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Left frontal lobe hypoperfusion and depressive symptoms in Alzheimer's disease patients taking cholinesterase inhibitors

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ABSTRACT

Depressive symptoms are common in patients with Alzheimer's disease (AD) and increase the caregiver burden. Many studies have reported dorsolateral prefrontal hypometabolism or hypoperfusion in AD patients with depressive symptoms, most of whom did not take acetylcholinesterase inhibitors (AChEI). It is not clear, however, whether a similar condition is present in patients taking AChEI medication. Fifty-seven consecutive AD patients taking AChEI were recruited at a memory clinic. Objective depressive symptoms were evaluated using the depression domain of the Neuropsychiatric Inventory (NPI-dep). All patients underwent brain single photon emission computed tomography (SPECT) with ^{99m}Tc-ethylcysteinate dimer, and the SPECT images were analyzed using the Statistical Parametric Mapping 8 program. No significant differences between groups with positive and negative NPI-dep scores were found with respect to age, sex, years of education, and cognitive function. Compared with patients with negative NPI-dep scores, patients with NPI-dep scores ≥ 1 showed significant hypoperfusion in the left middle frontal region. Our results indicate that the dorsolateral prefrontal area is significantly involved in the pathogenesis of depressive symptoms in AD patients being treated with AChEI. The area on the left side especially may be closely related to the depressive symptoms evaluated using the NPI.

1. Introduction

Depressive symptoms are common in patients with Alzheimer's disease (AD) and increase the caregiver burden (Akiyama et al., 2008; Kataoka et al., 2010). Although the etiology and pathologic mechanism of depressive symptoms in AD patients remain unclear, a biological marker that objectively evaluates depressive symptoms might be useful (Kataoka et al., 2010).

There have been eight studies on regional cerebral blood flow (rCBF) in AD patients with depressive symptoms (Hirono et al., 1998; Liao et al., 2003; Holthoff et al., 2005; Lee et al., 2006; Levy-Cooperman et al., 2008; Akiyama et al., 2008; Terada et al., 2014; Honda et al., 2014). Seven of eight studies focused on the depressive symptoms as objectively estimated by observers, and only one dealt with subjective depressive signs scored by the patients themselves (Honda et al., 2014). Four of those studies identified left dorsolateral prefrontal hypometabolism or hypoperfusion in AD patients with depressive symptoms (Holthoff et al., 2005; Akiyama et al., 2008; Terada et al., 2014; Honda et al., 2014), two reported bilateral dorsolateral prefrontal hypometabolisim or hypoperfusion (Hirono et al., 1998; Levy-Cooperman et al., 2008), and one study showed right dorsolateral prefrontal hypometabolism (Lee et al., 2006). Only one report did not find a dorsolateral prefrontal abnormality in depressed AD patients (Liao et al., 2003). Besides the dorsolateral prefrontal area, hypometabolism or hypoperfusion in the anterior cingulate was reported in two of the eight studies (Hirono et al., 1998; Liao et al., 2003).

As stated above, many studies on the functional imaging of AD patients with depressive symptoms have been reported in the medical literature. However, six of the eight reports excluded patients who took acetylcholinesterase inhibitors (AChEI), and the other two included some patients taking AChEI. Holthoff et al. (2005) included nine subjects taking AChEI among 53, and Levy-Cooperman et al. (2008) studied 18 subjects taking AChEI among 56. In the real world, most AD patients are treated with AChEI, but it is not clear whether a similar relationship between depressive symptoms and rCBF is present in patients being treated with AChEI medication. Therefore, in this study, we tried to clarify the relationship between depressive symptoms and rCBF in AD patients treated with AChEI.

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2. Methods

2.1. Ethics

This study was approved by the Internal Ethical Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences. After a complete description of the study to the subjects and their relatives, written informed consent was obtained.

2.2. Subjects

Fifty-seven consecutive patients with AD were recruited from the outpatient units of the Memory Clinic of Okayama University Hospital between September 2008 and April 2012 according to the following criteria. They all (1) underwent general physical and neurological examinations and extensive laboratory testing, including thyroid function tests, serum vitamin B12, and syphilis serology; (2) were evaluated with the revised Addenbrooke's Cognitive Examination (ACE-R; Yoshida et al., 2012), Mini-Mental State Examination (MMSE; Folstein et al., 1975), Frontal Assessment Battery (FAB; Kugo et al., 2007), and Geriatric Depression Scale (GDS; Muraoka et al., 1996); (3) underwent single photon emission computed tomography (SPECT) with ^{99m}Tc-ethylcysteinate dimer of the brain as well as magnetic resonance imaging (MRI) or computed tomography (CT) of the head; (4) were diagnosed with probable AD according to the criteria formulated by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984); (5) were judged by the chief clinician to have the ability to answer the GDS questions accurately; (6) were being treated with cholinesterase inhibitors. The exclusion criteria were (1) complications from other neurological diseases or illnesses; (2) history of mental illness or substance abuse before the onset of dementia; (3) evidence of focal brain lesions on head MRI or CT; (4) current treatment with memantine, antipsychotics, antidepressants, or anxiolytic drugs; and (5) left handedness or ambidexterity. The history of mental illness or substance abuse was ascertained from a family caregiver.

The profile of each patient (age, sex, months of disease duration, and years of education) was obtained. Scores on the Neuropsychiatric Inventory (NPI), Barthel Index of Activities of Daily Living (ADL), and Functional Assessment Questionnaire (FAQ) were calculated by a trained clinical psychologist based on information from family caregivers. The Clinical Dementia Rating (CDR) (Hughes et al., 1982) score was rated by the chief clinician.

This study included only patients who took cholinesterase inhibitors. Therefore, no patients in this project were included in our previous reports (Honda et al., 2014; Terada et al., 2014).

2.3. Instruments

The NPI is a valid and reliable instrument for measuring non-cognitive symptoms in dementia (Cummings et al., 1994; Hirono et al., 1997). It is a caregiver-based tool that assesses 10 different domains in dementia. The NPI gives a composite score for each domain, which is the product of frequency multiplied by severity subscores: scores from 1 to 4 (with 4 being the most frequent) for the frequency and from 1 to 3 (with 3 being the most severe) for the severity of each behavior (Akiyama et al., 2008). The maximum attainable score was 12. In this study, subjects were divided into two groups according to the NPI depression scores: an NPI-dep (+) group with an NPI-depression score ≥ 1 and an NPI-dep (−) group with an NPI depression score of 0.

The GDS, which has been extensively used in the fields of psychiatry and public health, was used to assess depression in the elderly (Yesavage and Blink, 1983). The GDS has two different versions: a standard version with 30 items and a shortened version with 15 items. For the present study, the short 15-item version of the GDS was administered (Muraoka et al., 1996). A higher score means more subjective depressive signs.

The ACE-R was developed to provide a brief test sensitive to early stage dementia, and is capable of differentiating between dementia subtypes including AD, frontotemporal dementia, progressive supranuclear palsy, and other parkinsonian syndromes (Mioshi et al., 2006). The ACE-R includes the MMSE, but extends it to encompass important areas not covered by the MMSE, such as frontal-executive function and visuospatial skills. For this study, we used the Japanese version of the ACE-R described by Yoshida et al. (2012).

The FAB consists of six items, and the score on each item ranges from 0 to 3. A lower score indicates a greater degree of executive dysfunction. The six subtests of the FAB explore (1) similarities (conceptualization), (2) lexical fluency (mental flexibility), (3) Luria motor sequences (programming), (4) conflicting instructions (sensitivity to interference), (5) a go/no-go test (inhibitory control), and (6) prehension behavior (environmental autonomy). For this study, we used the Japanese version of the FAB described elsewhere (Dubois et al., 2000; Kugo et al., 2007).

The Barthel Index consists of 10 items that measure a person's daily functioning, specifically the activities of daily living and mobility (Wade and Collin, 1988). The total Barthel Index score ranges from 0 to 100. A higher score indicates a better performance. The Functional Assessment Questionnaire (FAQ) measures functional activities of older adults using the patient's partner as an informant (Pfeffer et al., 1982). The FAQ consists of 10 items, and the score on each item ranges from 0 to 3. A higher score indicates more severe impairment.

2.4. Brain perfusion SPECT imaging

All subjects were examined by brain perfusion SPECT. Patients were examined in a comfortable supine position with their eyes closed in quiet surroundings. Ten minutes after intravenous administration of ^{99m}Tc-ethylcysteinate dimer (ECD, 600 MBq, Daiichi Radioisotope Laboratories Ltd., Tokyo, Japan), SPECT images were obtained using a triple-headed, rotating gamma camera interfaced to a minicomputer (GCA9300A/DI; Toshiba, Tokyo, Japan) equipped with a fanbeam, low-energy, high-resolution collimator. Sixty projection images over a 360° angle in a 128×128 matrix were acquired. All images were reconstructed using ramp-filtered back-projection and then three-dimensionally smoothed with a Butterworth filter (order 8, cutoff 0.12 cycles/cm). The reconstructed images were corrected for gamma ray attenuation using the Chang method ($\mu = 0.09$).

2.5. Data analysis

Spatial reprocessing and statistical analysis of images were performed on a voxel-by-voxel basis using Statistical Parametric Mapping 8 (SPM8, Wellcome Department of Imaging Neuroscience, University College, London, UK) running on MATLAB (The Mathworks, Inc., Natick, MA, USA). All SPECT images of each subject were normalized to the standard brain of the Montreal Neurological Institute (MNI), and spatial normalization was performed with 12-parameter affine and non-linear transformations (Friston et al., 1995). The voxel sizes of the reslice option were 2 mm × 2 mm × 2 mm. The non-linear parameters were set at 25 mm cut-off basis functions and 16 iterations. All the normalized SPECT images were then smoothed with an isotropic Gaussian kernel filter (12 mm full-width at half-maximum).

To examine the images for specific regions showing differences in perfusion, two-sample *t*-tests were performed. Global normalization was performed by proportional scaling with the mean voxel value. Masking was applied using the threshold method (0.8 times the global value). The analysis used a threshold of $p < 0.001$ (uncorrected) at the voxel level, and results were considered significant at 100 voxels at the cluster level.

2.6. Statistical analysis

Statistical analysis of images was performed on a voxel-by-voxel basis using SPM8. The detailed method has been described in Section 2.5. Other statistical analyses were performed using the SPSS 19.0J software program (SPSS Inc., Chicago, IL). Comparisons between two groups were performed by independent sample *t*-tests. A value of $p < 0.05$ was accepted as significant.

3. Results

3.1. Demographic characteristics

Demographic characteristics are shown in Table 1. Among the 57 AD patients, 39 were women and 18 were men. For dementia severity, 33 patients had CDR scores of 0.5, 20 had CDR scores of 1, and four had CDR scores of 2. On the NPI-depression scale, 34 patients had a score of 0, eight patients scored 1, eight patients scored 2, five patients scored 3, one patient scored 4, and one patient had a score of 6. For GDS scores, six patients scored 0, 31 scored from 1 to 5, 18 patients scored from 6 to 10, and two patients scored 11 or more.

Comparison between NPI-dep (+) and (−) groups showed that there were no significant differences in sex ratio, age, education, disease duration, cognition test scores, or ADL scores (Tables 1 and 2). The patients of the NPI-dep (+) group had higher scores on the GDS and on the irritability subscales of NPI than those of the NPI-dep (−) group (Table 2).

3.2. rCBF

A group comparison between the SPM results of the NPI-dep (+) and (−) groups was performed. Specific voxels with significantly lower perfusion in the NPI-dep (+) group than in the NPI-dep (−)

Table 1
Clinical characteristics and neuropsychological tests. Please check the head columns also.

	Not depressed	Depressed	t	p
Total (*n*)	34	23		
Sex (male/female)	10/24	8/15	0.669 (♯)	0.443
Age (mean years ± S.D.)	76.0 ± 6.0	76.0 ± 6.2	0.026	0.979
Education (mean years ± S.D.)	11.2 ± 2.1	10.7 ± 3.0	0.772	0.443
Duration (mean years ± S.D.)	39.5 ± 26.0	38.4 ± 17.9	0.173	0.863
ACE-R score	63.5 ± 14.8	64.5 ± 11.9	−0.264	0.793
Attention	13.2 ± 3.5	13.0 ± 2.9	0.267	0.790
Memory	9.3 ± 5.3	8.8 ± 5.0	0.316	0.754
Fluency	6.9 ± 3.3	6.5 ± 2.3	0.486	0.629
Language	20.8 ± 4.5	22.1 ± 4.0	−1.181	0.243
Visuospatial	13.3 ± 3.3	14.0 ± 2.2	−0.871	0.388
MMSE score	20.7 ± 4.8	21.0 ± 3.9	−0.261	0.795
FAB score	11.3 ± 3.3	11.5 ± 2.4	−0.319	0.751

Test scores, mean ± S.D.; S.D., standard deviation; Duration, disease duration.
ACE-R, Revised Addenbrooke's Cognitive Examination.
MMSE, Mini Mental State Examination; FAB, Frontal Assessment Battery.
♯, χ^2.

Table 2
Instrumental ADL and BPSD.

Group	Not dep	Dep	t	p
Total (*n*)	34	23		
FAQ score	14.0 ± 9.2	14.6 ± 7.1	−0.269	0.789
Barthel index	96.3 ± 8.9	96.7 ± 5.4	−0.200	0.842
GDS score	2.3 ± 2.4	5.8 ± 3.5	−3.281	0.002
NPI	4.0 ± 4.9	8.7 ± 7.5	−2.841	0.006
Delusion	0.5 ± 1.6	0.9 ± 1.8	−0.815	0.419
Hallucination	0.3 ± 0.7	0.2 ± 0.7	0.490	0.626
Agitation/aggression	0.1 ± 1.3	0.7 ± 1.6	−1.968	0.054
Depression	0	2.1 ± 1.2	−10.248	< 0.001
Anxiety	0.7 ± 2.4	0.4 ± 1.0	0.527	0.600
Euphoria	0	0	0	1.000
Apathy	1.3 ± 1.7	1.9 ± 2.2	−1.277	0.207
Disinhibition	0.3 ± 0.7	0.3 ± 0.9	0.090	0.929
Irritability	0.2 ± 0.5	0.7 ± 1.1	−2.293	0.026
Aberrant motor behavior	0.7 ± 2.5	1.5 ± 3.0	−1.162	0.250

Test scores, mean ± S.D.; S.D., standard deviation.
FAQ, Functional Assessment Questionnaire; ADL, Activities of Daily Living.
GDS, Geriatric Depression Scale; NPI, Neuropsychiatric Inventory.

group are shown in Fig. 1. No voxels with a significantly lower perfusion in the NPI-dep (−) group than in the NPI-dep (+) group were found.

Fig. 1 shows the *z* score for each voxel in this cluster superimposed on a three-way glass brain view. Fig. 1 shows a significant cluster of voxels in the middle frontal lobe in the left side (Brodmann area 6). Table 3 shows the probability results of the SPM analysis and the location of peak *z* scores in terms of MNI coordinates.

4. Discussion

We found a significant decrease of rCBF in the left middle frontal lobe of mild AD patients with depressive symptoms compared with mild AD patients without depressive symptoms under AChEI treatment. The results are in line with previous reports that found a significant decrease of rCBF in the left prefrontal dorsolateral areas in depressive AD patients not being treated with AChEI (Holthoff et al., 2005; Akiyama et al., 2008; Terada et al., 2014). The middle frontal gyrus is located in the dorsolateral prefrontal cortex (DLPFC), and the DLPFC circuit is one of the prefrontal-subcortical circuits, some of which are involved in the regulation of affect (Peng et al., 2012).

It is difficult to interpret the laterality of right or left dominance in depressive AD patients. In general, depression is reported to show perfusion abnormalities located in either frontal lobe. Left frontal damage has been commonly associated with depressive mood, namely feelings of despair and hopelessness (Levy-Cooperman et al., 2008). Meanwhile, patients with depression related to lesions in the right frontal area have more psychological symptoms, namely irritability, loss of interest, and difficulty in concentration (Levy-Cooperman et al., 2008). In the three reports controlling for the effect of coexisting apathy and anxiety, a decrease of rCBF in the left prefrontal area of depressed AD patients was found, and the NPI was used to estimate the depressive signs (Holthoff et al., 2005; Akiyama et al., 2008; Terada et al., 2014). Meanwhile, in the reports showing a bilateral or right prefrontal decrease, coexisting apathy and anxiety were not reported (Hirono et al., 1998; Liao et al., 2003; Lee et al., 2006; Levy-Cooperman et al., 2008), and various scales other than the NPI were used to measure depressive signs except for the study of Hirono et al. (Liao et al., 2003; Lee et al., 2006; Levy-Cooperman et al., 2008). AD patients with apathy symptoms were reported to show lower rCBF in the right superior or right inferior and middle frontal gyrus (Benoit et al., 2002; Lanctôt et al., 2007; Kang et al., 2012). Coexisting apathy and depressive symptoms might lead to lower rCBF in the right prefrontal areas of depressive AD patients.

The anterior cingulate gyrus is one of the neural circuits that connect the frontal lobe and subcortical structures, and dysfunction of these circuits is associated with many psychiatric, including depression (Liao et al., 2003). Hirono et al. (1998) reported that the depression score significantly correlated with glucose hypometabolism in the left anterior cingulate cortex as well as the bilateral superior frontal cortices among 53 AD patients. Liao et al. (2003) reported that depressed AD patients ($n=8$) had hypoperfusion in the bilateral anterior and posterior cingulate gyri and precuneus, compared with non-depressed AD patients ($n=35$). Neither study excluded the effect of coexisting apathy. AD patients with apathy are often reported to show hypoperfusion in the anterior cingulate compared with those without apathy (Benoit et al., 2002; Lanctôt et al., 2007; Kang et al., 2012). The mean MMSE score of AD patients in the study of Liao et al. was 12.9, while the mean MMSE score in most other studies was over 20 (Hirono et al., 1998; Holthoff et al., 2005; Levy-Cooperman et al., 2008; Terada et al., 2014; Honda et al., 2014). The effect of coexisting apathy and the difference in dementia severity might contribute to the different findings reported.

In the period between September 2008 and April 2012, we examined 79 AD patients not taking cholinesterase inhibitors and 57 AD patients taking cholinesterase inhibitors at the Memory Clinic of Okayama University Hospital. Twenty-eight (35.4%) of 79 patients not taking AChEI and 23 (40.4%) of 57 patients taking AChEI showed NPI-dep scores ≥ 1. The proportions of patients with NPI-dep scores >1 were not significantly different between cholinesterase positive and negative groups ($\chi^2=0.340$, $p=0.560$).

AChEI medication is usually prescribed to alleviate the cognitive dysfunction of AD patients. Treatment with AChEI has been reported to increase rCBF perfusion in AD patients, especially in the frontal areas (Tateno et al., 2008; Chaudhary et al., 2013). Irrespective of an increase of cortical perfusion due to AChEI treatment, a similar mechanism may produce depressive symptoms in both patients with and without AChEI. Based on the above, we suppose that the dorsolateral prefrontal area is significantly related to depressive symptoms in AD and that depressive symptoms in AD are due to a specific pathogenesis rather than a reactive phenomenon (Liao et al., 2003).

This study has limitations. First, we compared rCBF of two groups, namely NPI-dep (+) and (−) groups, but did not examine the relationship between the severity of depressive symptoms and rCBF. Secondly, we examined the AD outpatients at one memory

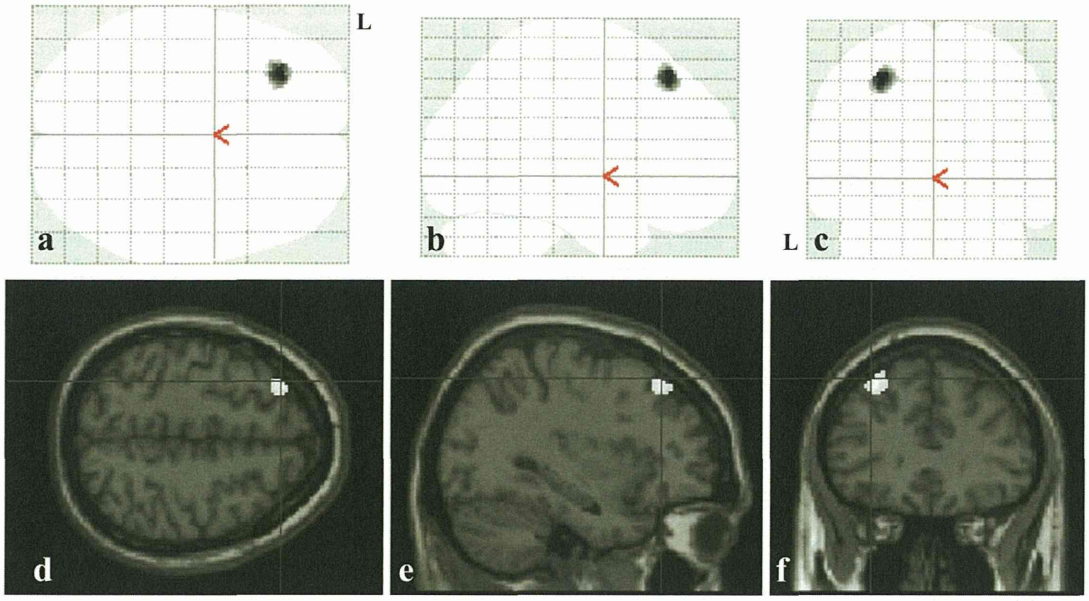

Fig. 1. (a–f) SPM (*z*) map of rCBF decrease in AD patients with NPI-depression scores ≥ 1 compared with AD patients with negative NPI-depression scores. (a–c) Three-way glass view of the area of significant hypoperfusion. (d–f) Superimposed on head MRI T1 weighed images: (d) $x=-32$; (e) $y=32$; and (f) $z=52$; L—left.

Table 3
Significant regional uptake differences between patients with NPI-dep score ≥ 1 and NPI-dep score=0 in AD taking AchE-I.

Direction of difference	Number of voxels	Peak *Z* scores	*p*	Coordinates (MNI)			Anatomical location
				x	*y*	*z*	
Decreased uptake in AD patients with NPI-dep score ≥ 1	125	4.11	< 0.001	−32	32	52	lt mid frontal

MNI, Montreal Neurological Institute; NPI-dep score, neuropsychiatric inventory-depression score.
AD, Alzheimer's disease patients; AchE-I, acetylcholine esterase inhibitors lt, left; mid, middle; frontal, frontal lobe.

clinic of a university hospital. Therefore, we cannot exclude possible selection bias. Thirdly, irritability in depressed subjects was significantly more frequent than in non-depressed subjects, and this also seems to be the case for aggression/agitation with a tendency towards significance. It is possible that the trend-level differences in irritability and/or aggression/agitation might have affected the results. However, irrespective of those limitations, this is the first study showing left dorsolateral prefrontal hypoperfusion among AD patients with depressive symptoms under AChEI medication.

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References

Akiyama, H., Hashimoto, H., Kawabe, J., Higashiyama, S., Kai, T., Kataoka, K., Shimada, A., Inoue, K., Shiomi, S., Kiriike, N., 2008. The relationship between depressive symptoms and prefrontal hypoperfusion demonstrated by eZIS in patients with DAT. Neuroscience Letters 441, 328–331. http://dx.doi.org/10.1016/j.neulet.2008.06.053.

Benoit, M., Koulibaly, P.M., Migneco, O., Darcourt, J., Pringuey, D.J., Robert, P.H., 2002. Brain perfusion in Alzheimer's disease with and without apathy: a SPECT study with statistical parametric mapping analysis. Psychiatry Research: Neuroimaging 114, 103–111. http://dx.doi.org/10.1016/S0925-4927(02)00003-3.

Chaudhary, S., Scouten, A., Schwindt, G., Janik, R., Lee, W., Sled, J.G., Black, S.E., Stefanovic, B., 2013. Hemodynamic effects of cholinesterase inhibition in mild Alzheimer's disease. Journal of Magnetic Resonance Imaging 38, 26–35. http://dx.doi.org/10.1002/jmri.23967.

Cummings, J.L., Mega, M., Gray, K., Rosenberg-Thompson, S., Carusi, D.A., Gornbein, J., 1994. The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. Neurology 44, 2308–2314.

Dubois, B., Slachevsky, A., Litvan, I., Pillon, B., 2000. The FAB: a frontal assessment battery at bedside. Neurology 55, 1621–1626.

Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. Mini-mental state. A practical method for grading the cognitive state of patients for the clinician. Journal of Psychiatric Research 12, 189–198.

Friston, K.J., Ashburner, J., Frith, C.D., Poline, J.B., Heather, J.D., Frackowiak, R.S.J., 1995. Spatial realignment and normalization of images. Human Brain Mapping 3, 165–189.

Hirono, N., Mori, E., Ikejiri, Y., Imamura, T., Shimomura, T., Hashimoto, M., Yamashita, H., Ikeda, M., 1997. Japanese version of the Neuropsychiatric Inventory: a scoring system for neuropsychiatric disturbance in dementia patients. Brain and Nerve 49, 266–271 (in Japanese with English abstract).

Hirono, N., Mori, E., Ishii, K., Ikejiri, Y., Imamura, T., Shimomura, T., Hashimoto, M., Yamashita, H., Sasaki, M., 1998. Frontal lobe hypometabolism and depression in Alzheimer's disease. Neurology 50, 380–383.

Holthoff, V.A., Beuthien-Baumann, B., Kalbe, E., Lüdecke, S., Lenz, O., Zündorf, G., Spirling, S., Schierz, K., Winiecki, P., Sorbi, S., Herholz, K., 2005. Regional cerebral metabolism in early Alzheimer's disease with clinically significant apathy or depression. Biological Psychiatry 57, 412–421. http://dx.doi.org/10.1016/j.biopsych.2004.11.035.

Honda, H., Terada, S., Sato, S., Oshima, E., Ikeda, C., Nagao, S., Yokota, O., Uchitomi, Y., 2014. Subjective depressive mood and regional cerebral blood flow in mild Alzheimer's disease. International Psychogeriatrics 26 (5), 817–823.

Hughes, C.P., Berg, L., Danziger, W.L., Coben, L.A., Martin, R.L., 1982. A new clinical scale for the staging of dementia. British Journal of Psychiatry 140, 566–572.

Kang, J.Y., Lee, J.S., Kang, H., Lee, H.W., Kim, Y.K., Jeon, H.J., Chung, J.K., Lee, M.C., Cho, M.J., Lee, D.S., 2012. Regional cerebral blood flow abnormalities associated with apathy and depression in Alzheimer disease. Alzheimer Disease and Associated Disorders 26, 217–224. http://dx.doi.org/10.1097/WAD.0b013e318231e5fc.

Kataoka, K., Hashimoto, H., Kawabe, J., Higashiyama, S., Akiyama, H., Shimada, A., Kai, T., Inoue, K., Shiomi, S., Kiriike, N., 2010. Frontal hypoperfusion in depressed patients with dementia of Alzheimer type demonstrated on 3DSRT. Psychiatry and Clinical Neuroscience 64, 293–298. http://dx.doi.org/10.1111/j.1440-1819.2010.02083.x.

Kugo, A., Terada, S., Ata, T., Ido, Y., Kado, Y., Ishihara, T., Hikiji, M., Fujisawa, Y., Sasaki, K., Kuroda, S., 2007. Japanese version of the frontal assessment battery for dementia. Psychiatry Research 153, 69–75. http://dx.doi.org/10.1016/j.psychres.2006.04.004.

Lanctôt, K.L., Moosa, S., Herrmann, N., Leibovitch, F.S., Rothenburg, L., Cotter, A., Black, S.E., 2007. A SPECT study of apathy in Alzheimer's disease. Dementia and Geriatric Cognitive Disorders 24, 65–72. http://dx.doi.org/10.1159/000103633.

Lee, D.Y., Choo, I.H., Jhoo, J.H., Kim, K.W., Youn, J.C., Lee, D.S., Kang, E.J., Lee, J.S., Kang, W.J., Woo, J.I., 2006. Frontal dysfunction underlies depressive syndrome in Alzheimer disease: a FDG-PET study. American Journal of Geriatric Psychiatry 14, 625–628. http://dx.doi.org/10.1097/01.JGP.0000214541.79965.2d.

Levy-Cooperman, N., Burhan, A.M., Rafi-Tari, S., Kusano, M., Ramirez, J., Caldwell, C., Black, S.E., 2008. Frontal lobe hypoperfusion and depressive symptoms in Alzheimer disease. Journal of Psychiatry and Neuroscience 33, 218–226.

Liao, Y.C., Liu, R.S., Lee, Y.C., Sun, C.M., Liu, C.Y., Wang, P.S., Wang, P.N., Liu, H.C., 2003. Selective hypoperfusion of anterior cingulate gyrus in depressed AD patients: a brain SPECT finding by statistical parametric mapping. Dementia and Geriatric Cognitive Disorders 16, 238–244. http://dx.doi.org/10.1159/000072808.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., Stadlan, E.M., 1984. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34, 939–944.

Mioshi, E., Dawson, K., Mitchell, J., Arnold, R., Hodges, J.R., 2006. The Addenbrooke's Cognitive Examination Revised (ACE-R): a brief cognitive test battery for dementia screening. International Journal of Geriatric Psychiatry 21, 1078–1085. http://dx.doi.org/10.1002/gps.1610.

Muraoka, Y., Ikuchi, S., Ihara, K., 1996. The physical, psychological and social background factors of elderly depression in the community. Japanese Journal of Geriatric Psychiatry 7, 397–407 (in Japanese with English abstract).

Peng, H., Zheng, H., Li, L., Liu, J., Zhang, Y., Shan, B., Zhang, L., Yin, Y., Liu, J., Li, W., Zhou, J., Li, Z., Yang, H., Zhang, Z., 2012. High-frequency rTMS treatment increases white matter FA in the left middle frontal gyrus in young patients with treatment-resistant depression. Journal of Affective Disorders 136, 249–257. http://dx.doi.org/10.1016/j.jad.2011.12.006.

Pfeffer, R.I., Kurosaki, T.T., Harrah, C.H., Chance, J.M., Filos, S., 1982. Measurement of the functional activities in older adults in the community. Journal of Gerontology 37, 323–329.

Tateno, M., Kobayashi, S., Utsumi, K., Morii, H., Fujii, K., 2008. Quantitative analysis of the effects of donepezil on regional cerebral blood flow in Alzheimer's disease by using an automated program, 3DSRT. Neuroradiology 50, 723–727. http://dx.doi.org/10.1007/s00234-008-0401-y.

Terada, S., Oshima, E., Sato, S., Ikeda, C., Nagao, S., Hayashi, S., Hayashibara, C., Yokota, O., Uchitomi, Y., 2014. Depressive symptoms and regional cerebral blood flow in Alzheimer's disease. Psychiatry Research: Neuroimaging 221, 86–91. http://dx.doi.org/10.1016/j.pscychresns.2013.11.002.

Wade, D.T., Collin, C., 1988. The Barthel ADL Index: a standard measure of physical disability? International Disability Studies 10, 64–67.

Yesavage, J.A., Blink, T.L., 1983. Development and validation of a geriatric depression scale: a preliminary report. Journal of Psychiatric Research 17, 37–49.

Yoshida, H., Terada, S., Honda, H., Kishimoto, Y., Takeda, N., Oshima, E., Hirayama, K., Yokota, O., Uchitomi, Y., 2012. Validation of the revised Addenbrooke's Cognitive Examination (ACE-R) for detecting mild cognitive impairment and dementia in a Japanese population. International Psychogeriatrics 24, 28–37. http://dx.doi.org/10.1017/S1041610211001190.

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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

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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

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