CLINICAL STUDY

Preproghrelin Leu72Met polymorphism predicts a lower rate of developing renal dysfunction in type 2 diabetic nephropathy

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Abstract

Objective: Ghrelin is a novel peptide hormone, which exerts somatotropic, orexigenic and adipogenic effects. Recent studies have shown that the preproghrelin Leu72Met polymorphism is associated with serum creatinine (Scr) concentration in type 2 diabetes; 72Met carriers exhibited lower Scr levels as compared with the 72Met non-carriers. We hypothesized that the preproghrelin Leu72Met polymorphism is associated with a lower rate of developing renal dysfunction in patients with type 2 diabetic nephropathy.

Design: The preproghrelin Leu72Met polymorphism was investigated using PCR techniques in 138 patients with diabetic nephropathy divided into two groups, one with normal renal function and the other with renal dysfunction.

Methods: Determination of the frequency of the preproghrelin Leu72Met polymorphism was the main outcome measure.

Results: The frequency of the Leu72Met polymorphism in diabetic nephropathy was significantly lower in patients with renal dysfunction (15.9%, \( P < 0.01 \)) than in patients with normal renal function (42.0%) or in the diabetes control group (40.6%). The Leu72Met polymorphism was also associated with serum total cholesterol levels in diabetic nephropathy patients with renal dysfunction; the 72Met carriers had lower total cholesterol levels than the 72Met non-carriers (\( P < 0.05 \)).

Conclusion: These data suggest that 72Met carrier status may be used as a marker predicting a lower chance of developing renal dysfunction in diabetic nephropathy.

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Introduction

Ghrelin is a novel endogenous ligand, which binds specifically to the growth hormone secretagog receptor (1). In addition to its demonstrated effects on the release of growth hormone (GH) from the pituitary gland, ghrelin plays a prominent role in the physiologic regulation of appetite and body weight (2). Although ghrelin has been reported to have many actions, its role in various renal diseases has not yet been well characterized. According to recent studies, ghrelin has been found to modulate glucose and insulin metabolism (3, 4). Also, markedly elevated plasma ghrelin levels are found in advanced renal failure and are correlated with fat mass, plasma insulin and serum leptin levels (5, 6).

Mutations in the ghrelin gene may potentially cause defects or inactivation of the ghrelin protein and also alter secretion of GHs and energy balance. One common preproghrelin Leu72Met polymorphism is associated with both obesity and glucose-induced insulin secretion (7, 8). Recent studies have shown that the preproghrelin Leu72Met polymorphism is associated with low serum creatinine (Scr) concentration in type 2 diabetes (9, 10).

In this study, we have examined the role of the preproghrelin Leu72Met polymorphism in the development of renal dysfunction in type 2 diabetes and its relationship to various physical/biochemical parameters.

Subjects and methods

Subjects

A total of 138 subjects with diabetic nephropathy were enrolled in this study. Type 2 diabetes was determined according to the WHO criteria (11), and diabetic nephropathy was diagnosed by the presence of overt proteinuria (random urinary protein/creatinine ratio > 500 mg/g creatinine or 24-h urinary protein > 500 mg) (12). Patients with diabetic nephropathy were divided into two groups on the basis of their Scr levels (Table 1). Group I (normal renal function group) consisted of 69 patients
(30 men, 39 women) with Scr levels < 1.5 mg/dl. Group II (renal dysfunction group) also consisted of 69 patients with Scr levels > 1.5 mg/dl, and 21 of them had hemodialysis. Both groups were comparable in age, sex and body mass index (BMI). Duration of illness is defined as the time elapsed from the onset of diabetes to the time of the study, while renal function remained normal (Scr < 1.5 mg/dl). Duration of illness in groups I and II was comparable when their creatinine levels were less than 1.5 mg/dl (11.4 ± 5.5 vs 12.0 ± 5.1 years). Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg (the average of two readings taken by the examining physician) or receiving medication for treatment of hypertension. The control group consisted of 69 diabetes patients without nephropathy matched for age, sex and BMI to the study groups. The control group was used to compare its preproghrelin Leu72Met genotype with that of the study groups I and II. A written informed consent was obtained from all subjects. Chonbuk National University Hospital Ethical Committee approved the protocols used for this study.

**DNA analyses**

Genomic DNA from leukocytes was prepared by standard techniques. The detection of the Leu72Met polymorphism using the PCR technique was performed as previously described (10). The forward and reverse primer sequences were as follows: 5'-agcagagaaag-0 and 5'-agaggtggcagtgaaca-3' respectively.

**Statistical methods**

All analyses were conducted using the SPSS Statistical Software Package (version 11.0; SPSS, Inc., Chicago, IL, USA), and all data are expressed as means ± S.D. Differences in physical/biochemical parameters between groups were assessed by Student’s t-test. Differences in the frequency of hypertension, retinopathy, anti-hypertensive agent use, and polymorphism between the groups were assessed using Chi-square tests. A P-value of < 0.05 was regarded as significant.

**Results**

Clinical characteristics of the study populations are shown in Table 1. The frequency of hypertension in the
diabetic nephropathy patients with normal renal function (group I) was 59.3%, whereas that of patients with the diabetic nephropathy with abnormal renal function (group II) was 90.5%. Scr and 24-h urinary protein levels were significantly higher in group II as compared with group I ($P<0.001$). In contrast, creatinine clearance was significantly lower in group II than in group I ($P<0.001$). Analyses of allele frequency of preproghrelin Leu72Met/Met72Met polymorphism revealed that there were no significant differences in the frequency of the polymorphism between the diabetes control group and diabetic nephropathy with normal renal function group (group I) (Table 2). However, the frequency of the Leu72Met polymorphism was significantly lower in the diabetic nephropathy with renal dysfunction group (group II) than in the diabetic nephropathy with normal renal function group (group I) (15.9 vs 42%, $P=0.001$).

To determine which of the given physical/biochemical parameters in 72Met carriers (Leu72Met + Met72Met genotypes) is different from that of 72Met non-carriers (Leu72Leu), we analyzed various parameters deemed to be affected in diabetic nephropathy with renal dysfunction (Table 3). There were no differences in physical parameters between the two groups, but one biochemical parameter, serum total cholesterol level, was significantly higher in the 72Met non-carriers ($175.5 \pm 49.2$ mg/dl, $P=0.022$) than in the 72Met carriers ($139.0 \pm 35.2$ mg/dl).

**Discussion**

In this study, we found that the frequency of the preproghrelin Leu72Met polymorphism was significantly lower in patients with diabetic nephropathy with renal dysfunction than in patients with normal renal function.

Ghrelin is a novel hormone that possesses GH-releasing, cardiovascular and metabolic activities (1). The acylation of the ghrelin peptide is a prerequisite for its biological activity; this occurs not only in the stomach (1), but also in the kidney (14). In addition, preproghrelin and ghrelin receptor genes are expressed in both the kidney and glomerulus of rodents (14). These findings indicate that ghrelin performs endocrine and/or paracrine functions in the kidney, which is one of the possible targets for direct ghrelin action. However, there is little information on the role of ghrelin in various renal diseases including diabetic nephropathy.

A common polymorphism at codon 72 of the preproghrelin gene (Leu72Met) is located outside the region where the mature ghrelin product is encoded (15). Although this polymorphism does not appear to induce any change in the sequence of the mature ghrelin, the resulting alterations in mRNA stability or protein processing may cause modified ghrelin secretion or activity as has been described for a number of other hormones and proteins (16, 17). Pöykö et al. (18) 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 (7) and reduction in glucose-induced insulin secretion (8). In our previous study (10), the frequency of the Leu72Met polymorphism was found to be similar in both the type 2 diabetes and the healthy control groups (34.5 vs 32.5%). However, the diabetic 72Met carriers had lower Scr levels than the diabetic 72Met non-carriers.

In this study, we have found that the frequency of preproghrelin Leu72Met polymorphism was significantly lower in patients with diabetic nephropathy with renal dysfunction compared with patients with normal renal function or the diabetes control group. In group II in this study, the Leu72Met polymorphism was associated with serum cholesterol level; the 72Met carriers had lower serum cholesterol levels compared with the 72Met non-carriers ($139.0 \pm 35.2$ vs $175.5 \pm 49.2$ mg/dl). These data may suggest that the preproghrelin 72Met carrier status is generally beneficial in maintaining normal renal function, and it may be a predictable marker for a lower rate of development of renal dysfunction in patients with diabetic nephropathy. There is a possibility that diabetes without nephropathy will develop into diabetic nephropathy in the future.

In conclusion, the Leu72Met polymorphism is associated with the development of renal dysfunction in diabetic nephropathy. Further studies are required to elucidate the mechanism responsible for this phenomenon and the functional significance of the Leu72Met polymorphism in type 2 diabetes.

**Table 2** Distribution of preproghrelin Leu72Met genotype in study subjects. Numbers in parentheses are percentages.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Wild (Leu72Leu)</th>
<th>Mutated</th>
<th>Total</th>
<th>Heterozygote (Leu72Met)</th>
<th>Homozygote (Met72Met)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes control ($n=69$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild (Leu72Leu)</td>
<td>41 (59.4)</td>
<td></td>
<td></td>
<td>28 (40.6)</td>
<td>26 (37.7)</td>
</tr>
<tr>
<td>Diabetic nephropathy, group I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Scr &lt; 1.5 mg/dl, $n=69$)</td>
<td>40 (58.0)</td>
<td></td>
<td></td>
<td>29 (42.0)</td>
<td>26 (37.7)</td>
</tr>
<tr>
<td>Diabetic nephropathy, group II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Scr &gt; 1.5 mg/dl, $n=69$)</td>
<td>58 (84.1)*</td>
<td>11 (15.9)*</td>
<td>10 (14.5)*</td>
<td></td>
<td></td>
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</table>

*P<0.01 compared with diabetes control or group I.
Table 3 Phenotypic characteristics of patients with diabetic nephropathy with renal dysfunction associated with genotype.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Wild (Leu72Leu)</th>
<th>Total</th>
<th>Leu72Met</th>
<th>Met72Met</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>58</td>
<td>62.6±6.4</td>
<td>61.9±6.3</td>
<td>69.0</td>
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<tr>
<td>Age (years)</td>
<td>61.1±10.8</td>
<td>22.0±4.8</td>
<td>22.1±5.1</td>
<td>21.0</td>
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<tr>
<td>Male sex (%)</td>
<td>27.58 (46.6)</td>
<td>11.8±5.1</td>
<td>12.7±5.7</td>
<td>12.1±5.6</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>192.9±80.3</td>
<td>117.8±50.0</td>
<td>122.3±50.8</td>
<td>77.0</td>
</tr>
<tr>
<td>Duration of illness (Scr &lt;1.5 mg/dl, years)</td>
<td>175.5±49.2</td>
<td>139±35.2</td>
<td>138.9±37.1</td>
<td>140.0</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>96.1±37.9</td>
<td>89.1±51.4</td>
<td>89.7±55.5</td>
<td>85.0</td>
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<tr>
<td>LDL (mg/dl)</td>
<td>38.7±23.0</td>
<td>35.5±11.4</td>
<td>35.8±12.0</td>
<td>33.0</td>
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<tr>
<td>HDL (mg/dl)</td>
<td>6.07±3.12</td>
<td>6.02±2.60</td>
<td>5.92±2.71</td>
<td>7.12</td>
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<tr>
<td>Total cholesterol (mg/dl)</td>
<td>183.9±84.9</td>
<td>199.7±35.5</td>
<td>199.7±35.5</td>
<td>198.0</td>
</tr>
<tr>
<td>eGFR (ml/min per 1.73m²)</td>
<td>9.9±4.0</td>
<td>7.9±1.8</td>
<td>8.1±1.8</td>
<td>6.1</td>
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<tr>
<td>FBG (mg/dl)</td>
<td>213.9±13.5</td>
<td>245.8±14.9</td>
<td>27.4±13.9</td>
<td>6.8</td>
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<tr>
<td>Medication of ACE inhibitor/ARB (%)</td>
<td>46.6</td>
<td>54.5</td>
<td>60.0</td>
<td>0</td>
</tr>
</tbody>
</table>

BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; Scr, serum creatinine; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker. *P<0.05 compared with wild genotype.

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References


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