

This is the peer reviewed version of the following article:

C. Ruffmann, F. C. F. Calboli, I. Bravi, D. Gveric, L. K. Curry, A. de Smith, S. Pavlou, J. L. Buxton, A. I. F. Blakemore, P. Takousis, S. Molloy, P. Piccini, D. T. Dexter, F. Roncaroli, S. M. Gentleman, L. T. Middleton (2015) *Neuropathology and Applied Neurobiology* Cortical Lewy bodies and A β burden are associated with prevalence and timing of dementia in Lewy body diseases.

which has been published in final form at

<http://onlinelibrary.wiley.com/doi/10.1111/nan.12294/abstract>.

This article may be used for non-commercial purposes in accordance with [Wiley Terms and Conditions for Self-Archiving](#).

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

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

AUTHORS: Claudio Ruffmann, MD^{1,2*}, Federico C.F. Calboli, PhD^{1*}, Ilaria Bravi, MD³, Djordje Gveric, MD, PhD³, Lisa K. Curry, MSc¹, Adam de Smith, PhD^{5,6}, Stelios Pavlou^{5,7}, Jessica L Buxton, PhD⁴, Alexandra I.F. Blakemore, PhD⁴, Petros Takousis, PhD¹, Sophie Molloy, MD, FRCP³, Paola Piccini, FRCP, PhD³, David T. Dexter, PhD³, Federico Roncaroli, MD⁸, Steve M. Gentleman, PhD³ and Lefkos T. Middleton, MD, FRCP¹.

* These authors contributed equally

¹ Neuroepidemiology and Ageing Research Unit, School of Public Health, Imperial College London

² Centro Parkinson, Istituti Clinici di Perfezionamento di Milano, Italy

³ Division of Brain Sciences, Department of Medicine, Imperial College, London

⁴ Section of Investigative Medicine, Department of Medicine, Imperial College London

⁵ Genomics of Common Disease, School of Public Health, Imperial College London

⁶ Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA, USA.

⁷ Department of Molecular Virology, Cyprus Institute of Neurology and Genetics

⁸ Institute of Brain Behaviour and Mental Health, University of Manchester

Corresponding Author:

Dr. Claudio Ruffmann
Department of Neuropathology
University of Oxford
Level 1, West Wing, John Radcliffe Hospital
Oxford OX3 7PD
UK
Tel: +44 01865223047
Email: claudio.ruffmann@ndcn.ox.ac.uk

KEYWORDS: Parkinson's disease; Lewy Bodies; Dementia; A β ; Neuropathology; alpha-synuclein

Word Count

Abstract: 249; Main Body: 4155; Acknowledgements: 153

Number of Figures: 5 Number of Tables: 4

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), A β 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 A β 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 $\epsilon 4$ 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 A β 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 $\epsilon 4$ allele.

Abbreviations:

A β : Beta-amyloid
 α SN: 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), 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 one 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 (α SN) containing intra-neuronal 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 α SN/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 A β plaque deposition has been reported in PDD in most [20] or a subset of patients [14,23]. Other studies have reported a higher A β 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 A β 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 α SN and tau pathologies [12,21], α SN and A β plaque deposition [12,32-35] and between the three pathologies of tau, A β and α SN [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, A β 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 α SN deposition in DLB [35,39] but not in PDD [14,20]. The APOE ϵ 4 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 ϵ 4 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 α SN, tau and A β 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 (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 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 (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 (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 A β deposition was performed in the frontal and entorhinal cortex with categories of 0 = absent, 1 = mild; 2 = moderate and 3= severe A β 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 sub-pial nor intra-cytoplasmic staining were considered for grading (see Supplementary Figure 1). For data analysis, the individual cortical A β plaque score of the frontal and entorhinal regions, as well as the sum of scores from both regions (total Cortical A β score) were used. Due to the high collinearity between frontal and entorhinal A β 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 A β 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 'Online Supporting Information'.

Genetic Characterization

For all individuals, DNA was extracted from 25 to 50 mg of frozen 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 ϵ 2/ ϵ 3/ ϵ 4 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 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, A β 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 is available in the 'Online Supporting Information'.

Neuropathology of Dementia

The regional and the overall cortical mean LB scores and the proportion of cases with α SN-Braak stage 6 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 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 A β 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 A β 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 A β score (OR 1.95, $p < 0.001$); entorhinal A β score (OR 1.86, $p = 0.003$); total cortical A β plaque score (OR 1.40, $p = 0.001$); α SN Braak stage (OR 3.23, $p = 0.034$); and striatal A β 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 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, A β , 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 a lower age at onset (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, $p=0.004$); frontal (beta = -1.91, $p=0.006$), entorhinal (beta = -2.59, $p=0.001$), total cortical (beta = -1.94, $p=0.01$) and striatal (beta = -2.97, $p=0.006$) A β 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 A β plaque score emerged as the only independent predictor of latency to dementia (beta = -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 5 regions was a reliable predictor of a faster progression to dementia (OR=3.08, $p<0.01$; Figure 4b). Higher A β scores also predicted a faster progression to dementia (OR 1.19, $p=0.02$; Figure 4a), but neither higher Braak α SN 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 demented and non-demented patients in the proportion of either APOE ϵ 4 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 ϵ 4 allele was observed in DLB (Fisher's Exact Test, $p=0.06$).

The association between APOE ϵ 4 allele carrier status and cortical A β 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 ϵ 4 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 A β score) using linear regression analysis (standardized beta: 0.187, $p=0.02$), where total cortical A β 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 ϵ 4 carrier status as the independent variable, showed that the ϵ 4 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 $\epsilon 4$ 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 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 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 A β plaque burden compared to PDD, in agreement with previous studies [24-26,28] (Figure 2b). Although we observed a trend suggesting a greater striatal A β 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 A β burden compared to PDD has been reported (87% and 43%, respectively) together with an inverse correlation between cortical burden of both LB and A β 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 A β plaque load in DLB compared to PDD [56-57]. In our multivariate model using stepwise linear regression, the total cortical A β 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 neuropathological 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 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 [18,19,36,58]. 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 α SN 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 α SN 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 α SN 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 A β and α SN 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 ϵ 4 in a large series of AD+ and AD- synucleinopathies with dementia has indicated

an increased frequency of $\epsilon 4$ in all forms, even in the absence of AD pathology [36]. We did not observe a direct effect of the APOE $\epsilon 4$ allele on dementia in our sample.

Interestingly, the APOE $\epsilon 4$ allele was associated with higher cortical A β 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 $\epsilon 4$ allele and CLB burden. Thus, the APOE $\epsilon 4$ allele may mediate increased neocortical LB deposition either indirectly, possibly through a greater abundance of fibrillization-promoting A β plaques, or directly through yet unexplored mechanisms. Gearing and coworkers found a similar prevalence of the APOE $\epsilon 4$ 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 $\epsilon 4$ dosage. They did not however demonstrate a significant increase in cortical Lewy pathology with increasing APOE $\epsilon 4$ 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 $\epsilon 4$ carriers but no association between APOE $\epsilon 4$ 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 $\epsilon 4$ 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 $\epsilon 4$ carriers in a post-mortem PD cohort, compared to subjects without the APOE $\epsilon 4$ 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 $\epsilon 4$ allele and cortical tau pathology predicted a higher CLB burden [14]. We have confirmed and enforced the concept that the APOE $\epsilon 4$ 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 $\epsilon 4$ allele might exert its influence on CLB, as opposed to the allocortical cingulate and entorhinal cortex, 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 α SN 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 neo-cortical LB pathology with APOE ϵ 4, which remains significant after adjusting for A β pathology. DLB shares important features with AD in the form of prominent A β (but not tau) pathology and APOE ϵ 4 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 α SN and A β , as well as the modulating mechanism of APOE ϵ 4 on LB neocortical pathology.

Acknowledgements

Tissue samples and associated clinical and neuropathological data were supplied by the Parkinson's UK Brain Bank, funded by Parkinson's UK, a charity registered in England and Wales (258197) and in Scotland (SC037554).

The authors are grateful to the more than 650 patients who, by donating brain tissue to the Parkinson's UK Brain Bank, have made this study possible. The authors thank the technicians and students of the Parkinson's UK Brain Bank and the Department of Genomics of Common Disease for their technical assistance. This study was supported by Parkinson's UK grant (G0909) and The Michael J. Fox Foundation for Parkinson's Research (LTM). FC and CR were further supported by a grant from UCB Pharma to LTM and CR was partly funded by the "Fondazione Grigioni per la lotta al Morbo di Parkinson".

Dr Roncaroli's work has partly been supported by the the European Union's Seventh Framework Program (FP7/2007-2013) under grant agreement HEALTH-F2-2011-278850 (INMiND)

Author Contributions

Study Design: LTM, CR, FCC, SMG, AIFB

Drafting of manuscript: CR

Manuscript editing: LTM, SMG, CR, FCC

Data collection: CR, IB, DG, FR, SMG, DD, AIFB, 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, Dubois 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. Parkkinen 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 A β 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, Lleó 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 ϵ 4 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, Lleó 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. Apolipoprotein 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, Rogava 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. Apolipoprotein 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, Kavantzias 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
α SN Braak Stage 6	16 (94%)	52 (95%)	40 (82%)	68 (94%) ^g
α SN Braak Stage 5	1 (6%)	3 (5%)	6 (12%)	4 (6%)
α SN 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 \pm 1.12 ^c	1.86 \pm 1.04 ^f	1.10 \pm 0.71	1.99 \pm 1.07 ^h
AC LB Score	2.81 \pm 0.54 ^b	2.60 \pm 0.85 ^e	2.00 \pm 0.89	2.66 \pm 0.78 ^h
TC LB Score	2.57 \pm 0.76 ^{c,d}	1.61 \pm 1.08 ^f	0.75 \pm 0.63	1.87 \pm 1.09 ^h
PC LB Score	1.91 \pm 1.14 ^{c,d}	1.09 \pm 0.90 ^e	0.44 \pm 0.60	1.26 \pm 1.02 ^g
EC LB Score	2.47 \pm 0.83 ^c	2.36 \pm 1.09 ^e	1.52 \pm 1.11	2.38 \pm 1.02 ^h
Mean Cortical LB Score	2.51 \pm 0.68 ^c	2.03 \pm 0.87 ^f	1.22 \pm 0.67	2.14 \pm 0.85 ^h
Striatal A β Plaque Score	2.43 \pm 1.13 ^{c,d}	1 \pm 1.14	0.52 \pm 0.98	1.40 \pm 1.29 ^g
Frontal A β Plaque Score	2.24 \pm 0.90 ^{c,d}	1.27 \pm 1.10	0.80 \pm 0.93	1.51 \pm 1.12 ^h
Entorhinal A β Plaque Score	2.06 \pm 1 ^{c,d}	1.11 \pm 0.91	0.78 \pm 0.92	1.34 \pm 1.00 ^g
HC A β plaque score	2.29 \pm 0.92 ^{c,d}	1.33 \pm 1.09	0.90 \pm 0.94	1.56 \pm 1.12 ^g
Total Cortical A β Score	4.13 \pm 1.85 ^{c,d}	2.43 \pm 1.92 ^e	1.57 \pm 1.79	2.81 \pm 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 \pm 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 α SN stage	Braak tau stage	Mean Cortical LB score	Striatal A β score	Cortical A β score
Braak α SN stage	<i>r</i>	1	0.12	0.44	-0.07	0.03
	P-value	-	0.18	<0.001	0.64	0.71
Braak tau stage	<i>r</i>		1	0.35	0.46	0.44
	P-value		-	<0.001	0.001	<0.001
Mean Cortical LB score	<i>r</i>			1	0.48	0.54
	P-value			-	<0.001	<0.001
Striatal A β score	<i>r</i>				1	0.85
	P-value				-	<0.001
Cortical A β score	<i>r</i>					1
	P-value					-

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

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 A β score	1.95	1.35 – 2.90	<0.001
Entorhinal A β score	1.86	1.26 – 2.84	=0.003
Total Cortical A β score	1.40	1.13 – 1.69	=0.001
Striatal A β score	2.58	1.40 – 5.42	=0.005
α SN Braak stage	3.23	1.30 – 11.79	=0.034
Multivariate Logistic Regression			
Mean CLB score	4.23	2.19 – 8.98	<0.001

B.

Univariate Linear Regression			
Variable	Beta Coefficient	95% CI	P-value
Tau Topographical Staging	-3.91	-6.51 - -1.30	0.004
Frontal A β plaque score	-1.91	-3.26 - -0.55	0.006
Entorhinal A β plaque score	-2.59	-4.10 - -1.08	0.001
Striatal A β plaque score	-2.97	-2.97 - -0.94	0.006
Total Cortical A β plaque score	-1.94	-3.30 - -0.59	0.01
AC LB Score	-2.21	-4.37 - 0.06	0.04
TC LB Score	-2.11	-3.73 - -0.50	0.01
Multivariate Linear Regression			
Total Cortical A β score	- 1.37	-2.36 - -0.38	<0.001

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

In the multivariate models, we included the mean CLB score rather than the individual regional LB scores and the total cortical A β plaque score to circumvent collinearity issues of LB load in the 5 cortical regions and of the A β burden between the frontal and entorhinal regions, respectively.

Figure Captions

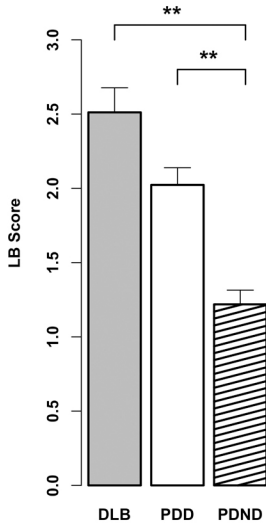
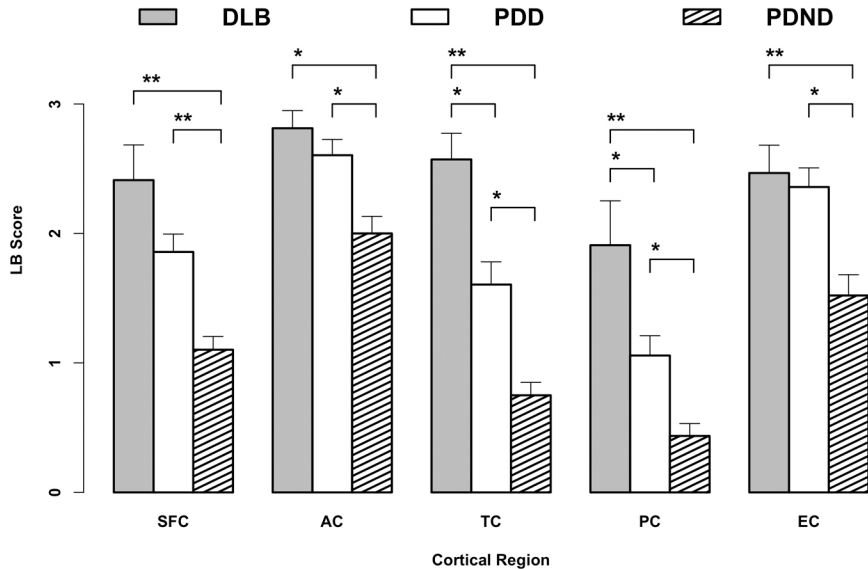
FIGURE 1: Distribution of mean overall (a) and regional (b) cortical Lewy body scores. DLB is in solid grey, PDD in white and PDND in striped bars. The columns represent mean values \pm SEM. Asterisks denote significance level (* $p < 0.05$; ** $p < 0.01$). SFC: superior frontal cortex; AC: anterior 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 A β 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 \pm SEM. Asterisks denote significance level (* $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$).

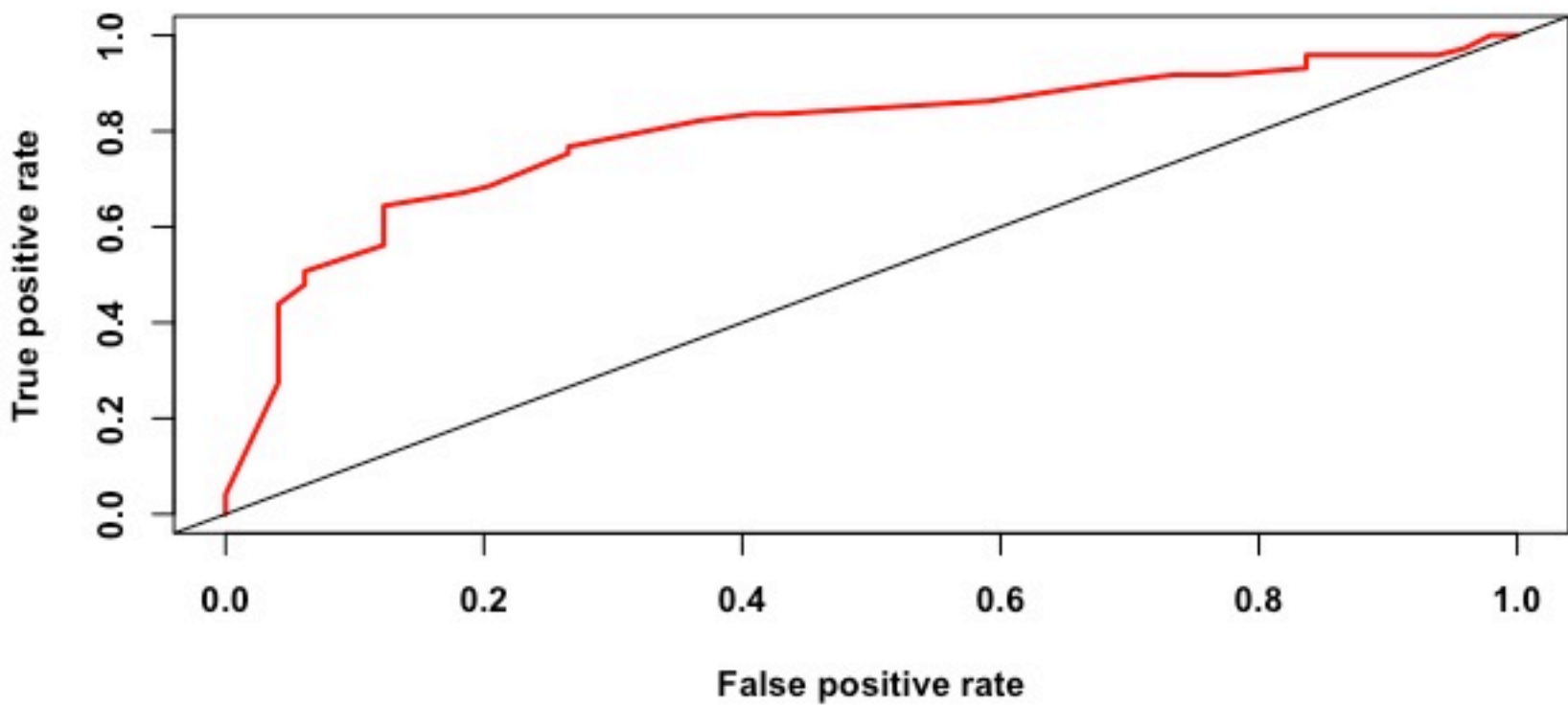
FIGURE 3: Receiver Operator Characteristic (ROC) curves for ability of pathology to classify LBD cases in those with and without dementia, as estimated by logistic regression models, with dementia as outcome and the three pathologies as covariates, individually (a,b,c) or jointly (d). (a) Mean CLB score 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 A β 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 A β cortical plaque scores: score of 0 to 3 vs. score of 4 to 6. Higher A β 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 \geq III vs stage \leq II. (OR 1.69, $p = 0.08$). (d) Braak α SN staging: stage 6 vs stage ≤ 5 (OR 2.40, $p = 0.09$).

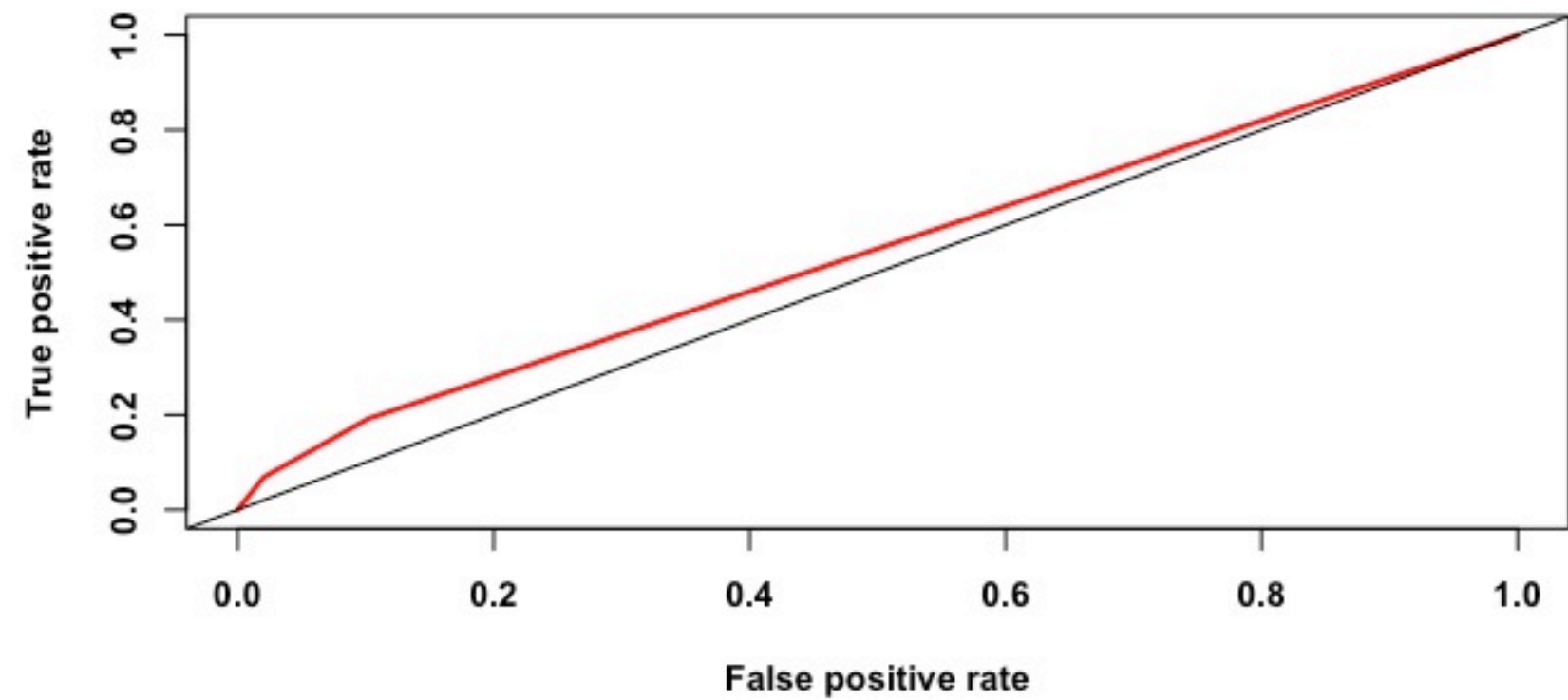
FIGURE 5: a) Boxplot of Total Cortical A β score distribution comparing individuals carrying at least one APOE ϵ 4 allele (homozygote or heterozygote) and individuals not carrying ϵ 4 at all. b) Cortical LB score distribution across brain regions, comparing patients carrying at least one APOE ϵ 4 allele (homozygote or heterozygote) and non-carriers of ϵ 4. SFC: superior frontal cortex; AC: anterior cingulate; TC: temporal cortex; PC: parietal cortex; Ent: entorhinal cortex; for both panels E4 = ϵ 4

a**b**

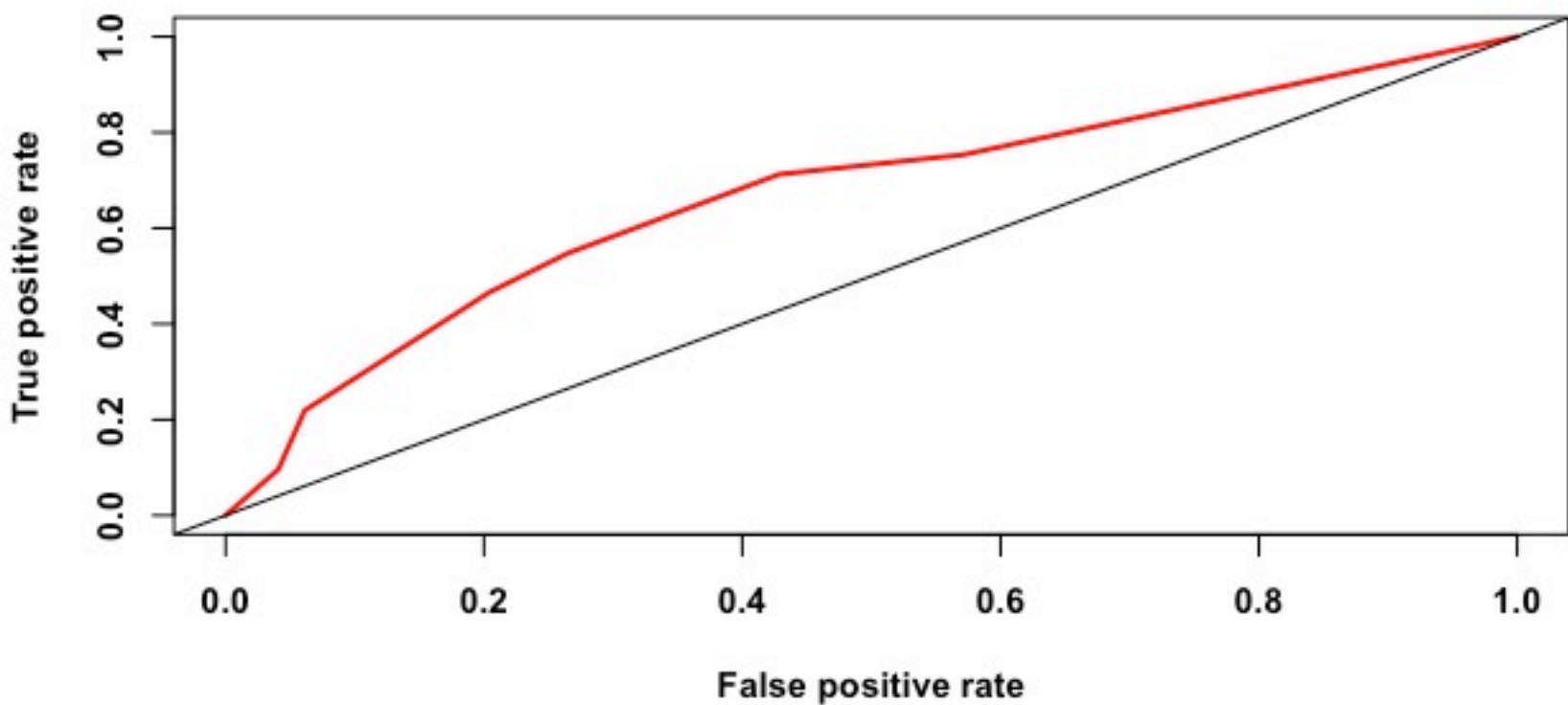
a Mean Cortical LB Score



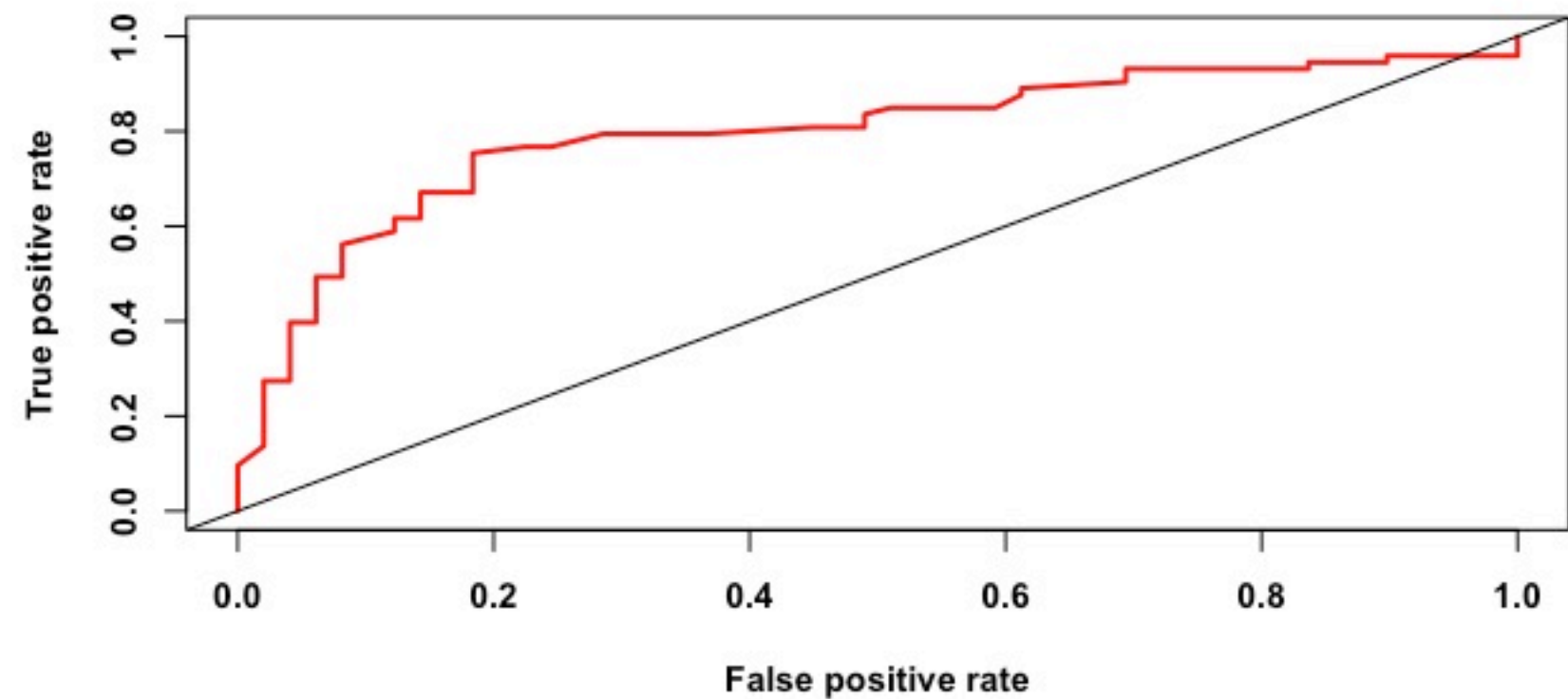
b Braak Tau Score



c Total Cortical A β Score



d ROC for all three pathologies together



a Total Cortical A β Score**b SFC LB Score****AC LB Score****TC LB Score****PC LB Score****Ent LB Score**