

Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: which is more important?

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The relative importance of Lewy- and Alzheimer-type pathologies to dementia in Parkinson's disease remains unclear. We have examined the combined associations of α -synuclein, tau and amyloid- β accumulation in 56 pathologically confirmed Parkinson's disease cases, 29 of whom had developed dementia. Cortical and subcortical amyloid- β scores were obtained, while tau and α -synuclein pathologies were rated according to the respective Braak stages. Additionally, cortical Lewy body and Lewy neurite scores were determined and Lewy body densities were generated using morphometry. Non-parametric statistics, together with regression models, receiver-operating characteristic curves and survival analyses were applied. Cortical and striatal amyloid- β scores, Braak tau stages, cortical Lewy body, Lewy neurite scores and Lewy body densities, but not Braak α -synuclein stages, were all significantly greater in the Parkinson's disease-dementia group ($P < 0.05$), with all the pathologies showing a significant positive correlation to each other ($P < 0.05$). A combination of pathologies [area under the receiver-operating characteristic curve = 0.95 (0.88–1.00); $P < 0.0001$] was a better predictor of dementia than the severity of any single pathology. Additionally, cortical amyloid- β scores ($r = -0.62$; $P = 0.043$) and Braak tau stages ($r = -0.52$; $P = 0.028$), but not Lewy body scores ($r = -0.25$; $P = 0.41$) or Braak α -synuclein stages ($r = -0.44$; $P = 0.13$), significantly correlated with mini-mental state examination scores in the subset of cases with this information available within the last year of life ($n = 15$). High cortical amyloid- β score ($P = 0.017$) along with an older age at onset ($P = 0.001$) were associated with a shorter time-to-dementia period. A combination of Lewy- and Alzheimer-type pathologies is a robust pathological correlate of dementia in Parkinson's disease, with quantitative and semi-quantitative assessment of Lewy pathology being more informative than Braak α -synuclein stages. Cortical amyloid- β and age at disease onset seem to determine the rate to dementia.

Keywords: lewy bodies; amyloid- β ; tau; Parkinson's disease; dementia

Abbreviations: MMSE = mini-mental state examination; PDD = Parkinson's disease with dementia; PDND = Parkinson's disease without dementia

Introduction

Dementia is frequent in the terminal phases of Parkinson's disease (Aarsland *et al.*, 2003), but its anatomical and pathological substrate remains unclear. Limbic and neocortical Lewy body pathology has been claimed to be the main determinant of the development of cognitive impairment in a number of studies (Hurtig *et al.*, 2000; Mattila *et al.*, 2000; Harding and Halliday, 2001; Kövari *et al.*, 2003; Aarsland *et al.*, 2005). A synergistic interaction between the Alzheimer's disease-related protein aggregates (amyloid- β and tau) and α -synuclein-containing inclusions (perikaryal Lewy bodies and Lewy neurites) has also been proposed as a critical determinant (Masliah *et al.*, 2001; Apaydin *et al.*, 2002; Giasson *et al.*, 2003; Pletnikova *et al.*, 2005; Lashley *et al.*, 2008; Clinton *et al.*, 2010), and a link between concomitant Alzheimer's disease pathology and faster progression to dementia has been suggested (Ballard *et al.*, 2006; Halliday *et al.*, 2008; Sabbagh *et al.*, 2009). Subcortical amyloid- β pathology has also been reported in patients with Parkinson's disease and cognitive impairment (Kalaitzakis *et al.*, 2008, 2009), but it remains uncertain whether the striatal amyloid- β deposition alone could drive the progression of dementia in Parkinson's disease independently of cortical amyloid- β lesions and cortical Lewy body burden (Lashley *et al.*, 2008; Dickson *et al.*, 2009).

Variants of the *APOE* and the *MAPT* gene have been respectively associated with the presence of amyloid- β aggregates (Polvikoski *et al.*, 1995) and with neuronal degeneration related to increased tau protein expression (Laws *et al.*, 2007), and both genes have been linked with an increased risk of dementia in Parkinson's disease (Goris *et al.*, 2007; Williams-Gray *et al.*, 2009); but a connection between these genetic variants and the burden of Lewy body- and Alzheimer's disease-type pathologies in Parkinson's disease has not been studied in detail.

Given that cognitive impairment usually occurs in the later phase of the disease and that advanced age increases the risk of multiple pathologies (Kovacs *et al.*, 2008; Matthews *et al.*, 2009), brains of patients with Parkinson's disease with dementia (PDD) are likely to show various degrees of amyloid- β and tau pathologies, in addition to α -synuclein pathology leading to difficulties in determining their relative contribution to the clinical picture.

In this study, we investigated the combined effect of Lewy body- and Alzheimer's disease-type (amyloid- β and tau) pathologies on the presence of and progression to dementia in Parkinson's disease.

Materials and methods

Case selection

Two hundred and three patients with an initial clinical diagnosis of parkinsonism and a pathologically proven diagnosis of Parkinson's

disease, according to widely accepted neuropathological criteria (Ince *et al.*, 2008), were identified from the records of donors to the Queen Square Brain Bank for Neurological Disorders, UCL Institute of Neurology (QSBB) from the period 2000 to 2008. Cases were collected using a protocol approved by a London Multicentre Research Ethics Committee and are stored under a licence issued by the Human Tissue Authority. The main selection criterion was the availability of reliable clinical information on the presence or the absence of cognitive impairment and features associated with dementia in Parkinson's disease, such as visual hallucinations and fluctuating confusion. Cases were excluded if the clinical records were incomplete as to whether the cognitive changes were related to alternative causes (i.e. drug-related) rather than to disease progression. Subsequently, the cases were retrospectively classified as Parkinson's disease without dementia (PDND) or PDD by consensus between three neurologists with expertise in movement disorders (Y.C., S.O.S., A.J.L.); who independently applied the functional rule of the presence of substantial and apparently permanent impairment of ability to perform tasks of daily living because of cognitive disability (Diagnostic and Statistical Manual of Mental Disorders, DSM-IV, 1995), and the recently proposed Movement Disorder Society clinical criteria for patients with PDD, which partly rely on the afore-mentioned dementia-associated features (Emre *et al.*, 2007).

Demographic and clinical data included gender, age at onset, age at death and disease duration, along with time to dementia for patients with PDD cases. The score on mini-mental state examination (MMSE) within the last year of life was also included, where available (Folstein *et al.*, 1975).

Neuropathological assessment

After fixation in 10% buffered formalin, the brains were examined by a neuropathologist and sampled in accordance with the standardized protocols of the Queen Square Brain Bank. In compliance with established criteria for the neuropathological diagnosis of Parkinson's disease (Ince *et al.*, 2008) and Alzheimer's disease (Mirra *et al.*, 1991; Ball *et al.*, 1997; Braak *et al.*, 2006), brain samples from selected regions were embedded in paraffin. Tissue sections (8 μ m thick) were cut, deparaffinized and rehydrated, followed by pretreatment with formic acid and/or pressure-cooking in citrate buffer at pH 6.0, as appropriate. Following epitope unmasking, monoclonal antibodies to amyloid- β , recognizing residues 17–26, (clone 4G8, dilution 1:3000; Cell Sciences Inc), phospho-tau recognizing phosphorylated serine 202/threonine 205 (clone AT8, dilution 1:600; Autogen Bioclear) and α -synuclein (clone KM51, dilution 1:1000; Novocastra) were applied and incubated at room temperature for 1 h or at 4°C overnight for α -synuclein. Subsequently, the secondary antibody (Dako, 1:200) was applied, followed by incubation with avidin-biotin peroxidase complex (ABC) kit (Elite PK-6100 Standard Vectastain® ABC kit, Vector Laboratories). Colour was developed with diaminobenzidine/H₂O₂ (DAB, Sigma) and with Romulin AEC chromogen (Biocare Medical) for α -synuclein.

Using tau immunohistochemistry, neurofibrillary tangle and neuropil thread pathology was staged (Braak stages I–VI) by Y.C., J.L.H. and T.R. in sections from the medial temporal lobe comprising the hippocampal formation with the entorhinal and transentorhinal cortices and

fusiform gyrus, first and second temporal gyri and occipital cortex (including striate, peri- and para-striate cortices), according to recommended criteria (Braak *et al.*, 2006).

Semi-quantitative assessment of α -synuclein-immunoreactive Lewy body-type pathology was carried out in five cortical regions (frontal, temporal, parietal, entorhinal and cingulate cortices) based on the recommendation by the consensus diagnostic criteria for dementia with Lewy bodies (McKeith *et al.*, 2005) and rated by L.P. and C.C., as follows: 1 = mild (sparse Lewy bodies at $\times 100$ magnification); 2 = moderate (1–3 Lewy bodies at $\times 100$ magnification); 3 = severe (≥ 4 Lewy bodies at $\times 200$ magnification); 4 = very severe (numerous Lewy bodies and neurites at $\times 200$ magnification). The α -synuclein-immunoreactive Lewy neurites were rated semi-quantitatively as: 0 = absent, 1 = sparse; 2 = moderate; and 3 = frequent. Each subject was given a cortical Lewy body and Lewy neurite score, calculated as the sum of all semi-quantitative regional scores in the above mentioned cortical areas (McKeith *et al.*, 2005). In addition, all Lewy bodies were systematically counted within the same five cortical areas of interest and these counts were adjusted to the surface area (Lewy body/mm²) using Image-Pro Plus software package (Media Cybernetics). Finally, each case was also classified according to Braak Parkinson's disease stage (ranging from 0 to 6) depending on the topographic distribution of α -synuclein-immunoreactive inclusions (Braak *et al.*, 2003).

Analysis of cortical amyloid- β immunoreactive plaques was performed by Y.C., J.L.H. and T.R. using a previously described protocol, based on the principles of 'The Consortium to Establish a Registry for Alzheimer's disease' (CERAD) (Mirra *et al.*, 1991; Lashley *et al.*, 2008). Both amyloid- β immunoreactive diffuse and mature plaques were scored semi-quantitatively as: 0 = absent, 1 = sparse; 2 = moderate; and 3 = frequent in the six selected cortical regions (frontal, temporal, parietal, occipital and cingulate cortices, and entorhinal gyrus). The corresponding subtotal score, ranging from 0 to 18, for both diffuse and mature plaques was calculated as the sum of the semi-quantitative scores of the six areas. We subsequently calculated the total (diffuse + mature) cortical amyloid- β score (from 0 to 36). In addition, the diffuse amyloid- β immunoreactive plaques were assessed in the striatum (with caudate and putamen being rated separately and together) and the claustrum by L.P. using a semi-quantitative scale ranging from 0 to 3 (absent to frequent).

The extent and severity of amyloid- β -cerebral amyloid angiopathy was examined as described elsewhere (Lashley *et al.*, 2008) by Y.C., J.L.H. and T.R. In brief, a semi-quantitative approach was used in the same six cortical areas used to determine the severity of amyloid- β plaque load as follows: 0 = none; 1 = mild (non-circumferential involvement of the vessel wall); 2 = moderate (involving the whole vessel wall circumference); 3 = severe (degenerative changes as vessel wall disruption or blood leakage out of the vessel). Leptomeningeal and parenchymal cerebral amyloid angiopathy were scored separately in each cortical area and, as with cortical plaques, the subtotal and total scores were subsequently calculated.

Genetic studies

For the genetic analysis of the tau (*MAPT*) and *APOE* genes, DNA was available for 46 cases. The *MAPT* H1 and H2 haplotypes were determined by genotyping the 238 bp insertion-deletion in intron 9 as previously described (Baker *et al.*, 1999). *APOE* was genotyped for the ϵ_2 , ϵ_3 and ϵ_4 variants following a published protocol using polymerase chain reaction amplification and HhaI restriction digestion and fragment analysis on a 6% Tris-borate-EDTA-polyacrylamide gel (Hixson and Vernier, 1990).

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences 15.0 software (SPSS Inc). Intra- and inter-rater reliability was examined for all neuropathological procedures with Cohen's κ coefficient and intra-class correlation. To assess univariate associations, chi-square test was used for comparisons of proportions, and Student's *t*-test or Mann-Whitney U test for comparison of frequencies depending on the applicability conditions. Bivariate correlations between the pathological variables were assessed by means of Spearman's coefficient. To investigate how well the three pathologies could classify cases as demented or not, the area under the receiver operating characteristic was used. Firstly, a binary regression model was used to determine the estimated probability for each case to belong to the dementia group, according to the burden of the three studied types of pathologies, both separately and altogether. Then, the estimated probabilities were used to obtain the area under the receiver operating characteristic curves (the closer to 1, the stronger the ability to classify cases) and its 95% confidence interval (CI) for cortical Lewy body scores, Braak tau stages, and cortical amyloid- β plaque scores, both separately and in combination.

Two types of analysis were undertaken to assess the possible differential relationship of the Alzheimer's disease and Lewy body pathologies with clinical variables related to dementia. Firstly, we performed correlation analyses between the severity of each pathology type with MMSE scores where available (Spearman's test). Secondly, Kaplan-Meier survival curves and Cox regression models (which provide estimates of the risk for the outcome with the corresponding 95% CI), were used to assess the effect of the cortical pathologies on time to dementia.

The statistical significance level was established at $P \leq 0.05$ (two-tailed analyses). All results were uncorrected for multiple comparisons, as the study was driven by *a priori* biologically and clinically plausible hypotheses (Perneger, 1998).

Results

Demographic, clinical and genetic information

Of the 203 potentially eligible cases, 147 were excluded due to incomplete medical records or inadequate brain sampling not meeting the criteria of this study. Of the 56 studied cases, 23 had been partially assessed in a previous study on the correlation between cortical α -synuclein and amyloid- β burden in Parkinson's disease (Lashley *et al.*, 2008). The included and excluded cases did not significantly differ in either gender distribution, age at onset, age at death or disease duration (Supplementary Table 1). Of the 56 included patients with Parkinson's disease, 29 (52%) were classified as demented (PDD) and 27 as non-demented (PDND), with no significant demographical or clinical differences between these two groups (Table 1). The H1/H2 haplotypes and *APOE* status were determined in 46 cases. The proportion of these genotypes in PDND and PDD groups are shown in Table 1 as descriptive information only, as the sample size was too small to perform any comparative statistical analysis of the genetic data.

Univariate analysis of α -synuclein, tau and amyloid- β pathologies

Intra- and inter-rater Cohen's κ and intra-class correlation coefficients were >0.75 for all the ratings performed, thus reflecting high reliability.

The proportion of subjects with Braak Alzheimer's disease Stage $>II$ (median value) was greater in the PDD group (Table 2; Fig. 1A). In most of the cases (90%) with moderate to severe Alzheimer's disease tau pathology and widespread cortical Lewy pathology the clinical information indicated cognitive decline. This contrasted with the group with neocortical Lewy pathology and mild or absent Alzheimer's disease tau pathology, in which a statistically smaller proportion of cases ($<30\%$) ($P = 0.0002$) had dementia (Table 3). No distinction could be made between the PDD and the PDND groups on the basis of the Braak Parkinson's disease stages as the median value calculated in the entire cohort was 6 and the inter-quartile range was 3–6 for both groups (Table 2), with Stage 5 being more frequent in PDND and Stage 6 in PDD cases ($P = 0.001$) (Fig. 1B). However, the semi-quantitative cortical Lewy body and Lewy neurite scores,

together with the Lewy body densities, were more sensitive measures of cognitive decline in life as these were significantly higher in the PDD than in the PDND group (Table 2; a detailed regional assessment is shown for cortical Lewy body and Lewy neurite scores and Lewy body densities in Fig. 2 and Supplementary Table 2). Similarly, both cortical and striatal amyloid- β scores, together with total cerebral amyloid angiopathy scores were significantly higher in the PDD than in the PDND cases (Table 2; a detailed regional assessment is shown for cortical and subcortical amyloid- β plaque scores in Fig. 3 and Supplementary Tables 3 and 4; and for leptomeningeal, parenchymal and combined cerebral amyloid angiopathy scores in Supplementary Fig. 1 and Supplementary Table 5). Overall, diffuse plaques were more common than mature plaques in the entire cohort (the mean of the sum of diffuse plaque scores: 9.05 ± 7.02 , the mean of the sum of mature plaque scores 4.39 ± 4.40 ; $P = 0.0035$), whereas there was no significant difference between the means of cerebral amyloid angiopathy leptomeningeal and parenchymal scores (3.05 ± 4.67 versus 1.49 ± 3.00 ; $P = 0.16$).

The sensitivity and specificity calculated for each pathological variable using a series of cut-off points of varying stringency are

Table 1 Basic demographic and clinical data of patients with Parkinson's disease ($n = 56$) classified according to lack or presence of dementia status upon their death

	PDND ($n = 27$)	PDD ($n = 29$)	P-value
Gender (%)	12 (44) female	8 (38) female	0.19
Age at onset (years)	59.41 ± 13.66	60.06 ± 11.37	0.98
Age at death (years)	73.22 ± 9.85	75.75 ± 8.23	0.25
Disease duration (years)	13.78 ± 6.85	15.69 ± 7.50	0.25
Age at dementia (years)	–	72.92 ± 8.43	NA
Time to dementia (years)	–	12.99 ± 7.57 (2–27.4)	NA
H1H1 carriers (%)	13/21 (62)	12/24 (50)	NA
APOE4 allele carriers (%)	6/22 (27)	10/24 (42)	NA

Data expressed as mean \pm SD, except for gender as n (percentage) females. Mann–Whitney test (PDND versus PDD) was applied, except for gender (chi-square test). NA = not applicable.

Table 2 Summary of the comparisons between PDND and PDD cases of the severity of the three pathologies: total cortical and striatal plaque scores along with total cerebral amyloid angiopathy score for amyloid- β ; Braak tau stage; and semi-quantitative scoring of the Lewy body, Lewy neurite assessment and Lewy body densities for α -synuclein

	Total cortical amyloid- β plaque score	Total striatal amyloid- β plaque score	Total cerebral amyloid angiopathy score	Braak Alzheimer's disease stage	Braak Parkinson's disease stage	Total cortical Lewy body score	Total cortical Lewy neurite score	Total cortical Lewy body densities
n	17	24	18	23	23	21	21	23
PDND	6.65 ± 7.66	1.21 ± 1.86	2.00 ± 5.15	0–III (3)	3–6 (NA)	4.71 ± 2.43	1.67 ± 2.29	1.29 ± 1.95
n	21	23	23	28	26	25	25	26
PDD	18.95 ± 10.41	2.74 ± 2.47	6.52 ± 8.5	I–V (20)	3–6 (NA)	9.17 ± 3.81	3.64 ± 3.21	4.86 ± 6.19
P-value	0.0005*	0.018*	0.009*	0.0001*	NA	0.001*	0.015*	0.002*

Data expressed as mean \pm SD except for Braak Alzheimer's disease and Parkinson's disease stages, as inter-quartile range with the number of cases above the median value (Stages II and 6, respectively), within parentheses. Mann–Whitney test (PDND versus PDD) was applied, except for chi-square test for high versus low Braak Alzheimer's disease and Parkinson's disease stages.

*Statistically significant values.

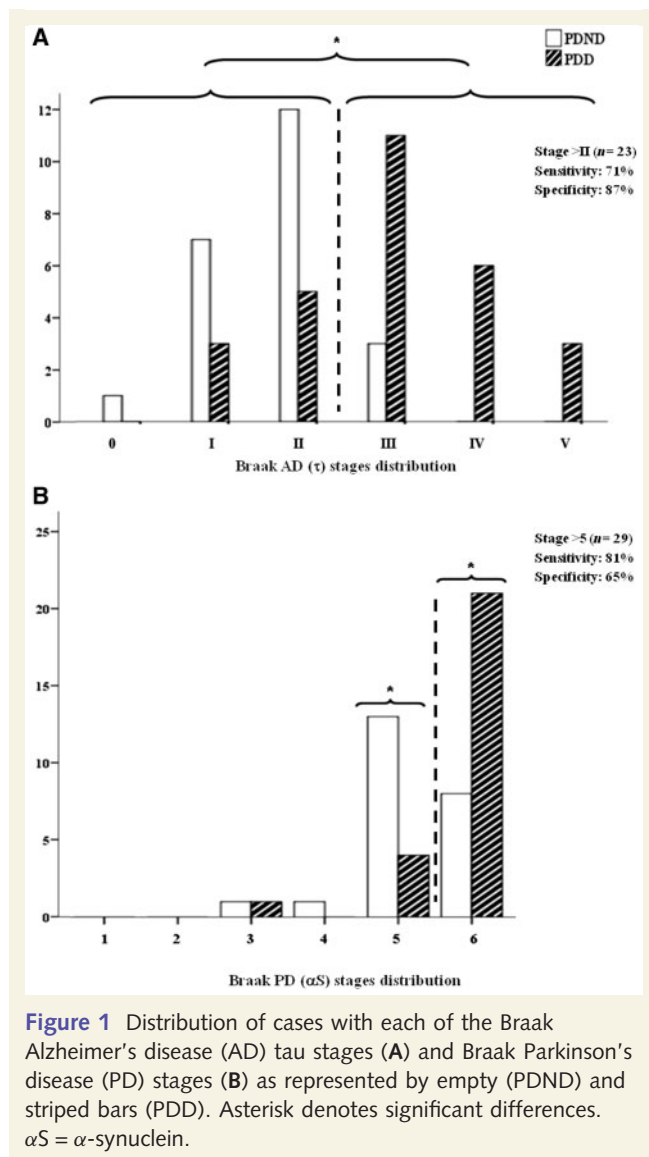


Figure 1 Distribution of cases with each of the Braak Alzheimer's disease (AD) tau stages (A) and Braak Parkinson's disease (PD) stages (B) as represented by empty (PDND) and striped bars (PDD). Asterisk denotes significant differences. α S = α -synuclein.

illustrated in Figs 1–3. These cut-offs were chosen on the basis of their degree of overlap or lack of overlap between PDND and PDD when analysing the raw data, as described elsewhere (Lashley *et al.*, 2008).

Correlations and combined analysis of α -synuclein, tau and amyloid- β pathologies

The severity of the studied pathologies (Lewy body and Lewy neurite densities, amyloid- β plaque and cerebral amyloid angiopathy scores, and tau stages) showed a significant correlation with each other, except total cortical Lewy body densities with cerebral amyloid angiopathy and cortical Lewy neurite scores with both cortical and striatal amyloid- β scores and cerebral amyloid angiopathy (Table 4). Confirming similar previous observations (Lashley *et al.*, 2008; Dickson *et al.*, 2009), when cases were separated into two groups according to the severity of the cortical amyloid- β score (cut-off value of total cortical amyloid- β score = 18) (Fig. 3C), there was a significant correlation between cortical amyloid- β score and cortical α -synuclein load (Lewy body densities) in the group with high total cortical amyloid- β scores in contrast to the group with low cortical amyloid- β scores (Supplementary Fig. 2).

The binary regression model with dementia as an outcome and Braak Alzheimer's disease tau stages and total cortical amyloid- β scores as covariates showed that the severity of both tau [odds ratio (OR) = 3.92; immunoreactive = 292%; 95% CI = 1.29–11.91; P = 0.016] and amyloid- β plaque (OR = 1.12; increased risk (IR) = 12%; 95% CI = 1.01–1.24; P = 0.037) pathologies significantly increased the risk of belonging to the PDD group, with age at onset having no significant influence (OR = 0.92; 95% CI = 0.85–1.00; P = 0.061). This effect was lost when cortical Lewy body scores were added as a further covariate, due to the

Table 3 Likelihood that pathological findings of dementia are associated with a Lewy body pathology and not Alzheimer's disease (according to McKeith *et al.*, 2005)

McKeith stage	Braak Alzheimer's disease stage		
	Braak stage 0–II n = 24	Braak stage III–IV n = 17	Braak stage V–VI n = 3
Brainstem predominant n = 5 (4 PDND + 1 PDD)	Low n = 4 (3 PDND + 1 PDD)	Low n = 1 (1 PDND)	Low n = 0
Limbic transitional n = 22 (14 PDND + 8 PDD)	High n = 11 (10 PDND + 1 PDD)	Intermediate n = 6 (all 6 PDD)	Low n = 0
Diffuse neocortical n = 22 (5 PDND + 17 PDD)	High n = 9 (4 PDND + 5 PDD)	High n = 10 (1 PDND + 9 PDD)	Intermediate n = 3 (all 3 PDD)
Proportion of demented cases* (%)	7 out of 24 (29)	18 out of 20 (90)	

*chi-square test; P = 0.0002.

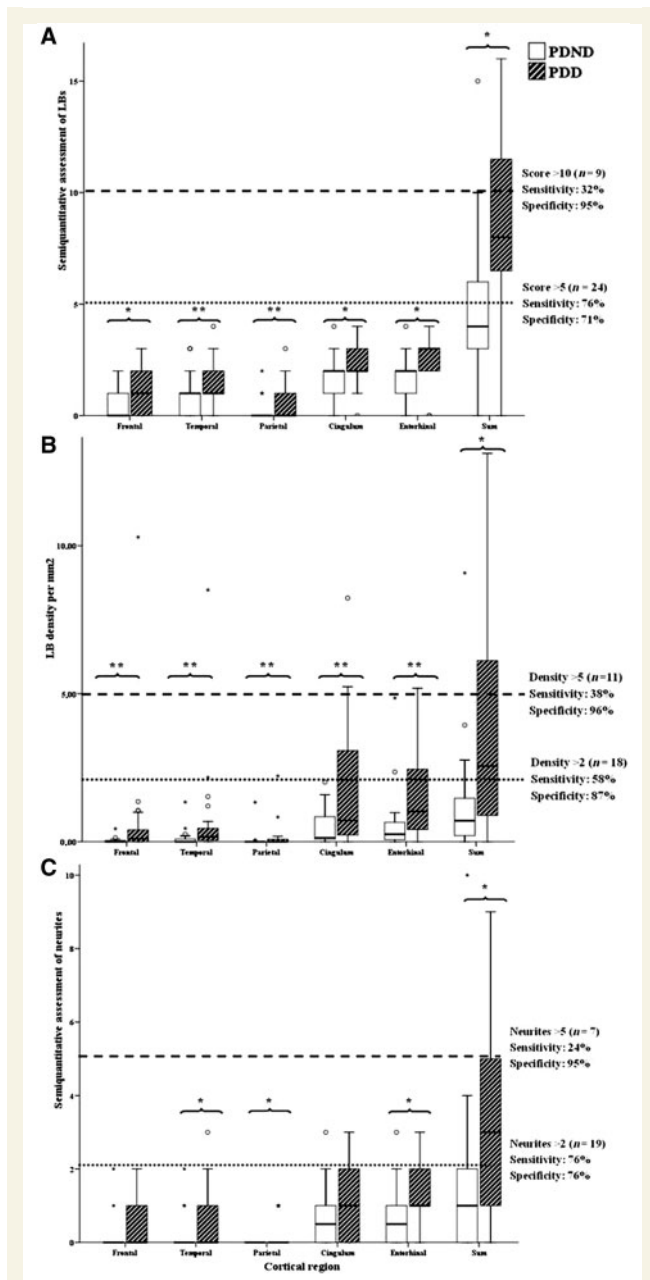


Figure 2 (A) Bar diagrams of regional and total semi-quantitative Lewy body (LB) scores with dashed and dotted lines, respectively, delineating cut-off values of 10 and 5 (score > 10: 88% PDD; score > 5: 79% PDD). (B) Bar diagrams of regional and total Lewy body (LB) densities with dashed and dotted lines, respectively, delineating cut-off values of 5 and 2 (scores > 5: 91% PDD; scores > 2: 83% PDD). (C) Bar diagrams of the regional and total semi-quantitative Lewy neurite scores with dashed and dotted lines, respectively, delineating cut-off values of 5 and 2 (scores > 5: 86% PDD; scores > 2: 74% PDD). * $P < 0.005$; ** $P < 0.05$.

observed correlation between all these variables, with such colinearity precluding further multivariate analyses (Harrell, 2001).

Using the estimated probabilities for each case to belong to the dementia group as calculated with the binary regression model,

we created the receiver operating characteristic curves for the currently accepted and most widely used staging or scoring systems for each pathology type: cortical Lewy body scores alone [area under the curve with 95% CI: 0.83 (0.70–0.97), $P = 0.001$]; Braak Alzheimer's disease tau stages alone [0.82 (0.70–0.93); $P = 0.0001$]; cortical total amyloid- β scores alone [0.83 (0.69–0.97), $P = 0.001$]; all the three pathologies in combination [0.95 (0.88–1.00); $P < 0.0001$]. In short, the ability of any of these pathologies alone to classify a given case as PDD was improved when all three pathologies were taken into consideration (Fig. 4A–D). The receiver operating characteristic curves for the other pathological variables related to Lewy-type and amyloid- β pathologies did not add any further information as a means of classifying cases as demented or not [total Lewy body densities: 0.76 (0.63–0.89), $P = 0.002$; Lewy neurite scores: 0.71 (0.56–0.86), $P = 0.016$; striatal amyloid- β scores: 0.69 (0.53–0.84), $P = 0.028$; total cerebral amyloid angiopathy scores: 0.72 (0.56–0.88), $P = 0.017$] (Supplementary Fig. 3).

Association of α -synuclein, tau and amyloid- β pathologies with the severity of cognitive impairment and progression to dementia

The subset of cases with MMSE performed within the year before death ($n = 15$; seven PDND and eight PDD) did not significantly differ in any demographic or clinical features from the other 41 cases (Supplementary Table 6) and showed pathological findings in line with those from the entire cohort (Supplementary Table 7). The analysis of this subset revealed that the Braak tau stages along with the parietal, cingulate, entorhinal and total cortical amyloid- β scores negatively correlated with MMSE score ante-mortem (that is, the higher the pathological stage or burden, the lower the MMSE score), whereas such correlation was not found for the Lewy body score or Braak Parkinson's disease stage (Table 5).

On survival analysis with time to dementia as time variable and dementia as outcome, high cortical amyloid- β scores implied shorter time to dementia than low scores ($P = 0.017$). In contrast, cases with Braak Alzheimer's disease tau stages > II, and those with high cortical Lewy body scores were not associated with significantly faster progression to dementia ($P = 0.83$ and 0.055 , respectively) (Table 6, Fig. 5). Of the demographic data, only older age at onset of Parkinson's disease had a significant effect (estimated relative hazard = 1.09; increased risk = 9%; 95% CI = 1.04–1.14; $P = 0.001$). When both amyloid- β and age at onset were included in the analysis as covariates, such significant effect was lost due to the fact that they were related to each other, as further confirmed by means of a *post hoc* Mann-Whitney comparison, which revealed that cases with greater cortical amyloid- β burden were those with higher age at onset ($P = 0.048$).

Genetic pathology association studies

Tau gene (*MAPT*) H1 homozygotes and *APOE* ϵ_4 carriers did not show higher Braak Alzheimer's disease tau stages or cortical Lewy

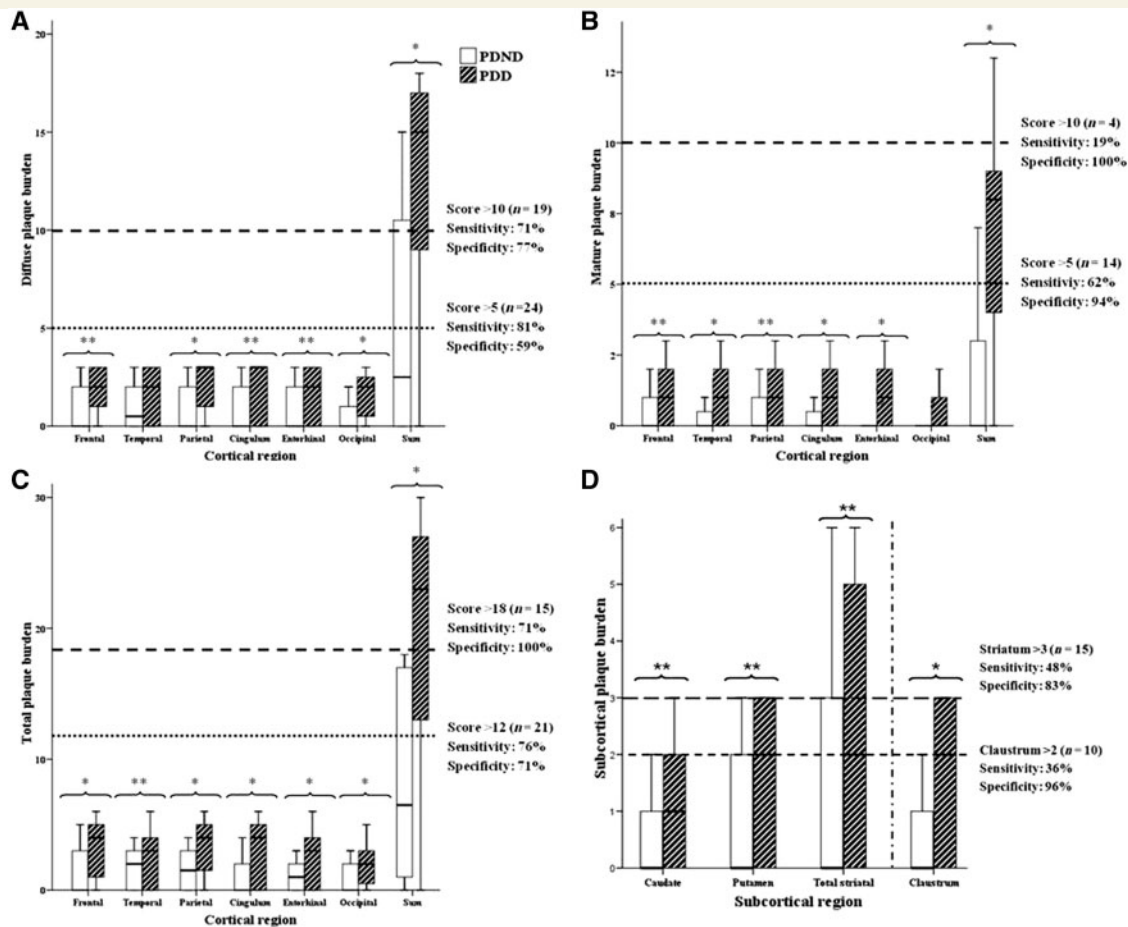


Figure 3 (A) Bar diagrams of regional and total amyloid- β diffuse plaque scores with dashed and dotted lines, respectively, delineating 10 and 5 cut-offs (score >10: 79% PDD; score >5: 71% PDD). (B) Bar diagrams of regional and total amyloid- β mature plaque scores with dashed and dotted lines, respectively, delineating 10 and 5 cut-offs (score >10: 100% PDD; score >5: 93% PDD). (C) Regional and total amyloid- β diffuse + mature plaque scores with dashed and dotted lines, respectively, delineating 18 and 12 (score >18: 100% PDD; score >12: 76% PDD). (D) amyloid- β diffuse plaque scores in the caudate and the putamen separately and together (striatum) with a dashed line delineating cut-off value of 3 for the total striatal score (73% PDD), as well as in the caudate, with a dotted line delineating cut-off value of 2 (90% PDD). * $P < 0.005$; ** $P < 0.05$.

body scores. In contrast, *APOE* ϵ_4 carriers featured higher total cortical amyloid- β plaque scores in entorhinal, temporal and parietal cortices, with no differences in striatal amyloid- β plaque scores, and higher entorhinal, occipital and total cerebral amyloid angiopathy scores (Supplementary Fig. 3, Supplementary Tables 8 and 9).

Discussion

We have explored the individual and combined roles of Lewy body- and Alzheimer's disease-type pathologies in Parkinson's disease-related dementia in a single large cohort. Our study indicates that a combined high burden of all three (Lewy body, amyloid- β and tau) pathologies is the best neuropathological correlate of patients with PDD, although marked severity of any one of these three cardinal pathological features in isolation also showed a good statistical association. Our study has also confirmed that quantitative and semi-quantitative measures of

α -synuclein pathology had a better discriminatory ability than currently used staging criteria. Additionally, we were able to show that higher cortical amyloid- β scores were associated with a faster progression to dementia.

In our study, we have found that both Lewy body (α -synuclein) and Alzheimer's disease- (amyloid- β and tau) type pathologies are increased in cortical areas of patients with PDD compared to patients with PDND. A number of studies have proposed that limbic and cortical Lewy body pathology is the main and specific pathological correlate of dementia in Parkinson's disease (Hurtig *et al.*, 2000; Mattila *et al.*, 2000; Harding and Halliday, 2001; Kövari *et al.*, 2003; Aarsland *et al.*, 2005), but other surveys have challenged this view (Apaydin *et al.*, 2002; Colosimo *et al.*, 2003; Pletnikova *et al.*, 2005; Ballard *et al.*, 2006; Kalaitzakis *et al.*, 2008, 2009; Lashley *et al.*, 2008). Furthermore, some clinicopathological studies favour a role for Alzheimer's disease-type pathological changes driving clinical disease progression to cognitive impairment in Parkinson's disease (Apaydin *et al.*, 2002; Jellinger and Attems, 2008). However,

Table 4 Correlations between the different pathologies across the entire Parkinson's disease cohort

	Total cortical amyloid- β score	Total striatal amyloid- β score	Total cerebral amyloid angiopathy score	Braak Alzheimer's disease stage	Braak Parkinson's disease stage	Lewy body Score	Lewy neurite score	Lewy body densities
Total cortical A β score	<i>r</i> 0.88 P-value <0.001*	0.40	0.56	0.43	0.51	0.19	0.42	
Total striatal A β score	<i>r</i> 0.36 P-value 0.032*	0.01*	<0.001*	0.11*	0.002*	0.24	0.008*	
Total cerebral amyloid angiopathy score	<i>r</i> 0.57 P-value <0.001*	0.47	0.001*	0.43	0.46	0.17	0.35	
Braak Alzheimer's disease stage	<i>r</i> 0.35 P-value 0.02*	0.27	0.001*	0.004*	0.003*	0.25	0.015*	
Braak Parkinson's disease stage	<i>r</i> 0.12 P-value <0.001*	0.12	<0.001*	0.27	0.47	0.19	0.23	
Lewy body score	<i>r</i> 0.66 P-value <0.001*	0.54	0.005*	0.35	0.54	0.27	0.15	
Lewy neurite score	<i>r</i> 0.69 P-value <0.001*	0.06	0.02*	0.02*	<0.001*	0.06	0.39	
Lewy body densities	<i>r</i> 0.75 P-value <0.001*	0.8	0.52	0.8	0.8	0.52	0.66	

Bivariate, pair-wise parametric (Spearman) correlation analysis between the three types of pathologies across the whole Parkinson's disease sample (PDND + PDD). *r* = correlation coefficient; * significant correlations.

the topographical distribution of α -synuclein- and tau-immunoreactive lesions behaved differently in our cohort. Tau pathology was most prominent in the entorhinal gyrus in PDND and with increasing Braak Alzheimer's disease tau stages, it spread out to the rest of the hippocampal formation, fusiform gyrus and the lateral temporal neocortex in patients with PDD. It is of note that with advancing Braak Alzheimer's disease tau stages there is both topographical progression and an increase in density of tau-positive lesions in affected regions (Braak *et al.*, 2006). Our finding that the higher the Braak Alzheimer's disease tau stages, the worse the cognitive performance was on the MMSE test, is in keeping with previous studies in Alzheimer's disease (Giannakopoulos *et al.*, 2003) and is in compliance with findings indicating that the severity of neurofibrillary tangle pathology is a good pathological indicator of dementia in the elderly (Matthews *et al.*, 2009).

In contrast, it was not possible to separate the non-demented patients with Parkinson's disease from the patients with PDD cases with the help of Braak Parkinson's disease stages, as most of our Parkinson's disease cases (94%) had reached the highest cortical Braak Parkinson's disease stages (Stages 5 and 6). This also explains why we were unable to find a significant correlation between Braak Parkinson's disease stages and MMSE scores, in contrast to a previous clinicopathological study (Braak *et al.*, 2005), where higher Braak Parkinson's disease stages were associated with lower MMSE scores. This is in line with studies suggesting an inconstant association between cortical Lewy body pathology and dementia (Colosimo *et al.*, 2003; Parkkinen *et al.*, 2005). In particular, and in contrast to our group, Braak's study had as many as 50% of cases in Stages 3 and 4, some of whom, nevertheless, displayed very low MMSE scores (Braak *et al.*, 2005). It is of note that one of the only two cases of the cohort with Braak Parkinson's disease Stage 3 was demented despite having Braak Alzheimer's disease Stage II, total cortical amyloid- β score as low as 13, and striatal amyloid- β score of 0, confirming that, although significant cortical Lewy pathology with conjoint Alzheimer-type pathology is the characteristic feature of the majority of patients with PDD cases, it is not an obligate post-mortem finding in all such cases. Other contributors including vascular damage (Jellinger and Attems, 2008; Matthews *et al.*, 2009), neuronal loss (Harding *et al.*, 2002), or specific neurotransmitter deficiencies (Cummings, 1988), might play a role in such instances, but their assessment was beyond the scope of our study.

Conversely, using semi-quantitative (Lewy bodies and Lewy neurites) and quantitative measures (Lewy body densities per mm²) of cortical α -synuclein lesions, we observed a higher burden of α -synuclein pathologies in patients with PDD than in non-demented patients with Parkinson's disease (PDND). This suggests that quantitative or semi-quantitative measures of α -synuclein-immunoreactive inclusions are more informative than the topographical assessment of Lewy bodies and Lewy neurites, perhaps because such quantitative approaches can overcome the ceiling effect of the Braak Parkinson's disease staging system, in which a single Lewy body in first order sensory association areas of the neocortex or premotor areas is enough to upgrade a given case from Stage 5 to 6 (Braak *et al.*, 2003). In our cohort, there

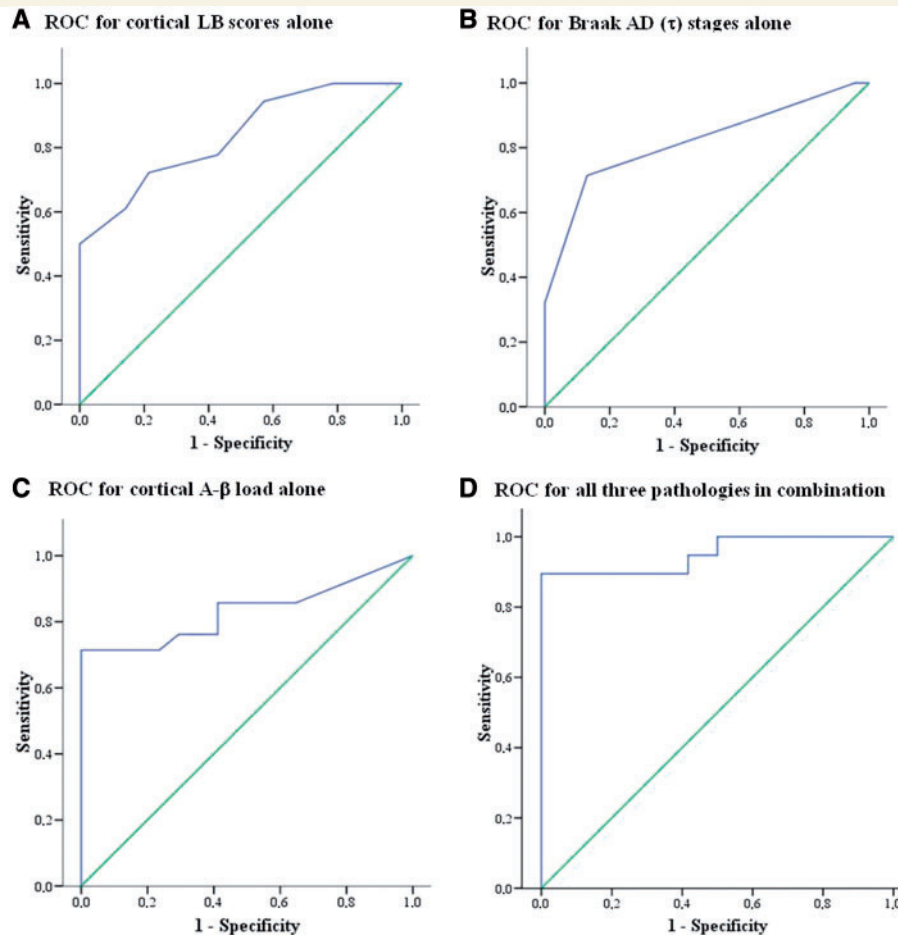


Figure 4 Receiver operating characteristic (ROC) curves for ability of pathology to classify cases as demented or non-demented created using the probabilities obtained in the binary regression models. (A) Cortical Lewy body (LB) scores alone (area under the curve = 0.83, 95% CI = 0.70–0.97, $P = 0.001$); (B) tau stages alone (area under the curve = 0.82, 95% CI = 0.70–0.93, $P = 0.0001$); (C) cortical amyloid- β (A- β) scores alone (area under the curve = 0.83, 95% CI = 0.69–0.97, $P = 0.001$); and (D) all three pathologies in combination (area under the curve = 0.95, 95% CI = 0.88–1.00, $P = 0.000003$). AD = Alzheimer's disease.

Table 5 Correlation between amyloid- β , tau and α -synuclein pathology with MMSE scores

	<i>r</i>	<i>P</i>
Braak Alzheimer's disease (tau) stage	−0.565	0.028*
Cortical amyloid- β score		
Mature parietal	−0.575	0.031*
Subtotal parietal	−0.558	0.035*
Diffuse cingulate	−0.595	0.032*
Mature cingulate	−0.579	0.038*
Subtotal cingulate	−0.61	0.027*
Mature entorhinal	−0.56	0.030*
Total cortical amyloid- β load	−0.62	0.043*
Total cortical Lewy body (α -synuclein) score	−0.25	0.41
Braak Parkinson's disease (α -synuclein) stage	−0.44	0.13

Spearman's non-parametric correlation analysis between each pathological variable and the MMSE scores within the last year of life ($n = 15$).

*Significant correlations.

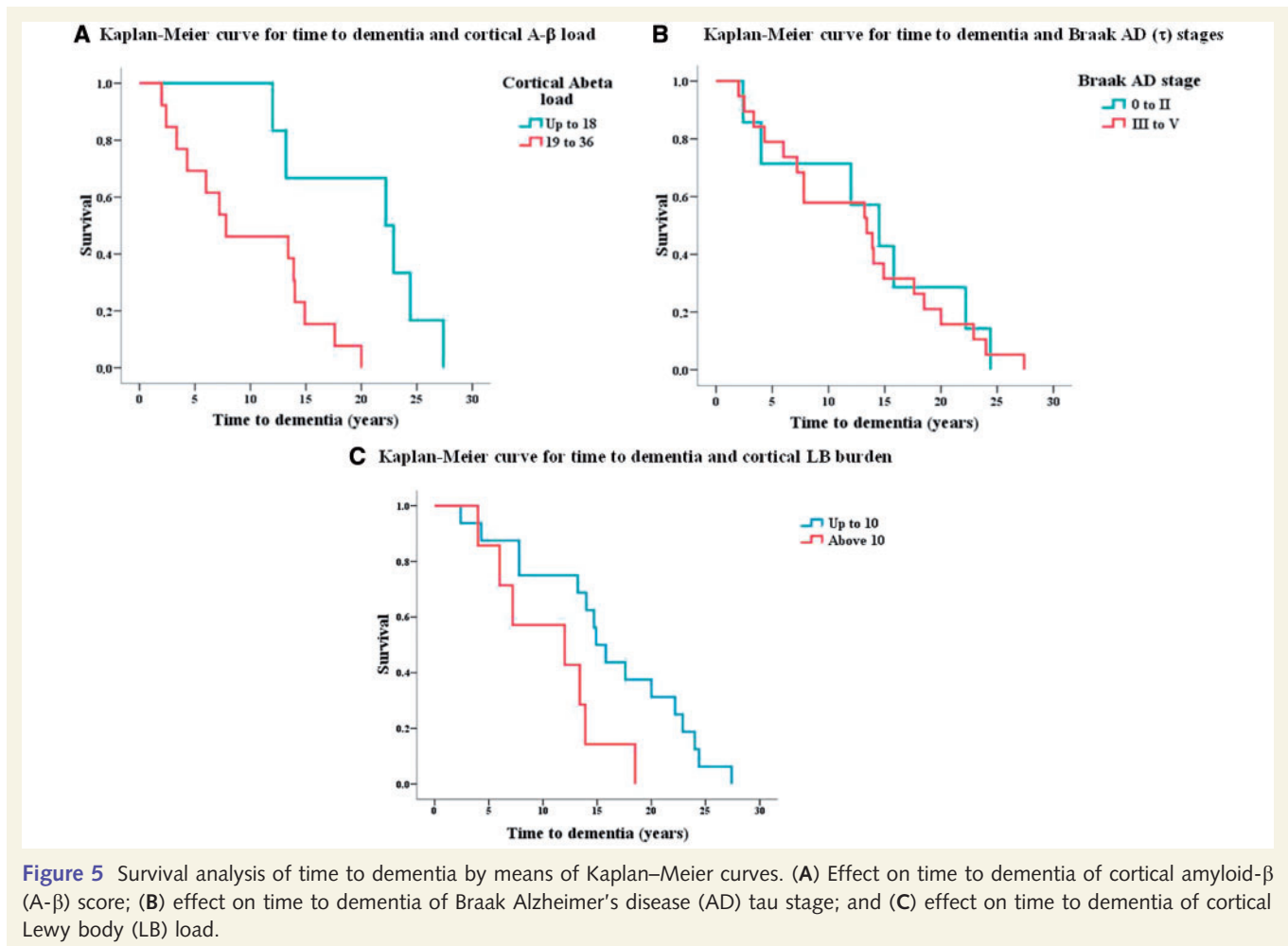
was no correlation between the cortical Lewy body scores and the MMSE scores either, which is in contrast to a recent survey where quantitative assessment and an alternative staging system for α -synuclein were used, showing a significant but mild correlation between α -synuclein lesion densities and MMSE scores in Parkinson's disease, with no mention of the association with concurrent Alzheimer's disease pathology, present in up to 62% of the studied patients with PDD (Beach *et al.*, 2009).

We found higher amyloid- β scores in cortical and subcortical structures in the PDD group compared to PDND cases, along with a significant correlation between the MMSE scores and the amyloid- β scores in several different cortical areas as well as the total cortical amyloid- β score. In Alzheimer's disease, some studies have suggested that insoluble amyloid- β load, inferred by plaque density at post-mortem, is a poor correlate of the degree of cognitive dysfunction (Giannakopoulos *et al.*, 2003; Guillozet *et al.*, 2003), while the cerebral levels of soluble amyloid- β (McLean *et al.*, 1999) as well as the topographical progression and the

Table 6 Cox regression analyses for the effect of cortical amyloid- β scores, Braak Alzheimer's disease and Parkinson's stages and cortical Lewy body scores on time to dementia

	<i>n</i> (high versus with low burden or stage)	Estimated relative hazard	95% CI (lower–upper)	<i>P</i> -value
Total cortical amyloid- β score >18	13 versus 6	6.43	1.33–111.67	0.017*
Braak Alzheimer's disease stage >II	19 versus 7	1.10	0.46–2.66	0.83
Braak Parkinson's disease stage >5	19 versus 5	2.15	0.76–6.07	0.15
Total cortical Lewy Body score >10	10 versus 13	2.53	0.98–6.52	0.055

*Significant findings.



severity of tau pathology are better correlates of dementia (Yamaguchi *et al.*, 2001). However, there is also evidence from recent studies using positron emission tomography probes for amyloid- β parenchymal amyloid deposits that the degree of uptake of the tracer ^{11}C -Pittsburgh Compound-B in several cortical areas and the putamen is closely associated with the cognitive performance in Alzheimer's disease (Grimmer *et al.*, 2009). In Parkinson's disease, the association between the severity of amyloid- β pathology and cognitive performance might be further explained by an apparent interaction between amyloid- β and

α -synuclein (Masliah *et al.*, 2001; Pletnikova *et al.*, 2005; Lashley *et al.*, 2008; Dickson *et al.*, 2009), but such an interaction could not be tested further by statistical means in our sample due to the colinearity between these variables (Harrell, 2001).

We also found a strong correlation between cortical and striatal amyloid- β burden suggesting that these are closely related pathological processes, which contrasts with the conclusions of a previous study (Kailatzakis *et al.*, 2008). Interestingly, the burden of diffuse plaques was higher than that of mature plaques, but showed more overlap between PDND and PDD. It has been

suggested that diffuse plaques represent an earlier event in amyloid- β aggregation preceding mature plaque formation (Yamaguchi *et al.*, 2001), so that diffuse plaques could already be seen in Parkinson's disease in the absence of dementia, whereas mature ones would be mostly observed in the setting of Parkinson's disease-dementia.

All the cortical pathologies were significantly correlated to each other in the bivariate analysis. We also replicated our earlier finding of significant correlation between cortical Lewy body densities and total cortical amyloid- β scores in the subset of Parkinson's disease patients with high cortical amyloid- β burden (Lashley *et al.*, 2008). This raises the possibility that there may be two pathological subsets of Parkinson's disease cases—one with severe cortical Lewy body in close correlation with the cortical amount of amyloid- β pathology, and another where both Lewy body and amyloid- β pathologies are relatively mild and unrelated to each other (Lashley *et al.*, 2008).

The receiver operating characteristic curves and the descriptive cut-offs for each pathological variable reflect the fact that a single pathology is not as reliable as a combination of all three in classifying Parkinson's disease cases as demented or not. This finding underlines the heterogeneous pathological substrate of Parkinson's disease-related dementia and the importance of the detailed assessment of all three pathologies. Large epidemiological-pathological surveys have also emphasized the importance of multiple pathologies in dementia (Kovacs *et al.*, 2008; Matthews *et al.*, 2009).

Genetic association studies have shown that common variation in the α -synuclein gene (*SNCA*) and the *MAPT* H1 haplotype contribute to sporadic Parkinson's disease risk. This has been confirmed in recent genome-wide association studies, which have confirmed *SNCA* gene polymorphisms and the *MAPT* H1 haplotype as the strongest genetic risk factors for Parkinson's disease (Pankratz *et al.*, 2009; Simon-Sanchez *et al.*, 2009). We did not find any association between the *MAPT* H1/H2 haplotype status and presence of dementia or the degree of tau or α -synuclein pathology probably due to underpowered analysis in the setting of numbers too small for a genetic association study. However, the *MAPT* H1 haplotype could be implicated in Parkinson's disease and related dementia through mechanisms influencing neuronal vulnerability without the necessary formation of tau fibrillar aggregates or independent of α -synuclein aggregation. In contrast, and not surprisingly, we have replicated the association between the *APOE* ϵ_4 allele and the severity of amyloid- β pathology (Lashley *et al.*, 2008). The fact that this was limited to the entorhinal, temporal and parietal cortices and did not occur in the striatum might suggest that factors other than *APOE* ϵ_4 may also play a role in the increased cortical and striatal amyloid- β pathology in Parkinson's disease. Perhaps α -synuclein could be one such factor, in light of the suggested synergism between the aggregation of these proteins (Masliah *et al.*, 2001; Gallardo *et al.*, 2008; Pletnikova *et al.*, 2008; Clinton *et al.*, 2010).

The wide range of time to dementia in our PDD cases reflected the presence of different rates of progression to dementia and allowed us to perform a survival analysis between the three variables of pathology (tau, α -synuclein and amyloid- β) and time to dementia. Besides a borderline significant association with cortical

Lewy body burden, the main finding was that of greater cortical amyloid- β and older age at disease onset significantly accelerating the rate of dementia in Parkinson's disease. This result reinforces the notion that there are two subsets of patients with PDD, as suggested by previous clinicopathological studies (Ballard *et al.*, 2006; Halliday *et al.*, 2008; Kempster *et al.*, 2010): those who are older at disease onset, develop dementia earlier in the disease course and have more mixed Alzheimer's disease-Lewy body pathology, as opposed to those who are younger at disease onset, present with cognitive decline after a much longer duration of disease and have a more pure Lewy body phenotype. The fact that amyloid- β and age at onset separately influenced the rate to dementia, but their significant effect was lost when assessed in combination suggests that amyloid- β deposition and ageing are closely related and together influence the time course to dementia. In contrast, age at onset did not imply higher risk of dementia in the binary regression model, in agreement with previous studies, where it was the general effect of age that was associated with dementia in Parkinson's disease (Aarsland *et al.*, 2007), whereas age at onset rather influenced the time interval between disease onset and recording of milestones as dementia (Kempster *et al.*, 2010).

The strict inclusion of a large number of cases for which detailed clinical information was available, the independent evaluation of clinical notes by neurologists experienced in Parkinson's disease by applying an accepted definition of Parkinson's disease-related dementia (Emre *et al.*, 2007), and the comprehensive and combined assessment of different pathologies potentially relevant to dementia in Parkinson's disease in a single group of patients represent strengths of the present study. Although findings from community- and hospital-based studies demonstrating the presence of these pathologies in cognitively intact elderly individuals (Esiri *et al.*, 2001; Parkkinen *et al.*, 2005) cast some doubt on their primary pathogenic role, there is growing evidence that amyloid- β , tau and α -synuclein act synergistically to enhance the aggregation of each other and to promote cognitive decline (Masliah *et al.*, 2001; Gallardo *et al.*, 2008; Pletnikova *et al.*, 2008; Clinton *et al.*, 2010). The retrospective nature of the study and the lack of assessment of other potentially relevant contributors, as discussed above, are shortcomings.

In summary, data from our study suggest that a combination of Lewy body- and Alzheimer's disease-type pathologies is the most robust pathological correlate of dementia in Parkinson's disease and that semi-quantitative and quantitative assessment of Lewy body-type pathology are more informative than the currently used topographical staging criteria. All this, along with the relevance of amyloid- β pathology to the rate of dementia in Parkinson's disease, probably in association with ageing, suggest that both Lewy- and Alzheimer-type pathologies are important in Parkinson's disease-related dementia.

Acknowledgements

The authors would like to thank the patients and their families for their generosity and goodwill, as without their support none of this research would have been possible. This work was undertaken

at UCLH/UCL with a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme. The Queen Square Brain Bank, UCL Institute of Neurology is supported by the Reta Lila Weston Institute of Neurological Studies and the Progressive Supranuclear Palsy (Europe) Association.

Funding

Y.C. was supported by a grant from the Spanish Neurological Society (Sociedad Española de Neurología; SEN). J.L.H., T.R. and A.J.L. are supported by research grants from the Multiple System Atrophy Trust and the Alzheimer's Research Trust. T.R. and J.L.H. are recipients of a project grant from Parkinson's UK. J.L.H. is supported by the Reta Lila Weston Institute for Neurological Studies. R.d.S. is supported by the Medical Research Council UK [G0501560], Cure PSP+ (the Irene & Abe Pollin Fund for CBD Research and the Charles D. Peebler Jr P.S.P. and CBD Genetics Program), the Brain Research Trust, the Alzheimer's Research Trust and Research into Ageing. This work was supported in part by the Wellcome/MRC Parkinson's Disease Consortium grant to UCL Institute of Neurology, the University of Sheffield and the MRC Protein Phosphorylation Unit at the University of Dundee.

Supplementary material

Supplementary material is available at *Brain* online.

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