Caregivers of patients with urinary incontinence have higher levels of stress and depression than those caring for people with other conditions, and incontinence leads to early institutionalization.[33–35]

Regarding IADL, deficits in handling domestic finances and transportation were common factors associated with CB in men and women. It is time-consuming and often stressful for caregivers to take over the financial responsibilities of the household.[29,36] Non-compliance with medication in women would cause caregivers mental stress. Regarding BADL, dressing was a candidate factor for CB in AD patients with more severe cognitive dysfunction. AD patients might often refuse to be helped with personal care including changing their clothes, which causes stress to their caregivers.

In cognitive stage of AD17–12, BPSD, IADL and geriatric syndrome were also associated with CB. BPSD were worse and care burden became more severe. Regarding IADL, caregivers were annoyed with the patients' deficits in their own personal tasks, such as in the use of transportation (in men and women) and in self-medication (men). Deficit in handling finances was still shown to be an associated factor in women. This could have been related to a lack of awareness by patients of a deficit.[36] Patients who are unaware of functional deficits often overestimate their ability and believe they are capable of activities beyond their capabilities, which can cause problems and stress in caregivers. As comorbidity, falls indicate further deterioration of motor function, and fatigue might reflect and accelerate passiveness.

In AD11–0, prominent factors for CB were BPSD, including Motor aggressiveness and Behavior disturbance. The frequency of BPSD related to CB markedly increased at this stage. For instance, agitation is a symptom related to frontal lobe dysfunction, with a prevalence of nearly 50% in AD.[37,38] It can be triggered by physical problems, such as pain and lack of sleep; psychiatric problems, such as anger, aggressiveness, anxiety and depression; environmental stresses, such as noisiness and inadequate temperature; and as a side-effect of medication. Agitation can also be a single determinant of early institutionalization.[25–28]

Although sleep disturbance and ringing in the ears could be associated with CB in this stage, the contribution of geriatric syndrome to CB was not obvious (Table 3). In this connection, it should be noted that participants in the present study were outpatients without serious physical complications. Alternatively, the increment of BPSD might have obscured the role of geriatric syndrome as a burden factor in the analysis with a relatively small number of participants ($n = 87$).

This study clearly indicated that various differential factors were cognitive stage-dependently associated with CB. It should be stressed that the higher prevalence of BPSD, geriatric syndrome and impairment of life function in particular cognitive stages was not always a burden factor. For instance, symptoms of Behavior disturbance in AD29–24 were not as frequent as in AD11–0, but were factors responsible for CB. Urinary incontinence was markedly increased in cognitive stages of AD11–0, but was associated with CB even in AD23–18. It seems likely that caregivers are surprised and embarrassed by their first experience of problematic symptoms of dementia in patients who have moderate cognitive dysfunction. It is therefore important to know and predict these burden factors in advance. Second, even if certain factors showed an association with CB in one cognitive stage, they did not always remain burden factors in subsequent cognitive stages. Different activities of IADL were shown to be burden factors in particular cognitive stages.

The results of the present study suggested that prevention of BPSD and comorbidity of geriatric syndrome is an essential consideration in the management of AD. At the same time, life care support for deteriorated IADL should be considered even for patients belonging to AD29–24. Treatment of BPSD and comorbidity could be beneficial in ameliorating CB, as comorbidity can cause various BPSD, and BPSD increase the risk of geriatric syndrome, such as falls and muscle weakness, and vice versa. It was reported that half of BPSD were caused by comorbidity and medication; in AD, 23% of BPSD are caused by medication, 18.3% by comorbidity and 6.7% by a combination of the two.[39] It is well established that physical rehabilitation is effective for not only the prevention of falls/motor disturbance, but also improvement of mood, apathy and day–night reversal.

Previous studies have shown that individualized educational and support programs for caregivers are effective to ameliorate CB.[40,41] Educational programs should provide prognostic information on the disease of dementia, as well as factors associated with CB. In this respect, the findings of the present study might be informative for caregiver education.

The present study had several limitations. It was a cross-sectional study. A second limitation was selection bias of the study participants, although the participants were composed of a large number of patients consecutively selected in the Medical Center for Dementia at the NCGG. All data were obtained from outpatients, and inpatients suffering from various physical complications, such as recurrent pneumonia and fractures, were not included. Finally, CB is comprised of multidimensional factors including patient factors, such as the severity of disease, premorbid characteristics, and financial and social status, caregiver factors, and other environmental factors, all of which are highly individualized.[42] The present study mainly analyzed burden factors on the patients' side. To clarify the multifactorial mechanisms of CB, more detailed information on

© 2014 Japan Geriatrics Society

demographics, socioeconomic conditions and use of several care services need to be analyzed. However, our observation provides important information on CB, which might reflect general attitudes of caregivers to demented older adults, when they first attended a medical center for consultation on dementia. Longitudinal follow-up studies of demented older adults with detailed information on CB are required.

Acknowledgements

This study was financially supported by grants from Chojyu (24–24), Japan's Ministry of Education, Culture, Sports, Science and Technology (22590654), and Japan's Ministry of Health, Labor and Welfare (H25-Ninchisho-008). We also thank the BioBank at NCGG for quality control of the clinical data.

Disclosure statement

The authors declare no conflict of interest.

References

1 American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*. Washington, DC: American Psychiatric Association, 2013.
2 Joling KJ, van Hout HP, Schellevis FG *et al.* Incidence of depression and anxiety in the spouses of patients with dementia: a naturalistic cohort study of recorded morbidity with a 6-year follow-up. *Am J Geriatr Psychiatry* 2010; **18**: 146–153.
3 Wimo A, Jonsson L, Bond J, Prince M, Winblad B. The worldwide economic impact of dementia 2010. *Alzheimers Dement* 2013; **9**: 1–11.e3.
4 Epstein-Lubow G, Gaudiano B, Darling E *et al.* Differences in depression severity in family caregivers of hospitalized individuals with dementia and family caregivers of outpatients with dementia. *Am J Geriatr Psychiatry* 2012; **20**: 815–819.
5 Romero-Moreno R, Marquez-Gonzalez M, Mausbach BT, Losada A. Variables modulating depression in dementia caregivers: a longitudinal study. *Int Psychogeriatr* 2012; **24**: 1316–1324.
6 Norton MC, Smith KR, Ostbye T *et al.* Greater risk of dementia when spouse has dementia? The Cache County study. *J Am Geriatr Soc* 2010; **58**: 895–900.
7 Oken BS, Fonareva I, Wahbeh H. Stress-related cognitive dysfunction in dementia caregivers. *J Geriatr Psychiatry Neurol* 2011; **24**: 191–198.
8 Ankri J, Andrieu S, Beaufils B, Grand A, Henrard JC. Beyond the global score of the Zarit Burden Interview: useful dimensions for clinicians. *Int J Geriatr Psychiatry* 2005; **20**: 254–260.
9 Ropacki SA, Jeste DV. Epidemiology of and risk factors for psychosis of Alzheimer's disease: a review of 55 studies published from 1990 to 2003. *Am J Psychiatry* 2005; **162**: 2022–2030.
10 Lopez OL, Becker JT, Sweet RA *et al.* Psychiatric symptoms vary with the severity of dementia in probable Alzheimer's disease. *J Neuropsychiatry Clin Neurosci* 2003; **15**: 346–353.
11 Richardson TJ, Lee SJ, Berg-Weger M, Grossberg GT. Caregiver health: health of caregivers of Alzheimer's and other dementia patients. *Curr Psychiatry Rep* 2013; **15** (7): 367.
12 Hebert R, Dubois MF, Wolfson C, Chambers L, Cohen C. Factors associated with long-term institutionalization of older people with dementia: data from the Canadian Study of Health and Aging. *J Gerontol A Biol Sci Med Sci* 2001; **56**: M693–M699.
13 Petersen RC, Doody R, Kurz A *et al.* Current concepts in mild cognitive impairment. *Arch Neurol* 2001; **58**: 1985–1992.
14 McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984; **34**: 939–944.
15 Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; **12**: 189–198.
16 Yesavage JA, Brink TL, Rose TL *et al.* Development and validation of a geriatric depression screening scale: a preliminary report. *J Psychiatr Res* 1982; **17**: 37–49.
17 Mahoney FI, Barthel DW. Functional evaluation: the barthel index. *Md State Med J* 1965; **14**: 61–65.
18 Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. *Gerontologist* 1969; **9**: 179–186.
19 Baumgarten M, Becker R, Gauthier S. Validity and reliability of the dementia behavior disturbance scale. *J Am Geriatr Soc* 1990; **38**: 221–226.
20 Zarit SH, Reever KE, Bach-Peterson J. Relatives of the impaired elderly: correlates of feelings of burden. *Gerontologist* 1980; **20**: 649–655.
21 Brown PJ, Devanand DP, Liu X, Caccappolo E. Alzheimer's Disease Neuroimaging Initiative. Functional impairment in elderly patients with mild cognitive impairment and mild Alzheimer disease. *Arch Gen Psychiatry* 2011; **68**: 617–626.
22 Koyano W, Shibata H, Nakazato K, Haga H, Suyama Y. Measurement of competence: reliability and validity of the TMIG Index of Competence. *Arch Gerontol Geriatr* 1991; **13**: 103–116.
23 Nygård LSS. Telephone use among noninstitutionalized persons with dementia living alone: mapping out difficulties and response strategies. *Scand J Caring Sci* 2003; **17**: 239–249.
24 Youn JC, Lee DY, Jhoo JH, Kim KW, Choo IH, Woo JI. Prevalence of neuropsychiatric syndromes in Alzheimer's disease (AD). *Arch Gerontol Geriatr* 2011; **52**: 258–263.
25 Ferrara M, Langiano E, Di Brango T, De Vito E, Di Cioccio L, Bauco C. Prevalence of stress, anxiety and depression in with Alzheimer caregivers. *Health Qual Life Outcomes* 2008; **6** (6): 93.
26 Hurt C, Bhattacharyya S, Burns A *et al.* Patient and caregiver perspectives of quality of life in dementia. An investigation of the relationship to behavioural and psychological symptoms in dementia. *Dement Geriatr Cogn Disord* 2008; **26**: 138–146.
27 Craig D, Mirakhur A, Hart DJ, McIlroy SP, Passmore AP. A cross-sectional study of neuropsychiatric symptoms in

© 2014 Japan Geriatrics Society

435 patients with Alzheimer's disease. *Am J Geriatr Psychiatry* 2005; **13**: 460–468.
28 Matsumoto N, Ikeda M, Fukuhara R *et al.* Caregiver burden associated with behavioral and psychological symptoms of dementia in elderly people in the local community. *Dement Geriatr Cogn Disord* 2007; **23**: 219–224.
29 Razani J, Kakos B, Orieta-Barbalace C *et al.* Predicting caregiver burden from daily functional abilities of patients with mild dementia. *J Am Geriatr Soc* 2007; **55**: 1415–1420.
30 Boyle PA, Malloy PF. Treating apathy in Alzheimer's disease. *Dement Geriatr Cogn Disord* 2004; **17**: 91–99.
31 Levy R, Dubois B. Apathy and the functional anatomy of the prefrontal cortex-basal ganglia circuits. *Cereb Cortex* 2006; **16**: 916–928.
32 Starkstein SE, Petracca G, Chemerinski E, Kremer J. Syndromic validity of apathy in Alzheimer's disease. *Am J Psychiatry* 2001; **158**: 872–877.
33 Drennan VM, Cole L, Iliffe S. A taboo within a stigma? A qualitative study of managing incontinence with people with dementia living at home. *BMC Geriatr* 2011; **11**: 75.
34 Luppa M, Luck T, Brahler E, Konig HH, Riedel-Heller SG. Prediction of institutionalisation in dementia. A systematic review. *Dement Geriatr Cogn Disord* 2008; **26**: 65–78.
35 Olazaran J, Reisberg B, Clare L *et al.* Nonpharmacological therapies in Alzheimer's disease: a systematic review of efficacy. *Dement Geriatr Cogn Disord* 2010; **30**: 161–178.
36 Seltzer B, Vasterling JJ, Yoder JA, Thompson KA. Awareness of deficit in Alzheimer's disease: relation to caregiver burden. *Gerontologist* 1997; **37**: 20–24.
37 Okura T, Plassman BL, Steffens DC, Llewellyn DJ, Potter GG, Langa KM. Prevalence of neuropsychiatric symptoms and their association with functional limitations in older adults in the United States: the aging, demographics, and memory study. *J Am Geriatr Soc* 2010; **58**: 330–337.
38 Senanarong V, Cummings JL, Fairbanks L *et al.* Agitation in Alzheimer's disease is a manifestation of frontal lobe dysfunction. *Dement Geriatr Cogn Disord* 2004; **17**: 14–20.
39 Nakano M, Miyamura T, Hirai S. Investigation of the actual condition of medical care for behavioral and psychological symptoms of dementia. *Jpn J Geriatr Psychiatry* 2011; **22**: 313–324.
40 Pinquart M, Sorensen S. Correlates of physical health of informal caregivers: a meta-analysis. *J Gerontol B Psychol Sci Soc Sci* 2007; **62**: 126–137.
41 Hepburn KW, Tornatore J, Center B, Ostwald SW. Dementia family caregiver training: affecting beliefs about caregiving and caregiver outcomes. *J Am Geriatr Soc* 2001; **49**: 450–457.
42 Pearlin LI, Mullan JT, Semple SJ, Skaff MM. Caregiving and the stress process: an overview of concepts and their measures. *Gerontologist* 1990; **30**: 583–594.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1 Prevalence of Dementia Behavior Disturbance Scale (DBD) subitems in subjects with normal cognition (NC), amnestic Mild cognitive impairment (aMCI) and Alzheimer's disease (AD)29–24, AD23–18, AD17–12, and AD11–0.

Figure S2 Prevalence of symptoms of geriatric syndrome in participants with normal cognition (NC), amnestic mild cognitive impairment (aMCI) and varying stages of Alzheimer's disease (AD).

© 2014 Japan Geriatrics Society

ORIGINAL ARTICLE

Differential subtypes of diabetic older adults diagnosed with Alzheimer's disease

Takashi Sakurai,[1] Shuji Kawashima,[1] Shosuke Satake,[1] Hisayuki Miura,[1] Haruhiko Tokuda[2] and Kenji Toba[1]

[1]*Center for Comprehensive Care and Research on Memory Disorders, and* [2]*Department of Diabetes and Endocrinology, National Center for Geriatrics and Gerontology, Obu, Japan*

Aim: The clinical management of diabetic elderly patients with Alzheimer's disease (AD) is hindered by several difficulties. The present study aimed to clarify the clinical characteristics and pathophysiological properties of AD in diabetic older adults.

Methods: A total of 91 patients with type 2 diabetes mellitus and 161 non-diabetic individuals who were diagnosed with AD were recruited. Diabetic patients were classified into two groups with glycated hemoglobin (HbA1c) <7.0% or ≥7.0%. The demographics, cognition, daily-life function, metabolic changes, treatment, and behavioral and psychological symptoms of dementia (BPSD), as well as brain pathophysiology, were compared among the three groups.

Results: Patients with higher HbA1c had increased diabetic vascular complications and impaired activities of daily living with decreased levels of serum high-molecular-weight adiponectin and 25-hydroxyvitamin D. Although cognitive status was similar among the three groups, BPSD, including apathy, overeating and excessive daytime sleeping appeared to be increased in the patients with HbA1c ≥7.0%. The frequency of apolipoprotein E4 carriers and of posterior cerebral hypoperfusion (AD-pattern) on single-photon emission computed tomography in poorly controlled diabetic subjects was similar to that in non-diabetic AD patients, whereas diabetic patients with HbA1c <7.0% included fewer apolipoprotein E4 carriers and fewer patients with an AD pattern on single-photon emission computed tomography.

Conclusion: Subtypes of older diabetic patients with AD were identified based on clinical features and brain pathophysiology. Physical and psychological complications of dementia are prevalent in patients with higher HbA1c. It seems likely that difficulties in the management of diabetes with AD are due not only to non-adherence to diabetes treatment, but also several symptoms and pathophysiological characteristics of dementia. **Geriatr Gerontol Int 2014; ••: ••–••.**

Keywords: Alzheimer's disease, behavioral and psychological symptoms of dementia, diabetes, glycemic control, pathophysiology.

Introduction

Diabetes increases the risk of dementia, including Alzheimer's disease (AD) and vascular dementia.[1] Once an older diabetic patient begins to experience cognitive decline, treatment of diabetes becomes difficult despite intensive care and education. Physical exercise and dietary changes are feasible treatment options, but adherence to diabetic medicine is usually impaired, even in the early course of AD. When serious hyperglycemia continues, the more powerful antidiabetic medicines are prescribed, which in turn increases the risk of hypoglycemia. Hyperglycemia and hypoglycemia, as well as acute fluctuation of glucose, further worsen cognitive impairment.[2–4] Behavioral and psychological symptoms of dementia (BPSD) also cause difficulties in the management of diabetes. To overcome these problems, a coordinated treatment plan that addresses both the AD and diabetes is required.

There is currently no consensus as to the pathophysiology of AD in diabetes; studies have alternatively reported that AD-associated pathology is increased,

Accepted for publication 26 December 2013.

Correspondence: Dr Takashi Sakurai MD PhD, 35 Gengo, Morioka-cho, Obu, Aichi 474-8511, Japan. Email: tsakurai@ncgg.go.jp

© 2014 Japan Geriatrics Society

unchanged or even decreased in diabetic older adults.[5–8] Other studies have found that cerebral vascular disease (CVD) is more likely to be involved in diabetes.[9,10] Metabolic factors of diabetes might have a profound impact on the clinical course of AD, resulting in a variety of clinical pictures.[11–14] Because of the complex nature of AD, the true reasons for the difficulties in managing diabetic elderly patients with AD have remained unclear.

The purpose of the present study was to clarify the clinical characteristics of diabetic older adults with AD from the standpoint of demographics, cognition, activities of daily living (ADL), complications of dementia, metabolic changes, treatment and pathophysiology of the brain. We hypothesized that clinical symptoms related to AD would depend largely on glycemic control and brain pathophysiology. We therefore compared these variables among three patient groups: diabetic patients with AD and good glucose control, diabetic patients with AD and poor glucose control, and non-diabetic patients with AD. The present study was designed to identify subtypes of dementia with differential clinical properties and pathophysiology in diabetic patients with AD.

Methods

Participants

The study protocol was approved by the institutional review board of the National Center for Geriatrics and Gerontology (NCGG), Japan. Candidate patients and their caregivers submitted informed consent before participation in the study.

A total of 252 elderly patients (age 65–85 years) who had been diagnosed with AD and treated in the NCGG were enrolled consecutively. The total Barthel Index score for each of the 252 patients was 80 or over.[15] Patients with severe cardiac failure, renal disorder, liver dysfunction or other neurological and psychiatric disorders, such as depression or alcohol abuse, and patients with symptomatic cerebral infarction or cortical lesions on brain magnetic resonance imaging (MRI) were excluded from the present study.

The final participant groups thus consisted of 91 patients with type 2 diabetes and 161 non-diabetic individuals. Diabetic patients were classified into two groups based on whether their glycated hemoglobin (HbA1c) was <7.0% or ≥7.0%. All diabetic participants had a history of diabetes, and were receiving pharmacological treatment for diabetes that included oral antihyperglycemic agents and/or insulin.

All participants underwent the standardized and reliable diagnostic procedures for dementia disorders.[16] AD was diagnosed as probable AD or possible AD according to the criteria from the National Institute of Neurological and Communicative Disorders and Stroke, and the Alzheimer's Disease and Related Disorders Association.[17]

Comprehensive assessment

Information about previous diseases and medication was obtained from the clinical charts. Polypharmacy was defined as taking five or more types of oral medicine.[18] The Barthel Index and the Lawton Index were used to evaluate basic and instrumental ADL, respectively.[15,19] Cognitive status was measured by using a psychiatric assessment battery that included the Mini-Mental State Examination (MMSE), Alzheimer's Disease Assessment Scale (ADAS), Digit Span Forward and Backward trials, Frontal Assessment of the Brain (FAB), Raven's Colored Progressive Matrices (RCPM), and Logical Memory I and II subtests from the Wachster Memory Scale-Revised.[20–24] Depressive mood and BPSD were estimated by the Geriatric Depression Scale-15 (GDS) and Dementia Behavior Disturbance Scale (DBD), respectively.[25,26] The Zarit burden interview (ZBI) was used for measurement of the caregivers' burden.[27] Risks for falls were evaluated by the Fall Risk Index (FRI).[28] Geriatric syndrome was assessed by administering a questionnaire to patients and their caregivers; the questionnaire included questions on dyspnea, cough, chest oppression, edema, fatigue, nausea, abdominal pain, diarrhea, constipation, dysphasia, numbness, tremor, syncope, dizzying, chewing troubles, polyuria, incontinence of urine, decubitus ulcer, itching, lumbago, back pain, lower limb pain, upper limb pain and sleeping problems.

Laboratory measurements

Apolipoprotein (Apo) E phenotypes were determined in plasma specimens by using isoelectric electrophoresis and immunoblotting methods.[29] Vitamin D insufficiency was assessed by the serum concentration of 25-hydroxyvitamin D, as currently recommended.[30] Levels of high-molecular-weight adiponectin were analyzed by enzyme immunoassay as described elsewhere.[31]

Neuroimaging studies

Brain MRI and single-photon emission computed tomography (SPECT) were used to elucidate the pathophysiology of dementia. A standard series of axial T1-weighted (repetition time [TR], 485 ms; echo time [TE], 11 ms), T2-weighted (TR, 3800 ms; TE, 93 ms) and fluid-attenuated inversion recovery (TR, 8000 ms; TE 101 ms; inversion time, 2500 ms; a 256 × 256 matrix). MR sequences were carried out in a 1.5T MR system (Siemens Avanto, Munich, Germany). Scans were in parallel with the anterior commissura–posterior commissura line, with 6-mm thick slices and an interslice gap of 1.2 mm.

© 2014 Japan Geriatrics Society

MRI data were processed to measure the total volumes of the intracranial space (IC), parenchyma, ventricles and white matter regions (WML) by a fully automatic segmentation program (Software for Neuro-Image Processing in Experimental Research: SNIPER), which was developed at the Department of Radiology, Leiden University Medical Center, Leiden, the Netherlands. Detailed procedures of the MRI post-processing by SNIPER have been described elsewhere.[32]

SPECT scanning was carried out by using a two-head rotating GCA 7200DI gamma-camera (Toshiba, Otabara, Japan). Imaging was started 15~45 min after injection of 222 MBq (6 mCi) of N-isopropyl-p-[^{123}I] iodoamphetamine (Nihon Mediphysics, Tokyo, Japan), while the participants rested in a supine position with their eyes closed. The data were acquired in 128 × 128 matrices through an 18° rotation at an angle interval of 4°. The projection data were prefiltered and reconstructed, and Chang's attenuation and scattering corrections were applied.[33]

SPECT data were processed using the three-dimensional stereotactic surface projection (3D-SSP) method (Neurostat Software Library; Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA).[34] To assess perfusion deficits, the normalized brain activity of each patient was compared with that of 18 normal participants by using a pixel-by-pixel z-score analysis.[33] Qualitative z-score image analysis was carried out by two specialists without any knowledge of the clinical data. An image was defined as showing an AD pattern if the perfusion was decreased in the bilateral parietal association areas and posterior cingulate cortices, with relative sparing of the sensorimotor cortex, occipital cortex and cerebellum. The 3D-SSP technique, together with SPECT and positron emission tomography, provide a high diagnostic accuracy for AD.[34]

Statistical analysis

Statistical analysis was carried out using SPSS 19.0 for Windows (SPSS, Chicago, IL, USA). Comparisons of variables among the three patients groups were carried out by χ^2-test and analysis of covariance (ANCOVA), followed by post-hoc analysis (Bonferroni) to detect statistically significant differences. The association between BPSD and HbA1c was analyzed by Spearman's correlation analysis. Independent risks for BPSD were analyzed by multivariate logistic regression. Differences were considered significant at $P < 0.05$.

Results

Clinical profiles of the study participants

Age and education level were similar among the three groups of patients, whereas male sex was more prevalent in diabetic patients with HbA1c <7% (Table 1). The Barthel Index was lower in diabetic patients with HbA1c ≥7.0% than those with HbA1c <7.0%. Impaired dressing ability and urinary incontinence were apparent in diabetic patients with higher HbA1c (data not shown). Depressive mood and vitality, as well as caregivers' burden were not different among the three patient subgroups. The FRI was significantly increased in diabetic individuals with HbA1c ≥7.0%, although the incidence of falls in the previous year was unchanged. Polypharmacy was prevalent in diabetic participants. Use of sulfonylurea and insulin was increased in patients with HbA1c ≥7.0%, whereas biguanide was used more frequently in patients with HbA1c <7.0% (Table 1).

Apo E4 carriage, a genetic risk for AD, was seen in 52.5% (n = 122) of non-diabetic AD patients, which was compatible with the previous reports. However, among diabetic patients, the frequency of Apo E4 carriage was 39.4% and 47.7% in those with HbA1c <7% (n = 33) and ≥7.0% (n = 44).

As for physical complications, numbness was significantly increased in patients with HbA1c ≥7.0%, compared with non-diabetic individuals (25.0% and 11.8%, respectively). Dysphagia and diarrhea/constipation were also increased in poorly controlled diabetic participants (data not shown).

Biochemical properties

Blood glucose and HbA1c were significantly elevated in diabetic individuals (Table 2). Serum alkaliphosphatase tended to be increased in diabetic patients, and a significant increase was seen in diabetic patients with HbA1c ≥7.0%. Serum creatinine and estimated glomerular filtration rate were not changed among the three subgroups, whereas persistent proteinuria (>1 g protein/gCr) tended to be prevalent in patients with higher HbA1c (P = 0.056). The serum level of adiponectin was significantly reduced in patients with HbA1c ≥7.0%. 25-Hydroxyvitamin D, which reflects the activity of vitamin D, was significantly decreased in poorly controlled diabetic participants.

Cognitive impairment

Global brain function as measured by MMSE, ADAS and RCPM was substantially impaired in all three groups, but the degree of impairment was not significantly different among them (Table 3). Verbal fluency was significantly impaired in diabetic patients with HbA1c ≥7.0%. Performance on the recent memory and digit span tests, the latter of which is used as a measure of attention, was not different among the groups.

BPSD

The total score of DBD was significantly elevated in patients with HbA1c ≥7.0% (Table 4). DBD is a

© 2014 Japan Geriatrics Society

Table 1 Clinical profiles of study participants

	Diabetes HbA1c <7.0% (n = 39)		Diabetes HbA1c ≥7.0% (n = 52)		Diabetes total (n = 91)		Non-diabetes (n = 161)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	76.9	4.7	76.7	5.3	76.8	5.0	77.1	5.3
Male (%)	53.8*		40.4		46.2*		24.8	
Education (years)	10.5	2.6	10.4	2.7	10.5	2.7	10.2	2.7
BMI (kg/m^2)	23.4*	3.4	22.7	3.4	23.0*	3.4	21.8	3.1
Heart rate (b.p.m.)	80.0*	13.0	80.2*	15.4	80.1*	14.3	75.4	14.1
Systolic blood pressure (mmHg)	158.9	24.3	158.0	24.9	158.4	24.5	158.2	25.0
Diastolic blood pressure (mmHg)	80.5	10.7	81.8	13.5	81.3	12.3	85.2	13.7
Barthel Index	98.8	3.1	95.7**	7.9	97.0	6.5	97.3	6.1
Lawton Index								
Male	3.2	1.3	3.2	1.4	3.2	1.3	3.3	1.4
Female	5.7	1.9	5.3	2.0	5.4	1.9	5.8	1.8
Geriatric depression scale	3.9	2.2	3.9	2.7	3.9	2.5	4.6	2.7
Zarit burden interview	17.8	13.1	21.2	14.8	19.8	14.1	19.6	14.6
Fall risk index	5.3	3.4	5.5*	3.6	5.4	3.5	4.9	3.6
Polypharmacy (%)	63.2*		54.9*		58.4*		32.5	
Insulin user (%)	0.0		21.2**		12.1		–	
Use of sulfonylurea (%)	25.6		51.9**		40.7		–	
Use of biguanide (%)	33.3		13.5**		22.0		–	
Use of thiazolidines (%)	25.6		23.1		24.2		–	
Apoprotein E4 carrier (%)	39.4		47.7		44.2		52.5	

*P < 0.05 versus non-diabetes, **P < 0.05 versus glycated hemoglobin (HbA1c) <7.0%, ANCOVA adjusted for age and sex.

Table 2 Biochemical and metabolic analysis

	Diabetes HbA1c <7.0%		Diabetes HbA1c ≥7.0%		Diabetes total		Non-diabetes	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Blood glucose (mg/dL)	141.4*	38.6	197.7*,**	102.0	174.4*	86.2	105.9	15.4
HbA1c (%)	6.5*	0.4	8.7*,**	1.5	7.7*	1.5	5.5	1.2
Serum albumin (g/dL)	4.3	0.3	4.4	0.3	4.4	0.3	4.4	0.3
ALP	274.3	59.4	332.5*,**	136.6	307.7*	113.7	244.4	69.3
AST (IU/L)	25.5	7.5	26.2	14.4	25.9	11.9	24.9	8.6
ALT (IU/L)	21.6	9.6	27.0*	22.5	24.7*	18.3	19.4	11.2
γ-GT (IU/L)	31.7	30.4	42.2*	61.2	37.8*	50.5	26.0	22.0
Creatinine (mg/dL)	0.9	0.3	0.8	0.3	0.8	0.3	0.7	0.2
eGFR (mL/min/1.73m^2)	59.6	17.5	67.0	19.7	63.8	19.1	67.3	17.8
Total cholesterol (mg/dL)	194.6*	40.7	211.2	46.0	204.2*	44.4	221.7	37.2
HDL cholesterol (mg/dL)	51.4*	13.4	54.1*	14.4	52.9*	14.0	66.7	16.7
Non-HDL cholesterol (mg/dL)	140.3*	35.0	158.2	44.8	150.7	41.7	158.2	32.8
LDL cholesterol (mg/dL)	109.6	31.2	125.4	37.6	118.7	35.7	127.7	31.1
Triglyceride (mg/dL)	138.9	70.5	164.7*	120.2	153.8*	102.5	117.4	64.8
Adiponectin (mg/mL)	8.2	8.5	6.0*	3.8	7.0	6.5	9.0	5.4
25-Hydroxyvitamin D (ng/mL)	27.1	8.8	21.0*,**	6.9	23.9	8.4	23.9	6.7
Persistent proteinuria (%)	16.7		18.4		17.6*		7.6	

*P < 0.05 versus non-diabetes, **P < 0.05 versus glycated hemoglobin (HbA1c) <7.0%, ANCOVA adjusted for age and sex. γ-GT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

© 2014 Japan Geriatrics Society

Table 3 Neuropsychiatric assessment

	Diabetes HbA1c <7.0%		Diabetes HbA1c ≥7.0%		Diabetes total		Non-diabetes	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
MMSE	18.7	3.8	19.6	4.1	19.2	4.0	19.3	5.1
ADAS	19.0	5.6	17.8	7.1	18.4	6.4	16.8	6.1
RCPM	21.2	5.3	23.5	4.8	22.3	5.1	22.4	6.3
FAB	8.8	2.3	9.2	2.6	9.0	2.5	9.6	2.5
Verbal fluency	2.6	2.1	2.3*	2.1	2.4*	2.1	3.2	2.0
Digit span: Forward	4.9	1.0	5.1	1.0	5.0	1.0	5.1	1.0
Backward	3.1	1.0	3.1	1.0	3.1	1.0	3.2	1.1
Logical memory 1	2.8	2.8	3.0	2.8	2.9	2.8	3.5	3.6
Logical memory 2	0.3	0.7	0.5	1.4	0.4	1.1	.4	1.1

*$P < 0.05$ versus non-diabetes, ANCOVA adjusted for age, sex and education. ADAS, Alzheimer's Disease Assessment Scale; FAB, Frontal Assessment of the Brain; HbA1c, glycated hemoglobin; MMSE, Mini-Mental State Examination; RCPM, Raven's Colored Progressive Matrices.

Table 4 Dementia Behavior Disturbance scale

	Diabetes HbA1c <7.0%		Diabetes HbA1c ≥7.0%		Diabetes total		Non-diabetes	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total score of DBD	12.8	8.3	20.1*,**	11.1	17.0	10.6	15.9	10.6
Exhibits lack of interest in daily activities	1.5*	1.4	2.1*	1.2	1.9	1.3	1.8	1.3
Sleeps excessively during the day	1.5	1.5	1.8*	1.3	1.7*	1.4	1.1	1.2
Is verbally abusive, curses	0.4	0.8	0.8*	1.2	0.6	1.1	0.4	0.9
Dresses inappropriately	0.3	0.6	0.9**	1.0	0.6	0.9	0.6	0.9
Hoards things for no obvious reason	0.5	1.1	1.2**	1.4	0.9	1.3	0.8	1.2
Overeats	0.7	0.9	1.3*,**	1.4	1.0*	1.3	0.5	0.9
Is incontinent of urine	0.2	0.6	0.7**	1.1	0.5	0.9	0.5	0.9

*$P < 0.05$ versus non-diabetes, **$P < 0.05$ versus glycated hemoglobin (HbA1c) <7.0%, ANCOVA adjusted for age and sex. DBD, Dementia Behavior Disturbance scale.

questionnaire composed of 28 items and similar comparisons were carried out for each subitem. Lack of interest, excessive daytime sleeping, verbal abuse, inappropriate dress, hoarding, overeating and urinary incontinence were significantly elevated in diabetic individuals with higher HbA1c. Significant correlations were observed between HbA1c and each of inappropriate dress, hoarding and urinary incontinence ($P = 0.023$, $P = 0.031$, $P = 0.014$, respectively). Because overeating is a crucial problem that can induce hyperglycemia in diabetes, we attempted to identify factors that might have been independently associated with hyperphagia by multivariate logistic regression. The results showed that male sex, excessive daytime sleeping, elevated levels of HbA1c and elevated triglyceride were independently associated with overeating ($P = 0.013$, $P = 0.005$, $P = 0.007$ and $P = 0.005$, respectively). Interestingly, overeating was significantly increased in patients with daytime sleep >3 h (38.5%), and also in those with daytime sleep of 0–3 h (16.0%), compared with those without daytime sleep (7.2%) (ANOVA).

Brain MRI and SPECT

Finally, we evaluated the morphological and functional changes of the brain (Table 5). The total volumes of the IC, parenchyma, ventricles and WML were determined by automatic segmentation on brain MRI. We found that the parenchyma/IC ratio, as an index for brain atrophy, was significantly decreased in patients with HbA1c ≥7.0%, whereas the WML/IC ratio was unchanged.

The 3D-SSP technique with SPECT provides a high diagnostic accuracy for AD.[34] Posterior cerebral hypoperfusion on SPECT (AD pattern) was observed in 60.6% of diabetic patients with HbA1c <7.0% ($n = 36$), which was significantly smaller than the percentage of

© 2014 Japan Geriatrics Society

Table 5 Magnetic resonance imaging and single-photon emission computed tomography analysis

	Diabetes HbA1c <7.0%		Diabetes HbA1c ≥7.0%		Diabetes total		Non-diabetes	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
MRI								
Parenchyma (mL)	1040.1	105.4	1028.0	112.0	1032.7	108.9	1024.8	101.0
WML (mL)	21.5	24.6	14.7	13.8	17.4	18.9	19.7	20.7
IC (mL)	1418.4	133.5	1400.4	140.3	1407.4	137.0	1377.9	125.6
Parenchyma/IC	73.3	3.3	73.4*	3.0	73.4	3.1	74.4	3.2
WML/IC	1.5	1.8	1.1	1.0	1.2	1.3	1.4	1.4
SPECT								
AD pattern (%)	60.6**		81.3		67.0*		81.3	

*$P < 0.05$ versus non-diabetes, ANCOVA adjusted for age and sex. **$P < 0.03$ versus diabetes glycated hemogobin (HbA1c) >7.0% and non-diabetes, χ^2-test. AD, Alzheimer's disease; IC, intracranial space; MRI, magnetic resonance imaging; SPECT, single-photon emission computed tomography; WML, white matter regions.

diabetic patients with HbA1c ≥7.0% ($n = 46$) and non-diabetic patients with AD ($n = 144$) who showed an AD pattern.

Discussion

The present study clearly identified that diabetic older adults diagnosed as having AD with HbA1c <7% have differential clinical features and pathophysiology from those with HbA1c ≥7.0%. Patients with higher HbA1c have increased vascular complications of diabetes, insulin resistance, impaired ADL, and altered bone and muscle metabolism. Although cognitive function was similar between the two groups, BPSD such as lack of interest, overeating and excessive daytime sleeping apparently increased in patients with higher HbA1c, which might have contributed to difficulties in the management of diabetes with dementia. Although the frequencies of Apo E4 carriage and of posterior cerebral hypoperfusion on SPECT in poorly controlled diabetic subjects were similar to those in non-diabetic AD patients, the group of diabetic patients with lower HbA1c had a lower incidence of Apo E4 carriage and an AD pattern on SPECT, suggesting the involvement of non-AD pathophysiology in this group. It seems plausible that difficulties in the management of diabetes with AD are due not only to non-adherence to diabetes treatment, but also several symptoms and pathophysiological characteristics of dementia.

Patients with AD show a variety of problematic behaviors during the course of the disease. One of these behaviors, increased food intake has been described in 9–26% of AD cases.[35] The neuroanatomical basis for overeating in AD remains unclear, but hyperphagia is often accompanied by forgetfulness and hyperorality.[36] Overeating has been of less concern in non-diabetic AD, because weight loss and malnutrition are more important in the late stages of dementia. However, in the case of diabetic elderly patients, overeating can lead directly to hyperglycemia. Food intake is controlled by a complex regulatory network in the brain. The hypothalamus plays a particularly important role in regulating appetite and energy expenditure. It has been postulated that interactions between adiposity and the central neuropeptidergic cascade are impaired in obesity.[37] Poor glycemic control is associated with overeating, even in adolescents with type 2 diabetes,[38] suggesting that eating disorders in our diabetic participants could be related to not only dementia disease, but also diabetes. In addition, we found a correlation between HbA1c and urinary incontinence. Thus, an interactive relationship should be considered between hyperglycemia and some problematic symptoms of dementia.

In this connection, the present study showed a link between daytime sleep and overeating. Recent studies suggest that insufficient sleep can facilitate feeding behavior by changing circulating hormones involved in feeding, glucose metabolism and appetite.[39] Thus, whether a lifestyle intervention to reduce excessive daytime sleep could prevent overeating should be tested in diabetic patients with AD in the future.

In patients with HbA1c ≥7.0%, FRI increased and basic ADL decreased. FRI is a surrogate marker for falls, but also for frailty in older adults.[28] Interestingly, the serum level of 25-hydroxyvitamin D was significantly reduced in patients with higher HbA1c. A recent meta-analysis showed that the serum concentration of 25-hydroxyvitamin D is decreased in AD.[40] Hypovitaminosis D is also associated with insulin resistance, and 25-hydroxyvitamin D levels are inversely related to HbA1c in type 2 diabetic patients.[41,42] The present study found low concentrations of serum high-molecular-weight adiponectin in patients with HbA1c ≥7.0%, which strongly suggested elevated insulin resistance.[43] Insulin resistance and hypovitaminosis D play a major

© 2014 Japan Geriatrics Society

role in the development of muscle loss, leading to frailty in elderly individuals.[44,45] It seems likely that diabetic patients with higher HbA1c are more prone to have muscle weakness and impaired life function, compared with those in patients with HbA1c <7.0%.

Apo E4 carriage is the strongest genetic risk factor for AD so far identified. It has been reported that the Apo E4 allele is highly prevalent among individuals with AD, with 64% of all patients with sporadic AD carrying at least one copy of the Apo E4 allele.[46] In contrast, the presence of an AD pattern on SPECT has 87% sensitivity and 90% specificity for discriminating AD from other dementias.[47] The lowered incidence of Apo E4 carriage and AD-pattern in diabetic patients with HbA1c <7% implies the possible involvement of non-AD dementia disorders. It should be noted that subcortical WML were unchanged in this group of diabetic patients.

In this respect, it is interesting to note the report of Fukazawa *et al.* where the frequency of Apo E4 carriers was 30~40% in a subgroup of diabetic patients with AD.[48] As for the pathological background, age-associated tauopathies, such as argyrophilic grain disease (AGD) and neurofibrillary tangle-predominant dementia, have been suggested. AGD is characterized by progressive amnesia, with other cognitive functions being relatively spared.[49] Because there are no specific criteria for the clinical diagnosis of AGD, it is still difficult to discriminate AGD from AD.[50,51] The metabolic effects of diabetes and invisible microinfarctions could affect cognitive function in some subtypes of dementia in diabetic older adults.[48]

To date, several organizations for the management of diabetes in the USA, Europe, Canada and Japan have introduced their guidelines for the treatment of older diabetes patients.[52–55] These guidelines share the concept that glycemic targets should be determined individually, with a targeted range of 6.5~7.5% for HbA1c in robust diabetic older adults, versus 7.5~8.5% for HbA1c in frail older adults. However, there is still no convincing data on which to base a target for glycemic control to maintain brain function in older adults, particularly for individuals with dementia. In this respect, the present study shows that patients in the lower HbA1c group had fewer BPSD. A positive correlation of BPSD with HbA1c was shown for inappropriate dressing, hoarding and urinary incontinence. Conversely, the present results showed that diabetic participants with mild AD had several properties of physical frailty and ADL impairment, and suggested that diabetic patients with more advanced stages of dementia should be treated less stringently, if we consider the guidelines described here. To address these controversies, prospective studies will be required to clarify the glycemic control levels for prevention of physical and psychological complications of dementia in diabetic older adults.

The present study had several limitations. It was a cross-sectional study, and therefore no causality can be inferred between HbA1c and the several clinical manifestations studied. Identification of non-AD dementia disorders in diabetic patients with HbA1c <7.0% should be investigated in future. Second, the impact of hypoglycemia on brain function was not directly examined. Because hypoglycemic episodes are often atypical or absent in older adults, it is difficult to obtain reliable information on hypoglycemia from patients with dementia and their caregivers.[56] We hypothesize that hypoglycemic episodes might occur more frequently in diabetic patients with HbA1c <7.0%, because all diabetic participants were receiving antihyperglycemic agents and/or insulin. However, fewer clinical problems were observed in patients with lower HbA1c.

Based on the present findings, it can be stated conclusively that there are subtypes of diabetic patients with AD from the viewpoint of clinical features and brain pathophysiology. Physical and psychological complications of dementia are largely dependent on glucose control levels. Although these distinctions require further corroboration, it seems clear that a comprehensive approach to diabetes and dementia will be needed in order to achieve reasonable control in older diabetic patients with AD.

Acknowledgements

This study was financially supported by grants from the Chojyu (24-24, 25-6), the Ministry of Education, Culture, Sports, Science, and Technology (22590654) and the Ministry of Health, Labor and Welfare (H25-Ninchisho-008), Japan. We also thank the BioBank at NCGG for quality control of the clinical data.

Disclosure statement

The authors declare no conflict of interest.

References

1 Biessels GJ, Staekenborg S, Brunner E, Brayne C, Scheltens P. Risk of dementia in diabetes mellitus: a systematic review. *Lancet Neurol* 2006; **5**: 64–74.
2 Whitmer RA, Karter AJ, Yaffe K, Quesenberry CP Jr, Selby JV. Hypoglycemic episodes and risk of dementia in older patients with type 2 diabetes mellitus. *JAMA* 2009; **301**: 1565–1572.
3 Rizzo MR, Marfella R, Barbieri M *et al.* Relationships between daily acute glucose fluctuations and cognitive performance among aged type 2 diabetic patients. *Diabetes Care* 2010; **33**: 2169–2174.
4 Yaffe K, Blackwell T, Whitmer RA, Krueger K, Barrett Connor E. Glycosylated hemoglobin level and development of mild cognitive impairment or dementia in older women. *Nutr Health Aging* 2006; **10**: 293–295.

© 2014 Japan Geriatrics Society

5 Peila R, Rodriguez BL, Launer LJ. Type 2 diabetes, ApoE gene, and the risk for dementia and related pathologies. The Honolulu-Asia Aging Study. *Diabetes* 2002; **51**: 1256–1262.
6 Matsuzaki T, Sasaki K, Tanizaki Y *et al.* Insulin resistance is associated with the pathology of Alzheimer disease: the Hisayama study. *Neurology* 2010; **75**: 764–770.
7 Heitner J, Dickson D. Diabetics do not have increased Alzheimer-type pathology compared with age-matched control subjects. A retrospective postmortem immunocytochemical and histofluorescent study. *Neurology* 1997; **49**: 1306–1311.
8 Beeri MS, Silverman JM, Davis KL *et al.* Type 2 diabetes is negatively associated with Alzheimer's disease neuropathology. *J Gerontol A Biol Sci Med Sci* 2005; **60**: 471–475.
9 Sonnen JA, Larson EB, Brickell K *et al.* Different patterns of cerebral injury in dementia with or without diabetes. *Arch Neurol* 2009; **66**: 315–322.
10 Ahtiluoto S, Polvikoski T, Peltonen M *et al.* Diabetes, Alzheimer disease, and vascular dementia. A population-based neuropathologic study. *Neurology* 2010; **75**: 1195–1202.
11 Mielke MM, Rosenberg PB, Tschanz J *et al.* Vascular factors predict rate of progression in Alzheimer disease. *Neurology* 2007; **69**: 1850–1858.
12 Sanz C, Andrieu S, Sinclair A, Hanaire H, Vellas B, REAL.FR Study Group. Diabetes is associated with a slower rate of cognitive decline in Alzheimer disease. *Neurology* 2009; **73**: 1359–1366.
13 Regan C, Katona C, Walker Z, Hooper J, Donovan J, Livingston G. Relationship of vascular risk to the progression of Alzheimer disease. *Neurology* 2006; **67**: 1357–1362.
14 Helzner EP, Luchsinger JA, Scarmeas N *et al.* Contribution of vascular risk factors to the progression in Alzheimer disease. *Arch Neurol* 2009; **66**: 343–348.
15 Wade DT, Collin C. The Barthel ADL Index: a standard measure of physical disability? *Int Disabil Stud* 1988; **10**: 64–67.
16 Kawai Y, Miura R, Tujimoto M *et al.* Neuropsychological differentiation of Alzheimer's disease and dementia with Lewy bodies in a memory clinic. *Psychogeriatrics* 2013; **13**: 157–163.
17 McKhann G, Drachman D, Folstein M *et al.* Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984; **34**: 939–944.
18 Kojima T, Akishita M, Nakamura T *et al.* Polypharmacy as a risk for fall occurrence in geriatric outpatients. *Geriatr Gerontol Int* 2012; **12**: 425–430.
19 Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. *Gerontologist* 1969; **9**: 179–186.
20 Folstein MF, Folstein SE, McHugh PR. "Mini-mental state." A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; **12**: 189–198.
21 Mohs R, Rosen W, Davis K. The Alzheimer's disease assessment scale: an instrument for assessing treatment efficacy. *Psychopharmacol Bull* 1983; **19**: 448–450.
22 Raven JC, Court JH, Raven J. *Manual for Raven's Progressive Matrices and Vocabulary Scales, The Coloured Progressive Matrices*. London: HK Lewis, 1977.
23 Wechsler D. *Wechsler Memory Scale-Revised*. San Antonio, TX: Psychological Corp, 1981.
24 Dubois B, Slachevsky A, Litvan I, Pillon B. The FAB: a Frontal Assessment Battery at bedside. *Neurology* 2000; **55**: 1621–1626.
25 Yesavage JA, Brink T, Rose T *et al.* Development and validation of a geriatric depression screening scale. *J Psychiatr Res* 1983; **17**: 37–49.
26 Baumgarten M, Becker R, Gauthier S. Validity and reliability of the dementia behavior disturbance scale. *J Am Geriatr Soc* 1990; **38**: 221–226.
27 Zarit S, Reever K, Bach-Peterson J. Relatives of the impaired elderly: correlates of feelings of burden. *Gerontologist* 1980; **20**: 649–655.
28 Ishimoto Y, Wada T, Kasahara Y *et al.* Fall Risk Index predicts functional decline regardless of fall experiences among community-dwelling elderly. *Geriatr Gerontol Int* 2012; **12**: 659–666.
29 Kataoka S, Paidi M, Howard BV. Simplified isoelectric focusing/immunoblotting determination of apoprotein E phenotype. *Clin Chem* 1994; **40**: 11–13.
30 Su Z, Slay BR, Carr R, Zhu Y. The recognition of 25-hydroxyvitamin D2 and D3 by a new binding protein based 25-hydroxyvitamin D assay. *Clin Chim Acta* 2013; **417**: 62–66.
31 Hayama S, Higuchi T, Miyakoshi H, Nakano Y. Analytical evaluation of a high-molecular-weight (HMW) adiponectin chemiluminescent enzyme immunoassay. *Clin Chim Acta* 2010; **411**: 2073–2078.
32 Admiraal-Behloul F, van den Heuvel DM, Olofsen H *et al.* Fully automatic segmentation of white matter hyperintensities in MR images of the elderly. *Neuroimage* 2005; **28**: 607–617.
33 Nihashi T, Yatsuya H, Hayasaka K *et al.* Direct comparison study between FDG-PET and IMP-SPECT for diagnosing Alzheimer's disease using 3D-SSP analysis in the same patients. *Radiat Med* 2007; **25**: 255–262.
34 Burdette J, Minoshima S, Borght TV, Tran DD, Kuhl DE. Alzheimer disease: improved visual interpretation of PET images by using three-dimensional stereotactic surface projections. *Radiology* 1996; **198**: 837–843.
35 Ikeda M, Brown J, Holland A, Fukuhara R, Hodges J. Changes in appetite, food preference, and eating habits in frontotemporal dementia and Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 2002; **73**: 371–376.
36 Bhagavati S. Marked hyperphagia associated with total loss of satiety in Alzheimer's disease. *J Neuropsychiatry Clin Neurosci* 2008; **20**: 248–249.
37 Amitani M, Asakawa A, Amitani H, Inui A. The role of leptin in the control of insulin-glucose axis. *Front Neurosci* 2013; **7**: 51.
38 Rothman RL, Mulvaney S, Elasy TA *et al.* Self-management behaviors, racial disparities, and glycemic control among adolescents with type 2 diabetes. *Pediatrics* 2008; **121**: e912–e919.
39 Hanlon EC, Van Cauter E. Quantification of sleep behavior and of its impact on the cross-talk between the brain and peripheral metabolism. *Proc Natl Acad Sci U S A* 2011; **108** (Suppl 3): 15609–15616.
40 Annweiler C, Llewellyn DJ, Beauchet O. Low serum vitamin D concentrations in Alzheimer's disease: a systematic review and meta-analysis. *J Alzheimers Dis* 2013; **33**: 659–674.
41 Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr* 2004; **79**: 820–825.
42 Kostoglou-Athanassiou I, Athanassiou P, Gkountouvas A, Kaldrymides P. Vitamin D and glycemic control in diabetes mellitus type 2. *Ther Adv Endocrinol Metab* 2013; **4**: 122–128.
43 Heidemann C, Sun Q, van Dam RM *et al.* Total and high-molecular-weight adiponectin and resistin in relation to

© 2014 Japan Geriatrics Society

the risk for type 2 diabetes in women. *Ann Intern Med* 2008; **149**: 307–316.
44 Morley JE, Malmstrom TK. Frailty, sarcopenia, and hormones. *Endocrinol Metab Clin North Am* 2013; **42**: 391–405.
45 Krentz AJ, Viljoen A, Sinclair A. Insulin resistance: a risk marker for disease and disability in the older person. *Diabet Med* 2013; **30**: 535–548.
46 Farrer LA, Cupples LA, Haines JL *et al.* APOE and Alzheimer Disease Meta Analysis Consortium. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease: a meta-analysis. *JAMA* 1997; **278**: 1349–1356.
47 Bonte FJ, Harris TS, Hynan LS, Bigio EH, White CL, 3rd. Tc-99m HMPAO SPECT in the differential diagnosis of the dementias with histopathologic confirmation. *Clin Nucl Med* 2006; **31**: 376–378.
48 Fukazawa R, Hanyu H, Sato T *et al.* Subgroups of Alzheimer's disease associated with diabetes mellitus based on brain imaging. *Dement Geriatr Cogn Disord* 2013; **35**: 280–290.
49 Togo T, Isojima D, Akatsu H *et al.* Clinical features of argyrophilic grain disease: a retrospective survey of cases with neuropsychiatric symptoms. *Am J Geriatr Psychiatry* 2005; **13**: 1083–1091.
50 Braak H, Braak E. Argyrophilic grain disease: frequency of occurrence in different age categories and neuropathological diagnostic criteria. *J Neural Transm* 1998; **105**: 801–819.
51 Tolnay M, Clavaguera F. Argyrophilic grain disease: a late-onset dementia with distinctive features among tauopathies. *Neuropathology* 2004; **24**: 269–283.
52 Brown AF, Mangione CM, Saliba D *et al.* California Healthcare Foundation/American Geriatrics Society Panel on Improving Care for Elders with Diabetes. Guidelines for improving the care of the older person with diabetes mellitus. *J Am Geriatr Soc* 2003; **51** (5 Suppl Guidelines): S265–S280.
53 Qaseem A, Vijan S, Snow V *et al.* Clinical Efficacy Assessment Subcommittee of the American College of Physicians. Glycemic control and type 2 diabetes mellitus: the optimal hemoglobin A1c targets. A guidance statement from the American College of Physicians. *Ann Intern Med* 2007; **147**: 417–422.
54 Meneilly GS, Tessier D. Diabetes in elderly adults. *J Gerontol A Biol Sci Med Sci* 2001; **56**: M5–13.
55 Sinclair AJ, Paolisso G, Castro M *et al.* European Diabetes Working Party for Older People. European Diabetes Working Party for Older People 2011 clinical guidelines for type 2 diabetes mellitus. Executive summary. *Diab Metab* 2011; **37**: S27–38.
56 Araki A, Ito H. Diabetes mellitus and geriatric syndromes. *Geriatr Gerontol Int* 2009; **9**: 105–114.

© 2014 Japan Geriatrics Society

LOWER VITAMIN D IS ASSOCIATED WITH WHITE MATTER HYPERINTENSITY IN ELDERLY WOMEN WITH ALZHEIMER'S DISEASE AND AMNESTIC MILD COGNITIVE IMPAIRMENT

To the Editor: White matter hyperintensity (WMH) on brain magnetic resonance imaging (MRI) is prevalent in the aging brain and is associated with cognitive decline and mobility impairment.[1,2] It is a clinical challenge to establish a prevention strategy for WMH. Although believed to be of vascular origin, the exact etiology of WMH remains unclear. Age and hypertension are consistent risk factors for WMH. High homocysteine levels, diabetes mellitus, hyperlipidemia, smoking, obesity, low vitamin B12 levels, and alcohol consumption are likely to increase WMH.[3]

There is growing evidence of potential roles of vitamin D in sustaining healthy brain function.[4] Low serum vitamin D has been reported in Alzheimer's disease (AD), which is often associated with WMH,[5] but little is known about the link between vitamin D and WMH in elderly adults with AD. This study aimed to clarify the interaction between vitamin D, WMH, brain atrophy in elderly adults with AD and amnestic mild cognitive impairment (aMCI).

Two hundred fifty-three women aged 65 and older diagnosed with aMCI (n = 39) or AD (n = 214) were recruited. AD was diagnosed as possible or probable AD according the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association, and aMCI was diagnosed based on previously defined criteria.[2,6] Individuals with severe cardiac failure, renal disorder, liver dysfunction, or musculoskeletal disease or with cortical lesions on MRI were excluded.

Cognitive function was evaluated using the Mini-Mental State Examination (MMSE). Hypertension, diabetes mellitus, lipid abnormalities, and chronic kidney disease were defined as having a history of these diseases or use of medication to treat them. Information on current smoking and drinking habits was obtained from clinical charts. Vitamin D insufficiency was assessed according to serum concentration of 25-hydroxyvitamin D (25(OH)D).

A standard series of axial T2-weighted (repetition time (TR), 3,800 ms; echo time (TE), 93 ms) and fluid-attenuated inversion recovery (TR, 8,000 ms; TE, 101 ms; inversion time, 2,500 ms; a 256 × 256 matrix) MR sequences were obtained using a 1.5-T MR scanner (Siemens Avanto, Munich, Germany). Scans in parallel with the anterior commissure–posterior commissure line were performed with 6-mm-thick slices and an interslice gap of 1.2 mm.[2] MRI data were processed to measure the total volume of the intracranial space (IC), parenchyma, ventricles, and WMH using a fully automatic segmentation program (Software for Neuro-Image Processing in Experimental Research).[7]

Statistical analysis was performed using SPSS 19.0 for Windows (SPSS, Inc., Chicago, IL). Because WMH were not normally distributed, their values were converted to rank variables. The association between WMH and clinical variables was analyzed using partial Spearman rank order correlation analysis and multivariate regression (stepwise). Differences were considered significant at $P < .05$.

Table 1. Clinical Profile and Magnetic Resonance Imaging Results of Study Participants (N = 253)

Characteristic	Value
Demographic characteristics and biochemical measurements	
Age, mean ± SD	77.5 ± 5.0
Education, years, mean ± SD	**10.0 ± 2.1**
Mini-Mental State Examination score, mean ± SD	19.9 ± 5.0
Hypertension, %	**56.9**
Diabetes mellitus, %	21.6
Lipid abnormality, %	**41.7**
Chronic kidney disease, %	32.3
Current smoking, %	**2.0**
Drinking habit, %	7.7
Systolic blood pressure, mmHg, mean ± SD	**150.1 ± 21.2**
Diastolic blood pressure, mmHg, mean ± SD	80.9 ± 11.9
Body mass index, kg/m^2, mean ± SD	**21.8 ± 3.6**
Glycosylated hemoglobin, %, mean ± SD	6.1 ± 0.8
Total cholesterol, mg/dL, mean ± SD	**221.5 ± 37.5**
High-density lipoprotein cholesterol, mg/dL, mean ± SD	63.5 ± 15.7
Triglyceride, mg/dL, mean ± SD	**126.5 ± 71.9**
Estimated glomerular filtration rate, mL/min per 1.73 m^2, mean ± SD	68.5 ± 18.4
Cystatin C, mg/L, mean ± SD	**1.0 ± 0.4**
25-hydroxyvitamin D, ng/mL, mean ± SD	23.6 ± 7.2
Homocysteine, nmol/mL, mean ± SD	**11.1 ± 5.6**
Vitamin B12, pg/mL, mean ± SD	674.2 ± 476.3
Cranial magnetic resonance imaging, mean ± SD	
IC, mL	1,324.2 ± 96.4
WMH, mL	**17.2 ± 18.1**
WMH/IC, %	1.3 ± 1.4
Parenchyma, mL	**991.1 ± 78.3**
Parenchyma/IC, %	74.9 ± 3.0

SD = standard deviation; IC = intracranial space; WMH = white matter hyperintensity.

Table 1 shows the demographic characteristics, biochemical measurements, and results of MRI analyses of the participants. Serum 25(OH)D levels in individuals receiving vitamin D supplementation (n = 20) were significantly lower than in those who were not ($P = .03$).

Partial Spearman rank order correlation revealed a possible correlation between WMH/IC and age ($r_s = 0.252$, $P < .001$), hypertension ($r_s = 0.179$, $P = .001$), MMSE score ($r_s = -0.157$, $P < .001$), cystatin-C ($r_s = 0.166$, $P = .004$), 25(OH)D ($r_s = -0.171$, $P = .006$), and homocysteine ($r_s = 0.146$, $P = .01$), whereas the other clinical indices were not correlated (data not shown). Multivariate regression analysis using these possible factors, together with education, as variables revealed that older age ($\beta = 0.208$, $P = .001$), hypertension ($\beta = 0.162$, $P = .009$), and lower 25(OH)D ($\beta = -0.163$, $P = .008$) were independently associated with WMH/IC. In contrast, 25(OH)D was not related to parenchyma/IC as an index of global brain atrophy (data not shown).

The present study clearly demonstrates that 25(OH)D is negatively correlated with WMH volume in elderly women with aMCI or AD, even after adjusting for classic risk factors for WMH, whereas 25(OH)D was not

associated with brain atrophy. A previous study suggested that individuals with 25(OH)D deficiency had greater WMH volume.[8] A correlation between vitamin D and WMH in multiple sclerosis has been demonstrated.[9] Another study recently reported an association between WMH and low 25(OH)D in independent outpatients.[10] The current study indicates that hypovitaminosis D is a predictor of WMH in individuals with AD or aMCI.

WMH has been found to have clinical relevance in cognitive decline and several geriatric syndrome conditions,[1,2] and vitamin D plays central roles in muscle weakness and physical frailty in elderly individuals. Taken together, the results of the current study suggest that vitamin D controls two pathways toward cognitive decline and frailty by means of white matter burden and sarcopenia in elderly adults with dementia. Prospective studies are needed to clarify the effects of vitamin D supplementation in prevention of WMH in individuals with AD.

Takashi Sakurai, MD, PhD
Noriko Ogama, MA
Kenji Toba, MD, PhD
Center for Comprehensive Care and Research on Memory Disorders, National Center for Geriatrics and Gerontology, Obu, Japan

ACKNOWLEDGMENTS

We thank BioBank, NCGG for quality control of clinical data.

Conflict of Interest: Research funding for Longevity Sciences (24–24, 25–5) was received from the National Center for Geriatrics and Gerontology, Japan (TS) and Health and Labor Sciences Research Grants (H25-Ninchisho-008) (KT).

Author Contributions: Sakurai: study concept and design, acquisition of subjects and data, analysis and interpretation of data, preparation of manuscript. Ogama: data acquisition, analysis and interpretation of data. Toba: study concept and design, manuscript editing.

Sponsor's Role: None.

REFERENCES

1. Wakefield DB, Moscufo N, Guttmann CR et al. White matter hyperintensities predict functional decline in voiding, mobility, and cognition in older adults. J Am Geriatr Soc 2010;58:275–281.
2. Ogama N, Sakurai T, Shimizu A et al. Regional white matter lesions predict falls in patients with amnestic mild cognitive impairment and Alzheimer's disease. J Am Med Dir Assoc 2014;15:36–41.
3. Rostrup E, Gouw AA, Vrenken H et al.; LADIS study group. The spatial distribution of age-related white matter changes as a function of vascular risk factors—results from the LADIS study. Neuroimage 2012;60:1597–1607.
4. Llewellyn DJ, Lang IA, Langa KM et al. Vitamin D and risk of cognitive decline in elderly persons. Arch Intern Med 2010;170:1135–1141.
5. Annweiler C1, Llewellyn DJ, Beauchet O. Low serum vitamin D concentrations in Alzheimer's disease: A systematic review and meta-analysis. J Alzheimers Dis 2013;33:659–674.
6. Petersen R, Doody R, Kurz A et al. Current concepts in mild cognitive impairment. Arch Neurol 2001;58:1985–1992.
7. Admiraal-Behloul F, van den Heuvel DM, Olofsen H et al. Fully automatic segmentation of white matter hyperintensities in MR images of the elderly. Neuroimage 2005;28:607–617.
8. Buell JS, Dawson-Hughes B, Scott TM et al. 25-hydroxyvitamin D, dementia, and cerebrovascular pathology in elders receiving home services. Neurology 2010;74:18–26.
9. Mowry EM, Waubant E, McCulloch CE et al. Vitamin D status predicts new brain magnetic resonance imaging activity in multiple sclerosis. Ann Neurol 2012;72:234–240.
10. Prager JM, Thomas C, Ankenbrandt WJ et al. Association of white matter hyperintensities with low serum 25-hydroxyvitamin D levels. AJNR Am J Neuroradiol 2014;35:1145–1149.

IMPROVING CARE TRANSITIONS IN INDIVIDUALS FREQUENTLY ADMITTED TO THE HOSPITAL

To the Editor: Individuals who are frequently admitted to the hospital have high levels of multimorbidity, polypharmacy, and functional impairment,[1] and the sudden reduction in intensity of care in the posthospital period may contribute to high rates of hospital readmission.[2–5] A multidisciplinary follow-up clinic for frequently admitted patients was piloted to address demonstrated gaps in transitional care.[6] This report describes the patient perspective of care in this model.

METHODS

Participants were recruited from general medical wards of a metropolitan hospital in Brisbane, Australia, from June to December 2012. Consecutive inpatients aged 60 and older with at least one unplanned hospitalization in the previous 6 months were identified through the admissions database. Individuals were ineligible if they lived outside Brisbane, were discharged to residential care, were receiving palliative care, could not consent, or were receiving care from the heart failure service. Eligible individuals were invited to participate if their treating team agreed. The study received ethical approval.

Participants were scheduled for clinic review within 2 weeks of discharge. The first visit included comprehensive assessment of social and medical services, symptoms, care goals, activities of daily living, malnutrition, and depression by a registered nurse. A clinical pharmacist undertook review of medication use after discharge, current medication understanding, and adherence. The physician then reviewed medical conditions and treatments and recommended a treatment plan, including referrals as needed. A physiotherapist or exercise physiologist assessed each participant and prescribed an individualized exercise program in a supervised weekly group exercise class or at home. A comprehensive summary with recommendations was sent to the general practitioner within 5 days, and at least two further reviews were scheduled over 12 weeks to assess progress and adjust management, with telephone support as required.

Participant perceptions were measured using the 15-item Care Transitions Measure (CTM-15), a reliable measure of quality of transitional care.[7,8] The CTM-15 was administered at the initial visit (reflecting hospital discharge) and at 12 weeks (reflecting clinic experience). Items were scored from 1 (strongly disagree) to 4 (strongly agree), and the CTM-15 score was calculated by averaging and linear transformation as originally described.[8] Baseline and 12-week CTM-15 scores were compared using paired *t*-tests; mean nontransformed scores for each item were compared using paired *t*-tests.

Novel plasma biomarker surrogating cerebral amyloid deposition

By Naoki KANEKO,[*1] Akinori NAKAMURA,[*2] Yukihiko WASHIMI,[*3] Takashi KATO,[*2,*3] Takashi SAKURAI,[*3] Yutaka ARAHATA,[*3] Masahiko BUNDO,[*3] Akinori TAKEDA,[*3] Shumpei NIIDA,[*4] Kengo ITO,[*2,*3] Kenji TOBA,[*3] Koichi TANAKA[*1] and Katsuhiko YANAGISAWA[*2,†]

(Contributed by Koichi TANAKA, M.J.A.)

Abstract: Alzheimer's disease (AD) is the most common and devastating dementia. Simple and practical biomarkers for AD are urgently required for accurate diagnosis and to facilitate the development of disease-modifying interventions. The subjects for the study were selected on the basis of PiB amyloid imaging by PET. Forty PiB-positive (PiB+) individuals, including cognitively healthy controls (HC), and mild cognitive impairment and AD individuals, and 22 PiB-negative (PiB−) HC participated. Employing our novel highly sensitive immunoprecipitation-mass spectrometry, we measured plasma amyloid β-proteins (Aβs; Aβ1-40 and Aβ1-42) and Aβ-approximate peptides (AβAPs), which were cleaved from amyloid precursor protein (APP). Among the AβAPs, APP669-711 appeared to be a good reference for deciphering pathological change of Aβ1-42. We evaluated the performance of the ratio of APP669-711 to Aβ1-42 (APP669-711/Aβ1-42) as a biomarker. APP669-711/Aβ1-42 significantly increased in the PiB+ groups. The sensitivity and specificity to discriminate PiB+ individuals from PiB− individuals were 0.925 and 0.955, respectively. Our plasma biomarker precisely surrogates cerebral amyloid deposition.

Keywords: Alzheimer's disease, amyloid β-protein, biomarker, mass spectrometry, immunoprecipitation, PiB amyloid imaging

Introduction

Alzheimer's disease (AD) is the most common and devastating form of dementia that ultimately causes death. The searching for biomarkers to identify people at risk of AD development has been intensified because disease-modifying interventions are likely effective in the presymptomatic stage.[1),2)] The decreased levels of amyloid β-protein (Aβ)1-42 in cerebrospinal fluid (CSF) and the increased retention of positron emission tomography (PET) tracers are reliable signatures of cerebral amyloid deposition, which occurs early in the pathophysiological development of AD[3)–8)] or may accelerate the antecedent tauopathy[9)] before the clinical onset of dementia by 10 years or more in case of AD development. However, given that CSF examination is invasive and PET imaging is costly and hardly available, these biomarkers are not appropriate for screening people at risk of AD development. Thus, plasma Aβs have been extensively studied for their usefulness as alternative biomarkers.[1),2)] Although measuring plasma Aβs may be valuable in longitudinal and pharmacodynamic studies,[10)–13)] so far it has not satisfactorily been informative on the state of cerebral amyloid deposition in cross-sectional studies.[10),14),15)]

In the investigation of plasma Aβs in healthy individuals by our novel highly sensitive immunoprecipitation-mass spectrometry (IP-MS),[16)] we have recently detected various Aβ-approximate peptides (AβAPs), which were cleaved from the amyloid precursor protein (APP) close to the amino- and/or carboxyl-terminus of Aβs.[17)] In this study, we aim to examine whether plasma Aβs and/or AβAPs can be used as biomarkers surrogating cerebral amyloid

[*1] Koichi Tanaka Laboratory of Advanced Science and Technology, Shimadzu Corporation, Kyoto, Japan.
[*2] Center for Development of Advanced Medicine for Dementia, National Center for Geriatrics and Gerontology, Obu, Japan.
[*3] Hospital, National Center for Geriatrics and Gerontology, Obu, Japan.
[*4] BioBank, National Center for Geriatrics and Gerontology, Obu, Japan.
[†] Correspondence should be addressed: K. Yanagisawa, Department of Alzheimer's Disease Research, Center for Development of Advanced Medicine for Dementia, National Center for Geriatrics and Gerontology, 35 Gengo, Morioka, Obu, Aichi 474-8522, Japan (e-mail: katuhiko@ncgg.go.jp).

doi: 10.2183/pjab.90.353
©2014 The Japan Academy

deposition. Classifying study participants solely on the basis of clinical diagnosis can bias the performance of biomarkers[18] because a large proportion of cognitively normal aged individuals exhibit AD pathologic features, including cerebral amyloid deposition.[19)–21)] Thus, we carried out amyloid imaging by PET using Pittsburgh Compound B (PiB) on all the participants, and then carefully analyzed the performances of levels of Aβs and/or AβAPs as biomarkers. Here, we report that the ratio of AβAP (APP669-711) to Aβ1-42 is a reliable plasma biomarker precisely surrogating cerebral amyloid deposition.

Materials and methods

Subject selection and clinical classification. The subjects of the present study, between the ages of 65 and 85 years, were recruited from the community-dwelling aged individuals and outpatients of the National Center for Geriatrics and Gerontology (NCGG) Hospital. On the basis of results of PiB amyloid imaging by PET and a set of neuropsychological examinations, 40 PiB-positive (PiB+) and 22 PiB-negative (PiB−) individuals were selected. The PiB+ group consisted of 11 cognitively healthy controls (HC+), 12 individuals with mild cognitive impairment (MCI), and 17 patients with AD. The PiB− group included only cognitively healthy controls (HC−). Comprehensive neuropsychological batteries, including Mini-Mental State Examination (MMSE), Alzheimer's Disease Assessment Scale Cognitive Component Japanese version (ADAS-Jcog), Logical Memory II from the Wechsler Memory Scale–Revised (LM2), and Geriatric Depression Scale (GDS) were administered to all the subjects. The clinical diagnoses of AD and MCI were made in accordance with the criteria developed by NIA-AA.[22),23)] PiB positivity on amyloid imaging by PET was visually determined as described below (*PiB PET visual rating*). All the sets of examinations were carried out within about one year. Individuals with any significant medical, neurologic or psychiatric diseases other than AD and MCI were excluded from the selection based on medical history, neurologic examination, appropriate laboratory tests, neuropsychological examinations, and MRI. This study was approved by the Ethics Committee of NCGG and Shimadzu Corporation. All the subjects, proxies of AD and MCI individuals as well, provided written informed consent prior to examination to participate in the clinical studies of cognitive impairment and AD at NCGG.

Brain imaging. *MR image acquisition.* Brain MR images were obtained using a Trio 3T scanner (Siemens, Germany), an Avanto 1.5T scanner (Siemens), or an Ingenia 1.5T scanner (Philips, Netherland). The protocol consisted of 3D T1-weighted, T2-weighted, and FLAIR (fluid attenuated inversion recovery) imaging.

PiB PET image acquisition. 3D static PET imaging for 50–70 min after intravenous injection of 555+/−185 MBq PiB was carried out using a PET-CT camera, Biograph True V (Siemens). X-ray CT for attenuation correction was performed before PET imaging.

PiB PET visual rating. The visual rating of PiB PET images reported by Rabinovici *et al.*[24)] was slightly modified for the present study. PiB PET images were visually read by two experienced nuclear medicine physicians (K.I. and T.K.) who were blind to the clinical data. The obtained static images were displayed with a rainbow scale and an inverse gray scale. PiB images were rated as "PiB-positive (PiB+)" when the tracer binding in the cortical gray matter was deemed equal to or greater than that in the white matter, and as "PiB-negative (PiB−)" when only nonspecific tracer binding in the white matter was observed. The judgments for the PiB positivity should agree 100% between the two experts. The results of the visual rating were used for the grouping of the subjects (PiB− and PiB+), and also used as the gold standard for the receiver operating characteristics (ROC) analyses (see Statistical analyses).

PiB PET data analysis. The reconstructed static PET images ($168 \times 168 \times 111$ matrices, $2.036 \times 2.036 \times 2.036$ mm voxel size) were spatially normalized in NMI stereotactic space with parameters obtained from individual 3D-T1 MR images coregistered to PiB PET images by Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL). The normalized PiB PET images were masked with the grey-matter-segmented MR images to exclude the white matter and regions outside the brain. The region of interest (ROI) values were obtained from the PiB images of the grey matter using the Automated Anatomical Labeling Atlas.[25)] All the ROI values were transformed into standardized uptake value ratio (SUVR) by dividing them by the average ROI value in the cerebellar hemispheres. Mean cortical SUVR (PiB-mcSUVR)[26)] was obtained by averaging the SUVRs of the frontal, parietal, and temporal ROIs. PiB SUVR images were generated by dividing the masked PiB images by the

average value in each of the cerebellar hemispheres on a pixel-by-pixel basis. The PiB SUVR images were spatially smoothed using a Gaussian kernel filter of 8 mm at full width at half maximum. Using these smoothed SUVR images, voxelwise regression analyses for plasma biomarkers as covariates were performed using Statistical Parametric Mapping (SPM8, Wellcome Trust Centre for Neuroimaging, University College, London, UK).

Immunoprecipitation-mass spectrometry. The levels of plasma Aβs and AβAPs were measured by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) followed by immunoprecipitation (IP).[16] We used F(ab′) fragments of two monoclonal antibodies (6E10 and 4G8) that are specific to different Aβ epitopes, coupling to polyethylene glycol (PEG) on magnetic beads [hetero-F(ab′)-$(PEG)_{24}$ beads] for IP.[16] The procedure was slightly modified from that described previously.[17] In brief, 250 µL of plasma was mixed with an equivalent amount of binding buffer [100 mM Tris-HCl, 800 mM *N*-acetyl-D-glucosamine, 0.2% w/v n-dodecyl-β-D-maltoside (DDM), 0.2% w/v n-nonyl-β-D-thiomaltoside (NTM), 300 mM NaCl; pH adjusted to 7.4] with 10 pM stable-isotope-labeled (SIL) Aβ1-38. After filtration by centrifugation, the plasma sample was pretreated with 500 µL of Protein G Plus Agarose (50% slurry; Pierce, Rockford, IL) at 4 °C for 1 h. Then, the hetero-F(ab′)-$(PEG)_{24}$ beads were washed twice with 50 mM glycine-HCl buffer (pH 2.8) containing 1% OTG and then three times with washing buffer (50 mM Tris-HCl, 0.1% w/v DDM, 0.1% w/v NTM, and 150 mM NaCl; pH adjusted to 7.4). The beads were incubated with the plasma sample at 4 °C for 1 h, and then washed five times with washing buffer, twice with 50 mM ammonium acetate (pH 7.4), and once with H_2O. The bound Aβs and AβAPs were eluted with 2.5 µL of acetonitrile/H_2O (7 : 3 v/v) containing 5 mM HCl. Each eluate (0.5 µL) was immediately applied onto four wells of a 900 µm µFocus MALDI plate™ (Hudson Surface Technology, Inc., Fort Lee, NJ) with an equal volume of the α-cyano-4-hydroxycinnamic acid (CHCA) solution containing methanediphosphonic acid (MDPNA). Mass spectra were acquired using a MALDI-linear TOF mass spectrometer (AXIMA Performance, Shimadzu/KRATOS, Manchester, UK) equipped with a 337 nm nitrogen laser in the positive ion mode. The limit of detection was established at an S/N of 3 : 1. The peptide mass tolerance for quantification was set within 2.5 Da of the theoretical mass. One immunoprecipitation preparation produced four mass spectra and consequently yielded four peak intensities per analyte peptide. The levels of plasma Aβ and AβAP were obtained by averaging the four intensity ratios of each of the Aβ and AβAP peaks to an internal standard (SIL-Aβ1-38) peak. Through our exploratory assessments, we found that one of the AβAPs, APP669-711, could be a good reference for deciphering the individual change of Aβ1-42 level. Thus, we analyzed performances of the ratio of plasma Aβ1-42 to APP669-711 as a biomarker, comparing with those of Aβ1-42, and the ratio of Aβ1-42 to Aβ1-40. We verified the preciseness of the assay using human EDTA plasma from healthy individuals, purchased from Tennessee Blood Services (Memphis, TN). The intra- and inter-day assay coefficients of variants obtained were 9.93% (n = 4) and 19.41% (n = 4) for Aβ1-40, 1.67% and 9.77% for Aβ1-42, 8.48% and 12.13% for APP669-711, respectively.

Statistical analyses. We pre-assigned the sample size to be approximately 60 subjects. We considered that the sample size would be adequate because of the following reasons. The CSF Aβ1-42, which is one of the most promising biomarkers reflecting the cerebral Aβ deposition, significantly correlates with the quantitative measure of PiB-PET, and the reported correlation coefficients (r) were about -0.57 to -0.73.[27),28] Because we aimed to establish a novel plasma biomarker with comparable clinical usefulness to the CSF biomarkers, we expected that the coefficient of determination (r^2) of our biomarker should be more than 0.25. With this value, we would have 95% power to detect a useful biomarker at a significance level of 5% with a total sample size of 47. Statistical analyses were performed using SPSS ver. 21 (IBM, New York, USA) and JMP software ver. 8 (SAS Institute, Cary, USA). For the subjects' demographics, categorical data such as gender and *APOE* ε4 carrier distributions were analyzed using the Chi-square test, and the Bonferroni correction was applied for multiple comparisons. Normally distributed continuous data were analyzed using the Student t-test or one-way ANOVA with Tukey-Kramer post-hoc comparisons. Not normally distributed data were analyzed using the Kruskal Wallis test followed by the Steel-Dwass post-hoc test. For the analyses of the biomarker and PiB-mcSUVR data, when the values were not normally distributed, the reciprocal transformation was applied and normality was ensured. Analysis of covariance (ANCOVA) was used to assess the group differences, and the Bonferroni correction was

Table 1. Demographics of subjects classified into groups

PiB classification	PiB− (n = 22)	PiB+ (n = 40)			Statistics (*P*-value)
sex (M : F)	8 : 14	19 : 21			0.397
age (y)	72.1 ± 2.9	75.2 ± 4.7			0.006
APOE ε4 (%)	3/22 (13.6)	26/40 (65.0)			<0.001
Clinical classification	HC− (n = 22)	HC+ (n = 11)	MCI (n = 12)	AD (n = 17)	Statistics (*P*-value)
sex (M : F)	8 : 14	7 : 4	8 : 4	4 : 13	0.054
age (y)	72.1 ± 2.9	73.5 ± 4.7	75.7 ± 4.0	76.0 ± 5.0	0.018[a]
education (y)	11.6 ± 2.2	12.0 ± 2.8	12.2 ± 3.2	11.4 ± 2.4	0.935
MMSE	28.5 ± 1.5	28.5 ± 1.3	26.9 ± 1.4	21.6 ± 3.9	<0.001[b]
ADAS-Jcog	6.1 ± 2.4	6.0 ± 2.3	9.8 ± 3.3	15.3 ± 5.8	<0.001[c]
LM2	9.1 ± 4.4	8.7 ± 4.0	2.5 ± 3.6	0.2 ± 0.6	<0.001[d]
GDS	1.7 ± 1.5	1.5 ± 1.3	3.0 ± 1.5	3.7 ± 2.4	0.004[e]
APOE ε4 (%)	3/22 (13.6)	5/11 (45.5)	8/12 (66.7)	13/17 (76.5)	<0.001[f]

For PiB classification, the PiB− group is the same as HC− group, and the PiB+ group includes all of the HC+, MCI and AD individuals. Values are presented as mean ± SD. Statistical analyses were performed using the chi square test (sex, *APOE* ε4), Student *t*-test or one-way ANOVA (age, ADAS), and the Kruskal Wallis test (education, MMSE, LM2, GDS). Post-hoc results were as follows: [a]HC− vs AD ($P < 0.05$); [b]HC−, HC+ and MCI vs AD ($P < 0.001$), HC− vs MCI ($P < 0.05$); [c]HC− and HC+ vs AD ($P < 0.001$), MCI vs AD ($P < 0.01$), and HC− vs MCI ($P < 0.05$); [d]HC− and HC+ vs AD ($P < 0.001$), HC− and HC+ vs MCI ($P < 0.01$); [e]HC− and HC+ vs AD ($P < 0.05$); [f]HC− vs AD ($P < 0.001$) and HC− vs MCI ($P < 0.01$).
MMSE = Mini-Mental State Examination; ADAS-Jcog = Alzheimer's Disease Assessment Scale-Cognitive Component-Japanese version; LM2 = Logical Memory II from the Wechsler Memory Scale–Revised (paragraph A only); GDS = Geriatric Depression Scale; *APOE* ε4 = positive for apolipoprotein E ε4.

applied for the post-hoc tests. Receiver operating characteristic (ROC) analyses were performed to estimate the capability of the plasma biomarkers to discriminate individuals with cortical Aβ deposition (PiB+) from those without deposition (PiB−). The area under the curve (AUC), sensitivity and specificity were calculated to assess the discriminative capability of a biomarker. The Pearson product-moment correlation coefficient analysis was conducted to evaluate the strength of the link between each biomarker and cortical Aβ deposition assessed using PiB-mcSUVR values. Multiple regression analysis and partial correlation analysis were also performed to assess the influence of possible confounders that may affect the correlation between each biomarker and PiB-mcSUVR. All the tests were two-tailed, and the significance level of difference was set at $P < 0.05$.

Results

Demographics. The demographics and clinical characteristics of the subjects are summarized in Table 1. In the PiB classification based on the visually rated PiB positivity, there were significant differences in age and allele frequency of *APOE* ε4. Therefore, we adjusted for age in the statistical analyses of the biomarkers. In the detailed classification based on the clinical category, age, the scores of MMSE, ADAS-Jcog, LM2 and GDS, and the allele frequency of *APOE* ε4 were significantly different among groups. No significant differences were observed in sex ratio and educational attainment among the groups.

Measurement of Aβs and AβAPs. Plasma samples obtained from the participants were spiked with SIL-Aβ1-38 at 10 pM and subsequently subjected to IP-MS as described previously.[17] The representative mass spectra of plasma Aβs and AβAPs from PiB− (HC−) and PiB+ (AD) subjects are shown in Fig. 1. Given that the signal intensity of hydrophobic peptides such as Aβ1-42 decreases in MALDI-TOF MS, the Aβ1-42/Aβ1-40 ratio in IP-MS was smaller than that in previous studies.[13] In addition to Aβ1-40 and Aβ1-42, the peaks of various AβAPs (Aβ1-38, Aβ3-40, Aβ1-39, OxAβ1-40, and APP669-711 indicated in Fig. 2) were generally detected in all subjects. There did not appear to be a distinct difference in the relative signal intensities of most of these peptides. However, note that the ratio of the signal intensity of Aβ1-42 to that of APP669-711 in AD subjects was remarkably different from that in HC- subjects (Fig. 1, arrows).

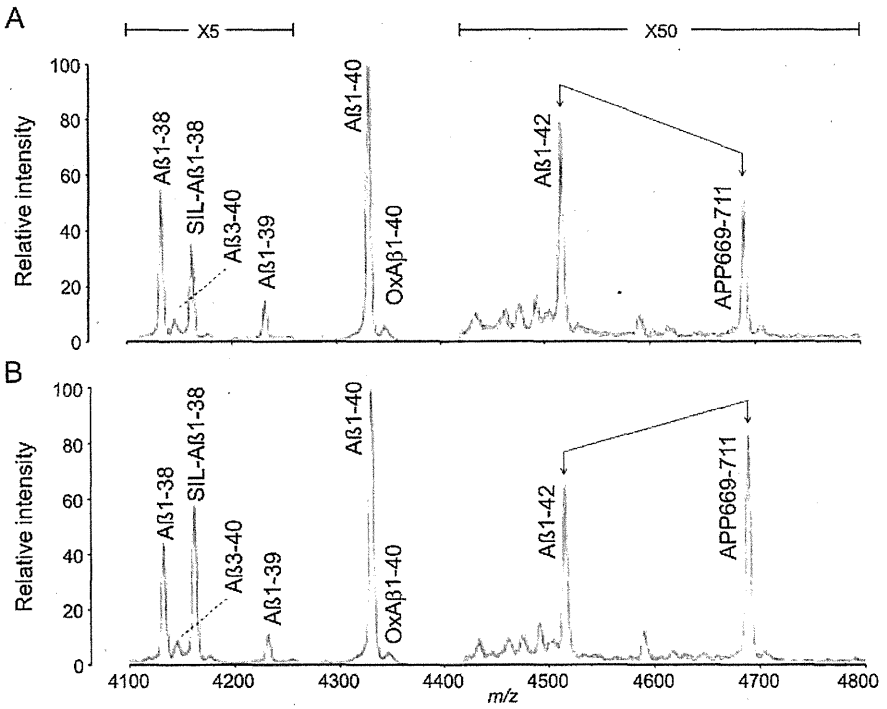

Fig. 1. MALDI-TOF mass spectra of plasma Aβs and AβAPs. Representative mass spectra obtained by IP-MS of plasma samples from the HC− (A) and AD (B) subjects are shown. In addition to Aβ1-40 and Aβ1-42, AβAPs including Aβ1-38, Aβ3-40, Aβ1-39 and APP669-711 were simultaneously measured by MALDI-TOF MS. Four mass spectra (represented in red, blue, green and orange) were obtained from one immunoprecipitation. The levels of Aβs and AβAPs were calculated by averaging the four intensity ratios of Aβs and AβAPs peak to SIL-Aβ1-38 peak. The arrows represent the difference in signal intensity between Aβ1-42 and APP669-711.

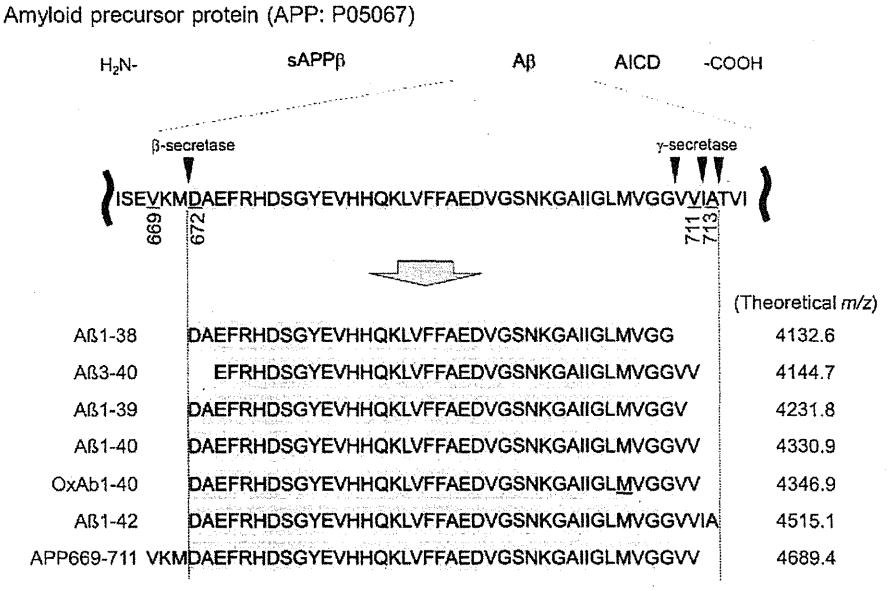

Fig. 2. Overview of Aβs and AβAPs detected by IP-MS. Seven Aβs and AβAPs were detected in plasma samples. The arrows above the sequence indicate the proteolytic processing sites of β- and γ-secretases. OxAβ1-40 represents Aβ1-40 with the oxidized methionine. sAPPβ = soluble APP; AICD = APP intracellular domain.

Table 2. Summary of statistical results for biomarkers

	Aβ1-42[a]	Aβ1-42/Aβ1-40[b]	APP669-711/Aβ1-42	PiB-mcSUVR
ANCOVA group comparisons				
PiB− (n = 22) mean (95% CI)	0.21 (0.18–0.24)	0.011 (0.009–0.012)	0.72 (0.63–0.82)	1.10 (0.98–1.21)
PiB+ (n = 40) mean (95% CI)	0.14 (0.12–0.15)	0.007 (0.006–0.008)	1.13 (1.07–1.19)	1.65 (1.57–1.72)
F-value	8.19	7.89	24.98	31.62
P-value	<0.001	0.001	<0.001	<0.001
coefficient of determination (η^2)	0.298	0.290	0.564	0.621
ROC analysis (PiB− vs PiB+, n = 62)				
area under the curve (AUC)	0.808	0.798	0.969	0.975
sensitivity	0.825	0.750	0.925	0.925
specificity	0.773	0.773	0.955	1.000
Multiple regression analysis (n = 62)				
model *R*	0.424	0.317	0.687	
F-value	6.48	3.30	26.44	
P-value	0.003	0.044	<0.001	
significance (*P*-value), mcSUVR/age	<0.001/0.094	0.016/0.841	<0.001/0.826	
single correlation (*r*), mcSUVR/age	−0.374/−0.060	−0.316/−0.085	0.687/0.217	
partial correlation (*r*), mcSUVR/age	−0.421/0.216	−0.307/0.026	0.668/−0.029	

Upper part: Results of analysis of covariance (ANCOVAs) for comparison between PiB− and PiB+ groups in biomarkers and PiB-mcSUVR. Values in parentheses represent 95% confidence interval (CI). All results are adjusted for age.
Middle part: Results of the receiver operating characteristic (ROC) analysis of biomarkers and PiB-mcSUVR to discriminate visually classified PiB− and PiB+ individuals (Fig. 3). The sensitivity indicates the true positive rate as calculated by (true positive)/((true positive) + (false negative)), and the specificity indicates the true negative rate as calculated by (true negative)/((false positive) + (true negative)). The AUC (the area under the ROC curve) express a kind of overall diagnostic accuracy. The cutoff values for Aβ1-42, Aβ1-42/Aβ1-40, APP669-711/Aβ1-42, and PiB-mcSUVR are 0.183, 0.009, 0.914, and 1.271, respectively.
Lower part: Results of the multiple regression analysis of biomarkers using PiB-mcSUVR and age as predictors. Also, results of the simple analysis of correlation (single correlation) to both PiB-mcSUVR and age, and results of partial correlation analysis adjusted for age or PiB-mcSUVR are shown.
[a]The values represent by the intensity of Aβ1-42 peak relative to that of SIL-Aβ1-38 peak as an internal standard.
[b]Note that the Aβ1-42/Aβ1-40 are markedly different from the reported values in other studies, because of methodological differences (see Results, Measurements of Aβs and AβAPs).

Performances of the plasma biomarkers. Since not Aβ1-42/APP669-711 but APP669-711/Aβ1-42 was normally distributed, the latter values were used for the statistical analyses. Therefore performances of the APP669-711/Aβ1-42 were compared with those of Aβ1-42 level and Aβ1-42/Aβ1-40. PiB-mcSUVR values were also analyzed as the ideal reference. All of the statistical results are summarized in Table 2.

Group comparisons between PiB− and PiB+ individuals. The upper part of Table 2 demonstrates the results of ANCOVA adjusted for age. Using our newly established method, the Aβ1-42 level showed a highly significant difference between the groups. Aβ1-42/Aβ1-40 did not improve the significant level; however, APP669-711/Aβ1-42 markedly enhanced the group-separation capability. The *F*-value and the effect size (η^2) of APP669-711/Aβ1-42 were comparable to those of PiB-mcSUVR.

Capability to discriminate PiB+ individuals from PiB− individuals. To evaluate the capability of APP669-711/Aβ1-42 to discriminate PiB+ individuals from PiB− ones, ROC analysis was performed. The results are shown in the middle part of Table 2 and Fig. 3. APP669-711/Aβ1-42 demonstrated an extremely high AUC (0.969). The sensitivity and specificity for the discriminative capability of APP669-711/Aβ1-42 were 0.925 (3 out of 40 were false negative) and 0.955 (1 out of 22 was false positive), respectively, with a cutoff value of 0.914. The performance indices of APP669-711/Aβ1-42 were also comparable to those of PiB-mcSUVR.

Correlation between our plasma biomarkers and PiB-mcSUVR. To evaluate the strength of the link between our plasma biomarkers and PiB-mcSUVR, we performed correlation and regression analyses. The results are shown in the lower part of Table 2 and Figs. 4A and 4B. All of the plasma biomarkers