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Neuropathological and genetic characteristics of a post-mortem series of cases with dementia with Lewy bodies clinically suspected of Creutzfeldt-Jakob's disease

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ABSTRACT

Introduction: The disease course of dementia with Lewy bodies (DLB) can be rapidly progressive, clinically resembling Creutzfeldt-Jakob's disease (CJD). To better understand factors contributing to this rapidly progressive disease course, we describe load and distribution of neuropathology, and the presence of possible disease-associated genetic defects in a post-mortem series of DLB cases clinically suspected of CJD.

Methods: We included pathologically confirmed DLB cases with a disease duration of 3.5 years or less from the Dutch Surveillance Center for Prion Diseases, collected between 1998 and 2014. Lewy body disease (LBD) and Alzheimer's disease (AD)-related pathology were staged and semi-quantitatively scored in selected brain regions. Whole exome sequencing analysis of known disease-associated genes, copy number analysis, *APOE* ε genotyping and *C9orf72* repeat expansion analysis were performed to identify defects in genes with a well-established involvement in Parkinson's disease or AD.

Results: Diffuse LBD was present in nine cases, transitional LBD in six cases and brainstem-predominant LBD in one case. Neocortical alpha-synuclein load was significantly higher in cases with intermediate-to-high than in cases with low-to-none AD-related pathology ($p = 0.007$). We found two *GBA* variants (p.D140H and p.E326K) in one patient and two heterozygous rare variants of unknown significance in *SORL1* in two patients.

Conclusion: A high load of neocortical alpha-synuclein pathology was present in most, but not all DLB cases. Additional burden from presence of concomitant pathologies, synergistic effects and specific genetic defects in the known disease-associated genes may have contributed to the rapid disease progression.

1. Introduction

Dementia with Lewy bodies (DLB) is a neurodegenerative dementia characterized by parkinsonism, fluctuating cognitive symptoms, visual hallucinations, REM-sleep behavior disorders and neuroleptic sensitivity [1]. Clinical symptoms of DLB are gradually progressive, with a median disease duration of seven to eight years [2]. However, DLB may occasionally present with a rapid deterioration leading to death within three years from the start of the first symptoms [3–9]. These rapidly progressive DLB cases frequently show additional focal neurological signs such as myoclonus, pyramidal and cerebellar signs, and akinetic mutism [3–9]. As these neurological signs are core symptoms of Creutzfeldt-Jakob's disease (CJD), a rapidly progressive prion disease [10], the clinical differential diagnosis often includes CJD. In autopsy-series of clinically possible and probable CJD cases, DLB was the neuropathological diagnosis in 2–8% of cases [3–5]. To date, it is unknown whether these cases should be regarded as a distinct entity within the Lewy body disease (LBD) spectrum. Studying the neuropathological and genetic correlates of this clinical phenotype may aid in a better understanding of factors contributing to the rapidly progressive disease

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course in DLB.

The main neuropathological lesions in DLB are alpha-synuclein immunoreactive Lewy bodies (LBs) and Lewy neurites (LNs). In most DLB cases, LB pathology is present in brainstem and limbic (transitional LBD), or in brainstem, limbic and neocortical areas (diffuse LBD) [11]. Additionally, Alzheimer's disease (AD)-related changes including amyloid-β plaques, neurofibrillary pathology and neuritic plaques are often present [12]. Neuropathological correlates of disease progression and survival in DLB have previously been assessed in prospectively [13] and retrospectively studied DLB [14] or combined Parkinson's disease (PD) dementia and DLB [15] cohorts. These studies revealed that patients with diffuse LBD had a shorter disease duration [13–15] and showed a more rapid cognitive decline [14] than patients with transitional LBD. Both a higher alpha-synuclein load and amyloid-β load, and to a lesser extent also neurofibrillary pathology load, were associated with a shorter disease duration [13].

To better understand neuropathological and genetic factors contributing to a rapidly progressive disease course in DLB, we describe here the load and distribution of alpha-synuclein pathology and concomitant pathology, and the presence of possible disease-associated genetic defects in a post-mortem series of DLB cases suspected of CJD from the Dutch Surveillance Center for Prion Diseases.

2. Methods

2.1. Patient selection

All cases clinically suspected of CJD with LBD at autopsy collected in the period from 1998 to 2014 by the Dutch Surveillance Center for Prion Diseases at the University Medical Center in Utrecht were included. The Dutch Surveillance Center obtained permission for brain autopsy and written informed consent for use of clinical, pathological and genetic data for research purposes from the patients during life or from their next of kin after death. The medical ethics committee (METC) of the University Medical Center Utrecht approved all procedures of autopsy. We retrieved information from medical records on symptoms and signs of DLB and sporadic CJD as described by the McKeith criteria and the criteria from the World Health Organization (WHO) [10,11]. Symptoms were only regarded as present or not present when explicitly stated in the clinical information. An experienced neurologist (AL) classified all patients to the clinical criteria for DLB [11] and WHO criteria for CJD [10].

Inclusion criteria for this study were: 1) presence of LBD at autopsy, 2) negative screen for prion protein at autopsy, 3) disease duration of 3.5 years or less from the start of first symptoms, 4) no other major neurological or systemic diseases that provided sufficient explanation for a rapid deterioration, and 5) presence of sufficient clinical data.

2.2. Neuropathological assessment

Post-mortem examination was performed within 4–8 h post-mortem. A total of 25 tissue blocks were taken from the following regions: frontal, parietal, temporal and occipital cortices, hippocampus, striatum, thalamus, mesencephalon, pons, medulla oblongata and cerebellum.

After decontamination using 98% formic acid for 5 min, brain tissue blocks were formalin-fixed, paraffin-embedded and cut into sections of 10 μm thickness. Routine histological stainings were performed with haematoxylin-eosin, Gallyas silver staining and combined Luxol fast blue-periodic acid-Schiff. All regions were examined for the presence of prion protein with the use of monoclonal antibody 3F4 (1:400, Signet labs, United States). Immunohistochemistry was performed using primary antibodies against alpha-synuclein (clone KM51; 1:500; Monosan, The Netherlands), hyperphosphorylated tau (clone AT8; 1:1000; Innogenetics, Belgium), and amyloid-β (clone 6f/3 d; 1:100; Dako, United States). For staging and semi-quantitative scores of alpha-synuclein pathology, Brain Net Europe (BNE) consensus guidelines [16] and modified McKeith criteria [17] were used. LBs and LNs were scored in brainstem regions, LNs in the CA2 regions and LBs in other limbic and neocortical regions. For pathological staging of neurofibrillary tangles, amyloid-β plaques and neuritic plaques, NIA-AA criteria were used [17]. Mean cerebral load of alpha-synuclein, neurofibrillary and amyloid-β pathology was calculated based on semi-quantitative load of pathology in selected regions used for pathological staging (Supplementary methods). Presence of age-related astrogliopathy (ARTAG) [18], argyrophilic grain disease [19], atherosclerosis, ischemic or hemorrhagic lesions and small vessel disease [17] was reported, and cerebral amyloid angiopathy (CAA) was classified [20]. Spongiform changes were assessed based on presence of vacuoles in the entorhinal and temporo-occipital cortex. Neuropathological assessment was performed by an experienced neuropathologist (AR), and semi-quantitative load of pathology was scored according to consensus criteria [16,17,21] by the same assessor (HG) in all cases.

Table 1
Basic demographic features, clinical symptoms, ancillary investigations and diagnoses of dementia with Lewy bodies and Creutzfeldt-Jakob's disease in sixteen cases with dementia with Lewy bodies clinically suspected of Creutzfeldt-Jakob's disease. PSWCs = periodic sharp wave complexes; EEG = electro-encephalogram; MR-DWI = magnetic resonance diffusion weighted imaging; CSF = cerebrospinal fluid.

Sex	9 M/7 F
Disease duration in months (median, range)	12 (4–42)
Age at onset (mean, SD)	77 ± 7.3
Age at death (mean, SD)	78 ± 7.3
Presence of clinical symptoms (yes/no/NA)	
- Dementia	16 (100%)/0/0
- Parkinsonism	14 (88%)/2/0
- Fluctuating cognition	11 (69%)/1/4
- Visual hallucinations	12 (75%)/0/4
- Delusions	9 (56%)/0/7
- Neuroleptic sensitivity	9 (56%)/1/6
- History of depression	5 (31%)/0/11
- Autonomic dysfunction	3 (19%)/0/13
- Myoclonus	10 (63%)/1/5
- Pyramidal symptoms	6 (38%)/5/5
- Cerebellar symptoms	4 (25%)/8/4
- Akinetic mutism	3 (19%)/4/9
Ancillary investigations	
- PSWCs on EEG	Present in 0 of 11 cases with a reported EEG (non-periodic bi- or triphasic complexes in 3 cases)
- Hyperintensities on MR-DWI	Present in 0 of 7 cases with a reported MRI
- 14-3-3 protein in CSF	2 negative; 1 inconclusive; 2 positive of 5 cases with a reported 14-3-3 CSF test
Clinical diagnosis of DLB [11]	0 no; 3 possible; 13 probable
Clinical diagnosis of CJD [10]	7 no; 8 possible; 1 probable

2.3. Genetic analysis

Fresh-frozen temporal cortex was available for eleven cases (Table 2). Genomic DNA was isolated from this tissue with the AllPrep DNA/RNA/miRNA Universal Kit of Qiagen. The Nimblegen SeqCap EZ Exome v.2.0 44 Mb kit (Roche Nimblegen, Inc., Madison, WI) was used on a HiSeq 2000 sequencer (paired-end 2 × 100) for the whole exome sequencing (WES). We selected non-synonymous, stop-gain or stop-loss variants in exons and variants near splice sites with a mean allele frequency of < 1% in the ExAC-NFE (*"Exome Aggregation Consortium-Non Finnish Europeans"*) database and the GoNL (*"Genome of the Netherlands"*) database in genes with a well-established involvement in Parkinson's disease (PD) (*SNCA, Parkin, PINK1, DJ1, LRRK2, GBA, VPS35, DNAJC6, RAB39B*), AD (*APP, PSEN1, PSEN2, SORL1, TREM2, APOE, ABCA7*) and frontotemporal dementia (*MAPT, GRN*). All variants in *GBA* were selected regardless the mean allele frequency reported in

Table 2
Staging of Lewy body disease and Alzheimer's disease related pathology, presence of vascular pathology and spongiform changes, genetic variants in disease-associated genes and *APOE ε* genotype in sixteen cases with dementia with Lewy bodies clinically suspected of Creutzfeldt-Jakob's disease. CERAD = Consortium to Establish a Registry for Alzheimer's Disease, ND = not done.

Case number	Sex	Age at death	Clinical criteria for DLB	Clinical criteria for CJD	Atrophy	Atherosclerosis	Infarctions	Braak alpha-synuclein stage	McKeith Lewy body stage
1	M	74	Probable DLB	No CJD	no	no	no	6	diffuse
2	M	79	Probable DLB	Possible CJD	moderate (global, wide ventricles)	no	no	5	transitional
3	F	87	Probable DLB	Possible CJD	no	no	no	6	diffuse
4	F	81	Probable DLB	No CJD	no	no	no	6	transitional
5	M	67	Probable DLB	No CJD	no	mild	no	6	diffuse
6	F	86	Probable DLB	Possible CJD	mild (frontal)	moderate	no	6	diffuse
7	M	75	Probable DLB	No CJD	no	no	no	6	transitional
8	M	71	Possible DLB	Possible CJD	mild (frontotemporal)	moderate	no	6	diffuse
9	M	85	Probable DLB	Possible CJD	no	moderate	no	5	transitional
10	F	76	Possible DLB	No CJD	mild (frontotemporal)	moderate	no	3	brainstem
11	M	75	Probable DLB	Possible CJD	mild (frontotemporal)	mild	no	6	diffuse
12	F	80	Probable DLB	Possible CJD	no	mild	no	5	transitional
13	M	84	Probable DLB	No CJD	moderate (frontal)	severe	microscopical infarctions: CA1/CA2 region hippocampus right, caudate nucleus right and left, occipital cortex	6	diffuse
14	F	62	Possible DLB	Possible CJD	mild (frontal, amygdala, brainstem)	mild	no	4	transitional
15	F	87	Probable DLB	Probable CJD	mild	severe	cerebellar left (2 cm), microscopical infarctions in temporal cortex and thalamus	6	diffuse
16	M	78	Probable DLB	No CJD	no	mild	no	6	diffuse

Case number	Braak neurofibrillary stage	Thal phase for amyloid-beta	CERAD age-related score	Level of AD-related pathology	Small vessel disease	Cerebral amyloid angiopathy	Spongiform changes	Genetic variants in disease-associated genes	*APOE ε* genotype
1	5	4	C	high	no	type 2 stage 2	moderate	ND	ND
2	3	1	O	low	yes	no	mild	*SORL1* (p.R1799Q)	2/3
3	2	3	O	low	no	type 2 stage 2	severe	-	3/3
4	2	2	O	low	no	no	moderate	ND	ND
5	3	3	B	intermediate	no	type 2 stage 1	none	ND	ND
6	4	3	B	intermediate	yes	no	mild	–	3/3
7	2	2	O	low	no	no	mild	*GBA* (p.D140H + p.E326-K)	3/3
8	3	3	B	intermediate	no	type 2 stage 1	mild	-	3/3
9	1	3	B	low	no	no	none	–	3/3
10	1	0	O	none	no	no	none	-	2/3
11	6	4	C	high	no	no	severe	ND	ND

(*continued on next page*)

Table 2 (*continued*)

Case number	Braak neurofibrillary stage	Thal phase for amyloid-beta	CERAD age-related score	Level of AD-related pathology	Small vessel disease	Cerebral amyloid angiopathy	Spongiform changes	Genetic variants in disease-associated genes	*APOE* ε genotype
12	1	0	O	low	no	no	mild	–	2/4
13	3	4	C	intermediate	yes	type 2 stage 1	severe	*SORL1* (p.D140 N)	3/4
14	1	0	O	none	no	no	none	–	3/3
15	3	5	C	intermediate	no	type 2 stage 1	mild	–	3/4
16	3	4	B	intermediate	no	type 1 stage 3	mild	ND	ND

public databases. Subsequently, Sanger sequencing was performed to validate selected variants and to exclude false-negative results in exons and intron-exon boundaries with low coverage (< 10 reads).

Furthermore, copy dosage analysis of *SNCA*, *Parkin*, *PARK7*, *PINK1* and *APP* was done using Multiplex Ligation-dependent Probe Amplification (MLPA, MRC Holland), *APOE* ε genotyping using Taqman Allelic Discrimination and *C9orf72* repeat expansions analysis using a repeat-primed PCR assay. Further details on genetic analyses techniques are provided in the Supplementary Methods.

2.4. Statistical analyses

Mean and standard deviation were calculated for normally distributed continuous data (age at onset, age at death), median and interquartile range was used for not normally distributed data (disease duration) and ordinal data (load of different types of pathology). Correlation between cerebral alpha-synuclein, neurofibrillary and amyloid-β load was assessed with Spearman's rank correlations, and the cerebral alpha-synuclein load in a group of cases with low-to-none versus a moderate-to-high level of AD-related pathology was compared using the Mann-Whitney *U* test. Data were considered significant if $p < 0.05$. All statistical analysis were done using IBM SPSS version 22.

3. Results

3.1. Neuropathological diagnoses in all cases suspected of CJD

Of 512 consecutive autopsy cases between 1998 and 2014, autopsy revealed a prion disease in 296 cases (58%), including 259 cases with sporadic CJD. Neurodegenerative diseases were the most frequent alternative diagnoses (48%), followed by immune diseases (17%), malignant diseases (10%), toxic metabolic disorders (10%), vascular diseases (9%) and other causes (6%) (Supplementary Table S1). LBD was found in 26 cases (12%), of whom sixteen cases fulfilled inclusion criteria (Supplementary methods).

3.2. Clinical diagnosis and demographics

All sixteen cases were diagnosed during life with rapidly progressive dementia. CJD was considered the most likely clinical diagnosis or was included in the differential diagnosis in all cases by the treating neurologists, and DLB was explicitly listed in the differential diagnosis in eight cases. In retrospect, thirteen cases fulfilled clinical criteria for probable DLB and three cases for possible DLB [11]. Clinical criteria categorized one case as probable CJD, eight cases as possible CJD, and seven cases as no CJD [10]. Median disease duration was 12 months (interquartile range 6.5–24 months), with a mean age at onset of 77 years and a mean age at death of 78 years (SD 7.3; range 62–87 years). Basic demographic features, clinical symptoms, ancillary investigations and clinical diagnoses of CJD and DLB are listed in Table 1.

3.3. Distribution and load of alpha-synuclein pathology

Mild to moderate atrophy was present in 50% of cases (Table 2). Moderate to severe neuronal loss in the substantia nigra was present in all cases. Brainstem-type LBD was present in one case (Fig. 1, 5a-c), transitional LBD in six cases and diffuse LBD in nine cases. Eleven cases showed Braak alpha-synuclein stage 6 (Table 2). A large proportion of the cases showed a moderate to severe load of alpha-synuclein pathology in the substantia nigra (81%), temporo-occipital cortex (75%), temporal cortex (56%), and frontal cortex (38%, Fig. 2).

3.4. Presence of concomitant AD-related pathology, vascular pathology and spongiform changes

All cases showed neurofibrillary pathology, with nine cases reaching

Braak neurofibrillary stage 3 or higher. One case (case 8) showed ARTAG, none of the cases showed argyrophilic grain disease. Diffuse and/or classical amyloid-β positive plaques were present in thirteen cases and neuritic plaques were present in the neocortex of nine cases (Table 2). The level of AD-related pathology was none in two cases, low in six cases, intermediate in six cases and high in two cases (case 1 and 11; Figs. 1, 2d-e, Table 2).

Case 16 showed severe capillary CAA, Thal stage 3 (Figs. 1 and 3e). Additionally, CAA type 2 was present in six cases. Signs of small vessel disease were present in three cases, and severe atherosclerosis of large vessels combined with multiple cortical and subcortical infarctions were present in two cases (case 13 and 15). Mild to severe spongiform cortical changes were present in twelve cases (75%) (Table 2).

3.5. Correlations between cerebral load of alpha-synuclein, neurofibrillary and amyloid-β pathology

Cases with an intermediate-to-high load of AD-related pathology (n = 8) had a significantly higher cerebral load of alpha-synuclein pathology compared to cases with no-to-low level of AD-related pathology (n = 8, p = 0.007). The load of alpha-synuclein pathology was significantly correlated to both the load of neurofibrillary (Spearman's rho 0.757; p = 0.001) and amyloid-β pathology (Spearman's rho 0.707; p = 0.002).

3.6. Presence of genetic defects and their neuropathological correlates

In three of the eleven cases (45%) analyzed, possible disease-associated variants were detected in genes with a well-established involvement in PD or AD (Supplementary Table S2).

In case 7, a rare variant and a common variant were detected in *GBA* (p.D140H and p.E326K) and confirmed by Sanger sequencing. E326K and E326K + D140H variants have been associated with a more rapid cognitive decline in PD [22,23]. At autopsy, case 7 showed a high load of neocortical alpha-synuclein and a low level of AD-related pathology (Figs. 1, 4a-e).

Additionally, the p.R1799Q and p.D140N variants in *SORL1* were found in case 2 and 13 respectively, and confirmed by Sanger sequencing. These variants have an allele frequency of 0.005% and 0.03% in the GnomAD respectively. The p.D140N variant is predicted to be pathogenic by half of the *in silico* programs, whilst the p.R1799Q variant is predicted to be likely benign by the majority of programs. The case with the p.D140N variant showed an intermediate level of AD-related pathology, whereas the case with the likely benign p.R1799Q variant showed only a low level of AD-type pathology.

No *SNCA, Parkin, PARK7, PINK1* or *APP* dosage abnormalities and no pathogenic *C9orf72* repeat expansions were found. Three cases carried an *APOE* ε4 allele, of which one had no AD-related pathology and two had an intermediate level of AD-related pathology (Table 2).

4. Discussion

Until now, few studies have reported the neuropathological characteristics of DLB cases suspected of CJD. Here, we describe neuropathological and genetic features in sixteen of these cases collected by the Dutch Surveillance Center for Prion Diseases. A high load of neocortical alpha-synuclein pathology was present in most, but not all DLB cases, and cases showed a variable load of AD-related pathology. We found two *GBA* variants in one patient and two heterozygous rare variants of unknown significance in *SORL1* in two patients.

In the current study, ten cases retrospectively fulfilled clinical criteria for possible or probable CJD, and all cases retrospectively fulfilled clinical criteria for possible or probable DLB, with a median disease duration of 12 months. The overlap of clinical symptoms in cases with rapidly progressive dementias is well-known, and it has been proven difficult to discriminate rapidly progressive DLB and CJD based on clinical symptoms and signs [3–5,7].

The neuropathological correlates of rapidly progressive DLB have only been studied in few case-studies and very small case-series. Transitional LBD was present in four cases, and diffuse LBD in 16 out of 20 cases described [4,7,8,24]. The level of AD-related pathology according to the National Institute on Aging – Alzheimer Association (NIA-AA) [17] ranged from none to severe [7–9]. Additionally, concomitant pathology included argyrophilic grain disease [7], TDP-43 positive inclusions [8], atherosclerosis [8], infarctions [8], small vessel disease [7–9], congophilic amyloid angiopathy (CAA) [7], and a subarachnoidal hemorrhage [8]. In conclusion, the neuropathology described in these cases was variable, but studies were small with heterogeneous inclusion criteria.

In 56% of DLB cases suspected of CJD in the current study, the neuropathology was characterized by diffuse LBD, with an intermediate to high load of alpha-synuclein pathology in neocortical regions. In comparison, a previous large post-mortem series of 807 DLB cases (mean disease duration 8.8 ± 4.0 years) revealed diffuse LBD in 57% of the cases [14]. However, due to differences in case selection, a direct comparison of different cohorts should be interpreted with caution [25]. Although diffuse LBD has been related to a more rapidly progressive disease course in DLB [13,14] and LBD [15], our results show that the pathological correlate of rapidly progressive DLB can be transitional LBD or even brainstem-predominant LBD.

The load of AD-related pathology varied from none to severe in this study. A negative correlation between load of AD-related pathology and survival in DLB has been shown by neuropathological studies [13,15] and a recent CSF biomarker study [26]. However, the current study shows that DLB symptoms can also progress rapidly in cases with little concomitant pathology. Also, concomitant vascular pathology was present in several cases, which may have added to disease progression.

Spongiform changes were found in 75% of cases in the current study, which is in line with a previous post-mortem study of 40 DLB cases, where 82% showed spongiform changes [27]. In this study, there was no correlation between spongiform changes and disease duration [27], which is corroborated by the low frequency of severe spongiform changes in the current series. The heterogeneous neuropathology in our cases is in line with previous reports on the presence of neuropathological hallmarks in DLB cases suspected of CJD [7–9], and with the neuropathology of DLB cases in general [13,14].

In this study, a higher alpha-synuclein load was associated with a higher load of AD-related pathology. This is in line with results from previous autopsy studies, that revealed a correlation between alpha-synuclein pathology and AD-related pathology in LBD [15], and DLB [13] cases. Together with evidence from in vitro and animal cross-seeding experiments, this suggests synergistic interactions between hyperphosphorylated tau, amyloid-β and alpha-synuclein aggregates [28].

The relation between genetic defects and a rapidly progressive phenotype in DLB has been understudied. In a few rapidly progressive DLB cases, a genetic screening has been performed, without finding evidence for presence of genetic defects [8,24].

Interestingly, in one diffuse LBD case with a low level of AD-related pathology, a combination of two genetic variants was found in *GBA* (p.D140H and p.E326K). This combination of variants has been described before in Gaucher disease [23] and PD [22]. PD carriers of the p.E326K variant and the D140H + E326K complex allele were shown to have a faster cognitive decline than non-GBA associated PD cases [22]. As *GBA* variants have been shown to be associated with more severe motor and cognitive dysfunction in DLB [29], these variants may

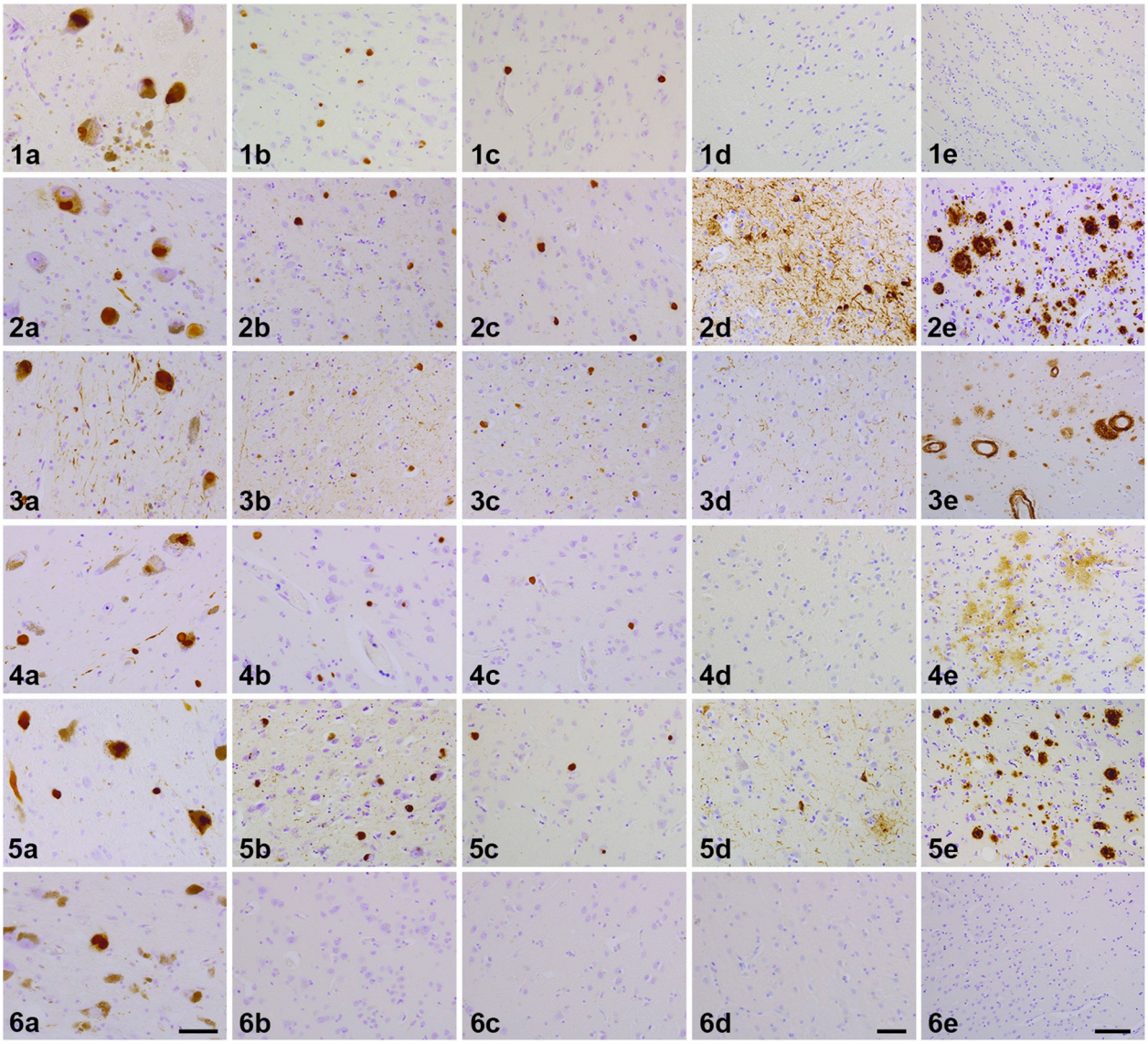

Fig. 1. Representative overview of the neuropathological features visualized with immunohistochemistry in sixteen cases with dementia with Lewy bodies clinically suspected of Creutzfeldt-Jakob's disease. Load of alpha-synuclein pathology was high in limbic and neocortical regions in most cases (**1b-4b, 1c-4c**), but a few cases only showed brainstem-predominant (**5a-c**) or transitional Lewy body disease (LBD). Neurofibrillary and amyloid-β pathology were absent or low in some (**1d-e, 4d-e, 5d-e**), but severe in other cases (**2d-e**). One case showed severe capillary cerebral amyloid angiopathy (**3e**). A case with two *GBA* variants (p.D140H and p.E326K) showed diffuse LBD (**4a-c**) and low levels of Alzheimer's disease (AD)-related pathology **(4d-e)**. One case showed nigral degeneration and brainstem-predominant LBD **(5a-c)** without signs of AD-related pathology (**5d-e**)**.**

Representative microscopy images from case 4 (**1a-e**), 11 (**2a-e**), 16 (**3a-e**), 7 (**4a-e**) and 10 (**5a-e**). Immunohistochemistry against alpha-synuclein (clone KM51) was performed on a: substantia nigra, **b**: trans-entorhinal cortex and c: temporal cortex. **d**: Immunohistochemistry against hyperphosphorylated tau (clone AT8) on temporal cortex. e: Immunohistochemistry against amyloid-β (clone 6f/3d) on temporal (**1e**, **2e**, **4e** and **5e**) and frontal (**3e**) cortex. The scale bar in 5a represents 50 μm and applies to **1a-5a**, **1b-5b** and **1c-5c**. The scale bar in **5d** represents 50 μm and applies to **1d-5d**. The scale bar in **5e** represents 100 μm and applies to **1e-5e**.

have contributed to the rapid cognitive decline.

Furthermore, two other heterozygous variants in the *SORL1* gene (p.D140N and p.R1799Q) were observed in two different cases. According to recent criteria for *SORL1* variants in AD, both variants are categorized as 'likely benign' [30]. However, as these criteria have been adopted for AD, the role of these variants in DLB disease progression is still uncertain.

Limitations of the current study are the small sample size, limited availability of tissue for genetic analysis, inclusion of cases based on clinical referrals, and the retrospective nature of the clinical data. Additionally, rapid clinical deterioration in DLB can be induced by a hypersensitive reaction to neuroleptic treatment, which may be misinterpreted as signs of CJD [6]. This iatrogenic cause of disease progression may play a role in some cases in this study. Studies in larger, prospectively followed cohorts of patients are needed to draw more generalizable conclusions on the neuropathological or genetic substrates of rapid disease progression in DLB, especially regarding the clinical and pathological heterogeneity in this group of patients. However, this may be difficult to realize in clinical practice, as DLB cases clinically suspected of CJD are very rare.

In short, a high load and neocortical distribution of alpha-synuclein pathology is present in some, but not all DLB cases in this post-mortem series. This suggests that a different set of factors contribute to the rapidly progressive disease course in cases with no or a low load of neocortical alpha-synuclein pathology. Additional burden from presence of concomitant pathologies, synergistic effects and specific genetic defects may have contributed to the disease progression in some cases. Understanding which factors contribute to a rapid disease

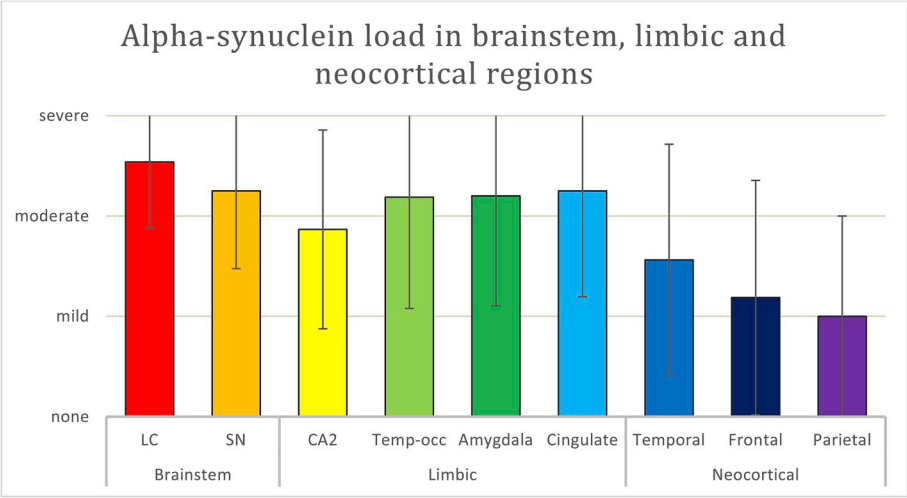

Fig. 2. Semi-quantitative load of alpha-synuclein pathology in brainstem, limbic and neocortical brain regions in sixteen cases with dementia with Lewy bodies clinically suspected of Creutzfeldt-Jakob's disease. Lewy bodies (LBs) and Lewy neurites (LNs) were scored in brainstem regions, LNs in the CA2 region and LBs in other limbic and neocortical regions. Bars represent mean values, error bars represent standard deviations. LC = locus coeruleus; SN = substantia nigra; CA2 = cornu ammonis region 2; temp-occ = temporo-occipital cortex; cingulate = posterior cingulate gyrus; temporal = medial temporal gyrus; frontal = frontal gyrus (Brodmann area 10); parietal = inferior parietal lobe.

progression in DLB could aid in the search for biomarkers that enable clinicians and researchers to select patients for therapeutic strategies.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.02.011.

References

[1] I.G. McKeith, B.F. Boeve, D.W. Dickson, G. Halliday, J.P. Taylor, D. Weintraub, et al., Diagnosis and management of dementia with Lewy bodies: fourth consensus report of the DLB Consortium, Neurology 89 (1) (2017) 88–100.
[2] M.M. Williams, C. Xiong, J.C. Morris, J.E. Galvin, Survival and mortality differences between dementia with Lewy bodies vs Alzheimer disease, Neurology 67 (11) (2006) 1935–1941.
[3] S. Haik, J.P. Brandel, V. Sazdovitch, N. Delasnerie-Laupretre, K. Peoc'h, J.L. Laplanche, et al., Dementia with Lewy bodies in a neuropathologic series of suspected Creutzfeldt-Jakob disease, Neurology 55 (9) (2000) 1401–1404.
[4] H.J. Tschampa, M. Neumann, I. Zerr, K. Henkel, A. Schroter, W.J. Schulz-Schaeffer, et al., Patients with Alzheimer's disease and dementia with Lewy bodies mistaken for Creutzfeldt-Jakob disease, J. Neurol. Neurosurg. Psychiatry 71 (1) (2001) 33–39.
[5] B. Van Everbroeck, I. Dobbeleir, M. De Waele, P. De Deyn, J.J. Martin, P. Cras, Differential diagnosis of 201 possible Creutzfeldt-Jakob disease patients, J. Neurol. 251 (3) (2004) 298–304.
[6] A.W. Lemstra, N. Schoenmaker, A.J. Rozemuller-Kwakkel, W.A. van Gool, The association of neuroleptic sensitivity in Lewy body disease with a false positive clinical diagnosis of Creutzfeldt-Jakob disease, Int. J. Geriatr. Psychiatry 21 (11) (2006) 1031–1035.
[7] K.A. Josephs, J.E. Ahlskog, J.E. Parisi, B.F. Boeve, B.A. Crum, C. Giannini, et al., Rapidly progressive neurodegenerative dementias, Arch. Neurol. 66 (2) (2009) 201–207.
[8] C. Gaig, F. Valldeoriola, E. Gelpi, M. Ezquerra, S. Llufriu, M. Buongiorno, et al., Rapidly progressive diffuse Lewy body disease, Mov. Disord. 26 (7) (2011) 1316–1323.
[9] O. Grau-Rivera, E. Gelpi, C. Nos, C. Gaig, I. Ferrer, A. Saiz, et al., Clinicopathological correlations and concomitant pathologies in rapidly progressive dementia: a brain bank series, Neurodegener. Dis. 15 (6) (2015) 350–360.
[10] I. Zerr, K. Kallenberg, D.M. Summers, C. Romero, A. Taratuto, U. Heinemann, et al., Updated clinical diagnostic criteria for sporadic Creutzfeldt-Jakob disease, Brain 132 (10) (2009) 2659–2668.
[11] I.G. McKeith, D.W. Dickson, J. Lowe, M. Emre, J.T. O'Brien, H. Feldman, et al., Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium, Neurology 65 (12) (2005) 1863–1872.
[12] D.H. Hepp, D.L. Vergoossen, E. Huisman, A.W. Lemstra, H.W. Berendse, A.J. Rozemuller, et al., Distribution and load of amyloid-beta pathology in Parkinson disease and dementia with Lewy bodies, J. Neuropathol. Exp. Neurol. 75 (10) (2016) 936–945.
[13] T.J. Ferman, N. Aoki, J.E. Crook, M.E. Murray, N.R. Graff-Radford, J.A. van Gerpen, et al., The limbic and neocortical contribution of alpha-synuclein, tau, and amyloid beta to disease duration in dementia with Lewy bodies, Alzheimer's Dementia : the journal of the Alzheimer's Association 14 (3) (2018) 330–339.
[14] J. Graff-Radford, J. Aakre, R. Savica, B. Boeve, W.K. Kremers, T.J. Ferman, et al., Duration and pathologic correlates of Lewy body disease, Neurol. 74 (3) (2017) 310–315.
[15] D.J. Irwin, M. Grossman, D. Weintraub, H.I. Hurtig, J.E. Duda, S.X. Xie, et al., Neuropathological and genetic correlates of survival and dementia onset in synucleinopathies: a retrospective analysis, Lancet Neurol. 16 (1) (2017) 55–65.
[16] I. Alafuzoff, P.G. Ince, T. Arzberger, S. Al-Sarraj, J. Bell, I. Bodi, et al., Staging/typing of Lewy body related alpha-synuclein pathology: a study of the BrainNet Europe Consortium, Acta Neuropathol. 117 (6) (2009) 635–652.
[17] T.J. Montine, C.H. Phelps, T.G. Beach, E.H. Bigio, N.J. Cairns, D.W. Dickson, et al., National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach, Acta Neuropathol. 123 (1) (2012) 1–11.
[18] G.G. Kovacs, I. Ferrer, L.T. Grinberg, I. Alafuzoff, J. Attems, H. Budka, et al., Aging-related tau astrogliopathy (ARTAG): harmonized evaluation strategy, Acta Neuropathol. 131 (1) (2016) 87–102.
[19] H. Braak, E. Braak, Argyrophilic grains: characteristic pathology of cerebral cortex in cases of adult onset dementia without Alzheimer changes, Neurosci. Lett. 76 (1) (1987) 124–127.
[20] D.R. Thal, W.S. Griffin, R.A. de Vos, E. Ghebremedhin, Cerebral amyloid angiopathy and its relationship to Alzheimer's disease, Acta Neuropathol. 115 (6) (2008) 599–609.
[21] I. Alafuzoff, T. Arzberger, S. Al-Sarraj, I. Bodi, N. Bogdanovic, H. Braak, et al., Staging of neurofibrillary pathology in Alzheimer's disease: a study of the BrainNet Europe Consortium, Brain Pathol. 18 (4) (2008) 484–496.
[22] G. Liu, B. Boot, J.J. Locascio, I.E. Jansen, S. Winder-Rhodes, S. Eberly, et al., Specifically neuropathic Gaucher's mutations accelerate cognitive decline in Parkinson's, Ann. Neurol. 80 (5) (2016) 674–685.
[23] M.E. Grace, P. Ashton-Prolla, G.M. Pastores, A. Soni, R.J. Desnick, Non-pseudogene-derived complex acid beta-glucosidase mutations causing mild type 1 and severe type 2 gaucher disease, J. Clin. Investig. 103 (6) (1999) 817–823.
[24] L. Saint-Aubert, J. Pariente, H. Dumas, P. Payoux, J.P. Brandel, M. Puel, et al., Case report of Lewy body disease mimicking Creutzfeldt-Jakob disease in a 44-year-old man, BMC Neurol. 16 (2016) 122.
[25] L. Parkkinen, H. Soininen, M. Laakso, I. Alafuzoff, Alpha-synuclein pathology is highly dependent on the case selection, Neuropathol. Appl. Neurobiol. 27 (4) (2001) 314–325.
[26] A.W. Lemstra, M.H. de Beer, C.E. Teunissen, C. Schreuder, P. Scheltens, W.M. van der Flier, et al., Concomitant AD pathology affects clinical manifestation and survival in dementia with Lewy bodies, J. Neurol. Neurosurg. Psychiatry 88 (2) (2017) 113–118.
[27] A. Sherzai, S.D. Edland, E. Masliah, L. Hansen, D.P. Pizzo, A. Sherzai, et al., Spongiform change in dementia with Lewy bodies and Alzheimer disease, Alzheimer Dis. Assoc. Disord. 27 (2) (2013) 157–161.
[28] T.L. Spires-Jones, J. Attems, D.R. Thal, Interactions of pathological proteins in neurodegenerative diseases, Acta Neuropathol. 134 (2) (2017) 187–205.
[29] T. Shiner, A. Mirelman, M. Gana Weisz, A. Bar-Shira, E. Ash, R. Cialic, et al., High frequency of GBA gene mutations in dementia with Lewy bodies among ashkenazi jews, Neurol. 73 (12) (2016) 1448–1453.
[30] H. Holstege, S.J. van der Lee, M. Hulsman, T.H. Wong, J.G. van Rooij, M. Weiss, et al., Characterization of pathogenic SORL1 genetic variants for association with Alzheimer's disease: a clinical interpretation strategy, Eur. J. Hum. Genet. : EJHG (Eur. J. Hum. Genet.) 25 (8) (2017) 973–981.