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Prevalence of mild cognitive impairment in type 2 diabetes mellitus is associated with serum galectin-3 level

Shizhan Ma[1,†], Shangbin Li[2,3,†], Renjun Lv[4], Xunyao Hou[3], Shanjing Nie[4], Qingqing Yin[4,*]

[1]Department of Endocrinology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, China, [2]Department of Geriatrics, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, China, [3]Department of Geriatric, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, China, and [4]Department of Geriatric Neurology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, China

Keywords
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***Correspondence**
Qingqing Yin
Tel.: +86-134-6591-0075
Fax: +86-531-6877-7833
E-mail address:
yinyunqing11@126.com

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ABSTRACT

Aims/Introduction: Galectin-3 (Gal3) contributes to insulin resistance, inflammation and obesity, the three risk factors for mild cognitive impairment (MCI) in type 2 diabetes mellitus patients.

Materials and methods: A total of 134 hospitalized type 2 diabetes mellitus patients were assessed by the Montreal Cognitive Assessment method, and divided into 65 MCI and 69 controls. Levels of variables, Gal3 and Aβ42, were investigated in relation with cognitive function in both type 2 diabetes mellitus patients with MCI and high-fat diet/streptozotocin induced type 2 diabetes mellitus rats.

Results: Significantly higher levels of serum Gal3 and lower levels of plasma Aβ42 (all $P < 0.05$) were found in the MCI type 2 diabetes mellitus group as compared with the non-MCI type 2 diabetes mellitus control. Partial correlation analysis showed that Gal3 is negatively correlated with both MMSE score ($r = -0.51$, $P < 0.01$) and Montreal Cognitive Assessment score ($r = -0.47$, $P < 0.001$) after adjustment for glycated hemoglobin, homoeostasis model assessment of insulin resistance and Aβ42 in all type 2 diabetes mellitus patients, with a stronger effect seen in the MCI type 2 diabetes mellitus group after further analysis with MCI strata. A simple logistic regression model showed that Gal3 and Aβ42 are significantly associated with MCI type 2 diabetes mellitus patients after adjustment with the covariates sex, age, body mass index, glycated hemoglobin, homoeostasis model assessment of insulin resistance and antidiabetic drugs. Serum and brain Gal3 levels were significantly increased in high-fat diet/streptozotocin diabetic rats, which correlate to the impairment of learning and memory ability. Gal3 inhibitor modified citrus pectin decreased serum and brain Gal3 levels in diabetic rats, accompanied by the amelioration of learning and memory impairment.

Conclusions: Gal3 might be associated with cognitive impairment in type 2 diabetes mellitus, and serum Gal3 level might be a new risk factor of MCI in type 2 diabetes mellitus patients.

INTRODUCTION

At least 90% of diabetes mellitus cases are type 2 diabetes mellitus, which typically begins in middle or older age, formerly known as adult-onset diabetes[1]. It is pathophysiologically characterized by high blood sugar, insulin resistance and relative lack of insulin[2]. The most prevalent neurodegenerative disease, Alzheimer's disease, (AD) occurs in old age, and is pathologically characterized by extracellular amyloid (Aβ) deposition and intraneuronal deposition of abnormally hyperphosphorylated tau, and is clinically shown by progressive cognitive impairment[3]. It is believed that approximately 10–15% of mild cognitive impairment (MCI) patients convert from normal to defective cognition with AD[4]. An increased risk of MCI and AD was found in patients diagnosed with type 2 diabetes

[†]These authors contributed equally to this study.
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mellitus, and the association is likely solidified through disease processes, such as AD[5]. It is hypothesized that the pathogenesis of cognitive impairment observed in both type 2 diabetes mellitus and AD is caused by the combination of one or more of the following well-characterized molecular mechanisms: inflammation, insulin resistance, Aβ deposition and tau abnormal hyperphosphorylation[6,7]. Further studies need to be carried out to understand the potential new molecular mechanisms underlying the pathogenesis of cognitive impairment in both type 2 diabetes mellitus and MCI/AD.

Galectin-3 (Gal3), a unique chimeric member of the galectin family, is highly expressed in both the intracellular and extracellular space of various cell types[8]. Gal3 is a pleiotropic protein implicated in cell activation, proliferation, migration, apoptosis, oxidative stress and inflammation of many pathological conditions, such as chronic kidney disease, obesity, tumor, cardiovascular disease, diabetes and AD[9-10]. Gal3 is secreted by macrophages in the peripheral system and can directly bind to the insulin receptor, inhibiting the downstream insulin receptor signaling pathway[11]. It has also been shown that Gal3 can promote peripheral insulin resistance *in vitro* (myocytes, adipocytes and hepatocytes) and *in vivo* (obesity mouse model)[11,12]. Gal3 is associated with the pathogenesis of diabetes and is reported to be a novel blood marker of prediabetes[13-14]. However, a central insulin signaling deficit is believed to be the major mechanism of diabetes-related cognitive impairment[15]. This is supported by our previous findings that an upregulated Gal3 in the brains of a fructose-drinking diabetic rat model co-occurs with insulin receptor signaling deficits, neuroinflammation and cognitive impairment[16]. Thus, Gal3 might serve as one of the main upstream mediators, contributing to a central insulin signaling deficit in accord with neuroinflammation, resulting in the outcome of cognitive decline in diabetes patients.

Gal3 is significantly upregulated in both the blood samples and brains from both people diagnosed with AD as well as 5xFAD transgenic mice[17,18]. It is reported that Gal3 acts as a novel endogenous ligand of the triggering receptor expressed on myeloid cells 2, activating microglia and regulating inflammatory response in AD[17,19]. In addition, Gal3 is found to be specifically expressed in microglia associated with Aβ plaques, and to activate microglia through the inflammatory response to fibrillar Aβ, along with fibrillar Aβ degradation and clearance disorders[17]. This evidence suggests a significant role of Gal3 in the pathogenesis of AD through the neuroinflammation mechanism. Recently, the association of Gal3 and diabetic microvascular complications has been studied. Gal3 has been considered as a prognostic biomarker and independently associated with the progression of diabetic nephropathy[20,21]. Gal3 was also identified as an independent risk factor of diabetic retinopathy and optic nerve disorders[9]. Diabetic microvascular complication is related to cognitive impairment[22], and risk factors of MCI and type 2 diabetes mellitus were not fully understood. To the best of our knowledge, whether the relationship between Gal3 and cognitive impairment is affected by diabetes is not known, and Gal3 level has not been examined in MCI type 2 diabetes mellitus patients.

Taken together, Gal3 might be associated with diabetic cognitive impairment. This hypothesis is addressed in the current study by exploring the potential relationship between serum Gal3 level and cognitive impairment in both MCI type 2 diabetes mellitus patients and diabetic rats. Our results will provide novel insights for the role of Gal3 as a biomarker and drug target for type 2 diabetes mellitus patients with MCI.

METHODS

Patients recruitment, clinical characteristics and ethical consideration

Hospitalized patients aged 50–80 years who meet the World Health Organization 1999 Criteria[25] for type 2 diabetes mellitus with a history > 3 years were recruited in the present study. The clinical characteristics, including sex, age, weight, height, blood pressure, duration of diabetes and drug use, such as antidiabetics, antihypertensives and antidyslipidemics, were collected from medical history. Body mass index (BMI) was calculated as bodyweight / height squared (kg/m^2). Physical activity was estimated in terms of the metabolic equivalent of task hours per week spent on work, transportation, housework and non-sedentary recreation. Diet score was calculated according to average consumption (grams per week) of several food items (including legumes, vegetables, fruits, fish, poultry and salt intake)[26]. Healthy diet was originally defined using the following five components: (i) legumes and cereals as basic food; (ii) ≥500 g fruits and vegetables daily; (iii) <100 g red meat/day; (iv) regular (in most weeks) intake of soybean products and/or unprocessed fish; and (v) preference for non-salty food, in accordance with the current "Dietary Guidelines for Chinese Residents"[27]. Diet score was classified as ideal (4–5 components), intermediate (2–3 components) or poor (0–1 components). Hypertension was defined as two blood pressure measurements ≥140/90 mmHg. All participants gave written consent to participate in the study. Ethical permission was approved for patients' recruitment, using their clinical data and blood assays, as well as assessment of cognitive status in the study by the Shandong Provincial Hospital Research Ethics Committee.

Blood assays

Fasting venous blood samples were collected by a research nurse, and then the serum and plasma of each sample were separated and stored at −80°C until use. The serum Gal3 level was determined by enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA), and plasma Aβ40 and Aβ42 levels were determined by ELISA kits (Millipore, Billerica, MA, USA) according to the manufacturer's instructions. Serum Gal3 levels, and plasma Aβ40 and Aβ42 levels were calculated in ng/mL and in pg/mL, respectively.

Glycated hemoglobin (HbA1c; %), fasting blood glucose (FBG) and fasting insulin (FINS), as well as fasting triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol

(LDL-C) and high-density lipoprotein cholesterol (HDL-C) were detected in the clinical laboratory of Shandong Provincial hospital, Jinan, China. HbA1c was tested using high-performance liquid chromatographic analysis (HLC-73G7; Tosoh, Tokyo, Japan) and FBG by the glucose oxidase method[28]. FINS was assayed by the electrochemiluminescence method (Roche Elecsys Insulin kit; Roche Diagnostics, Mannheim, Germany). TG, TC, LDL-C and HDL-C were determined using enzymatic colorimetry by an automatic analyzer (Beckman AU5800, Tokyo, Japan) according to the standard protocol[29]. Insulin resistance was estimated based on the homoeostasis model assessment of insulin resistance (HOMA-IR) formula: FBG (mmol/L) × FINS (mU/L) / 22.5.

Neuropsychological assessments

Both the Minni-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) were used to assess cognitive status in recruited type 2 diabetes mellitus patients based on the 2006 diagnostic criteria from the MCI Working Group of European Consortium[30]. Each participant was interviewed face-to-face by a trained neuropsychiatrist from the Department of Geriatric Neurology, Shandong Provincial Hospital, Jinan, China. The MMSE scale is widely used in cognitive function assessment, and its sensitivity and specificity for MCI detection are 0.4–1.0 and 0.7–1.0[31]. The MoCA, a screening tool of MCI with high sensitivity (0.8–1.0)[31], was used to evaluate the overall cognitive status of type 2 diabetes mellitus patients who were divided into MCI (65 cases MoCA <26; 35 men and 30 women) and healthy cognition (30 cases MoCA ≥26; 37 men and 32 women) groups (Table 1). The exclusion was carried out according to the criteria previously reported:[32] (i) suffering from hypoglycemia in the past 1 month before the neuropsychological tests; (ii) history of known stroke (Hachinski score ≥4), head trauma, alcoholism, neurodegenerative diseases containing Parkinson's disease and AD, epilepsy, major depression (assessed by the Self-Rating Depression Scale) or other neuropsychiatric diseases (e.g., anxiety, personality disorder and schizophrenia); (iii) major illness (e.g., hypoglycemic coma, hyperosmolar non-ketotic diabetic coma, cancer, thyroid dysfunction, anemia, hepatitis and serious infection); (iv) severe visual or hearing loss; and (v) use of drugs that might interfere with cognitive function tests (e.g., anti-Parkinson's drugs, benzodiazepines, neurosedative drugs or any drugs for AD).

Table 1 | Clinical and biochemical characteristics of the mild cognitive impairment and control groups

	MCI (n = 65)	Control (n = 69)
Female, n (%)	30 (46.2)	32 (46.4)
Age (years)	69.62 ± 6.53	68.35 ± 5.62
Body mass index (kg/m^2)	24.54 ± 1.87	23.27 ± 4.12
Physical activity (MET-h/week)	45.69 ± 14.5	49.45 ± 19.34
Diet score	2.21 ± 1.15	2.07 ± 1.25
Family history of diabetes, n (%)	25 (38.46)	27 (41.54)
Systolic BP (mmHg)	134.5 ± 18.2	133.8 ± 17.92
Diastolic BP (mmHg)	78.92 ± 14.15	77.82 ± 14.61
FBG (mmol/L)	8.07 ± 2.53	7.85 ± 3.47
HbA1c (%)	8.90 [6.90–9.68]	8.80 [6.30–9.15]**
Diabetes duration (years)	9.50 [7.00–14.00]	9.00 [6.00–14.00]
HOMA-IR	2.35 ± 0.42	1.88 ± 0.27**
Total cholesterol (mmol/L)	4.95 ± 1.52	4.83 ± 0.83
Triglycerides (mmol/L)	1.57 ± 0.37	1.39 ± 0.43
HDL-C (mmol/L)	1.58 ± 0.42	1.43 ± 0.48
LDL-C (mmol/L)	2.74 ± 0.92	2.79 ± 0.75
Gal3 (ng/mL)	7.45 ± 0.34	6.95 ± 0.51**
Aβ40 (pg/mL)	54.65 ± 21.34	57.69 ± 23.04
Aβ42 (pg/mL)	28.46 ± 17.24	31.53 ± 19.55**
Aβ42/40	0.58 ± 0.29	0.61 ± 0.33
MMSE scores	25.00 [23.0–26.0]	29.00 [27.0–30.0]*
MoCA scores	22.00 [19.0–24.0]	27.00 [26.0–29.0]**
Number and percentages of drug use patients, n (%)		
Antidiabetics	60 (92.31)	57 (82.61)
Antihypertensives	27 (41.54)	28 (40.58)
Antidyslipidemics	38 (58.46)	35 (50.72)

Physical activity was estimated in terms of metabolic equivalent of task (MET)-h/day spent on work, transportation, housework and non-sedentary recreation. *P < 0.01, **P < 0.001.

Diabetic rat experiments

Six-week-old male Wistar rats received from the Experimental Animal Center of Shandong University, Jinan, China, were used as the experimental animals, housed at 22 ± 2°C under a 12-h light/dark cycle with food and water ad libitum in a standard laboratory of Shandong Provincial Hospital. The control rats (group I, n = 8) were fed with a normal diet and injected intraperitoneally with 0.1 mL of 0.1 mol/L citrate buffer solution. The rats (group II, n = 8) were fed with a high-fat diet (HFD; Rodent diet D12492; Research diets Inc., New Brunswick, NJ, USA) for 4 weeks to induce insulin resistance, and then injected intraperitoneally once with low-dose streptozotocin (STZ; 30 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) according to the previous protocol[33]. Rats with FBG levels ≥16.7 mmol/L were considered as the type 2 diabetes mellitus rat model, and they were continuously fed with the HFD for an additional 4 weeks. Group III comprised of diabetic rats that were fed with the HFD and Gal3-activity inhibitor modified citrus pectin (MCP; 100 mg/kg/day; Centrax International Corporation, San Francisco, CA, USA) in drinking water for an additional 4 weeks. Group IV rats received MCP (100 mg/kg/day) only.

The animal experiments for the present study were approved by Shandong Provincial Hospital Council on Animal Care Committee, and were carried out according to the Provisions and General Recommendation of Chinese Experimental Animals Administration Legislation.

Blood assays and brain Gal3 level measurement in rats

After a neurobehavioral test, five rats from each group were fasted for 12 h, and 3-mL blood samples were collected from

the abdominal aorta 2 days before the rats were killed, then serum and plasma were separated and stored at −80°C for further detection of FBG and FINS levels. The FBG levels were determined by a glucose oxidase biochemistry analyzer, and the FINS levels measured by ELISA (Nanjing Jiancheng, China). Serum Gal3 level was determined by sensitive ELISA kits, specific for humans (R&D Systems). HOMA-IR was calculated by the following formula: FBG (mmol/L) × FINS (mU/L) / 22.5.

The brains were immediately removed and placed on the dry ice for the isolation of the cerebral cortex and hippocampus. The tissues of the cerebral cortex and hippocampus homogenates were prepared in 0.1 mol/L phosphate-buffered saline, pH 7.4 (1:5), centrifuged at 8,000 *g* 4°C for 10 min, aliquoted and stored at −80°C. The measurement of Gal3 in supernatant was carried out following the manufacturer's instructions by ELISA kits (Shanghai Blue Gene Biotech Co., Ltd., Shanghai, China). The results were expressed as ng/mg of protein.

Morris water maze test

The Morris water maze test was carried out with all eight rats according to the protocol previously reported[16]with small modifications. Briefly, the test consisted of 5-day training (visible [days 1–2] and invisible platform training [days 3–5] sessions) and a probe trial on day 6. Visible platform training was carried out for baseline differences in vision and motivation, and the escape latency of each rat during the 2-day visible platform test was recorded. Invisible platform training was carried out for spatial learning and retention memory to find the platform, the escape latency of each rat to reach the hidden platform during the 3-day invisible platform test was recorded. On day 6, the platform was removed and the probe trial started, and rats were placed at the quadrant that was opposite the target quadrant, and they had 90 s to search for the platform. The number of the cross times and time spent in the target platform area by each rat was recorded to evaluate the spatial memory. Time in the target platform area was also recorded by an overhead camera connected to a computerized tracking system (HVS Image, Hampton, UK). Then, we calculated the percentage of time and path length in the target platform area/all platform area (% of time and path in the target quadrant).

Statistical analysis

Student's *t*-tests were carried out for normally distributed variables (age, BMI, physical activity, diet score, SBP, DBP, FBG, HOMA-IR, TC, TG, HDL-C, LDL-C, Gal3, Aβ40, Aβ42 and Aβ40/Aβ42), and the non-parametric Mann–Whitney *U*-test was carried out for the remaining variables of HbA1c, diabetes duration, MMSE score and MoCA score.

The χ^2-test was used to analyze categorical data (sex, family history of diabetes, patients taking antidiabetics, antihypertensives or antidyslipidemics). The correlations between MMSE and MoCA, and Gal3, and between the three variables with clinical indictors were assessed using Pearson's correlation analysis for normally distributed variables (age, FBG, HbA1c, HOMA-IR, BMI, Aβ40, Aβ42, Aβ40/Aβ42, Gal3) and Spearman's rank correlation analysis for abnormally distributed variables (diabetes duration). The relationships of serum Gal3 level with MMSE and MoCA scores were examined using partial correlation adjusted for HbA1c, HOMA-IR and Aβ42.

The possible influences of explanatory variables Aβ42, or Gal3 on the outcome MCI type 2 diabetes mellitus versus non-MCI type 2 diabetes mellitus patients were analyzed by simple logistic regression, which were adjusted by covariates, such as sex, age, BMI, HbA1c, HOMA-IR and treatment for disease (antidiabetics, antihypertensives and antidyslipidemics), as listed in Table 1. Multicollinearity was tested using the variance inflation factor (VIF) method, with a VIF ≥5 showing the presence of multicollinearity. The resulting odds ratio (OR) and confidence interval (CI) are presented.

All data presented either as the mean ± standard error and median (interquartile range), or number and percentage and correlation efficiency (*r*) were analyzed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of participants and neuropsychological scores

No difference was observed between the MCI and control groups for clinical variables, such as sex, age, BMI, physical activity, diet score, systolic BP and diastolic BP (all $P > 0.05$; Table 1). Type 2 diabetes mellitus patients with MCI were confirmed with a significant deterioration in cognitive function assessed by both MMSE and MoCA, and showed a significant elevation for HbA1c and HOMA-IR (all $P < 0.05$), as compared with the type 2 diabetes mellitus controls. Type 2 diabetes mellitus patients with MCI showed higher serum Gal3 level and lower plasma Aβ42 level compared with the controls (all $P < 0.05$). The remaining variables, such as FBG, TG, TC, LDL-C, HDL-C, Aβ40, Aβ42/40, diabetes duration, and use of antidiabetics, antihypertensives and antidyslipidemics did not show a significant difference between the two groups.

Correlations of cognitive function and serum Gal3 with other clinical and blood assay variables

As shown in Table 2, the MMSE scores were negatively correlated with HbA1c ($r = -0.35$, $P < 0.05$), HOMA-IR ($r = -0.30$, $P < 0.01$) and serum Gal3 level ($r = -0.51$, $P < 0.01$), but positively correlated with plasma Aβ42 level ($r = 0.62$, $P < 0.05$) in the MCI group. No correlation was observed between the MMSE score and clinical or blood variables in the control group. The MoCA scores were negatively correlated with age ($r = -0.11$, $P < 0.05$), HbA1c ($r = -0.44$, $P < 0.01$), HOMA-IR ($r = -0.17$, $P < 0.01$) and serum Gal3 level ($r = -0.47$, $P < 0.001$), but positively with plasma Aβ42 level ($r = 0.56$, $P < 0.001$) and Aβ42/40 ($r = 0.23$, $P < 0.05$) in the MCI group. No correlation was observed between the MoCA score and other clinical or blood assay variables in the control group. The serum Gal3 level

Table 2 | Correlation of clinical and biochemical characteristics with cognitive function in type 2 diabetes mellitus patients

	MCI (n = 65)			Control (n = 69)			Total type 2 diabetes mellitus (n = 134)		
	MMSE	MoCA	Gal3	MMSE	MoCA	Gal3	MMSE	MoCA	Gal3
Age†	−0.08	−0.11*	0.18	−0.13	−0.19	0.11	−0.25	−0.29	0.37
Diabetes duration‡	−0.14	−0.07	0.14*	−0.09	−0.13	−0.05	−0.20	−0.17	0.28*
FBG†	−0.10	−0.13	0.20**	−0.17	−0.11	0.14	−0.19	−0.23	0.37**
HbA1c‡	−0.35*	−0.44**	0.47**	−0.28	−0.35	0.33	−0.30*	−0.39**	0.64*
HOMA-IR†	−0.30**	−0.17**	0.38***	−0.45	−0.14	0.32***	−0.42**	−0.20**	0.57**
BMI†	−0.20	−0.32	0.24**	−0.13	−0.10	0.07	−0.21	−0.29	0.61**
Aβ40†	−0.28	−0.24*	−0.08	−0.19	−0.19	−0.21	−0.25	−0.28	−0.12
Aβ42†	0.62*	0.56***	−0.15***	0.32	0.37	−0.26	0.54*	0.49**	−0.36***
Aβ42/40†	0.37	0.23*	0.07	0.24	0.28	0.25	0.31	0.30	0.17
Gal3§	−0.51**	−0.47***		−0.29	−0.35		−0.32*	−0.41***	

*P < 0.05, **P <= 0.01, ***P < 0.001. †Pearson's correlation analysis for normally distributed variables. ‡Spearman's rank correlation analysis for abnormally distributed variables. §Partial correlation analysis adjusted for glycated hemoglobin [HbA1c], homoeostasis model assessment of insulin resistance [HOMA-IR] and amyloid-β (Aβ)42. BMI, body mass index; FBG, fasting blood glucose; Gal3, galectin-3; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment.

was positively correlated with diabetes duration (r = 0.14, P < 0.05), FBG (r = 0.20, P < 0.01), HbA1c (r = 0.47, P < 0.01), HOMA-IR (r = 0.38, P < 0.001) and BMI (r = 0.24, P < 0.01) in the MCI group. Similar results to the MCI group were also obtained in all type 2 diabetes mellitus patients. No correlation was observed between Gal3 and clinical or blood assay variables in the control group, except for HOMA-IR.

Association between incident MCI and risk factors by simple logistic regression analysis in type 2 diabetes mellitus patients

Each variable in Table 1 with a VIF <5 was considered as a covariate, with the exception of Abeta42 and Gal3, as independent exposures, and MCI as the common dependent outcome variable. As shown in Table 3, findings from the simple logistic regression analysis showed that both plasma Aβ42 and serum Gal3 levels were significantly associated with incident MCI in type 2 diabetes mellitus patients after adjustment with covariates, such as sex, age, BMI, HbA1c, HOMA-IR and treatment for disease (antidiabetics, antihypertensives and antidyslipidemics). All factors in the logistic regression model had a VIF value <5.

Table 3 | Odds ratio and 95% confidence intervals of incident mild cognitive impairment associated with galectin-3 and amyloid-β42 in blood in patients with type 2 diabetes mellitus

Variables	OR	95% CI
Aβ42 (pg/mL)	1.76	1.27–1.94
Gal3 (ng/mL)	5.45	2.10–6.64

Total n = 134, incident mild cognitive impairment n = 65. Aβ, amyloid-β; CI, confidence interval; Gal3, galectin-3; HOMA-IR, homoeostasis model assessment of insulin resistance; MCI, mild cognitive impairment; OR, odds ratio.

Increased levels of Gal3 in both the brain and blood, and cognitive impairment in HFD/STZ diabetic rats

As shown in Table 4, levels of FBG, FINS, HOMA-IR and Gal3 showed significant alteration in HFD/STZ-induced type 2 diabetes mellitus rats as compared with control rats. Rats in the type 2 diabetes mellitus + MCP group showed significantly lower levels of FBG, HOMA-IR and Gal3 than that in the type 2 diabetes mellitus group. MCP-treated type 2 diabetes mellitus rats showed increased levels of FBG, FINS, HOMA-IR and Gal3 than that in the control group. No difference was found between control and MCP-treated control rats. In the probe trial, HFD/STZ diabetic rats showed spatial memory impairment compared with the control group. As shown in Figure 1, Gal3 level was significantly increased in the supernatants of both the cerebral cortex (P < 0.01) and hippocampus (P < 0.01) of HFD/STZ type 2 diabetes mellitus rats compared with the controls. Rats in the type 2 diabetes mellitus + MCP group showed significantly lower levels of Gal3 expression in the hippocampus (P < 0.05) and cerebral cortex (P < 0.05) than that in the type 2 diabetes mellitus group. MCP-treated type 2 diabetes mellitus rats showed increased levels of Gal3 expression in the hippocampus (P < 0.05) and cerebral cortex (P < 0.01) than that in the control group. No difference was found between control and MCP-treated control rats. As shown in Figure 2a, in the 2-day visible-platform test, no difference in vision or basal motivation was found in both the HFD/STZ and control groups, (P> 0.05); in the 3-day invisible platform test, rats in the HFD/STZ-induced type 2 diabetes mellitus rats showed spatial learning and retention memory impairment compared with the controls. MCP improved spatial learning and retention memory function compared with the type 2 diabetes mellitus group (Figure 2b).

Table 4 | Effects of galectin-3 inhibitor, modified citrus pectin, on the level of serum galectin-3 and related blood biochemical markers, fasting blood glucose, fasting insulin and homoeostasis model assessment of insulin resistance, as well as cognitive ability assessed during the probe trial test in high-fat diet/streptozotocin induced type 2 diabetes mellitus rats

	Control group	Type 2 diabetes mellitus group	Type 2 diabetes mellitus + MCP group	MCP group
FBG (mmol/L)	5.24 ± 0.45	19.23 ± 0.58‡	10.75 ± 1.34‡,§	5.11 ± 0.75
FINS (mU/L)	19.87 ± 2.53	13.35 ± 3.74‡	17.33 ± 1.69‡,§	21.19 ± 2.63
HOMA-IR	4.63 ± 0.58	11.41 ± 0.67‡	9.28 ± 0.77‡,¶	4.81 ± 0.78
Gal3 (ng/mg)	8.74 ± 2.47	21.33 ± 3.54‡	17.48 ± 2.79†,§	8.49 ± 1.75
Times of crossing the target quadrant	13.5 ± 0.45	4.73 ± 0.74‡	8.33 ± 0.77†,§	13.89 ± 0.69
Percentage of path in the target quadrant	0.51 ± 0.25	0.21 ± 0.07‡	0.45 ± 0.19†,¶	0.54 ± 0.11
Percentage of time in the target quadrant	0.39 ± 0.12	0.13 ± 0.09‡	0.32 ± 0.09‡,¶	0.41 ± 0.15

FBG, fasting blood glucose; FINS, fasting insulin; HFD, high-fat diet; HOMA-IR, homoeostasis model assessment of insulin resistance; MCP, modified citrus pectin; STZ, streptozotocin. $^{†}P < 0.05$, $^{‡}P < 0.01$ versus control group; $^{§}P < 0.05$, $^{¶}P < 0.01$ versus type 2 diabetes mellitus model group. Statistical significance was assessed with one-way analysis of variance (ANOVA) followed by the Tukey–Kramer test for post-hoc comparisons between groups.

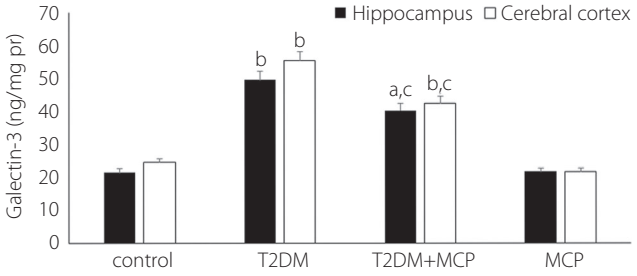

Figure 1 | Effects of galectin-3 inhibitor, modified citrus pectin (MCP), on the level of galectin-3 expression in the hippocampus and cerebral cortex of high-fat diet/streptozotocin-induced type 2 diabetes mellitus (T2DM) rats. Data are the mean ± standard deviation. $^{a}P < 0.05$, $^{b}P < 0.01$ versus control group; $^{c}P < 0.05$, versus type 2 diabetes mellitus model group.

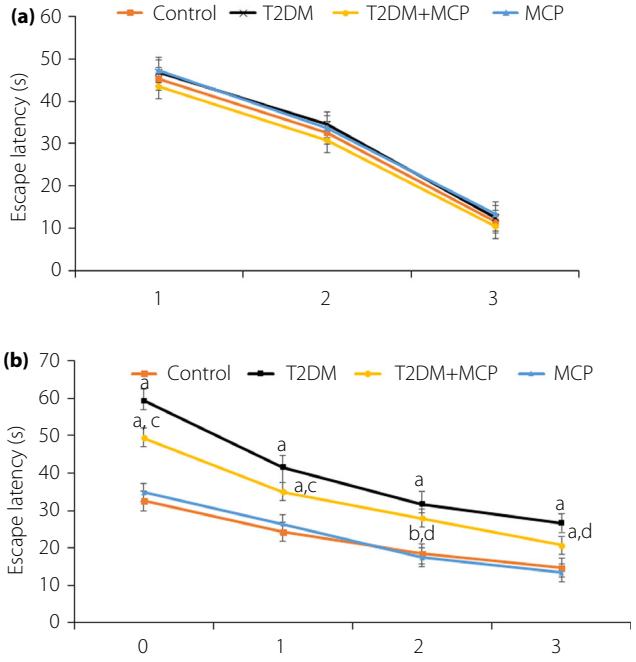

Figure 2 | Effects of galectin-3 inhibitor, modified citrus pectin (MCP), on the memory impairment of high-fat diet/streptozotocin-induced type 2 (T2DM) diabetes mellitus rats in the Morris water maze test. Day 0 represents performance on the first trail, and subsequent points show the average of all daily trials. (a) No differences were found in the escape latency between the two groups during the 2-day visible platform test. (b) Changes in escape latency to reach the hidden platform during the 3-day acquisition trails. $^{a}P < 0.05$, $^{b}P < 0.01$ versus control group; $^{c}P < 0.05$, $^{d}P < 0.01$ versus type 2 diabetes mellitus model group.

DISCUSSION

The majority of clinical studies of Gal3 have been carried out using blood or serum levels. It has been shown that Gal3 can be easily detected using an ELISA test[32], which makes it easily accessible to most practicing clinicians, and greater adoption is expected. Several previous studies showed that Gal3 has a similar positive correlation with cognitive impairment[17,33,34]. In a cross-sectional study, it was found that AD patients have higher serum Gal3 level compared with the controls[18]. In a proteomics study using serum samples from 35 amnestic MCI patients and 35 healthy controls[35], it was found that the MCI group actually had lower levels of Gal3-binding protein. It is possible that because more Gal3-binding proteins are bound by Gal3, a high level of biologically active Gal3 is generated[36]. In the present study, we consistently found a higher serum Gal3 level in the MCI type 2 diabetes mellitus group compared with the non-MCI type 2 diabetes mellitus control. Furthermore, we found that an increased Gal3 level is associated with an increased risk of MCI, suggesting that an increase of Gal3 in blood circulation is involved in the development of MCI in type 2 diabetes mellitus patients. Taken together, Gal3 might be an indicator for the early detection of diabetic cognitive impairment. Additionally,

the findings of the present study suggest that Gal3 plays an important role in the progression of diabetic cognitive impairment. We propose that Gal3 contributes to the development of diabetic cognitive impairment due to inflammation, insulin resistance and additional unidentified mechanisms, which likely include Aβ plaque formation. Assessment of the levels of the marker will be helpful in not only diagnosis, but also prognosis of MCI type 2 diabetes mellitus. Thus, Gal3 has the potential to be a novel biomarker in clinical practice, and could be considered a novel therapeutic target in efforts to combat diabetic cognitive impairment.

We found that plasma Aβ42 levels are lower in MCI type 2 diabetes mellitus patients compared with non-MCI type 2 diabetes mellitus controls, and decreased Aβ42 levels are associated with an increased risk of MCI. Low plasma Aβ42 levels in patients could be explained by increased Aβ42 deposition in the brain and decreased Aβ42 clearance from the brain to blood. The plasma Aβ42 level is closely correlated to its level in cerebrospinal fluid and the degree of Aβ deposition in the brain shown by 11C-labelled Pittsburgh compound-B positron emission tomography[37,38]. Aβ accumulation in brains is thought to contribute to the diabetes mellitus-associated cognitive impairment in both human and animal models[39,40]. Data from the present study also recommended Aβ42 as a key peripheral biomarker to detect MCI type 2 diabetes mellitus in addition to AD[41,42].

The current study is relatively unique, as the findings are from both clinical patients and experimental animals. There were several limitations. First, as we used the MoCA and MMSE as the assessment tools to assess the cognition function of the participants in the study, more accurate results would be obtained if more neuropsychological evaluation tests and neuroimaging examinations were included. Second, the small sample size and single ethnicity from the same race (Han Chinese) might limit the persuasion of the present results to a certain extent and application to other ethnic groups. Third, the present study was a cross-sectional design, and further longitudinal data are required to extend the results. Fourth, we only selected to detect the plasma Aβ level, because it is difficult to obtain cerebrospinal fluid from type 2 diabetes mellitus patients in a clinic. Finally, the pathological mechanism of cognitive impairment in type 2 diabetes mellitus patient is complex. The relationship between Gal3 and MCI might have been confounded by type 2 diabetes mellitus. Further studies need to be carried out to validate the role of Gal3 in type 2 diabetes mellitus related cognitive impairment.

In conclusion, we found that type 2 diabetes mellitus patients with MCI show significantly increased levels of serum Gal3 and decreased levels of plasma Aβ42 in association with cognitive impairment. Gal3 is a potential risk molecule involved in the development of MCI type 2 diabetes mellitus, and Aβ42 is a peripheral biomarker to help diagnose MCI in type 2 diabetes mellitus patients. We recommend large-scale multicenter studies to assess the clinical application of Gal3 as an independently novel biomarker for MCI type 2 diabetes mellitus patients. Whether Gal3 is simply a disease biomarker or is also a mediator of the development and progression of diabetic cognitive impairment warrants further investigation.

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DISCLOSURE

The authors declare no conflict of interest.

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