observation period and a larger number of intervention participants, increasing statistical power. In addition, our analyses adjusted for all these confounders and the protective effect of the supplementation intervention still remained significant.

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Memories of falling in elderly patients with dementia: response concordance rate and reproducibility.

Otaki M[1], Moriguchi K, Lebowitz A, Asada T.

Author information

Abstract

BACKGROUND: Although demented elderly patients have impaired memory, memories of falling are not uncommon. We aim to clarify reliability of memories of falling in such patients.

METHODS: Subjects included 62 patients (18 men, 44 women) diagnosed with dementia who resided in long-term care facilities. Mean age was 82.9 ± 7.8 years, mean Mini-Mental State Examination score was 16.4 ± 4.7 points, and mean Functional Independence Measure score was 67.9 ± 18.4 points. Subjects were asked a closed question about whether they were aware of having fallen (i.e. memories of falling) over the past year. Fear of falling was quantified using the visual analogue scale and FACES Pain Rating Scale. Scores were re-measured during retest approximately 10 days later to examine reproducibility of memories and fear of falling. Subjects whom staff had witnessed suffering a fall between baseline and retest session were excluded.

RESULTS: Fall memory concordance rate was 0.84, visual analogue scale reproducibility (correlation coefficient) was 0.98, and FACES Pain Rating Scale was 0.86. No differences in Mini-Mental State Examination scores were noted between groups for whom memories of falling were or were not reproducible. No correlation was observed among Mini-Mental State Examination, Functional Independence Measure scores, and intensity of fear of falling.

CONCLUSIONS: There was a high concordance rate for patients' memories of falling, which suggests that falls were retained as memories. No relationship was observed between memories of falling and degree of cognitive impairment, and severe dementia did not necessarily imply memories of falling were unreliable. The reproducibility of fear of falling suggested the intensity of fear of falling was not easily altered. It was possible memories of falling and fear of falling mutually interacted to reinforce and fixate with each other, leading to the observed phenomenon. Therefore, it appeared memories of falling were retained by patients; this fact can be used in fall prevention and vital function maintenance.

KEYWORDS: concordance in memories of falling; dementia; fear of falling; memories of falling; reproducibility of fear of falling

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Regular Article

Multicenter population-based study on the prevalence of early onset dementia in Japan: Vascular dementia as its prominent cause

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Aim: In Japan, the government and media have become aware of the issues of early onset dementia (EOD), but policies for EOD have not yet been established and support systems are inadequate. To provide practical data about EOD, a two-step postal survey was performed.

Methods: A questionnaire requesting information on EOD cases was sent to target institutions in five catchment areas in Japan. According to the answers from the institutions, we estimated the prevalence of EOD using census data and determined the illnesses causing EOD. As a quality control study, the authors reviewed every diagnosis in a quarter of the reported cases using the medical and psychiatric records and neuroimaging data. This study was conducted from 2006 to 2007.

Results: Information from 2469 patients was collected from 12 747 institutions, and 2059 subjects with EOD were identified. The estimated prevalence of EOD was 47.6 per 100 000 (95% confidence interval, 47.1–48.1) for all of Japan. Of the illnesses causing EOD, vascular dementia (VaD) was the most frequent (39.8%), followed by Alzheimer's disease.

Conclusions: The prevalence of EOD in Japan appeared to be similar to that in Western countries. However, unlike previously reported international experience, VaD was the most frequent cause of EOD in all catchment areas in Japan.

Key words: Alzheimer's disease, early onset dementia, prevalence, vascular dementia.

IN DEVELOPED COUNTRIES, dementia with onset before the age of 65 years, defined as early onset dementia (EOD), has presented a unique challenge to society and those who care for such individuals.[1]

In Japan, although several reports have described the prevalence of EOD and the frequency of illnesses causing EOD, their results differ depending on the study settings. Two university-hospital-based studies reported that the most common dementia diagnosis

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was Alzheimer's disease (AD).[2,3] On the other hand, one community-based study and one nationwide study, including five catchment areas, reported that the most frequent illness causing EOD was VaD.[4,5] Recently, we reported on a population-based study in a single catchment area with a population of 3 million.[6] Our study revealed also that vascular dementia (VaD) was the most common cause of EOD. Using the same methodology in a much larger population of over 9 million, we estimated the prevalence of EOD and examined the prominence of VaD among illnesses causing EOD.

METHODS

This study was conducted in five catchment areas in Japan: Ibaraki (population, 3 million), Gunma (2 million), Toyama (1 million), Ehime (1.5 million) and Kumamoto (1.8 million). These areas are representative of Japan's geographic, economic and educational composition. The productive-age population ratio of all Japan was 65.5 in 2006 and 65.0 in 2007, and in those five areas the average was 63.1 (range 61.3–66.0). Therefore, in order to reduce the influence of biased sample populations, prevalence in each area was adjusted using the standardized population. EOD subjects were defined as those whose age at onset and age on the census day was less than 65 years. The observation period in each area was 6 months: from 1 April to 31 October 2006 for Ibaraki and Gunma, from 1 April to 31 October 2007 for Toyama, and from 1 July to 31 December 2007 for Ehime and Kumamoto (Fig. 1). The reason why this period was employed was to allow direct comparison with a previous Japanese EOD study, which used 6 months.[5]

The survey was approved by the local ethics committees, including those of the University of Tsukuba, Kumamoto University, Ehime University, Gunma University, and Toyama Medical Association.

Step 1

A questionnaire was mailed to all of the following: medical institutions (including psychiatric and neurological hospitals and clinics), home-visit nursing services, long-term care insurance (LTCI)-related facilities, local branches of prefectural health, and local welfare commissioners. In Japan, all care services for community-dwelling individuals with EOD are provided by a publicly funded LTCI, which is separate from medical care insurance.

Each institution was asked, 'How many EOD patients did you care for in the last 6 months?' The criteria for the diagnosis of dementia were based on the DSM-III-R.[7]

Step 2

For the second step, respondent institutions with one or more cases were asked to provide additional patient data, including: initials, demographics, coexisting illnesses, duration and type of dementia, illnesses causing dementia (in the case of VaD, specifying the subtype of cerebrovascular disease [CVD]), severity of dementia, and functional status. Patients were then classified into subgroups according to the cause of dementia. AD, vascular dementia and alcohol-related dementia were defined according to the DSM-IV.[8] It is noteworthy that, in contrast to other VaD criteria, including National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherché et l'Enseignement en Neurosciences,[9] the DSM-IV criteria for VaD requires neither temporal relation between dementia and recognized stroke nor progressive cognitive decline. Dementia with Lewy bodies (DLB) and Parkinson's disease with dementia (PDD) were diagnosed according to the revised criteria for the clinical diagnosis of dementia with Lewy bodies,[10] and frontotemporal lobar degeneration (FTLD) was diagnosed according to the Lund and Manchester Criteria.[11] Finally, patients fulfilling the DSM-III-R criteria for dementia but not fulfilling criteria for any of the above diagnostic categories were designated 'Other'. Individuals with two or more comorbid diseases causing dementia, such as AD with VaD, were classified as 'overlap' and included in the 'Other' category.

The age at onset of disease was defined as the age of the patient at which the earliest conclusive dementia symptom was noticed by caregivers or other close informants.

Determination of dementia severity was based on the original manuals used by a previous Japanese EOD study[5] for comparison. Three stages of severity were defined as follows. Mild: the person can mostly live independently, with adequate personal hygiene and relatively intact judgment, but social activities and employment are both significantly impaired. Moderate: independent living is fraught with hazard to the extent that supervision is required. Severe: there is severe impairment of daily activities and continual supervision is needed.

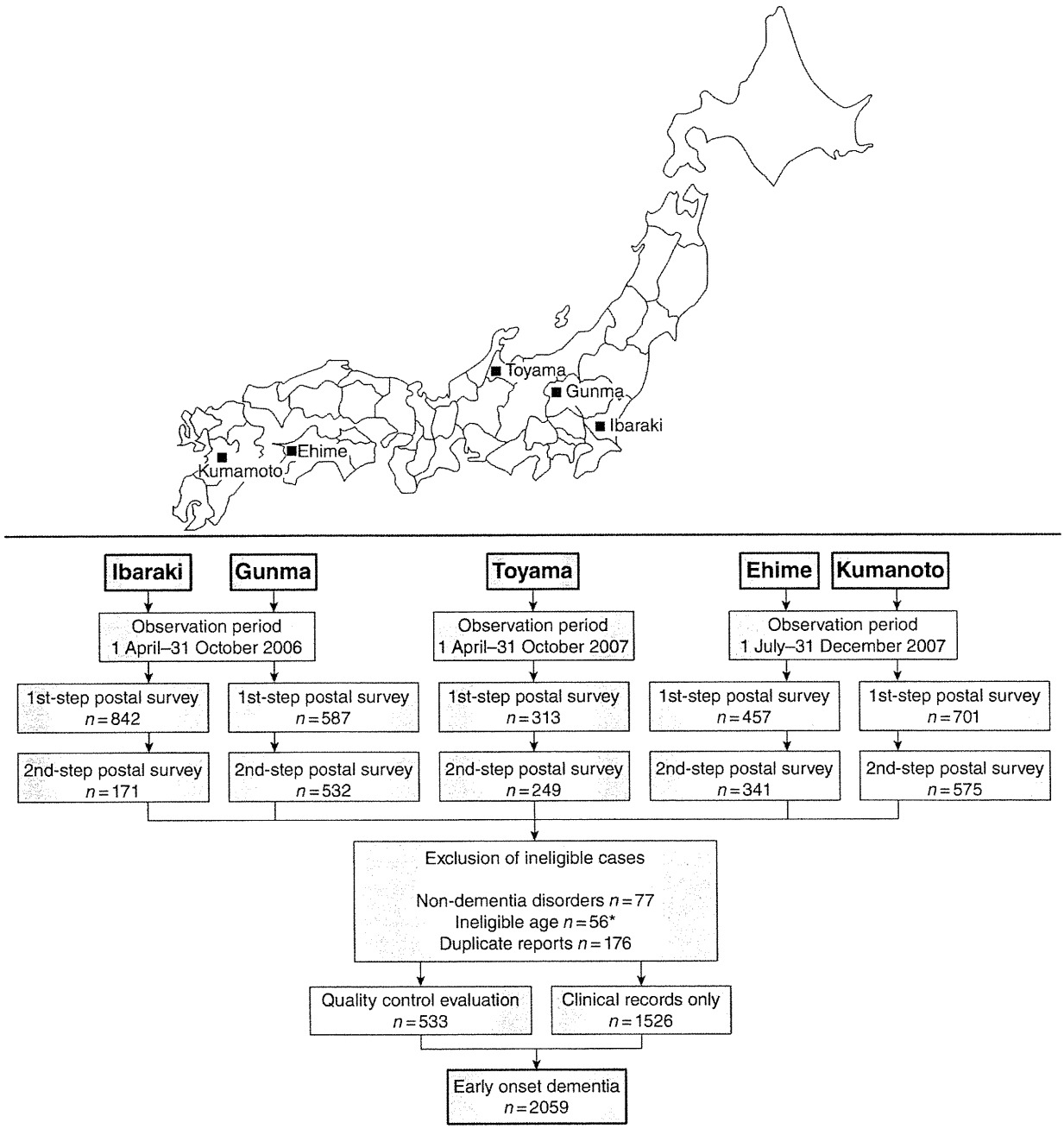

Figure 1. Map of Japan and schedule of each catchment area's survey.

Answers to the additional information for reported cases from non-medical institutions were based on comments by the consulting physicians.

It should be noted that in Japan acute illnesses, including stroke, are diagnosed and managed initially in hospitals then intensive rehabilitation units, prior to discharge home or to longer-term care in LTCI institutions. Degenerative illnesses are usually managed in specialist hospital outpatient clinics, prior to LTCI institutions for advanced stages. Hence,

almost all patients in this study would have received specialist evaluation at some stage of their illness, and hence their assigned diagnoses should be clinically accurate.

Quality control

In order to validate the accuracy of reported diagnoses, we conducted a quality control (QC) study using data from a quarter of the reported cases. We selected the institutions for this sub-study in descending order of reported case numbers. The authors of this paper visited such institutions and reviewed the patients' medical and psychiatric records and neuroimaging data, including magnetic resonance imaging (MRI), computed tomography (CT) and single photon emission computed tomography (SPECT). A separate diagnosis was made independently for each subject. In this way, the accuracy of the diagnosis of the attending physician from each institution could be evaluated.

Statistical analysis

The data to estimate the prevalence are based on the last governmental reports before the start of the observation period. The reports were published on 1 April 2006 for Ibaraki, on 1 October 2006 for Gunma and on 1 October 2007 for Toyama, Ehime, Kumamoto and the whole of Japan. The population denominators used were derived from census data of the target areas.

In each area, in order to reduce sampling bias due to case reporting failures, we adjusted using the response rates. The reciprocal of the product of the response rate for steps 1 and 2 (sample weight) was calculated, and the number of EOD patients was estimated using the sample weight multiplied by the reported number of cases as follows.

n_{ij} = reported number of dementia cases by area i and age strata j

w_i = sampling weight of area $_i$

P_{ij} = population of area i and age strata j.

We defined the estimated number of dementia cases of area i, age strata j as $m_{ij} = w_i n_{ij}$.

and the estimated prevalence per X as $\hat{\lambda}_{ij} = \frac{m_{ij}}{P_{ij}} X$.

Then, the estimated prevalence was adjusted by the standardized population, and the weighted average prevalence was calculated for the purpose of reducing the influence of different population distributions as follows.

Tj = all Japan population of age strata j at study period $S_j = \frac{Tj}{\sum_j Tj}$.

The estimated prevalence adjusted by the standardized population in area i was obtained by $\hat{T}_i = \sum_j S_j \hat{\lambda}_{ij}$.

We defined the population of area i as $Pi = \sum_j P_{ij}$.

The weighted average prevalence was obtained by $\hat{T} = \sum_i \Phi_i \hat{T}_i$

and $\Phi_i = \frac{\sum_j P_{ij}}{\sum_{ij} P_{ij}}$.

The EOD prevalence for the total Japanese population was estimated by integration of the adjusted prevalence in the five catchment areas. We regarded this prevalence as the Japanese standardized prevalence.

We calculated 95% confidence intervals (CI) based upon a standard normal distribution. The significance of differences between rates was estimated by χ^2-test or Fisher's exact tests. All analyses were carried out using SAS version 9.1 (SAS Institute, Cary, NC, USA) and R version 2.8.1 (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

As shown in Table 1, information from 2469 patients was collected from 12 747 institutions. Approximately 50% of the diagnoses were made in hospitals or clinics, and only 10% by general practitioners. For the remaining cases mainly cared for in LTCI institutions, diagnoses were made by either specialists or general practitioners to consider the appropriateness of their admission before the patients moved into their LTCI institutions.

After careful review of the answer sheets, patients with the following diagnoses were excluded: schizophrenia ($n = 8$), developmental disorder ($n = 38$), depression ($n = 6$), and other non-dementia disorders ($n = 25$). None of these patients were considered to have had concomitant EOD. Fifty-six patients were excluded because their age on the census day was over 65, although their age at onset of dementia was less than 65.

We received reports from two or more institutions for the same 157 cases. Consequently, 176 reports for

Table 1. Response rates of the postal surveys

Institutions	Step 1			Step 2			
	Target population	$n^{\dagger}$	Response rate (%)	Target population	$n^{\dagger}$	Response rate (%)	Reported cases
Hospitals	1 489	1 231	(82.7)	254	210	(82.7)	1429
Clinics	5 573	4 622	(82.9)	151	119	(78.8)	276
Health service facilities	385	326	(84.7)	95	81	(85.3)	185
Special nursing homes	919	847	(92.2)	137	112	(81.8)	214
Group homes	812	733	(90.3)	97	78	(80.4)	123
Welfare service center for disabled people	464	427	(92.0)	12	11	(91.7)	115
Day center	362	332	(91.7)	45	37	(82.2)	66
Home-visit nursing facilities	488	266	(54.5)	38	35	(92.1)	62
Welfare living centers	356	316	(88.8)	47	42	(89.4)	80
Government services	156	139	(89.1)	13	12	(92.3)	90
Local welfare commissioners	201	186	(92.5)	14	9	(64.3)	28
Care managers	1 542	1 156	(75.0)	174	147	84.5)	233
Total	12 747	10 582	(83.0)	1077	893	(82.9)	2901

†Number of respondent institutions.

the 157 cases were excluded. Among these cases, nine received different diagnoses according to the informants: AD and DLB for four cases, AD and brain infection for one, AD and Behçet's disease for one, AD and FTLD for one, AD and alcohol-related dementia for one, and VaD and alcohol-related dementia for one. Overall percent agreement of diagnosis for the 157 doubly or triply reported cases was 95.1%, and the percent for 40 of the 157 patients with diagnosis of VaD was as high as 97.5%.

For the cases lacking diagnostic agreement, we prioritized the diagnoses according to the following order: diagnosed by neurologists or psychiatrists at general hospitals, including university hospitals; diagnosed by psychiatrists or neurologists; diagnosed by physicians at general hospitals; diagnosed by physicians at clinics; and diagnosed by physicians from other health-care facilities. The final sample population comprised 2059 subjects (61.0% male). The mean age and age at dementia onset on the census day were 56.4 years (SD, 8.0; range, 18–64 years) and 51.3 years (9.8; 18–64 years), respectively.

As shown in Figure 2, of the illnesses causing EOD, VaD was the most frequent (40.1%), followed by AD (24.3%), head trauma (8.4%), FTLD (3.6%), alcohol-related dementia (3.2%), DLB/PDD (2.8%) and others (14.2%). The 'Other' category included seven subcategories: dementia secondary to neurodegenerative disorders (4.4%), for example, spinocerebellar degeneration, multiple system atrophy and progressive supranuclear palsy; infection (3.1%); surgery for brain tumor (1.9%); hypoxia (1.4%); other organic brain syndrome (2.9%), for example, normal pressure hydrocephalus and epilepsy; unknown dementia (3.4%); and overlap (0.5%). Six patients with both AD and VaD were included in the overlap category. The main subtypes of VaD were single large infarction (37.3%), intra-cerebral hemorrhage (35.7%), and subarachnoid hemorrhage (18.6%) (Fig. 2). Table 2 shows the prevalence rate of AD and VaD by sex for each catchment area. The most frequent illness causing EOD was VaD for men in all catchment areas, and AD for women in four areas. There was no significant difference in the distribution of VaD and AD for both sexes among the catchment areas. The prevalence of dementia in terms of dementia severity and the ratio for living places are shown in Table 2.

The QC evaluations were performed for 545 EOD individuals (26.5%). The percentage of agreement between the authors and doctors at the selected institutions for diagnosis of overall dementia was 98.9% and for VaD, it was 100%. The frequency of illnesses

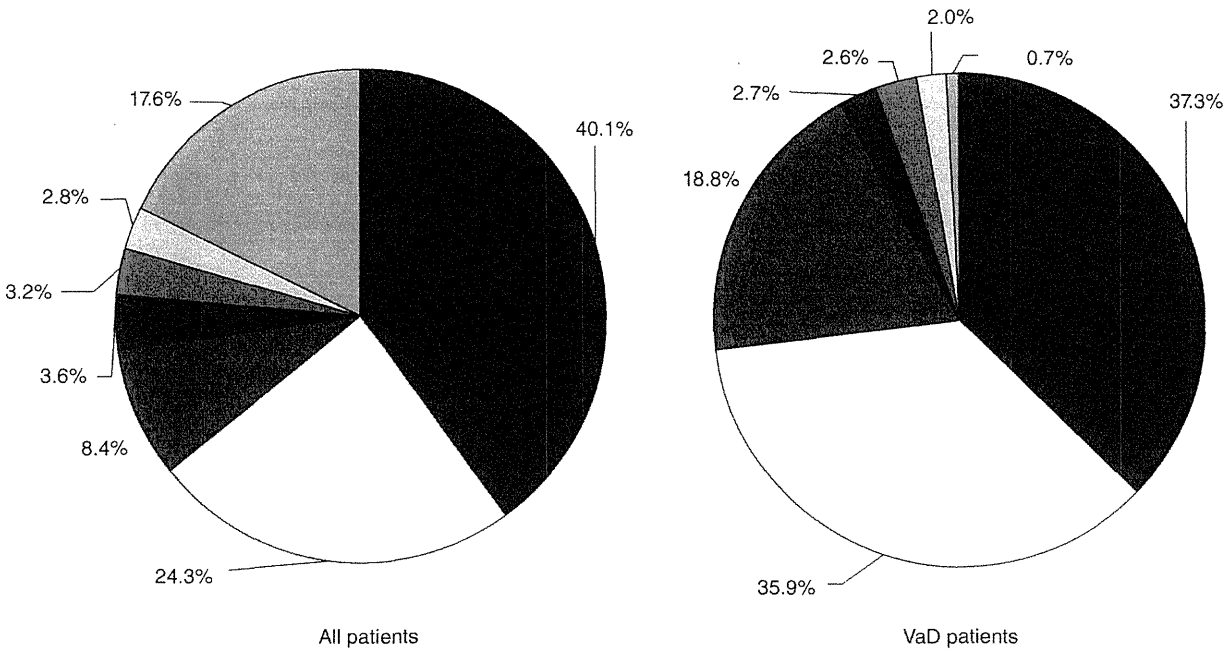

Figure 2. Distribution of diagnoses. All patients: (■) vascular dementia (VaD); (□) Alzheimer's disease; (■) Head trauma; (■) frontotemporal lobar degeneration; (■) alcohol-related dementia; (□) dementia with Lewy bodies/Parkinson's disease with dementia; and (■) Others. VaD patients: (■) Large cortical infarct; (□) Cerebral hemorrhage; (■) Subarachnoid hemorrhage; (■) Unspecified; (■) Mixed cerebrovascular disease; (□) Multiple infarction; and (■) Others.

causing EOD was calculated for two subgroups among the target individuals: university hospitals ($n = 252$) and others ($n = 293$). There were significant differences between the two groups ($P < 0.0001$): higher frequencies of AD (46.0%) and DLB (11.9%) and lower frequencies of VaD (6.3%) for the university hospital group. We reviewed CT or MRI images for 26.5% of patients during the 6-month study period and 18.6% after 6 months retrospectively because we offered quality control after we received the reports from institutions.

The total estimated number of patients adjusted by the standardized population of Japan was calculated to be 37 800. The prevalence rate in those aged 18–64 years was 47.6 per 100 000 (95%CI, 45.5–49.7). From the age of 30 onwards, the prevalence rate of dementia approximately doubled with each 5-year increase in age (Table 3).

DISCUSSION

To our knowledge, this is the largest population-based epidemiological study targeting EOD. There was no significant difference between our study and those from Western countries (Table 4) in the prevalence of all types of EOD combined.[4,5,12–17]

The proportion of illnesses causing EOD was quite different from the UK. Harvey *et al.*[16] reported causes there as AD 34%, VaD 18%, FTLD 12%, DLB 7%, alcoholic dementia 10%, and others 19%. Ratnavalli *et al.*[15] reported that primary degenerative dementias accounted for 71%, of which 35% were AD and 22% were FTLD. Namely, our study showed prominence of VaD, especially in men.

A nationwide study of Japanese EOD prevalence in 1997 also reported a higher prevalence of VaD (43.9%) than AD (16.8%).[5] The Strategies against Stroke Study for Young Adults in Japan (SASSY-Japan) used data from 7245 stroke patients from 18 centers and compared the salient features of stroke in younger (<50 years old) and older groups (<51 years old).[18] The SASSY-Japan study reported that male sex was a risk factor for the younger group. Even in Western countries, men have higher stroke prevalence than women, especially at young ages.[19]

Table 2. Comparison of five catchment areas

	Total	Ibaraki	Gunma	Toyama	Ehime	Kumamoto	*P*-value
Total population (all ages)	9 370 651	2 965 931	2 019 120	1 105 312	1 452 000	1 828 288	–
Target population aged 18–64 years, male (%)	5 664 741 (50.2)	1 862 942 (51.2)	1 238 395 (50.9)	654 646 (50.3)	848 641 (49.1)	1 060 137 (48.6)	–
Estimated number of patients	2 965	761	748	258	504	694	–
Prevalence† for age range 18–64	52.4	40.8	60.4	39.4	59.4	65.5	–
Prevalence† for age range 45–64	103.2	83.3	121.0	81.6	114.7	120.8	–
Prevalence† of AD and VaD by sex							
Male							
VaD	26.0	23.5	40.2	14.6	29.5	29.8	0.012
AD	9.7	9.0	11.8	12.1	14.3	8.0	0.448
Female							
VaD	11.9	12.0	14.1	7.4	12.6	16.7	0.675
AD	13.4	12.9	16.7	13.4	11.9	17.7	0.779
Both sexes							
VaD	19.1	18.1	27.4	11.0	21.3	23.1	0.113
AD	11.6	10.9	14.2	12.8	13.1	13.0	0.978
Severity of dementia							
Mild	24.3%	25.3%	24.3%	19.0%	22.8%	25.0%	–
Moderate	33.2%	29.0%	36.3%	29.9%	32.6%	37.9%	–
Severe	35.5%	36.0%	34.5%	46.0%	39.2%	29.4%	–
Living places							
Hospitalized and institutionalized	29.4%	36.8%	21.2%	30.8%	47.2%	35.8%	–
Living at home	38.3%	47.5%	62.1%	42.2%	40.5%	59.1%	–
Missing	32.3%	15.7%	16.7%	27.0%	12.3%	5.1%	

†Prevalence per 100 000 population.
AD, Alzheimer's disease; VaD, vascular dementia.

Although several explanations, including the role of estrogen, have been proposed, the true reason why Japanese men are more vulnerable to stroke than women remains an open question. At any rate, the high frequency of VaD in men accounts for the main result. On the other hand, it should be noted that AD prominence in women was observed in four of the five areas. Another important issue is the difference between presenile and senile populations in Japan in the pathogenesis of VaD. The SASSY-Japan reported that cerebral and subarachnoid hemorrhage were the major cause of presenile stroke, whereas lacunar infarction was the major cause in senile stroke victims. Our study also revealed that cerebral and subarachnoid hemorrhage were the major cause of EOD. Additionally, a population-based study of persons aged 65 years and older in a Japanese community found that the most frequent illness causing VaD was multiple lacunar infarction.[20] Taken together, the causes of stroke in the younger population appear to be quite different from those affecting the older population.

Our QC study and the examination of doubly or triply reported cases showed a high concordance between the diagnosis of illnesses causing EOD in general and VaD in particular. The QC also revealed that the most common EOD-causing illness was AD for all of the five university hospitals, which replicated the results of previous university-hospital-based EOD studies in Japan.[2,3] On the other hand, VaD was the leading cause for patients in the non-university hospitals. Considering the above-described Japanese medical system for acute and degenerative illnesses, this difference may be understandable. A possible reason for the discrepancy between the university-hospital-based diagnoses and those in other institu-

Table 3. Prevalence of early onset dementia in Japan

Japanese population (thousands)			All causes of dementia			Male			Female		
Age range, years	Total	Male	Female	$n^{\dagger}$	Prevalence	95%CI[‡]	n	Prevalence	95%CI	n	Prevalence	95%CI
18–19	2 618	1 341	1 277	21.6	0.8	(0.5–1.3)	21.9	1.6	(1.1–2.5)	0.0	0.0	0.0–0.3
20–24	7 238	3 716	3 521	367.3	5.1	4.6–5.6	289.5	7.8	6.9–8.7	78.6	2.2	1.8–2.8
25–30	7 795	3 967	3 828	451.6	5.8	5.3–6.4	330.5	8.3	7.5–9.3	120.3	3.1	2.6–3.8
30–34	9 363	4 748	4 615	552.6	5.9	5.4–6.4	434.9	9.2	8.3–10.1	117.0	2.5	2.1–3.0
35–39	9 426	4 763	4 663	839.8	8.9	8.3–9.5	539.2	11.3	10.4–12.3	301.8	6.5	5.8–7.2
40–44	8 220	4 141	4 079	1 218.4	14.8	14.0–15.7	766.3	18.5	17.2–19.9	455.6	11.2	10.2–12.2
45–49	7 733	3 879	3 854	2 094.9	27.1	26.0–28.3	1 303.7	33.6	31.8–35.5	795.5	20.6	19.3–22.1
50–54	8 051	4 018	4 033	4 163.6	51.7	50.2–53.3	2 737.3	68.1	65.6–70.7	1 407.9	34.9	33.1–36.8
55–59	10 433	5 162	5 271	12 006.8	115.1	113.0–117.2	7 460.2	144.5	141.3–147.8	4 492.8	85.2	82.8–87.8
60–64	8 473	4 130	4 343	16 036.9	189.3	186.2–192.1	9 173.5	222.1	217.6–226.7	6 740.3	155.2	151.5–158.9
18–64	79 350	39 865	39 484	37 753.5	47.6	47.1–48.1	23 056.9	57.8	57.1–58.6	14 509.8	36.7	36.2–37.4
45–64	34 690	17 189	17 501	34 302.2	98.9	97.8–99.9	20 674.7	120.3	118.7–121.9	13 436.5	76.8	75.5–78.1

[†]Estimated number of patients. [‡]95%CI: based on standard normal distribution. CI, confidence interval.

tions might be that cerebrovascular disease as an underlying illness of VaD is a common disease in middle age, so patients usually get medical treatment in general hospitals in Japan. On the other hand, early onset AD and DLB are still difficult to diagnose, so patients are referred from general hospitals or clinics to university hospitals for detailed examination.

The prevalence of FTLD in this study was lower than that in the UK (15.4%)[15,16] and the Netherlands (15.1%).[17] One possible reason is the rarity of familial FTLD cases in Japan, but otherwise the cause of this finding remains unknown.[21]

A limitation of the current study is that we could not confirm the accuracy of the diagnosis by neuropathological examination. Thus it remains possible that pathological diagnoses might alter the distribution due to mixed pathologies,[22] and vascular lesions might co-exist with other pathologies reducing the

Table 4. Comparison of prevalence of dementia per 100 000 in the 30–64-year-old age group among studies

Authors	Year	Country	Place	Age range	Population at risk	n	Prevalence	Target
Mölsä *et al.*[12]	1982	Finland	Turku	45–54	–	10	51.0	All dementia
				55–64		24	144.0	–
Kokmen *et al.*[13]	1989	USA	Rochester	45–49	–	2	77.0	All dementia
				50–54	–	1	40.0	–
				55–59	–	2	86.0	–
				60–64	–	5	249.0	–
Newens *et al.*[14]	1993	UK	Northern Health Region	45–64	655 800	227	34.6	AD
Ohshiro *et al.*[4]	1994	Japan	Tottori	40–64	209 621	100	81.4	All dementia
Ichinowatari *et al.*[5]	1997	Japan	5 catchment areas	18–64	3 729 706	1203	48.1	All dementia
Ratnavalli *et al.*[15]	2002	UK	London	45–64	326 019	59	81.0	All dementia
Harvey *et al.*[16]	2003	UK	–	30–64	240 766	130	54.0	All dementia
Rosso *et al.*[17]	2003	Netherlands	Zuid-Holland	30–59	1 435 769	21	1.5	FTLD
Present study	2009	Japan	5 catchment areas	18–64	9 370 651	2059	47.6	All dementia

AD, Alzheimer's disease; FTLD, frontotemporal lobar degeneration; VaD, vascular dementia.

overall significance of vascular disease as a sole cause of the cognitive impairment. In addition, although EOD is likely to come to medical attention, it is possible that a certain proportion of individuals with EOD might not have been detected. For the purpose of reducing such referral bias, case ascertainment was thoroughly made by surveying both medical institutions and non-medical (LTCI) facilities. As a result, the present study attained very high response rates.

Finally, in Japan the government and media have become aware of the issues of EOD, but policies for EOD have not yet been established and support systems for early onset dementia are inadequate. We hope this study may provide, not only for Japan but also policy-makers in other countries, basic data to estimate budgets for evaluating and enabling an optimal EOD health-care policy.

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Increased Levels of Plasma p3-Alcα35, a Major Fragment of Alcadeinα by γ-Secretase Cleavage, in Alzheimer's Disease

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Abstract. p3-Alcα is a metabolic fragment of Alcadeinα (Alcα). Similar to the generation of the p3 fragment from amyloid-β protein precursor (AβPP) processing, Alcα is cleaved by α- and γ-secretases, leading to the secretion of p3-Alcα peptides into cerebrospinal fluid (CSF). p3-Alcα is also detected in the plasma, similar to amyloid-β (Aβ), which is a metabolic fragment of AβPP cleaved by amyloidogenic β- and γ-secretases. Because p3-Alcα is a non-aggregatable and stable peptide, unlike aggregatable Aβ and metabolically labile p3 of AβPP, the changes of p3-Alcα in quality and/or quantity in CSF and plasma are expected to be a marker for assessing alteration of substrate cleavage by γ-secretase, such as Aβ generation from AβPP. The present study describes a sandwich enzyme-linked immunosorbent assay for quantifying levels of p3-Alcα35, the major form of the p3-Alcα species, and examines levels of p3-Alcα35 in the plasma of three independent Japanese cohorts. In two of the three cohorts, the p3-Alcα35 levels were significantly increased with a concomitant decrease in the Mini-Mental State Examination score, or in clinically diagnosed Alzheimer's disease (AD) patients, when compared with age-matched non-demented subjects. The values were significantly lower in AD subjects who were administered donepezil, when compared to AD subjects without donepezil treatment. The increase in plasma p3-Alcα35 levels may indicate an endophenotype in subjects in whom AD is due to a progressing cognitive impairment in subjects with a γ-secretase malfunction, or a disorder of the clearance of peptides.

Keywords: Alzheimer's disease, alcadein, diagnosis, donepezil, γ-secretase, p3-Alc, plasma biomarker

INTRODUCTION

Alcadeins (Alcα, Alcβ, and Alcγ, also called calsyntenin or XB31) constitute a family of neural type I transmembrane proteins, all of which are encoded by their respective genes, and are highly conserved among mammals [1, 2]. Both Alcα and the amyloid-β protein precursor (AβPP), which is involved in Alzheimer's

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disease (AD), function as cargo receptors for the kinesin-1 motor that transports membrane vesicles anterogradely in neurons [3–6]. Alc is subject to proteolytic processing by a combination of AβPP α- and γ-secretases, but not an amyloidogenic combination of β- and γ-secretases [7]. This processing of Alc secretes a large amino-terminal extracellular domain and small p3-Alc peptide, along with the intracellular release of the cytoplasmic domain fragments, AlcICDs [8]. In neurons, Alc and AβPP form a complex mediated by the cytoplasmic interaction of X11-like (X11L), a neural adaptor protein which stabilizes proteolytic cleavage of both proteins, and facilitate intracellular colocalization of both membrane proteins in the neuron [2, 9, 10]. X11L (also called X11β, Mint2, or APBA2) was recently reported as a possible candidate of ApoE4-related late-onset AD effector [11]. The integrated genomic approach of late-onset/sporadic-type AD suggests that the ApoE4 variant is involved in the intracellular trafficking of AβPP, in which X11L plays an important role [12–14].

In transporting membrane vesicles in the late secretory pathway, plasma membrane, or in the endocytotic recycling pathway, Alc and AβPP are likely to be cleaved by primary secretases. AβPP is cleaved by α-secretase ADAM 10 and ADAM 17, or β-secretase BACE, to leave the membrane associated AβPP carboxy-terminal fragment (AβPP CTFα or CTFβ) [15], while Alc is cleaved by only α-secretase to leave Alc CTF [7]. All CTFs are further cleaved by γ-secretase to secrete a p3 peptide from AβPP CTFα, Aβ peptide from AβPP CTFβ, and p3-Alc from Alc CTF into the exracellular milieu [7, 8]. Therefore, Alc and AβPP perform similar functions [5], show large colocalization in the neuron [2], and are subject to almost the same regulation of proteolytic processing [9]. This suggests that some alteration in the processing systems of the substrates in specific regions of the brain, or the malfunction of the clearance system for secreted short peptides, may appear in the qualitative and/or quantitative alteration of metabolic products derived from AβPP and Alc almost equivalently [16]. In fact, recent reports describe that γ-secretase dysfunction and/or malfunction of the Aβ clearance system are observed in AD patients [16–19], suggesting that some AD pathogenesis is due to an altered membrane substrate cleavage, or a clearance failure of the cleaved products.

The p3 of AβPP is metabolically labile to detect in the cerebrospinal fluid (CSF) and plasma, and Aβ (in particular the more AD-pathogenic $Aβ_{42}$) is progressively aggregatable to detect quantitative or qualitative changes in the plasma. Aβ is a causative metabolic peptide of AD, which detects qualitative and quantitative alterations in the CSF and/or plasma, and is very important in diagnosing AD patients [20]. However, the aggregatable form of this peptide, and various aggregated soluble Aβ oligomers, make it difficult to investigate the alteration of Aβ levels in body fluids precisely. Instead of Aβ, non-aggregatable p3-Alc can be available as a surrogate marker for the detection of changes in the quality and quantity of the γ-cleavage of substrates.

In the adult brain, the expression of Alcα and Alcβ is more prominent than Alcγ, and p3-Alcα and p3-Alcβ are also more prominent in the CSF than p3-Alcγ [7]. In human CSF, p3-Alcα35 is the major peptide among several p3-Alcα species, while p3-Alcβ37 and p3-Alcβ40 are the major products of Alcβ [7, 16]. We previously developed a sandwich ELISA (sELISA) system for quantifying the total amount of p3-Alcα [21]. This sELISA was constructed with pan-p3-Alcα antibodies that can quantify the total amount of all of the p3-Alcα species, including the major p3-Alcα35, and the minor p3-Alcα species in the CSF and plasma. However, the sELISA cannot selectively quantify specific species such as p3-Alcα35 or p3-Alcα38. Despite the restricted conditions of the sELISA, several trials using patients' samples have provided significant information about p3-Alcα: (a) the total p3-Alcα level in the plasma correlates with the level in the CSF of individuals, (b) the total p3-Alcα level correlates with the levels of $Aβ_{40}$, an Aβ species that is less aggregatable than $Aβ_{42}$, in the CSF and plasma samples of several cohorts, and (c) the CSF and total plasma p3-Alcα levels of several cohorts increases in AD patients, when compared to age-matched control subjects [21–23]. These observations suggest that changes in p3-Alcα levels in body fluids may be able to diagnose AD status. The present study develops a novel monoclonal antibody which specifically recognizes p3-Alcα35 and establishes a new sELISA system for quantifying p3-Alcα35 levels. The plasma p3-Alcα35 levels of AD patients, mild cognitive impaired (MCI), and non-demented subjects of three independent Japanese cohorts will be investigated, in addition to the fluctuation of p3-Alcα35 levels in AD subjects treated with donepezil.

METHODS

Antibodies and the ELISA system

p3-Alcα35 is a peptide that includes the sequence from Ala817 to Thr851 of the human Alcα1. The

monoclonal mouse antibody was raised against an antigen peptide containing the sequence between positions Asn839 and Thr851. The antibody reacts with the antigen peptide specifically, but not with another peptide containing the sequence between positions Asn839 and Ile854 (for amino acid sequence of p3-Alcα, see [7]). Clone 63A1 was selected among several clones showing specific reactivity to p3-Alcα35. The affinity-purified antibody 63A1 was used to capture p3-Alcα35. The horseradish peroxidase-conjugated pan-p3-Alcα rabbit polyclonal antibody 817, which was raised against a peptide containing the sequence between positions Ala817 and Val822 [21], and tetramethyl benzidine were used to detect the captured p3-Alcα35.

Total amount of p3-Alcα was quantified with an ELISA kit of pan-p3-Alcα monoclonal antibody to capture all p3-Alcα species, which was supplied from Immuno-Biological Laboratories Co., Ltd. This ELISA kit is different from our previous assay system in which pan-p3-Alcα polyclonal antibody 839 is used to capture all p3-Alcα species [21].

Blood samples were collected from the subjects into tubes containing EDTA and centrifuged. Two hundred microliters of plasma was used per duplicate assay. p3-Alcα was extracted from the plasma, as described for the total extraction of p3-Alcα for ELISA [21], and quantified for p3-Alcα35 using the new sELISA system, in duplicate.

Cohort information

The first cohort (Cohort 1, $n = 135$) is largely composed of MCI and AD patients, many of whom are not hospitalized and living in the countryside together with non-demented control subjects; the second cohort (Cohort 2, $n = 252$) is largely composed of inpatients with normal controls, MCI, AD, and other neurological diseases (OND); and the third cohort (Cohort 3, $n = 91$) is a mixture of inpatients and non-hospitalized subjects living in the city. Detailed descriptions of all subjects are shown in Supplementary Tables 1–3. The cohorts are different from those of previous studies for an analysis of total p3-Alcα [21, 23].

RESULTS

Characterization of the ELISA system with the monoclonal p3-Alcα35 C-terminal end-specific antibody

To develop the new sELISA, the 63A1 antibody was used to capture p3-Alcα35 specifically, instead of the polyclonal pan-p3-Alcα antibody 839 used in previous studies, to examine the total amount of p3-Alcα [21]. The new ELISA recognized p3-Alcα35 specifically, but not p3-Alcα39 (Fig. 1A), and does not react with the p3-Alcβ species (data not shown), indicating the establishment of a new sELISA system specific for p3-Alcα35 analysis. Previously, plasma samples were extracted, and total p3-Alcα was quantified using

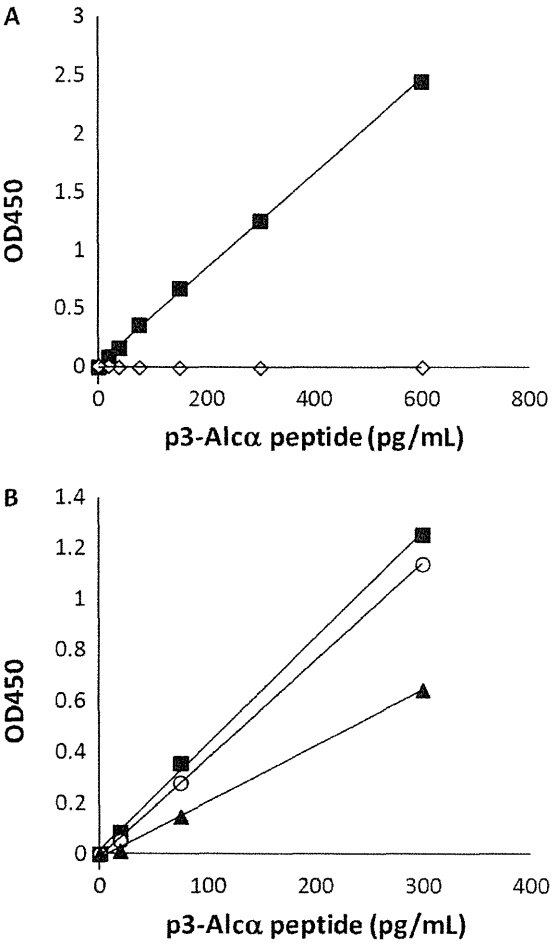

Fig. 1. Specificity of the sELISA. A) Specific reactivity of the sELISA system to p3-Alcα35. The indicated amount of synthetic p3-Alcα35 (closed square) and p3-Alcα39 (open diamond) were dissolved in buffer A (PBS containing 1% (w/v) bovine serum albumin and 0.05% (v/v) Tween-20), and assayed with the ELISA. B) Quantification of the p3-Alcα35 peptide in human plasma with or without an extraction process. The indicated amounts of synthetic p3-Alcα35 were dissolved in human plasma (prepared from a non-AD healthy volunteer; open circle and closed triangle) or buffer A (closed square). The plasma was subject to extraction with (open circle) or without (closed triangle) a standard organic extraction protocol prior to analysis by ELISA, as described [21]. The horseradish peroxidase-conjugated antibody and tetrametyl benzidine were used to detect the captured p3-Alcα35 colormetrically at OD_{450}.

the sELISA, because the treatment of plasma samples with a standard organic extraction protocol removed factor(s) that interfered with the immuno-detection of p3-Alcα [21]. This extraction procedure was also included in the new ELISA to allow significant recovery of p3-Alcα from the plasma, and therefore accurate quantification of p3-Alcα35 levels in the plasma. The ELISA system was performed to quantify the amount of synthetic p3-Alcα35 dissolved in the human plasma (Fig. 1B). After the extraction process, the slope of the standard curve was almost identical to that of the standard curve in which an identical amount of the synthetic p3-Alcα35 was dissolved in assay buffer. This procedure results in a yield of over 90% (compare closed square with open circle in Fig. 1B), which is sufficient to be considered quantitative, while the recovery of synthetic p3-Alcα35 from the plasma was approximately 50% to 60% when plasma samples were assayed without the extraction process (compare closed square with closed triangle in Fig. 1B). Therefore, plasma p3-Alcα35 quantification was carried out by sELISA after extraction of the endogenous p3-Alcα peptides, as described previously [21].

Plasma p3-Alcα35 levels of subjects in three independent Japanese cohorts

The levels of p3-Alcα35 in the plasma of subjects from three cohorts were investigated (Table 1). The correlation between p3-Alcα35 and total amount of p3-Alcα levels was first examined. The p3-Alcα35 levels were significantly correlated with the total amount of p3-Alcα in the three cohorts (Fig. 2 left). Furthermore, in all cohorts, plasma p3-Alcα35 levels significantly increased in an age-dependent manner (Fig. 2 right).

The relationship between p3-Alcα35 levels and MMSE scores was then investigated (Fig. 3). The increase in plasma p3-Alcα35 levels correlates with the decrease of the score significantly in two of the three cohorts (Fig. 3A, B). These analyses suggest that p3-Alcα35 levels increase during the aging process, and that subjects appearing to have cognitive impairment show higher levels of p3-Alcα35 in their plasma.

Table 1
Summary of subjects' data analyzed in Fig. 2

n	A) Cohort 1 135	B) Cohort 2 252	C) Cohort 3 91
Age (years)	74.9 ± 6.50	81.3 ± 10.1	76.6 ± 8.37
p3-Alcα35 (pg/mL)	164.6 ± 46.9	192.1 ± 60.1	140.5 ± 43.8
p3-Alcα total (pg/mL)	241.6 ± 45.0	240.0 ± 74.4	172.5 ± 51.5

Average age and average values of p3-Alcα35 and p3-Alcα total in three cohorts are summarized. Numbers indicate means ± standard deviation. Details of individual subjects are shown in Supplementary Tables 1–3.

The p3-Alcα35 levels of AD and MCI patients were then compared with those of non-demented control subjects or OND patients, in an age-matched population (Fig. 4 and Table 2). In all cohorts, AD and MCI were clinically diagnosed based on two major criteria: the Diagnostic and Statistical Manual of Mental Disorders: 4th Edition (DSM-IV) and the National Institute of Neurological and Communicational Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA), although we realize that there are many different definitions of MCI [24].

In the subjects of Cohort 1 (Fig. 4A and Table 2A), the p3-Alcα35 levels of AD patients were significantly higher than the values of the age-matched non-demented controls. The age-matched MCI subjects showed higher levels of p3-Alcα35 than non-demented controls, although this finding was not statistically significant.

In Cohort 2 (Fig. 4B and Table 2B), the subjects with OND were divided into two subgroups with (MMSE $\leq$22) or without (MMSE $\geq$23) remarkable cognitive impairment [25]. We examined the p3-Alcα35 levels of age-matched controls, MCI, AD, OND (MMSE $\geq$23), and OND (MMSE $\leq$22) subjects. The p3-Alcα35 level in AD subjects was significantly higher when compared to the values of the control subjects. OND patients who show cognitive impairment (MMSE $\leq$22) also presented higher p3-Alcα35 levels than subjects of OND without severe cognitive impairment (MMSE $\geq$23) and control subjects, although these were not significant. The present findings suggest that the increase in the p3-Alcα35 levels may be involved in neurodegeneration and cognitive impairment.

We also analyzed the p3-Alcα35 levels of 67 age-matched subjects between 63 and 83 years of age in Cohort 3 (Fig. 4C and Table 2C). The levels of p3-Alcα35 are statistically identical between non-demented controls, MCI, AD, and OND subjects.

The subjects of Cohorts 2 and 3 were also classified by other criteria for dementia, the Clinical Dementia Rating (CDR) scale [26]. The p3-Alcα35 levels were compared in age-matched populations with a CDR score of CDR 0, CDR 0.5, and CDR 1, 2, 3 (Fig. 5). CDR 0 subjects were selected from clinically diagnosed MCI and OND patients by criteria of non-demented subjects, and CDR 1, 2, 3 subjects were selected from OND and AD subjects. The CDR scores of some subjects did not agree with the clinical diagnosis. Therefore we removed subjects from analysis who

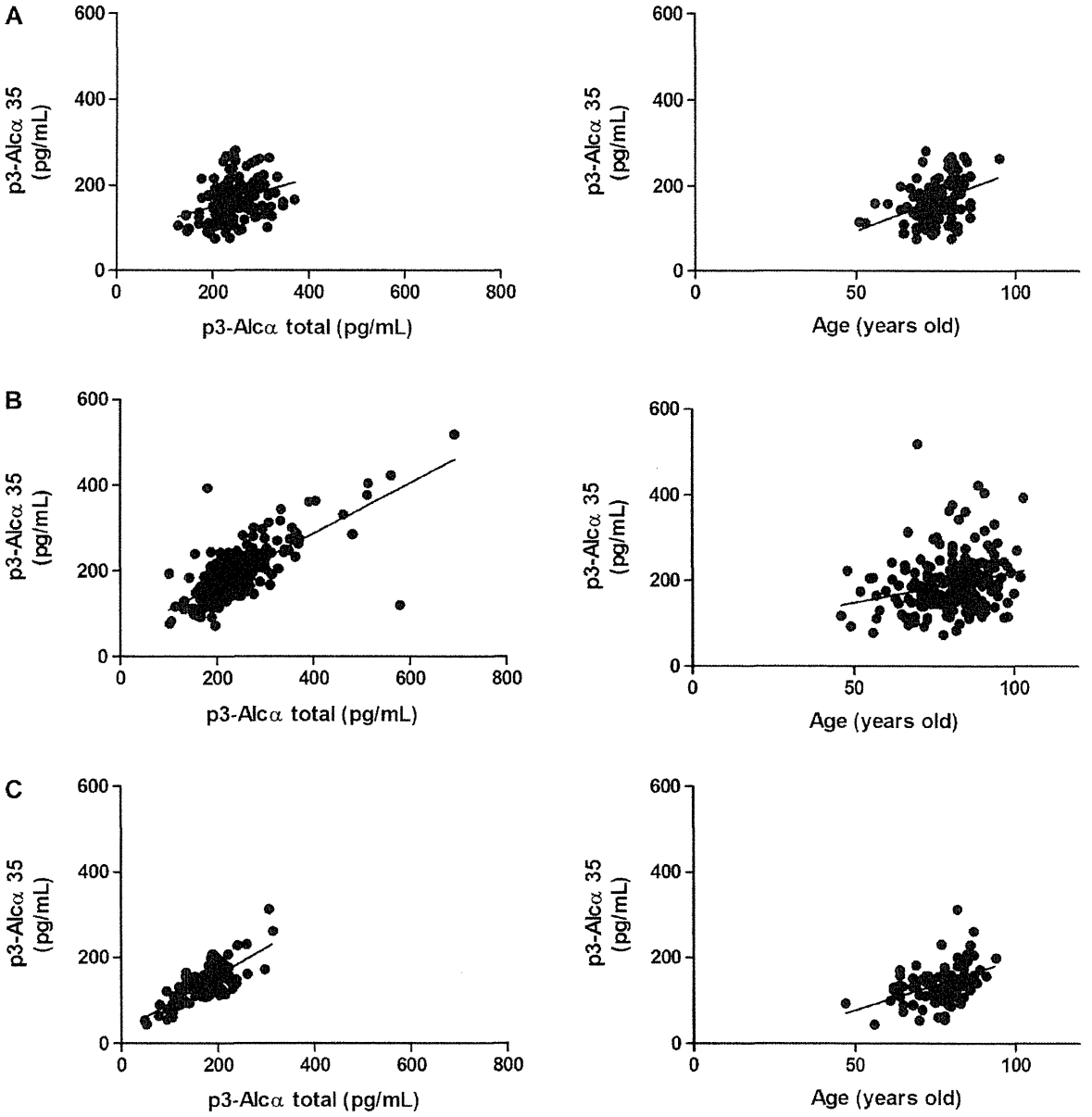

Fig. 2. Correlation of p3-Alcα35 levels with total p3-Alcα levels in plasma and age-dependency of plasma p3-Alcα35 levels. (Left) The correlation between p3-Alcα35 and total p3-Alcα levels are shown. A) Cohort 1 ($r^2 = 0.1009$, $p < 0.001$), B) Cohort 2 ($r^2 = 0.5362$, $p < 0.001$), C) Cohort 3 ($r^2 = 0.5790$, $p < 0.001$). (Right) The correlation between p3-Alcα35 levels and age are shown. A) Cohort 1 ($r^2 = 0.1512$, $p < 0.001$), B) Cohort 2 ($r^2 = 0.055912$, $p < 0.001$), C) Cohort 3 ($r^2 = 0.2005$, $p < 0.001$). Statistical analysis was performed by using the Pearson's correlation coefficient test. Subject numbers (n), average age, and p3-Alcα35 amounts of each cohort are summarized in Table 1.

had remarkably different diagnosis, such as a subject labeled as MCI presenting with CDR 2. In the present analysis of Cohort 2 (Fig. 5A), CDR 1, 2, 3 patients ($n = 197$, 198.0 ± 60.5 pg/mL) presented significantly high p3-Alcα35 levels when compared to those of CDR 0 subjects ($n = 17$, 152.8 ± 44.1 pg/mL) and CDR 0.5 subjects ($n = 6$, 153.1 ± 15.7 pg/mL). In Cohort 3 (Fig. 5B), there are no significant differences between the respective CDR subjects. Based on at least two criteria (Figs. 4 and 5), the p3-Alcα35 levels showed a tendency to be increased in AD and/or demented (CDR 1, 2, 3) subjects.

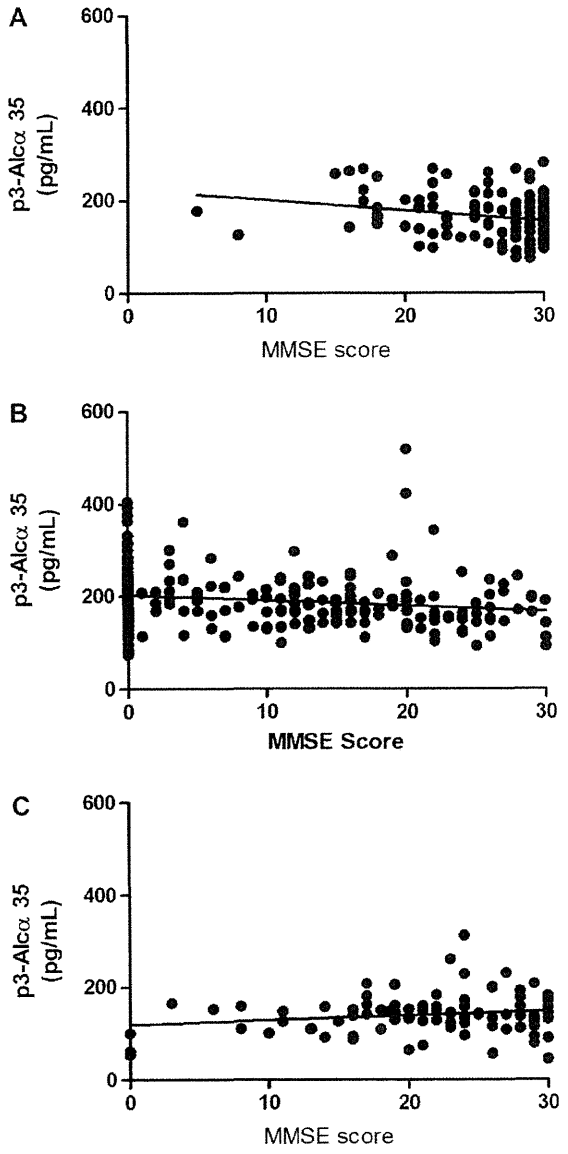

Fig. 3. The relationship between MMSE scores and plasma p3-Alcα35 levels. The correlation between Mini-Mental State Examination (MMSE) scores and p3-Alcα35 levels in the plasma of subjects is shown. In the graphs, statistical analysis was performed by using the Pearson's correlation coefficient test. A) Cohort 1 ($r^2 = 0.05303$, $p < 0.01$), B) Cohort 2 ($r^2 = 0.03525$, $p < 0.01$), C) Cohort 3 ($r^2 = 0.02934$, $p = 0.1045$).

We also investigated the p3-Alcα35 levels of AD patients with or without donepezil hydrochloride (Aricept) administration (Fig. 6). In 31 age-matched AD subjects with CDR 1 and CDR 2 in Cohort 2, non-treated subjects ($n = 18$, average age 80.4 ± 9.2) showed significantly higher levels of plasma p3-Alcα35 (182.7 ± 35.9 pg/mL) when compared to the

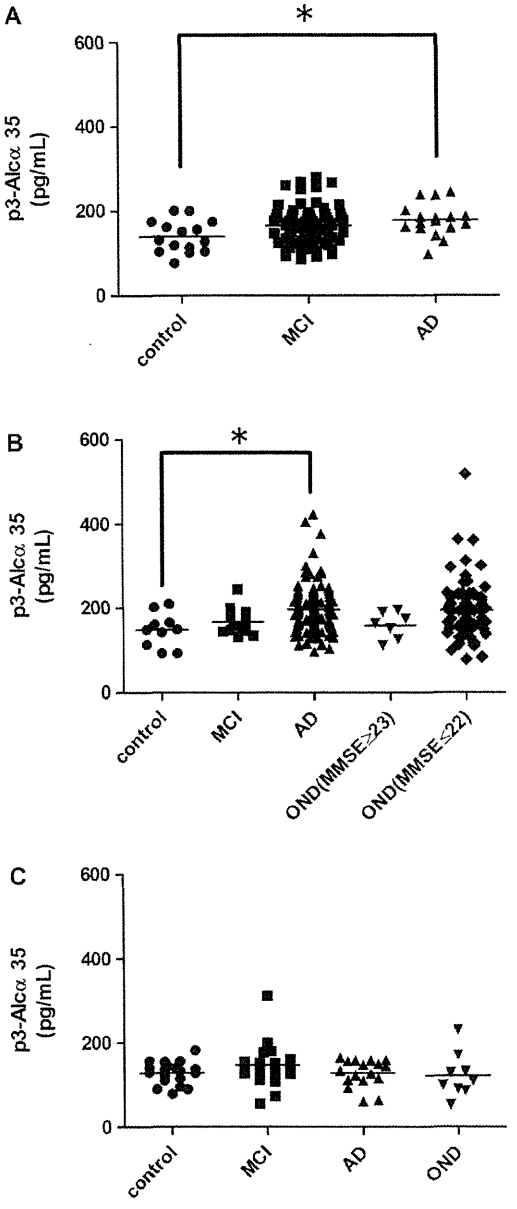

Fig. 4. Levels of plasma p3-Alcα35 following subgrouping into non-demented, MCI, AD, and OND. Subjects from three cohorts were clinically divided into four groups: non-demented subject (control), subjects with mild cognitive impairment (MCI), Alzheimer's disease (AD), and patients with other neurological diseases (OND). In Cohort 2 (B), OND are further distinguished into subjects with (MMSE ≤22) or without (MMSE ≥23) remarkable cognitive impairment. Plasma p3-Alcα35 levels of age-matched subjects in these subgroups were compared within the respective cohorts. A) Cohort 1 (subjects between 70 and 83 years old), B) Cohort 2 (subjects between 73 and 94 years old), C) Cohort 3 (subjects between 63 and 83 years old). Statistical analysis was performed using the Dunn's multiple comparison test following the Kruskal-Wallis test. *$p < 0.05$. Subject numbers (n), average age, and p3-Alcα35 amounts are summarized in Table 2.

Table 2
Summary of subjects' data analyzed in Fig. 4

A) Cohort 1					
n		Control	MCI	AD	
		15	70	17	
p3-Alcα35 (pg/mL)		140.1 ± 37.8	166.3 ± 45.1	178.7 ± 38.5	
Age (years)		74.3 ± 4.1	75.5 ± 3.7	77.1 ± 4.1	
B) Cohort 2					
n	Control	MCI	AD	OND (MMSE ≥ 23)	OND (MMSE ≤22)
	10	12	116	7	85
p3-Alcα35 (pg/mL)	148.0 ± 40.3	166.4 ± 32.6	196.5 ± 55.7	159.2 ± 31.7	194.8 ± 64.0
Age (years)	74.6 ± 10.4	81.5 ± 4.4	82.8 ± 7.7	79.3 ± 11.1	78.9 ± 10.1
C) Cohort 3					
n		Control	MCI	AD	OND
		19	22	17	9
p3-Alcα35 (pg/mL)		128.6 ± 26.8	146.0 ± 49.5	130.8 ± 32.7	122.9 ± 52.3
Age (years)		74.5 ± 4.4	76.2 ± 6.4	75.3 ± 6.6	74.4 ± 5.7

Average age and average values of p3-Alcα35 in three cohorts are summarized. Numbers indicate means ± standard deviation. MCI, mild cognitive impairment; AD, Alzheimer's disease; OND, other neurological diseases.

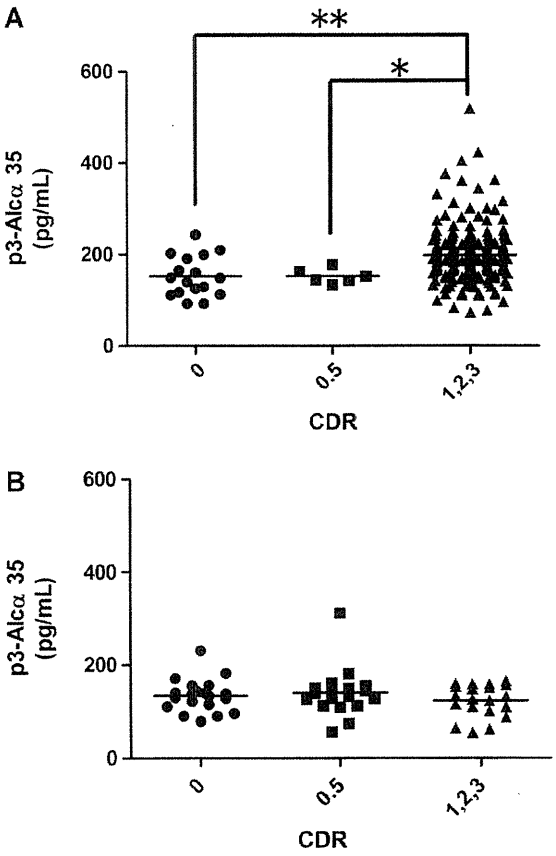

Fig. 5. Levels of plasma p3-Alcα35 of subjects divided into subgroups using the clinical dementia rating (CDR) scale. Age-matched subjects of cohort 2 (A) and cohort 3 (B) were divided into three groups based on CDR scales. Statistical analysis was performed using the Dunn's multiple comparison test following the Kruskal-Wallis test. *$p < 0.05$; **$p < 0.01$.

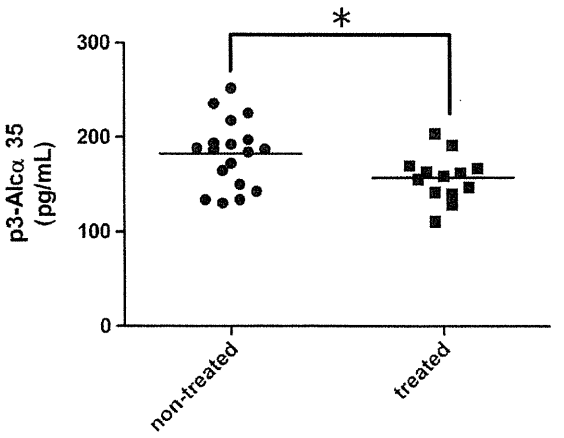

Fig. 6. Influence of donepezil hydrochloride administration on plasma p3-Alcα35 levels. Age-matched AD (CDR 1 and CDR 2) patients treated with Aricept (donepezil hydrochroride) were selected from Cohort 2, and their plasma p3-Alcα35 levels were compared to those of age-matched subjects who were not treated with Aricept (non-treated). Statistical analysis was performed using the Mann-Whitney test. *$p < 0.05$.

levels detected in subjects who were treated with the drug (157.1 ± 24.7 pg/mL; $n = 13$, average age 81.5 ± 5.3), suggesting that the increase in p3-Alcα35 levels may be slowed by the suppression of cognitive impairment by donepezil administration.

DISCUSSION

We previously showed that p3-Alcα35 is the major p3-Alcα species in human CSF by MALDI-TOF/MS spectrometric analysis of p3-Alcα peptides