whereas RD4 only labeled the 64/68 kDa doublet and some fragments at ~25 kDa (Fig. 1h). Anti-4R strongly stained the smears and fragments (Fig. 1i), suggesting that tau in these RD4-negative anti-4R-positive bands and smears is deamidated at N279. The weak RD4 and strong anti-4R immunoreactivities were completely abolished after trypsin or Pro-K treatment (Fig. 1h, i). This result is inconsistent with the immunohistochemistry, but protease sensitivity is likely different in fixed tissues. In contrast, the RD3 epitope was retained in the fragments, and RD3 strongly reacted with the protease-resistant 10–25 kDa bands after trypsin or Pro-K treatment (Fig. 1g). pS396 epitope was removed by Pro-K but not trypsin, suggesting a location outside the PHF core. Trypsin may not cleave the KSP site because of phosphorylation of Ser396. These results demonstrate reciprocal effects of protease treatment on RD3 and RD4 epitopes, indicating that RD4 epitope in tau in AD is susceptible to proteases, while RD3 epitope is highly resistant.

These results are consistent with previous findings. Wischik et al. identified two types of amino acid sequences, QPGGGKVQIVYK... (3R tau) and IKXVPGG... (4R tau), in 12-kDa tau fragment comprising the pronase-resistant core of PHFs [6] (see Fig. 1k). We identified HQPGGG... (3R tau) and HVPGGG... (4R tau) in 7–15 kDa trypsin-resistant fragments of PHF-tau in AD brains [5]. In both cases, 3R and 4R tau isoforms were detected, but the 4R tau N-terminus lacked the RD4 epitope. Based on these observations and a computed cross-section of PHF (Fig. 1k) [1], we propose a schematic model of tau folding in PHF (Fig. 1l). Analysis of the cross-sectional density in the PHF core on electron micrographs indicates the presence of two C-shaped morphological units, which correspond to the two strands of PHF, each with three domains (Fig. 1k) [1]. The RD3 epitope is buried in the PHF core and is normally masked by the N- or C-terminal region of tau, but is exposed in ghost tangles and/or in PHFs attacked by proteases. The RD4 epitope, which is mostly deamidated in PHF, is located slightly outside the core, where it can be digested by proteases (Fig. 1l). This model can explain the epitope masking of RD3 and RD4 and the reciprocal effects of degradation or protease treatment on the immunoreactivities.

This study indicates that differential presentation of epitopes can occur as a result of folding and processing, even when the epitopes are located in close proximity. Tau in PHFs appears to be processed gradually by intracellular proteases and more extensively in extracellular space during AD progression. We suggest that changes in immunoreactivity to antibodies reflect aging of tau in tangles or PHFs, which are composed of both 3R and 4R tau isoforms. We also show that Pro-K treatment of sections after Ac and FA treatment is useful for unmasking buried epitopes.

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ORIGINAL PAPER

Argyrophilic grain disease as a neurodegenerative substrate in late-onset schizophrenia and delusional disorders

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Abstract To study the relationship between neurodegenerative diseases including argyrophilic grain disease (AGD) and late-onset schizophrenia and delusional disorders (LOSD; onset ≥40 years of age), we pathologically examined 23 patients with LOSD, 71 age-matched normal controls, and 22 psychiatric disease controls (11 depression, six personality disorder, two bipolar disorders, and three neurotic disorders cases). In all LOSD cases (compared to age-matched normal controls), the frequencies of Lewy body disease (LBD), AGD, and corticobasal degeneration (CBD) were 26.1 % (11.3 %), 21.7 % (8.5 %), and 4.3 % (0.0 %), respectively. There was no case of pure Alzheimer's disease (AD). The total frequency of LBD, AGD, and CBD was significantly higher in LOSD cases than in normal controls. Argyrophilic grains were significantly more severe in LOSD than in controls, but were almost completely restricted to the limbic system and adjacent temporal cortex. In LOSD patients whose onset occurred at ≥65 years of age (versus age-matched normal controls), the frequencies of LBD and AGD were 36.4 % (19.4 %) and 36.4 % (8.3 %), respectively, and AGD was significantly more frequent in LOSD patients than in normal controls. In LOSD patients whose onset occurred at <65 years of age, the frequencies of LBD, AGD, and CBD were 16.7, 8.3, and 8.3 %, comparable to those of age-matched normal controls (10.2, 5.1, and 0.0 %). In all psychiatric cases, delusion was significantly more frequent in AGD cases than in cases bearing minimal AD pathology alone. Given these findings, LOSD patients may have heterogeneous pathological backgrounds, and AGD may be associated with the occurrence of LOSD especially after 65 years of age.

Keywords Argyrophilic grain · α-Synuclein · Corticobasal degeneration · Four-repeat tau · Late onset · Tauopathy

Introduction

Schizophrenia is most prevalent in early and middle life, before 40 years of age, but it is also known that this disorder is not infrequent in later life [1]. While there is no limitation regarding the age at onset in the current diagnostic criteria for schizophrenia, the International Classification of Diseases, Revision 10 (ICD-10) and Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) [2], it has been also considered that the pathogenic backgrounds of early- and late-onset schizophrenia may not be identical. Indeed, since a historic report on schizophrenia developing after 40 years of age, called late-onset schizophrenia, was published by Bleuler [3] in 1943, many studies have demonstrated that patients with late-onset schizophrenia have clinical features different from those in

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early-onset cases: less severe affective flattening, less severe thought disorder, and a more favorable prognosis [4–9]. In 2000, the International Late-Onset Schizophrenia Group proposed that patients who develop symptoms of schizophrenia after 40 and 60 years of age should be differentiated from early-onset cases and the diseases called late-onset schizophrenia and very-late-onset schizophrenia-like psychosis, respectively [10]. In the present paper, we call psychotic disorders that occurred in cognitively preserved people older than 40 years of age "late-onset schizophrenia and delusional disorders" (LOSD).

Potential pathological backgrounds in patients with late-life depression have been explored mainly by focusing on cerebrovascular lesions [11–15], Alzheimer's disease (AD) [16], and Lewy body disease (LBD) [17–19], although several pathological studies suggested that vascular lesions and AD pathology are usually unrelated to the occurrence of late-life depression [20, 21]. Several studies demonstrated that the frequencies of AD [22, 23] and LBD pathologies [23] were not increased in elderly patients with chronic or residual schizophrenia. On the other hand, the available pathological data regarding psychoses that have developed in elderly people are limited. A few studies demonstrated that LOSD patients often had mild to moderate neurofibrillary tangles (NFTs) in the limbic region [24] and that pyramidal neurons in the hippocampus were spared in number [25, 26]. It is also known that some LBD cases show paranoia as the first symptom [27]. It was also reported that some cases of corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP), common four-repeat tauopathies, show psychosis along with characteristic motor disturbance [28–31]. Argyrophilic grain disease (AGD) is another of the four-repeat tauopathies that increases in frequency with age [32]. It was reported that cases bearing extensively and intensively distributed argyrophilic grains frequently show dementia [33] and that some AGD cases with dementia additionally show prominent psychiatric symptoms, such as aggression, irritability, depression, and psychosis [31, 34–38]. However, to our knowledge, no study that comprehensively examined these neurodegenerative changes common in the elderly in patients developing LOSD has been reported.

The primary aims of this study were to systematically examine the neurodegenerative bases in LOSD cases and to clarify whether AGD is associated with the occurrence of LOSD. To address these, we examined 23 LOSD cases, 71 age-matched normal controls, and 22 cases of various psychiatric disorders as a disease control using modern sensitive and standardized pathological methods. In this paper, we demonstrated that AGD may be a common pathology in LOSD cases that is comparable to LBD in frequency and that AGD may be associated with the occurrence of LOSD especially after the age of 65 years.

Materials and methods

Subjects

We selected 39 LOSD cases and 71 age-matched normal control cases without neurological or psychiatric disorders, as well as 22 cases having psychiatric disorders other than LOSD as disease controls (11 depression, six personality disorder (personality change), two bipolar disorder, and three neurotic disorder cases). All psychiatric cases were selected from an autopsy case series registered with the Department of Neuropsychiatry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences. The common selection criteria for the psychiatric cases including LOSD cases were as follows: (1) the initial psychiatric symptoms occurred after 40 years of age, (2) the absence of a history of neurological or psychiatric disorders before 40 years of age, (3) the absence of dementia in the early to middle stage of the course, and (4) the absence of episodes suggesting evident memory impairment, including delusion of theft. In this study, LOSD was defined as psychosis that developed after 40 years of age, fit the criteria of schizophrenia or delusional disorders of ICD-10, and lacked dementia at least in the early to middle stage of the course. Some of the LOSD cases were originally diagnosed as presenile-onset schizophrenia or senile-onset psychosis. Nine LOSD cases were excluded from the study because they had alcohol dependence, respiratory diseases, liver diseases, renal diseases, neurosyphilis, Huntington disease, or pathological evidence of large cerebral infarction or dentatorubral–pallidoluysian atrophy, which may be associated with the development of psychotic symptoms. Seven LOSD cases without detailed clinical data were also excluded. Finally, we pathologically re-examined 23 LOSD cases, 71 age-matched normal controls, and 22 disease control cases (11 depression, six personality disorder, two bipolar disorder, and three neurotic disorder cases) using modern standardized methods including a panel of immunohistochemistry and sensitive silver stains. Two psychiatrists (SN and OY) reviewed the available clinical information, interviewed clinicians if necessary, and made a consensus diagnosis based on ICD10. All of the LOSD cases but four died in psychiatric hospitals. Age-matched normal control cases ($n = 71$) were selected from an autopsy case series registered with the Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology. Selection criteria for these normal control cases were as follows: (1) the absence of primary neurological and psychiatric disorders including dementia, stroke, and gait disturbance, (2) the absence of pathological evidence of large cerebral infarctions, and (3) the availability of medical and autopsy records and

Table 1 Demographic data of all cases

	Late-onset schizophrenia and delusional disorders	Normal controls	Other late-life psychiatric disorders			
			Depression	Bipolar disorders	Personality disorders	Neurotic disorders
N	23	71	11	2	6	3
Female, *n* (%)[a]	15 (65.2)	20 (28.2)	6 (54.5)	2 (100.0)	2 (33.3)	2 (66.7)
Age at onset, mean ± SD (years)	63.3 ± 12.9	–	62.3 ± 8.8	68.0	68.3 ± 10.9	65.7 ± 9.2
(Range, years)	(41–86)	–	(51–74)	(68)	(58–82)	(55–71)
Age at death, mean ± SD (years)	75.1 ± 7.5	72.3 ± 6.6	68.3 ± 7.2	78.5 ± 4.9	70.7 ± 11.0	73.0 ± 3.0
Disease duration, mean ± SD (years)	12.0 ± 7.7	–	7.1 ± 6.4	10.5 ± 4.9	2.3 ± 1.0	7.3 ± 6.8
Dementia in the last stage, *n* (%)[b]	7/19 (36.8)	0/71 (0.0 %)	4/9 (44.4)	0/2 (0.0)	1/6 (16.7)	1/3 (33.3)
Cause of death (*n*)						
Neoplasm	1	41	–	–	1	–
Acute myocardial infarction	2	5	–	–	–	–
Aortic aneurysm dissection	–	1	–	–	–	–
Acute respiratory distress syndrome	–	1	–	–	–	–
Heart failure	2	1	–	–	–	–
Pulmonary infarction	–	1	–	–	–	–
Pneumonia	5	3	5	–	3	1
Respiratory failure	4	4	–	–	1	–
Lung abscess	–	1	–	–	–	–
Gastrointestinal bleeding	1	1	1	–	–	–
Ileus	1	–	–	–	–	–
Hepatic failure	–	1	–	–	–	–
Liver cirrhosis	–	1	–	–	–	–
Goodpasture syndrome	–	1	–	–	–	–
Renal failure	–	3	–	–	–	–
Diabetes mellitus	–	2	–	–	–	–
Sepsis	1	1	–	1	–	–
Shock	1	1	–	–	–	–
Sudden death	1	2	1	1	1	1
Suicide	1	–	2	–	–	–
Not available	3	–	2	–	–	1

SD standard deviation

[a] The proportion of cases in each category of clinical diagnosis

[b] The proportion of cases that had dementia in the last stage of the course to all subjects which clinical data in the terminal stage was available

paraffin-embedded tissues. All subjects were autopsied after informed consent was obtained from family members. All experiments in this study were approved by the ethical committees of the Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, and the Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology. The demographic data of all subjects are shown in Table 1.

Conventional neuropathological examination

Brains tissue samples were fixed postmortem with 10 % formaldehyde and embedded in paraffin. The median fixation time was 141 days (range 35–3,918 days, 25–75th percentile range 72–270 days) in LOSD cases for which data were available ($n = 11$, 47.8 %), 90 days (range 16–2,647 days, 25–75th percentile range 34–464 days) in psychiatric disease control cases ($n = 13$, 59.1 %), and 19 days (range 7–65 days, 25–75th percentile range 14–38 days) in age-matched normal control cases ($n = 46$, 63.4 %), respectively. The fixation time in age-matched normal controls was significantly shorter than those in LOSD cases and psychiatric disease control cases, respectively ($P < 0.0001$, respectively. Mann–Whitney *U* test [$\alpha/2$]). Ten-μm-thick sections from the frontal, temporal, parietal, occipital, insular, and cingulate cortices,

hippocampus, amygdala, basal ganglia, midbrain, pons, medulla oblongata, and cerebellum were prepared. Sections of the left hemisphere in psychiatric cases including LOSD cases and standard size sections including each anatomical region examined in age-matched normal control cases were stained with hematoxylin–eosin (H&E) and Klüver–Barrera (KB) stains. Selected regions were stained with modified Bielschowsky silver, methenamine silver, Gallyas–Braak silver methods, and Holzer stain.

Immunohistochemistry

Paraffin sections were cut at 6 μm thickness from regions for the standard assessments described below and immunostained by the immunoperoxidase method using 3′3-diaminobenzidine tetrahydrochloride as reported previously [39]. Antibodies used were against phosphorylated tau (AT8, mouse, monoclonal, 1:1,000, Innogenetics, Ghent, Belgium), tau (T46: mouse, monoclonal, 1:1,000, Invitrogen, Carlsbad, CA, USA), three-repeat (3R) tau (RD3: mouse, monoclonal, 1:3,000, Upstate, Syracuse, NY, USA), and four-repeat (4R) tau (RD4: mouse, monoclonal, 1:200, Upstate), Aβ11-28 (12B2, mouse, monocloncal, 1:2,000, Immuno-Biological Laboratories, Fujioka, Japan), Aβ42 (Aβ42, rabbit, polyclonal, 1:100, Immuno-Biological Laboratories), phosphorylated α-synuclein (psyn#64, mouse, monoclonal, 1:5,000, Wako, Osaka, Japan), α-synuclein (anti-α-synuclein, mouse monoclonal, 1:10,000, Invitrogen, Burlington, ON, Canada), phosphorylated TDP-43 (pS409/410-2, rabbit polyclonal, 1:5,000, Cosmo Bio, Tokyo, Japan), TDP-43 (anti-TDP-43, rabbit polyclonal, 1:1,000, ProteinTech, Chicago, IL, USA), and phosphorylated neurofilament (SMI31, mouse, monoclonal IgG, 1:10,000, Sternberger Monoclonals, Baltimore, MD, USA). When using anti-Aβ antibody, sections were pretreated with 70 % formic acid for 10 min for antigen retrieval. When using psyn#64, SMI31, anti-TDP-43, and pS409/410-2, sections were pretreated in a pressure cooker for 3 min in 10 mM sodium citrate buffer pH 6.0 to enhance immunoreaction. When using phosphorylation-independent anti-α-synuclein antibody, RD3 and RD4, sections were pretreated with 70 % formic acid for 10 min and heated for 3 min in 10 mM sodium citrate buffer in a pressure cooker. Sections were lightly counterstained with hematoxylin.

Assessment of histopathological changes

The distribution and severity of histopathological changes were assessed according to standardized methods. (1) The distribution of NFTs was assessed according to the Braak NFT stage (stage 0–VI) using AT8 immunohistochemistry [40]. (2) The distribution of senile plaques was assessed according to the Braak senile plaque stage using Aβ immunohistochemistry [41]. In statistical analyses, the original stages (i.e., none, A, B, and C) were indicated as stages 0, 1, 2, and 3, respectively. The pathological diagnosis of AD was made according to the NIA-Reagan criteria [42–44] using the modified Bielschowsky silver method, and tau and Aβ immunohistochemistry. (3) Lewy body-related pathology was classified into four histological subtypes (i.e., brain stem type, limbic type, diffuse neocortical type, amygdala-predominant type) according to the Third Consensus Guidelines for DLB [45] and a more recent report [46] with α-synuclein immunohistochemistry. (4) The distribution of argyrophilic grains was classified into four stages (stage 0–III) using a system proposed by Saito et al. [33]. For this evaluation, sections from the ambient gyrus, amygdala, entorhinal cortex, hippocampus, temporal, cingulate, and insular cortex, and orbital gyrus were stained with the Gallyas–Braak silver method and tau immunohistochemistry. In cases having argyrophilic grains, four-repeat tau-predominant accumulation was confirmed by RD4 and RD3 immunostaining. (5) The diagnoses of CBD and PSP were made according to established criteria [47, 48]: astroglial lesions (i.e., tufted astrocytes and astrocytic plaques), NFTs, pretangles, neuropil threads, and ballooned neurons were examined in the posterior superior and middle frontal gyri, primary motor cortex, parietal and temporal cortices, hippocampus, amygdala, caudate nucleus, putamen, globus pallidus, subthalamic nucleus, oculomotor nucleus, substantia nigra, pontine nucleus, inferior olivary nucleus, and dentate nucleus in the cerebellum using both the Gallyas–Braak silver method and tau immunohistochemistry. In this study, cases having not only sufficient NFTs but also astroglial lesions (tufted astrocytes or astrocytic plaques) were diagnosed with CBD or PSP. (6) TDP-43-positive lesions were assessed in the amygdala, entorhinal cortex, hippocampus, frontal and temporal cortices, and hypoglossal nuclei using an anti-TDP-43 antibody. The distribution of TDP-43-positive inclusions in the limbic region was classified into three pathological subtypes using the following system [39], which is similar to that reported by Amador-Ortiz et al. [49]: the amygdala type, in which inclusions were present only in the amygdala; the limbic type, in which inclusions extend to the amygdala, hippocampal dentate gyrus, entorhinal cortex, and fusiform gyrus, but not into the occipitotemporal gyrus; and the temporal type, in which inclusions are also present in the occipitotemporal gyrus. (7) Neuronal loss associated with gliosis and vascular lesions were assessed on H&E- and KB-stained sections according to the grading system employed in our previous studies [50, 51]. Cerebrovascular lesions in the cerebral cortex and basal ganglia were assessed on sections including the whole left hemisphere in LOSD cases ($n = 23$) and psychiatric disease control cases ($n = 22$)

according to a four-point grading system, respectively: grade 0, no lacuna; grade 1, one small lacuna; grade 2, two or more small lacuna without large infarction; grade 3, one or more large infarction. Then, the severity of vascular lesions was compared between LOSD cases and psychiatric disease controls. The severity (the number and size) of vascular lesions in LOSD cases was not compared with that in age-matched normal controls because in the latter group, only sections on standard size slides that included each anatomical region (cortices and nuclei) with only adjacent white matter were available.

Statistical analysis

The Mann–Whitney *U* test and Fisher's exact test were used to compare two groups. In multiple comparisons, Bonferroni correction was done. The odds ratio was used as the measure of the strength of association between binary variables. A P value <0.05 was accepted as significant. Statistical analyses were performed using Excel and statistical package R (http://www.r-project.org/). Clinical diagnosis subgroups were compared with age (the age at death)-matched control cases that were serially extracted from all 71 normal control cases, respectively: LOSD cases with the onset of $\geq$65 years of age (the age at death: median 79 years of age, range 72–91 years) were compared with 59 age-matched normal controls (median 76 years of age, range 73–90 years), and LOSD cases with the onset at <65 years of age (median 70 years of age, range 58–77 years) were compared with 36 age-matched normal controls (median 72 years of age, range 58–77 years). The variation in the number of normal control cases is due to this procedure.

Results

Frequencies of neurodegenerative changes in all LOSD and control cases

The pathological diagnoses of all LOSD and age-matched normal control cases are shown in Table 2 and Fig. 4. Of 23 LOSD cases, six cases (26.1 %) had LBD, five (21.7 %) had AGD, and one (4.3 %) had CBD. Two cases (8.7 %) had moderate AD pathology alone (Braak stage III–IV/0–C), and nine (39.1 %) had mild AD pathology alone (Braak stage I–II/0–C). Argyrophilic grains in all LOSD cases were almost completely restricted to the amygdala, hippocampus, and adjacent temporal cortex, corresponding to Saito's stages I–II (i.e., mild to moderate AGD). A few TDP-43-positive inclusions in the limbic region were found in two LOSD cases (one diffuse neocortical type LBD and one limbic type LBD). Representative AGD, LBD, and CBD cases that clinically exhibited LOSD are shown in Figs. 1, 2, 3. No LOSD case had pathological evidence of demyelinating diseases, neoplasms, or infections in the central nervous system. In all age-matched normal controls, eight cases (11.3 %) had LBD, and six (8.5 %) had AGD, respectively. In addition, one case (1.4 %) had moderate AD pathology alone (Braak stage III–IV), 52 (77.8 %) had minimal AD pathology alone (Braak stage 0–II), and four (5.6 %) lacked any degenerative change. No normal control case had CBD pathology. A few TDP-43-positive inclusions in the limbic region were found in one age-matched control case having Saito's stage II AGD.

None of our subjects was pathologically diagnosed as having pure AD (Braak NFT stage V–VI [44]), PSP, Pick's disease (with tau-positive Pick bodies), white matter tauopathy with globular glial inclusions [52], or frontotemporal lobar degeneration with TDP-43-positive inclusions. In addition, no case had senile dementia of the neurofibrillary tangle type (SD-NFT), a form of tangle-only dementia characterized by abundant extracellular NFTs, severe neuronal loss in the hippocampus, and no or minimal Aβ deposits [53].

A comparison of LOSD and control cases demonstrated a significant relationship between LOSD and the distribution of pathological diagnoses [$P = 0.0015$, Fisher's exact test ($\alpha/7$)]. The frequencies of AGD, LBD, and CBD in all LOSD cases tended to be higher than those in normal controls, although statistically not significantly [$P = 0.09$, 0.09, and 0.24, Fisher's exact test ($\alpha/7$)]. On the other hand, the total frequency of cases having either AGD, LBD, or CBD was significantly higher in LOSD cases than in controls [$P = 0.0037$, Fisher's exact test ($\alpha/7$)]. The frequency of cases having no or mild AD pathology alone (Braak stage 0–II) was significantly lower in LOSD cases than in control cases [$P = 0.0006$, Fisher's exact test ($\alpha/7$)], while the frequency of cases having moderate AD pathology alone (Braak stage III–IV) was not significantly different between two groups [$P = 0.15$, Fisher's exact test ($\alpha/7$)]. Odds ratio analyses demonstrated that patients who developed LOSD after the age of 40 years had a significantly increased risk of having either AGD, LBD, or CBD pathology [odds ratio 4.44, 95 % confidence interval (CI), 1.62–12.1] compared with normal controls.

The AGD stages in all LOSD cases were (versus normal controls): 75th percentile 0.5 (0); median 0 (0); and 25th percentile 0 (0). The AGD stage was significantly higher in LOSD cases than in control cases ($P = 0.0225$, Mann–Whitney *U* test). The Braak NFT stages in all LOSD cases were (versus normal controls): 75th percentile 3 (1); median 2 (1); and 25th percentile 2 (1). The Braak NFT stage was also significantly higher in all LOSD cases than that in controls ($P < 0.0001$, Mann–Whitney *U* test). The Braak stages of Aβ-positive senile

Table 2 Pathological diagnoses in all cases

Pathological diagnosis	Late-onset schizophrenia and delusional disorders ($n = 23$)	Normal controls ($n = 71$)	Other late-onset psychiatric disorders			
			Depression ($n = 11$)	Bipolar disorders ($n = 2$)	Personality disorders ($n = 6$)	Neurotic disorders ($n = 3$)
Argyrophilic grain disease						
AGD2 + NFT2-4 + SPB	2	–	–	–	–	–
AGD2 + NFT2 + SP0	1	–	1	–	–	–
AGD2 + NFT2 + SPA + TDPlim	–	1	–	–	–	–
AGD1 + NFT3 + SPB	–	1	–	–	–	–
AGD1 + NFT1-2 + SP0-A	2	4	–	–	–	–
Total (n)	5	6	1	–	–	–
Lewy body disease						
LBDdn + NFT5 + SPC + TDPlim	1	–	–	–	–	–
LBDdn + NFT5 + SP0	1	–	–	–	–	–
LBDdn + NFT1-3 + SPC	–	1	1	–	–	–
LBDlim + NFT4 + SPC + TDPlim	1	–	–	–	–	–
LBDlim + NFT1-2 + SPC	1	1	1	–	–	–
LBDbs + NFT1-2 + SPA	2	6	1	–	–	–
Total (n)	6	8	3	–	–	–
Corticobasal degeneration						
CBD + AGD3 + NFT3 + SPC	–	–	–	–	1	–
CBD + AGD1 + NFT1 + LBDbs	–	–	1	–	–	–
CBD + AGD1 + NFT2	1	–	–	–	–	–
CBD + AGD1 + NFT1	–	–	–	–	1	–
Total (n)	1	–	1	–	2	–
Mild to moderate AD pathology						
NFT3-4 + SP0-C	2	1	1	1	–	1
NFT1-2 + SPB-C	1	11	1	–	1	–
NFT1-2 + SP0-A	8	38	4	1	3	2
NFT0 + SPA-C	–	3	–	–	–	–
Total (n)	11	53	6	2	4	3
No degeneration						
Total (n)	–	4	–	–	–	–

NFT1, 2, 3, 4 and 5, Braak NFT stage I, II, III, IV, and V; SPA, B, and C, Braak senile plaque stage A, B, and C; LBDbs, brain stem type Lewy body disease; LBDlim, limbic type Lewy body disease; LBDdn, diffuse neocortical type Lewy body disease; TDPlim, limbic type TDP-43 pathology, AGD1, argyrophilic grain disease stage I; AGD2, AGD stage II, AGD3, AGD stage III; CBD, corticobasal degeneration; PSP, progressive supranuclear palsy

plaques in all LOSD cases were (versus normal controls): 75th percentile 2 (1); median 1 (1); and 25th percentile 0 (0). The Braak Aβ-positive plaque stage was not statistically different between two groups ($P = 0.83$, Mann–Whitney U test).

Frequencies of neurodegenerative changes in LOSD cases with onset age of ≥65 years

Of 11 LOSD cases with onset at ≥65 years of age (median age at death, 79 years), four cases (36.4 %) had AGD, four (36.4 %) had LBD, two (18.2 %) had moderate AD pathology (Braak stage III–IV) alone, and one (9.1 %) had minimal AD pathology (Braak stage I–II) alone (Fig. 4). In contrast, in 36 age-matched normal controls (median age at death, 76 years), three cases (8.3 %) had AGD, seven (19.4 %) had LBD, one (2.8 %) had moderate AD pathology (Braak stage III–IV) alone, and 25 (69.4 %) had minimal AD pathology (Braak stage I–II) alone.

The frequency of AGD was significantly higher in LOSD cases with onset at ≥65 years of age than in normal controls ($P = 0.0424$, Fisher's exact test). LOSD patients with onset at ≥65 years of age had a significantly increased risk of having AGD (odds ratio 6.29; 95 % CI 1.14–34.6) compared to age-matched normal controls.

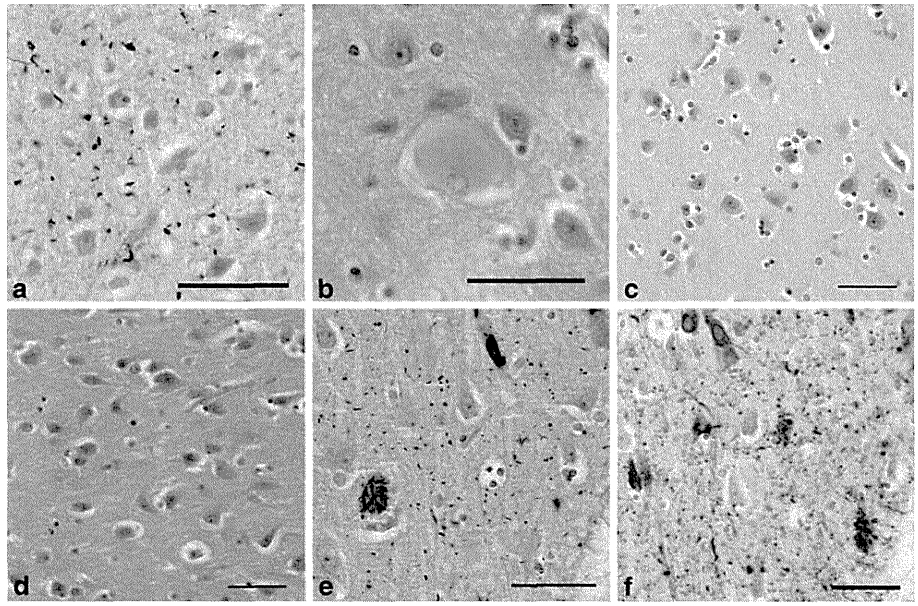

Fig. 1 Pathological findings in a representative argyrophilic grain disease case showing LOSD. Argyrophilic grain disease (AGD) seen in a 79-year-old woman who was diagnosed with senile-onset schizophrenia. She initially showed persecutory delusion and tactile hallucination at 68 years of age. Motor disturbance or dementia was absent throughout the course, and she died suddenly. The brain weighed 1,305 g. **a** Moderate argyrophilic grains in the ambient gyrus. **b** Ballooned neurons in the amygdala. **c** In contrast to the presence of argyrophilic grains, neuronal loss or gliosis is not noted in the amygdala. Pyramidal neurons in the hippocampal CA1 were well spared in number (**d**), but argyrophilic grains were densely distributed in the hippocampal CA1, corresponding to Saito's AGD stage II (**e**). **f** Tau-positive argyrophilic grains with a few neurofibrillary tangles in the hippocampus (Braak stage II). **a**, **e** Gallyas–Braak silver stain, **b**–**d** hematoxylin–eosin stain, **f** AT8 immunohistochemistry. All *scale bars* = 50 μm

Frequencies of neurodegenerative changes in LOSD cases with onset at <65 years of age

Of 12 LOSD cases with onset at <65 years of age (median age at death, 70 years), one had AGD (8.3 %), two (16.7 %) had LBD, and one (8.3 %) had CBD (Fig. 4). Of 59 age-matched normal controls (median age at death, 72 years), three (5.1 %) had AGD, and six (10.2 %) had LBD. The frequency of AGD was not significantly different between LOSD cases whose onset age was <65 years and control cases ($P = 0.53$, Fisher's exact test).

Comparison of severities of neurodegenerative changes between LOSD cases with and without dementia in last stage

The clinical data regarding the presence or absence of dementia in the last stage were available for 19 of 23 LOSD cases, and seven had dementia in the last stage. The Braak NFT stage (LOSD with vs. without dementia, median: 2 vs. 2), Braak Aβ stage (2 vs. 0.5), AGD stage (0 vs. 0), and the frequency of LBD (42.9 vs. 14.3 %) did not significantly differ between LOSD cases with and without dementia in the last stage ($P = 0.54$, 0.27, 0.33, and 0.12, Mann–Whitney U test and Fisher's exact test).

Frequencies of degenerative changes in cases of various psychiatric disorders with onset at ≥40 years of age

We additionally examined pathological changes in 22 patients who developed various psychiatric disorders other than LOSD after 40 years of age (Table 2).

AGD and LBD were found in some depression cases, but not in any case in the other clinical diagnosis groups. The frequencies of AGD (9.1 vs. 8.5 %) and LBD (27.3 vs. 11.3 %) were not statistically different between all depression cases and normal control cases, respectively [$P = 0.56$ and 0.12, Fisher's exact test (α/2), Table 2]. Of five depression patients with onset at ≥65 years of age, while no case had AGD, three had LBD. The frequency of LBD was significantly higher in depression patients whose onset age was ≥65 years of age than in normal controls ($n = 56$) (60.0 vs. 10.7 %, $P = 0.0198$, Fisher's exact test). On the other hand, of six depression patients whose onset age was <65 years of age, one had AGD and no case had LBD. The frequency of AGD in this subgroup of depression was not significantly different between LOSD and normal control cases ($n = 21$) [16.7 vs. 14.3 %, $P = 0.66$, Fisher's exact test (α/2)].

Pathological changes of CBD were found in one depression case and two cases of presenile-onset

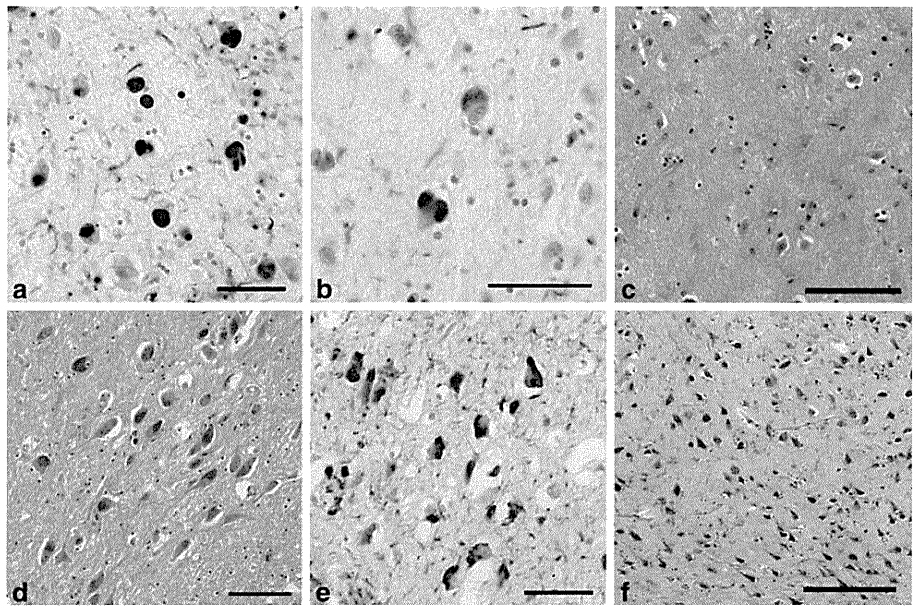

Fig. 2 Pathological findings in a representative Lewy body disease case showing LOSD. Limbic type Lewy body disease seen in a 82-year-old man diagnosed with senile-onset schizophrenia-like psychotic disorder. He initially developed delusion of observation and auditory hallucination at 72 years of age. His intelligence was mildly impaired after 77 years of age, but he did not exhibit dementia throughout the course. Although dysphasia was seen in the terminal stage, muscle rigidity or tremor was absent during the course. He died of pneumonia. The brain weighed 1,150 g. Many α-synuclein-positive Lewy bodies in the entorhinal cortex (**a**) and amygdala (**b**). **c** Moderate neuronal loss with astrocytosis in the amygdala. **d** Neurons in the substantia nigra were well spared in number. **e** Tau-positive neurofibrillary tangles (NFTs) in the hippocampal CA1. This case had NFTs in the insular cortex, corresponding to Braak NFT stage IV. **f** In contrast to NFTs, pyramidal neurons in the hippocampal CA1 were relatively well preserved in number. **a**, **b** Psyn#64 immunohistochemistry, **c**, **d** hematoxylin–eosin stain, **e** AT8 immunohistochemistry, **f** Klüver–Barrera stain. *Scale bars* = (**a**, **b**, **e**) 50 μm, (**c**, **d**, **f**) 100 μm

personality disorder. No psychiatric case had pathological evidence of demyelinating diseases, neoplasms, or infections in the central nervous system.

Comparison of vascular lesions between LOSD cases and psychiatric disease control cases

The severities of vascular lesions in the cerebral cortex in LOSD cases (versus psychiatric disease controls) were: 75th percentile 0.5 (0); median 0 (0); and 25th percentile 0 (0). The severity of vascular lesions in the cerebral cortex was not significantly different between the two groups ($P = 0.42$, Mann–Whitney U test). Likewise, the severities of vascular lesions in the basal ganglia in LOSD cases (versus psychiatric disease controls) were: 75th percentile 1 (1); median 0 (0); and 25th percentile 0 (0). The severity of vascular lesions in the basal ganglia was not significantly different between the two groups ($P = 0.78$, Mann–Whitney U test). The age at death in LOSD cases (the mean age at death 75.1 ± 7.5 years) was not significantly different from that in psychiatric disease control cases (70.5 ± 8.1 years) ($P = 0.082$, Mann–Whitney U test).

Clinical features in pathological diagnosis groups

The demographic data in four pathological diagnosis groups, i.e., AGD (AGD cases lacking LBD or CBD pathology), LBD (LBD cases lacking AGD or CBD pathology), CBD (cases having pathology of CBD), and non-degenerative disease groups (cases having minimal AD pathology of Braak stage I–II/0–A alone) are shown in Table 3. The ages of onset and death in CBD cases were about 10 years lower than those in AGD and LBD cases, respectively. Figure 5 shows the frequencies of clinical symptoms in each pathological diagnosis group. Delusion was significantly more frequent in the AGD group, and disinhibition was significantly more frequent in the CBD group than those in a non-degenerative disease group, respectively [$P = 0.0127$ and 0.0026, Fisher's exact test ($\alpha/3$)].

Discussion

To our knowledge, this is the first study that comprehensively examined the neurodegenerative bases in patients

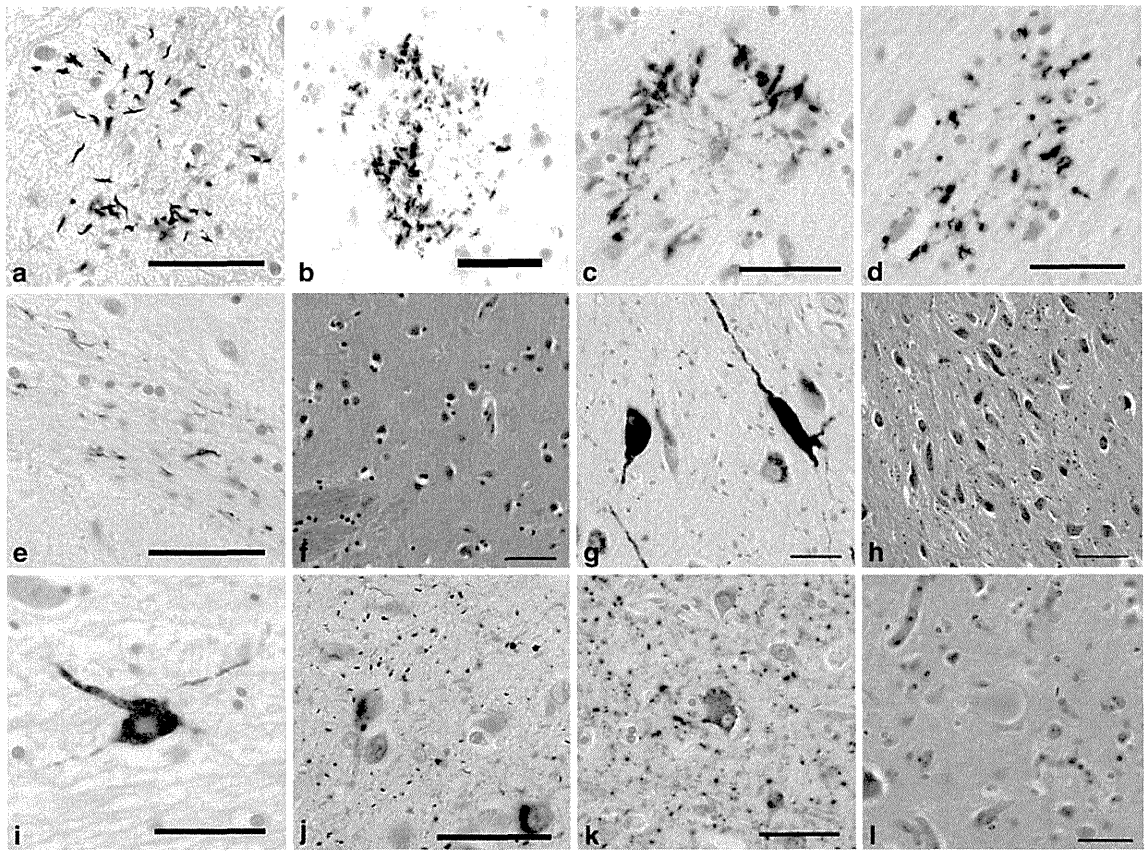

Fig. 3 Pathological findings in a representative corticobasal degeneration case showing LOSD. Corticobasal degeneration (CBD) seen in a 58-year-old man who was diagnosed with late-onset schizophrenia. His initial symptom was apathy at 41 years of age. Auditory hallucination and irritability occurred at 52 years of age. Motor disturbance, aphasia, or dementia was absent throughout the course. He died of chronic obstructive pulmonary disease. The brain weighed 1,480 g. Astrocytic plaques in the superior frontal gyrus (**a**), motor cortex (**b**), caudate nucleus (**c**), and putamen (**d**). **e** A small number of tau-positive threads were seen in the putamen. A small number of tau-positive and Gallyas-positive neurofibrillary tangles and threads were found in the frontal cortex, caudate nucleus, putamen, subthalamic nucleus, substantia nigra (**g**), oculomotor nucleus, pontine nucleus (**i**), and inferior olivary nucleus. Unlike classic CBD cases, neuronal loss with glial proliferation was not seen in the putamen (**f**) or substantia nigra (**h**). **j–l** This case also had Gallyas- and tau-positive argyrophilic grains (**j**, **k**), mild neurofibrillary tangles (Braak stage II), and ballooned neurons (**k**, **l**) in the ambient gyrus and amygdala. However, neuronal loss and gliosis were minimal in these regions (**l**). **a**, **j** Gallyas–Braak silver stain, **f**, **h**, **l** hematoxylin–eosin stain, (**b–e**, **g**, **i**, **k**) AT8 immunohistochemistry. *Scale bars* = (**a**, **c**, **d**, **e–g**, **i–l**) 50 μm, **b** 25 μm, **h** 100 μm

with LOSD and demonstrated a significant relationship between LOSD and AGD. The main findings in the present study were as follows: (1) AGD and LBD had comparably common pathological bases in our LOSD cases (21.7 and 26.1 %), frequencies about 2.5 times higher than those in normal controls (8.5 and 11.3 %). Argyrophilic grains in LOSD cases are almost completely restricted to the limbic system and adjacent temporal cortex. CBD was rarely found in LOSD but never in normal control cases. Consequently, LOSD patients who experienced onset after 40 years of age had about a fourfold increased risk of having either AGD, LBD, or CBD (odds ratio 4.44, 95 % CI 1.62–12.1) compared with normal controls. (2) AGD was significantly more frequent in LOSD patients whose onset occurred at ≥65 years of age than in normal controls, and the LOSD patients had about a sixfold increased risk of having AGD (odds ratio 6.29; 95 % CI 1.14–34.6) compared with normal controls. (3) In a psychiatric case series, the frequency of delusion in AGD cases was significantly more frequent than that in cases having minimal AD pathology alone. These findings suggest that LOSD cases may have heterogeneous pathological backgrounds, including AGD, LBD, and CBD and that mild to moderate argyrophilic grains may play an important role in the occurrence of LOSD, especially in elderly people.

It has been reported that some AGD cases with dementia show various psychiatric features, including delusion, hallucination, aggression, irritability, and obsession [31, 34–38]. However, as far as we know, there has been no study that demonstrated a significant relationship between AGD and LOSD in non-demented elderly people. In general, it is difficult to determine whether histopathological

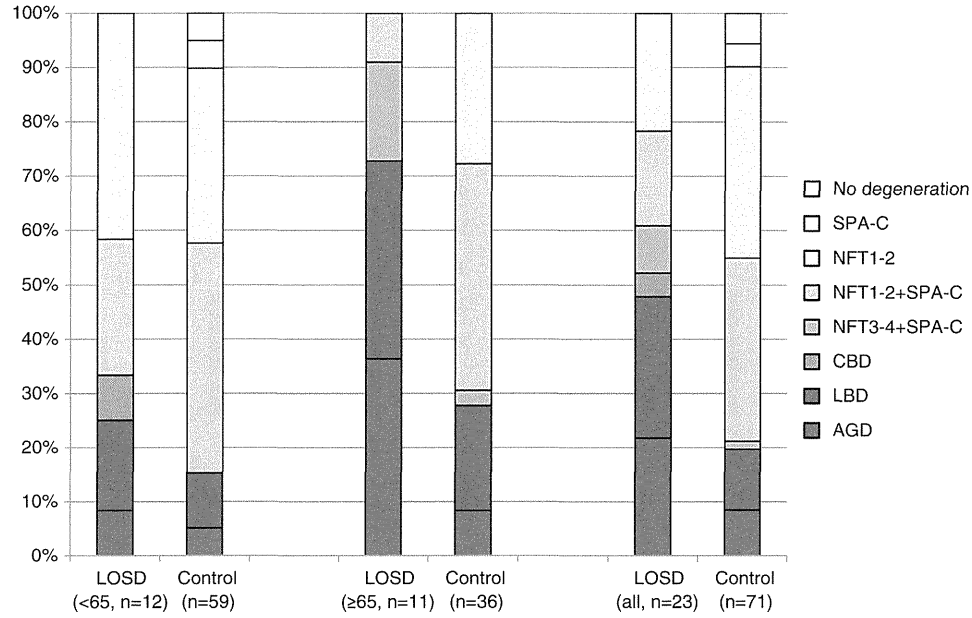

Fig. 4 Distribution of pathological diagnoses in LOSD cases and age-matched normal controls. *Right* A comparison of all LOSD and age-matched normal control cases. The frequencies of AGD and LBD in LOSD cases were about 2.5 times those in controls, and CBD was found only in LOSD cases. The total proportion of AGD, LBD, and CBD was significantly higher in LOSD cases than in controls [$P = 0.0037$, Fisher's exact test ($\alpha/7$)]. *Center* A comparison of LOSD patients ≥65 years of age at onset and age-matched normal controls. The frequency of AGD was significantly higher in LOSD cases than in controls ($P = 0.0424$, Fisher's exact test). *Left* A comparison of LOSD patients whose onset occurred at <65 years of age and age-matched normal controls. The frequency of AGD did not statistically differ between two groups (Fisher's exact test). *LOSD* late-onset schizophrenia and delusional disorders, *NFT* neurofibrillary tangles, *CBD* corticobasal degeneration, *LBD* Lewy body disease, *AGD* argyrophilic grain disease

Table 3 Demographic data by pathological diagnosis in cases with psychiatric disorders

Pathological diagnosis	*n*	Female *n* (%)	Age at onset (years) Mean ± SD	Age at death (years) Mean ± SD	Disease duration (years) Mean ± SD	Brain weight (g) Mean ± SD	Dementia in last stage *n* (%)[g]
Argyrophilic grain disease group[b]	6	4 (67.7)	67.0 ± 12.0	77.8 ± 7.3[f]	10.8 ± 5.9	1,179 ± 138.2	1/4 (25.0)
Lewy body disease group[a]	9	3 (33.3)	70.1 ± 8.3[e]	78.2 ± 5.7[e]	8.9 ± 6.1	1,273 ± 143.2	5/7 (71.4)
Corticobasal degeneration group[c]	4	1 (25.0)	60.5 ± 16.8	66.8 ± 12.4	6.3 ± 7.3	1,315 ± 124.8	2/4 (50.0)
Total	19	8 (42.1)	66.9 ± 11.6	75.7 ± 8.8	8.9 ± 6.1	1,240 ± 133.6	8/15 (53.3)
Non-degenerative group[d]	18	14 (77.8)	59.2 ± 9.4	68.3 ± 5.7	9.7 ± 9.2	1,204 ± 182.2	3/15 (20.0)

LBD Lewy body disease, *AGD* argyrophilic grain disease, *CBD* corticobasal degeneration, *PSP* progressive supranuclear palsy, *SD* standard deviation

[a] Lewy body disease (LBD) cases with variable degrees of Alzheimer's disease (AD) pathology but without argyrophilic grain disease (AGD), corticobasal degeneration (CBD), or progressive supranuclear palsy (PSP) pathology

[b] AGD cases without LBD, CBD, or PSP pathology

[c] All cases diagnosed pathologically as having CBD or PSP

[d] Cases having only minimal AD pathology (Braak NFT stage 0–II and/or Braak senile plaque stage 0–A)

[e] The onset age and age at death in the LBD group were significantly higher than those in the non-degenerative disease group, respectively [median age at onset: 71.5 vs. 61.0 years; median age at death: 77.0 vs. 69.0 years. $P = 0.0090$ and 0.00007. Mann–Whitney U test and Bonferroni correction ($P < 0.016$]

[f] The age at death in the AGD group was significantly higher than that in the non-degenerative disease group [median age at death: 76.0 vs. 69.0 years. $P = 0.0026$, Mann–Whitney U test and Bonferroni correction ($P < 0.016$)]

[g] The proportion of cases that had dementia in the last stage of the course of all subjects whose clinical data in the terminal stage was available

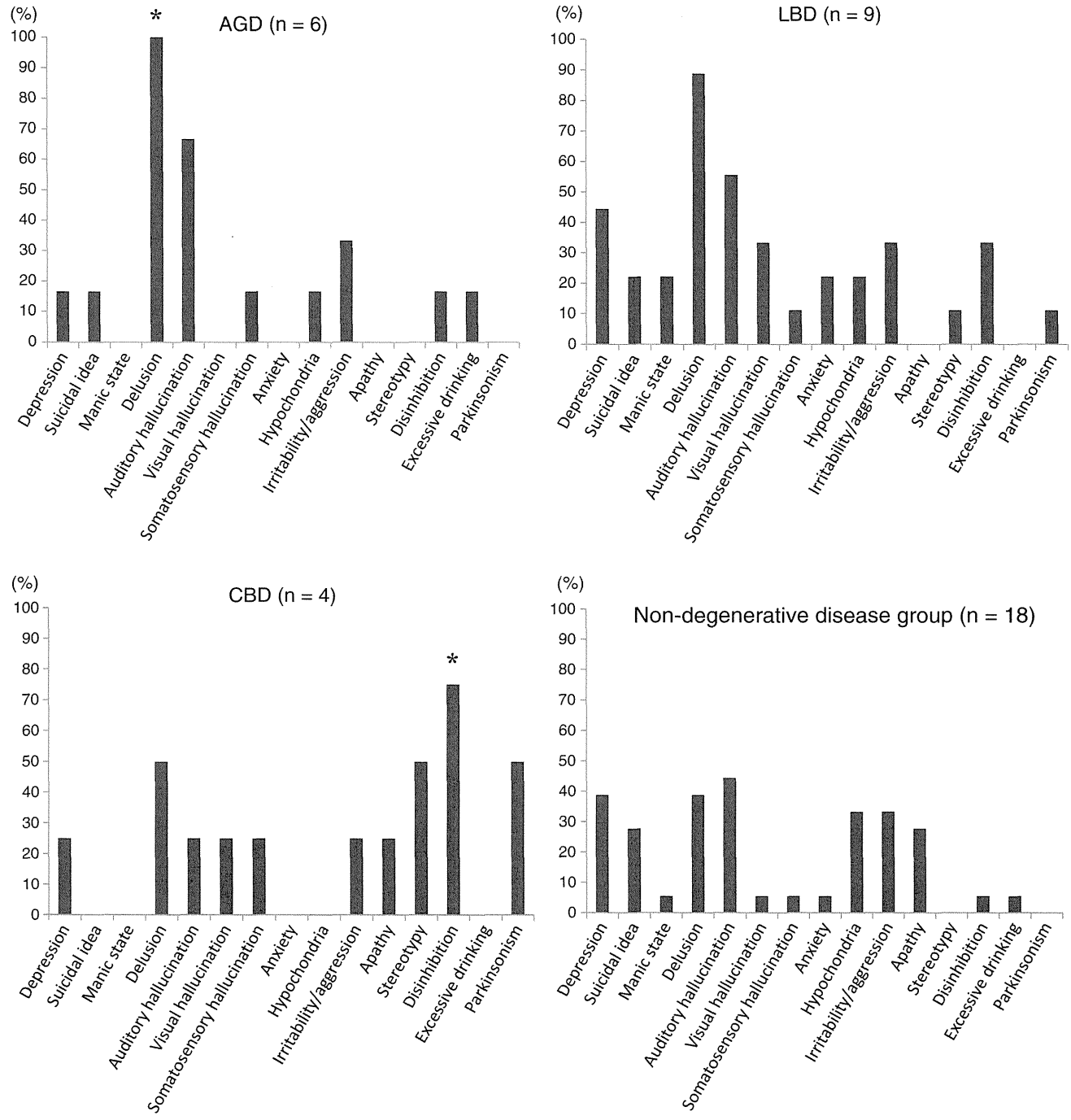

Fig. 5 Frequencies of clinical features by pathological diagnosis group. The frequencies of prominent clinical symptoms in the early stages of LBD ($n = 9$), AGD ($n = 6$), CBD ($n = 4$), and non-degenerative disease groups ($n = 18$). The frequency of delusion in the AGD group was significantly higher than those in a non-degenerative disease group [Fisher's exact test, $P = 0.0162$, Bonferroni correction ($\alpha/3$)]. The frequency of disinhibition in the CBD group was also significantly higher than that in a non-degenerative disease group [$P = 0.0026$, Fisher's exact test and Bonferroni correction ($\alpha/3$)]. See the definition of each pathological diagnosis group in the text. *LBD* Lewy body disease, *AGD* argyrophilic grain disease, *CBD* corticobasal degeneration

changes are causally related to young-onset psychiatric disorders because the initial symptoms usually occur several decades before death, and some major degenerative changes, e.g., argyrophilic grains [32], Lewy bodies [54], NFTs [55], and Aβ deposits [55], increase in frequency with age. However, our study demonstrated that the high frequency of AGD in LOSD cases may not be explained only by the age at death.

Argyrophilic grains in our LOSD cases tended to be less severe in topographical distribution (i.e., Saito's stage I–II)

compared with those in AGD cases showing dementia reported previously: some previous studies demonstrated that AGD cases with dementia frequently had argyrophilic grains more extensively distributed in the neocortex (i.e., Saito's stage III) [33]. In this context, it seems to be natural that our AGD cases lacking dementia, at least in the early to middle stage of the course, had less severe tau pathology. The impact of such mild to moderate argyrophilic grains on psychological functions, such as mood, anxiety, and thought, has hardly been explored. Our results suggest that LOSD without dementia at the onset may be one of the clinical presentations in elderly people having mild to moderate AGD. It is known that psychosis tends to occur secondarily when the limbic region and temporal cortex are involved in various diseases, such as cerebrovascular disease, traumatic brain injury, and epilepsy [56, 57]. These findings led us to consider that the occurrence of psychotic symptoms in AGD cases may be associated with the initial involvement of the limbic system by tau pathology in AGD [33].

It was reported that some LBD cases show systematized delusion [58]. The frequencies of delusion in LBD cases was reported to be 17–30 % in Parkinson's disease cases with or without dementia [59, 60] and 25–28.6 % in DLB cases [61, 62]. In our study also, although the difference did not reach statistical significance, the frequency of LBD in LOSD cases was about 2.5 times that in normal controls. Further, LBD was significantly more frequent in our depression cases with the onset at $\geq$65 years of age than in normal controls. On the other hand, AGD was not found in our depression cases. Because the number of cases examined in our study was small, whether the clinical spectrum in LBD cases is different from that in AGD cases cannot be concluded from these results. However, considering that the topographical distribution of degenerative changes, which is usually closely associated with clinical presentation, is different in AGD and LBD, it is plausible that these two degenerative diseases have different neuropsychiatric spectrums. For example, the limbic system and some brain stem nuclei (e.g., the raphe nuclei and locus coeruleus) frequently degenerate in LBD [45, 63], while argyrophilic grains consistently occur in the limbic regions but not in the brain stem nuclei. Dysfunction of the limbic system and brain stem nuclei was reported to be associated with depression [64]. Whether AGD is associated with the occurrence of depression or other psychiatric conditions should be explored by further studies using a larger sample.

In our study, although rare, some LOSD cases had CBD pathology. Several previous studies have also demonstrated that autopsy-confirmed CBD cases rarely showed psychotic symptoms [28–30, 65]. Interestingly, psychiatric symptoms occurred in all of our CBD cases younger than 65 years of age, in contrast to the relatively higher onset age in AGD and LBD cases (Table 3). Given these findings, in psychiatric practice, CBD should be considered one of the possible underlying pathologies in a patient who develops psychiatric symptoms before 65 years of age rather than after. The pathophysiological mechanism in the development of LOSD in CBD cases remains unclear. However, the coexistence of argyrophilic grains observed in all of our CBD cases might contribute at least partially to the occurrence of psychiatric symptoms.

Several previous studies, as well as this study, have consistently demonstrated that LOSD may not be associated with severe AD pathology (i.e., Braak NFT stage V–VI) [24, 25]. However, previous findings regarding the relationship between moderate NFTs in the limbic system and LOSD are not always consistent: while the severity of NFTs in the hippocampal CA1, entorhinal cortex, and temporal cortex was reported to be not significantly different between young-onset schizophrenia and LOSD cases [25], another study demonstrated that moderate NFTs distributed mainly in the limbic system and adjacent temporal cortex (Braak stage III–IV) may be associated with the development of LOSD [24]. In our study, the Braak NFT stage in LOSD cases was significantly higher than that in age-matched normal controls. However, our results cannot be simply compared with previous findings because various histological changes, including AD, LBD, AGD, and CBD pathologies, were not simultaneously evaluated in previous studies. For example, the high Braak NFT stage observed in our LOSD cases may be affected by the high proportions of LBD, AGD, and CBD cases having various severities of NFTs. Interestingly, in our LOSD cases, although the proportion of cases having only moderate NFTs (Braak stage III–IV) is not very large (8.7 %), it tends to be higher than that in normal controls (1.4 %). Considering our results together with previously reported findings, there may be LOSD patients whose onset is explained only by moderate numbers of NFTs distributed mainly in the limbic system; however, the proportion of such cases in all LOSD cases may not necessarily be large.

Limitations of our study are several. First, the sample sizes, especially those in clinical and pathological subgroups, are small. Therefore, our results may not always refute the possibility that LOSD is actually associated not only with AGD but also with LBD, CBD, and PSP. For example, in our study, although statistically not significant, the frequency of LBD in LOSD cases was over double that in age-matched normal controls (26.1 vs. 11.3 %), and CBD was found only in LOSD cases but not in age-matched normal controls. It is also known that LBD is found in some patients with paranoia [27, 59–61] and that clinically diagnosed PSP cases rarely show delusions [66]. Second, because almost all of our LOSD cases were psychiatric hospital inpatients, who probably had more severe clinical symptoms than outpatients, the case selection bias may

affect the frequency of each underlying pathology. Third, vascular lesions of the LOSD and age-matched normal control groups in this study could not be compared because the method of tissue sampling was different between these two groups. The impact of vascular changes on the occurrence of LOSD needs to be examined in the future. Fourth, in general, the immunoreactivity in tissue sections can be reduced by long fixation with formaldehyde, especially when using phosphorylation-dependent antibodies. However, in this study, AGD was explored by not only tau immunohistochemistry using phosphorylation-dependent and phosphorylation-independent anti-tau antibodies but also by Gallyas-Braak silver stain. Further, the fixation time in LOSD cases was longer than that in age-matched normal controls. Therefore, the significantly high frequency of AGD in LOSD cases observed in this study could not be explained by the effect of fixation time. Finally, whether neuronal loss and gliosis occur in AGD cases with LOSD are less severe than that in AGD cases with dementia should be also examined by further studies.

Although the present study demonstrated that LOSD patients have heterogeneous neurodegenerative backgrounds, including AGD, it may be still difficult to predict the underlying pathology in LOSD patients in life. Based on our results, biomarkers for tauopathies [67, 68] and α-synucleinopathies [67, 69], which continue to be developed for the precise clinical diagnosis of neurodegenerative dementias, might be useful to predict the pathogenic background in LOSD patients. Further clinicopathological studies are awaited to provide precise prognostic information to families based on biological findings and to develop novel therapeutic strategies for patients with LOSD.

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Conflict of interest None.

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