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

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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: One hundred and twenty-one 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 plaque deposition are both correlated with a faster progression to dementia. The higher CLB load in the temporal and parietal regions, which seems to be a distinguishing feature of DLB, may account for the shorter latency to dementia and could be mediated by the APOE ε4 allele.

Keywords: alpha-synuclein, Aβ, dementia, Lewy bodies, neuropathology, Parkinson’s disease
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 nonmotor complication in the course of Parkinson’s disease (PD), 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% [5]. The distinction between DLB and PDD is based on the occurrence of dementia before or within 1 year of motor signs of parkinsonism in DLB, but this is an arbitrary cut-off [1,6]. DLB, PDD and PD without dementia (PDND) share a common neuropathological feature consisting of alpha-synuclein (αSN) containing intraneuronal inclusions called Lewy bodies (LB) [1,7]. For this reason, these conditions have been grouped under the definition of ‘Lewy body diseases’ (LBD) [8–10]. There is growing evidence from several clinical [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 subcortical LB has been reported in autopsies of 11–18% of asymptomatic elderly individuals, a condition termed as ‘Incidental LB’, which has been reported to represent a presymptomatic 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 comorbid 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 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 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**

Subjects were donors of brain tissue to the Parkinson UK Brain Bank at Imperial College London who had provided written informed consent for inclusion in research studies. Ethical approval was obtained via the Parkinson’s UK Brain Bank [Wales Research Ethics Committee (ref. no. 08/MRE09/31+5)].

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 [4] and classify subjects into (i) PD without dementia within a year prior to death (PDND); (ii) DLB and (iii) PDD. The Diagnostic and Statistical Manual of the American Psychiatric Association (4th edn) 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 1 year of the onset of motor signs (although none actually had dementia onset prior to motor symptoms), while cases were defined as PDD if dementia occurred later in the disease course [1,3]. One hundred and twenty-one cases were included in the analysis (17 DLB, 55 PDD and 49 PDND).

**Neuropathological assessment**

Topographical staging of α-SN pathology was based on the Brain-Net Europe (BNE) Consortium guidelines for Braak staging (0–6) [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 (PC; sampled at the level of the superior parietal lobule, 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 (neuropil threads) 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 FC and EC 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 was considered for grading (see Figure S1). For data analysis, the individual cortical Aβ plaque score of the frontal and entorhinal regions, as well as 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.

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 ‘Data S1’.

**Genetic characterization**

For all individuals, DNA was extracted from 25 to 50 mg of frozen brain tissue. Six MAPT and two APOE SNPs were genotyped (see Data S1) allowing for the definition of the MAPT H1/H2 haplotypes and the APOE ε2/ε3/ε4 status.

**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’s exact test while, 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 vs. 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 Data S1).

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 summarized in Tables 1 and 2. The mean age at death of our sample was $77.8 \pm 7.4$ years (range = 58–93) and the mean age at onset of PD signs was $65.6 \pm 9.7$ years (range = 40–86), with a mean disease duration of $12.2 \pm 6.9$ years (range = 0–29). The mean age of dementia onset was $71.7 \pm 7.5$ years in DLB and $74.6 \pm 6.8$ years in PDD. More information on clinical, neuropathological and genetic characteristics is available in the ‘Data S1’.

**Neuropathology of dementia**

The regional and the overall cortical mean LB scores and the proportion of cases with αSN-Braak stage 6

| Table 1. Clinical, genetic and demographic characteristics |
|------------------------------------------------------------|
| **Table 1. Clinical, genetic and demographic characteristics** |
| | **DLB (n = 17)** | **PDD (n = 55)** | **PDND (n = 49)** | **Dementia (n = 72)** |
| **Females** | 3 (18%) | 17 (31%) | 15 (31%) | 20 (28%) |
| **Age at death (years)** | $75.88 \pm 7.30$ | $77.69 \pm 6.63$ | $78.53 \pm 8.19$ | $77.26 \pm 6.78$ |
| **Age at onset motor (years)** | $71.41 \pm 7.66$ | $62.95 \pm 8.75$ | $66.47 \pm 10.45$ | $64.94 \pm 9.20$ |
| **Disease duration (years)** | $4.47 \pm 1.87$ | $14.75 \pm 6.10$ | $12.06 \pm 6.72$ | $12.32 \pm 6.96$ |
| **Age onset dementia (years)** | $71.76 \pm 7.47$ | $74.60 \pm 6.80$ | - | $73.93 \pm 7.01$ |
| **Time to dementia (years)** | $0.35 \pm 0.49$ | $11.65 \pm 5.39$ | - | $8.99 \pm 6.74$ |
| **Dementia duration (years)** | $4.12 \pm 1.76$ | $3.09 \pm 2.26$ | - | $3.33 \pm 2.21$ |
| **Hallucinations** | 14 (82%) | 48 (87%) | 9 (18%) | 62 (86%) |
| **RBD** | 10 (59%) | 15 (27%) | 5 (10%) | 25 (35%) |
| **Cognitive fluctuations** | 5 (33%) | 11 (20%) | 0 (0%) | 16 (22%) |
| **APOE4 carrier** | 7 (47%) | 18 (33%) | 11 (22%) | 25 (35%) |
| **MAPT H1/H1** | 10 (67%) | 38 (69%) | 30 (61%) | 48 (67%) |

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.

RBD, REM sleep behaviour disorder; PDD, Parkinson’s disease with dementia; PDND, Parkinson’s disease without dementia.

*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.

RBD, REM sleep behaviour disorder; PDD, Parkinson’s disease with dementia; PDND, Parkinson’s disease without dementia.

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Table 2. Neuropathological characteristics

|                  | DLB       | PDD       | PDND      | Dementia* |
|------------------|-----------|-----------|-----------|-----------|
| zSN-Braak stage 6| 16 (94%)  | 52 (95%)  | 40 (82%)  | 68 (94%)† |
| zSN-Braak stage 5| 1 (6%)    | 3 (5%)    | 6 (12%)   | 4 (6%)    |
| zSN-Braak stage 3/4| 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‡‡ | 1.86 ± 1.04§ | 1.10 ± 0.71 | 1.99 ± 1.07¶ |
| AC LB score      | 2.81 ± 0.54** | 2.60 ± 0.85†† | 2.00 ± 0.89 | 2.66 ± 0.78‡‡ |
| TC LB score      | 2.57 ± 0.76‡‡ | 1.61 ± 1.08§ | 0.75 ± 0.63 | 1.87 ± 1.09‡‡ |
| PC LB score      | 1.91 ± 1.14‡‡ | 1.09 ± 0.90†† | 0.44 ± 0.60 | 1.26 ± 1.02†† |
| EC LB score      | 2.47 ± 0.83‡‡ | 2.36 ± 1.09†† | 1.52 ± 1.11 | 2.38 ± 1.02†† |
| Mean cortical LB score | 2.51 ± 0.68‡‡ | 2.03 ± 0.87§ | 1.22 ± 0.67 | 2.14 ± 0.85‡‡ |
| Striatal Aβ plaque score | 2.43 ± 1.13‡‡ | 1 ± 1.14    | 0.52 ± 0.98 | 1.40 ± 1.29†† |
| Frontal Aβ plaque score | 2.24 ± 0.90‡‡ | 1.27 ± 1.10 | 0.80 ± 0.93 | 1.51 ± 1.12‡‡ |
| Entorhinal Aβ plaque score | 2.06 ± 1.00‡‡ | 1.11 ± 0.91 | 0.78 ± 0.92 | 1.34 ± 1.00†† |
| HC Aβ plaque score | 2.29 ± 0.92‡‡ | 1.33 ± 1.09 | 0.90 ± 0.94 | 1.56 ± 1.12‡‡ |
| Total cortical Aβ score | 4.13 ± 1.85‡‡ | 2.43 ± 1.32†† | 1.57 ± 1.79 | 2.81 ± 2.02†† |
| CVP              | 3 (18%)   | 20 (36%)  | 14 (29%)  | 23 (32%)  |

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.

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; PDD, Parkinson’s disease with dementia; PDND, Parkinson’s disease without dementia.

*DLB vs PDD. †‡ vs. PDND P < 0.05.
‡‡vs. PDND P < 0.001.
§ vs. PDND P < 0.001.
‡‡‡vs. PDND P < 0.001.
**vs. PDD P < 0.05.
††† vs. PDND P < 0.05.
‡‡‡‡ vs. PDD P < 0.05.

were significantly higher in the dementia groups (DLB and PDD) compared to PDND (Table 2 and Figure 1a). Furthermore, a higher CLB load was observed in the TC and PC in DLB compared to PDD (Figure 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); frontal Aβ score (OR = 1.95, P < 0.001); entorhinal Aβ score (OR = 1.86, P = 0.003); total cortical Aβ plaque score (OR = 1.40, P = 0.001); zSN-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).

Receiver Operator Characteristic curves suggested 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 remained unchanged when all three pathologies of tau, Aβ, and CLB were analysed together (AUC: 0.798; 95%CI: 0.717–0.879) (Figure 2).
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 PC were associated with a lower age at onset (β coefficient = -4.66, P = 0.004) and, among the demographic factors, women had a later age of onset than men (β 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 (β = -3.91, P = 0.004); frontal (β = -1.91, P = 0.006), entorhinal (β = -2.59, P = 0.001), total cortical (β = -1.94, P = 0.01) and striatal (β = -2.97, P = 0.006) Aβ plaque scores; AC LB score (β = -2.21, P = 0.04) and TC LB score (β = -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 (β = -1.37, P < 0.001) (Table 4B). 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 five regions was a reliable predictor of a faster progression to dementia (OR = 3.08, P < 0.01; Figure 3b). Higher Aβ scores also predicted a faster progression to dementia (OR = 1.19, P = 0.02; Figure 3a), but neither higher Braak αSN stages nor higher Braak tau stages did (P = 0.20, and 0.55, respectively, Figure 3c and d).

Genetics of dementia

There were no significant differences between demented and nondemented patients 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 divided into DLB and PDD, a trend for a higher prevalence of the APOE ε4 allele was observed in DLB (Fisher’s exact test, P = 0.06).
Table 3. Pearson correlation analysis (taken as all possible pairs) of the three types of pathologies across the whole sample [DLB, Parkinson’s disease with dementia (PDD) and PD without dementia (PDND)].

| Variable         | $r$ | $P$-value | $r$ | $P$-value | $r$ | $P$-value |
|------------------|-----|-----------|-----|-----------|-----|-----------|
| Striatal Aβ score | 1   | 0.48      | 0.85 | 0.001     | 1   | 0.001     |
| Mean cortical LB score | 1   | 0.35      | 0.46 | 0.001     |     |           |
| Total cortical Aβ score | 1   | 0.48      | 0.54 | 0.001     |     |           |
| Total cortical LB score | 1   | 0.48      | 0.44 | 0.001     |     |           |
| Striatal tau stage | 1   | 0.35      | 0.46 | 0.001     |     |           |
| Braak SN stage     | 1   | 0.12      | 0.44 | 0.001     |     |           |
| Mean cortical LB score | 1   | 0.35      | 0.46 | 0.001     |     |           |
| Striatal Aβ score | 1   | 0.48      | 0.54 | 0.001     |     |           |
| Mean cortical LB score | 1   | 0.35      | 0.46 | 0.001     |     |           |

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

The association between APOE ε4 allele carrier status and cortical Aβ plaque burden was significant ($t = 2.31, P = 0.02$; Figure 4a) 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 AC or EC (Figure 4b).

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.

Table 4. Neuropathological variables associated with dementia (A) and with latency of dementia from onset of motor symptoms (B).

(A) Univariate logistic 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    |
| Frontal Aβ score | 1.95| 1.35–2.90 | <0.001    |
| Entorhinal       | 1.86| 1.26–2.84 | 0.003     |
| Aβ score         |     |       |           |
| Total cortical   | 1.40| 1.13–1.69 | 0.001     |
| Aβ score         |     |       |           |
| Striatal Aβ score| 2.58| 1.40–5.42 | 0.005     |
| SN-Braak stage   | 3.23| 1.30–11.79 | 0.034     |

(B) Univariate linear regression

| Variable         | Beta | 95% CI | $P$-value |
|------------------|------|--------|-----------|
| Tau topographical staging | $-3.91$ | $-6.51$ to $-1.30$ | 0.004 |
| Frontal Aβ plaque score | $-1.91$ | $-3.26$ to $-0.55$ | 0.006 |
| Entorhinal Aβ plaque score | $-2.59$ | $-4.10$ to $-1.08$ | 0.001 |
| Striatal Aβ plaque score | $-2.97$ | $-2.97$ to $-0.94$ | 0.006 |
| Total cortical Aβ plaque score | $-1.94$ | $-3.30$ to $-0.59$ | 0.01 |
| AC LB score      | $-2.21$ | $-4.37$ to $0.06$ | 0.04 |
| TC LB score      | $-2.11$ | $-3.73$ to $-0.50$ | 0.01 |

In the multivariate models, 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 five cortical regions and of the Aβ burden between the frontal and entorhinal regions, respectively.

LB, Lewy body; SFC, superior frontal cortex; AC, anterior cingulate; TC, temporal cortex; PC, parietal cortex; EC, entorhinal cortex; CLB, Cortical LB.

Discussion

We have conducted an integrated retrospective analysis of clinical, pathological and genetic characteristics of DLB, PDD and PDND in a large cohort of patients—tissue donors to the Parkinson’s UK Brain Bank. Our
results confirm the strong positive association previously reported between CLB burden and 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 the spectrum of LB 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 PC and TC 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 PC and TC 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 TC as a distinguishing characteristic of DLB cases [30].

Dementia with Lewy bodies cases were also characterized by a higher cortical and striatal Aβ plaque burden compared to PDD, in agreement with previous studies [24–26,28] (Figure 5). 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 have 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 the results from neuropa-thological analysis of a longitudinally followed cohort of PD cases with and without dementia [58]. Finally, survival analysis indicated that CLB burden is also a predictor of faster progression to dementia (Figure 3).

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 [18,19,36,58,59]. 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 2).

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 nonlinear disease progression, with milestones such as dementia heralding a stereotyped final phase of the disease, notwithstanding differences in the overall disease duration [60]. While 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 LBD 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...
Figure 4. (a) Boxplot of total cortical Aβ score distribution comparing individuals carrying at least one APOE e4 allele (homozygote or heterozygote) and individuals not carrying e4 at all. (b) Cortical LB score distribution across brain regions, comparing patients carrying at least one APOE e4 allele (homozygote or heterozygote) and noncarriers of e4. SFC, superior frontal cortex; AC, anterior cingulate; TC, temporal cortex; PC, parietal cortex; Ent, entorhinal cortex; for both panels E4 = e4.

Figure 5. 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, Parkinson’s disease with dementia (PDD) in white and Parkinson’s disease without dementia (PDND) in striped bars, while both dementia types (DLB and PDD) grouped together are in slate. The columns represent mean ± SEM. Asterisks denote significance level (*P < 0.05; **P < 0.01, ***P < 0.001).
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 e4 in a large series of AD+ and AD– synucleinopathies with dementia has indicated an increased frequency of e4 in all forms, even in the absence of AD pathology [36]. We did not observe a direct effect of the APOE e4 allele on dementia in our sample.

Interestingly, the APOE e4 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 e4 allele and CLB burden. Thus, the APOE e4 allele may mediate increased neocortical LB deposition either indirectly, possibly through a greater abundance of fibrillation-promoting Aβ plaques, or directly through yet unexplored mechanisms. Gearing and co-workers found a similar prevalence of the APOE e4 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 e4 dosage. They did not however demonstrate a significant increase in cortical Lewy pathology with increasing APOE e4 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 e4 carriers but no association between APOE e4 allele and density of cortical pathology [65]. Both of these studies predated the advent of alpha-synuclein-reactive antibodies and were based on ubiquitin staining, which may have caused underestimation of Lewy pathology [64,65]. Recently, the study by Compta and colleagues failed to find any correlations between APOE e4 allele and cortical LB 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 e4 carriers in a post mortem PD cohort, compared to subjects without the APOE e4 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 colleagues reported a multivariate model in which both the APOE e4 allele and cortical tau pathology predicted a higher CLB burden [14]. We have confirmed and enforced the concept that the APOE e4 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 e4 allele might exert its influence on CLB, as opposed to the allocortical cingulate and EC, where this association was not seen.

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. Nevertheless, in common with all retrospective studies our work has limitations. Symptoms and signs of dementia 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 sSN 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 semi-quantitative 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 sharing the same Braak score. Further studies based on computerized quantitative assessment of pathology could potentially substantiate and refine our findings [67].

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 neocortical 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 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

Study Design: LTM, CR, FCC, SMG, AlFB. Drafting of manuscript: CR. Manuscript editing: LTM, SMG, CR, FCC. Data collection: CR, IB, DG, FR, SMG, DD, AlFB, PP, SM, SP, PT, LC. Data analysis: FCC, CR, SMG, AdS, SP, PT.

Conflicts of interest

Nothing to report.

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61 Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Figure S1. Reference images for semiquantitative grading of plaques.

Data S1. Supplementary Text and Table.