TITLE: Cortical Lewy bodies and Aβ burden are associated with prevalence and timing of dementia in Lewy body diseases

Running Head: Neuropathology of dementia in the Lewy body disease spectrum

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ABSTRACT

Aims: Our main objective was to determine the neuropathological correlates of dementia in patients with Lewy body disease (LBD). Furthermore, we used data derived from clinical, neuropathological and genetic studies to investigate boundary issues between Dementia with Lewy bodies (DLB) and Parkinson's disease with (PDD) and without (PDND) dementia.

Methods: 121 cases with a neuropathological diagnosis of LBD and clinical information on dementia status were included in the analysis (55 PDD, 17 DLB and 49 PDND). We carried out topographical and semi-quantitative assessment of Lewy bodies (LB), Aβ plaques and tau-positive neuropil threads (NT). The APOE genotype and MAPT haplotype status were also determined.

Results: The cortical LB (CLB) burden was the only independent predictor of dementia (OR: 4.12, $p<0.001$). The total cortical Aβ plaque burden was an independent predictor of a shorter latency to dementia from onset of motor signs ($p=0.001$). DLB cases had a higher LB burden in the parietal and temporal cortex, compared to PDD. Carrying at least one APOE ϵ4 allele was associated with a higher cortical LB burden ($p=0.02$), particularly in the neocortical frontal, parietal, and temporal regions.

Conclusions: High CLB burden is a key neuropathological substrate of dementia in LBD. Elevated cortical LB pathology and Aβ plaques are both correlated with a faster progression to dementia. The higher LB load in the temporal and parietal regions, which distinguishes DLB cases, could have a role in the shorter latency to dementia and may be mediated by the APOE ϵ4 allele.

Abbreviations:

Aβ: Beta-amyloid
αSN: Alpha-synuclein
AC: Anterior Cingulate Cortex
AD: Alzheimer's disease
APOE: Apolipoprotein-E
BNE: BrainNet Europe
CLB: Cortical Lewy bodies
CVP: Concomitant Vascular Pathology
DLB: Dementia with Lewy Bodies
EC: Entorhinal Cortex
LB: Lewy bodies
LBD: Lewy Body disease
MAPT: Microtubule associated protein tau
PC: Parietal Cortex
PD: Parkinson's disease
PDD: Parkinson's disease with dementia
PDND: Parkinson's disease without dementia
NT: Neuropil threads
SFC: Superior Frontal Cortex
SNP: Single Nucleotide Polymorphism
TC: Temporal Cortex

INTRODUCTION

Dementia with Lewy Bodies (DLB) represents the second most frequent neurodegenerative form of late-onset dementia after Alzheimer's disease (AD), with a yearly incidence of 3.2% of all new dementia cases.[1-2] Dementia is also a common non-motor complication in the course of Parkinson's disease (PD), where it is known as "Parkinson's disease dementia" (PDD).[3] The estimated incidence of PDD in prevalence samples of PD reaches 100 per 100,000 patient- years,[4] and the cumulative prevalence of dementia in patients with PD may exceed 75% in the course of their disease.[5] The distinction between DLB and PDD is made on clinical grounds, with the arbitrary cut-off of dementia occurring before or within one year of motor signs of parkinsonism in DLB.[1,6] DLB, PDD and PD without dementia (PDND) share a common neuropathological substrate of alpha-synuclein (αSN) containing intra-neuronal inclusions called Lewy bodies (LB)[1,7] and have been labelled as "Lewy Body Diseases" (LBD).[8-10] There is growing evidence from several clinic-based[11-14] and population-based[15] studies of a strong association between neocortical αSN/LB pathology and cognitive decline/dementia, although this has not been a consistent finding.[16-17] Of note, a variable load of cortical and sub-cortical LB has been reported in autopsies of 11-18 % of asymptomatic elderly individuals, a condition termed as "Incidental Lewy body disease", which has been reported to represent a pre-symptomatic disease stage.[18-19]

Recent studies have also shown a positive association between AD-type pathology and DLB/PDD. Higher Braak tau stages have been reported in PDD[14,20-22] and Aβ plaque deposition has been reported in PDD in most[20] or a subset of patients.[14,23] Other studies have reported a higher Aβ plaque burden in DLB compared to both PDD and PDND patients in the neocortex[21,24-26] and in the striatum[27-28] and in both PDD and DLB cases compared to PD patients without dementia.[29] These observations led to suggestions that AD-type Aβ plaques and tau aggregates may also be important correlates of dementia in at least a significant portion of LBD patients,[21-22,30] that their presence may obscure a unique LB-related cognitive phenotype,[6] or that they must be present with CLB for dementia to occur.[31]

A positive correlation has been reported in LBD between the severity of αSN and tau pathologies,[12,21] αSN and Aβ plaque deposition[12,32-35] and between the three pathologies of tau, Aβ and αSN.[20,22] These observations, coupled with evidence from molecular and *in vitro* studies, have led to the hypothesis of a synergistic interaction[12,20] or an additive effect[15] of the two pathologies at the molecular level. However, others have found no difference in CLB counts between PDD cases with and without concomitant AD

pathology[23] and no significant association between LB, Aβ or tau pathological scores in 22 prospectively followed patients.[11] Several workers have proposed a classification of DLB and PDD based on the presence or absence of co-morbid AD-type pathology into DLB and PDD with AD pathology (DLB+AD and PDD+AD), as opposed to the "pure synucleopathic" forms (devoid of significant AD pathology) of pure DLB (pDLB) and PDD-AD.[15,16,23,26,36]

From a genetic standpoint, there have been reports of association between a faster cognitive decline and dementia in PD with the H1/H1 haplotype of the MAPT gene[37-38] and of H1/H1 with αSN (but not tau) deposition in DLB[35,39] but not in PDD.[14,20] The APOE ϵ4 allele, still the most validated susceptibility genetic factor for AD[40] and strongly associated with AD pathology,[41-43] has recently been reported to also have a significant association with DLB[36,41,44] and with PDD in some[14,36,45,47] but not all[47-48] studies. A higher CLB burden has been reported in carriers of the APOE ϵ4 allele[14,33,49] but this finding has not been confirmed by others.[20]

Although a considerable volume of data has been accumulated in recent years there is still uncertainty regarding the relationship between the neuropathology and clinical presentation due to the heterogeneity in sample size of cohorts, clinical criteria for recruitment and staining methodologies applied by different researchers. This study was designed to address these limitations by combining extensive neuropathological semi-quantitative assessment of αSN, tau and Aβ pathology with genetic characterization of APOE and MAPT status in one large sample of LBD patients with and without dementia of the DLB and PDD types. Importantly, selection of cases for our sample was based purely on neuropathological criteria, making this cohort unbiased for the presence of dementia.

SUBJECTS AND METHODS

All subjects were donors of brain tissue to the Parkinson UK Brain Bank at Imperial College London who had provided informed, written consent for inclusion in scientific research studies. Selection for this study was based on a neuropathological diagnosis of PD and the availability of reliable clinical data on the presence or absence of dementia. A retrospective review of patient medical records was conducted by a team of neurologists with expertise in movement disorders (CR, SM, PP, and LTM) to establish the clinical diagnosis of PD [20] and classify subjects into (1) PD without dementia within a year prior to death (PDND); (2) DLB and (3) PDD. The Diagnostic and Statistical Manual of the American Psychiatric Association (4th edition) DSM IV criteria[50] and the Clinical Diagnostic

Criteria for dementia associated with Parkinson's disease[3] were applied. Cases were classified as DLB when dementia occurred prior to or within one year of the onset of motor signs (although none actually had dementia onset prior to motor symptoms), whilst cases were defined as PDD if dementia occurred later in the disease course.[1,3] 121 cases were included in the analysis (17 DLB, 55 PDD, 49 PDND).

Neuropathological assessment

Topographical staging of αSN pathology was based on the Brain-Net Europe (BNE) Consortium guidelines for Braak staging (0-VI).[51] Semi-quantitative assessment of LB pathology was carried out in five cortical regions: superior frontal cortex (SFC), n = 121; inferior and middle temporal cortex (TC), n = 91; parietal cortex, sampled approximately 1 cm posterior to the central sulcus (PC), n = 84; entorhinal cortex (EC), n= 115; anterior cingulate (AC), n = 109. We used a score range of 0-4, as recommended by the DLB Consortium.[1] For each case, a score representing the average of the individual cortical regions was also calculated (mean Cortical LB score).

On the basis of severity and topographical distribution, neurofibrillary tangle (NT) pathology was divided into three categories (mild, moderate and severe) corresponding respectively to Braak stages I-II, III-IV, and V-VI of the BNE guidelines.[52]

Semi-quantitative assessment of Aβ deposition was performed in the frontal and entorhinal cortex with categories of 0 = absent, 1 = mild; 2 = moderate and 3= severe Aβ deposition. The individual scores were assigned by subjective assessment of the overall stained area in sections of entorhinal and frontal cortex. No distinction was made between cored and diffuse plaques. Neither subpial nor intracytoplasmic staining were considered for grading (see supplementary figure 1). For data analysis, both the individual cortical Aβ plaque score from the frontal and entorhinal regions and the sum of scores from both regions (total Cortical Aβ score) were used. Due to the high collinearity between frontal and entorhinal Aβ scores within the same subject (Pearson product-moment correlation 0.870; 95%CI 0.818 – 0.908), for the multivariate model we used total cortical Aβ score, which offered a wider range of assessment of overall cortical Aβ burden.

Vascular pathology was evaluated through retrospective review of the neuropathological reports, based on recently proposed criteria;[53] cases were categorically classified as 'without' or 'with' concomitant vascular pathology (CVP).

For more information on methods see the 'Online Supporting Information'.

Genetic Characterization

For all individuals, DNA was extracted from 25 to 50 mg of brain tissue. Six MAPT and 2 APOE SNPs were genotyped (see 'Online Supporting Information') allowing for the definition of the MAPT H1/H2 haplotypes and the APOE ε2/ε3/ε4 genotypes.

Statistical Analysis

Univariate comparisons between two groups [Dementia (including DLB and PDD) vs. PDND] were made using the t-test for independent samples or Fisher Exact test whilst, for comparisons between three groups (DLB, PDD and PDND), we used one way ANOVA followed by Fisher's Least Significant Difference Test (with Bonferroni correction for multiple comparisons). We used Pearson product-moment correlation analysis to evaluate correlations between specific variables of interest.

Univariate regression models were used to search for possible predictors of dementia, latency to dementia or higher neuropathological scores. We used logistic regression for binary outcomes (dementia) and linear regression for continuous outcomes (neuropathological scores, latency to dementia), all of which were adjusted for gender and age at death. Univariate regression analysis was followed by construction of multivariate models to explore the simultaneous effects of the factors that were significantly associated with dementia when analysed individually.

Kaplan Meier plots for survival analysis were generated, using a Cox Proportional Hazard model, to evaluate the effect of pathology on time to dementia (latency to dementia from onset of motor signs) by comparing high versus low scores of neuropathological staging. Receiver Operator Characteristic (ROC) curves were constructed to evaluate the ability of pathology (LB, tau, Aβ plaques or their combination) to predict dementia based on logistic regression (for more information see 'Online Supporting Information').

Significance was set at a p-value of 0.05, with Bonferroni correction in case of multiple testing in the same analysis. All analyses and graphs were carried out with R 2.15.3 (R Foundation for Statistical Computing).[54]

RESULTS

The basic demographic, clinical, neuropathological and genetic characteristics of our sample are summarised in Tables 1 and 2. The mean age at death of our sample was 77.8 ± 7.4 years (range 58 – 93) and the mean age at onset of PD signs was 65.6 ± 9.7 years (range 40 – 86), with a mean disease duration of 12.2 ± 6.9 years (range 0 – 29). The

mean age of dementia onset was 71.7 ± 7.5 years in DLB and 74.6 ± 6.8 years in PDD. More information on clinical, neuropathological and genetic characteristics of the sample is available in the 'Online Supporting Information'.

Neuropathology of Dementia

All LB scores (both the regional and the overall cortical mean) and the proportion of cases with αSN-Braak stage 6 were significantly higher in the dementia groups (DLB and PDD) compared to PDND (Table 2 and Figure 1). Furthermore, a higher CLB load was observed in the temporal and parietal cortex in DLB compared to PDD (Fig 1b). We did not observe any significant difference across the three groups for Braak tau staging, with 84% of all cases being in the mild (Braak stage 0-II) category. Cortical and striatal Aβ plaque scores were significantly higher in DLB compared to PDD and in the dementia group as a whole (DLB + PDD) compared to PDND, but no difference was noted between PDD and PDND. Correlation analysis showed a positive correlation between tau staging, LB score and Aβ plaques (Table 3).

The following neuropathological variables were associated with dementia in the univariate logistic regression models: SFC LB score (OR 2.65, p<0.0001); AC LB score (OR 2.45, p=0.001), TC LB score (OR 3.69, p<0.0001), PC LB score (OR 3.64, p<0.0014); EC LB score (OR = 2.04, p<0.001); mean Cortical LB score (OR 4.13, p<0.034); highest cortical Aβ plaque score (OR 1.85, p=0.001); total cortical Aβ plaque score (OR 1.40, p=0.001); αSN Braak stage (OR 3.23, p=0.034); and striatal Aβ plaque score (OR 2.58, p=0.005).. In the multivariate model, only the mean CLB score maintained a strong association with dementia (OR: 4.23, p<0.001) (Table 4).

ROC curves indicated that the mean CLB score (AUC: 0.802; 95%CI:0.722-0.883) was the best indicator of the presence of dementia, and this was not improved when all three pathologies of tau, Aβ, and CLB were analysed together (AUC: 0.798; 95%CI: 0.717-0.879) (Figure 3).

Timing of Dementia

Age of onset of dementia and latency of dementia from onset of motor signs were analysed using linear regression models. Among the neuropathological variables, only the LB scores in the parietal cortex were associated with an earlier age at onset of dementia (beta coefficient -4.66, p=0.004) and, among the demographic factors, women had a later age of onset than men (beta coefficient 6.96, p<0.001). A significant association of shorter

latency to dementia from the onset of motor signs was observed with the following neuropathological variables: tau topographical staging (beta = -3.91, p=0.004); total cortical (beta = -1.94, p=0.01) and striatal (beta = -2.97, p=0.006) Aβ plaque scores; AC LB score (beta = -2.21, p=0.04) and TC LB score (beta=-2.11, p=0.01). When these variables were included in a multivariate model using stepwise linear regression, the total cortical Aβ plaque score emerged as the only independent predictor of latency to dementia (beta =-1.37, p<0.001). No association was observed between CVP and the age of onset of dementia or the latency of dementia from onset of motor signs.

Survival analysis, with time to dementia (or death) as the time variable and dementia as the outcome, showed that the sum of CLB scores from all 5 regions was a good predictor of a faster progression to dementia (OR=3.08, p<0.01; Fig 4b). Higher Aβ scores also predicted a faster progression to dementia (OR 1.19, p=0.02; Figure 4a) but neither higher Braak αSN stages nor higher Braak tau stages did (p = 0.20, and p = 0.55, respectively, Figure 4c and 4d).

Genetics of Dementia

There were no significant differences between patients with and without dementia in the proportion of either APOE ϵ4 allele carriers (Fisher's Exact Test, p=0.16) or H1/H1 haplotype carriers (Fisher's Exact Test, p=0.64). However, when the dementia group was subdivided into DLB and PDD, a non-significant trend for a higher prevalence of the APOE ϵ4 allele was observed in DLB (Fisher's Exact Test, p=0.06).

The association between APOE ϵ4 allele carrier status and cortical Aβ plaque burden was significant (t=2.31, p=0.02; Figure 5a) in the overall sample. Furthermore, there was a strong correlation between carrying at least one APOE ϵ4 allele and having a higher cortical LB burden (t=3.38, p=0.001). This finding was confirmed in the multivariate model (covariates: age at death, gender, total cortical Aβ score), using linear regression analysis (standardized beta: 0.187, p=0.02), where total cortical Aβ score also emerged as a significant predictor of a higher cortical LB burden (standardized beta: 0.487, p<0.01). Linear regression, with individual regional cortical LB scores as the dependant variable and APOE ϵ4 carrier status as the independent variable, showed that the ϵ4 allele was associated with higher LB scores in the superior frontal (beta coefficient 0.73, p<0.001), parietal (beta coefficient 0.68, p=0.003) and temporal (beta coefficient 0.71, p=0.006) cortex but not in the anterior cingulate or entorhinal cortex (Figure 5b).

There was no correlation between APOE ϵ4 carrier status and higher tau stages, or between MAPT H1/H1 status and any of the clinical or neuropathological variables.

DISCUSSION

We have conducted an integrated retrospective analysis of clinical, pathological and genetic characteristics of DLB, PDD and PDND in one large sample of patients - tissue donors to the Parkinson's UK Brain Bank. Our findings confirm the previously reported strong positive association between CLB burden and the presence of dementia in PD.[6,11-14,18,20] CLB burden was higher in all five examined brain regions in patients with dementia (both DLB and PDD) compared to PDND individuals. This finding implies a widespread increase of cortical pathology in Lewy body dementias[12,14,18] rather than a selective deposition of cortical LB in the frontal and temporal neocortical regions as previously reported.[33,55] However, we did observe a higher CLB burden in the parietal and temporal cortex in DLB compared to PDD. Furthermore, our univariate analysis showed a significant correlation between the temporal LB load and the latency to dementia from the onset of motor signs and indicated that parietal LB scores were an independent predictor of a younger age at onset of dementia. Taken together, these findings support the hypothesis that higher CLB burden in the neocortical parietal and temporal cortex may, at least partly, account for the short latency (or lack thereof) between the onset of motor signs and dementia in DLB compared to PDD. Interestingly, a recent study reported higher overall pathological scores in the temporal cortex as a distinguishing characteristic of DLB cases.[30]

DLB cases were also characterized by a higher cortical and striatal Aβ plaque burden compared to PDD, in agreement with previous studies (Figure 2b).[24-26,28] Although we observed a trend suggesting a greater striatal Aβ plaque density in PDD compared to PDND, this difference did not reach statistical significance in our sample. A higher proportion of DLB cases with a moderate-high Aβ burden compared to PDD has been reported (87% and 43%, respectively) together with an inverse correlation between cortical burden of both LB and Aβ plaques and time to dementia in a sample of DLB and PDD cases.[24] *In vivo* amyloid [11C] PIB PET studies have also shown a higher Aβ plaque load in DLB compared to PDD.[56-57] In our multivariate model using stepwise linear regression, the total cortical Aβ plaque burden was the only independent predictor of shorter latency to dementia from onset of motor signs. This finding is in keeping with results from neuropathological analysis of a longitudinally followed cohort of PD cases with and without dementia. In this study, Halliday and coworkers reported a higher prevalence of significant

Aβ plaque pathology in cases with early onset of dementia (DLB) than that found in cases with a long disease duration and late onset cognitive impairment (PDD).[58] Finally, survival analysis indicated that CLB burden is also a good predictor of faster progression to dementia (Figure 4).

We confirm the previously reported positive correlation between CLB pathology and neuropathological features of AD in DLB and PDD.[12,20,21,32-34] However, the precise mechanism of this dynamic interaction cannot be elucidated solely through post-mortem studies. Disentangling the respective roles of these pathologies in DLB and PDD is further confounded by their relatively frequent occurrence in post-mortem studies of asymptomatic elderly individuals.[18,19,36,59] Of note, our analysis with ROC curves showed that CLB pathology by itself is the best predictor of dementia and that the combination of all three pathologies does not improve the area under the curve, which supports the dominant role of αSN deposition in the development of dementia in PD (Figure 3).

The finding of a similar age at onset of dementia and a similar interval between dementia onset and death in our series of patients with DLB and PDD is in agreement with the concept of a non-linear disease progression, with milestones such as dementia heralding a stereotyped final phase of the disease, notwithstanding differences in the overall disease duration.[60] Whilst disease progression in PDD seems to conform to the Braak staging theory of a predictable order of ascending caudo-rostral αSN deposition,[61] the timing of dementia in relation to the onset of motor signs in DLB is intriguingly discordant to this staging model. Progression of αSN pathology in DLB seems to follow a different and/or much faster 'map' of regional propagation, compared to PDD and PDND. Indeed, DLB is said to reside at the most severe end of the Lewy body disease spectrum, with "incidental LBD" at the other end.[10] Comorbid occurrence of Aβ and αSN in neocortical regions in DLB may give rise to reciprocal promotion of fibrillization, as has already been shown to occur *in vitro,*[62-63] thus initiating or accelerating the neurodegenerative cascade with progressive involvement of more caudal regions. This could account for the "inverse" clinical progression in DLB compared to PDND and PDD.

In our sample, the MAPT H1/H1 haplotype was not associated with dementia. Furthermore, in contrast with previous reports, we found no correlation between the MAPT H1/H1 and any of the neuropathological markers.[35,39] In our study, the overall prevalence of moderate or severe Braak tau stages (III – VI) was low (16%) and did not differ significantly between LBD patients with and without dementia. Thus, at variance with some studies[21-22,60] and in agreement with others,[11,13,23] we can only conclude that tau pathology (a key determinant of AD) does not seem to have an independent role in the occurrence of cognitive decline in LB disease. A recent report on the frequency of APOE ϵ4 in a large

series of AD+ and AD- synucleinopathies with dementia has indicated an increased frequency of ϵ4 in all forms, even in the absence of AD pathology.[36] We did not observe a direct effect of the APOE ϵ4 allele on dementia in our sample.

Interestingly, the APOE ϵ4 allele was associated with higher cortical Aβ plaque pathology and was strongly associated with high CLB burden, particularly in neocortical regions. Multiple linear regression adjusting for AD pathology did not alter the significant association between the APOE ϵ4 allele and CLB burden. Thus, the APOE ϵ4 allele may mediate increased neocortical LB deposition either indirectly, possibly through a greater abundance of fibrillization-promoting Aβ plaques, or directly through yet unexplored mechanisms. Gearing and coworkers found a similar prevalence of the APOE ϵ4 allele when comparing cases with pure AD pathology to cases with AD associated with a pathologic diagnosis of PD (AD + PD). They reported an increase in neurite density in the CA2-3 regions of the hippocampus in AD+PD cases, which correlated with APOE ϵ4 dosage. They did not however demonstrate a significant increase in cortical Lewy pathology with increasing APOE ϵ4 dosage, although there was a trend in that direction.[64] Similarly, in a small sample of pure LBD cases, Lippa and colleagues found more intense neuritic degeneration in the CA2-3 regions in APOE ϵ4 carriers but no association between APOE ϵ4 allele and density of cortical pathology.[65] Both of these studies pre-dated the advent of alpha-synuclein-reactive antibodies and were based on ubiquitin staining, which may have caused underestimation of Lewy pathology.[64-65] More recently, the study by Compta and colleagues failed to find any correlations between APOE ϵ4 allele and cortical Lewy body density, in a large sample comparing PD cases with and without dementia.[20] On the other hand, Mattila and co-workers reported greater numbers of Lewy bodies in several cortical regions in APOE ϵ4 carriers in a post-mortem PD cohort, compared to subjects without the APOE ϵ4 allele. However, they did not assess whether this held true after adjusting for concomitant AD pathology, which was present in 40% of cases in their sample.[33] More recently, Irwin and and colleagues reported a multivariate model in which both the APOE ϵ4 allele and cortical tau pathology predicted a higher CLB burden.[14] We have confirmed this, enforcing the concept that the APOE ϵ4 allele may have an independent role in increasing CLB burden, and we have extended these findings to include DLB cases. Furthermore, our linear regression models for each analysed cortical region point to the neocortical frontal, temporal and parietal regions as the site where the APOE ϵ4 allele might exert its influence on CLB, as opposed to the allocortical cingulate and entorhinal cortex, where this association was not seen.

This study has several limitations. In common with all retrospective studies, clinical symptoms and signs may have been underreported, due to negative ascertainment bias, resulting in potential errors of approximation of the timing of onset and duration of

symptoms but with little impact on the genetic-pathological correlations. Furthermore, neuropathological analysis of post-mortem data is not the appropriate methodology to evaluate the dynamic process of disease progression in DLB and PDD. Longitudinal neuroimaging studies using clinically validated αSN markers,[66] if and when they become available, would be a more suitable approach in this respect. Also, there was some asymmetry in sample size, with smaller numbers of DLB cases compared to the other two groups. This was due to our approach to case selection, which was based on a neuropathological diagnosis and unbiased towards the occurrence of dementia. Our sample was derived from donors to a movement disorders brain bank. Thus, our DLB cases were more likely to have parkinsonian syndromes and may represent a subtype of DLB. Finally, subjective semiquantitative assessment of pathology visualized with immunohistochemical techniques may have failed to capture more subtle effects of regional pathology. For example, our finding of a lack of association between the burden of fibrillary tau pathology and dementia is based on the established Braak staging scheme, which may fail to capture small but potentially significant differences in entorhinal pathological tau burden between cases with the same Braak score. Further studies based on computerized quantitative assessment of pathology can be expected to substantiate and refine our findings.[67] The main strength of our report resides in our integrated approach combining clinical, neuropathological and genetic analysis to study one large sample of PD patients with and without dementia of the DLB and PDD forms.

In summary, our data provide further support to the notion that neocortical LB burden is the key neuropathological substrate of DLB and PDD, and we report a strong association of a higher neo-cortical LB pathology with APOE ϵ4, which remains significant after adjusting for Aβ pathology. DLB shares important features with AD in the form of prominent Aβ (but not tau) pathology and APOE ϵ4 status and may represent a biological link between the two nosological entities of AD and PD. Further studies are required to better elucidate the modalities of interactions between αSN and Aβ, as well as the modulating mechanism of APOE ϵ4 on LB neocortical pathology.

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Author Contributions

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Drafting of manuscript: CR

Manuscript editing: LTM, SMG, CR, FCC

Data collection: CR, IB, DG, FR, SMG, DD, AIB, PP, SM, SP, PT, LC

Data analysis: FCC, CR, SMG, AdS, SP, PT

Potential Conflicts of Interest

Nothing to report.

References

1. McKeith IG, Galasko D, Kosaka K, Perry IK, Dickson DW, Hansen LA, Salmon DP, Lowe J, Mirra SS, Byrne EJ, Lennox G, Quinn NP, Edwardson JA, Ince PG, Bergeron C, Burns A, Miller BL, Lovestone S, Collerton D, Jansen ENH, Ballard C, de Vos RAI, Wilcock GK, Jellinger KA, Perry RH, for the Consortium on Dementia with Lewy Bodies. Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy Bodies (DLB): report of the consortium on DLB international workshop. Neurology 1996;47:1113–1124.

2. Zaccai J, McCracken C, Brayne C. A systematic review of prevalence and incidence studies of dementia with Lewy bodies. Age Ageing 2005;34:561–566.

3. Emre M, Aarsland D, Brown R, Burn DJ, Duyckaerts C, Mizuno Y, Broe GA, Cummings J, Dickson DW, Gauthier S, Goldman J, Goetz C, Korczyn A, Lees A, Levy R, Litvan I, McKeith I, Olanow W, Poewe W, Quinn N, Sampaio C, Tolosa E, Dubois B. Clinical diagnostic criteria for dementia associated with Parkinson's disease. Mov Disord 2007;22:1689–1707.

4. Aarsland D, Andersen K, Larsen JP, Lolk A, Nielsen H, Kragh-Sorensen P. Risk of dementia in Parkinson's disease: a community-based, prospective study. Neurology 2001;56:730-736.

5. Aarsland D, Andersen K, Larsen JP, Lolk A, Nielsen H, Kragh-Sorensen P. Prevalence and characteristics of dementia in Parkinson disease: an 8-year prospective study. Arch Neurol 2003;60:387-392.

6. McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H, Cummings J, Duda JE, Lippa C, Perry EK, Aarsland D, Arai H, Ballard CG, Boeve B, Burn DJ, Costa D, Del Ser T, Bubois B, Galasko D, Gauthier S, Goetz CG, Gomez-Tortosa E, Halliday G, Hansen LA, Hardy J, Iwatsubo T, Kalaria RN, Kaufer D, Kenny RA, Korczyn A, Kosaka K, Lee VMY, Lees A, Litvan I, Londos E, Lopez OL, Minoshima S, Mizuno Y, Molina JA, Mukaetova-Ladinska EB, Pasquier F, Perry RH, Schulz JB, Trojanowski JQ, Yamada M, for the Consortium on DLB. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology 2005;65:1863–1872.

7. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature 1997;388:839–840.

8. Baba M, Nakajo S, Tu PH, Tomita T, Nakaya K, Lee VM, Trojanowski JQ, Iwatsubo T. Aggregation of alpha-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. Am J Pathol 1998;152:879–884.

9. Gibb WRG, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1988;51:745-752.

10. Goedert M, Spillantini MG, Del Tredici K, Braak H. 100 years of Lewy pathology. Nat Rev Neurol 2013;9:13-24.

11. Aarsland D, Perry R, Brown A, Larsen JP, Ballard C. Neuropathology of dementia in Parkinson's disease: a prospective, community-based study. Ann Neurol 2005;58:773–776.

12. Apaydin H, Ahlskog JE, Parisi JE, Boeve BF, Dickson DW. Parkinson disease neuropathology: later-developing dementia and loss of the levodopa response. Arch Neurol 2002;59:102-112.

13. Hurtig HI, Trojanowski JQ, Galvin J, Ewbank D, Schmidt ML, Lee VM, Clark CM, Glosser G, Stern MB, Gollomp SM, Arnold SE. Alpha-synuclein cortical Lewy bodies correlate with dementia in Parkinson's disease. Neurology 2000;54:1916–1921.

14. Irwin DJ, White MT, Toledo JB, Xie SX, Robinson JL, VanDeerlin V, Lee VMY, Leverenz JB, Montine TJ, Duda JE, Hurtig HI, Trojanowski JQ. Neuropathologic substrates of Parkinson disease dementia. Ann Neurol 2012;72:587-598.

15. Schneider JA, Arvanitakis Z, Yu L, Boyle PA, Leurgans SE, Bennett DA. Cognitive impairment, decline and fluctuations in older community-dwelling subjects with Lewy bodies. Brain 2012;135:3005-3014.

16. Colosimo C, Hughes AJ, Kilford L, Lees AJ. Lewy body cortical involvement may not always predict dementia in Parkinson's disease. J Neurol Neurosurg Psychiatry 2003;74:852-856.

17. Parkinnen L, Kauppinen T, Pirttilä T, Autere JM, Alafuzoff I. Alpha-synuclein pathology does not predict extrapyramidal symptoms or dementia. Ann Neurol 2005;57:82-91.

18. Bennett DA, Wilson RS, Boyle PA, Buchman AS, Schneider JA. Relation of neuropathology to cognition in persons without cognitive impairment. Ann Neurol 2012;72:599-609.

19. Dickson DW, Fujishiro H, DelleDonne A, Menke J, Ahmed Z, Klos KJ, Josephs KA, Frigerio R, Burnett M, Parisi JE, Ahlskog JE. Evidence that incidental Lewy body disease is pre-symptomatic Parkinson's disease. Acta Neuropathol 2008;115:437-44.

20. Compta Y, Parkkinen L, O'Sullivan SS, Vandrovcova J, Holton JL, Collins C, Lashley T, Kallis C, Williams DR, de Silva R, Lees AJ, Revesz T. Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: which is more important? Brain 2011;34:1493-1505.

21. Jellinger KA, Attems J. Prevalence and impact of vascular and Alzheimer pathologies in Lewy body disease. Acta Neuropathol 2008;115:427-436.

22. Horvath J, Herrmann FR, Burkhard PR, Bouras C, Kovari E. Neuropathology of dementia in a large cohort of patients with Parkinson's disease. Park Rel Dis 2013;19:864-868.

23. Sabbagh MN, Adler CH, Lahti TJ, Connor DJ, Vedders L, Peterson LK, Caviness JN, Shill HA, Sue LI, Ziabreva I, Perry E, Ballard CG, Aarsland D, Walker DH, Beach TG. Parkinson disease with dementia: comparing patients with and without Alzheimer pathology. Alzheimer Dis Assoc Disord 2009;23:295-297.

24. Ballard C, Ziabreva I, Perry R, Larsen JP, O'Brien J, McKeith I, Perry E, Aarsland D. Differences in neuropathologic characteristics across the Lewy body dementia spectrum. Neurology 2006;67:1931-1934.

25. Fushijiro H, Iseki E, Higashi S, Kasanuki K, Murayama N, Togo T, Katsuse O, Uchikado H, Aoki N, Kosaka K, Arai H, Sato K. Distribution of cerebral amyloid deposition and its relevance to clinical phenotype in Lewy body dementia. Neuroscience Letters 2010;486:19-23.

26. Tsuboi Y, Hirotake U, Dickson DW. Neuropathology of Parkinson's disease dementia and dementia with Lewy bodies with reference to striatal pathology. Park Rel Dis 2007;S221-S224.

27. Jellinger KA, Attems J. Does striatal pathology distinguish Parkinson disease with dementia and dementia with Lewy bodies? Acta Neuropathol 2006;112:253–260.

28. Kalaitzakis ME, Graeber MB, Gentleman SM, Pearce RKB. Striatal beta-amyloid deposition in Parkinson disease with dementia. J Neuropathol Exp Neurol 2008;67:155–161.

29. Kalaitzakis ME, Walls AJ, Pearce RKB, Gentleman SM. Striatal Aβ peptide deposition mirrors dementia and differentiates DLB and PDD from other parkinsonian syndromes. Neurobiol Dis 2011;41:377-384.

30. Howlett DR, Whitfield D, Johnson M, Attems J, O'Brien JT, Aarsland D, Lai MKP, Lee JH, Chen C, Ballard C, Hortobagyi T, Francis PT. Regional multiple pathology scores are associated with cognitive decline in Lewy Body Dementias. Brain Pathol 2014;Aug 8: doi: 10.1111/bpa.12182.

31. Nelson PT, Kryscio RJ, Jicha GA, Abner EL, Schmitt FA, Xu LO, Cooper G, Smith CD, Markesbery WR. Relative preservation of MMSE scores in autopsy-proven dementia with Lewy bodies. Neurology 2009;73:1127-1133.

32. Lashley T, Holton JL, Gray E, Kirkham K, O'Sullivan SS, Hilbig A, Wood NW, Lees AJ, Revesz T. Cortical alpha-synuclein load is associated with amyloid-beta plaque burden in a subset of Parkinson's disease patients. Acta Neuropathol 2008;115:417-425.

33. Mattila PM, Rinne JO, Helenius H, Dickson DW, Roytta M. Alpha-synuclein-immunoreactive cortical Lewy bodies are associated with cognitive impairment in Parkinson's disease. Acta Neuropathol 2000;100:285–290.

34. Pletnikova O, West N, Lee MK, Rudow GL, Skolasky RL, Dawson TM, Marsh L, Troncoso JC. Abeta deposition is associated with enhanced cortical alpha-synuclein lesions in Lewy body diseases. Neurobiol Aging 2005; 26:1183-1192.

35. Colom-Cadena M, Gelpi E, Charif S, Belbin O, Blesa R, Marti MJ, Clarimon J, Lleo A. Confluence of α-synuclein, tau, and β-amyloid pathologies in Dementia with Lewy Bodies. J Neuropathol Exp Neurol 2013;72(12):1203-1212.

36. Tsuang D, Leverenz JB, Lopez OL, Hamilton RL, Bennett DA, Schneider JA, Buchman AS, Larson EB, Crane PK, Kaye JA, Kramer P, Woltjer R, Trojanowski JQ, Weintraub D, Chen-Plotkin AS, Irwin DJ, Rick J, Schellenberg GD, Watson GS, Kukull W, Nelson PT, Jicha GA, Neltner JH, Galasko D, Masliah E, Quinn JF, Chung KA, Yearout D, Mata IF, Wan JY, Edwards KL, Montine TJ, Zabetian CP. APOE ϵ4 increases risk for dementia in pure synucleinopathies. JAMA Neurol. 2013;70:223-228.

37. Goris A, Williams-Gray CH, Clark GR, Foltynie T, Lewis SJ, Brown J, Ban M, Spillantini MG, Compston A, Burn DJ, Chinnery PF, Barker RA, Sawcer SJ. Tau and alpha-synuclein in susceptibility to, and dementia in, Parkinson's disease. Ann Neurol 2007;62:145–153.

38. Williams-Gray CH, Evans JR, Goris A, Foltynie T, Ban M, Robbins TW, Brayne C, Kolachana BS, Weinberger DR, Sawcer SJ, Barker RA. The distinct cognitive syndromes of Parkinson's disease: 5 year follow-up of the Campaign cohort. Brain 2009;132:2958-2969.

39. Colom-Cadena M, Gelpi E, Martí MJ, Charif S, Dols-Icardo O, Blesa R, Clarimon J, Lleo A. MAPT H1 haplotype is associated with enhanced α-synuclein deposition in dementia with Lewy bodies. Neurobiol Aging 2013;34:936-42.

40. Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, Pericak-Vance MA, Goldgaber D, Roses AD. Increased amyloid β-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late onset Alzheimer disease. Proc Natl Acad Sci USA 1993;90:9649-9653.

41. Kobayashi S, Tateno M, Park TW, Utsumi K, Sohma H, Ito YM, Kokai Y, Saito T. Apolipoprotein E4 frequencies in a Japanese population with Alzheimer's disease and dementia with Lewy bodies. PLoS One 2011;6:e18569.

42. Lane R, He Y, Morris C, Leverenz JB, Emre M, Ballard C. BuChE-K and APOE e4 allele frequencies in Lewy body dementias, and influence of genotype and hyperhomocysteinemia on cognitive decline. Mov Disord 2009;24:392-400.

43. Polvikosky T, Sulkava R, Haltia M, Kainulainen K, Vuorio A, Verkkoniemi A, Niinisto L, Halonen P, Kontula K. Apoliprotein E, dementia, and cortical deposition of β-Amyloid protein. N Engl J Med 1995;333:1242-1247.

44. Bras J, Guerreiro R, Darwent L, Parkkinen L, Ansorge A, Escott-Price V, Hernandez DG, Nalls MA, Clark LN, Honig LS, Marder K, Van Der Flier WM, Lemstra A, Scheltens P, Rogaeva E, St George-Hyslop P, Londos E, Zetterberg H, Ortega-Cubero S, Pastor P, Ferman TJ, Graff-Radford NR, Ross OA, Barber I, Braae A, Brown K, Morgan K, Maetzler W, Berg D, Troakes C, Al-Sarraj S, Lashley T, Compta Y, Revesz T, Lees A, Cairns N, Halliday GM, Mann D, Pickering-Brown S, Dickson DW, Singleton A, Hardy J. Genetic analysis implicates APOE, SNCA and suggests lysosomal dysfunction in the etiology of dementia with Lewy bodies. Hum Mol Genet 2014;23(23):6139-6146.

45. Huang X, Chen P, Kaufer DI, Troster AI, Poole C. Apolipoprotein E and dementia in Parkinson disease: a meta-analysis. Arch Neurol 2006;63:189-193.

46. Pankratz N, Byder L, Halter C, Rudolph A, Shults CW, Conneally PM, Foroud T, Nichols WC. Presence of an APOE4 allele results in significantly earlier onset of Parkinson's disease and a higher risk with dementia. Mov Disord 2006;21:45–49.

47. Jasinska-Myga B, Opala G, Goetz CG, Tustanowski J, Ochudio S, Gorzkowska A, Tyrpa J. Apolipoprotein E gene polymorphism, total plasma cholesterol level, and Parkinson disease dementia. Arch Neurol 2007;64:261-265.

48. Williams-Gray CH, Goris A, Saiki M, Foltynie T, Compston DA, Sawcer SJ, Barker RA. Apoliprotein E genotype as a risk factor for susceptibility to and dementia in Parkinson's disease. J Neurol 2009;256:493-498.

49. Wakabayashi K, Kakita A, Hayashi S, Okuizumi K, Onodera O, Tanaka H, Ishikawa A, Tsuji S, Takahashi H. Apolipoprotein E epsilon4 allele and progression of cortical Lewy body pathology in Parkinson's disease. Acta Neuropathol 1998;95:450-454.

50. Diagnostic and statistical manual of mental disorders. 4th edition. Washington, DC: American Psychiatric Association, 2000.

51. Alafuzoff I, Ince PG, Arzberger T, Al-Sarraj S, Bell J, Bodi I, Bogdanovic N, Bugiani O, Ferrer I, Gelpi E, Gentleman S, Giaccone G, Ironside JW, Kavantzas N, King A, Korkolopoulou P, Kovacs GG, Meyronet D, Monoranu C, Parchi P, Parkkinen L, Patsouris E, Roggendorf W, Rozemuller A, Stadelmann N, Streichenberger N, Thal DR, Kretzschmar H. Staging/typing of Lewy body related α-synuclein pathology: a study of the BrainNet Europe Consortium. Acta Neuropathol 2009;117:635-652.

52. Alafuzoff I, Arzberger T, Al-Sarraj S, Bodi I, Bogdanovic N, Braak H, Bugiani O, Del-Tredici K, Ferrer I, Gelpi E, Giaccone G, Graeber MB, Ince P, Kamphorst W, King A, Korkolopoulou P, Kovacs GG, Larionov S, Meyronet D, Monoranu C, Parchi P, Patsouris E, Roggendorf W, Seilhean D, Tagliavini F, Stadelmann C, Streichenberger N, Thal DR, Wharton SB, Kretzschmar H. Staging of neurofibrillary pathology in Alzheimer's disease: a study of the Brain-Net Europe Consortium. Brain Pathol 2008;18:484-496.

53. Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, Duyckaerts C, Frosch MP, Masliah E, Mirra SS, Nelson PT, Shneider JA, Thal DR, Trojanowski JQ, Vinters HV, Hyman BT. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. Acta Neuropathol 2012;123:1-11.

54. The R project for statistical computing. Available at: http://www.r-project.org.

55. Harding AJ, Halliday GM. Cortical Lewy body pathology in the diagnosis of dementia. Acta Neuropathol 2001;102:355–363.

56. Edison P, Rowe CC, Rinne JO, S N, Ahmed I, Kemppainen N, Villemagne VL, O'Keefe G, Nagren K, Chaudhury KR, Masters CL, Brooks DJ. Amyloid load in Parkinson's disease dementia and Lewy body dementia measured with [11C]PIB positron emission tomography. J Neurol Neurosurg Psychiatry 2008;79:1331-1338.

57. Shimada H, Shinotoh H, Hirano S, Miyoshi M, Sato K, Tanaka N, Ota T, Fukushi K, Irie T, Ito H, Higuchi M, Kuwabara S, Suhara T. β-amyloid in Lewy body disease is related to Alzheimer's disease-like atrophy. Mov Disord. 2013;28:169-75.

58. Halliday G, Hely M, Reid W, Morris J. The progression of pathology in longitudinally followed patients with Parkinson's disease. Acta Neuropathol 2008;115:409-415.

59. Kok E, Haikonen S, Luoto T, Huhtala H, Goebeler S, Haapasalo H, Karhunen PJ. Apolipoprotein E-dependent accumulation of Alzheimer disease-related lesions begins in middle age. Ann Neurol. 2009;65:650-657.

60. Kempster PA, O'Sullivan SS, Holton JL, Revesz T, Lees AJ. Relationships between age and late progression of Parkinson's disease: a clinico-pathological study. Brain 2010;133:1755-1762.

61. Braak H, Del Tredici K, Rüb U, de Vos RAI, Jansen Steur ENH, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003;24:197-211.

62. Masliah E, Rockenstein E, Veinbergs I, Sagara Y, Mallory M, Hashimoto M, Mucke L. beta-amyloid peptides enhance alpha-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. Proc Natl Acad Sci U S A 2001;98:12245–12250.

63. Tsigelny IF, Crews L, Desplats P, Shaked GM, Sharikov Y, Mizuno H, Spencer B, Rockenstein E, Trejo M, Platoshyn O, Yuan JX, Masliah E. Mechanisms of hybrid oligomer formation in the

pathogenesis of combined Alzheimer's and Parkinson's diseases. PLoS One 2008;3:e3135.

64. Gearing M, Schneider JA, Rebeck GW, Hyman BT, Mirra SS. Alzheimer's disease with and without coexisting Parkinson's disease changes: Apolipoprotein E genotype and neuropathologic correlates. Neurology 1995;45:1985-1990.

65. Lippa CF, Smith TW, Saunders AM, Crook R, Pulaski-Salo D, Davies P, Hardy J, Roses AD, Dickson D. Apolipoprotein E genotype and Lewy body disease. Neurology 1995;45:97-103.

66. Vernon AC, Ballard C, Modo M. Neuroimaging for Lewy body disease: Is the in vivo molecular imaging of α-synuclein neuropathology required and feasible? Brain Res Rev 2010;65:28-65.

67. Attems J, Neltner JH, Nelson PT. Quantitative neuropathological assessment to investigate cerebral multi-morbidity. Alzheimers Res Ther 2014;6(9):85.

TABLE 1: Clinical, genetic and demographic characteristics

	DLB (n = 17)	PDD (n = 55)	PDND (n = 49)	Dementia (n=72)[a]
Females	3 (18%)	17 (31%)	15 (31%)	20 (28%)
Age at death (yrs)	75.88 ± 7.30	77.69 ± 6.63	78.53 ± 8.19	77.26 ± 6.78
Age at onset motor (yrs)	71.41 ± 7.66[c]	62.95 ± 8.75	66.47 ± 10.45	64.94 ± 9.20
Disease duration (yrs)	4.47 ± 1.87[b d]	14.75 ± 6.10	12.06 ± 6.72	12.32 ± 6.96
Age onset dementia (yrs)	71.76 ± 7.47	74.60 ± 6.80	-	73.93 ± 7.01
Time to dementia (yrs)	0.35 ± 0.49 [d]	11.65 ± 5.39	-	8.99 ± 6.74
Dementia duration (yrs)	4.12 ± 1.76	3.09 ± 2.26	-	3.33 ± 2.21
Hallucinations	14 (82%) [b]	48 (87%) [f]	9 (18%)	62 (86%) [g]
RBD	10 (59%) [b c]	15 (27%) [e]	5 (10%)	25 (35%) [g]
Cognitive Fluctuations	5 (33%) [b]	11 (20%) [f]	0 (0%)	16 (22%) [g]
APOE4 carrier	7 (47%)	18 (33%)	11 (22%)	25 (35%)
MAPT H1/H1	10 (67%)	38 (69%)	30 (61%)	48 (67%)

yrs: years; RBD: REM sleep Behavior Disorder; [a] DLB + PDD; [b] vs PDND $p < 0.001$; [c] vs PDD $p < 0.05$; [d] vs PDD $p < 0.001$; [e] vs PDND $p < 0.05$; [f] vs PDND $p < 0.001$; [g] vs PDND $p < 0.001$.

Dementia was compared to PDND with Mann-Whitney U test or Chi Square test, as appropriate. The three groups of DLB, PDD, and PDND were compared with One Way Anova, followed by Fisher's Least Significant Difference, with Bonferroni correction for multiple testing.

Values are expressed as either counts (%) or mean ± SEM.

TABLE 2: Neuropathologic Characteristics

	DLB	PDD	PDND	Dementia [a]
αSN Braak Stage VI	16 (94%)	52 (95%)	40 (82%)	68 (94%) [g]
αSN Braak Stage V	1 (6%)	3 (5%)	6 (12%)	4 (6%)
αSN Braak Stage III/IV	0	0	3 (6%)	0
Tau Severe (Braak V-VI)	4 (24%)	1 (2%)	1 (2%)	5 (7%)
Tau Moderate (Braak III-IV)	3 (18%)	6 (11%)	4 (8%)	9 (13%)
Tau Mild (Braak 0-II)	10 (58%)	48 (87%)	44 (90%)	58 (80%)
SFC LB Score	2.41 ± 1.12 [c]	1.86 ± 1.04 [f]	1.10 ± 0.71	1.99 ± 1.07 [h]
AC LB Score	2.81 ± 0.54 [b]	2.60 ± 0.85 [e]	2.00 ± 0.89	2.66 ± 0.78 [h]
TC LB Score	2.57 ± 0.76 [c d]	1.61 ± 1.08 [f]	0.75 ± 0.63	1.87 ± 1.09 [h]
PC LB Score	1.91 ± 1.14 [c d]	1.09 ± 0.90 [e]	0.44 ± 0.60	1.26 ± 1.02 [g]
EC LB Score	2.47 ± 0.83 [c]	2.36 ± 1.09 [e]	1.52 ± 1.11	2.38 ± 1.02 [h]
Mean Cortical LB Score	2.51 ± 0.68 [c]	2.03 ± 0.87 [f]	1.22 ± 0.67	2.14 ± 0.85 [h]
Striatal Aβ Plaque Score	2.43 ± 1.13 [c d]	1 ± 1.14	0.52 ± 0.98	1.40 ± 1.29 [g]
Frontal Aβ Plaque Score	2.24 ± 0.90 [c d]	1.27 ± 1.10	0.80 ± 0.93	1.51 ± 1.12 [h]
Ent. Aβ Plaque Score	2.06 ± 1 [c d]	1.11 ± 0.91	0.78 ± 0.92	1.34 ±1.00 [g]
Total Aβ Plaque Score	4.13 ± 1.85 [c d]	2.43 ± 1.92 [e]	1.57 ± 1.79	2.81 ± 2.02 [g]
CVP	3 (18%)	20 (36%)	14 (29%)	23 (32%)

LB: Lewy body; SFC: superior frontal cortex; AC: anterior cingulate; TC: temporal cortex; PC: parietal cortex; EC: entorhinal cortex; HC: Highest Cortical; CVP: Concomitant Vascular Pathology.

[a] DLB + PDD; [b] vs PDND $p < 0.05$; [c] vs PDND $p < 0.001$; [d] vs PDD $p < 0.05$; [e] vs PDND $p < 0.05$; [f] vs PDND $p < 0.001$; [g] vs PDND $p < 0.05$; [h] vs PDND < 0.001.

Dementia was compared to PDND with Mann-Whitney U test or Chi Square test, as appropriate. The three groups of DLB, PDD, and PDND were compared with One Way Anova, followed by Fisher's Least Significant Difference, with Bonferroni correction for multiple testing.

Values are expressed as either counts (%) or mean ± SEM.

TABLE 3: Pearson correlation analysis (taken as all possible pairs) of the three types of pathologies across the whole sample (DLB, PDD and PDND).

		Braak αSN stage	Braak tau stage	Mean Cortical LB score	Striatal Aβ score	Cortical Aβ score
Braak αSN stage	*r*	1	0.12	0.44	-0.07	0.03
	P-value	-	0.18	<0.001	0.64	0.71
Braak tau stage	*r*		1	0.35	0.46	0.44
	P-value		-	<0.001	0.001	<0.001
Mean Cortical LB score	*r*			1	0.48	0.54
	P-value			-	<0.001	<0.001
Striatal Aβ score	*r*				1	0.85
	P-value				-	<0.001
Cortical Aβ score	*r*					1
	P-value					-

R = correlation coefficient; correlation is significant for p-values < 0.05.

TABLE 4: Neuropathological variables associated with dementia

Univariate Regression			
Variable	OR	CI	P-value
SFC LB score	2.65	1.75 – 4.27	<0.0001
TC LB score	3.69	2.15 – 6.90	<0.0001
PC LB score	3.74	1.96 – 7.92	<0.0014
AC LB score	2.45	1.53 – 4.20	=0.001
EC LB score	2.04	1.44 – 2.99	<0.001
Mean CLB score	4.13	2.45 – 7.52	<0.034
HC Aβ score	1.85	1.26 – 2.64	=0.001
Total Cortical Aβ score	1.40	1.13 – 1.69	=0.001
αSN Braak stage	3.23	1.30 – 11.79	=0.034
Striatal Aβ score	2.58	1.40 – 5.42	=0.005
Multivariate Regression			
Mean CLB score	4.23	2.19 – 8.98	<0.001

LB: Lewy body; SFC: superior frontal cortex; AC: anterior cingulate; TC: temporal cortex; PC: parietal cortex; EC: entorhinal cortex; HC: Highest Cortical; CLB: Cortical Lewy body.
In the multivariate logistic regression model, we included the mean CLB score rather than the individual regional LB scores and the total cortical Aβ plaque score to circumvent collinearity issues of LB load in the 5 cortical regions and of the Aβ burden between the frontal and entorhinal regions, respectively.

Figure Captions

FIGURE 1: Distribution of mean overall (a) and regional (b) cortical Lewy body scores. DLB is in solid grey, PDD in white and PDND in striped bars. The columns represent mean values ± SEM. Asterisks denote significance level (* $p < 0.05$; ** $p < 0.01$). SFC: superior frontal cortex; AC: anterior cingulate; TC: temporal cortex; PC: parietal cortex; EC: entorhinal cortex.

FIGURE 2: Distribution of (a) highest cortical (highest between entorhinal and frontal region) and (b) striatal Aβ plaque scores with semi-quantitative staging (0 = none, 1 = mild, 2 = moderate, 3 = severe). DLB is in solid grey, PDD in white and PDND in striped bars, while both dementia types (DLB and PDD) grouped together are in slate. The columns represent mean values ± SEM. Asterisks denote significance level (* $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$).

FIGURE 3: Receiver Operator Characteristic (ROC) curves for ability of pathology to classify LBD cases in those with and without dementia, as estimated by logistic regression models, with dementia as outcome and the three pathologies as covariates, individually (a,b,c) or jointly (d). (a) Mean CLB score AUC: 0.802; 95%CI:0.722-0.883; (b) Braak tau score AUC: 0.548; 95%CI; 0.485-0.610; (c) Total Cortical Aβ AUC: 0.666; 95%CI: 0.571-0.761; (d) Combined pathologies AUC: 0.798; 95%CI: 0.717-0.879. AUC = Area under Curve, which is equal to the probability that the explanatory variable correctly identifies dementia cases (AUC of 50% correspond to a random finding). The neuropathological variable with the highest predictive value was the mean CLB score (a); the predictive ability of the model did not increase when the three pathologies were analysed together (d).

FIGURE 4: Kaplan-Meier survival analysis curves, using a Cox proportional hazard model, with dementia as outcome and neuropathological scores as predictors. (a) Sum of semi-quantitative Aβ cortical plaque scores: score of 0 to 3 vs. score of 4 to 6. Higher Aβ scores predicted a faster progression to dementia (OR 1.84, 95%CI: 1.14-2.97; p=0.01); (b) Total cortical LB score (sum of 5 cortical regions): overall score ≤ 9 vs score ≥ 10. A higher CLB score was a good predictor of earlier dementia (OR 3.85, 95%CI: 2.31-6.42; p<0.001); (c) Braak tau staging: stage ≥ III vs stage ≤ II. (OR 1.69, p = 0.08). (d) Braak αSN staging: stage VI vs stage ≤ V (OR 2.40, p = 0.09).

FIGURE 5: a) Boxplot of Total Cortical Aβ score distribution comparing individuals carrying at least one APOE ϵ4 allele (homozygote or heterozygote) and individuals not carrying ϵ4 at all. b) Cortical LB score distribution across brain regions, comparing patients carrying at least one APOE ϵ4 allele (homozygote or heterozygote) and non-carriers of ϵ4. SFC: superior frontal cortex; AC: anterior cingulate; TC: temporal cortex; PC: parietal cortex; Ent: entorhinal cortex; for both panels E4 = ϵ4

FIGURE 1

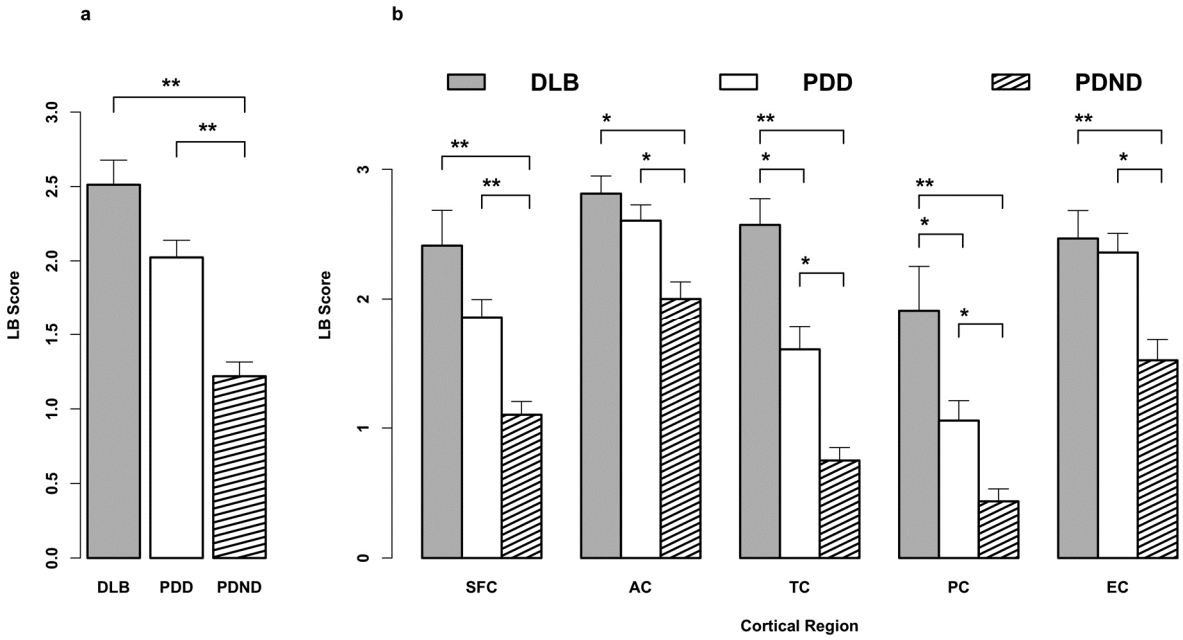

FIGURE 2

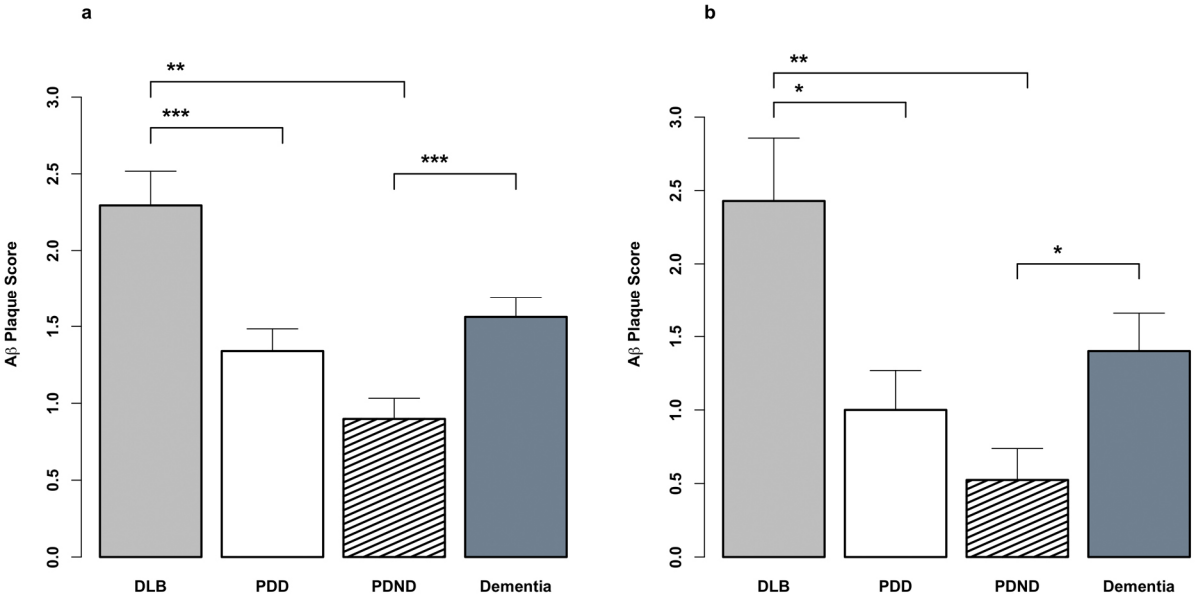

FIGURE 3

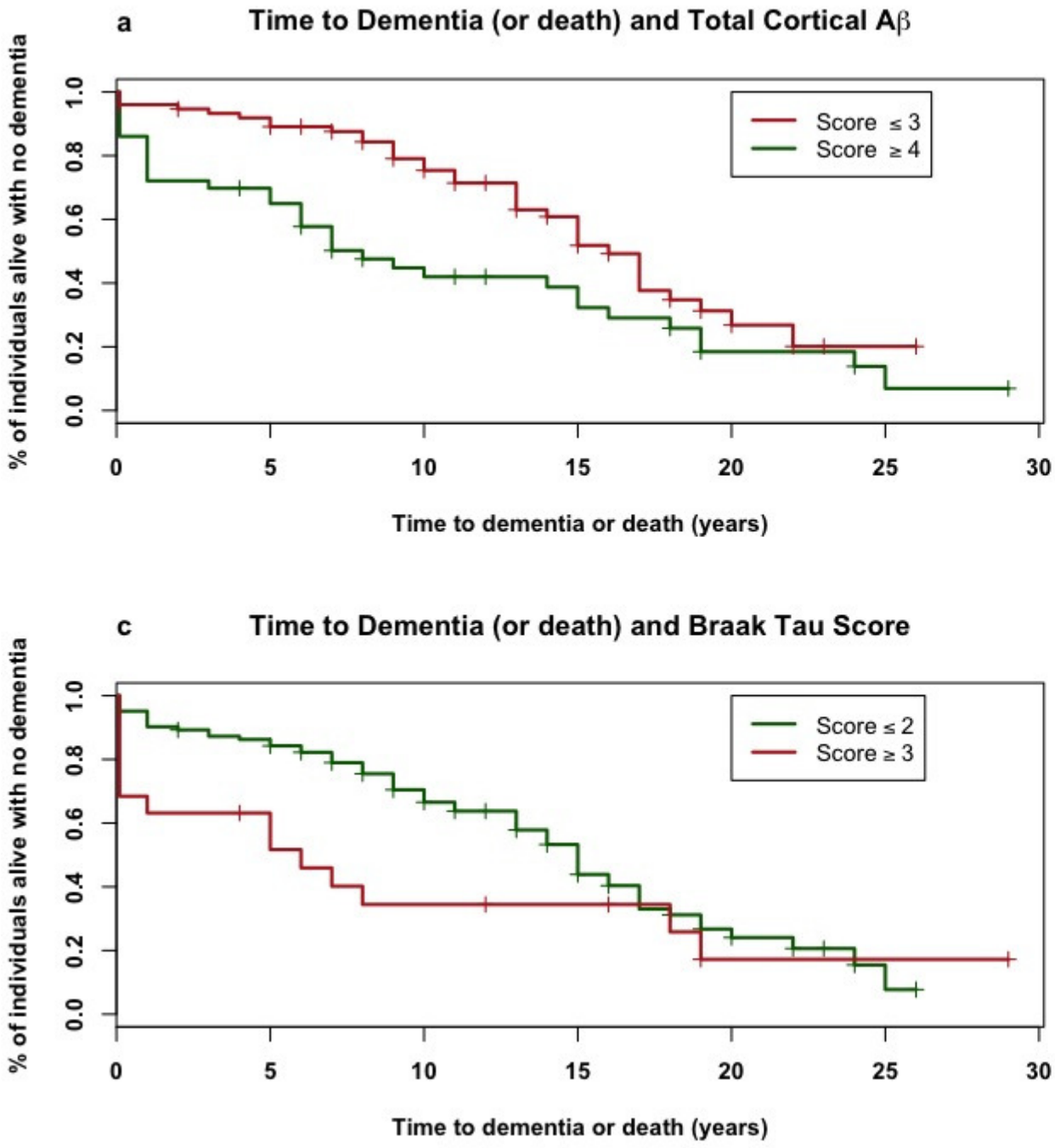
a
Time to Dementia (or death) and Total Cortical Aβ
% of individuals alive with no dementia
0.0
0.2
0.4
0.6
0.8
1.0
Score ≤ 3
Score ≥ 4
0
5
10
15
20
25
30
Time to dementia or death (years)
c
Time to Dementia (or death) and Braak Tau Score
% of individuals alive with no dementia
Score ≤ 2
Score ≥ 3
Time to dementia or death (years)

FIGURE 4

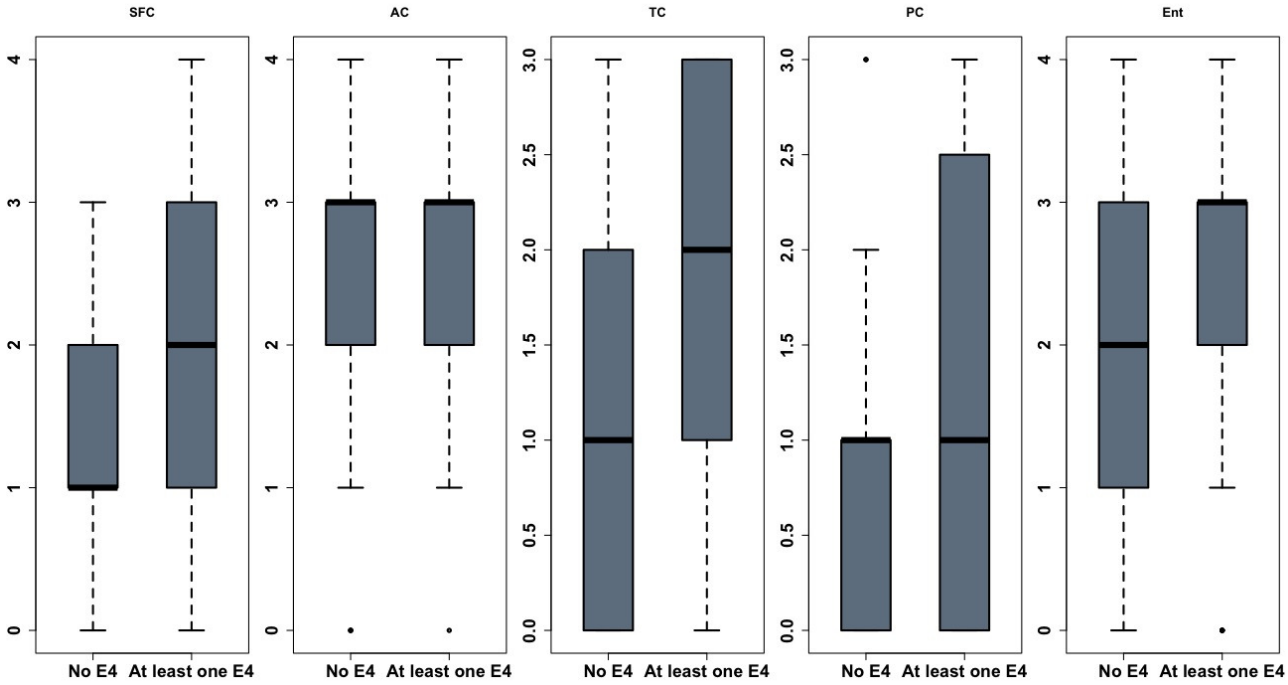

FIGURE 5