofibrillary tangle; SP = senile plaque.
The sample median (minimum, maximum) is given.

pathology” (n = 159), those with both Braak stage 0–III and Thal phase 3–5 were categorized as “moderate AD pathology” (n = 152), and those with both Braak stage IV–VI and Thal phase 3–5 were categorized as “high AD pathology” (n = 339) (table 2). Two cases had Braak stage IV–VI and Thal phase 0–2 and did not fit into the 3 AD pathology subgroups; these 2 patients were excluded from genetic disease-risk analyses, but were included in analysis of cortical LB counts. Of importance, the 3 categories of severity of AD pathology were chosen to allow for as detailed a categorization as possible (in particular, while attempting to limit the degree of AD pathology in the “low AD pathology” subgroup) while simultaneously having a reasonable sample size in each of the 9 pathologic subgroups. The low, moderate, and high AD pathology categories that we utilized do not reflect recent guidelines³⁸; our categorization is stricter regarding what constitutes low AD pathology, as appropriate given the rationale for our study.

Finally, the 3 LBD subtypes and the 3 AD pathology severity categories were combined into an overall LBD/AD pathology variable, resulting in a total of 9 different pathologic subgroups that were utilized in genetic disease-risk analyses. Characteristics of each pathologic subgroup are shown in table 3.

Genetic analysis

For controls, genomic DNA was extracted from peripheral blood lymphocytes using the AutoGen FlexSTAR (Holliston, MA). For the autopsy LBD cases, genomic DNA was extracted from frozen cerebellar tissue using the AutoGen 245T. APOE genotype was determined with 2 single nucleotide polymorphisms (SNPs), rs7412 and rs429358, using ABI SNP Genotyping TaqMan assays. Genetic analysis was completed using SDS 2.2.2 software (ABI, Applied Biosystems, Foster City, CA). There was no evidence of

a departure from Hardy-Weinberg equilibrium for either rs7412 or rs429358 in controls (p > 0.05).

Statistical analysis

In disease-risk analyses, for the entire LBD series and separately for each of the 9 LBD/AD severity pathologic subgroups, associations between APOE ε4 and risk of LBD (vs controls) were evaluated using logistic regression models that were adjusted for age and sex. We examined APOE ε4 under a dominant model in all analyses (presence or absence of the ε4 allele) because of the low number of patients with an ε4/ε4 genotype in some pathologic subgroups. Odds ratios (ORs) and 95% confidence intervals were estimated.

In analysis of only LBD cases, for the overall LBD series and also separately for cases with low, moderate, and high AD pathology, associations between APOE ε4 and LB counts in each brain region were assessed using negative binomial regression models that were adjusted for age at death, sex, Braak stage, and Thal phase. Multiplicative effects and 95% confidence intervals were estimated and are interpreted as the multiplicative increase on the mean LB count corresponding to presence of the ε4 allele. In secondary analysis, associations between measures of severity of LB and AD pathology were examined using the Spearman test of correlation, and associations of APOE ε4 with NFT and SP counts in each brain region were assessed using negative binomial regression models (or logistic regression models for brain regions with high concentrations of SP counts equal to 50) that were adjusted for age, sex, and LBD subtype (brainstem, transitional, or diffuse).

To adjust for multiple testing in our primary analysis, we utilized a Bonferroni correction. As tests of association with ε4 were performed in comparison to controls for the entire

Table 2 Distribution of Alzheimer disease pathology in the 652 cases of Lewy body disease

Braak neurofibrillary tangle stage	No. of patients					
	Thal amyloid phase					
	0	1	2	3	4	5
0	6	5	1	1	0	0
I	6	6	2	5	1	1
II	25	24	10	28	8	11
III	32	22	20	47	16	34
IV	0	1	1	38	21	49
V	0	0	0	8	12	84
VI	0	0	0	0	9	118

Patients with both a Braak stage of 0–III and a Thal phase of 0–2 were categorized as “low Alzheimer disease (AD) pathology” (n = 159); these patients are in the upper left quadrant. Patients with both a Braak stage of 0–III and a Thal phase of 3–5 were categorized as “moderate AD pathology” (n = 152); these patients are in the upper right quadrant. Patients with both a Braak stage of IV–VI and a Thal phase of 3–5 were categorized as “high AD pathology” (n = 339); these patients are in the lower right quadrant. The 2 patients in the lower left quadrant had both a Braak stage of IV–VI and Thal phase of 0–2 and did not fit into the 3 AD pathology subgroups; these 2 patients were excluded from analysis involving AD pathology subgroups but were included in analysis involving the entire Lewy body disease series.

Table 3 Characteristics of LBD and AD pathology subgroups

Variable	Low AD pathology (Braak stage 0–III and Thal phase 0–2)			Moderate AD pathology (Braak stage 0–III and Thal phase 3–5)			High AD pathology (Braak stage IV–VI and Thal phase 3–5)		
	Brainstem LBD (n = 46)	Transitional LBD (n = 80)	Diffuse LBD (n = 33)	Brainstem LBD (n = 10)	Transitional LBD (n = 27)	Diffuse LBD (n = 115)	Brainstem LBD (n = 19)	Transitional LBD (n = 111)	Diffuse LBD (n = 209)
Age at death, y	80 (55, 99)	77 (50, 92)	77 (61, 87)	87 (73, 96)	79 (69, 97)	78 (63, 93)	86 (59, 99)	82 (58, 97)	78 (54, 96)
Sex									
Male	32 (70)	58 (73)	23 (70)	4 (40)	21 (78)	70 (61)	11 (58)	52 (47)	109 (52)
Female	14 (30)	22 (28)	10 (30)	6 (60)	6 (22)	45 (39)	8 (42)	59 (53)	100 (48)
Braak NFT stage									
0	4 (9)	7 (9)	1 (3)	0 (0)	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)
I	5 (12)	8 (10)	1 (3)	3 (30)	1 (4)	3 (3)	0 (0)	0 (0)	0 (0)
II	17 (37)	28 (35)	14 (42)	3 (30)	10 (37)	34 (30)	0 (0)	0 (0)	0 (0)
III	20 (44)	37 (46)	17 (52)	4 (40)	15 (56)	78 (68)	0 (0)	0 (0)	0 (0)
IV	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (26)	15 (14)	88 (42)
V	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (16)	27 (24)	74 (35)
VI	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	11 (58)	69 (62)	47 (23)
Thal amyloid phase									
0	25 (54)	36 (45)	8 (24)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1	17 (37)	30 (38)	10 (30)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2	4 (9)	14 (18)	15 (46)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
3	0 (0)	0 (0)	0 (0)	9 (90)	13 (48)	59 (51)	2 (11)	8 (7)	36 (17)
4	0 (0)	0 (0)	0 (0)	0 (0)	4 (15)	21 (18)	2 (11)	11 (10)	29 (14)
5	0 (0)	0 (0)	0 (0)	1 (10)	10 (37)	35 (30)	15 (79)	92 (83)	144 (69)

Abbreviations: AD = Alzheimer disease; LBD = Lewy body disease; NFT = neurofibrillary tangle.

The sample median (minimum, maximum) is given for age at death. The remaining data represent n (%). There were 2 patients with LBD (Braak stage VI, Thal phase 1 and 2) who were not assigned a severity of AD pathology category; these 2 patients were excluded from analysis involving AD pathology subgroups but were included in analysis involving the entire LBD series.

LBD series and for each of the 9 pathologic subgroups, $p \leq 0.005$ was considered as statistically significant in disease-risk analysis. For associations between $\epsilon 4$ and cortical LB counts, 20 different statistical tests were performed, and therefore $p \leq 0.0025$ was considered as significant. All statistical tests were 2-sided. Statistical analyses were performed using SAS (version 9.2; SAS Institute, Inc., Cary, NC).

Data availability

Anonymized data will be shared by request from any qualified investigator.

Results

Of the 660 controls, 183 (28%) had a copy of the $\epsilon 4$ allele. When examining the entire LBD cohort, risk of LBD in comparison to controls was higher for $\epsilon 4$ carriers (OR = 3.52,

$p = 7 \times 10^{-19}$). The $\epsilon 4$ allele was present in 348 of the 652 LBD cases (53%).

Associations of *APOE* $\epsilon 4$ with risk of LBD (in comparison to controls) are displayed in table 4 for each of the 9 pathologic subgroups. Of note, *APOE* $\epsilon 4$ was associated with an increased risk of diffuse LBD with low AD pathology (OR = 3.46, $p = 0.001$). *APOE* $\epsilon 4$ was not associated with risk of brainstem or transitional LBD in patients with low AD pathology, or with brainstem LBD in patients with moderate AD pathology (table 4). There was an association between *APOE* $\epsilon 4$ and an increased risk of transitional and diffuse LBD with moderate AD pathology (ORs ≥ 3.42 , $p \leq 0.004$), and also with an increased risk of brainstem, transitional, and diffuse LBD with high AD pathology (ORs ≥ 5.58 , $p \leq 0.0002$) (table 4).

To examine whether *APOE* $\epsilon 4$ might modify LB pathology in LBD cases, the association of *APOE* $\epsilon 4$ with LB counts in

Table 4 Association between *APOE* ε4 and risk of LBD according to LBD subtype and severity of AD pathology

LBD subtype	Severity of AD pathology		
	Low AD pathology	Moderate AD pathology	High AD pathology
Brainstem LBD			
Median Braak stage	II	II	VI
Median Thal phase	0	3	5
Fraction (%) of ε4 carriers	4/46 (9)	3/10 (30)	13/19 (68)
OR (95% CI) vs controls	0.30 (0.10–0.87)	1.88 (0.37–9.49)	9.37 (2.90–30.24)
<i>p</i> Value vs controls	0.027	0.45	0.0002
Transitional LBD			
Median Braak stage	II	III	VI
Median Thal phase	1	4	5
Fraction (%) of ε4 carriers	16/80 (20)	14/27 (52)	75/111 (68)
OR (95% CI) vs controls	0.73 (0.40–1.34)	3.42 (1.48–7.92)	5.58 (3.38–9.20)
<i>p</i> Value vs controls	0.31	0.004	2×10^{-11}
Diffuse LBD			
Median Braak stage	III	III	V
Median Thal phase	1	3	5
Fraction (%) of ε4 carriers	18/33 (54)	60/115 (52)	144/209 (69)
OR (95% CI) vs controls	3.46 (1.66–7.22)	3.74 (2.34–5.97)	6.96 (4.70–10.29)
<i>p</i> Value vs controls	0.001	4×10^{-8}	3×10^{-22}

Abbreviations: AD = Alzheimer disease; CI = confidence interval; LBD = Lewy body disease; OR = odds ratio.

Comparisons are made to 660 controls, of whom 183 (28%) had a copy of the ε4 allele. ORs, 95% CIs, and *p* values result from logistic regression models (vs controls) adjusted for age and sex. *APOE* ε4 was considered under a dominant model (i.e., presence vs absence of the ε4 allele) given the limited sample size of some of the pathologic subgroups. Patients with both a Braak stage of 0–III and Thal phase of 0–2 were categorized as low AD pathology, patients with both a Braak stage of 0–III and a Thal phase of 3–5 were categorized as moderate AD pathology, and patients with both a Braak stage of IV–VI and a Thal phase of 3–5 were categorized as high AD pathology. There were 2 patients with LBD (Braak stage VI, Thal phase 1 and 2) who were not assigned a severity of AD pathology category; these 2 patients were excluded from analysis involving AD pathology subgroups but were included in analysis involving the entire LBD series. The *p* values ≤0.005 are considered as statistically significant after applying a Bonferroni adjustment for multiple testing (the 10th test involves comparing the entire LBD series to controls and is not shown here).

cortical regions was evaluated and results are displayed in table 5. In the overall LBD series (adjusting for age and sex), presence of ε4 was associated with more LB counts in superior temporal ($p = 0.0001$), inferior parietal ($p = 0.0002$), and entorhinal ($p = 3 \times 10^{-6}$) gyri. However, these associations dramatically weakened after additional adjustment for Braak stage and Thal phase (table 5).

When examining the association between ε4 and cortical LB counts for the low, moderate, and high AD pathology subgroups, no associations were observed in the moderate or high subgroups (table 5). However, we observed associations in all 5 brain regions for the low AD pathology subgroup in age- and sex-adjusted analysis and when additionally adjusting for Braak stage and Thal phase ($p \leq 0.002$, table 5). Specifically, mean LB count was between 1.45 and 2.12 times higher for cases with a copy of the ε4 allele (table 5 and figure). In a sensitivity analysis for the low

AD pathology group (with slightly smaller sample sizes because of missing SP count data), with additional adjustment for SP counts in the given brain region for the 4 brain regions where this was possible, ε4 was associated with greater superior temporal LB counts ($p = 0.0005$) and nominally ($p < 0.05$) associated with more inferior parietal ($p = 0.006$), middle frontal ($p = 0.020$), and entorhinal ($p = 0.005$) LB counts; mean LB counts were between 1.50 and 1.77 times higher for *APOE* ε4 carriers.

In secondary analysis, we examined the relationships between pathologic burden based on counts of LBs, NFTs, and SPs in the overall LBD series. High LB counts in the entorhinal and superior temporal gyri were correlated with high NFT counts in most of the 13 brain regions examined (median Spearman $r = 0.17$; 77% of $p \leq 0.05$), whereas correlations were generally not evident for LB counts in the cingulate, inferior parietal, and middle frontal gyri (median Spearman $r = -0.02$; 18% of

Table 5 Association of *APOE* ε4 with cortical LBs according to severity of AD pathology

Cortical region	No.	LB counts		Adjusting for age at death and sex		Additional adjustment for Braak stage and Thal phase	
		0 ε4 alleles	1 or 2 ε4 alleles	Multiplicative effect on mean LB counts (95% CI)	p Value	Multiplicative effect on mean LB counts (95% CI)	p Value
All LBD (n = 638)		n = 299	n = 339				
Middle frontal	638	2 (0, 1, 5, 30)	3 (0, 1, 6, 35)	1.18 (1.04–1.34)	0.011	1.04 (0.91–1.18)	0.59
Superior temporal	634	5 (0, 3, 12, 45)	9 (0, 4, 15, 51)	1.22 (1.11–1.35)	0.0001	1.04 (0.94–1.15)	0.45
Inferior parietal	634	1 (0, 1, 4, 15)	3 (0, 1, 5, 30)	1.30 (1.13–1.48)	0.0002	1.13 (0.98–1.30)	0.090
Cingulate	633	7 (0, 4, 14, 35)	9 (0, 5, 15, 45)	1.11 (1.01–1.22)	0.021	1.03 (0.94–1.14)	0.48
Entorhinal	594	10 (0, 5, 18, 45)	16 (0, 9, 24, 40)	1.24 (1.13–1.35)	3 × 10 ^{−6}	1.08 (0.98–1.18)	0.11
LBD with low AD pathology (n = 156)		n = 119	n = 37				
Middle frontal	156	1 (0, 1, 2, 7)	2 (0, 1, 3, 10)	1.88 (1.39–2.56)	5 × 10 ^{−5}	1.69 (1.23–2.33)	0.001
Superior temporal	154	3 (0, 2, 5, 12)	5 (1, 3, 8, 51)	2.09 (1.61–2.71)	4 × 10 ^{−8}	1.84 (1.40–2.42)	1 × 10 ^{−5}
Inferior parietal	154	1 (0, 0, 1, 7)	2 (0, 1, 3, 8)	2.53 (1.77–3.62)	4 × 10 ^{−7}	2.12 (1.46–3.07)	7 × 10 ^{−5}
Cingulate	155	5 (0, 3, 7, 20)	8 (2, 4, 10, 30)	1.64 (1.31–2.06)	2 × 10 ^{−5}	1.45 (1.15–1.83)	0.002
Entorhinal	151	5 (0, 3, 9, 22)	10 (1, 6, 14, 27)	1.80 (1.38–2.36)	2 × 10 ^{−5}	1.66 (1.25–2.21)	0.0005
LBD with moderate AD pathology (n = 148)		n = 73	n = 75				
Middle frontal	148	5 (0, 2, 8, 30)	5 (0, 2, 7, 26)	0.94 (0.71–1.23)	0.63	0.92 (0.70–1.22)	0.58
Superior temporal	148	10 (0, 5, 15, 40)	10 (0, 6, 15, 28)	0.91 (0.72–1.14)	0.40	0.89 (0.71–1.12)	0.33
Inferior parietal	148	3 (0, 2, 6, 14)	4 (0, 2, 6, 27)	1.09 (0.84–1.41)	0.51	1.13 (0.87–1.47)	0.37
Cingulate	147	12 (1, 7, 16, 30)	10 (0, 7, 16, 45)	0.93 (0.77–1.13)	0.48	0.92 (0.76–1.12)	0.39
Entorhinal	142	15 (1, 9, 22, 35)	16 (1, 9, 22, 40)	0.96 (0.79–1.16)	0.66	0.91 (0.75–1.09)	0.30
LBD with high AD pathology (n = 333)		n = 107	n = 226				
Middle frontal	333	4 (0, 1, 8, 16)	3 (0, 1, 6, 35)	0.89 (0.69–1.15)	0.37	1.01 (0.80–1.27)	0.96
Superior temporal	331	10 (0, 5, 18, 45)	12 (0, 5, 17, 50)	0.97 (0.81–1.16)	0.70	1.06 (0.89–1.26)	0.53
Inferior parietal	331	3 (0, 1, 6, 15)	3 (0, 1, 5, 30)	0.99 (0.76–1.30)	0.96	1.14 (0.88–1.46)	0.32
Cingulate	330	10 (0, 4, 16, 35)	9 (0, 4, 16, 30)	0.91 (0.75–1.09)	0.31	1.04 (0.87–1.25)	0.63
Entorhinal	300	15 (1, 8, 23, 45)	17 (0, 9, 24, 38)	1.07 (0.91–1.24)	0.42	1.15 (0.99–1.34)	0.069

Abbreviations: AD = Alzheimer disease; CI = confidence interval; LB = Lewy body; LBD = Lewy body disease.

For LB counts, the sample median (minimum, 25th percentile, 75th percentile, maximum) is given. Multiplicative effects, 95% CIs, and *p* values result from negative binomial regression models. *APOE* ε4 was considered under a dominant model (presence or absence of ε4 allele). Multiplicative effects are interpreted as the multiplicative increase on the mean LB count when the ε4 allele is present. The *p* values ≤0.0025 are considered as statistically significant after applying a Bonferroni adjustment for multiple testing. Sample sizes represent the total number of patients with LB counts in that region. Patients with both a Braak stage of 0–III and Thal phase of 0–2 were categorized as low AD pathology, patients with both a Braak stage of 0–III and a Thal phase of 3–5 were categorized as moderate AD pathology, and patients with both a Braak stage of IV–VI and a Thal phase of 3–5 were categorized as high AD pathology. There were 2 patients with LBD (Braak stage VI, Thal phase 1 and 2) who were not assigned a severity of AD pathology category; these 2 patients were excluded from analysis involving AD pathology subgroups but were included in analysis involving the entire LBD series.

$p \leq 0.05$). Greater cortical LB counts in all 5 cortical regions were more strongly associated with greater SP counts in the 12 brain regions in which they were measured (median Spearman $r = 0.25$; 92% of $p \leq 0.05$). LB and SP counts were positively correlated even in the low AD pathology subgroup (median Spearman $r = 0.23$; 84% of $p \leq 0.05$). In addition, as the distribution of LBD pathology increased (from brainstem

to diffuse), there were corresponding increases in both Braak stage ($r = 0.11$, $p = 0.004$) and Thal phase ($r = 0.29$, $p < 0.0001$) in the overall LBD series.

Also of note, as expected, *APOE* ε4 was strongly associated with higher NFT and SP counts in almost all brain regions (table 6).

Figure LB counts according to *APOE* $\epsilon 4$ status in LBD cases with low AD pathology

Box plots of LB counts in the middle frontal, superior temporal, inferior parietal, cingulate, and entorhinal gyri are displayed according to absence of the *APOE* $\epsilon 4$ allele (E4-) or presence of the *APOE* $\epsilon 4$ allele (E4+) for LBD cases with low AD pathology (Braak stage 0-III and Thal phase 0-2). Each box plot shows the minimum, 25th percentile, median, 75th percentile, and maximum LB counts. The y-axis was capped at a value of 30 to allow for better visual display, which did not allow for the presentation of the maximum superior temporal LB counts in $\epsilon 4$ carriers, which was equal to 51. AD = Alzheimer disease; LB = Lewy body; LBD = Lewy body disease.

Discussion

The *APOE* $\epsilon 4$ allele has been associated with an increased risk of DLB and PDD.¹⁰⁻¹⁷ Given the role of $\epsilon 4$ in AD, it seems logical that $\epsilon 4$ influences risk of these LB dementias at least in part via effects on comorbid AD pathology. An additional possibility is that *APOE* $\epsilon 4$ modulates disease risk through an independent association with LB pathology, and it is primarily this latter hypothesis that we set out to address. We found that for the LBD group with low AD pathology, *APOE* $\epsilon 4$ was more frequent in diffuse LBD cases when compared to controls, and $\epsilon 4$ was also associated with higher LB counts in all 5 brain regions examined, independently of severity of AD pathology. These findings suggest that $\epsilon 4$ may alter the risk of DLB and PDD by its effect on LB pathology in addition to its influence on AD pathology.

A recent study reported on a series of autopsy-confirmed cases with clinically diagnosed dementia that were classified as AD ($n = 244$), LBD-AD ($n = 224$), pure DLB ($n = 91$), and PDD ($n = 81$) based on severity of AD and LB pathology.¹⁴ Compared to controls, the association with $\epsilon 4$ was strongest for LBD-AD (OR = 12.6) followed by AD (OR = 9.9), pure DLB (OR = 6.1), and PDD (OR = 3.1). The associations with pure DLB and PDD seem to indicate that $\epsilon 4$ might be independently associated with severity of LB pathology, as both of these groups were neuropathologically classified as having no

or low levels of AD pathology (along with transitional/diffuse LBD). However, the patients classified into the PDD and pure DLB groups could still have a substantial degree of AD pathology. (Cases with Braak stage $\geq$ IV and CERAD [Consortium to Establish a Registry for Alzheimer's Disease] plaque score of moderate/frequent were included in the pure DLB and PDD subgroups; only if both characteristics were present was a different disease classification made.) In the current study, we used stricter criteria when defining which LBD cases have low AD pathology, and we still observed an association between *APOE* $\epsilon 4$ and increased risk of diffuse LBD with low AD pathology despite the relatively small sample size of this subgroup ($n = 33$), thus confirming the initial association. Furthermore, we noted that $\epsilon 4$ was also associated with greater cortical LB counts in the entire low AD pathology LBD subgroup. Taken together, these findings support that $\epsilon 4$ is independently associated with LB pathology, and we speculate that this may be responsible for a portion of the increased risk of DLB and PDD that has been noted in $\epsilon 4$ carriers.

An important issue to address is the degree of correlation between LB and AD pathologies. Specifically, LB counts were positively correlated with SP counts, and more widespread LBD distribution was associated with higher Thal amyloid phase. Correlations between LB pathology measures and NFT counts were also evident, but notably weaker. Similar findings

Table 6 Association of *APOE* ε4 with NFT and SP counts

Brain region	No.	NFT or SP counts		Association measure	Adjusting for age at death and sex		Additional adjustment for LBD subtype	
		0 ε4 alleles (n = 298)	1 or 2 ε4 alleles (n = 336)		Estimate (95% CI)	p Value	Estimate (95% CI)	p Value
NFT counts								
Middle frontal	632	0 (0, 0, 1, 30)	1 (0, 0, 4, 34)	Multiplicative effect	2.02 (1.47–2.76)	1 × 10 ^{−5}	2.04 (1.49–2.79)	8 × 10 ^{−6}
Superior temporal	632	1 (0, 0, 3, 36)	2 (0, 1, 8, 45)	Multiplicative effect	1.91 (1.49–2.44)	2 × 10 ^{−7}	1.94 (1.52–2.48)	1 × 10 ^{−7}
Inferior parietal	632	0 (0, 0, 1, 40)	2 (0, 0, 7, 30)	Multiplicative effect	2.18 (1.62–2.94)	3 × 10 ^{−7}	2.21 (1.64–2.99)	2 × 10 ^{−7}
Motor cortex	616	0 (0, 0, 0, 11)	0 (0, 0, 1, 12)	Multiplicative effect	2.04 (1.29–3.22)	0.002	2.25 (1.42–3.56)	0.0006
Visual cortex	618	0 (0, 0, 0, 12)	0 (0, 0, 1, 18)	Multiplicative effect	3.31 (2.06–5.31)	8 × 10 ^{−7}	3.73 (2.32–6.01)	6 × 10 ^{−8}
Hippocampus								
CA4	623	1 (0, 0, 2, 8)	2 (0, 1, 3, 22)	Multiplicative effect	2.30 (1.91–2.78)	3 × 10 ^{−18}	2.30 (1.90–2.78)	7 × 10 ^{−18}
CA2/3	622	1 (0, 0, 4, 50)	2 (0, 0, 6, 40)	Multiplicative effect	1.25 (1.00–1.56)	0.054	1.25 (1.00–1.57)	0.055
CA1	633	2 (0, 1, 7, 33)	7 (0, 2, 15, 60)	Multiplicative effect	1.82 (1.53–2.17)	1 × 10 ^{−11}	1.83 (1.53–2.18)	2 × 10 ^{−11}
Subiculum	633	2 (0, 1, 7, 60)	10 (0, 2, 25, 98)	Multiplicative effect	2.50 (2.07–3.01)	8 × 10 ^{−22}	2.51 (2.08–3.03)	7 × 10 ^{−22}
Entorhinal	518	7 (0, 3, 15, 50)	12 (0, 6, 20, 45)	Multiplicative effect	1.49 (1.29–1.73)	1 × 10 ⁸	1.47 (1.26–1.71)	6 × 10 ^{−7}
Basal nucleus	521	1 (0, 1, 2, 30)	3 (0, 1, 8, 45)	Multiplicative effect	2.06 (1.66–2.57)	1 × 10 ^{−10}	2.16 (1.73–2.68)	5 × 10 ^{−12}
Amygdala								
Basolateral	525	1 (0, 0, 3, 35)	2 (0, 1, 7, 35)	Multiplicative effect	2.06 (1.63–2.61)	2 × 10 ^{−9}	2.09 (1.65–2.64)	9 × 10 ^{−10}
Corticomedial	525	1 (0, 1, 4, 50)	6 (0, 2, 20, 80)	Multiplicative effect	2.56 (2.03–3.21)	1 × 10 ^{−15}	2.59 (2.06–3.25)	5 × 10 ^{−16}
SP counts								
Middle frontal	620	50 (0, 3, 50, 50)	50 (0, 50, 50, 50)	Odds ratio	4.97 (3.40–7.28)	5 × 10 ^{−16}	4.17 (2.77–6.27)	4 × 10 ^{−12}
		151 (52%)	274 (84%)					
Superior temporal	621	31 (0, 0, 50, 50)	50 (0, 50, 50, 50)	Odds ratio	4.90 (3.45–6.96)	5 × 10 ^{−19}	4.29 (2.99–6.15)	4 × 10 ^{−15}
		112 (38%)	246 (75%)					
Inferior parietal	621	50 (0, 1, 50, 50)	50 (0, 50, 50, 50)	Odds ratio	6.03 (4.06–8.97)	7 × 10 ^{−19}	5.20 (3.40–7.96)	3 × 10 ^{−14}
		151 (51%)	281 (86%)					
Motor cortex	605	6 (0, 0, 16, 50)	16 (0, 8, 26, 50)	Multiplicative effect	1.78 (1.46–2.17)	9 × 10 ^{−9}	1.80 (1.48–2.18)	3 × 10 ^{−9}
Visual cortex	606	10 (0, 0, 22, 50)	30 (0, 14, 48, 50)	Multiplicative effect	2.13 (1.77–2.57)	2 × 10 ^{−15}	2.12 (1.76–2.55)	1 × 10 ^{−15}

Continued

Table 6 Association of *APOE* ε4 with NFT and SP counts (*continued*)

Brain region	No.	NFT or SP counts		Association measure	Adjusting for age at death and sex		Additional adjustment for LBD subtype	
		0 ε4 alleles (n = 298)	1 or 2 ε4 alleles (n = 336)		Estimate (95% CI)	p Value	Estimate (95% CI)	p Value
Hippocampus								
CA4	607	0 (0, 0, 1, 25)	1 (0, 0, 6, 22)	Multiplicative effect	2.65 (1.93–3.64)	2 × 10 ^{−9}	2.60 (1.89–3.58)	5 × 10 ^{−9}
CA2/3	606	0 (0, 0, 1, 12)	1 (0, 0, 3, 16)	Multiplicative effect	1.85 (1.40–2.46)	2 × 10 ^{−5}	1.78 (1.34–2.35)	6 × 10 ^{−5}
CA1	607	1 (0, 0, 6, 36)	6 (0, 3, 12, 46)	Multiplicative effect	1.95 (1.59–2.41)	3 × 10 ^{−10}	1.89 (1.54–2.32)	9 × 10 ^{−10}
Subiculum	607	5 (0, 0, 12, 50)	17 (0, 10, 25, 50)	Multiplicative effect	2.22 (1.83–2.69)	4 × 10 ^{−16}	2.21 (1.84–2.66)	3 × 10 ^{−17}
Entorhinal	508	25 (0, 0, 50, 50)	50 (0, 33, 50, 50)	Odds ratio	4.75 (3.23–6.99)	5 × 10 ^{−15}	4.16 (2.80–6.17)	4 × 10 ^{−12}
≥50		88 (35%)	181 (71%)					
Amygdala								
Basolateral	507	8 (0, 0, 18, 50)	16 (0, 10, 27, 50)	Multiplicative effect	1.64 (1.34–2.02)	2 × 10 ^{−6}	1.65 (1.36–2.01)	6 × 10 ^{−7}
Corticomedial	506	11 (0, 0, 25, 50)	41 (0, 16, 50, 50)	Multiplicative effect	2.08 (1.69–2.56)	6 × 10 ^{−12}	2.06 (1.68–2.53)	3 × 10 ^{−12}

Abbreviations: CI = confidence interval; LBD = Lewy body disease; NFT = neurofibrillary tangle; SP = senile plaque.

The sample median (minimum, 25th percentile, 75th percentile, maximum) is given for NFT and SP counts in each different brain region. For all NFT and SP counts except middle frontal, superior temporal, inferior parietal, and entorhinal SP counts, *p* values result from negative binomial regression models. For middle frontal, superior temporal, inferior parietal, and entorhinal SP counts, because of the large number of counts equal to 50, we dichotomized these 4 outcomes as <50 or 50 and assessed associations of these dichotomous categorical variables with *APOE* ε4 using logistic regression models. *APOE* ε4 was considered under a dominant model (i.e., presence vs absence of the minor allele). Multiplicative effects are interpreted as the multiplicative increase on the mean NFT or SP count when the ε4 allele is present. Odds ratios are interpreted as the multiplicative increase in the odds of SP counts ≥50 when the ε4 allele is present. The sample sizes given represent the number of patients with an NFT or SP count measure in the given brain region.

have been previously reported.^{39–41} For our study, the association between distribution and counts of LBs and SPs creates a confounding situation, as *APOE* $\epsilon 4$ is also very strongly associated with SPs.²¹ We attempted to address this by stratifying our analysis by severity of AD pathology; however, for patients with low AD pathology for whom associations between $\epsilon 4$ and severity of LB pathology were observed, this greater degree of LB pathology was still accompanied by more SPs. Therefore, in analyses involving LB counts, we adjusted for Thal amyloid phase and Braak NFT stage and still observed higher levels of LB pathology in $\epsilon 4$ carriers in the low AD pathology subgroup. These findings were slightly weaker but still apparent with additional adjustment for SP counts in order to address the total burden of amyloid in the given brain region along with the overall distribution.

In addition to mechanisms that are based on an amyloid cascade hypothesis, there may be other plausible reasons to explain how *APOE* might influence LB pathology. The major protein component of LBs, α -synuclein, is a lipid-binding protein with an apolipoprotein-like domain in the amino-terminal half of the molecule,^{42,43} a domain in which most of the coding region mutations that cause PD and DLB are located.⁴⁴ There is evidence to suggest that a physiologic function of α -synuclein is to bind to and “bend” membranes⁴⁵ to promote synaptic vesicle release and recycling through this domain.⁴⁶ Furthermore, the specific role of ApoE4 protein isoform in α -synucleinopathy and possible roles in α -synuclein release, transmission, or uptake could support intracellular accumulation and thus aggregation. Like apolipoproteins, α -synuclein is linked to brain cholesterol metabolism,⁴⁷ with increases in cholesterol in α -synuclein knockout mice.^{48–50} The indirect influence of ApoE on membrane lipid composition may contribute to the pathophysiology of α -synuclein.

Several limitations of our study are important to bear in mind. First, our series of LBD cases was a sample of convenience and therefore may not be representative of the larger population of patients with LBD. Second, controls were diagnosed based on clinical rather than neuropathologic criteria. However, the lack of clinically diagnosed neurologic disease would likely correspond to a similar lack of notable LB or AD pathology, and the associations between $\epsilon 4$ and risk of the majority of the LBD pathologic subgroups that were examined were so strong that they would not be notably altered by any unlikely presence of significant LB or AD pathology in controls. Third, clinical diagnoses were not made consistently using uniform guidelines but were instead made retrospectively based on medical chart review, which is an inherent limitation of studying a series obtained from a brain bank. These clinical diagnoses were given only to provide an approximation as to what clinical syndromes these pathologically diagnosed LBD cases had, and they should be considered as exploratory and interpreted with caution.

Acknowledging the relatively small sample sizes of some of the pathologic subgroups, our results provide the strongest evidence to date of an independent association between *APOE* $\epsilon 4$

and severity of LB pathology. These findings increase our understanding of the mechanism behind reported associations of $\epsilon 4$ with risk of DLB and PDD. As *APOE* $\epsilon 4$ has generally not been associated with risk of PD, our findings suggest that it may function as a modifier of processes that favor LB spread rather than acting directly to initiate LB pathology.

Author contributions

Dennis W. Dickson: study concept and design, analysis or interpretation of data, sample preparations or genotyping, drafting/revising the manuscript. Michael G. Heckman: analysis or interpretation of data, drafting/revising the manuscript. Melissa E. Murray: sample preparations or genotyping, drafting/revising the manuscript. Alexandra I. Soto: sample preparations or genotyping, drafting/revising the manuscript. Ronald Walton: sample preparations or genotyping, drafting/revising the manuscript. Nancy N. Diehl: analysis or interpretation of data, drafting/revising the manuscript. Jay A. van Gerpen: contribution of patients, drafting/revising the manuscript. Ryan J. Uitti: contribution of patients, drafting/revising the manuscript. Zbigniew K. Wszolek: contribution of patients, drafting/revising the manuscript. Nilüfer Ertekin-Taner: analysis or interpretation of data, drafting/revising the manuscript. David S. Knopman: contribution of patients, drafting/revising the manuscript. Ronald C. Petersen: contribution of patients, drafting/revising the manuscript. Neill R. Graff-Radford: contribution of patients, drafting/revising the manuscript. Bradley F. Boeve: contribution of patients, drafting/revising the manuscript. Guojun Bu: analysis or interpretation of data, drafting/revising the manuscript. Tanis J. Ferman: contribution of patients, analysis or interpretation of data, drafting/revising the manuscript. Owen A. Ross: study concept and design, analysis or interpretation of data, drafting/revising the manuscript.

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