TABLE 1. Neuropsychiatric Symptoms in "Pure" AGD Cases

					Neuropsychiatric Symptoms[a]									
Case #	Age at Onset	Sex	Duration	Amnesia	Delusions	Hallucinations	Agitation	Dysphoria	Anxiety	Euphoria	Apathy	Disinhibition	Irritability	Aberrant Motor Behavior
1	74	M	4	*	1		2	2			*	2	2	2
2	84	F	8	*	1			*			*		*	
3	87	F	4	*				2					2	2
4	86	F	5	*				2			2		2	
5	75	F	2	*										
6	80	F	10	*	*		*	*					*	
7	80	M	3		3		*					*	*	*
8	79	M	9	3	3			*			*			7
9	90	F	7	5	*	*	*						*	5
10	87	F	6	*	*		*				1		*	

Note: Age at Onset and Duration are in years. * initial symptom. [a] year of onset after initial symptom.

were not applicable for all patients. The mean total ADL (activities of daily living) score in the 10 cases of pure-AGD was 86.4 (SD: 16.6), indicating well-preserved functional abilities to perform activities of daily living. Regarding each domain, the mean basic ADL score was 88.8 (SD: 13.1), and the mean Instrumental ADL score was 83.1 (SD: 20.2). The difference between basic and instrumental ADL scores was not significant, although the Basic ADL scores tended to be higher than the Instrumental ADL scores ($t = 2.17$; $p = 0.06$).

Neuroimaging and Brain Weight

The radial width of the temporal horn was measured by CT in 10 patients with pure-AGD and 107 patients with AD. Admission CT scan was used for this analysis. CT was not available for six patients with AD. The mean age in the 10 cases of AGD was 86.2 years (SD: 6.3), whereas the mean age in the 107 cases with AD was 80.7 years (SD: 8.2); this difference was significant ($t = 2.53$; $p = 0.01$). The mean radial width of the temporal horn was 5.75 mm (SD: 0.54) in the pure-AGD group, whereas the mean was 6.26 mm (SD: 0.75) in AD, showing a significant difference between the two groups ($t = -2.09$; $p = 0.04$). These findings indicate that enlargement of the temporal horn owing to shrinkage of the hippocampus is milder in AGD than in AD, despite the higher age in AGD. Neuropathologically, the mean brain weight of the 10 cases with pure-AGD was 1,128 g, as opposed to the 1,066 g observed in AD cases, showing a trend toward greater weight in AGD cases ($F_{[1]} = 3.42$; $p = 0.07$), after controlling for sex.

Case Report. Although there appears to be considerable variability in the clinical course of the pure-AGD patients, there appear to be several key findings that clinically characterize AGD. We present here a brief individual case report of a patient with pure-AGD and her pathology, which we consider to highlight the key findings.

Case 10: A woman with a 6-year curriculum of school education developed delusions of persecution and amnesia at the age of 87. She distrusted and got on badly with her neighbors. She was also disoriented and sometimes lost her way when she took a walk. These symptoms worsened, and she became irritable and often angry with her family. She could read, write, and speak well, and needed little assistance with basic activities of daily living. One year later, she was admitted to a nursing home because of the persistence of these neuropsychiatric symptoms. She often ran away from the home on impulse, and she was hospitalized at Fukushimura Hospital at the age of 88. On admission, she was disoriented to time, and but not place and person. Memory disturbance, especially of immediate and recent memory, was remarkable. Her abilities in calculation, naming, and verbal fluency (she could name 9 animals in 30 seconds) were relatively well preserved, and her MMSE score was 22/30. She could eat, dress, and use the toilet without assistance, but needed some assistance with bathing. Her Basic ADL score was 94.1, and Instrumental ADL score was 93.8, suggesting that functional abilities to perform both Basic and Instrumental ADLs were well-preserved. She was both apathetic and irritable, and sometimes behaved violently toward the nursing staff. She died of heart failure at the age of 93. Her diagnosis was senile dementia of the Alzheimer type.

Neuropathology of Case 10: The brain weight was 1,140 grams. The sulci and gyri revealed mild cortical atrophy. The medial temporal lobe had no significant atrophy. Sequential sections through the supratentorial tissues revealed the ventricular system to be mildly dilated, especially the frontal horn of the lateral ventricle. The hippocampal formation and amygdala were both minimally atrophic. Microscopically, the neocortex had a relatively unremarkable appearance on HE staining, with minimal neuronal loss and gliosis. Many plaques, but no neurofibrillary tangles (NFT), were noted with Gallyas staining. The plaques were diffuse amyloid deposits without neuritic elements. The hippocampus had a normal neuronal population in all sectors of Ammon's horn and only a few NFT in Sommer's sector. Tau immunostaining showed many AGs in CA-1 and the subiculum, and there were a few tau-positive processes in the temporal white matter, and a few coiled bodies. No neurons in the dentate fascia had NFT, but there was granular tau immunoreactivity of pretangles in some of the dentate neurons. The degree of neurofibrillary pathology was consistent with Braak Stage II. Ballooned neurons were present in the entorhinal cortex, amygdala, and cingulate gyrus, but not in the convex cortices.

DISCUSSION

AGD, including both pure and mixed cases, was found in 8.5% of samples in our brain bank, suggesting that AGD is not a rare disease, although a retrospective survey of this type cannot give accurate information regarding prevalence. Because the brains in our brain bank were from mental hospitals, a bias in this prevalence is possible. Nevertheless, this is in line with previous reports, which showed the prevalence of AGD to be 5%–10% in consecutive autopsies.[3,7,8] It is also worth noting that a recent report described the prevalence of AGD as over 40% when the very early stage of AGD is included.[9] In our series, the average age at onset was over 80 years, suggesting that AGD is a late-onset disease and corroborating the view that AGD is an age-associated disease.[25] AGD, on the other hand, was accompanied by a variety of other neurodegenerative or vascular diseases, which is also in accordance with previous reports.[8,26] In such AGD cases with complications, the age at onset tended to be lower than in "pure" cases. These findings suggest that AGD is a late-onset disease frequently accompanied by other neurological diseases and that an initial symptom of AGD could often be worsened by the complications.

Neuropsychiatric symptoms in AGD seemed to be more frequent and conspicuous than in cases of AD or mild cognitive impairment.[21,27] These characteristics may be essentially the same as the descriptions of "personality change" in AGD.[7,10,11] However, it must be noted again that these results should be interpreted with caution, given that the subjects reported here were hospitalized because of the appearance of behavioral or neuropsychiatric symptoms, suggesting a possible bias in the neuropsychiatric features. It may be reasonable to speculate that these features are neuropsychiatric symptoms that lead to difficulty in care and require medical management during the clinical course of AGD. On the other hand, cognitive functions other than memory appeared to be well preserved in comparison with AD,[28] although this needs to be confirmed in future longitudinal studies.

The clinical features of AGD can be attributed to the pathological lesions in the limbic system. Pathological lesions in this area have been implicated in amnesia and emotional disorders caused by various neurological disorders. Gascon and Gilles reported a case of herpes simplex encephalitis with selective limbic lobe destruction, which presented with amnesia and behavioral changes similar to Klüver-Bucy syndrome, whereas the primary functions associated with intelligence were relatively spared. They termed this patient's syndrome "limbic dementia."[29] Similarly, patients with paraneoplastic limbic encephalitis often exhibit amnesia, with other cognitive functions relatively intact.[30,31] In our series of pure-AGD, amnesia and emotional disorders were frequently present, whereas other cognitive functions seemed to be spared relative to the severity of amnesia. These symptoms resemble those reported in neurological disorders with limbic lesions, as described above, and, therefore, it may be reasonable to consider the symptoms in AGD to be a clinical variation of "limbic dementia," although the actual distribution of AGs and their clinical correlations cannot be determined from this study because staining for AGs was limited to the medial temporal lobe and basal forebrain.

Brain imaging of the AGD cases showed relative preservation of brain volume. Above all, measurement of the temporal horn of the lateral ventricle showed mild enlargement in AGD, indicating less shrinkage of the medial temporal lobe, including the hippocampus, than in AD. These CT results need to be confirmed by future MRI study. The average brain weight in pure-AGD cases tended to be greater than in AD cases, corroborating the findings from brain-imaging studies. This is also consistent with the neuropathological finding that neuronal loss in the hippocampus is mild in AGD, as compared with AD.[8]

Although we have presented the clinical features of AGD here, it must be noted that most cases in this study were admitted to mental hospitals, indicating a possible bias toward neuropsychiatric symptoms, as mentioned previously. Therefore, although this study has shown that the clinical features of AGD are amnesia with other cognitive functions relatively spared, and neuropsychiatric features with emotional disorders, it is possible that amnesia is the only prominent feature in case series of AGD not derived from neuropsychiatric referrals. It is also possible that the symptoms in these AGD cases are different from dementia in a general sense, even though dementia has been reported in approximately half of AGD patients.[32,33] The clinical features of AGD from other referrals need to be revealed through future studies.

Several limitations of the present study deserve attention. Although neuropsychiatric symptoms were categorized retrospectively by clinicians in accordance with the items in the NPI, this method has not been validated. Therefore, the results obtained in this study cannot be compared directly with the frequency of symptoms evaluated by NPI in other studies. The evaluation of ADLs was also performed retrospectively. There may be concern about categorizing cases with Braak tangle Stage III as pure-AGD, since a considerable number of cases with Braak Stage III have exhibited memory impairment.[34] Actually, two cases (Case 4 and Case 8) had a neurofibrillary pathology consistent with Braak Stage III, and it is possible that this affected their cognitive decline. This article focuses, rather, on the clinical features of "pure" cases, in an attempt to define the core features of AGD, and clinical features of AGD cases with complications remain to be clarified. Although we certainly recognize other limitations of this retrospective investigation, we are also aware of its potential implications. We hope that the observations reported here will stimulate further clinical research into this poorly understood pathological entity.

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Original Article

Vascular complications in dementia with Lewy bodies: A postmortem study

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The effects of cerebrovascular lesions on DLB are not yet fully understood, whereas the development of Alzheimer's disease (AD) is known to be associated with cerebrovascular lesions. In this study, we investigated the frequency of concomitant cerebrovascular pathologies in autopsy-proven DLB cases ($n = 25$) in comparison with AD cases ($n = 63$). We also investigated the correlation between cerebrovascular pathologies and the clinical features of DLB cases. On gross inspection, five cases of DLB and seven cases of AD were complicated by cerebral hemorrhages and the difference was significant; most of the lesions in DLB were subdural hemorrhages, possibly related to trauma. Nine cases of DLB and 25 cases of AD had grossly identified infarctions, but no significant difference was observed. Three cases of DLB and four cases of AD had concomitant hemorrhages, while 10 cases of DLB and 43 cases of AD had infarcts on microscopic inspection. There was a significant difference in the frequency of microscopic infarcts between DLB and AD, whereas no significant difference was noted in the frequency of microscopic hemorrhages. In DLB cases without vascular complications, memory disturbance was common as the initial symptom, while parkinsonism was more common in those with vascular complications. However, no significant difference was observed between DLB cases with and without vascular complications with respect to the frequency of individual clinical symptoms over the whole clinical course. These findings suggest that grossly identified hemorrhages are more common in DLB because of trauma, while microinfarcts are less common in DLB than AD, although the reason remains unclear. Such vascular complications might affect the clinical manifestations, in particular, the initial symptom, of DLB.

Key words: Alzheimer's disease, cerebral hemorrhage, cerebral infarction, dementia with Lewy bodies, vascular complication.

INTRODUCTION

Although neuronal degeneration due to neurofibrillary tangle and senile plaque formation is considered to be central in the pathogenesis of Alzheimer's disease (AD), the development of the disease might also be associated with cerebrovascular lesions. Alzheimer's disease and vascular dementia share some of the risk factors for atherosclerosis, such as diabetes mellitus or hypertension,[1] and a history of cerebral infarction might increase the incidence of AD by as much as 50%.[2] Atherosclerosis, as indicated by vessel wall thickness and plaques in the carotid arteries, has been shown to be associated with the development of AD.[3] Cerebrovascular lesions also play an important role in determining the appearance and severity of the symptoms of AD.[4] These findings strongly support the hypothesis that cerebrovascular lesions are implicated in the development of AD, at least in part.

However, the effects of cerebrovascular lesions on other neurodegenerative diseases are not yet fully understood. Several studies have investigated the relationship between stroke and Parkinson's disease (PD), but the results are conflicting as some show a reduced risk of ischemic and hemorrhagic stroke, while others indicate an increased

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likelihood of stroke-related death.[5-11] Interestingly, Jellinger recently investigated the prevalence of vascular lesions in DLB, the second most frequent cause of degenerative dementia, and showed that the frequency was lower in DLB than in either PD or a control.[12]

In the present study, we investigated the frequency of concomitant cerebrovascular pathologies in autopsy-proven DLB cases in comparison with AD cases. We also compared the clinical features of DLB cases with and without vascular complications on the hypothesis that vascular complications might affect the clinical course of DLB.

METHODS

Brains from 25 cases with DLB (Braak tangle stages ranging from 2–4,[13] 12 males and 13 females, age at death between 67 and 94 years, mean = 80.8 ± 6.6 years) and 63 age-matched and sex-matched cases with AD (Braak tangle stages 5 and 6, 22 males and 41 females, age at death between 67 and 94 years, mean = 83.2 ± 6.2 years) were employed in this study, which was approved by the Human Subjects Review Committees of both Yokohama City University and Fukushimura Hospital. For routine neuropathological evaluation, formalin-fixed, paraffin-embedded sections from each area of the brain were stained with HE, KB, and methenamin-silver stains. In addition, α-synuclein immunostaining was routinely used to detect Lewy bodies. All cases of DLB fulfilled the post-mortem criteria for DLB:[14] 12 cases were the neocortical-type and 13 cases were the transitional-type. All AD cases fulfilled the criteria of definite AD, using the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) protocol,[15] with neurofibrillary changes ranging from Braak tangle stages 5–6.

One hemisphere of the brain was fixed in 4% paraformaldehyde in a 0.1 mol/L phosphate buffer after autopsy, while the other hemisphere was deep-frozen for biochemical analysis. Coronal slices of 1 cm thickness were cut through the hemisphere fixed in paraformaldehyde and transverse slices were cut through the brainstem and cerebellum. The brain slices were initially examined for the presence of vascular lesions, including cerebral hemorrhages and infarcts, by gross inspection. The slices were then embedded in paraffin and cut into 7-μm-thick sections for microscopic examination; sections were taken every 1 cm from that hemisphere. The presence of vascular lesions was microscopically investigated, mainly using sections stained with HE and by the KB method.

The clinical symptoms of all cases were assessed through an evaluation of clinical records with respect to the presence of vascular risk factors including hypertension, hyperlipidemia, heart disease, diabetes mellitus, and tobacco use. The presence of visual hallucinations, delusions, wandering, and parkinsonism also was investigated in the DLB cases. Cognitive fluctuation, one of the core features of DLB, was not evaluated in the present study because it was difficult to assess this symptom retrospectively from the clinical records.

The difference in brain weight between DLB and AD cases and the difference in the frequency of vascular complications between DLB cases with mild neurofibrillary pathology (Braak tangle stage 2 or 3) and those with relatively severe pathology (Braak tangle stage 4) were analyzed by two-sample t-tests or χ^2-tests. The difference in the frequency of clinical symptoms between DLB with and without vascular lesions also was evaluated by χ^2-tests. Logistic regression was used to evaluate the differences between DLB and AD in age, onset and duration of the disease, and frequency of hemorrhages, infarctions, and vascular risk factors. The data were analyzed using SPSS for Windows, and $P < 0.05$ was considered to be statistically significant.

RESULTS

Concomitant vascular risk factors for dementia with Lewy bodies and Alzheimer's disease

As some reports have argued that the difference in frequency of vascular complications between PD and AD might be related to the difference in concomitant vascular risk factors,[5] the difference in the frequency of the risk factors for DLB and AD was assessed (Tables 1,2). In the present study, there was no difference in the frequency of the major vascular risk factors, including hypertension (odds ratio [OR] = 1.18, $P = 0.80$), hyperlipidemia (OR = 1.09, $P = 0.96$), diabetes mellitus (OR = 0.66, $P = 0.68$), and tobacco use (OR = 1.36, $P = 0.62$).

Frequency of vascular complications in dementia with Lewy bodies and Alzheimer's disease

On gross inspection, five cases (20%) of DLB and seven cases (11%) of AD were complicated by cerebral hemorrhages, and most of the lesions were subdural hemorrhages possibly related to trauma (Tables 1,2). Nine cases (36%) of DLB and 25 cases (40%) of AD had grossly identified infarctions. There was a significant difference between DLB and AD in the frequency of hemorrhages (OR = 6.44, $P = 0.03$), while the difference was not significant with respect to infarctions (OR = 1.96, $P = 0.39$). Three cases (12%) of DLB and four cases (6%) of AD had concomitant hemorrhages, while 10 cases (40%) of DLB and 43 cases (68%) of AD had infarcts on microscopic inspection. The cases with hemorrhages on gross inspection outnum-

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Table 1 Backgrounds, vascular risk factors, and vascular complications in dementia with Lewy bodies (DLB) and Alzheimer's disease (AD)

Variable	DLB ($n=25$)	AD ($n=63$)
Age at death (years)	80.8 ±6.6	83.2 ±6.2
Age of onset (years)	75.1 ±6.9	75.4 ±7.4
Duration (months)	69.4 ±50.5	92.8 ±55.1
Hypertension	11 (44%)	27 (43%)
Hyperlipidemia	2 (8%)	2 (3%)
Heart disease	4 (16%)	11 (17%)
Diabetes mellitus	2 (8%)	10 (16%)
Tobacco use	10 (40%)	18 (29%)
Brain weight	1164 ±124	1048 ±118
Gross hemorrhage	5 (20%)	7 (11%)
Gross infarction	9 (36%)	25 (40%)
Microscopic hemorrhage	3 (12%)	4 (6%)
Microscopic infarction	10 (40%)	43 (68%)

Table 2 Logistic regression analysis of the differences between dementia with Lewy bodies and Alzheimer's disease

Factor	*P*-value	Odds ratio	95%CI
Basic factors			
Age at death (years)	0.11	0.33	0.85–1.27
Age of onset (years)	0.12	2.90	0.76–11.1
Duration (months)	0.17	1.08	0.97–1.21
Risk factors			
Hypertension	0.80	1.18	0.34–4.12
Hyperlipidemia	0.96	1.09	0.05–24.2
Heart disease	0.72	0.72	0.12–4.43
Diabetes mellitus	0.68	0.66	0.10–4.52
Tobacco use	0.62	1.36	0.40–4.65
Pathological factors			
Gross hemorrhage	0.03*	6.44	1.26–32.8
Gross infarction	0.39	1.96	0.42–9.13
Microscopic hemorrhage	0.81	0.76	0.78–7.35
Microscopic infarction	0.02*	0.15	0.03–0.70

*$P < 0.05$.

Table 3 Clinical pictures of dementia with Lewy bodies cases with and without vascular complications

Variable	Vascular complication ($n=17$)	Vascular complication ($n=8$)	*t*-value	χ^2	*P*-value
Age at death (years)	80.5 ±7.5	81.5 ±4.2	−0.34	–	0.74
Age at onset (years)	74.4 ±7.4	77.8 ±4.6	−1.17	–	0.26
Duration (months)	79.9 ±55.0	48.6 ±40.4	1.42	–	0.18
Memory disturbance as the initial symptom	5 (29%)	6 (75%)	–	4.59	0.03*
Visual hallucinations	13 (76%)	5 (63%)	–	0.53	0.47
Delusions	10 (76%)	4 (50%)	–	0.17	0.68
Wandering	6 (35%)	4 (50%)	–	0.49	0.48
Parkinsonism	7 (41%)	4 (50%)	–	0.17	0.68

*$P < 0.05$.

bered those with hemorrhages on microscopic inspection, as most of the hemorrhages identified grossly were subdural hemorrhages and were not observed on thin sections. There was a significant difference in the frequency of microscopic infarcts between DLB and AD (OR = 0.15, $P = 0.02$), but no significant difference was found in the frequency of microscopic hemorrhages (OR = 0.76, $P = 0.81$). In the DLB cases, no significant difference in vascular complications was found between the cases with mild neurofibrillary pathology of a Braak tangle stage of 2 or 3 and those with relatively severe pathology, with a Braak tangle stage of 4 ($\chi^2 = 0.414$, $P = 0.52$).

Clinical symptoms of dementia with Lewy bodies with and without vascular complications

On the hypothesis that the core symptoms of DLB might be affected by vascular complications, such as hemorrhage and infarction, differences in the frequency of the core symptoms were compared between the DLB cases with and without vascular complications (Table 3). In contrast to our assumption, there was no significant difference in the frequency of parkinsonism ($\chi^2 = 0.17$, $P = 0.68$), wandering ($\chi^2 = 0.49$, $P = 0.48$), delusions ($\chi^2 = 0.17$, $P = 0.68$) or visual hallucinations ($\chi^2 = 0.53$, $P = 0.47$). Likewise, there was no significant difference in the age of onset ($t = -1.17$, $P = 0.26$) or duration of the disease ($t = 1.42$, $P = 0.18$). However, the frequency of the cases whose initial symptom was memory disturbance was 29% in DLB cases with vascular complications and 75% in those without vascular complications; this difference was significant ($\chi^2 = 0.59$, $P = 0.03$). Parkinsonism was more common as the initial symptom in DLB cases with vascular complications.

DISCUSSION

In the present study, we investigated the frequency of concomitant vascular pathologies in DLB and showed that the frequency of microinfarcts in DLB is lower than in AD. This result is consistent with the findings reported by Jellinger, who found the frequency of cerebrovascular lesions in DLB to be lower than in PD and AD.[12,16] No significant differences in the major vascular risk factors, including hypertension, hyperlipidemia, diabetes mellitus, and tobacco use, were found between DLB and AD in this study, although some reports have argued that the differ-

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ence in frequency of vascular complications between PD and AD might be related to differences in concomitant vascular risk factors.[5] Our results indicate that the different frequency of vascular risk factors is not the only explanation for the difference in vascular complications observed in DLB and AD, although the reason remains unclear. However, the frequency of grossly indentified hemorrhages in DLB was higher than in AD; most of the lesions were subdural hemorrhages, possibly related to head trauma. This seems to be explained by the fact that falls are common among DLB patients, together with the exacerbation of parkinsonism.

The precise mechanism of less frequent concurrent microinfarcts in DLB is not known, but an increased frequency of vascular pathologies in AD seems to explain this discrepancy, at least in part. Amyloid deposition within cerebral vessels, or cerebral amyloid angiopathy (CAA), is common in advanced age and even more common in AD.[17] Although sporadic CAA is usually clinically silent, it can be associated with a number of clinical manifestations, including cerebral hemorrhage and infarction.[18–20] Other possible explanations of the higher frequency of vascular lesions in AD include vascular nitric oxide (NO) release disorder and nitric oxide synthase (NOS) dysfunction.[21] Chronic cerebral hypoperfusion resulting from aging and vascular risk factors, such as hypertension, hyperglycemia or hyperlipidemia, can stimulate a rapid release of NO via activation of NOS on the endothelium, inducing further basement membrane thickening. This progressive change in the microvasculature can then promote a decrease in glucose and oxygen delivery to neuronal and glial cells, which results in neurodegeneration, including the accumulation of abnormal proteins and neuronal cell death. This mechanism via NO is possibly involved in the development of AD pathologies,[22] although it remains unclear if this also promotes the development of DLB pathologies.

Despite the lower incidence of microinfarcts in DLB, the difference in frequency of large or gross infarcts was not significant between DLB and AD. This seems to be related to the differing frequency of gross infarcts and microinfarcts in AD; gross infarcts were less frequently found in AD cases. Although the specific risk factors for lacunar strokes are broadly similar to those in large ischemic stroke patients, it is likely that hypertension is significantly more common among lacunar stroke patients than among those with other forms of ischemic stroke.[23] The difference in vascular risk factors, however, was not significant between AD cases with gross infarction and those with microinfarction (data not shown). The reason for this discrepancy remains to be elucidated.

This study corroborated some clinical features of DLB. The mean duration of DLB tends to be shorter than AD. Studies comparing DLB and AD suggest that the mean duration of illness is shorter in DLB patients than in AD patients, although this is still controversial.[24] However, the mean brain weight was significantly greater in DLB than in AD. This is consistent with the results of neuroimaging studies showing the preservation of the hippocampus and medial temporal lobe volume in DLB.[25]

In DLB cases, vascular complications are associated with the initial symptoms, although no apparent association was found in this study between the clinical symptoms and vascular pathologies over the whole clinical course. Memory disturbance was common as the initial symptom in DLB cases without vascular complication, while parkinsonism was more common in those with vascular lesions. The basal ganglia are vulnerable to vascular changes in general and might be responsible for parkinsonism in DLB cases with vascular complications.

In conclusion, grossly identified hemorrhages, mainly related to trauma, were more common in DLB, while microinfarcts were less common in DLB than AD, although the reason remains unclear. These vascular complications might affect the clinical manifestations of DLB, in particular the initial symptoms: memory disturbance is seen in cases without vascular complications and parkinsonism in those with complications.

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A novel alternative splice variant of nicastrin and its implication in Alzheimer disease

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Abstract

Nicastrin interacts with γ-secretase complex components predominantly via the N-terminal third of the transmembrane domain. The authentic transmembrane domain is critically required for the interaction with γ-secretase complex components and for formation of an active γ-secretase complex. In this study, we have identified a novel alternatively spliced transcript of nicastrin in human brain tissue. This transcript (NCSTN-ΔE16) lacks exon 16 of nicastrin mRNA, which leads to deletion of 71 amino acids just upstream of its transmembrane domain. Its expression pattern was analyzed in the hippocampus of patients with pathologically diagnosed Alzheimer disease (cases) and non-Alzheimer dementia (controls). In patients with the APOE-ε4 allele, the frequency of Alzheimer disease appeared to be increased in the NCSTN-ΔE16-positive group, but the association was not statistically significant. In conclusion, the expression of NCSTN-ΔE16 transcript may confer some additional risk for developing Alzheimer disease beyond the risk due to ApoE-ε4 allele. Further investigation in larger scale population would be necessary to address its potential implication in Alzheimer disease.
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Keywords: Alzheimer disease; Nicastrin; Apolipoprotein E; Alternative splicing

Introduction

Accumulation of amyloid plaques in the brain is a key component of the pathology of Alzheimer disease (AD). Amyloid β-peptide (Aβ), the main component of amyloid plaques, is released from the β-amyloid precursor protein by β- and γ-secretases (Hardy and Selkoe, 2002). Recent studies revealed that nicastrin is a component of γ-secretase complex, which also contains presenilin-1/presenilin-2, APH-1 and PEN-2 (Takasugi et al., 2003).

Yu et al. first reported that artificial deletion mutants of the conserved hydrophilic DYIGS domain in nicastrin decreased Aβ production, whereas a double-missense mutation (D336A+Y337A) increased Aβ production (Yu et al., 2000). Capel et al. reported that a decrease of nicastrin expression by RNAi in HEK293 cells was accompanied by reduced expression of presenilin-1, APH-1aL, and PEN-2 and reduced Aβ generation. Overexpression of wild-type nicastrin restored their reductions, while expression of nicastrin lacking the transmembrane domain did not (Capell et al., 2003). These results suggest that nicastrin plays an important role in activation of γ-secretase complex, production of Aβ peptide and onset of Alzheimer disease.

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Materials and methods

Subjects

All subjects were Japanese ($n=23$, 74% female, all clinically demented, age range at death 69–98 years). They were inpatients at Fukushimura Hospital (Toyohashi, Aichi, Japan), and were cognitively evaluated by neuropsychological tests such as the Mini-Mental State Examination during hospitalization.

Treatment of autopsied brain

When they died, autopsy and pathological diagnosis were carried out according to the criteria of the Consortium to Establish a Registry for Alzheimer's disease (Mirra et al., 1991). Written consent of the patients' guardians for diagnosis and biochemical, molecular biological and genomic research was obtained. The autopsied brain was weighed, and cut midsagitally. One half of the brain was divided into several portions (frontal, temporal, parietal, occipital cortex, hippocampus, etc.), snapped frozen in liquid nitrogen, and stored at −80 °C. The other half was fixed and used for pathological diagnosis, as described previously (Akatsu et al., 2002). Based on this pathological diagnosis, subjects were divided into AD group and non-Alzheimer dementia (non-AD) group.

Genotyping

APOE genotyping was performed using DNA samples extracted from dissected brain tissues, according to the procedure described previously (Yoshiiwa et al., 1997).

Screening for novel splicing variants and sequencing

Total RNA was extracted from the frozen hippocampus using Trizol (Invitrogen, Carlsbad, CA, USA.), according to the manufacturer's protocol, and first strand cDNAs were synthesized from 5 μg total RNA with an oligo(dT)$_{12-18}$ primer using 50 units superscript II RNase H$^-$ reverse transcriptase (Invitrogen) in a total volume of 20 μl, according to the manufacturer's protocol. The cDNAs were diluted at 1:5 with distilled water, and then 2 μl was used as a template for PCR with Platinum Taq DNA polymerase (Invitrogen) and the sense and anti-sense primers listed in Table 1. Sequencing was performed by direct sequencing method with a dye terminator cycle sequencing FS kit (PE Biosystems) following the manufacturer's protocol.

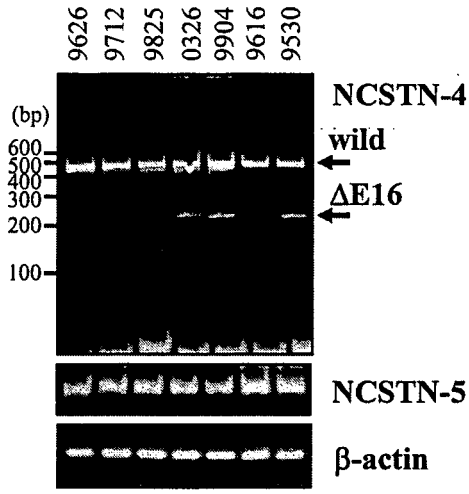

Fig. 1. Identification of a novel alternatively spliced variant of NCSTN. Upper panel; RT-PCR from human hippocampus with primers, NCSTN4-F and NCSTN4-B. Note the 427 bp band (wild-type) exists in all patients, while the 214 bp band (ΔE16) exists in some patients (0326, 9904, and 9530). Middle panel; RT-PCR from hippocampus with primers, NCSTN5-F and NCSTN5-B (see Table 1) as intra-molecular control. Lower panel; RT-PCR from hippocampus with β-actin primers as external control.

Reverse-transcription PCR (RT-PCR)

RT-PCR was performed with the cDNAs from the hippocampus, the primers; NCSTN4-F and NCSTN4-B, and

Table 1
Primers used for screening for splicing variants of nicastrin

	Name	Sequences	Position
Sense primer	NCSTN1-F	GCTAACAGACAGGAGCCGAACG	94–115
	NCSTN2-F	TGGGCAATGGTTTGGCTTATG	642–662
	NCSTN3-F	GAGAAGAGTGGTGCTGGTGTCC	1346–1367
	NCSTN4-F	GCCCCACCAACACCACTTATG	1818–1838
	NCSTN5-F	TGGACTGAGAGCCGCTGGAAAG	2084–2105
	NCSTN6-F	GGGTTCCTGATTAAAGCCAACAAC	1712–1735
	NCSTN7-F	TCATGGTTCCAGTCTATCCTCAGG	1736–1759
	NCSTN8-F	GCCTTGTCTCCTGCCTTTGAAC	2030–2051
Anti-sense primer	NCSTN1-B	CTTCATAAGCCAAACCATTGCC	665–644
	NCSTN2-B	TGAGGATGACAGCAGGGACACC	1382–1361
	NCSTN3-B	AAGTGGTGTTGGTGGGGCTGGAGAC	1835–1811
	NCSTN4-B	GGAGCAATGAAAAGGACATCAGC	2244–2222
	NCSTN5-B	AGCACGCCCACCCTAATGTG	2806–2787
	NCSTN6-B	GCATTGATGCAGTAGGTGACGATG	2217–2194
	NCSTN7-B	CAGTGGGACAGATGCTCTAGGAAG	2333–2310
	NCSTN8-B	CTGAAGGGCAAATTAGGGTGG	2584–2564
	NCSTN9-B	AAAAGTAGAAGGGTCCTGAAGGG	2600–2577

The number depicts the position of the sequence in NCSTN cDNA (Genbank Accession # AF240468).

Platinum Taq DNA polymerase at 95 °C for 0.5 min, 55 °C for 1 min, at 72 °C for 1 min, for 40 cycles.

Statistical analysis

AD group (cases) and non-AD group (controls) were further divided by the presence of APOE-ε4 allele into APOE-ε4-positive and APOE-ε4-negative groups. The frequency of NCSTN-ΔE16 transcript was compared between AD and non-AD groups by χ^2 analysis. Differences with p values of <0.05 were considered significant.

Results

With the primers, NCSTN4-F and NCSTN4-B, two major bands were detected in some brain samples (Fig. 1). The most frequent was a 427 bp band (wild-type), followed by a 214 bp band. Sequencing analysis revealed that this latter transcript was an in-frame splicing variant that lacks exon 16, and it was designated "NCSTN-ΔE16". The exact result of sequencing analysis is described in Fig. 2A. The schematic structure of NCSTN-ΔE16 is illustrated in Fig. 2B.

Out of the 23 patients examined in this study, 10 were diagnosed pathologically with AD (mean age at onset of dementia: 72.2±7.4 years, mean age at death: 81.7±8.2 years, mean brain weight at death: 1024±105 g) and 13 were diagnosed as non-AD (mean age at onset of dementia: 83.2±8.4 years, mean age at death: 89.2±7.5 years, mean brain weight at death: 1099±109 g). Non-AD included normal physiological aging, multiple infarctions, diffuse Lewy-body disease, Parkinson disease, etc. (data not shown). 13 patients carried the APOE-ε4 allele (APOE-ε4-positive group), while

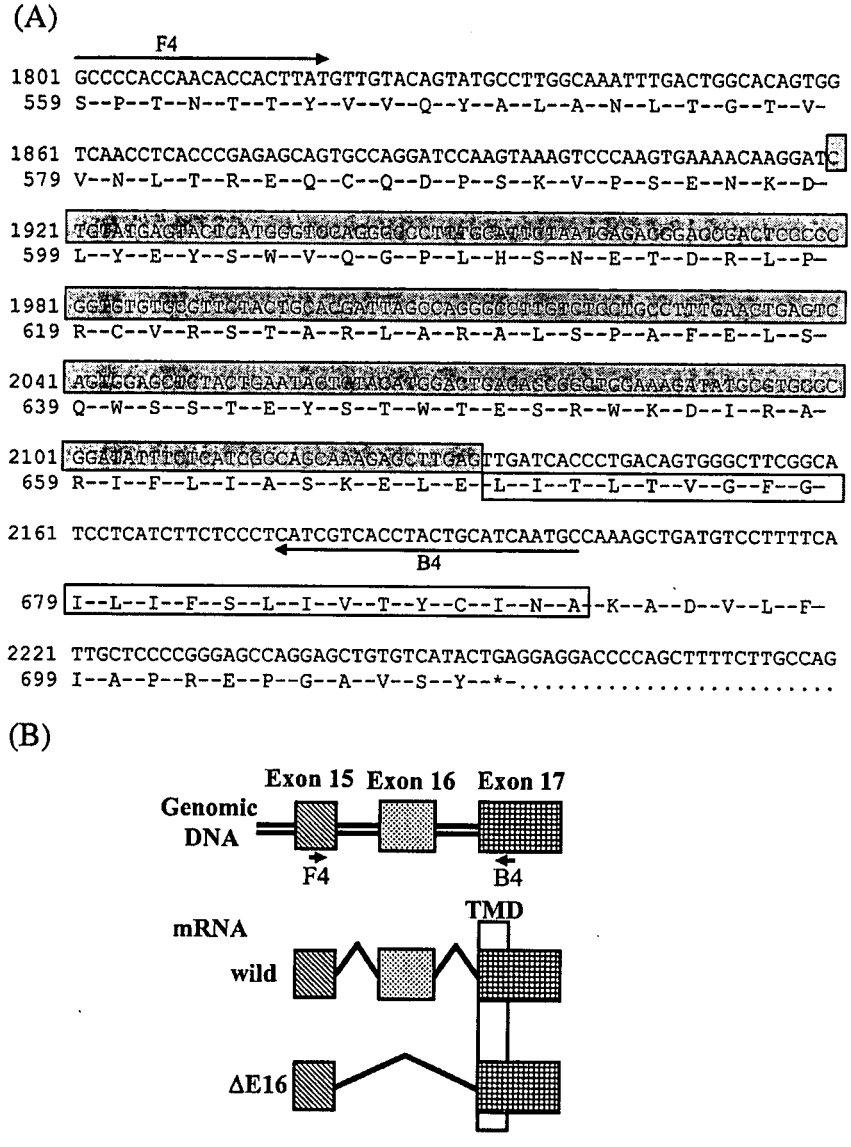

Fig. 2. Structure of the splicing variant of NCSTN. (A) Sequencing analysis revealed that NCSTN-ΔE16 is an in-frame splicing variant lacking exon 16. Open box corresponds to amino acid sequence of the transmembrane domain. Gray box corresponds to exon 16 sequence, which is deleted in NCSTN-ΔE16. F4 (NCSTN4-F) and B4 (NCSTN4-B) are the primers used to detect NCSTN-ΔE16. (B) Schematic representation of NCSTN gene and its wild-type (wild) and alternatively spliced transcript (ΔE16).

Table 2
Summary of the characteristics of AD and non-AD subjects by detection of NCSTN-ΔE16 transcript

	Pathological diagnosis		
	Overall	AD	Non-AD
Age at onset of dementia			
NCSTN-ΔE16(−)	79.5±9.9(11)	71.5±9.1(4)	84.0±7.4(7)
NCSTN-ΔE16(+)	75.6±9.3(9)	72.7±7.0(6)	81.3±12.1(3)
P value	0.38	0.84	0.75
Age at death			
NCSTN-ΔE16(−)	85.8±9.5(12)	79.5±9.3(4)	88.9±8.4(8)
NCSTN-ΔE16(+)	86.2±7.8(11)	83.2±7.9(6)	89.8±6.7(5)
P value	0.90	0.54	0.83
Brain weight at death			
NCSTN-ΔE16(−)	1071±96(12)	1073±138(4)	1071±80(8)
NCSTN-ΔE16(+)	1060±131(11)	991±71.6(6)	1143±144(5)
P value	0.81	0.34	0.35

Values are means±S.D. (number of cases). No significant difference was detected between NCSTN-ΔE16(−) and NCSTN-ΔE16(+) groups. AD: Alzheimer disease, non-AD: non-Alzheimer dementia.

10 patients did not (APOE-ε4-negative group). RT-PCR analysis with the primers, NCSTN4-F and NCSTN4-B, detected the wild-type NCSTN transcript in the hippocampus in all 23 patients, while it also detected NCSTN-ΔE16 in 11 patients.

The age at onset of dementia, age at death and brain weight at death were not significantly different between NCSTN-ΔE16(−) group and NCSTN-ΔE16(+) group in overall, AD, and non-AD patients, as described in Table 2. NCSTN-ΔE16 transcript was detected in 6 out of 10 AD patients and 5 out of 13 non-AD patients. The difference in the frequency of NCSTN-ΔE16 transcript between AD cases and non-AD controls was not significant ($p=0.55$) (Table 3). When analysis was limited to APOE-ε4-negative patients, the NCSTN-ΔE16 transcript was detected in 1 out of 2 AD patients, and 4 out of 8 non-AD patients. The frequency of NCSTN-ΔE16 transcript was not significantly different between AD patients and non-AD patients, either ($p=1.00$). Likewise, when analysis was limited to APOE-ε4-positive patients, the NCSTN-ΔE16 transcript was detected in 5 out of 8 AD patients, and 1 out of 5 non-AD patients. The frequency of NCSTN-ΔE16 transcript was not significantly different between AD patients and non-AD patients, either ($p=0.35$).

Discussion

Assembly of nicastrin into γ-secretase complex is essential for activation of γ-secretase and generation of Aβ. In molecular and cellular biological studies, Capell et al. reported that nicastrin interacts with γ-secretase complex components predominantly via the N-terminal third of the transmembrane domain (670–692 amino acids). The authentic transmembrane domain of nicastrin is critically required for the interaction with γ-secretase complex components and for formation of an active γ-secretase complex (Capell et al., 2003).

In this study, in the human hippocampus, we identified a novel alternatively spliced transcript lacking exon 16, which encodes the 71 amino acid sequence just upstream of this functional transmembrane domain (see Fig. 2A). This transcript was detected in some patients, but not in others. The cause of this dissociation is unknown. It is not clear if this endogenous deletion may affect the function of nicastrin and the activity of γ-secretase in the human brain or even in vitro. Change in the activity of γ-secretase may influence the risk of AD. Accordingly, the implications of the expression of this transcript and AD pathology were examined here.

When we analyzed overall patients, the difference in the frequency of NCSTN-ΔE16 transcript between AD cases and non-AD controls was not significant. As described in most other studies, APOE-ε4 allele is a major risk factor for developing AD. It is estimated to account for about 40–50% of the genetic variation in late-onset AD (Roses, 1996). To examine the association between the existence of NCSTN-ΔE16 transcript and the development of AD independently of APOE genotype, we further categorized AD and non-AD patients by the presence of APOE-ε4 allele into APOE-ε4-negative and APOE-ε4-positive groups. In APOE-ε4-negative group, the difference between AD cases and non-AD controls was not significant, either. In APOE-ε4-positive group, the frequency of NCSTN-ΔE16 transcript appeared to be higher in AD cases than in non-AD controls. However, the association was not statistically significant because of the small population size. This suggests the possibility of interaction between NCSTN-ΔE16 and APOE-ε4, so that NCSTN-ΔE16 only influences risk if an individual carries APOE-ε4; however, statistical tests for interaction were not significant.

Several genetic studies have focused on the association between nicastrin polymorphisms and the onset of AD. Helisalmi et al. reported that one haplotype of nicastrin significantly increased the risk of AD in patients without an APOE-ε4 allele in the Finnish population (Helisalmi et al., 2004). Dermaut et al. reported that one SNP haplotype of nicastrin is increased in patients with familial early-onset AD without the APOE-ε4 allele in the Dutch population (Dermaut et al., 2002). On the contrary, Orlacchio et al. (2004) and Cousin et al. (2003) reported no such associations. Thus, there is disagreement in opinion, and further investigation of this matter is necessary.

In conclusion, the expression of NCSTN-ΔE16 transcript may confer some additional risk for developing Alzheimer disease beyond the risk due to ApoE-ε4 allele. Further

Table 3
The number of subjects with or without NCSTN-ΔE16 transcript in overall AD and non-AD patients, and by ApoE genotype subgroups

	Overall[a]		APOE-ε4-negative[b]		APOE-ε4-positive[c]	
	AD	Non-AD	AD	Non-AD	AD	Non-AD
	(*n*=10)	(*n*=13)	(*n*=2)	(*n*=8)	(*n*=8)	(*n*=5)
NCSTN-ΔE16(−)	4	8	1	4	3	4
NCSTN-ΔE16(+)	6	5	1	4	5	1

a: $\chi^2=0.36$, $p=0.55$, b: $\chi^2=0.00$, $p=1.00$, c: $\chi^2=0.85$, $p=0.35$.

investigation in larger scale population would be necessary to address its potential implication in AD.

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Variations in the *BDNF* Gene in Autopsy-Confirmed Alzheimer's Disease and Dementia with Lewy Bodies in Japan

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Key Words
Brain-derived neurotrophic factor · Alzheimer's disease · Parkinson's disease · Dementia with Lewy bodies · Single-nucleotide polymorphism

Abstract
Background/Aim: Brain-derived neurotrophic factor (BDNF) is associated with the hippocampus and the nigrostriatal dopaminergic function. Data showing that its level was reduced in Alzheimer's disease (AD) and Parkinson's disease (PD) suggested that the BDNF function must play an important role in the pathogenetics of these diseases. Indeed, variation in the *BDNF* gene may confer susceptibility to AD and PD development. Recently, a functional *BDNF* Val66Met polymorphism has been found to be associated with episodic memory and hippocampal function, with intracellular trafficking, and with activity-dependent secretion of BDNF. To date, there have been several conflicting reports on the correlation between AD or PD and Val66Met or C270T polymorphism in the *BDNF* promoter region, although no data on this relationship have been published with respect to dementia with Lewy bodies (DLB). In the present study, we investigated a possible association between such *BDNF* polymorphisms and susceptibility to AD or DLB. ***Methods:*** *BDNF* genotyping was carried out by the polymerase chain reaction-restriction fragment length polymorphism method in autopsy-confirmed human samples. ***Results and Conclusion:*** On comparing patients and controls, the distribution of *BDNF* genotypes and alleles did not differ significantly. Our findings suggest that it is unlikely that these *BDNF* polymorphisms play a major role in the pathogenesis of AD and DLB in the Japanese population. Copyright © 2006 S. Karger AG, Basel

Introduction

Brain-derived neurotrophic factor (BDNF) is a small dimeric protein which is a member of the nerve growth factor family and is widely expressed in the adult mammalian brain, especially in the hippocampus [1]. It has been found to promote the survival of hippocampal and neocortical neurons. Moreover, the gene product has been involved in development, regeneration, and maintenance of neuronal systems [2]. A selective reduction in

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the BDNF mRNA expression [3] as well as in the protein level [2, 4, 5] was first demonstrated in the hippocampal formation of Alzheimer's disease (AD) patients. In addition, a later study [6] showed that the proBDNF protein level is decreased in the parietal cortex of AD patients. These findings implicated BDNF in the pathogenesis of AD and suggested that the *BDNF* gene is a predisposing factor in AD development.

In a recent study performed in an Italian AD population [7], the authors investigated a functional single-nucleotide polymorphism (SNP; G196A) in the coding region of the *BDNF* gene which results in an amino acid change (Val66Met). In addition, Egan et al. [8] demonstrated that a SNP at G196A in the *BDNF* gene could affect intracellular processing and secretion of BDNF, leading to impairment in the hippocampal function. Moreover, these authors demonstrated the impact of this polymorphism on human brain function. In addition, Hariri et al. [9] showed that this SNP affects the human memory-related hippocampal activity and predicts the memory performance in healthy individuals. However, Nacmias et al. [10] analyzed the cognitive performance in AD patients with the *BDNF* Val66Met polymorphism and found it not to be an AD susceptibility factor, although these authors supported the effect of the apolipoprotein E (ApoE) ε4 allele in increasing the risk of developing AD. On examining a potential role for the *BDNF* Val66Met polymorphism as an AD risk factor in Chinese [11] and Spanish [12] populations, the researchers found no association between this SNP and AD.

Furthermore, Kunugi et al. [13], who studied a group of Japanese AD patients, described another functional SNP (C-270T) in the promoter region of the *BDNF* gene. Subsequently, this allele was found to be frequently ocurring in German AD patients [14] and a relevant risk factor for AD development. However, a group studying a Brazilian population did not obtain similar findings [15]. Finally, in another Italian study [16], these *BDNF* SNPs were rejected as having any association with AD. All of these patients had been diagnosed clinically as having probable AD. Therefore, with differential diagnoses, it is extremely important to distinguish dementia with Lewy bodies (DLB) from AD.

As for the relationship between BDNF and DLB, this factor has been strongly correlated with dopaminergic neurons [17], especially in patients having the disease. The substantia nigra of Parkinson's disease (PD) patients also showed reduced levels of BDNF mRNA [18] and protein as compared with control brains [19, 20]. Interestingly, one group [21] reported that glial BDNF was elevated in the substantia nigra of PD patients. Three genes, *α-synuclein, parkin,* and *DJ-1*, have been clearly linked with familial PD [22], and, based on the findings mentioned above, it is reasonable to suggest that the *BDNF* gene may be linked with both PD and DLB pathogeneses. Momose and coworkers [23, 24] found that homozygous possession of the *BDNF* polymorphism, Val66Met, was associated with PD in Japanese patients as compared with unaffected controls. However, Swedish [25] and Chinese [26] groups were unable to replicate these findings in their own populations. The results of yet another Japanese group [27] were inconclusive. Although PD is a type of DLB, there have been no data as yet on the association between DLB and *BDNF* polymorphism.

All previous genetic analyses were based on clinical findings. By using neuropathologically diagnosed cases, we were able to clearly study the correlation between *BDNF* polymorphisms and major neurodegenerative dementias such as AD and DLB.

Patients and Methods

Patients

In the present study, the 267 cases examined consisted of patients hospitalized in the Fukushimura Hospital. All of these patients were cognitively evaluated by neuropsychological testing, using such tests as the Mini-Mental State Examination [28] and Hasegawa's dementia scale [29] or its revised version [30] which is commonly utilized in Japan. We also recorded interviews employing a comprehensive questionnaire covering psychological and medical symptoms, chronic conditions, treatments, and activities of daily living. Autopsies were carried out at Fukushimura Hospital, Japan, from October 1990, and *BDNF* genotyping was performed using DNA samples extracted from dissected brain tissues obtained between January 1993 and March 2003, after obtaining the agreement of the patients' guardians for use of these tissues for the purpose of diagnosis, research, and genetic analysis. This work was approved by the Ethical Committee of the Fukushimura Hospital, February 30, 2004, and assigned application No. 177.

These samples are currently stored in the Fukushimura Brain Bank. The patients consisted of 126 males and 141 females, with a mean age at the time of death of 82.7 ± (SD) 8.49 (range 44–105) years.

As a nondemented group, 108 elderly individuals were recruited from the Ehime University Graduate School of Medicine, and we have previously used this group as controls [31]. These population-based nondemented controls represented 87 females and 21 males with mean age at the time of blood drawing of 81.6 ± (SD) 6.95 years and an age range at the time of death of 70–101 years (table 1).

Autopsy and Sampling of Brain Tissues

The brain was removed at autopsy, weighed, cut midsagittally, and examined for vascular and other macroscopically detectable

Table 1. Summary of the main neuropathological subgroup diagnoses

	Males	Females	Total	Age at death years (mean ± SD)
Fukushimura Brain				
Bank samples	126	141	267	82.7 ± 8.49
AD	37	58	95	83.5 ± 7.50
LNTD	2	2	4	95.0 ± 5.72
FTD	1	3	4	75.3 ± 4.50
DLB	16	18	34	81.3 ± 9.21
CVD	55	46	101	82.5 ± 8.37
Control brains	10	11	21	87.3 ± 6.70
Population based control samples	21	87	108	81.6 ± 6.95[a]

AD = Alzheimer's disease; LNTD = limbic neurofibrillary tangle dementia; FTD = frontotemporal dementia; DLB = dementia with Lewy bodies; CVD = cerebrovascular disorders.

[a] Age at the time blood was drawn.

lesions. Specimens for diagnostic examination were taken from the hemisphere showing abnormalities on CT scanning, or from the left hemisphere when no difference between the left and the right hemispheres was found, and fixed in 4% paraformaldehyde as a hemisphere block. The other hemisphere was divided into several regions. Some lesion samples were frozen for further analyses and stored at –80°C, while others were removed and fixed in 4% paraformaldehyde for immunohistochemical analysis.

Samples for diagnostic purposes were taken from several portions of the brain, as described previously [32]. The specimens were embedded in paraffin and processed into 5-μm sections for conventional histological and immunohistochemical examination.

Neuropathological Diagnostic Criteria

The specimens were stained using hematoxylin-eosin and Klüver-Barrera methods. Methenamine-silver staining was used to detect senile plaques, cerebral amyloid angiopathy, and neurofibrillary tangles (NFTs) [33]. Ubiquitin, α-synuclein, Aβ, and tau immunostaining methods were also used when necessary.

In addition to scoring according to CERAD criteria, senile plaques and NFTs as indicative of AD pathology were quantified as described by Mölsa et al. [34]. We have previously reported diagnostic criteria for limbic NFT dementia [32, 35–38] as well as other disease criteria which we have used previously [32].

Clinical neuropathological diagnoses of DLB and subtype differentiation were based on DLB guidelines [39] and on the findings described in other reports [32, 40, 41]. Among our control brains, there were no pathological findings other than the physiological changes of aging.

BDNF Subtyping

DNA of the autopsied cases was extracted from brain tissues by the phenol-chloroform method. The peripheral blood from the population-based nondemented group of elderly subjects was collected into tubes containing EDTA, and DNA was extracted using a QIAamp DNA Blood Mini kit (Qiagen, Valencia, Calif., USA) and stored at 4°C. *BDNF* genotyping was carried out by means of the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method, according to a procedure used by Ventriglia et al. [7] to analyze Val66Met and by Kunugi et al. [13] to analyze C270T. As for the analysis of ApoE in AD and DLB patients, we have described the methods used and the results obtained in a previous report [32].

Statistics

Statistical analysis was carried out with both the χ^2 test with Yates' correction and Fisher's exact test using 2 × 2 tables. A difference was considered significant, if $p < 0.05$ and the odds ratio had a 95% confidence interval (CI).

Results

Frequencies of Neuropathological Findings

Frequencies and mean ages at the time of death of the neuropathologically diagnosed subgroups are summarized in table 1. The main neuropathological findings of the Brain Bank samples were cerebrovascular disorders (cerebral infarcts and hemorrhages with or without dementia; 38%), AD (36%), and DLB (13%). Two or three types of diagnostic changes were used as criteria for more than one disease. Percentages of each of the main neuropathological diagnoses were similar to those described in our previous report [35]. The age distribution of the Brain Bank and population-based samples was similar.

Frequencies of BDNF Alleles and Genotypes in the Main Neuropathological Disorders

Since only 21 (8%) of the Fukushimura Brain Bank samples showed signs of physiological aging alone, we used the population-based nondemented group of elderly subjects as a control in comparing alleles and genotype frequencies of the *BDNF* gene (table 2a). The genotype distribution for any patient or control group did not deviate significantly from the Hardy-Weinberg equilibrium, excluding Val66Met in the AD group. We do not know whether theVal66Met heterozygotes in AD exceeded the theoretical Hardy-Weinberg equilibrium ratio. Along with this population-based nondemented group of elderly subjects, groups representative of the main neurological diseases, AD, limbic NFT dementia, frontotemporal dementia, DLB, and cerebrovascular disorders, were analyzed for C270T and Val66Met polymorphisms of the *BDNF* gene. Our data show that no group had TT at

Table 2a. Association between C270T and Val66Met polymorphisms of the *BDNF* gene and neuropathological findings compared with those of the population-based control group

	AD	LNTD	FTD	DLB	CVD	Control brains	Population-based controls
C270T							
CC (%)	89 (94)	3 (75)	4 (100)	31 (91)	98 (97)	20 (95)	101 (94)
CT (%)	6 (6)	1 (25)	0 (0)	3 (9)	3 (3)	1 (5)	7 (6)
TT (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Allele C (%)	184 (97)	7 (88)	8 (100)	65 (96)	199 (99)	41 (98)	209 (97)
Allele T (%)	6 (3)	1 (12)	0 (0)	3 (4)	3 (1)	1 (2)	7 (3)
Odds ratio (95% CI)	1.03 (0.34–3.11)	4.26 (0.46–39.53)	–	1.38 (0.35–5.48)	2.22 (0.18–2.89)	/	reference
Val66Met							
Val/Val (%)	25 (26)	1 (25)	1 (25)	12 (35)	28 (28)	6 (29)	35 (32)
Val/Met (%)	58 (61)	3 (75)	1 (25)	17 (50)	55 (54)	10 (48)	53 (49)
Met/Met (%)	12 (13)	0 (0)	2 (50)	5 (15)	18 (18)	5 (23)	20 (19)
Allele Val (%)	108 (57)	5 (63)	3 (37)	41 (60)	111 (55)	22 (52)	123 (57)
Allele Met (%)	82 (43)	3 (37)	5 (63)	27 (40)	91 (45)	20 (48)	93 (43)
Odds ratio (95% CI)	1.00 (0.67–1.48)	1.26 (0.29–5.41)	2.20 (0.51–9.46)	1.15 (0.66–2.00)	1.08 (0.74–1.60)	/	reference

For explanation of abbreviations see table 1.

As compared with population-based controls, no group showed a statistically significant difference ($p < 0.05$, odds ratio with a 95% CI which included 1.0).

Table 2b. Genotype distribution of BDNF C270T and Val66Met polymorphisms in patients with DLB subtypes

	DLB total	Brain stem type	Transient type	Neocortical type	Population-based controls
C270	34	10	14	10	108
CC (%)	31 (91)	9 (90)	12 (86)	10 (100)	101 (94)
CT (%)	3 (9)	1 (10)	2 (14)	0 (0)	7 (6)
TT (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Allele C (%)	65 (96)	19 (95)	26 (93)	20 (100)	209 (97)
Allele T (%)	3 (4)	1 (5)	2 (7)	0 (0)	7 (3)
Odds ratio (95% CI)	1.38 (0.35–5.48)	1.57 (0.18–13.45)	2.30 (0.45–11.65)	–	reference
Val66Met					
Val/Val (%)	12 (35)	4 (40)	5 (36)	3 (30)	35 (32)
Val/Met (%)	17 (50)	5 (50)	7 (50)	5 (50)	53 (49)
Met/Met (%)	5 (15)	1 (10)	2 (14)	2 (20)	20 (19)
Allele Val (%)	41 (60)	13 (65)	17 (61)	11 (55)	123 (57)
Allele Met (%)	27 (40)	7 (35)	11 (39)	9 (45)	93 (43)
Odds ratio (95% CI)	1.45 (0.66–2.00)	1.40 (0.54–3.66)	1.17 (0.52–2.61)	1.08 (0.43–2.72)	reference

As compared with population-based controls, no group showed a statistically significant difference ($p<0.05$, odds ratio with a 95% CI which included 1.0).

C270T (table 2a). For Val66Met, three PCR-FRLP patterns emerged (data not shown). Val/Met comprised around 50% of each group, except for the frontotemporal dementia group, and the distribution pattern was similar to that of the population-based controls. When comparing each main neurological disease group with the findings in the population-based controls, the odds ratio had a 95% CI which included 1.0 (table 2a).

Table 3. Analysis of BDNF SNP patterns with ApoE ε4 status and age at AD and DLB onset

ApoE ε4	AD		DLB		Population-based controls
	+	–	+	–	
C270T					
CC (%)	45 (92)	45 (96)	11 (100)	20 (87)	101 (94)
CT (%)	4 (8)	2 (4)	0 (0)	3 (13)	7 (6)
TT (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Allele C (%)	94 (96)	92 (98)	22 (100)	43 (93)	209 (97)
Allele T (%)	4 (4)	2 (2)	0 (0)	3 (7)	7 (3)
Odds ratio (95% CI)	1.27 (0.36–4.44)	1.54 (0.31–7.56)	–	2.08 (0.52–8.38)	reference
Val66Met					
Val/Val (%)	12 (24)	13 (28)	3 (27)	9 (39)	35 (32)
Val/Met (%)	29 (59)	30 (64)	5 (46)	12 (52)	53 (49)
Met/Met (%)	8 (17)	4 (8)	3 (27)	2 (9)	20 (19)
Allele Val (%)	53 (54)	56 (60)	11 (50)	30 (65)	123 (57)
Allele Met (%)	45 (46)	38 (40)	11 (50)	16 (35)	93 (43)
Odds ratio (95% CI)	1.12 (0.69–1.81)	1.11 (0.68–1.82)	1.32 (0.55–3.18)	1.42 (0.73–2.75)	reference

As compared with the population-based controls, no group showed a statistically significant difference ($p < 0.05$, odds ratio with a 95% CI which included 1.0).

Frequencies of BDNF Alleles and Genotypes in DLB

The DLB group as a whole was not significantly different from the population-based controls with respect to C270T and Val66Met. In a previous report, harboring a Val66Met polymorphism in the *BDNF* gene was described as a genetic risk factor for PD, as in the brain stem type of DLB. Our 34 DLB cases were classified according to their subclass following DLB guidelines [39], supported by information gathered in previous studies [32, 40, 41]. No subgroup showed any remarkable odds ratio inclination (table 2b).

Analysis of BDNF SNP Patterns with ApoE ε4 Status and Age at AD and DLB Onset

From the above, we conclude that having a C270T or Val66Met polymorphism in the *BDNF* gene or in a gene nearby represents a relevant genetic risk factor for developing AD or DLB, particularly in patients with lacking the ApoE ε4 allele, as determined using our Japanese samples. For our AD and DLB patients, we determined whether the ApoE ε4 status was positive or negative, but, as shown in table 3, we could not detect any statistical correlation with the *BDNF* polymorphisms. AD/DLB samples with or without the ApoE ε4 allele were compared with samples from population-based controls using a mean odds ratio (95% CI which included 1.0).

Discussion

BDNF appears to be a trophic factor for mesencephalic dopaminergic neurons and increases their survival, including that of neuronal cells which degenerate in PD [17]. This factor provides for long-term neuronal adaptation by controlling the responsiveness of its target neurons to the important neurotransmitter, dopamine. BDNF has been shown to control dopamine D3 receptor expression and to trigger behavioral sensitization [42]. BDNF also modulates hippocampal plasticity and hippocampus-dependent memory in cell models and in animals [8]. Moreover, the decrease in the transcript level of BDNF mRNA in hippocampi of individuals with AD was verified with an RNAase protection assay, suggesting that BDNF may contribute to the progression of cell death in AD patients [3].

A report appearing in 2001 [13] showed that C270T functional promoter polymorphism in the *BDNF* gene represented an AD risk factor. In 2002, other groups [7, 23] reported independently that an SNP at Val66Met in BDNF raises the likelihood of AD [7] or PD [23]. Moreover, a functional analysis demonstrated a role for BDNF and its Val66Met polymorphism in human episodic memory and hippocampal function and suggested that Val66Met exerts these effects by impacting intracellular trafficking and activity-dependent secretion of BDNF [8].