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Correlation of Glypican-4 Level with Basal Active Glucagon-Like Peptide 1 Level in Patients with Type 2 Diabetes Mellitus

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Background: Previous studies have reported that glypican-4 (GPC4) regulates insulin signaling by interacting with insulin receptor and through adipocyte differentiation. However, GPC4 has not been studied with regard to its effects on clinical factors in patients with type 2 diabetes mellitus (T2DM). We aimed to identify factors associated with GPC4 level in T2DM.

Methods: Between January 2010 and December 2013, we selected 152 subjects with T2DM and collected serum and plasma into tubes pretreated with aprotinin and dipeptidyl peptidase-4 inhibitor to preserve active gastric inhibitory polypeptide (GIP) and glucagon-like peptide 1 (GLP-1). GPC4, active GLP-1, active GIP, and other factors were measured in these plasma samples. We performed a linear regression analysis to identify factors associated with GPC4 level.

Results: The subjects had a mean age of 58.1 years, were mildly obese (mean body mass index [BMI], 26.1 kg/m^2), had T2DM of long-duration (mean, 101.3 months), glycated hemoglobin 7.5%, low insulin secretion, and low insulin resistance (mean homeostatic model assessment of insulin resistance [HOMA-IR], 1.2). Their mean GPC4 was 2.0±0.2 ng/mL. In multivariate analysis, GPC4 was independently associated with age (β=0.224, P=0.009), and levels of active GLP-1 (β=0.171, P=0.049) and aspartate aminotransferase (AST; β=–0.176, P=0.043) after being adjusted for other clinical factors.

Conclusion: GPC4 was independently associated with age, active GLP-1, and AST in T2DM patients, but was not associated with HOMA-IR and BMI, which are well known factors related to GPC4. Further study is needed to identify the mechanisms of the association between GPC4 and basal active GLP-1 levels.

Keywords: Glypicans; Active glucagon-like peptide 1; Diabetes mellitus

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a disease characterized by a combination of insulin resistance and relative insulin insufficiency [1,2]. Obesity is one of the most important factors associated with insulin resistance, which is the initial step in the development of T2DM. The recent rapidly increasing prevalence of T2DM and obesity has been identified as a major worldwide health crisis [3]. Therefore, improving the understanding of the various factors associated with this health issue is a very important focus of research.

Glypican-4 (GPC4) is a member of the glycosylphosphatidylinositol (GPI)-anchored heparin sulfate proteoglycans [4]. GPC4 is expressed in visceral and subcutaneous adipose tissues

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and was identified recently as a novel adipokine [5]. GPC4 was demonstrated to regulate insulin signaling through interaction with the insulin receptor and by inducing differentiation of adipocytes, indicating a potentially important role in the regulation of body fat [6,7]. GPC4 also acts as an essential modulator of key regulatory proteins, including Wnt, bone morphogenetic proteins, fibroblast growth factor, and sonic hedgehog [4]. GPC4 was also shown recently to play an important role in fat distribution, an effect that was modified by rosiglitazone through differential regulation of GPC4 mRNA in subcutaneous and visceral fat tissues [7]. In addition, studies of GPC4 expression in human white adipose tissue showed a positive correlation with body fat content and insulin resistance [6], while the circulating plasma GPC4 level was increased in women but not in men with nonalcoholic fatty liver disease (NAFLD) [8]. In another study, GPC4 was higher in patients with prediabetes than that in normal subjects [9]. These studies showed that GPC4 level was significantly elevated in states of metabolic dysfunction, including obesity, insulin resistance, and NAFLD.

There has been no definitive study of GPC4 level and its correlation with other clinical factors in typical T2DM patients. In this study, we aimed to investigate the associations of circulating GPC4 level with various factors in patients with T2DM.

METHODS

This study included 152 patients with T2DM who were admitted to Jeju National University Hospital from July 2010 to July 2013. The participants were part of the Korea Diabetes Cohort Study, which was conducted after approval from the Jeju National University Hospital Institutional Review Board (IRB No. 2010-06-033). Written informed consent was obtained from all subjects in this study. The inclusion criteria were patients with T2DM, aged with 18 to 80 years, who had given informed consent. Exclusion criteria for our study were: type 1 diabetes mellitus (fasting serum C-peptide <0.6 ng/mL and a history of diabetic ketoacidosis); patients treated with a dipeptidyl peptidase-4 (DPP4) inhibitor, α-glucosidase inhibitor, glucagon-like peptide-1 (GLP-1) agonist, or a thiazolidinedione, all drugs known to affect plasma GLP-1 or gastric inhibitory polypeptide (GIP) level; severe renal dysfunction (a glomerular filtration rate [GFR] less than 30 mL/min/1.73 m^2); severe hepatic dysfunction (Child-Pugh score B or C); and patients who were diabetic because of secondary causes.

We collected serum in plain tubes and plasma in ethylenediaminetetraacetic acid tubes coated with aprotinin (25 μL/mL blood; Trasylol, SRL Inc., Tokyo, Japan) and DPP4 inhibitor (10 μL/mL blood; Millipore, St. Charles, MO, USA) from the enrolled patients to measure active GLP-1 and active GIP levels. All blood samples were cooled on ice immediately and within 20 minutes of collection were centrifuged at 4ºC, after which serum/plasma was stored at –70ºC until analysis.

Physical measurements (height, weight, waist circumference, and hip circumference), blood pressure (BP), and biochemical laboratory test results were performed for all participating patients. Waist circumference was measured twice at the narrowest part between the chest and iliac crest parallel to the ground while maintaining normal breathing. Measurements of insulin, C-peptide, glucose, glycated hemoglobin (HbA1c), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, blood urea nitrogen, high-sensitivity C-reactive protein (hsCRP), total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides (TG), apolipoprotein A1 (ApoA1), and apolipoprotein B (ApoB) were performed on blood samples obtained after 12 hours fasting. By interview or reviewing medical records, we assessed the participants' histories of medication, including antihypertensive medication, glucose-lowering agents, and lipid-lowering agents, and the durations of their diabetes. We additionally measured adiponectin, GPC4, and interleukin 6 (IL-6) in the collected plasma samples. We also measured active GLP-1 and active GIP as biologically active forms in the collected plasma samples.

GPC4 level was assayed using a commercially available ELISA kit (USCNK Life Science, Houston, TX, USA), as were levels of adiponectin (Abcam, Cambridge, UK), IL-6 (Abcam), active GLP-1 (Immuno-Biological Laboratories, Takasaki, Japan), and active GIP (Immuno-Biological Laboratories).

Analysis

The results are presented as mean±standard error of the mean. The stratified quartile groups were analyzed using analysis of variance (ANOVA). We used correlation analysis followed by linear regression analysis to identify the factors that were independently associated with GPC4 level. All analyses were performed with SPSS software version 18.0 (SPSS Inc., Chicago, IL, USA), and $P<0.05$ was defined as significant.

RESULTS

Baseline characteristics of T2DM patients

The mean age of the subjects in this study was 58.1 years, and

23.8% were women. The subjects were mildly obese with an average body mass index (BMI) of 26.1 kg/m^2 and a waist-hip ratio of 1.0, with a mean disease duration of 101.3 months. Their mean glucose status was 140.7 mg/dL fasting glucose and 7.5% HbA1c, and their mean insulin status was 8.4 μU/L of insulin, 2.2 ng/mL fasting C-peptide, 1.2 homeostatic model assessment of insulin resistance (HOMA-IR), and 44.5% HOMA-β. They had reduced insulin resistance and decreased ability for insulin secretion. The baseline characteristics of the participants in this study are typical for Asian patients with T2DM (Table 1). The average GPC4 concentration was 2.0 ng/mL, that of adiponectin 2.8 μg/mL, and that of IL-6 9.2 pg/mL. The levels of active GIP and GLP-1 were 3.2 and 5.2 pmol/L, respectively. Most participants were being treated with metformin, and more than half of them with sulfonylurea, lipid-lowering agents, and antihypertensive medications.

Table 1. Baseline Characteristics of Patients

Factor	Value
Age, yr	58.1±0.7
Female sex, %	23.8
Body mass index, kg/m^2	26.1±0.3
Waist hip ratio	1.0±0.0
Systolic blood pressure, mm Hg	142.5±1.4
Diastolic blood pressure, mm Hg	84.0±0.9
DM duration, mo	101.3±7.1
Glucose, mg/dL	140.7±2.9
Insulin, μU/mL	8.4±0.5
C-peptide, ng/mL	2.2±0.1
HbA1c, %	7.5±0.1
HOMA-IR	1.2±0.1
HOMA-β, %	44.5±2.4
hsCRP, mg/dL	0.2±0.0
Blood urea nitrogen, mg/dL	16.4±0.4
Creatinine, mg/dL	1.1±0.0
GFR, mL/min/1.73 m^2	71.2±0.8
Total cholesterol, mg/dL	167.6±2.7
Triglyceride, mg/dL	133.7±6.6
HDL-C, mg/dL	48.1±1.0
LDL-C, mg/dL	99.5±2.5
AST, IU/L	27.1±1.22
ALT, IU/L	33.1±1.9
γGT, IU/L	32.3±1.6
GLP-1, pmol/L	5.2±0.3
GIP, pmol/L	3.2±0.3
Glypican-4, ng/mL	2.0±0.2
Adiponectin, μg/mL	2.8±0.0
IL-6, pg/mL	9.2±2.5
Sulfonylurea, %	58.7
Metformin, %	77.4
Insulin, %	15.5
Statin, %	51.0
Antihypertensive medication, %	52.3

Values are expressed as mean±SEM.
DM, diabetes mellitus; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; GFR, glomerular filtration rate; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, γ glutamyl transpeptidase; GLP-1, glucagon-like peptide 1; GIP, gastric inhibitory polypeptide; IL-6, interleukin 6.

Factors associated with GPC4 level

The GPC4 level is known to differ between healthy men and women [8]. Therefore, we compared the GPC4 level of male participants with that of female participants (Supplemental Table S1). The GPC4 level did not differ between men and women with T2DM; nor did the level of adiponectin, a surrogate marker of insulin sensitivity, or of IL-6, a marker of inflammation. In the correlation analysis, adiponectin (r=0.005, P=0.952) and IL-6 (r=–0.083, P=0.337) were not significantly associated with GPC4 (data not shown).

Correlation analysis showed that GPC4 level in patients with T2DM was not significantly correlated with any factor except age (Fig. 1). No other factors, including fasting glucose, AST, ALT, active GLP-1, BP, glucose-lowering agents, antihypertensive drugs, waist circumference, smoking, disease duration, fasting insulin, C-peptide, HbA1c, HbA1c quartile, BMI quartile, HOMA-β quartile, HOMA-β (%), hsCRP, creatinine, estimated GFR, total cholesterol, TG, HDL-C, LDL-C, ApoB, ApoA1, the ApoB/A1 ratio, and γ-glutamyl transpeptidase were significantly associated with GPC4 in patients with T2DM.

We stratified the patients into quartiles based on BMI, HOMA-IR, and HOMA-β (Supplemental Fig. 1S). The GPC4 did not differ significantly among quartile groups stratified based on BMI. However, GPC4 level differed significantly among the quartile groups stratified based on HOMA-IR and HOMA-β (P=0.040 and P=0.049, respectively). By linear regression, GPC4 also showed a decreasing trend in the higher HOMA-IR quartiles (P=0.033), but not in the HOMA-β quartiles (P=0.146).

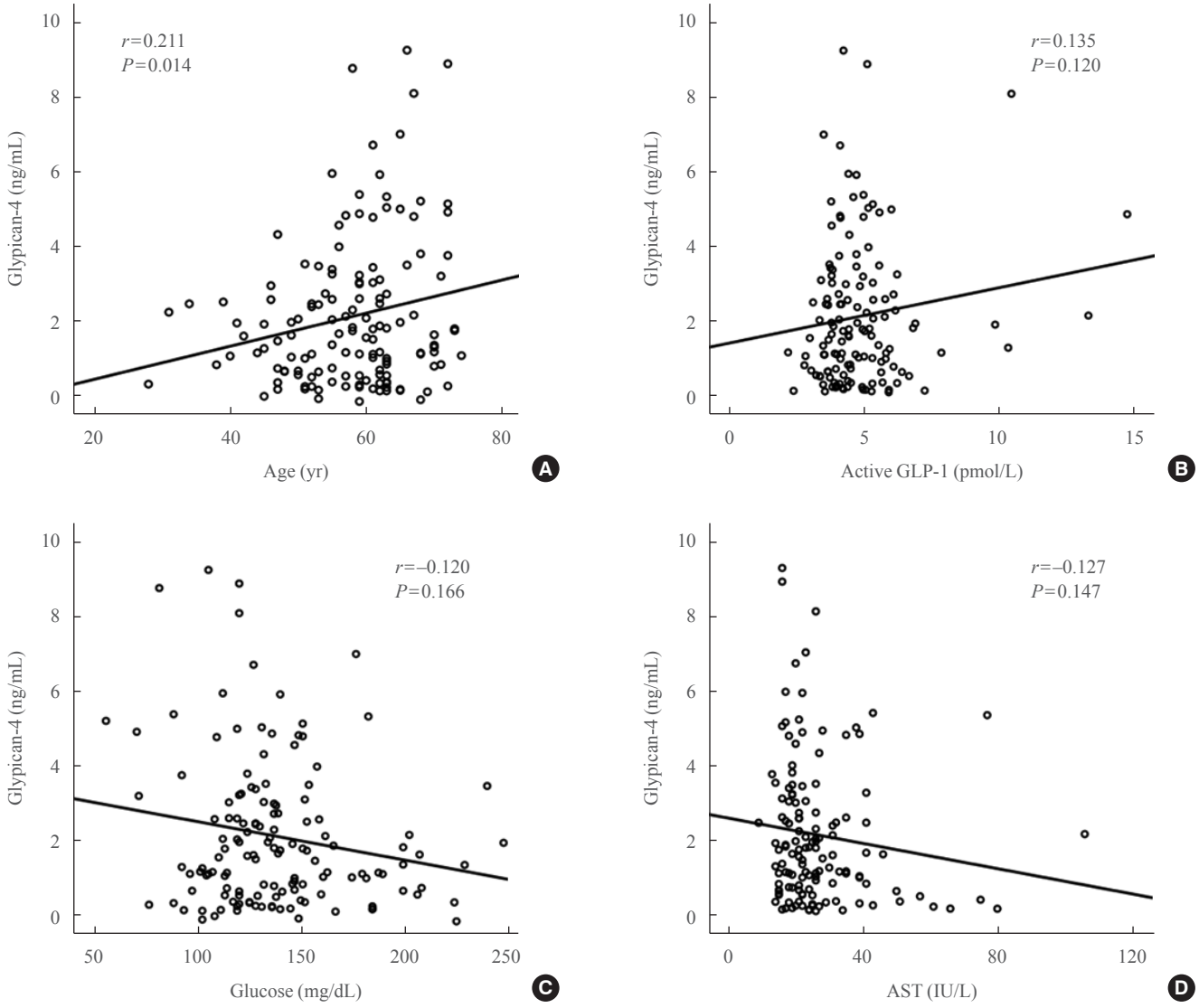

Fig. 1. Correlation curves of plasma glypican-4 concentration with (A) age, (B) active glucagon-like protein 1 (GLP-1), (C) fasting glucose, and (D) aspartate aminotransferase (AST) in patients with type 2 diabetes mellitus. Spearman correlation coefficients and corresponding *P* values are displayed.

Multivariate analysis of factors related to GPC4 level

The only factor in the correlation analysis that was significantly associated with GPC4 level in T2DM patients was age. Therefore, multivariate analysis was performed to identify factors independently associated with GPC4 by adjusting for various factors. We included age, sex, and BMI as basic factors and added variables with a $P<0.2$ in the correlation analysis: fasting glucose, AST, ALT, active GLP-1, and HOMA-IR quartile. We found that age (β=0.224, $P=0.009$), active GLP-1 (β=0.171, $P=0.049$), and AST (β=–0.176, $P=0.043$) were independently associated with GPC4 level after adjusting for sex, BMI, fasting glucose, ALT, and HOMA-IR quartile (Table 2).

DISCUSSION

In this study, we found that age and active GLP-1 level were positively associated and AST was negatively associated with GPC4 level in patients with T2DM.

Previous studies have shown that GPC4 level was associated with insulin resistance because it acts as an insulin sensitizer [6,8,10]. In those studies, GPC4 was investigated in obese patients with or without mild metabolic dysfunction such as impaired fasting glucose, impaired glucose tolerance, or newly diagnosed diabetes. The GPC4 level was shown to be increased in subjects with obesity, prediabetes, newly diagnosed diabetes,

Table 2. Multivariate Analysis to Identify Factors Associated with Glypican-4

Factor	β	*P* value
Age, yr	0.224	0.009
Female sex	–0.015	0.862
BMI, kg/m^2	–0.057	0.515
Glucose, mg/dL	–0.097	0.258
HOMA-IR (quartiles)	–0.123	0.185
AST, IU/L	–0.176	0.043
ALT, IU/L	–0.040	0.779
Active GLP-1, pmol/L	0.171	0.049

BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GLP-1, glucagon-like peptide 1.

and fatty liver disease compared with that in normal subjects. The GPC4 level was also consistently positively correlated with a marker of obesity, such as the waist-hip ratio, BMI, and fat distribution. Therefore, GPC4 was associated with insulin resistance or metabolic disorders. The mechanisms of this association may originate from the expression of GPC4 on cell membranes, especially in visceral fat, and the release of circulating GPC4 from the cell surface by an enzymatically regulated process mediated by the GPI lipase family [6]. Obese subjects or those with fatty liver disease have higher insulin level, which augments the activity of GPI-specific phospholipase D (GPLD-1), a member of the GPI lipase family. This higher activity of GPLD-1 enzyme induced by higher insulin level cleaves more GPC4 from cell surfaces, resulting in more circulating GPC4. Therefore, elevated GPC4 in obesity is related to insulin resistance.

An animal study has shown that GPC4 was elevated in *ob*/*ob* mice on a high-fat diet that were normoglycemic and normoinsulinemic, whereas *ob*/*ob* mice with elevated glucose and insulin levels showed reduced GPC4 level [6]. This indicates that T2DM patients have different associations between GPC4 and other clinical factors than do nondiabetic patients. In this study, we identified a correlation between GPC4 and basal active GLP-1 in patients with T2DM. GLP-1 is an incretin hormone that increases insulin secretion and reduces glucagon production in the pancreas. The physiological role of GLP-1 was suggested to be the reduction of blood glucose level after meals, but this proposal was based on analysis of the postprandial GLP-1 response, not of basal GLP-1 level [11,12]. In fact, the clinical meaning of basal GLP-1 level is unclear. GLP-1 and GPC4 have similar effects in improving hyperglycemia. In previous studies, GLP-1 responses were decreased in patients with T2DM [13], while another study suggested that low GLP-1 level was associated with a risk of T2DM [14], and a third study showed that basal active GLP-1 decreased in patients with T2DM [15]. A study in mice found that GPC4 was elevated in normoglycemia mice on a high fat diet, but decreased in mice with hyperglycemia [6]. The fact that active GLP-1 and GPC4 have similar effects may be why GPC4 and active GLP-1 are both decreased in T2DM patients and are positively correlated with each other. Although the mechanism behind this phenomenon should be investigated further, we demonstrated an interesting association between GPC4 and basal active GLP-1 levels.

We found that GPC4 level in patients with T2DM was associated with age and AST level. GPC4 level was higher in older patients than in younger patients; this could be related to decreased renal function in older patients. Faerch et al. [13] reported that GLP-1 responses were positively correlated with age, milder obesity, and better insulin sensitivity. They also suggested that the role of age in GLP-1 responses might be related to the general reduction in renal clearance in older patients, which could explain the higher basal active GLP-1 and GPC4 levels in older patients with T2DM seen in this study. In addition, in this study, the AST level was negatively associated with GPC4 level in patients with T2DM. Yoo et al. [8] reported that GPC4 level was correlated with AST level and suggested that GPC4 level was increased in women with NAFLD. We obtained the opposite result, showing a negative correlation between GPC4 and AST in T2DM patients. The differences between the two studies might be due to different subjects. Our patients had lower BMI and lower insulin resistance than those in the study by Yoo et al. [8], which might have affected the association between GPC4 and AST levels. However, we showed that GPC4 was independently associated with basal active GLP-1, age, and AST level, which suggested that GPC4 plays a different role in T2DM patients than that proposed previously.

In contrast, in this study, we found that increasing HOMA-IR quartile was negatively associated with GPC4 level using ANOVA, which is the opposite result to those of previous studies [6,8,10]. This phenomenon, i.e., that the level of a hormone increases in prediabetes or metabolic syndrome and decreases in diabetes mellitus, is also observed for other hormones. For example, insulin level increases during the prediabetes and metabolic syndrome stages, but decreases in full-blown T2DM. This decrease, together with the concomitant insulin resistance, is involved in the pathophysiologic mechanisms of progress to

T2DM [16]. The tendency for decreasing GPC4 level in the highest HOMA-IR quartile group might reflect the failure of patients with T2DM to overcome insulin resistance. Alternatively, this difference might be because the patients enrolled in this study were not typically obese T2DM patients, but were only mildly obese, and did not have increased HOMA-IR, quite different characteristics from Caucasian patients. These characteristics of patients in this study might be causes to show a weak relationship between GPC4 and other disease parameters, while previous studies showed other factors to be more strongly associated with GPC4 level. These differences might have caused the different results in the previous and this study.

In conclusion, active GLP-1 and AST levels were associated with GPC4 level in patients with T2DM. Further experimental studies in models of T2DM are necessary to clarify the mechanisms behind the correlation between GPC4 and active GLP-1 and the role of GPC4 in patients with T2DM.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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