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ORIGINAL ARTICLE

Observational Study

Inverse relationship between platelet Akt activity and hippocampal atrophy: A pilot case-control study in patients with diabetes mellitus

Haruhiko Tokuda, Takamitsu Hori, Daisuke Mizutani, Tomoyuki Hioki, Kumi Kojima, Takashi Onuma, Yukiko Enomoto, Tomoaki Doi, Rie Matsushima-Nishiwaki, Shinji Ogura, Hiroki Iida, Toru Iwama, Takashi Sakurai, Osamu Kozawa

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Abstract

BACKGROUND

Akt plays diverse roles in humans. It is involved in the pathogenesis of type 2

diabetes mellitus (T2DM), which is caused by insulin resistance. Akt also plays a vital role in human platelet activation. Furthermore, the hippocampus is closely associated with memory and learning, and a decrease in hippocampal volume is reportedly associated with an insulin-resistant phenotype in T2DM patients without dementia.

AIM

To investigate the relationship between Akt phosphorylation in unstimulated platelets and the hippocampal volume in T2DM patients.

METHODS

Platelet-rich plasma (PRP) was prepared from the venous blood of patients with T2DM or age-matched controls. The pellet lysate of the centrifuged PRP was subjected to western blotting to analyse the phosphorylation of Akt, p38 mitogen-activated protein (MAP) kinase and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Phosphorylation levels were quantified by densitometric analysis. Hippocampal volume was analysed using a voxel-based specific regional analysis system for Alzheimer's disease on magnetic resonance imaging, which proposes the Z-score as a parameter that reflects hippocampal volume.

RESULTS

The levels of phosphorylated Akt corrected with phosphorylated p38 MAP kinase were inversely correlated with the Z-scores in the T2DM subjects, whereas the levels of phosphorylated Akt corrected with GAPDH were not. However, this relationship was not observed in the control patients.

CONCLUSION

These results suggest that an inverse relationship may exist between platelet Akt activation and hippocampal atrophy in T2DM patients. Our findings provide insight into the molecular mechanisms underlying T2DM hippocampal atrophy.

Key Words: Akt; Platelet; Hippocampal atrophy; Magnetic resonance imaging; Diabetes mellitus

Core Tip: Type 2 diabetes mellitus (T2DM) is caused by insulin resistance, and Akt is involved in this process. Akt also plays an important role in human platelet activation. The hippocampus, associated with memory and learning, can decrease in volume in patients with an insulin-resistant phenotype. We investigated the relationship between Akt phosphorylation in unstimulated platelets and hippocampal volume-reflecting Z-score in T2DM patients. The levels of phosphorylated Akt corrected for phosphorylated p38 mitogen-activated protein kinase were inversely correlated with Z-scores in T2DM patients. These results suggest an inverse relationship between Akt activation in platelets and hippocampal atrophy in patients with T2DM.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a global concern with a significant human health burden and serious economic implications[1]. Patients with T2DM have an elevated risk of vascular complications, such as cardiovascular disease, stroke, and vascular dementia[2-4]. It is well known that platelets play an important role in the pathogenesis of atherosclerosis and thrombosis. Various stimuli, such as thrombin, adenosine diphosphate, collagen, thromboxane A2, shear stress, and hyperglycaemia cause platelet activation[5-7]. Activated platelets perform several functions, including aggregation. We previously reported that T2DM patients exhibit spontaneous microaggregation of platelets and that the P2Y12 receptor plays a pivotal role in this process[8]. Phosphatidylinositol 3-kinase (PI3K) plays a vital role in platelet activation[6,9-14]. Akt, also known as protein kinase B, is the main downstream effector of PI3K[15], and is known to undergo phosphorylation first at Thr-308 by phosphatidylinositol 3,4,5-triphosphate-bound phosphoinositide-dependent kinase-1 and then at Ser-473 by the cytosolic mammalian target of rapamycin complex2, resulting in full activation[15]. Contrarily, it is also well established that the PI3K-Akt pathway plays a central role in insulin signalling, particularly in the process of glucose uptake[16]. Insulin resistance is recognised as the pathophysiological centre of T2DM, which means that insulin action is not sufficiently exerted in sensitive organs such as the liver and skeletal muscles, which are engaged in PI3K-Akt

pathway impairment[16,17].

The hippocampus is a vulnerable and elongated structure located deep within the medial temporal lobe of the human brain[18]. The human hippocampus plays a pivotal role in memory and learning because of its intrinsic circuitry; therefore, hippocampal atrophy is frequently observed in memory-disturbed patients, including those with Alzheimer's disease (AD), which is one of the major causes of cognitive impairment[18,19]. A voxel-based specific regional analysis system for AD (VSRAD) using magnetic resonance imaging (MRI) was developed as an adjunct technique to discriminate early AD patients from controls[20]. The VSRAD presents a Z-score that reflects the volume loss of medial temporal areas, including the hippocampus and entorhinal cortex, and has been approved as a supportive method for AD diagnosis, in Japan[20,21]. In addition, the Z-score of the target volume of interest (VOI) (VSRAD Z-score) is a useful tool for both cross-sectional and longitudinal evaluations of AD patients[22,23]. T2DM is a risk factor for AD[24,25]. In T2DM patients without dementia, hippocampal atrophy evaluated with the VSRAD has been reported to be associated with insulin resistance phenotypes such as abdominal visceral fat, high-sensitivity C-reactive protein, and plasma homocysteine levels [26-28]. However, the mechanisms underlying hippocampal atrophy in T2DM patients remain unclear.

In the present study, we investigated the relationship between Akt activity in unstimulated resting platelets and hippocampal atrophy, as measured using the VSRAD Z-score, in patients with T2DM.

MATERIALS AND METHODS

Study design

This case-control study investigated the relationship between Akt activity in unstimulated resting platelets and hippocampal atrophy in patients with T2DM.

Materials

Phospho-specific Akt (Thr-308) and phospho-specific p38 mitogen-activated protein (MAP) kinase antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Other materials and chemicals were obtained from commercial sources.

Patients

Patients who were neither diagnosed with dementia nor impaired instrumental activities of daily living were recruited from those admitted or outpatient at the National Center for Geriatrics and Gerontology. The inclusion criterion for the study group was the presence of T2DM according to the criteria of the World Health Organization. For the control group, we recruited age-matched patients with well-controlled hyperlipidaemia and hyperuricaemia but without diabetes. Patients with a past history of symptomatic cerebrovascular disease; significant cortical damage such as hydrocephalus, brain tumour or cerebral contusion evaluated by MRI; or atrial fibrillation were excluded. Patients with malignancy, infectious diseases, including hepatitis B and hepatitis C, or autoimmune disorders were also excluded. All participants were requested to avoid blood donation or sleep deprivation.

Blood sampling and protein preparation

After 15 min of bed rest to maintain steady-state conditions, 10 mL of venous blood was drawn between 8:00 AM and 9:00 AM post overnight starvation, and 14 μmol/L sodium citrate was added immediately as an anticoagulant. Platelet-rich plasma (PRP) was obtained by centrifugation at 155 × *g* for 12 min at room temperature, and ice-cold ethylenediaminetetraacetic acid (10 mmol/L) solution was immediately added to avoid platelet activation. The mixture was collected and centrifuged at 10000 × *g* at 4 °C for 2 min. The pellet was washed twice with phosphate-buffered saline (PBS) and lysed by boiling in lysis buffer containing 62.5 mmol/L Tris-HCL (pH 6.8), 2% sodium dodecyl sulphate (SDS), 50 mmol/L dithiothreitol and 10% glycerol for western blot analysis.

Western blot analysis

Western blotting was performed as previously described[29]. Briefly, SDS-polyacrylamide gel electrophoresis was performed on a 10% polyacrylamide gel, according to Laemmli[30]. The proteins were fractionated and transferred onto a polyvinylidene difluoride (PVDF) membrane, which was then blocked with 5% fat-free dry milk in PBS with 0.1% Tween 20 (PBS-T; 10 mmol/L Na_2HPO_4, 1.8 mmol/L KH_2PO_4, pH 7.4, 137 mmol/L NaCl, 2.7 mmol/L KCL and 0.1% Tween 20) for 2 h before incubation with phospho-specific Akt (Thr-308) antibodies, GAPDH antibodies and phospho-specific p38 MAP kinase antibodies as primary antibodies. Peroxidase-labelled anti-rabbit or anti-mouse IgG antibodies were used as the secondary antibodies. Primary and secondary antibodies were diluted to the optimal concentrations using 5% fat-free dry milk in PBS-T. Peroxidase activity on the PVDF membrane was visualised on an X-ray film using an ECL western blotting detection system (GE Healthcare, Buckinghamshire, United Kingdom) as described in the manufacturer's protocol. The bands were analysed by densitometry using a scanner and imaging software (ImageJ version 1.50; NIH, Bethesda, MD, United States). The quantitative data for each sample were obtained as pixel counts.

Brain MRI

MRI was performed using a 1.5-T system (Philips Ingenia, Eindhoven, Netherlands). Three-dimensional volumetric imaging of a T1-weighted gradient echo sequence at 9.3/3.9/1 (repetition time/echo time/excitation) was carried out for

Table 1 Characteristics of the study subjects

Parameters	DM group	Control group
Total number (F/M)	40 (20/20)	15 (8/7)
Age (years)	75.0 ± 7.6	73.4 ± 7.0
DM duration (years)	13.6 ± 11.3	None
Height (cm)	156.7 ± 8.8	158.5 ± 8.3
Weight (kg)	57.0 ± 10.5	57.8 ± 7.1
BMI	23.2 ± 3.8	23.0 ± 2.6
sBP (mmHg)	124.5 ± 19.3	130.0 ± 15.7
dBP (mmHg)	67.8 ± 13.6	74.4 ± 11.6
HbA1c (%)	8.1 ± 1.4[d]	5.9 ± 0.2
Glu (mg/dL)	175.0 ± 54.2[d]	99.3 ± 13.3
TC (mg/dL)	185.1 ± 33.3	201.6 ± 29.4
TG (mg/dL)	119.1 ± 53.7	110.7 ± 57.0
HDL (mg/dL)	53.6 ± 14.4	61.9 ± 13.7
Plt (× 10^4/μL)	21.4 ± 5.9	23.8 ± 3.7
VSRAD Z-score	0.841 ± 0.462	0.900 ± 0.489

[d]$P < 0.01$, compared to the control group.

F: Female; M: Male; DM: Diabetes mellitus; BMI: Body mass index; sBP: Systolic blood pressure; dBP: Diastolic blood pressure; HbA1c: Hemoglobin A1c; Glu: Plasma glucose; TC: Total cholesterol; TG: Triglyceride; HDL: High-density lipoprotein; Plt: Platelet counts; VSRAD: Voxel-based specific regional analysis for Alzheimer's Disease. The data are presented as the mean ± SD.

voxel-based morphometry analysis with the following parameters: Flip angle, 10 °; acquisition matrix, 256 × 256; field of view, 24.0 cm; and section thickness, 1.00 mm.

Voxel-based morphometry

A voxel-based specific regional analysis system for AD (VSRAD) (VSRAD advance 2, Eisai Co., Ltd., Tokyo, Japan) segmented MRI images into grey matter, white matter, and cerebrospinal fluid using a unified tissue segmentation procedure after image intensity nonuniformity correction. These linearly transformed and segmented images were then nonlinearly transformed using Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) procedures and modulated to a customised template for DARTEL, followed by smoothing using an 8-mm full width at half-maximum kernel. Each processed segmented image was compared to the mean and standard deviation of the grey matter or white matter images of 80 healthy volunteers using voxel-by-voxel Z-score analysis: Z-score = (control mean - individual value)/(control standard deviation). These Z-score maps were displayed as overlays on tomographic sections of each brain. The VSRAD registers the target VOI in the medial temporal structures, including the hippocampus and entorhinal cortex[20,21], defined by comparing patients with early AD with healthy controls.

Statistical analysis

Quantitative variables were presented as the mean ± standard deviations. The statistical significance of the correlation between the two variables and linear regression analysis was adopted. All statistical analyses were performed using SPSS version 19.0 (IBM Japan Ltd., Tokyo, Japan). A value of $P < 0.05$ was statistically significant. The biomedical statistician, Dr. Reo Kawano (National Center for Geriatrics and Gerontology) reviewed the statistical methods used in this study.

RESULTS

Characterization of the patients using western blotting and VSRAD

The clinical and biochemical characteristics of the participants in the DM (n = 40) and control (n = 15) groups are presented in Table 1. The haemoglobin A1c (HbA_{1c}) levels of all participants in the DM group were higher than the upper limit of the normal range (5.9%). Anthropometric indices were within normal ranges in the Japanese population, and there were no significant changes in metabolic variables. There were no statistically significant differences between the DM and control groups, except for plasma glucose or HbA1c levels. The list and number of antidiabetic medications used by the patients in the DM group are presented in Table 2. The numbers of patients who received metformin, glucagon-like peptide 1 receptor agonist (GLP-1RA) and exogeneous insulins, which can affect Akt activity[31-33], was 3, 2 and 7,

Table 2 The list and number of anti-diabetic medications in the subjects of diabetes mellitus group

Agent	Cases
Metformin	3
GLP-1RAs	2
Insulins	7
DPP-4 inhibitors	22
α-Glucosidase inhibitors	4
SGLT2 inhibitors	5
Sulfonylureas	10

GLP-1RA: Glucagon-like peptide-1 receptor agonist; DPP-4: Dipeptidyl peptidase 4; SGLT2: Sodium glucose transporter-2.

Table 3 Voxel-based specific regional analysis for Alzheimer's Disease Z-scores and the medication (exogenous insulin, glucagon-like peptide-1 receptor agonist or metformin) of the diabetes mellitus subjects

Patient	Z-score	Patient	Z-score	Patient	Z-score	Patient	Z-score
1	1.33	11	0.25	21[2]	0.94	31	1.95
2	0.38	12	0.82	22[3]	0.39	32	0.71
3	1.01	13	0.67	23[1,3]	0.65	33	1.65
4[1]	0.34	14	0.38	24	0.59	34	0.63
5[1]	0.35	15	0.41	25	1.14	35	0.72
6	0.68	16	0.31	26	0.67	36	1.65
7	0.51	17	0.54	27	1.18	37	1.49
8	1.04	18[1]	1.93	28	0.72	38	0.79
9	0.23	19	1.62	29	0.58	39	0.87
10	0.49	20	1.05	30	0.95	40	1.06

[1]Exogenous insulin-medicated.
[2]Glucagon-like peptide-1 receptor agonist-medicated.
[3]Metformin-medicated.
Voxel-based specific regional analysis system for AD (VSRAD) registered the target volume of interest (VOI) in the medial temporal lobe including hippocampus. The individual Z-scores in the target VOI (VSRAD Z-score) are presented.

respectively.

VSRAD Z-scores of the participants and diabetic medications in the DM group

Individual VSRAD Z-scores and the use of insulin, GLP-1 RA or metformin in the DM group are presented in Table 3. Z-scores for the VSRAD ranged from 0.23 to 1.93. There were no cases with VSRAD Z-scores ≥ 2.0, which primarily indicates significant hippocampal atrophy[20,21]; however, 13 patients presented with scores between 1.0 and 2.0, in the borderline range[20,21]. We investigated the relationship between age and VSRAD Z-scores in the study group; however, no statistically significant relationship (r = 0.181, P = 0.263) was observed. Individual VSRAD Z-scores of the control group are shown in Table 4. Z-scores for the VSRAD ranged from 0.43 to 1.86. There were no cases with VSRAD Z-scores ^{3} 2.0; however, five patients presented scores between 1.0 and 2.0, in the borderline range.

Relationship between individual levels of phosphorylated Akt and the VSRAD Z-scores

It is well known that the PI3K-Akt pathway plays a vital role in human platelet activation[6,9-14]. Thus, we first examined the individual levels of Akt phosphorylation corrected by the levels of GAPDH using western blot analysis. The results of western blotting and densitometric analyses are presented in Figure 1A and B for the DM group and Figure 1C and D for the control group. The individual levels of Akt phosphorylation were upregulated in some cases despite the results obtained from unstimulated platelets; however, the levels differed among samples in both groups. At least in part, low levels of Akt-phosphorylation were observed in cases with high VSRAD Z-scores, such as in patient No. 1. However, high levels of phosphorylation were observed in patients with low VSRAD Z-scores, such as in patient No. 2. The individual levels of phosphorylated-Akt/GAPDH against the VSRAD Z-scores were plotted, and a tendency toward

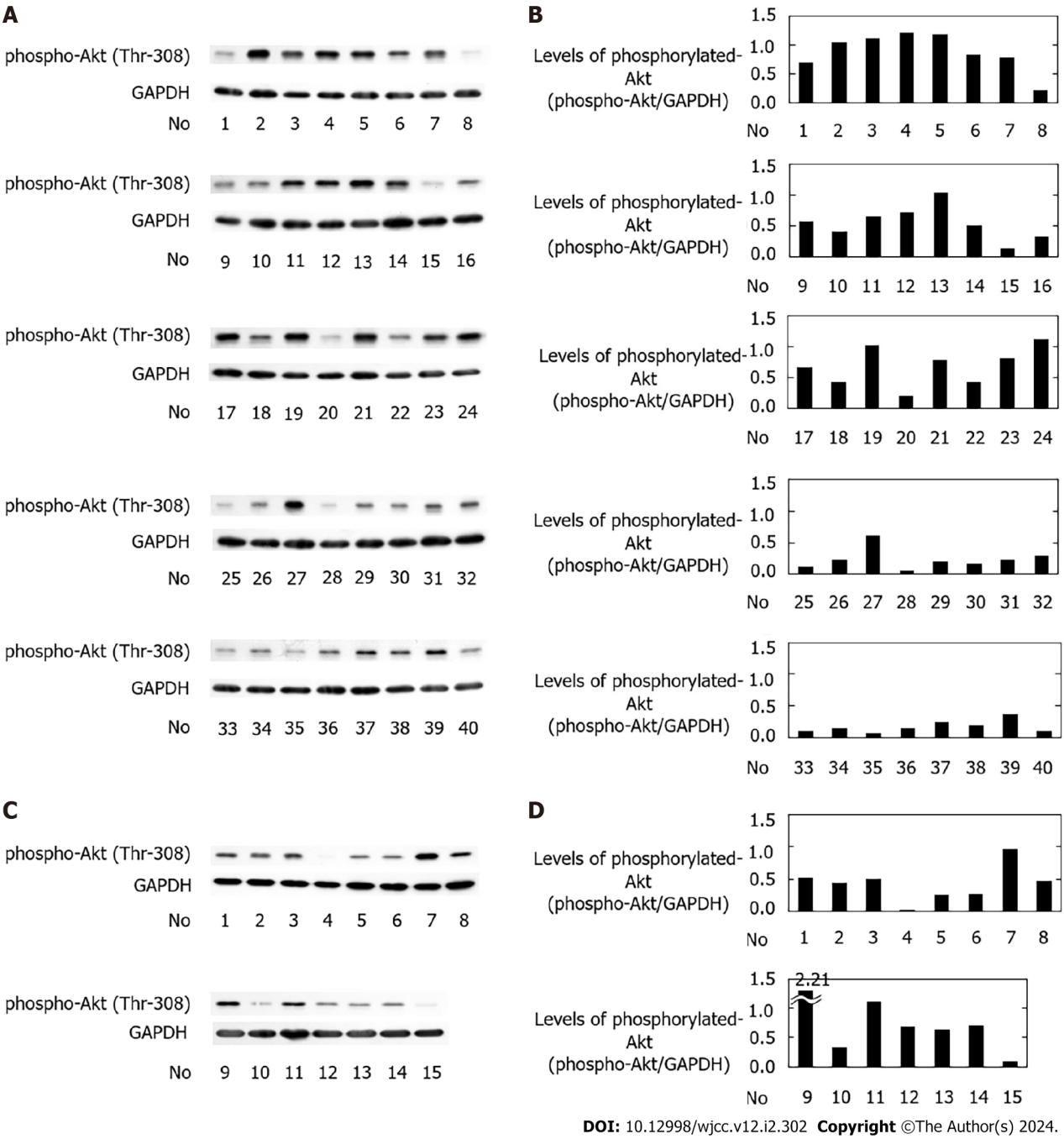

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Figure 1 Phosphorylated-Akt in the platelets. A and B: Type 2 diabetes mellitus (T2DM) group; C and D: The control group. To avoid platelet activation, ice-cold ethylenediaminetetraacetic acid (10 mmol/L) solution was added immediately to the platelet-rich plasma obtained from the patients in the T2DM group (A and B) and the control group (C and D). The lysates of the platelets were then subjected to western blot analysis using antibodies against phospho-specific Akt (Thr-308) or glyceraldehyde 3-phosphate dehydrogenase (GAPDH). All the results are presented collectively (A: n = 40; C: n = 15). The levels of GAPDH and phosphorylated Akt were determined using the ImageJ software program. The bar graphs show the levels of phosphorylated Akt corrected by those of GAPDH in the T2DM group (B) and the control group (D). GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

an inverse correlation between phosphorylated-Akt/GAPDH levels and VSRAD Z-scores was observed. In contrast, no statistical significance was observed in their relationship (R^2 = 0.072, P = 0.094, n = 40) (Figure 2A). In the control group, the individual levels of phosphorylated-Akt/GAPDH had no significant association with VSRAD Z-scores (R^2 = 0.003, P = 0.841) (Figure 2B).

Relationship between individual levels of phosphorylated Akt corrected by the levels of phosphorylated p38 MAP kinase (phosphorylated Akt/phosphorylated p38 MAPK) and VSRAD Z-scores

In addition to Akt, it has been reported that p38 MAP kinase is activated not only by environmental stress but also by inflammatory cytokines[34], playing an essential role in platelet activation, including in patients with T2DM[35]. Therefore, we examined individual levels of p38 MAP kinase phosphorylation. The results of western blot analysis of phosphorylated p38 MAP kinase and densitometric analyses for all participants are shown in Figure 3A and B for the DM

Table 4 Voxel-based specific regional analysis for Alzheimer's Disease Z-scores in the control subjects

Patient	Z-score	Patient	Z-score
1	0.53	9	0.51
2	1.63	10	0.63
3	1.86	11	1.27
4	0.50	12	1.54
5	0.43	13	0.89
6	0.70	14	0.48
7	1.32	15	0.77
8	0.45		

Voxel-based specific regional analysis for Alzheimer's Disease (VSRAD) registered the target volume of interest (VOI) in the medial temporal lobe including hippocampus. The individual Z-scores in the target VOI (VSRAD Z-score) are presented.

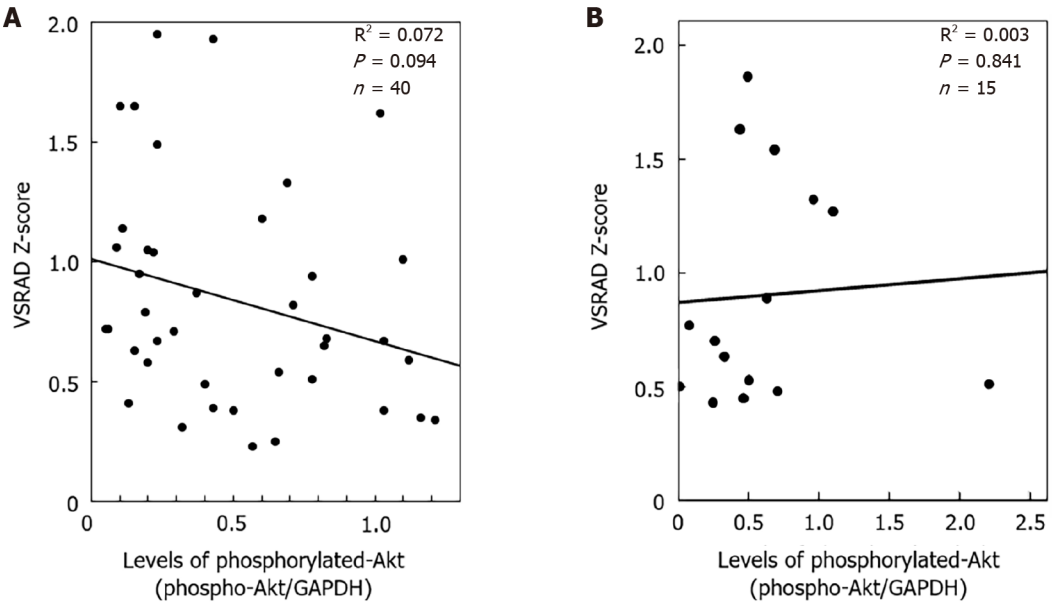

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Figure 2 The relationship between the individual levels of phosphorylated-Akt and the Voxel-based specific regional analysis system for Alzheimer's disease Z-score. A: Type 2 diabetes mellitus (T2DM) group; B: The control group. The levels of phosphorylated-Akt corrected with those of glyceraldehyde 3-phosphate dehydrogenase in the T2DM group (A) and the control group (B) were plotted against Voxel-based specific regional analysis system for Alzheimer's disease Z-scores obtained by magnetic resonance imaging. The plotted data were analysed by linear regression analysis. VSRAD: Voxel-based specific regional analysis system for Alzheimer's disease; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

group, and Figure 3C and D for the control group. Similar to the levels of Akt phosphorylation, the individual levels of p38 MAP kinase phosphorylation were quite different among the samples in both groups, and were significantly upregulated in some cases despite the presence of resting platelets. There was no correlation between the phosphorylation levels of p38 MAP kinase and VSRAD Z-scores (data not shown). For further analysis, we plotted individual phosphorylated Akt/phosphorylated p38 MAP kinase levels against VSRAD Z-scores. In the DM group, the levels of phosphorylated Akt/phosphorylated p38 MAP kinase were inversely correlated with the VSRAD Z-scores (R^2 = 0.140, P = 0.018, n = 40) (Figure 4A); however, there was no significant relationship between them in the control group (R^2 = 0.018, P = 0.841, n = 15) (Figure 4B).

DISCUSSION

The present study investigated the possible mechanisms underlying hippocampal atrophy in T2DM patients without cognitive impairment. We evaluated the VSRAD Z-scores mainly reflecting the hippocampus volume[20,21] in the study participants, the DM and control groups, and confirmed that cases defined as significant atrophy were not included. We

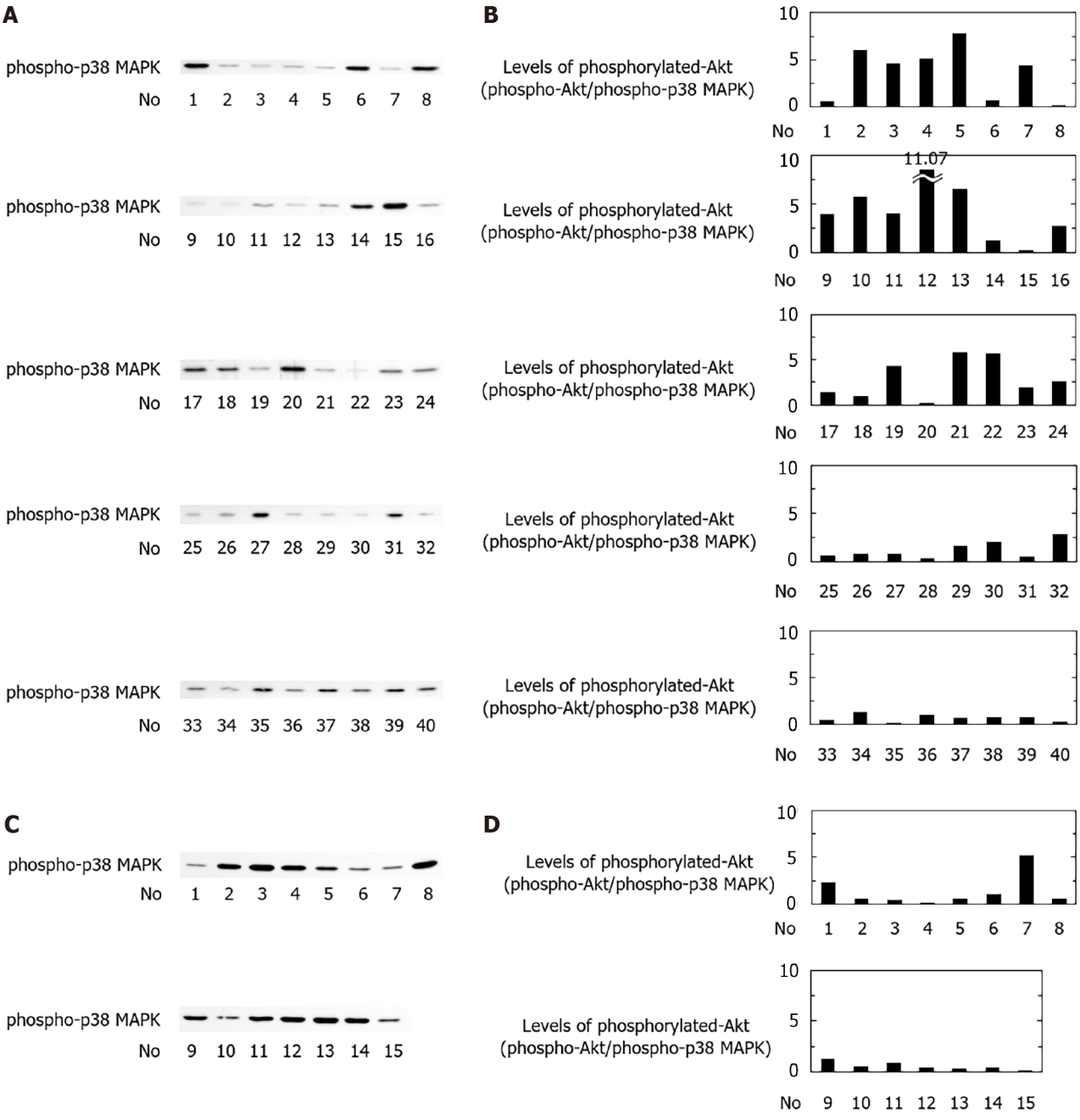

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Figure 3 Phosphorylated p38 mitogen-activated protein kinase in the platelets and the levels of phosphorylated Akt corrected with those of phosphorylated p38 mitogen-activated protein kinase. A and B: Type 2 diabetes mellitus (T2DM) group; C and D: The control group. To avoid platelet activation, ice-cold ethylenediaminetetraacetic acid (10 mmol/L) solution was added immediately to the platelet-rich plasma obtained from patients in the T2DM group (A and B) and the control group (C and D). The lysates of the platelets were then subjected to western blot analysis using antibodies against phospho-specific p38 Mitogen-activated protein kinase (MAPK). All the results are presented collectively (A: n = 40; C: n = 15). The levels of phosphorylated-p38 MAPK were determined using the ImageJ software program. The bar graphs show the levels of phosphorylated-Akt corrected with those of phosphorylated p38 MAPK in the T2DM group (B) and the control group (D). MAPK: Mitogen-activated protein kinase.

previously found that spontaneous aggregation occurred, at least partially, in the platelets of T2DM patients[8]. Akt is known to play an important role in human platelet activation[6,9-14], and impairment of Akt signalling is closely related to insulin resistance, a central pathology of T2DM[16,17]. We examined the levels of Akt phosphorylation in the platelets of T2DM patients under unstimulated conditions compared to control subjects with no diabetes. We found that the individual levels of Akt phosphorylation were upregulated in some cases in both groups, but the levels were different. It is likely that Akt in the platelets is spontaneously activated in some T2DM and non-T2DM patients. Thus, we investigated the relationship between the VSRAD Z-scores and Akt phosphorylation levels after adjusting for GAPDH levels. We did not find statistical significance in the relationship between them; however, there was likely a tendency for an inverse correlation only in the DM group. Although ageing is known to impact hippocampal volume significantly, we did not find any significant relationship between age and VSRAD Z-scores. It is unlikely that individual hippocampal volume is

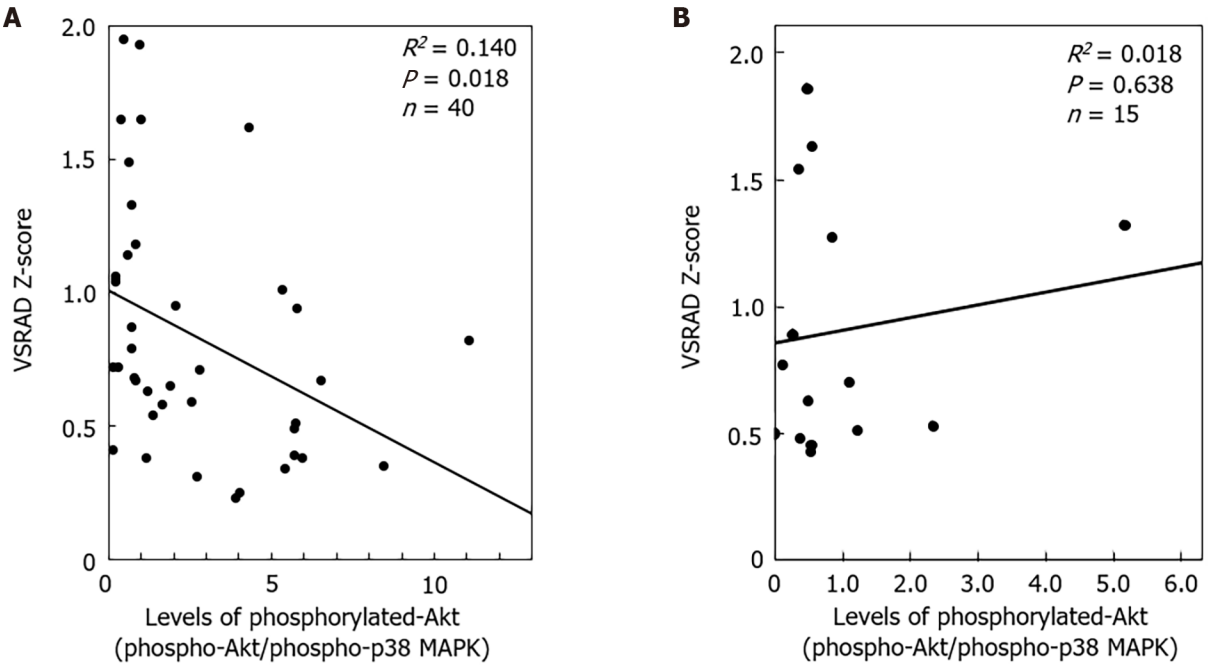

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Figure 4 The relationship between the individual levels of phosphorylated Akt corrected with phosphorylated p38 mitogen-activated protein kinase (phosphorylated Akt/phosphorylated p38 mitogen-activated protein kinase) and the voxel-based specific regional analysis system for Alzheimer's disease Z-score. A: Type 2 diabetes mellitus (T2DM) group; B: The control group. The levels of phosphorylated Akt corrected with phosphorylated-p38 mitogen-activated protein kinase (MAPK) (phosphorylated-Akt/phosphorylated-p38 MAPK) in the T2DM group (A) and the control group (B) were plotted against the voxel-based specific regional analysis system for Alzheimer's disease Z-scores. The plotted data were analysed by linear regression analysis. VSRAD: Voxel-based specific regional analysis system for Alzheimer's disease; MAPK: Mitogen-activated protein kinase.

closely related to age, at least in the patients with DM in this study.

The activation of environmental stress-related p38 MAP kinase[34], in addition to Akt, plays a role in the excessive activation of platelets in T2DM patients[35]. Therefore, we examined the individual levels of p38 MAP kinase phosphorylation in the unstimulated platelets of the study participants. We found that the levels of p38 MAP kinase phosphorylation were different in each sample and were upregulated in some cases in both groups. It is likely that p38 MAP kinase in platelets could also be spontaneously activated in some T2DM and non-T2DM patients. We confirmed no correlation between the phosphorylation levels of p38 MAP kinase and VSRAD Z-scores in the study participants. However, the individual levels of p38 MAP kinase phosphorylation were quite different from those of Akt phosphorylation and appeared to be contrary in some cases in the DM group.

Finally, we investigated the relationship between the levels of Akt phosphorylation corrected for p38 MAP kinase phosphorylation levels and VSRAD Z-scores in unstimulated platelets of T2DM patients. We found a significant inverse correlation between the levels of phosphorylated Akt/phosphorylated p38 MAP kinase and the VSRAD Z-scores, suggesting that the increased ratio of Akt/p38 MAP kinase activity in unstimulated platelets could negatively contribute to the decline in hippocampal volume in T2DM patients. In contrast, we found no significant relationship between the phosphorylated Akt/phosphorylated p38 MAP kinase ratio and VSRAD Z-scores in the control group. Likely, an inverse relationship between individual VSRAD-Z scores and the levels of Akt phosphorylation to p38 MAP kinase could be observed specifically in T2DM patients. To the best of our knowledge, this is the first report to show a relationship between hippocampal atrophic changes and Akt/p38 MAP kinase activity in unstimulated platelets. The ratio of phosphorylated Akt to phosphorylated p38 MAP kinase indicates the relative activity of individual platelets. The lower the ratio, the greater the decline in hippocampal volume in patients with T2DM. Therefore, the activity of Akt in platelets, in contrast to that of p38 MAP kinase, may have a protective effect against hippocampal atrophy associated with T2DM. Although we evaluated platelet activity only under non-stimulated conditions, the unstimulated activation status of Akt to p38 MAP kinase might be more important in reflecting the pathological platelet status than that observed in ex vivo-stimulated platelets. A limitation of this study is that our analysis probably included biases, such as experimental conditions, randomization, and sampling, which may have affected the observed trends.

A decrease in the hippocampal volume in T2DM patients is observed in comparison with the non-diabetic population [36]. It has been reported that a decline in hippocampal volume could be associated with the insulin resistance phenotype in T2DM patients without dementia[26-28]. Impairment of Akt signalling plays a pivotal role in the pathogenesis of insulin resistance, which is closely associated with the onset of T2DM[16,17]. Conversely, decreased levels of PI3K subunits and blunted Akt kinase phosphorylation have been reported in the brains of patients with AD[37], suggesting that impairment of the PI3K-Akt pathway related to insulin signalling in the brain may play a role in the pathogenesis of AD[37]. Therefore, our present findings regarding the Akt/p38 MAP kinase phosphorylation ratio in platelets may

provide a novel indicator of the risk of hippocampal atrophy in T2DM patients. Further research is required to clarify the mechanisms underlying hippocampal atrophy in T2DM patients.

CONCLUSION

In patients with T2DM, there may be an inverse relationship between Akt activation in platelets and hippocampal atrophy. Our findings provide an important step towards understanding the molecular mechanisms underlying hippocampal atrophy in T2DM.

ARTICLE HIGHLIGHTS

Research background

Type 2 diabetes mellitus (T2DM) is caused by insulin resistance, a condition in which Akt is involved. Akt plays an important role in human platelet activation. A decrease in hippocampal volume is associated with an insulin-resistant phenotype in T2DM patients.

Research motivation

If a relationship exists between Akt phosphorylation in unstimulated platelets and hippocampal volume, Akt phosphorylation could be a risk indicator of hippocampal atrophy in T2DM patients.

Research objectives

Patients with T2DM in the study group and age-matched patients with metabolic disorders without diabetes in the control group were recruited to obtain platelet-rich plasma from venous blood.

Research methods

Protein phosphorylation in platelets was analysed by western blotting, followed by densitometry. The Z-score by a voxel-based specific regional analysis system for Altzheimer's disease on magnetic resonance imaging was adopted as a parameter reflecting hippocampal volume.

Research results

The levels of phosphorylated Akt corrected for phosphorylated p38 mitogen-activated protein (MAP) kinase were inversely correlated with Z-scores in the study group but not in the control group.

Research conclusions

In patients with T2DM, there may be an inverse relationship between Akt activation in platelets and hippocampal atrophy.

Research perspectives

The Akt/p38 MAP kinase phosphorylation ratio in platelets may be a novel indicator of the risk of hippocampal atrophy in T2DM patients.

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FOOTNOTES

Author contributions: Tokuda H contributed to project administration, funding acquisition, and patient recruitment; Tokuda H and Kozawa O contributed to conceptualisation, methodology, resources, and data curation; Tokuda H, Hori T, and Mizutani D conducted investigation; Hori T, Mizutani D, Hioki T, and Kojima K performed formal analysis; Hioki T, and Kojima K performed visualization; Tokuda H, Kozawa O, and Sakurai T performed validation; Tokuda H, Hori T, Mizutani D, Hioki T, Onuma T, and Kozawa O contributed to the writing and the editing of the manuscript; Enomoto Y, Doi T, Matsushima-Nishiwaki R, Ogura S, Iida H, and Iwama T contributed to supervision; All the authors have read and agreed to the published version of the manuscript.

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