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TDP-43 is associated with a reduced likelihood of rendering a clinical diagnosis of dementia with Lewy bodies in autopsy-confirmed cases of transitional/diffuse Lewy body disease

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Abstract

Background—Trans-acting response DNA-binding protein of 43kDa (TDP-43) can be detected in up to 63% of autopsy confirmed Lewy body disease (LBD) cases. It is unclear whether TDP-43 is associated with a decreased likelihood of a clinical diagnosis of probable dementia with Lewy bodies (pDLB) during life.

Methods—In an autopsy cohort of 395 cognitively impaired patients from the Mayo Clinic Alzheimer's Disease Research Center, we determined the presence of TDP-43 in the hippocampus (hTDP-43(+)) and examined associations between hTDP-43 and an antemortem pDLB clinical diagnosis with multiple regression analyses. For this study, given our specific question, we only counted transitional and diffuse Lewy body disease as LBD positive (LBD+).

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Marina Buciuc, Jennifer L. Whitwell & Keith A. Josephs contributed to the study conception and design. Material preparation and data collection were performed by Melissa E. Murray, Bradley F. Boeve, Jonathan Graff-Radford, Rodolfo Savica, Joseph E. Parisi, Kejal Kantarci, Julie A. Fields, Tanis J. Ferman, Jennifer L. Whitwell, David S. Knopman, Ronald C. Petersen, Dennis W. Dickson, and Keith A. Josephs; Marina Buciuc organized, revised and performed analysis of the data. The first draft of the manuscript was written by Marina Buciuc and all authors reviewed for intellectual content and commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethical standards All procedures were performed in accordance with the institutional ethics committee and the Declaration of Helsinki.

Results—One-hundred forty-five cases (37%) were hTDP-43(+) and 156 (39%) were LBD+; co-pathology was noted in 63 (16%) cases. Patients with pDLB-LBD+ were more likely to be older, hTDP-43(+) and have high Braak neurofibrillary tangle (NFT) status compared to the pDLB+LBD+ patients. After accounting for older age at death and high Braak NFT status, hTDP-43(+) status was associated with the absence of a clinical diagnosis of pDLB despite LBD+ status ($p<0.05$).

Conclusion—The absence of a diagnosis of pDLB during life in patients with LBD is associated with older age, high Braak NFT stage and hTDP-43, each feature contributing independently to a lower likelihood of a clinical diagnosis of pDLB during life.

Keywords

TDP-43; Lewy body disease; dementia with Lewy bodies; pathology; clinical diagnosis

Introduction

The process of ageing renders the brain and other tissues more susceptible to degeneration and accumulation of abnormal proteins and other pathologies[1]. Moreover, the presence of one abnormal protein appears to be associated with the increased chance of co-pathologies, potentially due to synergistic interactions between the proteins, as shown for amyloid-beta ($A\beta$), tau and α -synuclein [2,3]. Supporting this hypothesis are the findings of several studies that report mixed pathologies on autopsy to be more frequent than isolated proteinopathies [4–8]. Certain genetic constituents, such as the apolipoprotein E (*APOE*) $\epsilon 4$ allele carriership, are also known risk factors for multiple proteinopathies [9,4,10–12]. Furthermore, assessment of co-pathologies and their degree of severity has been considered when it comes to the establishment of the likelihood of one diagnosis versus another and has been included in the 4th consensus diagnostic criteria for dementia with Lewy bodies (DLB) with regard to concomitant presence of Alzheimer's disease neuropathological change (ADNC) and Lewy body disease (LBD) [13].

Whereas $A\beta$, tau and α -synuclein co-occurrence is acknowledged and widely reported [6,14,8,15], little is known about other proteins. With the advancement in the fields of molecular biology and immunohistochemistry, another protein, trans-active response DNA-binding protein of 43kDa (TDP-43), has more recently come into play in the neurodegenerative field. Hyperphosphorylated TDP-43 is frequently found in the brains of cognitively unimpaired individuals [16–18], as well as in a variety of neurodegenerative disorders. Whereas amyotrophic lateral sclerosis and frontotemporal lobar degeneration (FTLD) are considered to be primary TDP-43-proteinopathies [19,20], TDP-43 immunoreactive inclusions are also found in up to 74% of patients with ADNC [21,5,22,4,23]. In addition, TDP-43 has been found in 29% - 63% of LBD cases depending on the region screened for the presence of TDP-43 [24,21,25,26,4]. Hence, it is important to try to better understand the relationships between TDP-43 and other pathologies and whether these pathologies might add complexity and variability to perceived clinical phenotypes.

The possibility of modification of the clinical presentation and progression of the disease by co-pathology has been reported [27,4]. Multiple studies have assessed TDP-43 in context of

ADNC and have found associations between TDP-43 aggregation in the hippocampus and more advanced memory impairment [27,28,5], as well as a faster rate and a greater degree of hippocampal atrophy [29] in ADNC patients. However, there are limited data available regarding TDP-43 and Lewy body disease (LBD). Taking into account the implication of TDP-43 pathology with memory loss, it is possible that the presence of TDP-43 may lead to more uncertainty in rendering a DLB clinical diagnosis if the clinical syndrome is less distinct and may help explain the variability in cognitive profiles in patients with DLB, in that some have more predominant attention/executive and visuospatial dysfunction whereas others may also have prominent memory impairment. Given that LBD is strongly associated with the clinical syndrome of DLB [30], we were specifically interested in testing the hypothesis that a higher frequency of TDP-43 will be associated with not receiving a clinical diagnosis of DLB albeit LBD being diagnosed pathologically. This hypothesis was driven by the notion that since the presence of TDP-43 is associated with greater memory loss, the presence of TDP-43 would blur the classic signs and symptoms associated with the clinical syndrome of DLB.

Materials and Methods

Study design and participants

We conducted a clinical-pathological study using autopsy cohort participants that had TDP-43 in the hippocampus. All participants were enrolled in the Mayo Clinic Alzheimer's Disease Research Center and were prospectively followed. The patients died between May 12th, 1999, and December 31st, 2015. From this cohort we identified all participants who met the following inclusion criteria: were cognitively impaired by the time of their last neurological evaluation prior to death and had an established clinical diagnosis. All patients who had died without any cognitive impairment by the time of their last evaluation were excluded. We identified 395 patients that met our inclusion and exclusion criteria. The clinical diagnosis and cognitive status of all 395 cases in this study had been assigned based on evaluations by a behavioral neurologist and neuropsychologist on the last visit prior to death. The medical records of all patients who had received a clinical diagnosis of DLB prior to death were reviewed and a final clinical diagnosis of probable DLB (pDLB) was established according to the guidelines published in the 4th consensus report of DLB consortium [13]. Specifically, all patients had to have a dementia, as well as two or more of the four following clinical features well documented in the medical records: Parkinsonism, hallucinations, rapid eye movement sleep behavior disorder and/or fluctuations. At the time of enrollment participants' demographics (age, sex, etc.) were collected and apolipoprotein-E (*APOE*) genotyping was performed on all participants, as previously described [31,9].

Standard protocols approvals, registrations and patient consents

This study has been approved by the Mayo Clinic institutional review board, and all patients and/or their proxies signed a written informed consent form before taking part in any research activities in accordance with the 1964 Declaration of Helsinki and its late amendments.

Pathological analysis

All cases had undergone pathological examination according to the recommendations of the National Institute on Aging and Alzheimer's Association (NIA-AA)[32]. Each individual had been assigned a Braak neurofibrillary tangle (NFT) stage 0-VI [33,34] and A β neuritic plaque stage (absent, sparse, moderate, frequent) according to the Consortium to Establish a Registry for AD (CERAD) staging [35]. For further analysis, the Braak NFT stage was collapsed to two stages: subcortical tangles included Braak NFT stages I – III and neocortical tangles included Braak NFT stages IV – VI. We did not have any cases with a Braak NFT stage 0. We also collapsed the CERAD stage into two stages: those with absent to sparse neuritic A β plaques and those with moderate to frequent plaques. Given that only limbic/transitional and diffuse LBD is associated with a clinical DLB diagnosis we collapsed the LBD staging scheme proposed by Braak *et al* [36] into LBD-positive versus LBD-negative status. Specifically, cases with Braak LBD stage 4 and above which correspond to limbic/transitional and diffuse LBD were considered LBD-positive (LBD+), while subcortical Braak LBD stages 1–3 were considered LBD negative (LBD-)[36].

In this study, we focused on TDP-43 reaching the hippocampus since hippocampal TDP-43 is associated with loss of episodic memory. Cases with TDP-43 in the hippocampus were designated as hTDP-43(+) if TDP-43 immunoreactive neuronal cytoplasmic inclusions, dystrophic neurites, fine neurites or neuronal intranuclear inclusions were identified in the hippocampus which would correspond to TDP-43 stage 2 and above [37,38]. Those without TDP-43 deposition or TDP-43 deposition limited to the amygdala (TDP-43 stage 1) were designated as TDP-43-negative in hippocampus (hTDP-43(-)) for this study.

We also document the presence of hippocampal sclerosis[39], diagnosed if there was neuronal loss in the CA1 and/or the subiculum of the hippocampus out of proportion to the observable burden of extracellular neurofibrillary tangle pathology, based on consensus recommendations[40] and argyrophilic grains disease (AGD) if silver and tau-positive spindle-shaped lesions in transentorhinal and entorhinal cortex, amygdala or temporal allocortex were identified[41].

Statistical analyses

For this study all patients were classified into one of four groups depending on whether they had received a clinical diagnosis of pDLB and whether they had received a pathological diagnosis of LBD, as defined above. Those that received a clinical pDLB diagnosis and also had pathological LBD were designated (pDLB+LBD+), those that received a clinical pDLB diagnosis but did not have LBD as (pDLB+LBD-), those that did not receive a clinical pDLB diagnosis but had pathological LBD as (pDLB-LBD+), and those that did not receive a clinical pDLB diagnosis and also did not have LBD as (pDLB-LBD-). All statistical analysis was performed in JMP Pro 14 software (https://www.jmp.com/en_us/software/predictive-analytics-software.html) and figures have been generated in GraphPad Prism 8 software (<https://www.graphpad.com/scientific-software/prism/>). The Kruskal-Wallis and Wilcoxon rank sum tests were used to compare continuous variables, while chi-squared test was used for categorical variables such as sex. Fisher's exact test was used where subcategories included small numbers. Multivariable logistic regression was performed with

the four groups as the outcome with the following covariates: age, sex, Braak NFT status, CERAD A β status, *APOE* ϵ 4 status, AGD and hTDP-43(+) status. We also performed a logistic regression analysis with pDLB diagnosis (pDLB+ vs pDLB-) as the outcome and another regression with TDP-43 status as the outcome adjusting for the same covariates. We provide associations between covariates and outcomes as odds ratio with confidence intervals. We show the age effect for the models a by genarian (age by decade) older at death regardless of onset of the cognitive impairment. The *P* value was set at 0.05 to be considered significant.

Results

General pathological characteristics of the cohort

Out of all 395 patients included in this study 145 (37%) were hTDP-43(+) and 156 (39%) were LBD+; mixed LBD and hTDP-43 co-pathology was noted in 63 (16%) cases. The *APOE* ϵ 4 allele frequency was higher in those with LBD versus those without LBD (60% [93/156] vs. 46% [111/239]; $p=0.0135$), as well as higher in those with hTDP-43 versus those without hTDP-43 (59% [86/145] vs. 47% [118/250]; $p=0.0249$). Hippocampal sclerosis was observed in 67 cases (17%), of which 53 (79%) were hTDP-43(+). AGD was observed in 39 cases (10%), of which 12 (31%) were hTDP-43(+).

Pathological and clinical correlations

Demographic, genetic and pathological characteristics of the study participants subdivided into the four groups are summarized in Table 1. Of the 395 study participants, 56 (14%) had a clinical diagnosis of pDLB, of which 47 (84%) had LBD on autopsy. Of the remaining 339 patients without a clinical pDLB diagnosis, 111 (33%) had LBD. A breakdown of the clinical diagnoses in these 339 cases are shown in Table 2.

When we combine the two pDLB+ subgroups and the two pDLB- subgroups we found the frequency of hTDP-43 was significantly lower among pDLB+ patients compared to pDLB- patients ($p=0.0002$). The frequency of hTDP-43(+) across the four groups however was also significantly different ($p<0.0001$), with the highest frequency (53%) occurring in the pDLB-LBD+ group. Furthermore, the hTDP-43 (+) frequency was higher in the pDLB-LBD+ group compared to the pDLB+LBD+ group ($p<0.0001$) and the pDLB-LBD- group ($p=0.0009$) (Table 1). The four groups also differed in sex, age at death, Braak NFT, CERAD, and hippocampal sclerosis statuses (Table 1). The pDLB-LBD+ group were also more likely to have been diagnosed with probable Alzheimer's dementia (AD) as opposed to mild cognitive impairment (MCI) ($p=0.0089$) compared to the pDLB-LBD- group (Table 2) despite no significant differences among these two groups in terms of age, *APOE* ϵ 4 carriership and other pathologies (Braak NFT, CERAD, AGD and hippocampal sclerosis statuses, ($p>0.05$) (Table 1).

Multivariate and multivariable analyses

In our regression model with clinical pDLB as an outcome (pDLB+ vs pDLB-), older age at death, Braak NFT status and hTDP-43(+) status were inversely associated with pDLB+ whereas LBD+ status was 12X more likely to be associated with pDLB+ (Fig.1, Table 3.A).

In the multivariable logistic regression model with age, sex, Braak NFT, CERAD, *APOE* $\epsilon 4$ allele and hTDP-43 + statuses as covariates, there was a significant difference in hTDP-43 status between the pDLB-LBD+ and pDLB+LBD+ groups. Patients with pDLB-LBD+ were more likely to be older, hTDP-43(+) and have neocortical tangles compared to the pDLB+LBD+ patients (Fig.2, Table 3.B). Alternatively, with hTDP-43(+) as the outcome we found hTDP-43(+) status to be associated with older age at death, presence of neocortical tangles, LBD+ status as well as being inversely associated with the clinical diagnosis of pDLB (Fig.3, Table 3.C). Results were unchanged in all three regression models when limiting cases to those with amnesic MCI and probable AD (n=322); hTDP-43 was positively associated with pDLB-LBD+ ($p=0.0167$) whereas hTDP-43 and pDLB were inversely associated (for pDLB as outcome, $p=0.0338$; for hTDP-43(+) status as outcome, $p=0.0238$).

Discussion

In this clinical-pathological study of 395 cognitively impaired older adults, we found that the frequency of TDP-43 pathology was different across the four groups stratified by a pDLB clinical diagnosis and LBD pathological diagnosis. As we hypothesized, the frequency of TDP-43 in those that did not receive a clinical diagnosis of pDLB yet had LBD pathology (i.e. pDLB-LBD+) was significantly greater than those that did receive a clinical diagnosis of pDLB and were also found to have LBD pathology (pDLB+LBD+).

To our knowledge, this is the first study that assessed for associations between TDP-43 pathology and a establishment of clinical diagnosis of DLB using international clinical criteria[13]. Investigation of this association is warranted given that mixed pathologies are now considered key to the understanding of neurodegenerative dementias. It is even more pertinent when it comes to co-pathologies without a specific *in vivo* biomarker, such as TDP-43 and LBD proteinopathies. We chose to evaluate implication of hippocampal TDP-43 in clinical diagnosis of DLB since available literature reports that TDP-43 limited to amygdala (TDP stage 1) might be clinically silent and/or not sufficient to have a currently detectable clinical phenotype[28,29,18,42,43]. We had hypothesized that the frequency of hTDP-43 would be higher in the pDLB-LBD+ group compared to the pDLB+LBD+ group given that TDP-43 is associated with memory loss and hence may blur the classic signs and symptoms associated with the clinical syndrome of DLB. Indeed, in our pDLB-LBD+ patients, the frequency of hTDP-43 co-pathology was significantly greater when compared to DLB+LBD+ patients. It should be pointed out that the pDLB-LBD+ patients were also older at the time of death than the DLB+LBD+ cases, which may not be surprising since we know that TDP-43 frequency increases with age [4, 21, 22]. We also found that the pDLB-LBD+ cases were more likely to have neocortical tangles compared to the pDLB+LBD+ cases, suggesting that tau may also be contributing to not making a clinical diagnosis of pDLB when LBD is present. Several studies have reported that LBD with ADNC, particularly neocortical tangles, has lower diagnostic sensitivity for pDLB than LBD without neocortical tangles due to more prominent memory impairment and decreased frequency of core clinical features of DLB in these patients [44,45]. The finding of neocortical tangles in the pDLB-LBD+ group given a hTDP-43 (+) status is in keeping with there being an association between higher Braak NFT stage and increased frequency of TDP-43 pathology

[27]. Nevertheless, after controlling for both age and Braak NFT status, we still found an association between hTDP-43 (+) status and the pDLB-LBD+ group. This association, although indirect, is evidence to support the hypothesis that hTDP-4, independent of ADNC, might be playing a role in influencing clinicians not making a clinical diagnosis of pDLB when LBD is present.

Hippocampal TDP-43 was also significantly more frequent in those with pDLB-LBD+ compared to pDLB-LBD-, which is consistent with other studies showing positive association between the co-occurrence of LBD and TDP-43[28,24]. Higher frequency of TDP-43 pathology in patients with LBD can implicate synergic interaction between these proteins [2,3] and possibly a specific TDP-43/ α -synuclein interaction taking into account no differences in tau and A β pathology between these groups. A higher likelihood of AD diagnosis versus MCI in DLB-LBD+ group compared to DLB-LBD- may imply aggravating effect of TDP-43 on memory[27,28], higher genetic predisposition[9] as well as a contributing effect of LBD co-pathology[2,4].

Our findings of hTDP-43 frequency across all LBD+ cases was 40%, which is comparable to previous studies [24,26,4] reporting on the frequency of TDP-43 and LBD co-pathology. The range of TDP-43 positivity reported by different groups is quite wide, varying from 30% to 63%. Some possible explanations for such variability is the region screened (amygdala vs hippocampus) as well as due to differences in age at death of the cohorts, as TDP-43 is more common in older individuals. Another reason for differences could be the inclusion and exclusion of cases with incidental Lewy bodies, amygdala-only Lewy bodies and brainstem-only Lewy body disease. We did not include incidental or amygdala only cases in this study and those with brainstem-only LBD were considered LBD- for purposes discussed above. Another possibility could be due to the sensitivity and specificity of the anti-TDP-43 antibodies utilized for the study. It is noteworthy that our cohort included only cognitively impaired patients, which potentially could also affect the frequency since the frequency of TDP-43 is known to be lower in cohorts of cognitively normal individuals compared to those with cognitive impairment [46,47,28]. Lastly, there may be differences related to the relative number of patients with MCI vs dementia that make up the cognitively impaired group.

Ample clinical and pathological data, as well as consideration of clinical diagnosis, strengthen the results of the present study. We considered only patients with cognitive impairment to increase clinical relevance of our findings since cases of cognitive resilience in the context of advanced pathology on autopsy have been reported [48] and would have confounded our results. Whereas several studies looked at degree and progression of cognitive decline [4,21] in cases of mixed pathologies, in this study we investigated whether hTDP-43 had an association with the establishment of the clinical diagnosis of pDLB. Our findings, however, should be interpreted with some caution. There is an imbalance in terms of size between our main clinical groups, pDLB+ and pDLB-, which might undermine statistical analysis. However, it is also noteworthy that DLB is much less common than AD, the leading diagnosis of pDLB- group, making our cohort representative of the general population. Some of the subgroups of our cohort had small numbers of patients, which limited fair comparison between certain groups and statistical power. We used staging as a

scale of severity of A β , tau, TDP-43 and α -synuclein proteinopathies whereas using quantitative data (burden) instead might have had an impact on our findings and should be explored in the future. In addition, our designation of having a positive clinical sign or symptoms was based on a clinical designation of whether the sign was present or absent. Further studies should include more detailed clinical data (test scores, specific test batteries) where the data can be analyzed continuously.

It is known that mixed neuropathologies can lead to “misdiagnosis,” thus affecting the epidemiology of distinct neurodegenerative diseases. Mixed pathologies are also important to consider when it comes to evaluating outcomes of targeted therapies since multiple co-pathologies might hinder potentially beneficial effects of the treatment. Since TDP-43 immunoreactivity has been more routinely assessed on autopsy, it is now becoming apparent that TDP-43 is a common co-pathology and here we confirm a high prevalence of TDP-43 co-pathology. Moreover, the range of frequency of hippocampal TDP-43 and LBD mixed pathology seems to be associated with clinical presentation. An older patient who is hTDP-43(+), LBD+ and with severe tau pathology is more likely to not present with typical features of DLB, and more likely to be given an alternative diagnosis, most likely Alzheimer’s dementia. Although this may be thought to be mainly due to ADNC, in fact it is just as likely, maybe even more likely, to be due to TDP-43 given that TDP-43 is strongly associated with hippocampal atrophy[49,28,43,23,17,29], and, hence, memory loss. Furthermore, whereas associations between TDP-43 and tau have been reported in relation to clinical AD features [27–29], it is now apparent that TDP-43 and LBD, either interacting or merely co-occurring, is having a similar effect in the establishment of the clinical diagnosis of DLB, making it more challenging for the physicians to diagnose.

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Conflict of Interest/ Disclosure Statements

Marina Buciuc, Jennifer L. Whitwell, Melissa E. Murray, Bradley F. Boeve, Jonathan Graff-Radford, Julie A. Fields, Joseph E. Parisi, Dennis W. Dickson, and Keith A. Josephs declare that they have no conflict of interest. David S. Knopman serves on a Data Safety Monitoring Board for the DIAN study; is an investigator in clinical trials sponsored by Biogen, Lilly Pharmaceuticals and the University of Southern California. Ronald C. Petersen has served on the National Advisory Council on Aging and on the scientific advisory boards of Pfizer, GE Healthcare, Elan Pharmaceuticals, and Janssen Alzheimer Immunotherapy, has received publishing royalties from Oxford University Press, and has been a consultant for Roche Incorporated, Merck, Genentech, Biogen, and Eli Lilly. Bradley F. Boeve has served as an investigator for clinical trials sponsored by Biogen and Alector. He serves on the Scientific Advisory Board of the Tau Consortium. He receives research support from the NIH, the Mayo Clinic Dorothy and Harry T. Mangurian Jr. Lewy Body Dementia Program, the Little Family Foundation, and the Lewy Body Dementia Functional Genomics Program. Rodolfo Savica receives research support from the National Institute on Aging, the National Institute of Neurological Disorders and Stroke, and the Mayo Clinic Small Grants Program National Center for Advancing Translational Sciences (NCATS) and unrestricted research grants from Acadia Pharmaceutical, Inc.

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Fig. 1. Odds ratio and 95% confidence intervals for multivariate logistic regression model with clinical probable dementia with Lewy bodies diagnosis as outcome

The age effect shown for both models is for a 10-year older age at death. Confidence intervals that do not cross one indicate significance at $p < 0.05$. LBD – Lewy body disease; moderate/frequent CERAD – moderate or frequent neuritic amyloid plaques; hTDP-43+ – TDP-43 in hippocampus

Fig. 2. Odds ratio and 95% confidence intervals for pDLB-LBD+ vs pDLB+LBD+ from multivariable logistic regression model

The age effect shown is for a 10-year older age at death. Confidence intervals that do not cross one indicate significance at $p < 0.05$. pDLB-LBD+ – clinical diagnosis of probable dementia with Lewy bodies and with present Lewy body disease; pDLB+LBD+ – clinical diagnosis of probable dementia with Lewy bodies and with present Lewy body disease; moderate/frequent CERAD – moderate or frequent neuritic amyloid plaques; hTDP-43+ – TDP-43 in hippocampus

Fig. 3. Odds ratio and 95% confidence intervals for multivariate logistic regression model with hTDP-43(+) status as outcome

The age effect shown is for a 10-year older age at death. Confidence intervals that do not cross one indicate significance at $p < 0.05$. pDLB – probable dementia with Lewy bodies; LBD – Lewy body disease; AGD – argyrophilic grains disease; moderate/frequent CERAD – moderate or frequent neuritic amyloid plaques

Table 1

Demographic and pathological characteristics of study participants with different combinations of clinical and pathological diagnoses

No. (% or interquartile range) ^a					
	pDLB-LBD ^b (n=228)	pDLB-LBD ^c (n=111)	pDLB+LBD ^d (n=9)	pDLB+LBD ^e (n=47)	p-value ^f
Demographic and genetic					
Male	109 (48%)	49 (44%)	5 (56%)	36 (77%)	<0.0010 ^{†‡}
Education, years	14 (12 – 16)	14 (12 – 16.5)	13(12 – 17)	14 (12 – 16)	0.9715
APOE ε4 carrier	107 (47%)	70 (63%)	4 (44%)	23 (49%)	0.0576
Age at death, years	86 (82 – 91)	86 (78 – 92)	85 (75 – 88)	76 (71 – 80)	<0.0001 ^{†‡}
Pathological					
TDP-43 positive in hippocampus	78 (34%)	59 (53%)	2 (22%)	6 (13%)	<0.0001 ^{λ†‡}
Hippocampal sclerosis positive	40 (18%)	26 (23%)	0 (0%)	1 (2%)	0.0024 ^{†‡ϕ}
Neocortical tangles (Braak IV – VI)	188 (82%)	97 (87%)	9 (100%)	23 (49%)	<0.0001 ^{†‡¥}
Moderate or frequent CERAD	176 (77%)	89(80%)	9 (100%)	27 (57%)	0.0066 ^{†‡¥§}
Argyrophilic grains disease positive	26 (11%)	10 (9%)	1 (11%)	2 (4%)	0.4544

^aData shown are n (%) or median (interquartile range)

^bpDLB-LBD- = No clinical diagnosis of probable dementia with Lewy bodies and with absent Lewy body disease

^cpDLB-LBD+ = No clinical diagnosis of probable dementia with Lewy bodies and with present Lewy body disease

^dpDLB+LBD- = Clinical diagnosis of probable dementia with Lewy bodies and with absent Lewy body disease

^epDLB+LBD+ = Clinical diagnosis of probable dementia with Lewy bodies and with present Lewy body disease

^fFor categorical variables, *p*-values are from Fisher's Exact Test. For continuous variables, *p*-values are from Wilcoxon rank sum test

^λ = Post hoc differences between pDLB-LBD+ and pDLB-LBD- (*p*<0.05)

[†] = Post hoc differences between pDLB+LBD+ and pDLB-LBD- (*p*<0.05)

[‡] = Post hoc differences between pDLB-LBD+ and pDLB+LBD+ (*p*<0.05)

[¥] = Post hoc differences between pDLB+LBD- and pDLB+LBD+ (*p*<0.05)

^ϕ = Post hoc differences between pDLB-LBD+ and pDLB+LBD- (*p*<0.05)

[§] = Post hoc differences between pDLB-LBD- and pDLB+LBD- (*p*<0.05)

Table 2

Distribution of clinical diagnoses of patients without clinical diagnosis of probable dementia with Lewy bodies

Clinical diagnosis	All (n=339)	pDLB-LBD ^{-a} (n=228)	pDLB-LBD ^{+b} (n=111)
Mild cognitive impairment [*]	79 (23%)	60 (26%, 76%)	19 (17%, 24%)
Alzheimer's –type dementia ^{**}	206 (61%)	128 (56%, 62%)	78 (70%, 38%)
Vascular dementia	8 (2%)	7 (3%, 88%))	1 (1%, 12%))
Dementia, unclassifiable	7 (2%)	3 (1%, 43%)	4 (4%, 57%)
Frontotemporal dementia	7 (2%)	7 (3%, 100%)	0 (0%, 0%)
Other clinical diagnoses ^c	23 (7%)	15(7%, 65%)	8 (7%, 35%)
Uncertain	9 (3%)	8 (4%, 89%)	1(1%, 11%)

Data is presented a n (%). In pDLB-LBD- and pDLB-LBD+ groups first percentage represent the frequency of the diagnosis within the group and the second percentage represents the distribution of clinical diagnosis by LBD status between these two groups, e.g. MCI 60 (60/228, 26%, 60/79, 76%)

^a pDLB-LBD- = No clinical diagnosis of probable dementia with Lewy bodies and with absent Lewy body pathology

^b pDLB-LBD+ = No clinical diagnosis of probable dementia with Lewy bodies and with present Lewy body pathology

^c Other clinical diagnoses included Parkinson's disease (n=1), progressive supranuclear palsy (n=1), corticobasal syndrome (n=10), progressive non-fluent aphasia (n=3), progressive fluent aphasia/ semantic aphasia (n=3), posterior cortical atrophy (n=5)

Difference in the frequency of the diagnosis between the groups is statistically significant:

^{*} $p < 0.05$

^{**} - $p < 0.01$

Table 3Odds ratio, 95% confidence intervals and *p*-values for logistic regression models

	Odds ratio	Lower 95% CI	Upper 95% CI	<i>p</i> -value ^a
A. Multivariate logistic regression model with clinical dementia with Lewy bodies as outcome				
Male sex	2.09	0.97	4.53	0.0600
Age at death, 10y increase	0.53	0.37	0.77	0.0007
<i>APOE</i> ε4 +	0.64	0.30	1.35	0.2413
Neocortical tangles +	0.30	0.09	0.94	0.0386
Moderate/frequent CERAD +	1.47	0.44	4.96	0.5355
LBD(+)	12.60	5.36	29.63	<0.0001
hTDP-43(+)	0.39	0.16	0.98	0.0424
B. pDLB-LBD+ vs pDLB+LBD+ from multivariable logistic regression model				
Male sex	0.68	0.44	1.04	0.0829
Age at death, 10y increase	2.08	1.39	3.15	0.0004
<i>APOE</i> ε4 +	1.11	0.73	1.68	0.6211
Neocortical tangles +	2.46	1.34	4.64	0.0043
Moderate/frequent CERAD +	0.96	0.51	1.77	0.8916
hTDP-43(+)	1.75	1.07	3.02	0.0314
C. Multivariate logistic regression model with hTDP-43(+) status as outcome				
Male sex	0.78	0.49	1.25	0.3016
Age at death, 10y increase	2.06	1.54	2.76	<0.0001
<i>APOE</i> ε4 +	1.55	0.95	2.53	0.0787
Neocortical tangles +	3.47	1.49	8.10	0.0026
Moderate/frequent CERAD +	1.10	0.53	2.28	0.7906
AGD+	1.01	0.42	2.39	0.9878
LBD+	2.13	1.30	3.51	0.0025
pDLB+	0.41	0.17	0.99	0.0388

^aThese *p*-values need to be interpreted in the context of Fig.1, Fig.2, Fig.3