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ORIGINAL ARTICLE

Involvement of *ApoE4* in dementia with Lewy bodies in the prodromal and demented stages: evaluation of the Strasbourg cohort

Olivier Bousiges · Benjamin Cretin · Candice Muller · Anne Botzung · Lea Sanna · Pierre Anthony · Nathalie Philippi · Catherine Demuynck · Frédéric Blanc

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Abstract *ApoE4* as a risk factor for dementia with Lewy bodies (DLB) is still an issue. We sought to determine the involvement of *ApoE4* according to different clinical parameters in our cohort of patients from Strasbourg, France. ApoE genotyping was performed on the AlphaLewyMA cohort. In this cohort, 197 patients were genotyped: 105 DLB patients, 37 Alzheimer's disease (AD) patients, 29 patients with AD/DLB comorbidity, and 26 control subjects (CS). The groups of patients were also classified according to the stage of evolution of the disease: prodromal or demented. We analyzed other parameters in relation to *ApoE4* status, such as years of education (YOE) and Alzheimer CSF biomarkers. We observed a higher proportion of *ApoE4* carriers in the AD (51.4%) and AD/DLB (72.4%) groups compared to the DLB (25.7%) and CS (11.5%) groups ($p < 0.0001$). We found a correlation between age at disease onset and YOE in the AD group ($p = 0.039$) but not in the DLB group ($p = 0.056$). Interestingly, in the DLB group, the subgroup of patients with high YOE (≥ 11) had significantly more patients with *ApoE4* than the subgroup with low YOE (< 11). AD biomarkers did not seem to be impacted by the presence of *ApoE4*, except for Aβ42: DLB *ApoE4*-positive demented patients showed a more marked Aβ42 decrease. *ApoE4* does not appear to be a risk factor for "pure" DLB patients. These results suggest a strong link between *ApoE4* and amyloidopathy and consequently with AD. Trial registration: AlphaLewyMa, Identifier: NCT01876459, date of registration: June 12, 2013.

O. Bousiges (✉) · B. Cretin · A. Botzung · N. Philippi · F. Blanc
ICube Laboratory UMR 7357 and FMTS (Fédération de Médecine Translationnelle de Strasbourg), IMIS Team and IMAGeS Team, University of Strasbourg and CNRS, Strasbourg, France
e-mail: olivier.bousiges@chru-strasbourg.fr

O. Bousiges
Laboratory of Biochemistry and Molecular Biology, University Hospital of Strasbourg, Strasbourg, France

B. Cretin · C. Muller · A. Botzung · L. Sanna · C. Demuynck · F. Blanc
CM2R (Research and Resources Memory Center), Geriatrics Department, Geriatric Day Hospital, Neurogeriatric Service, University Hospital of Strasbourg, Strasbourg, France

P. Anthony · N. Philippi
CM2R, Neuropsychology Unit, Head and Neck Department, Neurology Department, University of Strasbourg, Strasbourg, France

P. Anthony
CM2R, Geriatrics Department and Neurology Department, Colmar General Hospital, Colmar, France

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Introduction

Alzheimer's disease (AD) and dementia with Lewy bodies (DLB) are the two main age-related cognitive neurodegenerative diseases. The differential diagnosis between these two pathologies is difficult. Many symptoms of DLB are close to those of AD, especially at the onset of the pathology: deficits in executive functions, visual memory, and visuo-constructive and visuospatial abilities, with weaknesses for episodic memory, short-term and working memory, verbal initiation, praxis, and language, as well as social cognition [1]. DLB is also close to Parkinson's disease (PD) due to the presence of parkinsonism (bradykinesia, rigidity, and postural instability), which is often discrete, especially at the onset of the disease [2, 3]. DLB is also close to PD because of its pathophysiology, with the presence of positive α-synuclein (α-syn) aggregates in the brain, forming Lewy bodies [4]. While α-syn aggregates are mostly localized in the brainstem and in the substantia nigra at the onset of PD, they are rather diffuse throughout the brain in the early stages of DLB.

Regarding genetic risk factors, however, AD and DLB are relatively different. AD and DLB have been the subject of numerous genome-wide association studies (GWAS), revealing many risk factors for AD, such as TREM2, PICALM, BIN1, CLU, CR1, SORL1, and CD33 [5, 6], some of which are even correlated with Alzheimer biomarkers [7], but different risk factors for DLB: such as SNCA, GRN, LRP10, SNCB, LRRK2, and GBA [8–11].

Of all the genetic risk factors so far identified for AD, *apolipoprotein E4* (*ApoE4*) appears to be the most important [12, 13]. For DLB, there is a substantial literature suggesting that *ApoE4* may also be a risk factor, including autopsy studies [14–16] and GWAS [9–11, 17]. However, recent neuropathologic studies have questioned this, pointing out that this risk factor is only associated with DLB when there is an important AD comorbidity [18–22] and calling into question the direct impact of *ApoE4* on synucleinopathy (studies agreeing with the view that *ApoE4* is not a risk factor for PD, e.g., [14]). In the past, some clinical studies have also questioned the notion of *ApoE4* as a risk factor for DLB [23–25].

Based on the neuropathologic studies, it seems important to be able to identify AD/DLB comorbidities if we want to determine whether *ApoE4* is truly a genetic risk factor for DLB or more globally for comorbidity. From a clinical point of view, the dual diagnosis is not simple and is rarely made in practice even though anatomopathological studies indicate that AD/DLB comorbidities is a frequent occurrence. Indeed, 77% of autopsy-confirmed AD/DLB comorbidity patients were clinically diagnosed with AD [26]. Some authors even reported that 87% of DLB patients had moderate to abundant cortical amyloid plaques [27]. Thus, it seems important in studies without autopsy verification to be able to diagnose AD/DLB comorbidity, in particular by using clinical and biomarkers, as we endeavor to do in our studies [28].

We analyzed *ApoE4* as a risk factor in a cohort of AD, DLB, and AD/DLB patients followed for at least 5 years. This cohort is very well described from a clinical and biological point of view [28, 29] so these patients are quite well characterized. The study of *ApoE4* in this cohort allowed us to conclude that *ApoE4* is probably not a direct risk factor for DLB. Interestingly, DLB patients with a high number of years of education were more likely to be *ApoE4* carriers than DLB patients with a low number of years of education. Lastly, CSF Aβ42 levels of DLB patients tended to be lower in *ApoE4*-positive demented patients.

Methods

Patients

All patients included in the present study had been enrolled in a hospital clinical research protocol called AlphaLewyMA (registered in ClinicalTrials.gov: https://clinicaltrials.gov/ct2/show/NCT01876459) at the tertiary Memory Clinic (CM2R) of Alsace, France, by an experienced team of neurologists, geriatricians, and neuropsychologists between June 2013 and June 2018 (but whose follow-up is still ongoing). The CM2R of Alsace comprises three different centers, two at the University Hospitals of Strasbourg (*CHU Hautepierre* and *Hôpital de la Robertsau*) and

one at *Hôpitaux Civils de Colmar*. Patients in the AlphaLewyMA cohort underwent detailed clinical evaluation, an extensive neuropsychological evaluation, blood examination, brain MRI (3 T), and lumbar puncture for CSF biomarkers, as previously described [28]. This study is therefore a retrospective study on data collected prospectively.

DLB patients were selected for the AlphaLewyMA cohort according to McKeith's criteria (probable DLB, based on the existence of two core symptoms in addition to cognitive decline) for DLB demented (DLB-d) patients and for patients with prodromal DLB (pro-DLB), also called mild cognitive impairment with Lewy bodies (MCI-LB) ([2, 30]). Fluctuations were assessed with the Mayo Clinic Fluctuations Scale [31]. The hallucination Parkinson's disease–associated psychotic symptom questionnaire was used to evaluate the presence of hallucinations [32]. Rapid eye movement (REM) sleep behavior disorder (RBD) was evaluated using a questionnaire based on the article by Gjerstad et al. [33], simplified into two questions for the patient and the caregiver, one concerning movements during sleep and the other concerning vivid dreams and nightmares.

Patients with AD were selected for the AlphaLewyMA cohort according to Albert's criteria [34] and Dubois' criteria [35] for patients with pro-AD and McKhann's criteria [36] and Dubois' criteria [35] for demented AD patients.

Patients were considered to have DLB and AD (AD/DLB) when they met both the Dubois' criteria and the McKeith's criteria concurrently. For example, a patient with memory storage disorders, CSF findings in favor of AD, and two of the four clinical criteria for DLB was considered to have both DLB and AD.

Table 1 summarizes the main clinical information of the patients included in the present study. This clinical information was collected throughout the follow-up of the patients. A total of 197 patients were ApoE genotyped for this study: 26 control subjects (CS group), 68 patients with DLB at the prodromal stage (pro-DLB group), 37 patients with DLB at the demented stage (DLB-d group), 12 AD patients at the prodromal stage (pro-AD group), 25 AD patients at the demented stage (AD-d group), and 29 patients with both the criteria of AD and criteria of probable DLB (30), divided into two groups (pro-AD/DLB group ($n=5$) and AD/DLB-d group ($n=24$) (see flowchart in Fig. 1). The CS group consisted of patients originally included in the study with cognitive disorders as found in AD and DLB, who, after follow-up in the study, were found to have neither AD nor DLB. The CS group had various diagnoses, defined according to international criteria (for details, see Table 1).

ApoE genotyping

To perform ApoE genotyping, a blood tube (EDTA) was collected at the inclusion visit and centrifuged upon receipt in the laboratory. DNA extraction was performed on QIAcube Connect Blue (Qiagen) using a QIAamp DNA mini kit.

After DNA extraction, the apolipoprotein E (APOE) gene polymorphism was determined by PCR using 200 ng of genomic DNA amplified using the following primers: s2: GGG CAC GGC TGT CCA AGG AGC TG; as22: TTC GCG GGC CCC GGC CTG GTA CAC T. The polymerase chain reaction (PCR) was initiated with a 94 °C pre-denaturation for 5 min followed by pre-hybridization at 60 °C during 2 min, then an amplification for 35 cycles (2 min 72 °C ramp for 1° C/s, 30 s 95 °C ramp for 1 °C/s, 2 min 60 °C ramp for 1 °C/s), and a final extension for 10 min at 72 °C using 2.5 µL Taq 10X Master Mix. The PCR product (4 µL) was digested at 0.2 µL HhaI overnight at 37 °C, and the digested PCR product was resolved on an 8% non-denaturing acrylamide gel, which was electrophoresed at 105 V for 65 min. The gel was visualized under ultraviolet light to determine the APOE genotype (37). All participants were categorized into six groups according to the allele pattern of the APOE gene (E2/E2; E2/E3; E3/E3; E3/E4; E2/E4; E4/E4).

Statistical analysis

Statistical analyses were carried out using Graph-Pad PRISM, V.8 (GraphPad, San Diego, CA, USA). Normally distributed data were analyzed using one-way analysis of variance with Tukey's post hoc analyses to determine between-group differences. In the case of non-Gaussian-distributed parameters, we used the Kruskal–Wallis test with Dunn's multiple comparison test. In the case of contingency analyses, a χ^2 test was used with post hoc Fisher's exact test when appropriate. In the case of a Gaussian distribution of the

Table 1 Clinical and demographic characteristics of patients

		DLB $N=105$		AD $N=37$		AD + DLB $N=29$				
		Pro-DLB $N=68$	DLB-d $N=37$	Pro-AD $N=12$	AD-d $N=25$	Pro-AD/DLB $N=5$	AD/DLB-d $N=24$	CS[f] $N=26$	Test statistic, P	Post hoc[g]
Age, years[a]		68.3 (8.9)	75.0 (8.7)	74.6 (7.7)	76.6 (7.3)	67.2 (9.3)	73.2 (9.6)	66.3 (9.5)	$H=31.05$, $p<0.0001$	CS < DLB-d, AD-d; Pro-DLB < DLB-d, AD-d
Age at onset, years		62.9 (9.9) (17ND)	70.6 (10.7) (7ND)	73.3 (7.4) (5ND)	73.2 (8.2) (7ND)	63.3 (11.3) (1ND)	68.2 (12.0) (9ND)	56.7 (9.9) (9ND)	$F=6.635$, $p<0.0001$	Pro-DLB, CS < DLB-d, pro-AD, AD-d; CS < AD/DLB-d
Gender (F/M)		38/29	20/17	5/7	18/7	2/3	13/11	16/10	$\chi^2=4.468$, $p=0.6136$	
MMSE score[b]		27.7 (1.6)	21.3 (3.4)	27.1 (1.3)	21.1 (2.9)	28.0 (1.4)	21.5 (5.8)	27.5 (2.1)	$H=134.1$, $p<0.0001$	CS, pro-DLB, pro-AD and pro-AD/DLB > DLBd, ADd, and AD/DLBd
Years of education		12.7 (4.1) (4ND)	10.4 (4.5) (2ND)	13.7 (5.0) (1ND)	10.7 (3.4) (3ND)	10.4 (2.6)	11.3 (3.3) (5ND)	12.7 (3.2) (3ND)	$H=11.42$, $p=0.0763$	
Hallucinations[c]		72.1%	73.0%	8.3%	30.4% (2ND)	40.0%	41.7% (2ND)	26.9%	$\chi^2=38.12$, $p<0.0001$	CS, pro-AD, ADd, AD/DLBd < pro-DLB; CS, pro-AD, ADd < DLBd
Fluctuations[c]		89.7%	97.3%	16.7%	30.4%	100%	86.3% (2ND)	42.3%	$\chi^2=75.52$, $p<0.0001$	CS, pro-AD, ADd < pro-DLB, DLBd, pro-AD/DLB, AD/DLBd;
Parkinsonism	Rigidity 0/1/2/3/4	11/47/9/1/0	4/15/16/2/0	11/1/0/0/0	18/4/0/1/0 (2ND)	1/4/0/0/0	5/16/1/0/0 (2ND)	14/8/1/2/1	$H=57.41$, $p<0.0001$	DLB-d > CS, pro-AD and AD-d; pro-DLB > pro-AD and AD-d; AD/DLB-d > pro-AD

Table 1 (continued)

		DLB $N=105$		AD $N=37$		AD + DLB $N=29$					
		Pro-DLB $N=68$	DLB-d $N=37$	Pro-AD $N=12$	AD-d $N=25$	Pro-AD/DLB $N=5$	AD/DLB-d $N=24$	CS[f] $N=26$		Test statistic, P	Post hoc[g]
	Akinesia 0/1/2/3/4	12/40/13/2/1	2/12/13/10/0	11/1/0/0/0	19/3/0/1/0 (2ND)	3/2/0/0/0	9/10/2/1/0 (2ND)	16/5/2/2/1		$H=67.89$, $p<0.0001$	DLB-d > CS, pro-DLB, pro-AD, AD-d, pro-AD/DLB and AD/DLB-d; pro-DLB > pro-AD, ADd
	Tremor at rest 0/1/2/3/4	31/36/1/0/0	23/14/0/0/0	12/0/0/0/0	22/0/0/0/0 (3ND)	2/3/0/0/0	16/5/0/0/0 (3ND)	20/6/0/0/0		$H=39.29$, $p<0.0001$	Pro-DLB > pro-AD, and ADd
RBD[c]		66.7% (2ND)	54.1%	0.0%	4.3% (2ND)	60%	45.5% (2ND)	23.1%		$\chi^2=44.94$, $p<0.0001$	CS, pro-AD, ADd < pro-DLB, DLBd, AD/DLBd; pro-AD, ADd < pro-AD/DLB, AD/DLBd
Hippocampi atrophy[d] 0/1/2/3/4	Left hippocampus	19/18/15/10/1 (5ND)	2/7/9/6/5 (8ND)	2/2/3/0/1 (4ND)	2/2/5/6/2 (8ND)	0/2/0/3/0	2/6/8/2/2 (4ND)	7/11/3/2/0 (3ND)		$H=22.92$, $p=0.0008$	DLB-d, ADd > CS; DLB-d > pro-DLB
	Right Hippocampus	19/21/16/7/0 (5ND)	5/8/6/5/5 (8ND)	2/2/3/0/1 (4ND)	2/5/4/5/1 (8ND)	0/2/1/2/0	1/7/8/2/2 (4ND)		7/9/6/1/0 (3ND)	$H=15.96$, $p=0.014$	
FCSRT[e]		50%	83.8%	66.7%	100%	80%	95.5% (2ND)	44% (1ND)		$\chi^2=41.36$, $p<0.0001$	CS, pro-DLB < DLBd, ADd, AD/DLBd; pro-AD < ADd, AD/DLBd

Age at time of lumbar puncture and cognitive evaluation. Mean (standard deviation). [b]Mean (standard deviation). [c]Percentage. [d]According to Scheltens et al., JNNP, 1992. [e]Percentage of deficient patients

[f]The group included patients suffering from depression ($n=1$), neurosis ($n=1$), vascular dementia and depression ($n=1$), sleep apnea syndrome and primary age-related tauopathy (PART) ($n=1$), traumatic brain injury and left parietal meningeal hemorrhage ($n=1$), corticobasal degeneration (CBD) ($n=1$), low-grade glioma left-antero-fronto insula ($n=1$), cognitive impairment due to diabetes and depression ($n=1$); insulotemporale cavernome ($n=1$); vacsular dementia and frontotemporal dementia (FTD) ($n=1$), mild cognitive impairment in the context of inflammatory rheumatism ($n=1$), temporal epilepsy and limbic encephalitis ($n=1$), bipolar ($n=2$), neuroborreliosis ($n=1$), temporal epilepsy ($n=2$), progressive supranuclear palsy (PSP) ($n=4$), primary age-related tauopathy (PART) ($n=1$), stroke ($n=1$), stable mild cognitive impairment ($n=1$), toxic white matter lesions ($n=1$), and 1 healthy control

[g]Kruskall-Wallis post hoc test (H) with Dunn's multiple comparisons test, ordinary one-way ANOVA (F) with Tukey's multiple comparisons test

MMSE Mini-Mental Status Examination, *N* number, *ND* no data, *RBD* rapid eye movement sleep behavior disorder, *FCSRT* Free and Cued Selective Reminding Test

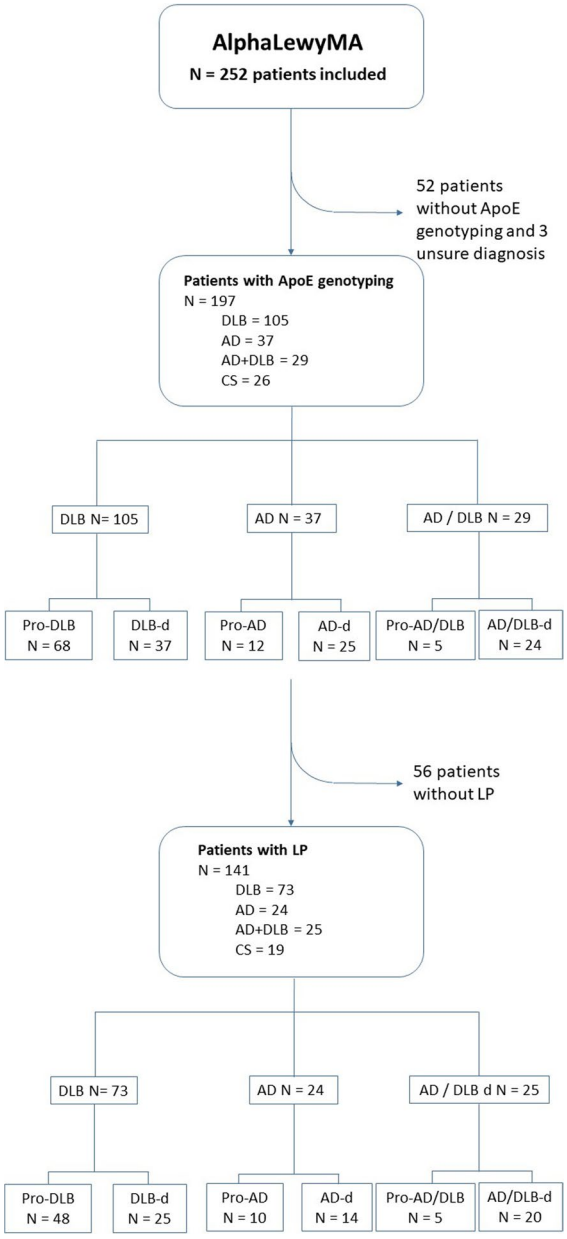

Fig. 1 Flowchart of patient selection from the AlphaLewyMA cohort. AD Alzheimer's disease, DLB dementia with Lewy bodies, LP lumbar puncture, Pro-AD prodromal-AD, Pro-DLB prodromal-DLB, AD-d AD-demented, DLB-d DLB-demented

results, then an analysis of the standard deviations (SDs) is performed using a Brown-Forsythe test. In the case of a difference in SDs, then a Brown-Forsythe and Welsh ANOVA correction is performed. Note that no correction was necessary in this study.

Results

The study population's demographic characteristics are presented in Table 1. The results are in line with those obtained in our previous results on this cohort [28, 29].

ApoE4 statistics

Table 2 shows the genotyping frequency in each group (no statistical analysis was performed because some genotypes such as E2/E2 were absent in some of the groups) and the percentage of E4 carriers and the allelic frequency (E2, E3, E4) in each diagnostic group. Statistical analyses showed that there were significantly more E4 carriers (DLB vs AD vs AD/DLB vs CS: $\chi^2 = 32.22$, $p < 0.0001$) in the AD and AD/DLB groups than in the CS group (CS vs AD: $p = 0.0012$; CS vs AD/DLB: $p < 0.0001$), but also in the DLB (DLB vs AD: $p = 0.0073$; DLB vs AD/DLB: $p < 0.0001$) groups. There was no significant difference between the AD/DLB group and the AD group (AD vs AD/DLB: $p = 0.1274$). Allelic frequency analysis confirmed these results (DLB vs AD vs AD/DLB vs CS: $\chi^2 = 33.43$, $p < 0.0001$): there were significantly more E4 carriers among the AD patients (AD and AD/DLB) than among the other patients (CS and DLB) (CS vs AD: $\chi^2 = 15.33$, $p = 0.0005$; CS vs AD/DLB: $\chi^2 = 16.33$, $p = 0.0003$; DLB vs AD: $\chi^2 = 11.71$, $p = 0.0029$; DLB vs AD/DLB: $\chi^2 = 17.53$, $p = 0.0002$). No significant difference was found between the CS group and pure DLB patients, either in terms of E4 carriers or in terms of allele frequency (E4 carriers $p = 0.1911$; allele frequency $\chi^2 = 4.62$, $p = 0.10$).

Years of education and age at disease onset according to *ApoE4* genotyping

The number of years of education (YOE) was assessed in the cohort. No differences were observed between the different groups; all groups of patients had on average the same YOE (Table 1). In a second step, we studied the correlation between the YOE and the reported age at onset of the disease (DLB, AD, AD/DLB). Note that for a number of patients, the number of years before the onset of symptoms was not known; we decided to exclude these patients from the analysis rather than

Table 2 *ApoE* genotyping, carrier status, and frequency (analysis)

		Genotype frequency *N* (%)						E4 carrier *N* (%)	Allele frequency *N* (%)		
Group	Patients *N*	2/2	2/3	2/4	3/3	3/4	4/4		2	3	4
Pro-DLB	68	0 (0.0)	9 (13.2)	1 (1.5)	42 (61.8)	15 (22.1)	1 (1.5)	17 (25.0)	10 (7.4)	108 (79.4)	18 (13.2)
DLB-d	37	0 (0.0)	3 (8.1)	1 (2.7)	24 (64.9)	8 (21.6)	1 (2.7)	10 (27.0)	4 (5.4)	59 (79.7)	11 (14.9)
DLB (pro + d)	**105**	**0 (0.0)**	**12 (11.4)**	**2 (1.9)**	**66 (62.9)**	**23 (21.9)**	**2 (1.9)**	**27 (25.7)**	**14 (6.7)**	**167 (79.5)**	**29 (13.8)**
pro-AD	12	0 (0.0)	1 (8.3)	0 (0.0)	6 (50.0)	4 (33.3)	1 (8.3)	5 (41.7)	1 (4.2)	17 (70.8)	6 (25.0)
AD-d	25	0 (0.0)	1 (4.0)	0 (0.0)	10 (40.0)	11 (44.0)	3 (12.0)	14 (56.0)	1 (2.0)	32 (64.0)	17 (34.0)
AD (pro + d)	**37**	**0 (0.0)**	**2 (5.4)**	**0 (0.0)**	**16 (43.2)**	**15 (40.5)**	**4 (10.8)**	**19 (51.4)**	**2 (2.7)**	**49 (66.2)**	**23 (31.1)**
AD/DLB	**29**	**0 (0.0)**	**3 (10.3)**	**1 (3.5)**	**5 (17.2)**	**19 (65.5)**	**1 (3.5)**	**21 (72.4)**	**4 (6.9)**	**32 (55.2)**	**22 (37.9)**
CS	**26**	**1 (3.9)**	**5 (19.2)**	**0 (0.0)**	**17 (65.4)**	**3 (11.5)**	**0 (0.0)**	**3 (11.5)**	**7 (13.5)**	**42 (80.8)**	**3 (5.8)**
								DLB vs AD vs AD/DLB vs CS: $\chi^2 = 32.22$, $p < 0.0001$ **Post hoc: Fisher's exact test** CS vs DLB: $p = 0.1911$ **CS vs AD: $p = 0.0012$** **CS vs AD/DLB: $p < 0.0001$** **DLB vs AD: $p = 0.0073$** **DLB vs AD/DLB: $p < 0.0001$** AD vs AD/DLB: $p = 0.1274$	**DLB vs AD vs AD/DLB vs CS: $\chi^2 = 33.43$, $p < 0.0001$** **Post hoc: Chi-square** CS vs DLB: $\chi 2 = 4.62$, $p = 0.10$ **CS vs AD: $\chi^2 = 15.33$, $p = 0.0005$** **CS vs AD/DLB: $\chi^2 = 16.33$, $p = 0.0003$** **DLB vs AD: $\chi^2 = 11.71$, $p = 0.0029$** **DLB vs AD/DLB: $\chi^2 = 17.53$, $p = 0.0002$** AD vs AD/DLB: $\chi^2 = 2.35$, $p = 0.3085$		

Values in bold indicate complete groups, i.e. prodromal and demented

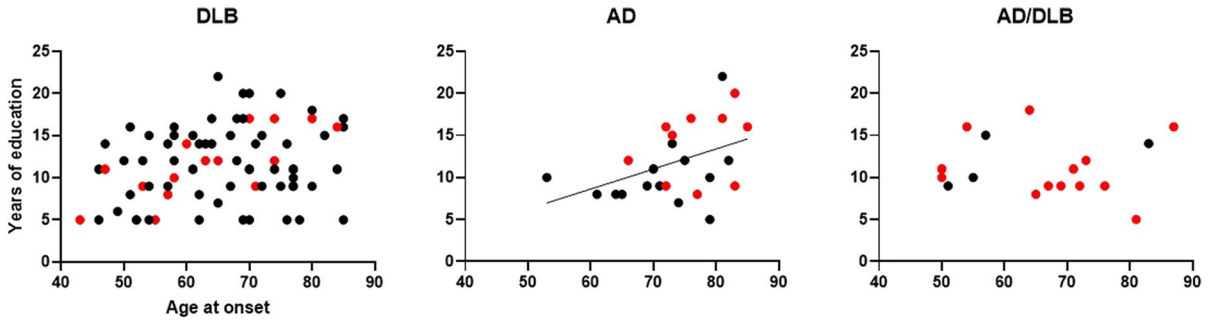

Fig. 2 Correlation between years of education and age at onset for pure DLB, pure AD, and AD/DLB. Years of education was correlated with age at onset for AD patients only ($r=0.4247$, $p=0.039$), although for DLB patients, the trend was strong ($r=0.2203$, $p=0.056$), whereas for the AD/DLB group, no correlation was observed ($r=-0.02693$, $p=0.918$). Black dots represent non-*ApoE4*-carrier patients; red dots represent *ApoE4*-carrier patients. DLB dementia with Lewy bodies, AD Alzheimer's disease, AD/DLB dementia with Lewy bodies and Alzheimer's disease

using the age at the time of the first consultation (DLB $N=76$; AD $N=24$; AD/DLB $N=17$). In AD patients, there was a positive correlation between age at onset and YOE ($r=0.425$, $p=0.039$). For DLB patients, although the correlation was not significant, there was a relatively strong positive trend between age at onset and YOE ($r=0.22$, $p=0.056$). For patients with AD/DLB comorbidity, there was no correlation between age at onset and YOE ($r=-0.0269$, $p=0.918$) (Fig. 2).

In Fig. 2, *ApoE4* patients are indicated with a red circle. As Boot and colleagues showed that DLB patients more commonly had higher YOE than AD patients [25], we wanted to further analyze the YOE according to the presence or not of *ApoE4*. For this purpose, we calculated the median YOE in the DLB group in order to have balanced groups (median education = 11 years; i.e., 1 year before Baccalaureat level). Thus, we separated patients with a YOE higher than or equal to 11 and patients with a YOE less than 11 (Table 3). We observed that the group with a high YOE had significantly more *ApoE4*-carrier patients than the group with a low YOE (Fisher's exact test $p=0.03$). Interestingly, DLB patients with a high YOE had a strong tendency to have more *ApoE4*-carriers than controls ($p=0.064$) and had a strong tendency to have fewer *ApoE4* carriers than AD patients ($p=0.057$).

Age at onset according to *ApoE4* presence

We sought to determine whether the presence of *ApoE4* in patients was associated with an earlier onset of the disease. We found that, for each of the diseases, *ApoE4* carriers did not have an earlier onset of the disease when compared to non-carriers. However, it was notable that DLB *ApoE4*-carrier patients started their disease significantly earlier than AD *ApoE4*-carrier patients (Fig. 3; $p=0.044$).

Alzheimer biomarkers and *ApoE4*

For a part of the cohort (141 patients), we had the results of the Alzheimer biomarker analysis (see flowchart in Fig. 1 and Table 4). These biomarkers had been analyzed previously [29, 38], but without

Table 3 *ApoE4* and years of education in DLB patients

	CS, $N=26$ (%)	DLB with YOE < 11, $N=35$ (%)	DLB with YOE ≥ 11, $N=64$ (%)	AD, $N=37$ (%)	AD/DLB, $N=29$ (%)	Test statistic, p	Post hoc
ApoE4 carrier	3 (11.5)	4 (11.4)	20 (31.25)	19 (51.4)	21 (72.4)	$\chi^2=37.39$, $p<0.0001$	CS < AD and AD/DLB; DLB < 11 < DLB ≥ 11, AD, and AD/DLB
Non-*ApoE4* carrier	23 (88.5)	31 (88.6)	44 (68.75)	18 (48.6)	8 (27.6)		

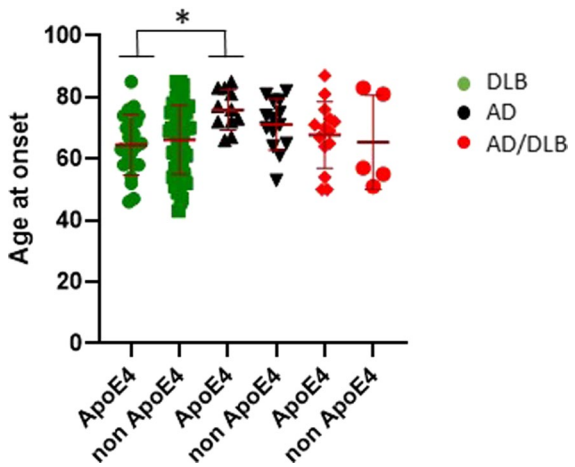

Fig. 3 Age at onset of disease as a function of *ApoE4*. Green labeling, DLB patients; black labeling, AD patients; red labeling, AD/DLB patients. DLB dementia with Lewy bodies, AD Alzheimer's disease, AD/DLB dementia with Lewy bodies and Alzheimer's disease

including the *ApoE4* results. Figure 4 shows that the presence or absence of *ApoE4* did not seem to significantly influence the levels of the different biomarkers. Note that the results of the Aβ42/Aβ40 ratio are not presented for the AD patients because we only had the results of one *ApoE4* carrier and 4 non-carriers, which was not sufficient to perform statistical analysis.

We decided to take the analysis a step further. Indeed, we had previously shown an Aβ42 decrease between the prodromal and demented stages of DLB patients [29, 38]. We therefore analyzed Aβ42 levels between prodromal and demented DLB patients according to the presence or absence of *ApoE4*. Aβ42 levels were significantly lower in demented *ApoE4* carriers than in prodromal non-*ApoE4* carriers (Fig. 5; $p = 0.015$). The one-way ANOVA did not reveal any significant difference between the demented and prodromal *ApoE4* groups ($p = 0.151$); however, a *t*-test between these two groups showed a significant difference ($p = 0.016$) and a *t*-test between demented non-*ApoE4* carriers and demented *ApoE4* carriers indicated a strong trend ($p = 0.068$). A larger group would most likely be needed in order to confirm these variations. Overall, we can conclude that the Aβ42 decrease at the demented stage seemed to be more prominent in *ApoE4* carrier patients.

As the *ApoE4* carrier patients in the DLB group were mainly patients with a high YOE, we wanted to check whether the Aβ42 levels were lower in this group compared to patients with a low YOE and compared to the non-*ApoE4*-carrier patients. We found that this was not the case: we found no significant difference (*ApoE4* high YOE; non-*ApoE4* high YOE; *ApoE4* low YOE; non-*ApoE4* low YOE; ANOVA, $p = 0.461$; data not shown).

Discussion

Is *ApoE4* really a risk factor for pure DLB?

Despite very different percentages of *ApoE4* presence between CS (11.5%) and DLB (25.7%), we did not find a significant difference between the two groups (see also the discussion in the "YOE and ApoE4" section below). This result suggests that *ApoE4* is finally not a risk factor for pure DLB, a view that is therefore in agreement with a number of recent publications [18–21, 23–25].

In a second step, we showed that there were significantly more *ApoE4* carriers in the AD group (51.4%) than in the CS group (11.5%) but also in the DLB group (25.7%). Interestingly, we showed that the proportion of ApoE4 carriers was even higher in the AD/DLB group (72.4%). Allelic frequency assessment confirmed these results, with a higher frequency of *ApoE4* carriers in the AD (31.1%) and AD/DLB (37.9%) groups compared to the CS (5.8%) and DLB (13.8%) groups. While the presence of *ApoE4* as a risk factor for AD is no longer debated, we show, as expected, that *ApoE4* is also a risk factor for AD/DLB comorbidity. van Steenoven and colleagues also found that AD/DLB patients were more often *ApoE4* carriers than pure DLB patients (39). Interestingly, in a more global way, an autopsy study with 766 patients showed that *apoE4* was a risk factor for copathologies independently of the neurodegenerative disease [40].

How can conclusions about *ApoE4* as a risk factor in DLB be so different between studies? As mentioned in the introduction, the diagnosis of AD/DLB comorbidity is rarely made and this group of patients is almost never represented in the clinical studies. Indeed, at no time have patients with comorbidities been included in the GWAS studies [9–11, 17]. If these comorbidities are not indicated, it is perhaps

Table 4 Biomarker values

		DLB $N=73$		AD $N=24$		AD/DLB $N=25$				
		Pro-DLB $N=48$	DLB-d $N=25$	Pro-AD $N=10$	AD-d $N=14$	Pro-AD/DLB $N=5$	AD/DLB-d $N=20$	CS $N=19$	Test statistic, p	Post hoc
CSF biomarkers	t-Tau (ng/L)	268 (113)	308 (98)	551 (134)	776 (290)	795 (314)	644 (229)	268 (81)	$H=87.44$ $p<0.0001$	CS, pro-DLB, DLB-d < pro-AD, AD-d, pro-AD/DLB, AD/DLB-d
	P-Tau (ng/L)	44 (17)	47 (14)	85 (21)	96 (27)	101 (44)	91 (31)	42 (14)	$H=82.32$ $p<0.0001$	CS, pro-DLB < pro-AD, AD-d, pro-AD/DLB, AD/DLB-d; DLB-d < pro-AD, AD-d, AD/DLB-d;
	Aβ42 (ng/L)	927 (290)	734 (249)	703 (288)	523 (148)	770 (345)	583 (212)	1040 (199)	$F=11.23$ $p<0.0001$	CS > DLB-d, pro-AD, AD-d, AD/DLB-d; pro-DLB > DLB-d, AD-d, AD/DLB-d
	Aβ40 assays	**DLB $N=38$**		**AD $N=5$**		**AD/DLB $N=14$**				
		Pro-DLB $N=27$	**DLB-d $N=11$**					**CS[f] $N=8$**		
	Aβ40 (ng/L)	9165 (3462)	8536 (2844)	15,639 (4280) 0.0466 (0.0188)		13,396 (5554) 0.0466 (0.0154)		11,519 (4711)	$H=15.82$ $p=0.0033$	Pro-DLB < AD, AD/DLB
	Aβ42/Aβ40	0.1068 (0.0355)	0.1003 (0.0413)					0.1004 (0.0181)	$F=11.96$ $p<0.0001$	CS, pro-DLB, DLB-d > AD, AD/DLB

Kruskall-Wallis post hoc test (H)

Ordinary one-way ANOVA (F)

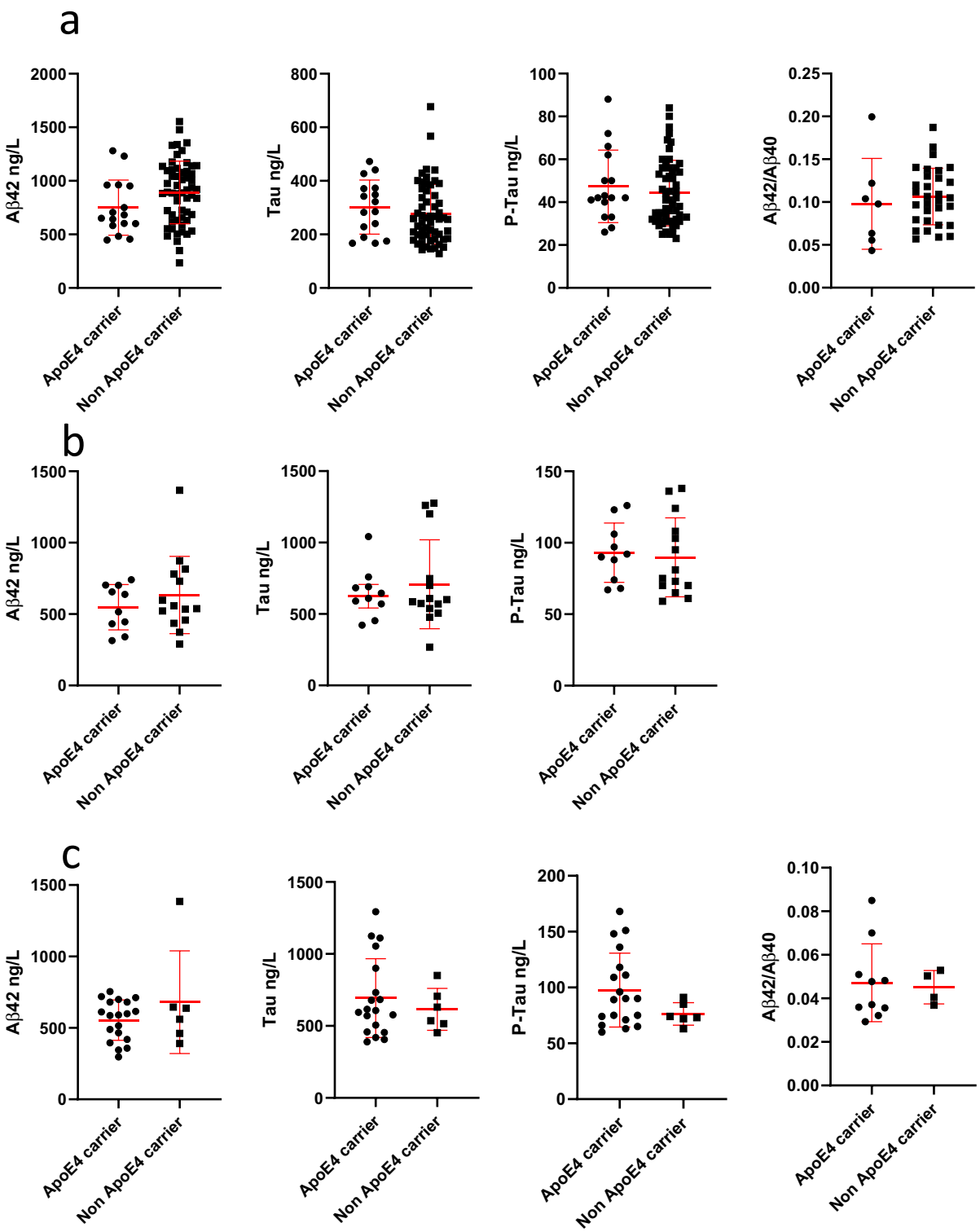

Fig. 4 Biomarker levels according to *ApoE4* presence. **a** DLB. **b** AD. **c** AD/DLB

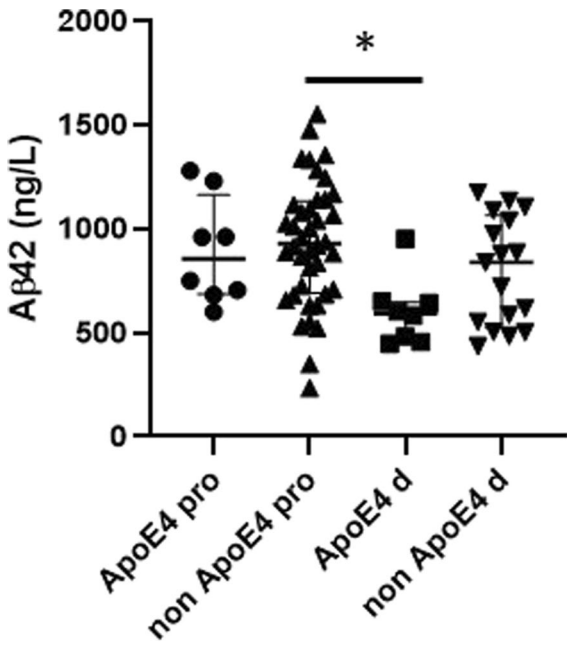

Fig. 5 Difference in Aβ42 levels between prodromal DLB and demented DLB for *ApoE4* carriers and non-carriers

because they are not looked for. Therefore, it is possible that these studies have unknowingly included patients with AD copathology in their DLB group, which would not be very surprising as it has been shown that mixed AD/DLB pathologies have a more visible and therefore more easily detectable DLB symptomatology [41]. Thus, we need to look at autopsy studies and try to understand why some support the notion that *ApoE4* is a risk factor for DLB [14–16] while others do not [18–22]. What is noteworthy is that the publications showing that *ApoE4* is a risk factor for DLB consider the pathology to be pure, where there are obviously Lewy bodies, but accept Braak stages 0 to III. Thus, a patient with synucleinopathy and Braak stage III is not considered to have AD/DLB comorbidity. This is an important bias as demonstrated by Raunio and colleagues [20], Kaivola and colleagues [21], and Schaffert and colleagues [19] who, by selecting patients with synucleinopathy but limiting Braak stages from 0 to II, proved that *ApoE4* was not a risk factor for pure DLB. Kaivola et al. even showed that DLB patients with intermediate AD copathology, i.e., patients with Braak stage III, had significantly more *ApoE4* patients in their group, thus demonstrating that Braak stage III is already AD and that it is important to select truly pure forms of DLB to show the involvement of *ApoE4* in this disease. Similarly, an older study indicated that an association was found between *ApoE4* and the presence of neocortical Lewy bodies, but this association was no longer significant when the pathological variable AD was included in the multiple regression model [42]. This suggests that *ApoE4* remains primarily a risk factor for AD, and thus, DLB patients with *ApoE4* are likely to be at greater risk of developing an AD comorbidity.

Age at onset and YOE

In our group of AD patients, we found, as described in the literature, an impact of the YOE on the age at onset of the disease ($p=0.039$). There is indeed an effect of cognitive reserve that allows the age at AD onset to be delayed [43, 44]. For the AD/DLB group, there was no significant correlation between YOE and age at onset ($p=0.918$ for AD/DLB), which suggests that in the case of AD/DLB comorbidity, the cognitive reserve allowing the slowing of the entry into this comorbidity no longer played a role.

Concerning DLB patients, we found a strong trend towards a correlation between YOE and age at disease onset ($p=0.056$), suggesting, as in AD, that cognitive reserve helps to slow down the onset of the disease. However, some studies indicate rather an inverse correlation between YOE and age at disease onset; thus, the higher the patients' YOE, the higher the risk of starting DLB at a young age [19, 25]. The authors justify their result by indicating that cognitive disorders are detected more easily in patients with a high YOE than those with a low YOE. Our results rather indicate an opposite tendency even if we did not find a significant correlation between YOE and age at DLB onset. It should be noted that in DLB, the disease is not purely cognitive as in AD, and the fact that the disease is characterized by fluctuations does not facilitate the estimation of pathology onset. Estimating the age at DLB onset is likely to be less accurate than in AD and most likely depends on whether or not the symptoms are taken into account.

YOE and *ApoE4*

Interestingly, we showed that the group of DLB patients with a high YOE was more likely to have

ApoE4 than DLB patients with a low YOE ($p = 0.03$). Thus, even if the high-YOE group of patients did not reach the frequency of *ApoE4* patients in AD, this result is a challenge and one might think that part of the high-YOE patients developing DLB was due to the presence of *ApoE4*. Nevertheless, before coming to this conclusion, it would be interesting to follow these patients to determine whether they are at an increased risk of developing AD/DLB comorbidity. It should be noted that our patients had a relatively long follow-up since they were followed for 5 to 8 years and already a number of patients had been reclassified as AD/DLB. However, as we have seen, *ApoE4*-carrier DLB patients were younger than *ApoE4*-carrier AD patients and were therefore likely to develop AD comorbidity at a later stage. Furthermore, as previously mentioned, numerous publications have indicated that *ApoE4* is more a risk factor for comorbidity than for pure DLB [18–25, 40]. However, this does not explain why in the DLB group, patients with a high YOE were more likely to be *ApoE4* carriers.

Comparing low YOE and high YOE, we saw that in our DLB cohort, there was a strong tendency for YOE to correlate with the age at onset of the disease ($p = 0.056$). This suggests that the high-YOE patients "resist" a little better than the low-YOE patients thanks to their cognitive reserve. It can therefore be hypothesized that high-YOE patients are more "resistant" than low-YOE patients, except for those who are *ApoE4* carriers. Among the high-YOE patients, it is conceivable that *ApoE4* carriers are more easily identified because of greater cognitive impairment (e.g., related to the onset of amyloidopathy, as discussed below). Conversely, high YOE non-*ApoE4*-carrier patients may be relatively protected from the disease due to their cognitive reserve. DLB is a mainly "functional" disease at the beginning, there is indeed very little neurodegeneration (compared to AD). Thus, if there are more *ApoE4*-carrier patients in the high-YOE group, this is possibly related to the amyloidopathy and to the neurodegenerative phenomena caused by *ApoE4*, making them more easily detectable. Low-YOE patients, however, irrespective of their genotype, do not have enough cognitive reserve to fight the disease. The initial "functional" disease is thus sufficient to cause deficits detectable by the clinician. To conclude on this hypothesis, we can consider that *ApoE4* is not a risk factor for DLB, but *ApoE4* causes additional cognitive deficits due to amyloidopathy in patients with a high YOE that the cognitive reserve is not sufficient to compensate.

Another hypothesis is to imagine that there is potentially a different environment between patients with a high YOE (e.g., city) and those with a low YOE (e.g., countryside) and that this environment in the case of high YOE interacts with *ApoE4* to promote the development of DLB. In a separate study, it would be interesting to determine where these patients live and their levels of exposure to pollutants (such as fine particles, mainly in the city) and toxic substances (such as pesticides, herbicides, and insecticides, mainly in the countryside). However, according to a recent publication, there does not seem to be an interaction between pollution and *ApoE4*, as this risk factor does not modify the association between air pollution and dementia [45].

Another interesting hypothesis is that *ApoE4*-carrier individuals have a better cognitive performance at a young age than other individuals. Indeed, many studies have investigated the *ApoE4* effect in young people on cognition [46–51]. Thus, *ApoE4* would have beneficial effects in youth and would be linked to neurodegenerative diseases in the elderly. The different effects of genes on health during different stages of life have been called antagonistic pleiotropy [52]. The missing link in this hypothesis is that no study has succeeded in demonstrating that having better cognition predisposes individuals to having a higher YOE. This would imply that in people with a high YOE, there would be more people carrying *ApoE4*. In any case, this assumption would explain the higher number of *ApoE4*-carrier patients in the higher YOE group of DLB patients.

ApoE4 and amyloidopathy

We had previously shown that the Aβ42 decrease in CSF, classically observed in DLB patients, appeared only at the demented stage [29, 38]. Our results suggest that this decrease at the demented stage is most noticeable in *ApoE4* patients. Similarly, van Steenoven and colleagues showed that Aβ42 levels decrease in DLB patients depending on the *ApoE4* copy number, with a significant decrease in patients with double *ApoE4* [39]. Thus, the Aβ42 decrease (which is most often translated into the presence of brain amyloidopathy) observed in DLB-d patients would be related to the presence of *ApoE4*. This means that DLB patients

who progress to dementia and are *ApoE4* carriers are more likely to develop amyloidopathy. This result reinforces the link between *ApoE4* and amyloidopathy, also explaining why *ApoE4* is likely to be more a risk factor for AD than for DLB.

Limitations

Our study does not have strong limitations. The patients were recruited prospectively, but we deplore the imbalance in the numbers of patients in the groups, with the DLB group much larger than the AD and AD/DLB groups. We also regret the loss of clinical information that prevented us from using all patients for each analysis (e.g., YOE information and age at onset). The advantage of this cohort, which was initiated in 2013, is that it is still relatively well described, it has been the subject of a few publications [28, 29, 53, 54], and the follow-up has allowed us to have fairly accurate diagnoses despite the absence of autopsy confirmation. Moreover, the results obtained in our study are quite consistent with part of the literature, for example, where we found that *ApoE4* was a risk factor for AD and AD/DLB comorbidity. We are also quite confident that the DLB group was relatively "pure," as also suggested in an international collaboration [55] which concluded that "the Strasbourg cluster might reflect the purest DLB subtype of the cohort, because of normal AD CSF biomarkers and a very low burden of cerebrovascular disease and moreover almost all patients have cognitive fluctuations which is one of the most typical characteristics of DLB" [56].

Conclusion

In conclusion, our results seem to point to an absence of involvement or at the very most a weak action of ApoE4 in the development of DLB. Furthermore, it appears that DLB patients with high YOE are more likely to be *ApoE4* carriers than those with low YOE. Finally, the Alzheimer biomarkers do not seem to be affected by the presence or absence of *ApoE4*, with the exception of Aβ42, which appears lower in *ApoE4*-carrier DLB patients at the demented stage. A next step could be to determine the impact of *ApoE4* on MRI findings in these DLB patients.

Abbreviations ***AD*: Alzheimer's disease; *AD-d*: AD demented; *CS*: Control subjects; *CSF*: Cerebrospinal fluid; *DLB*: Dementia with Lewy bodies; *DLB-d*: DLB demented; *FCSRT*: Free and Cued Selective Reminding Test; *MMSE*: Mini Mental State Examination; *Pro-AD*: Prodromal AD; *Pro-DLB*: Prodromal DLB; *RBD*: Rapid eye movement sleep behavior disorder; *YOE*: Years of education; *t-Tau*: Total Tau**

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Author contribution OB and FB: study concept and design, analysis of the results, and drafting the manuscript. OB: ApoE genotyping and statistical analyses, analyses of biological measurements, and contribution to data interpretation and revision of the manuscript for important intellectual content. AB, LS, and FB: study protocol design. NP, PA, CD, CM, BC, and FB: clinical work, organization of lumbar punctures, diagnosis confirmation, and contribution to data interpretation and revision of the manuscript for important intellectual content.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

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Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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