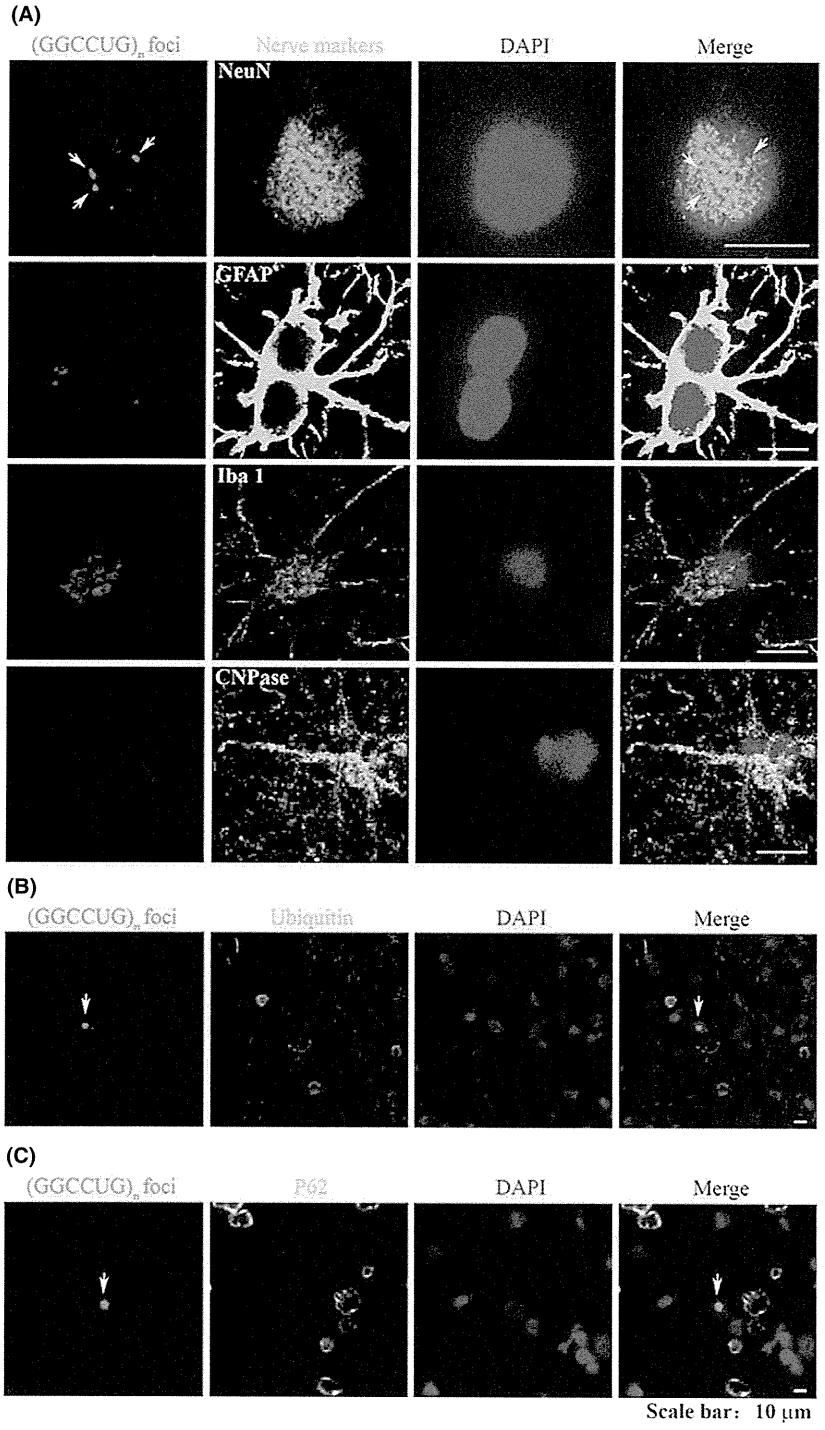

Figure 4 Combination of FISH and IFA in the Asidan patient. (A) Double IFA of probe A (red) with nerve cell markers (green) in the Asidan patient cerebral frontal cortex. (B) Probe A (red) with ubiquitin (green) in the inferior olivary nucleus of the Asidan patient. (C) Probe A (red) with p62 (green) in the inferior olivary nucleus of the Asidan patient. Arrows indicate RNA foci in the nuclei. Scale bar 10 μm. The full colour version of this figure may be viewed online.

in neurons (Fig. 3). The morphology of the observed RNA foci was classified into three types according to their diameter and number in one cell nucleus, i.e. single small, multiple small and giant (Fig. 5). The present study also describes for the first time giant RNA foci in Asidan patients, which has not been reported thus far in other repeat expansion diseases.

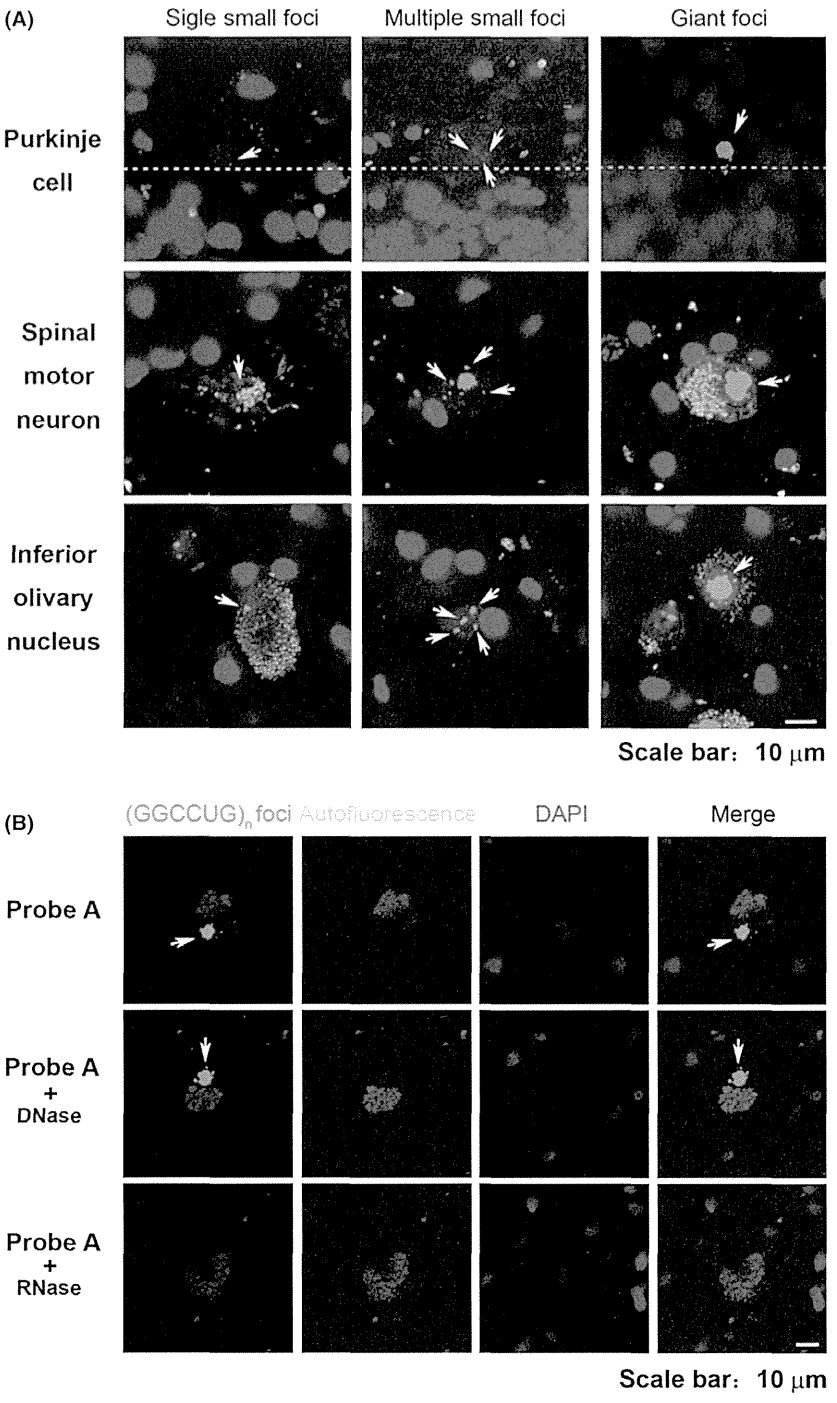

Figure 5 Variety of morphologies of $(GGCCUG)_n$ RNA foci in the Asidan patient. (A) Three types of RNA foci (single small, multiple small and giant) are shown in a Purkinje cell (top panels), a spinal motor neuron (middle panels) and an inferior olivary nucleus (bottom panels). (B) RNA foci of probe A in the Asidan patient's inferior olivary nucleus (top panels), stained positive after DNase treatment (middle panels), but with no signal after RNase treatment (bottom panels), proving that the giant foci are RNA foci. Scale bar 10 μm.

Polyubiquitinated and p62-positive intranuclear inclusions are characteristic of intronic expansion mutation diseases such as SCA [7]. Ubiquitinated inclusions within spinal motor neurons are also a representative pathology of motor neuron disease (MND) [8,9]. A 43 kDa transactive response DNA-binding protein (TDP-43) was reported as the main component of ubiquitinated aggregations found in amyotrophic

lateral sclerosis patients [10]. Expansion of an intronic GGGGCC hexanucleotide repeat in the *C9orf72* gene displayed p62-positive/TDP-43-negative neuronal cytoplasmic inclusions in the cerebellum, hippocampus and frontotemporal neocortex [11]. In our previous study, abnormal TDP-43 aggregation was not found in the cytoplasm of an Asidan patient [4]. In the present study, p62-positive neuronal cytoplasmic inclusions were found (Fig. 1A), in particular in the inferior olivary nucleus. In addition, in comparison with three healthy controls, a tendency to neuronal loss in the inferior olivary nucleus of the Asidan patient was found (Fig. 1B), indicating that the site of predilection was the inferior olivary nucleus, but not the cerebrum or cerebellum. p62/ubiquitin inclusions that appeared in the *C9orf72* hexanucleotide expansion are related to no-ATG-initiated translation [11], and the appearance of the p62/ubiquitin-positive neuronal inclusion in the Asidan patient suggests that there is a considerable mutual pathomechanism between Asidan and *C9orf72* mutations with a similar intronic hexanucleotide expansion. A further study on the no-ATG-initiated translation of Asidan is needed.

Various neurological diseases, including Asidan, show intranuclear RNA inclusions [12]. As was shown in our previous study within lymphoblastoid cells by RNA FISH analysis [2], the present study detected $(GGCCUG)_n$ RNA foci in most nerve cells and muscles (Fig. 3). Neurological diseases show different numbers (1–50), shapes (discrete and vagiform) and sizes (<3 μm in diameter) of repeated RNA foci in the nucleus [12,13]. In the present study, on the other hand, three types of RNA foci in the Asidan patient were observed: single small, multiple small and giant (Fig. 5). Single small foci are the most frequent whilst one nucleus typically has three to seven multiple small type RNA foci. Much to our surprise, giant RNA foci nearly 10 μm in diameter were detected in Purkinje cells, spinal motor neurons and most frequently in the inferior olivary nucleus (Fig. 5). These may be responsible for pivotal clinical symptoms of Asidan, but a double stain of FISH and ubiquitin/p62 showed no colocalization in nerve cells in the inferior olivary nucleus in the present study (Fig. 4B, C).

In conclusion, the present study showed both ubiquitin- and p62-positive inclusions in the cytoplasm of the inferior olivary nucleus of an Asidan patient, $(GGCCUG)_n$ RNA foci in neuronal nuclei of the cerebrum, cerebellum, inferior olivary nucleus, spinal cord and temporal muscle, and three types of RNA foci, i.e. single small, multiple small and giant. Of interest is that the giant RNA foci nearly 10 μm in diameter were detected in Purkinje cells, spinal motor neurons and most frequently in the inferior olivary nucleus. The relationships between the giant RNA foci and neurodegeneration need to be studied in the future.

Acknowledgements

This work was partly supported by a Grant-in-Aid for Scientific Research (B) 21390267, (C) 24591263 and Challenging Research 24659651, and by Grants-in-Aid from the Research Committees (Mizusawa H, Nakano I, Nishizawa M, Sasaki H and Aoki M) from the Ministry of Health, Labour and Welfare of Japan.

Disclosure of conflicts of interest

The authors declare no financial or other conflicts of interest.

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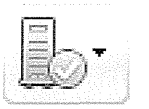

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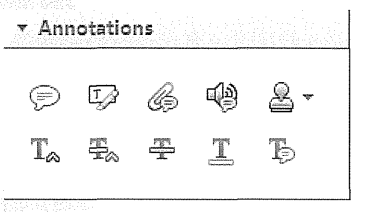

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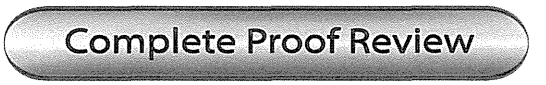

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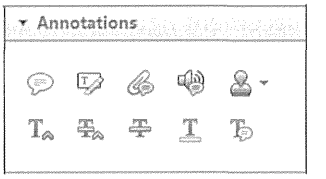

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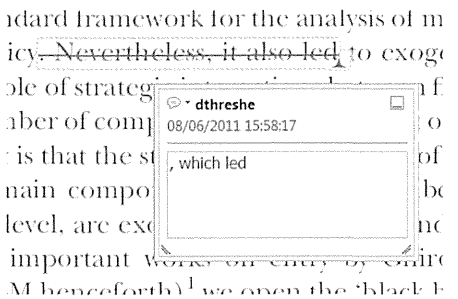

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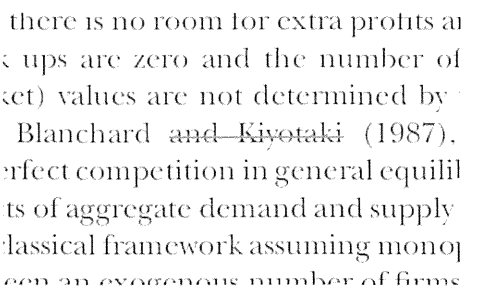

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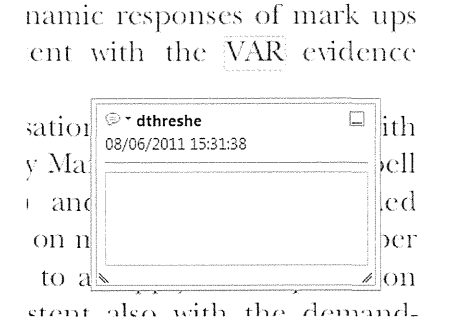

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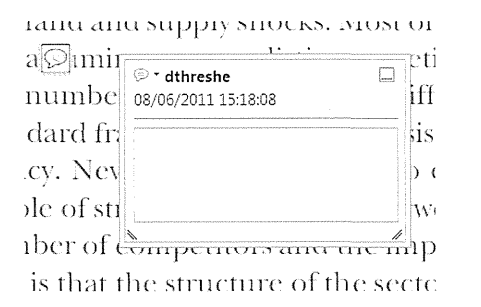

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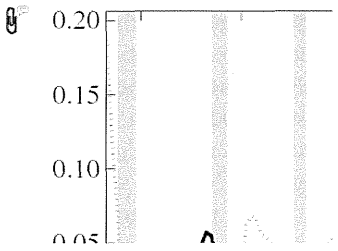

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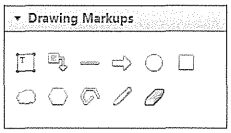

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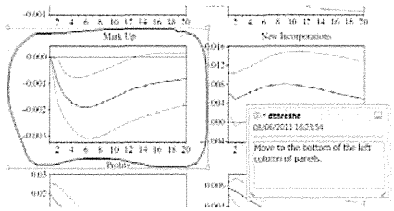

doi:10.1111/ene.12568

Cognitive and affective functions in diabetic patients associated with diabetes-related factors, white matter abnormality and aging

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Keywords:
cognitive and affective functions, diabetes mellitus, HbA1c, insulin resistance, MRI

Received 26 March 2014
Accepted 1 August 2014

Background and purpose: Diabetes mellitus (DM) is associated with a decline in cognitive and affective functions.

Methods: In all, 182 outpatients with DM were investigated for associations of cognitive and affective functions with diabetes-related factors and cerebral white matter abnormalities. In addition, the difference in cognitive decline of age-matched late elderly normal subjects and DM patients was investigated.

Results: The present study revealed that cognitive and affective functions declined in some DM patients. Furthermore, the decline in these functions was unrelated to fasting blood sugar level but was related to glycosylated hemoglobin (HbA1c) and insulin resistance. Poor HbA1c control was associated with a significant decline in the 'calculation' subscale and insulin resistance for 'naming', 'read list of letters' and 'delayed recall' Montreal Cognitive Assessment (MoCA) subscale scores. Magnetic resonance imaging scans showed that both periventricular hyperintensity (PVH) and deep white matter hyperintensity were associated with Mini Mental State Examination (MMSE) and MoCA scores, but only PVH was related to homeostasis model assessment of insulin resistance scores. Compared with age-matched late elderly normal subjects, 'orientation to time' and 'registration' MMSE subscales declined in late elderly DM patients.

Conclusions: These results suggest that cognitive and affective decline in DM patients was mostly related to glucose control and insulin resistance, whilst amongst late elderly subjects the impairment of 'attention' and 'orientation' were characteristic features of DM patients.

Introduction

As a rapidly aging country, Japan is facing increases in patients with both dementia and diabetes mellitus (DM). DM is an important vascular risk factor (VRF) not only for cardiovascular and cerebrovascular diseases but also for cognitive and affective impairments such as vascular dementia [1]. VFRs are also associated with the occurrence and progression of Alzheimer's disease (AD). However, treatment of VRFs is effective for preventing cognitive decline in AD patients [2,3]. It is presumed that in DM patients chronic hyperglycemia, arteriosclerosis, insidious ischaemia and insulin resistance could lead to generalized atrophy, cerebral white matter changes, accumulation of advanced glycation end-products, and metabolic disturbance of amyloid-β and tau, which probably leads to vascular dementia, AD and acceleration of 'aging' [4].

Cognitive impairment of DM patients is correlated with glucose control, postprandial hyperglycemia, severe hypoglycemia, acute glucose fluctuation, hyperinsulinemia and insulin resistance [5–11]. A close relationship has been shown between poor glucose control and cognitive decline [6], and between oral anti-hyperglycemic therapy and delaying dementia [12,13]. It is important to detect cognitive/affective decline at an early stage in DM patients to allow for interventional treatment.

In this clinic-based cross-sectional study, cognitive and affective functions in DM patients were examined by assessing the ischaemic changes in serum glucose, glucose control, insulin resistance and cerebral

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white matter. In addition, as the world is rapidly aging, cognitive functions between late elderly DM patients and age-matched healthy individuals were compared.

Subjects and methods

Subjects

In this cross-sectional study, 182 outpatients with DM who attended the diabetes clinic of our hospital were recruited. The patients' average age was 64.7 ± 18.0 (mean ± SD) years. The 182 DM patients included 19 late elderly individuals over 75 years of age (78.1 ± 2.4). The average duration of DM was 6.7 ± 6.1 years (range 1–11 years). Physical, cognitive and affective functions of all patients were measured.

For late elderly controls, healthy out-clinic age-matched individuals who took annual check-ups at the hospital were included ($n = 75$; age 78.8 ± 3.2 years). Their physical, cognitive and affective functions were examined using serological tests. None of the control subjects had any past or present central nervous system diseases, psychiatric disorders, DM or serological dysfunctions.

All patients and control subjects gave written informed consent, and the study protocol was approved by the Ethics Committee of Okayama University, Japan.

Cognitive and affective functions

Cognitive function was assessed using the Mini Mental State Examination (MMSE), Hasegawa dementia score – revised (HDS-R), frontal assessment battery (FAB) and the Montreal Cognitive Assessment (MoCA). Affective functions were also examined using the geriatric depression scale (GDS) and apathy scale (AS).

Serological laboratory tests

Serological laboratory data of fasting blood sugar (FBS) level, fasting insulin and glycosylated hemoglobin (HbA1c) were measured, and homeostasis model assessment of insulin resistance (HOMA-IR) score was calculated on the same day as cognitive and affective assessments. Patients were divided into three groups according to FBS (<110 mg/day, 110–199 mg/day, ≥200 mg/day) and HOMA-IR (<1.2, 1.2–11.9, ≥12.0), and into four groups according to HbA1c level (<6.5%, 6.5%–6.9%, 7.0%–7.9%, ≥8.0%) pragmatically, in order to find certain results in each serological DM-related factor.

Neuroimaging study

Brain magnetic resonance imaging (MRI) scans were obtained from 106 DM patients using a 3.0-T scanner. Qualitative visual MRI analysis was performed by three independent investigators. Assessment of periventricular hyperintensity (PVH) and deep white matter hyperintensity (DWMH) lesions was measured by using the Fazekas rating scale, and graded as 0–III for PVH and 0–III for DWMH [14].

Statistical analysis

Non-parametric Mann–Whitney U tests were performed to compare cognitive and affective functions between the two groups divided by DM-related factors, and to compare the MMSE subscales between the late elderly DM patients and late elderly normal subjects. For the dichotic subscales of the MMSE, the Pearson χ^2 test was used to evaluate differences. Trend analyses, using the Cochran–Armitage test and the Jonckheere–Terpstra test, were conducted to examine the relationship between DM-related factors and subscales of cognitive tests. Additionally, Spearman correlations were calculated to assess the relationships of PVH and DWMH with cognitive and affective functions and serological DM-related factors. A value of $P < 0.05$ was considered statistically significant.

Results

The demographic and characteristic data of the 182 outpatients with DM are shown in Fig. 1a and Table 1. For major VRFs, 53.3% of patients did not have hypertension (HT) and hyperlipidemia (HL) (Fig. 1b). In addition to DM, 24.2% of patients suffered from HT, 6.6% HL, and 15.9% both HT and HL (Fig. 1b). A total of 138 subjects (75.8%) had a high MMSE score considered to be in the normal range (28–30 points), 32 subjects (17.6%) were suspected to be in slight cognitive decline (24–27 points) and 12 subjects (6.6%) showed mild cognitive dysfunction (≤23 points) (Fig. 1c), even though none of them had been diagnosed with dementia before the study. A total of 106 of 182 DM patients who underwent MRI were classified according to the Fazekas grades for PVH (Fig. 1d) and DWMH (Fig. 1e).

Figure 2 shows the cognitive and affective functions of the 182 DM patients, and displays each cognitive score as a percentage. The scores for the MMSE, HDS-R, FAB and MoCA were 28.4 ± 3.7 (mean ± SD), 28.3 ± 3.6, 15.6 ± 3.2 and 24.6 ± 6.1

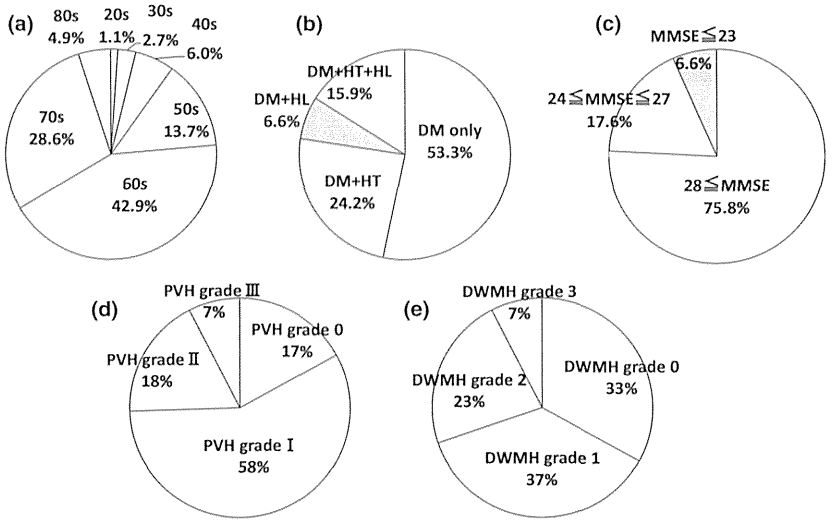

Figure 1 Demographic data of 182 outpatients with DM (a), and the complication ratio of other VRFs (b). Patients were subdivided according to MMSE score (c). MRI findings were classified in accordance with the Fazekas score of PVH (d) and DWMH (e).

Table 1 Characteristics of the DM patients

All DM patients	n = 182
Age (years)	64.7 ± 18.0
Male (%)	86 (45.1)
Duration of DM (years)	6.7 ± 6.1 (range 1–11 years)
Education (years)	12.3 ± 2.4
Complications	
DM + HT (%)	40.1
DM + HL (%)	22.5
DM + HT + HL (%)	15.9
Metabolic syndrome (%)	28.6
CKD (%)	10.4
Previous cerebrovascular event (%)	1.6
Previous cardiovascular event (%)	8.2
Body mass index	
Male	24.7 ± 4.1 (range 18.5–43.4)
Female	25.0 ± 5.4 (range 16.1–45.7)
Medical treatments for complications	
DM (n = 182) (%)	100
HT (n = 73) (%)	95.9
HL (n = 41) (%)	87.8
HT stage under medical treatment	
Normal range (%)	79.5
Stage 1 (%)	19.2
Stage 2 (%)	1.4
Stage 3 (%)	0
Hematological examinations	
Tcho ≥ 220 mg/dl (%)	6.6
LDL ≥ 140 mg/dl (%)	4.9
TG ≥ 150 mg/dl (%)	15.4
HDL ≤ 40 mg/dl (%)	9.9

DM, diabetes mellitus; HT, hypertension; HL, hyperlipidemia; CKD, chronic kidney disease; Tcho, total cholesterol; LDL, low-density lipoprotein; TG, triglyceride; HDL, high-density lipoprotein.

points, respectively (Fig. 2a, left). Although 76.4% of the 182 DM patients had a normal GDS score, 19.2% presented a depressive state (GDS, 5–9 points) and 4.4% had depression (GDS, 10–15 points) (Fig. 2a, right). Most DM patients showed normal vitality, i.e. no apathy (AS, 0–15 points), but 11.5% displayed apathy (AS, 16–42 points) (Fig. 2a, right).

DM patients were divided into two opposite groups according to DM-related factors (Fig. 2b–g). There were no differences between cognitive and affective functions in the FBS normal group (FBS < 110 mg/dl; n = 25) and the FBS high group (FBS ≥ 200 mg/dl; n = 21) (Fig. 2b and c).

There were no significant differences between the good-control DM group with HbA1c levels <6.5% (n = 29) and the poor-control group (≥8.0% HbA1c, n = 18) in MMSE score (28.7 ± 1.8 vs. 27.5 ± 2.9), HDS-R score (28.6 ± 1.5 vs. 28.1 ± 2.6), FAB score (16.0 ± 2.6 vs. 14.9 ± 2.4) or MoCA score (24.7 ± 3.9 vs. 23.3 ± 4.2) (Fig. 2d). However, the affective function GDS score was significantly increased in the poor-control group (3.9 ± 4.0, $*P < 0.05$) compared with the good-control group (1.2 ± 1.5). There was a trend for an increase in AS score in the poor-control group but this was not significantly different from the good-control group (7.7 ± 8.9 vs. 3.1 ± 3.9) (Fig. 2e).

Cognitive and affective differences became more evident when DM patients were divided into low HOMA-IR (< 1.2, n = 13) and high HOMA-IR (≤ 12, n = 19) groups. MMSE, HDS-R and FAB scores were not significantly different between the two groups (MMSE score, high group 29.1 ± 1.2 vs. low group 27.9 ± 3.4; HDS-R score, 29.3 ± 0.8 vs. 28.2 ± 2.8; FAB score, 16.1 ± 1.9 vs. 15.3 ± 2.1), but the MoCA score was significantly decreased in the high HOMA-IR group (23.5 ± 4.40, $*P < 0.05$) compared with the low HOMA-IR group (26.2 ± 3.0) (Fig. 2f). Significant differences were also found for both affec-

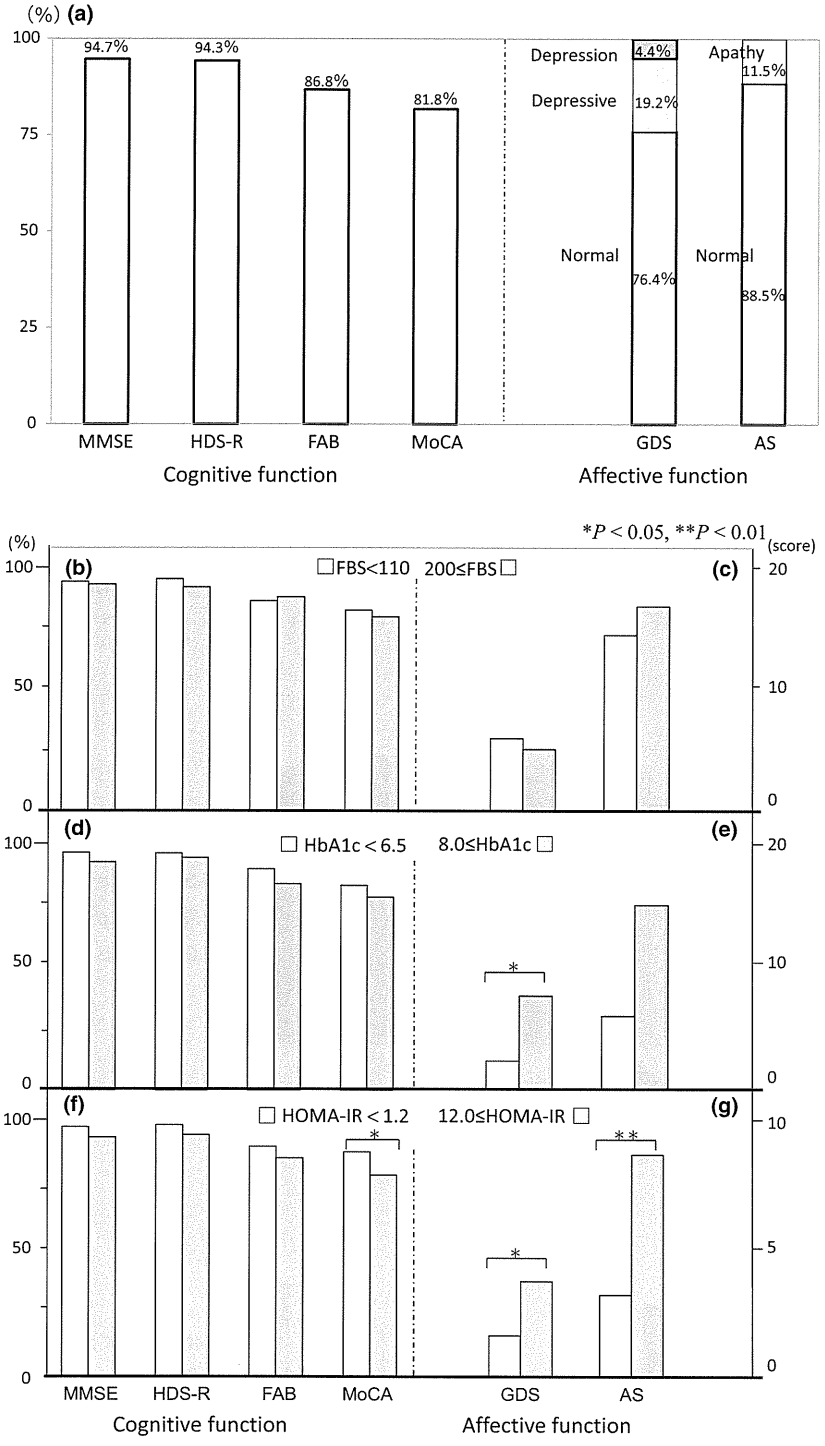

Figure 2 Cognitive and affective functions in the 182 DM patients. Each cognitive score is shown as a mean percentage (a, left), and affective scores for GDS and AS are shown on the right side as percentages of normal and disease conditions. The relevance of FBS/HbA1c/HOMA-IR for cognitive (b, d, f) and affective (c, e, g) functions in 182 DM patients, comparing two opposite groups of three DM-related factors: FBS (b, c), HbA1c (d, e) and HOMA-IR (f, g). Note the significant decline in the poor-control HbA1c (e, $*P < 0.05$) and high HOMA-IR (f and g, $*P < 0.05$, $**P < 0.01$) groups relative to the good-control groups.

tive functions. GDS and AS scores of the high HOMA-IR group were higher compared with the low group (GDS score, low group 1.5 ± 1.9 vs. high group 3.5 ± 3.7, $*P < 0.05$; AS score, 3.0 ± 3.7 vs. 8.2 ± 7.3, $**P < 0.01$) (Fig. 2g).

MMSE subscale analysis revealed significant decreases associated with HbA1c level increases ($\#P < 0.05$, Fig. 3a, arrow). MoCA subscale analysis also revealed significant decline in three scores ('naming', 'read list of letters' and 'delayed recall') with

three deteriorating HOMA-IR groups (#$P < 0.05$, Fig. 3b, arrows).

Increasing white matter changes in both PVH and DWMH were correlated with the decline in MMSE and MoCA total scores (Fig. 4a–d). The MMSE score in PVH grades 0–III was 29.6 ± 1.0, 28.4 ± 2.2, 26.3 ± 3.1 and 25.0 ± 3.6 points, respectively (grade 0 vs. II and III, I vs. III, **$P < 0.01$; I vs. II, *$P < 0.05$). The MoCA score in PVH grades 0–III was 25.9 ± 2.5, 24.9 ± 3.2, 22.6 ± 3.1 and 20.3 ± 4.7 points, respectively (grade 0 vs. II and III, I vs. III, **$P < 0.01$; I vs. II, *$P < 0.05$). The MMSE score in DWMH grades 0–3 was 29.3 ± 1.7, 28.3 ± 1.9, 26.8 ± 3.2 and 24.1 ± 2.9 points, respectively (grade 0 vs. 2 and 3, 1 vs. 3, **$P < 0.01$; 2 vs. 3, *$P < 0.05$). The MoCA score in grades 0–3 was 26.1 ± 2.2, 24.4 ± 3.1, 23.2 ± 3.7 and 19.6 ± 4.1 points, respectively (grade 0 vs. 2 and 3, 1 vs. 3, **$P < 0.01$; 2 vs. 3, *$P < 0.05$). In contrast, GDS and AS scores were not different according to PVH and DWMH grades (Fig. 4e–h). Although there was no correlation between serological HbA1c and PVH/DWMH (Fig. 4i and j), the serological HOMA-IR level of PVH grade III (18.7 ± 20.6) vs. I and II (*$P < 0.05$) was significantly higher compared with grade I (6.8 ± 5.7) and II (4.0 ± 3.6) (Fig. 4k).

Although total MMSE scores were not significantly different between late elderly normal subjects (27.6 ± 1.8) and late elderly DM patients (26.8 ± 2.6), subscale analysis revealed a significant decrease in late DM patients in 'orientation to time' (4.6 ± 0.6, *$P < 0.05$) and 'registration' (2.9 ± 0.2, *$P < 0.05$) subscales compared with normal subjects ('orientation to time', 4.9 ± 0.4; 'registration', 3.0 ± 0.0) (Fig. 5).

Discussion

The present study revealed that cognitive and affective functions of a selection of DM patients declined

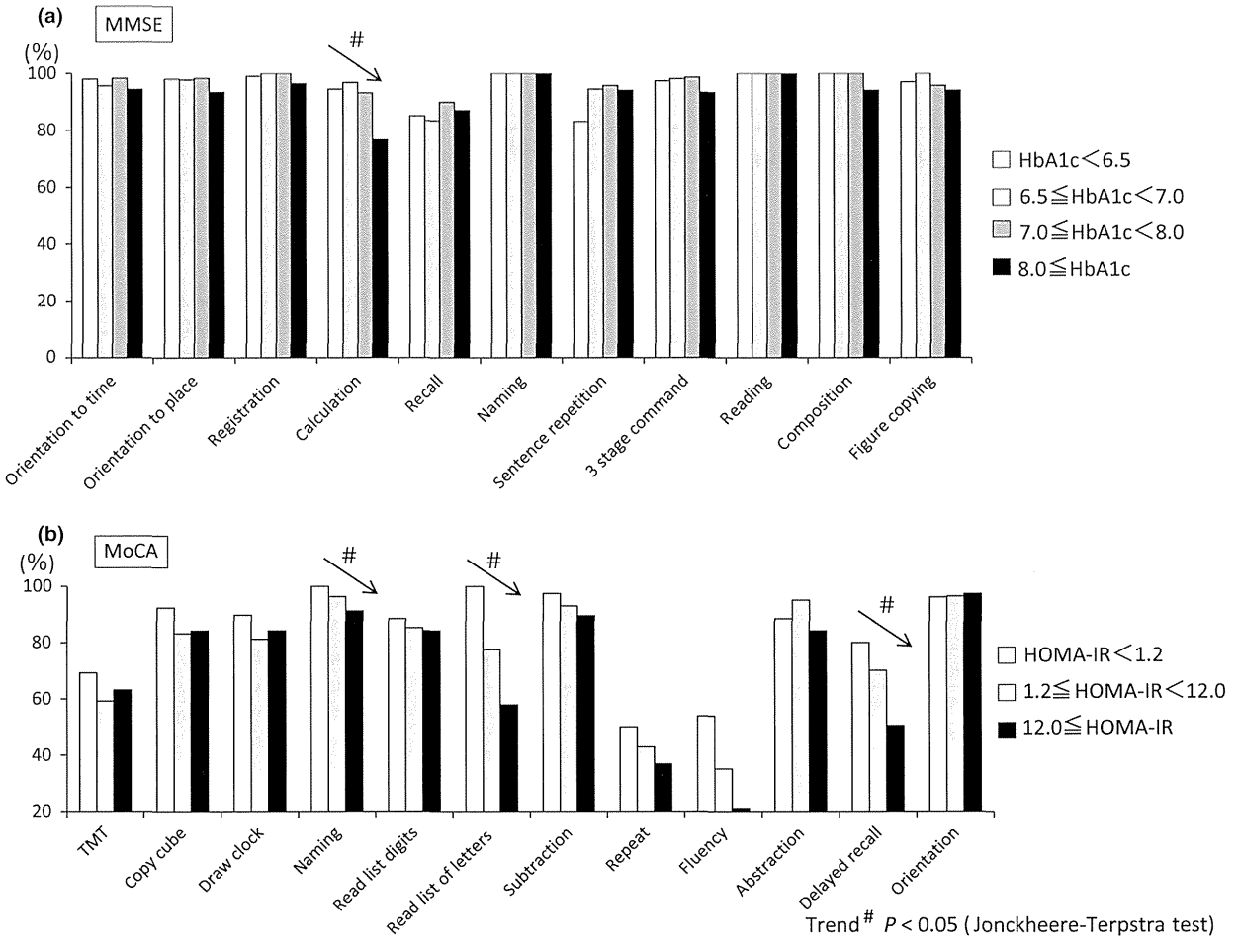

Figure 3 Subscale analysis of the MMSE (a) and MoCA (b) in the 182 DM patients according to level of HbA1c (a) and HOMA-IR (b). The results are expressed as an average score and as a percentage (trend #$P < 0.05$).

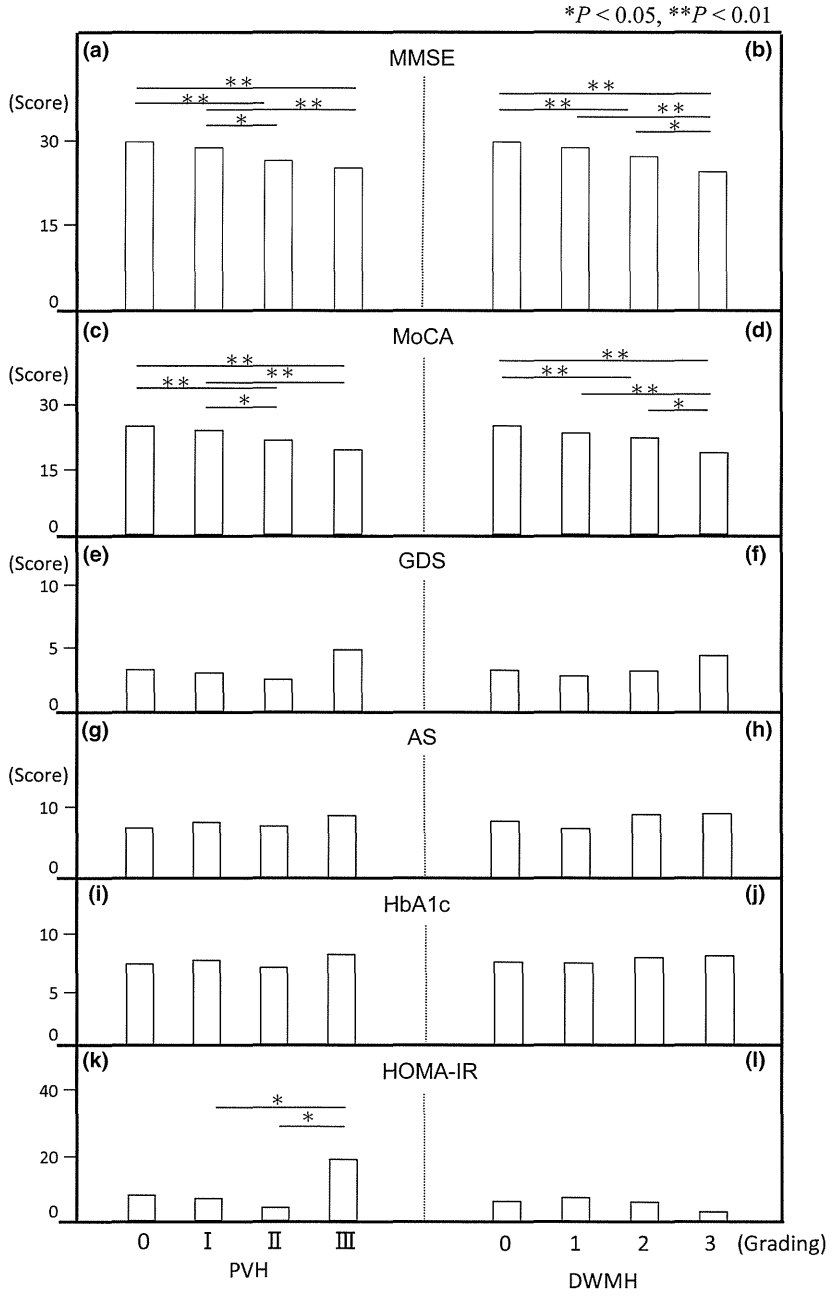

Figure 4 Association between cognitive (MMSE, MoCA) and affective (GDS, AS) functions, serological DM-related factors (HbA1c, HOMA-IR) and PVH (a, c, e, g, i, k) or DWMH (b, d, f, h, j, l) in the 182 DM patients. Note the significant differences between the MMSE (a, b) and MoCA (c, d) scores and the progressive PVH and DWMH scores (*$P < 0.05$, **$P < 0.01$), but no such associations with affective functions (e–h) and HbA1c level (i, j). Only HOMA-IR was related to severe PVH (k) but was not related to DWMH (l).

slightly (MMSE 24–27, 17.6%) and moderately (MMSE ≤ 23, 6.6%), although they and/or their families did not express concern or knowledge of such decline (Figs 1, 2). Cognitive decline was detected more clearly with the FAB and MoCA scales, which are used for executive and frontal lobe assessments, than with MMSE and HDS-R, which are used for global cognitive screening. Cognitive and affective functions were unrelated to FBS but were related to glucose control (HbA1c) and insulin resistance (HOMA-IR) (Fig. 2), which became clearer after subscale analysis of the MMSE and MoCA (Fig. 3a, b). Poor glucose control was significantly associated with decline in the 'calculation' subscale of the MoCA (Fig. 3a), and insulin resistance was significantly associated with 'naming', 'read list of letters' and 'delayed recall' subscale decreases (Fig. 3b).

Previous studies have reported that DM patients showed decreases in attention, executive function, working memory and psychomotor speed, which were related to glucose control impairments such as chronic

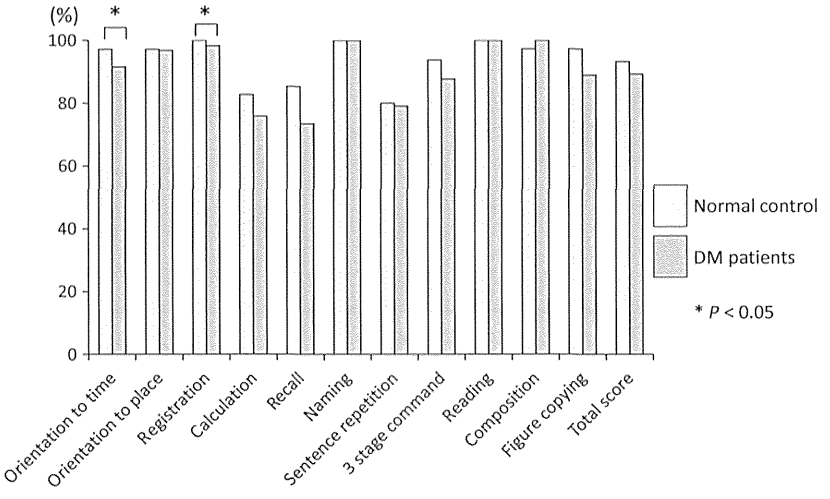

Figure 5 Comparison of MMSE subscale scores (percentages) between late elderly normal controls and late elderly DM patients, showing a significant decrease in 'orientation to time' and 'registration' in late elderly DM patients.

hyperglycemia [5–7], postprandial glycemia [8] and acute glucose fluctuation [9]. Insulin resistance and hyperinsulinemia are not only associated with cognitive dysfunction [10,11] but also with pathological degeneration in AD [4]. In contrast, HbA1c was not reported to be associated with cognitive decline [15,16]. The present study showed only a trend for cognitive decline in the HbA1c poor-control group (Fig. 2d), but a significantly positive trend for the 'calculation' subscale (Fig. 3a). The MoCA subscale analysis showed cognitive decline in 'naming', 'read list of letters' and 'delayed recall' subscales in the insulin-resistant group (Fig. 3b). Thus, cognitive subscale analysis may be useful to detect early cognitive decline in DM patients.

This study also showed that affective functions were more strongly correlated with HbA1c levels and HOMA-IR scores compared with FBS (Fig. 2). Of 182 DM patients, 24.6% presented a depressive state or depression, and 11.5% displayed apathy (Fig. 2a, right). Two previous studies [17,18] showed that depression and other psychological conditions in DM patients were related to sociological factors such as female gender, low education level, obesity and duration of diabetes, and the level of depression was much higher than in the DM patients in the present study. This may be due to differences in the condition of the DM controls, drug treatment for DM, race or cultural differences.

White matter abnormality is related to many VRFs, such as obesity, DM, HT and HL. PVH is an independent factor associated with motor speed but not with cognitive impairment [7]. However, the present study revealed that in DM patients both PVH and DWMH were related to cognitive decline (Fig. 4a–d) but not affective functions and HbA1c levels (Fig. 4e–j). PVH was related to high HOMA-IR score (Fig. 4k). Our data also showed that DWMH was associated with cognitive dysfunction but not with glucose control and insulin resistance (Fig. 4b, d, j and l) and that PVH may be more affected by VRFs (Fig. 4k).

Relative to the age-matched late elderly normal subjects, 'orientation to time' and 'registration' subscale declined in late elderly DM patients (Fig. 5). Specifically, 'attention/concentration' and 'recent and working memory' declined in both groups of elderly people, but impairments to 'attention' and 'orientation' were characteristic of late elderly DM patients, probably because of significant hippocampal atrophy [19] and vascular complications in DM.

In summary, the present study demonstrated cognitive and affective declines in DM patients mainly related to glucose control and insulin resistance. In particular, 'attention' and 'working memory' were worse in DM patients within our restricted cognitive tests. In addition, both PVH and DWMH were significantly associated with cognition in DM patients. Cognitive subscale analysis of DM patients may be effective in detecting early decline in relation to affective assessments and white matter abnormalities. Moreover, further study is needed to elucidate the underlying pathophysiological mechanisms relating cognitive and affective functions in DM, hypertension and hyperlipidemia.

Acknowledgements

This work was partly supported by Grants-in-Aid from the Research Committees (Mizusawa H, Nakano I, Nishizawa M, Sasaki H and Aoki M) of the Ministry of Health, Labour and Welfare of Japan.

Disclosure of conflicts of interest

Shikata has received research grants and/or payment for lectures from Astellas, MDS, Novartis, Takeda, Taisyo-Toyama, Kowahakko-Kirin, Ono, Tanabe-Mitsubishi, Boehringer-Ingelheim, Eli-Lilly, Dainippon-Sumitomo, Novo-Nordisk and Sanofi-Aventis. Makino has received grants and/or payment for lectures from Astellas, Boehringer-Ingelheim, Daiichi-Sankyo, Dainippon-Sumitomo, Kowahakko-Kirin, Mochida, MSD, Novartis, Novo-Nordisk, Pfizer, Takeda, Tanabe-Mitsubishi, AbbVie, Chugai and Teijin.

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DOI: 10.3727/096368915X686869

CT-2537 Accepted 01/28/2015 for publication in the special PPSSCC issue of "Cell Transplantation"

Time-dependent Profiles of MicroRNA Expression Induced by Ischemic Preconditioning in the Gerbil Hippocampus

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Running header: MicroRNA Expression Profiles in Ischemic Gerbil Hippocampus

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ABSTRACT

MicroRNAs (miRNAs) are critically important in both normal neuronal development and neurological diseases. Although cerebral ischemia has been shown to alter the miRNA profiles of rats, the role of miRNA in the cornu ammonis 1 (CA1) region of the gerbil hippocampus under ischemic tolerance has not been studied. In the present study, Mongolian gerbils were subjected to one or three times the non-lethal dose of 2 min-transient common carotid artery occlusion (tCCAO). miRNA microarray technology detected 251 miRNAs, and the expression of seven of these in terms of ischemic tolerance. They were compared at different time points: 1 day (d), 7 d, 1 month (M) and 6 M. Mmu-miR-15a-5p, related to neurogenesis, showed increased expression after one dose of 2 min-tCCAO and was much higher after three doses. An increase in sha-miR-24 and oan-let-7b-3p, related to transactivation response DNA-binding protein (TDP43), was observed after one dose of 2 min-tCCAO, but the peak was accelerated to an earlier period of reperfusion after three doses. In contrast, mmu-miR-125b-5p and mmu-miR-132-5p, related to fused in sarcoma/translocated in liposarcoma (FUS/TLS), showed similar increases at both doses. Mmu-miR-181c-5p and mmu-miR-378a-5p, related to heat shock protein 70 (HSP70), also showed accelerated expression after three doses. This data set provides new insight about miRNA expression during neurogenesis, and related to TDP43, FUS/TLS, and HSP70, which may be useful when pursuing further studies on the possible use of miRNAs as biomarkers in cerebral ischemic tolerance and neuroregeneration.

Key words: microRNA, ischemic preconditioning, CA1, gerbil

INTRODUCTION

MicroRNAs (miRNAs) are small non-coding ribonucleic acids (RNAs) that regulate gene expression at the post-transcriptional level (19,45). They function by binding to complementary sites on the 3′ untranslated region (UTR) of genes and promote the recruitment of protein complexes responsible for degrading messenger RNA (mRNA) and/or impairing translation (31). The human genome has been estimated to contain up to 1000 miRNAs, and each miRNA affects approximately 200 species of mRNAs (32). However, gerbil miRNAs have not yet been examined.

Several miRNAs have been found to target transcription factors regulating embryonic stem (ES) cell self-renewal and differentiation (44, 50), and other miRNAs have been shown to facilitate critical reprogramming towards induced pluripotent stem (iPS) cells (4,22,47). Recent studies showed that various pathological conditions significantly altered cerebral miRNA profiles, which could affect the disease outcome (7) in Alzheimer's disease (AD) (20), stroke (21,34), tumor growth (40), Down syndrome (25), and schizophrenia (6).

We previously reported that a single 2-min ischemia induced no neuronal damage, but 3 reperated-2 min ischemic insults caused neuronal damage and significant neuronal cell recovery subsequently occurred in the selectively vulnerable region-CA1 in the hippocampus (48). Short period transient ischemia can induce ischemic tolerance (23), which is a protective response against several subsequent

lethal ischemias, and can induce change in the cerebral miRNAome (13,43). Ischemic preconditioning offers endogenous neuroprotection by changing gene expression and altering protein synthesis (46). miRNAs are critically important to both normal neuronal development and in many neurological diseases. The gerbil model of transient global cerebral ischemia is well studied, particularly the underlying mechanisms of ischemic tolerance mainly to the pyramidal neurons in the hippocampal CA1 region. Increased induction of neuronal apoptosis is known to occur in this model (30). However, the role of miRNA in the CA1 region of the gerbil hippocampus in ischemic tolerance and following neurogenesis has not yet been studied.

We hypothesized that miRNAs may serve as important mediators of RNA processing and resulting protein synthesis required for ischemic tolerance as well as neurogenesis. Thus we examined changes in miRNA expression in the preconditioned gerbil hippocampal CA1 sector after transient common carotid artery occlusion (tCCAO).

EXPERIMENTAL PROCEDURES

Experimental model

Experiments were performed with male Mongolian gerbils (Japan SLC Inc., Shizuoka, Japan). The 10-12 week-old gerbils weighing about 80 g were maintained in a temperature-regulated room (23-25°C) at a 12-h light/dark cycle for at least 7 days (d). The gerbils were fasted, but were allowed free access to water overnight before