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Preproghrelin Leu72Met polymorphism is not associated with type 2 diabetes mellitus

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Abstract

Ghrelin is a novel gut-brain peptide, which exerts somatotropic, orexigenic, and adipogenic effects. Genetic variants of ghrelin have been associated with both obesity and insulin metabolism. In this study, we determined a role of preproghrelin Leu72Met polymorphism on type 2 diabetes mellitus and its relationship to variables studied. Genotypes were assessed by polymerase chain reaction. Frequencies of the Leu72Met polymorphism were found to be 35.4% in the type 2 diabetic patients and 32.5% in the normal controls. The Leu72Met polymorphism was not associated with hypertension, macroangiopathy, retinopathy, serum cholesterol, triglyceride, blood urea nitrogen, HbA_{1c}, lipoprotein (a), fasting insulin, or 24-hour urinary protein levels in the type 2 diabetic group. However, the Leu72Met polymorphism was clearly associated with serum creatinine levels in the diabetic group, as the Met72 carriers exhibited lower serum creatinine levels than the Met72 noncarriers. Our data indicate that the preproghrelin Leu72Met polymorphism is not associated with type 2 diabetes mellitus. However, the Leu72Met polymorphism is associated with serum creatinine levels. These data suggest that Met72 carrier status may be a predictable marker for diabetic nephropathy or renal impairment in type 2 diabetes mellitus.

1. Introduction

Ghrelin is a novel endogenous ligand, which binds specifically to the growth hormone (GH) secretagogue receptor [1]. In addition to its demonstrated effects on the release of GH from the pituitary gland, ghrelin plays a prominent role in the physiological regulation of appetite and body weight [2]. The most abundant source of ghrelin production is the stomach, but various tissues including kidney synthesize ghrelin [3,4]. Gastric ghrelin production seems to be inhibited by leptin, insulin, GH, insulin-like growth factor I (IGF-I), and a high-fat diet, whereas fasting and low-protein diets have been associated with increased plasma concentrations of ghrelin [5,6].

Although ghrelin possesses a variety of biological actions, its role in diseases has not been well characterized. Based on the recent studies, ghrelin has been thought to play a role in both glucose and insulin metabolism [7,8]. Yoshimoto et al [9] have also reported that plasma ghrelin is significantly correlated with the serum creatinine level in chronic renal failure.

Mutations in the ghrelin gene may potentially cause defects or inactivation of the ghrelin protein and also alter secretion of GHs and energy balance. A common preproghrelin Leu72Met polymorphism is associated with both obesity and glucose-induced insulin secretion [10,11]. Recently, Ukkola and Kesanniemi [12] have demonstrated the association between the Leu72Met polymorphism and serum creatinine and lipoprotein (a) levels in type 2 diabetes mellitus. In this study, we have examined the role of

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preproghrelin Leu72Met polymorphism on type 2 diabetes mellitus and its relationship to variables, specifically, such as serum total cholesterol, triglyceride, creatinine, lipoprotein (a), insulin, fasting blood glucose, HbA_{1c}, or 24-hour urinary protein.

2. Materials and methods

2.1. Subjects

A total of 206 subjects with type 2 diabetes mellitus were enrolled in this study (Table 1). Type 2 diabetes mellitus was determined according to the World Health Organization criteria [13]. Patients who showed more than 1.5 mg/dL of serum creatinine concentrations were excluded from the study. Before undergoing physical examinations, all subjects completed a series of questionnaires, which were prepared from collected data on hypertension, stroke, smoking, drinking, duration of illness, and family history of diabetes. Hypertension, smoking, or cardiovascular and cerebrovascular diseases were predicated on the self-reported data collected from the questionnaires. Screening for diabetic retinopathy was performed by an ophthalmologist via direct ophthalmoscopy after pupillary dilation, and these screenings were documented electronically. The nondiabetic control group consisted of 80 healthy adults who were matched to age and sex with the diabetic patients (Table 1). The control group was used to compare preproghrelin Leu72Met genotype distribution with type 2 diabetic patients. Written informed consent was obtained from all subjects, and the protocols of this study were approved by the Chonbuk National University Hospital Ethical Committee.

2.2. Anthropometric measurements

Weights were measured to the nearest 0.1 kg using calibrated balances or electronic scales. Heights were measured to the nearest 1 mm. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters. The waist was measured at the narrowest point between the rib cage and iliac crest, and the hips were measured at the maximal width of the buttocks.

Table 1
Anthropometric data of subjects enrolled in this study

Group	Diabetes	Controls
Age (y)	56.4 ± 10.1	54.9 ± 9.5
Male sex (%)	111/206 (53.6)	43/80 (53.8)
BMI (kg/m^2)	24.5 ± 2.9	23.8 ± 5.6
WHR	0.91 ± 0.05	0.90 ± 0.06
Duration of diabetes	7.4 ± 6.1	
Systolic blood pressure (mm Hg)	130.9 ± 21.7	121.4 ± 15.6
Diastolic blood pressure (mm Hg)	82.8 ± 13.6	80.2 ± 11.7
Smoking (%)	21.9	23.8
Hypertension (%)	41.1	0.0*

WHR indicates waist-hip ratio.
* $P < .05$ compared with diabetes.

Table 2
Distribution of preproghrelin Leu72Met genotype in study subjects

Genotype	Wild (Leu72Leu)	Mutated		
		Total	Leu72Met	Met72Met
Controls (n = 80)	54 (67.5%)	26 (32.5%)	23 (28.8%)	3 (3.8%)
Type 2 diabetes mellitus (n = 206)	133 (64.6%)	73 (35.4%)	65 (31.5%)	8 (3.9%)

Waist-hip ratios were calculated as the waist measurement divided by the hip measurement.

2.3. Laboratory investigations

Blood samples were taken early in the morning after 12 hours of fasting. Concentrations of glucose, total cholesterol, triglycerides, low-density lipoprotein cholesterol, blood urea nitrogen, and creatinine were measured using a Hitachi 7600-110 analyzer (Hitachi High Technologies, Tokyo, Japan). Apolipoprotein (Apo) A-I, ApoB, and lipoprotein (a) levels were analyzed by immunoturbidimetric assay using a Roche-Hitachi Modulator Chemistry analyzer (Mannheim, Germany). HbA_{1c} was analyzed by ion-exchange high-performance liquid chromatography using a Bio-Rad VARIENT II TURBO (Bio-Rad Laboratories, Hercules, CA). Serum insulin concentrations were determined with an immunoradiometric assay kit (Abbott Japan, Tokyo, Japan). Twenty-four–hour urine samples were collected from the patients, and urinary protein concentrations were measured by an immunoturbidimetric assay (Asan Pharmaceutical, Seoul, Korea).

2.4. DNA analyses

Genomic DNA was extracted from leukocytes. A 207–base pair DNA fragment encompassing exon 2 was amplified by standard polymerase chain reaction. The forward and reverse primer sequences were as follows: 5′ -agcagagaaaggagtcg-3′ and 5′-agaggtggcagtgaaca-3′, respectively. The Leu72Met polymorphism was identified on the basis of the restriction endonuclease, *Bsr*I, which retains the mutated site (leucine replaced by methionine) at base 108 in exon 2 of the preproghrelin gene undigested. The amplified product was digested overnight with 5 U of *Bsr*I at 65°C, and the resultant fragments were separated on 2% agarose gel and visualized under ultraviolet light after staining with ethidium bromide.

2.5. Statistical analysis

All analyses were conducted using the SPSS Statistical Software Package (version 11.0, SPSS, Chicago, IL), and all data are expressed as means ± SD. Differences in physical/biochemical parameters between groups (Leu72-Leu vs Leu72Met + Met72Met subjects and Leu72Met vs Met72Met subjects) were assessed by Student *t* tests. If their distribution was not symmetric, Mann-Whitney *U* test was used. Differences in polymorphism frequencies between

Table 3
Characteristics of subjects by preproghrelin Leu72Met genotype in type 2 diabetes mellitus

Genotype	Wild (Leu72Leu)	Mutated		
		Total	Leu72Met	Met72Met
No. of patient	133	73	65	8
Age (y)	56.9 ± 9.4	55.6 ± 11.2	55.4 ± 11.6	57.3 ± 7.6
Male sex (%)	74/133 (55.6)	37/73 (50.7)	35/65 (53.5)	2/8 (25)
BMI (kg/m^2)	25.5 ± 2.9	24.3 ± 3.0	24.3 ± 3.1	24.1 ± 2.5
WHR	0.90 ± 2.99	0.91 ± 0.05	0.91 ± 0.05	0.91 ± 0.04
Duration of illness (y)	7.2 ± 6.4	7.9 ± 5.8	8.1 ± 5.9	5.7 ± 5.6
SBP (mm Hg)	137.9 ± 23.7	135.1 ± 17.4	134.2 ± 17.7	142.1 ± 17.9
DBP (mm Hg)	83.4 ± 14.5	81.6 ± 11.7	81.7 ± 11.4	80.6 ± 14.7
Total cholesterol (mg/dL)	190.3 ± 45.5	195.8 ± 40.9	197.4 ± 41.9	183.6 ± 32.7
Triglyceride (mg/dL)	166.4 ± 80.9	161.3 ± 77.5	162.4 ± 80.7	153.1 ± 49.7
HDL (mg/dL)	44.8 ± 12.0	45.3 ± 11.5	45.0 ± 11.3	47.4 ± 13.5
LDL (mg/dL)	107.1 ± 33.8	114.3 ± 32.3	115.4 ± 32.9	105.9 ± 20.1
Serum creatinine (mg/dL)	1.04 ± 0.19	0.91 ± 0.15*	0.91 ± 0.15	0.92 ± 0.20
Blood urea nitrogen (mg/dL)[a]	18.7 ± 8.9 (n = 116)	16.9 ± 7.5 (n = 62)	16.8 ± 7.8 (n = 56)	18.3 ± 3.9 (n = 6)
24-h urinary protein (mg)[a]	430.6 ± 1288.8 (n = 103)	144.2 ± 208.1 (n = 57)	147.2 ± 212.9 (n = 54)	90.7 ± 79.6 (n = 3)
HbA_{1c} (%)	8.2 ± 2.1	8.2 ± 2.1	8.3 ± 2.2	8.2 ± 2.0
FBS (mg/dL)	180.0 ± 63.6	183.1 ± 63.6	185.7 ± 63.2	163.1 ± 67.5
Lipoprotein (a) (mg/dL)	25.1 ± 17.4	23.9 ± 20.3	23.7 ± 20.8	26.2 ± 18.1
ApoA-1	1.24 ± 0.33	1.37 ± 0.34	1.38 ± 0.35	1.33 ± 0.36
ApoB	1.09 ± 0.33	1.12 ± 0.34	1.12 ± 0.33	1.13 ± 0.42
Fasting insulin (μU/mL)	11.3 ± 10.1	11.1 ± 9.2	11.3 ± 9.5	10.1 ± 6.9

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FBS, fasting blood glucose.

[a] Data from some patients were not available.

* $P < .05$ compared with wild genotype.

groups were assessed using χ^2 tests. $P < .05$ was regarded as significant.

3. Results

Anthropometric data, except hypertension, between type 2 diabetic and nondiabetic controls were similar with each other. Hypertensive type 2 diabetes mellitus was found in 41.1% of type 2 diabetic subjects, whereas none of control subjects were hypertensive (Table 1). Analyses of distribution and allele frequency of preproghrelin Leu72Met/Met72Met polymorphism revealed that there are no significant differences in the frequency of the preproghrelin polymorphism between diabetes and nondiabetes (Table 2). Thus, these phenotypes were present in 34.5% of type 2 diabetic subjects and 32.5% of the controls.

To determine whether any physical/biochemical parameters in 72Met carriers (Leu72Met/Met72Met genotypes) are different from 72Met noncarriers (Leu72Leu), we analyzed various parameters that may change in diabetes (Table 3). There were no differences in physical parameters between 2 groups, and only 1 biochemical parameter, serum creatinine level, among 13 parameters examined was significantly higher (1.04 ± 0.19 mg/dL, $P < .05$) in the 72Met noncarriers than in the 72Met carriers (0.91 ± 0.15 mg/dL).

Analysis of complication of type 2 diabetes mellitus showed that macroangiopathic patient population was slightly higher in the noncarrier than in the carrier (16.7% vs 13.7%), whereas retinopathic patient population was slightly lower in the noncarriers than in the carriers (22.7% vs 29.6%) without statistical significances.

4. Discussion

In this study, we were not able to correlate the preproghrelin Leu72Met polymorphism with type 2 diabetes mellitus. However, we did find evidence of an association between the Leu72Met polymorphism and serum creatinine level in type 2 diabetes mellitus (Table 4).

Table 4
Complications according to the Leu72Met genotype in type 2 diabetes mellitus

Genotype	Wild (Leu72Leu)	Mutated		
		Total	Leu72Met	Met72Met
Macroangiopathy (n = 205)	22/132 (16.7%)	10/73 (13.7%)	8/65 (12.3%)	2/8 (25%)
Retinopathy (n = 199)	29/128 (22.7%)	21/71 (29.6%)	20/63 (31.7%)	1/8 (12.5%)
Hypertension (n = 205)	54/132 (40.9%)	30/73 (41.1%)	25/65 (38.5%)	5/8 (62.5%)

Ghrelin is a 28–amino acid acylated peptide, which exhibits a potent ability to stimulate the release of GH from the pituitary gland, an ability that has been demonstrated in both rats and humans [1]. The acylation of the ghrelin peptide is a prerequisite for its biological activity, and this occurs not only in the stomach [1], but also in the kidney [3]. Recently, Mori and Ahihiro Yoshimoto [3] have reported that preproghrelin gene is expressed in cultured rat mesangial and mouse podocytes. In addition, preproghrelin and ghrelin receptor genes are expressed in both the kidney and glomerulus of rodents [3]. These findings indicate that ghrelin performs both endocrine and/or paracrine functions in the kidney, and the kidney is one of possible targets of direct ghrelin action. Growth hormone stimulates the secretion of IGF-I in the liver, and both GH and IGF-I increase renal blood flow, glomerular filtration rates, and tubular phosphate and sodium reabsorption [14]. They are also involved in the normal growth of the kidney and have been implicated in renal hypertrophy [14,15]. These findings have indicated that ghrelin may not only have physiological, but also pathophysiological significance in the kidney.

Preproghrelin may be processed in a variety of ways, resulting in generation of several different products, depending upon the stimulus. A common polymorphism at codon 72 of the preproghrelin gene (Leu72Met) is located outside the region in which the mature ghrelin product is encoded [16]. Although this polymorphism does not appear to induce any changes in the sequence of the mature ghrelin, the resulting alterations in messenger RNA stability or protein processing may cause modified ghrelin secretion or activity as has been described for a number of other hormones and proteins [17,18]. Alterations in the activity of the ghrelin peptide may modulate both GH secretion and energy balance. Pöykkö et al [19] have reported that the hypertensive subjects with Leu72Met genotype have lower ghrelin concentration than the subjects with Leu72Leu genotype.

The Leu72Met polymorphism is associated with early-onset obesity [10] and reduction in glucose-induced insulin secretion [11]. A recent report has also uncovered an association between the Leu72Met polymorphism and serum creatinine and lipoprotein (a) levels in patients with type 2 diabetes mellitus [12]. In this study, we have found that the Leu72Met polymorphism is similar with each other in both the type 2 diabetic and control groups. However, in the type 2 diabetic group, this polymorphism is associated with serum creatinine level, as the diabetic Met72 carriers evidenced lower serum creatinine level than the diabetic Met72 noncarriers. In contrast, we have not been able to find any association between the Leu72Met polymorphism and levels of serum lipoprotein (a). Recent studies have reported that plasma ghrelin concentrations are markedly elevated in cases of advanced renal failure and can be correlated with BMI, fat mass, plasma insulin, and serum leptin and creatinine levels [9,20]. These data suggest that preproghrelin Met72 carrier status generally may be beneficial for the maintenance of renal function and a predictable marker of diabetic nephropathy or renal impairment in patients with type 2 diabetes mellitus.

In conclusion, the Leu72Met polymorphism of the preproghrelin gene is not related with type 2 diabetes mellitus or with its complications. However, the Leu72Met polymorphism is clearly associated with serum creatinine level. The mechanism responsible for this phenomenon may relate to changes in ghrelin production and its endocrine and/or paracrine renal effects. However, further studies are required to elucidate the functional significance of the Leu72Met polymorphism in type 2 diabetes mellitus.

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