OBJECTIVE — Recent studies have proved that blockade of the renin-angiotensin system (RAS) retards the progression of diabetic nephropathy, whereas hyporeninemia is known as a typical state in diabetic subjects. The purpose of this study is to determine whether expression levels of RAS differ between nondiabetic and diabetic renal tissues with accurate quantitative method.

RESEARCH DESIGN AND METHODS — Subjects were 66 nondiabetic and 8 diabetic patients with biopsy-proven renal diseases. The eight diabetic subjects suffered from type 2 diabetes with overt proteinuria. Renal histology revealed typical diffuse or nodular lesions with linear IgG deposit on immunofluorescent staining and thickened basement membrane on electronic microscopy. Total RNA from a small part of the renal cortical biopsy specimens was reverse-transcribed, and the resultant CDNA was amplified for new major components of RAS (i.e., renin, renin receptor, angiotensinogen, ACE, ACE2, angiotensin II type 1 receptor, and angiotensin II type 2 receptor) and measured.

RESULTS — Among these components, a significant upregulation was observed in the ACE gene in diabetic renal tissue.

CONCLUSIONS — The results suggest that renal tissue RAS might be activated in the respect that ACE gene expression is upregulated in spite of a tendency to low renin expression in type 2 diabetic nephropathy.

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RESEARCH DESIGN AND METHODS — Subjects were 66 nondiabetic and 8 diabetic patients with biopsy-proven renal diseases. The study was approved by the ethics committee of Fukui University (number 17-12), and consent was obtained from all individuals for inclusion onto the study. Salt intake was standardized to 10 g daily during hospitalization. The nondiabetic patients consisted of 8 with minor abnormalities, 8 benign nephrosclerosis, 38 primary glomerulonephritis including 4 minimal change nephrotic syndrome, and 12 lupus nephritis. Major clinical characteristics are listed in Table 1. Significant difference was observed in age, systolic blood pressure (sBP), and serum creatinine concentration between the two groups. The total patient numbers of administered depressors at renal biopsy were as follows: calcium channel block-
ers, five in nondiabetic subjects and four in diabetic subjects; α-blockers, zero in nondiabetic subjects and one in diabetic subjects; diuretics, eight in nondiabetic subjects and one in diabetic subjects; ACE inhibitors, one in nondiabetic subjects and zero in diabetic subjects; and ARBs, zero in nondiabetic subjects and zero in diabetic subjects. Administered ACE inhibitors and ARBs were replaced by calcium channel blockers or α-blockers before biopsy. Creatinine clearance (Ccr) was determined with serum creatinine concentration (s-Cr) and urinary creatinine concentration (u-Cr) and urinary creatinine (Cr) clearance (Ccr) before biopsy. Creatinine clearance (Ccr) was calculated for all genes and additional analysis was performed with the use of SPSS Version 11.0J (SPSS Japan). All data are expressed as means ± SD. Data for clinical characteristics were evaluated by ANOVA. Differences of gene expressions were calculated by ANCOVA with three covariance (age, sBP, and serum creatinine) for all genes and additionally with four covariance (age, sBP, serum creatinine, and proteinuria) for ACE, since ACE upregulation in the rat

### Table 1—Clinical characteristics of subjects at renal biopsy

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Type of diabetes</th>
<th>Duration of diabetes (years)</th>
<th>Treatment</th>
<th>A1C (%)</th>
<th>Renal histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>70</td>
<td>Type 2</td>
<td>7</td>
<td>Glibenclamide</td>
<td>7.3</td>
<td>Nodular</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>67</td>
<td>Type 2</td>
<td>23</td>
<td>Glibenclamide</td>
<td>8.2</td>
<td>Nodular</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>74</td>
<td>Type 2</td>
<td>19</td>
<td>Insulin</td>
<td>5.1</td>
<td>Nodular</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>32</td>
<td>Type 2</td>
<td>6</td>
<td>Diet therapy only</td>
<td>8.7</td>
<td>Nodular</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>64</td>
<td>Type 2</td>
<td>26</td>
<td>Insulin</td>
<td>7.2</td>
<td>Nodular</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>59</td>
<td>Type 2</td>
<td>25</td>
<td>Glibenclamide</td>
<td>7.4</td>
<td>Nodular</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>61</td>
<td>Type 2</td>
<td>6</td>
<td>Insulin</td>
<td>4.0</td>
<td>Nodular</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>55</td>
<td>Type 2</td>
<td>2</td>
<td>Diet therapy only</td>
<td>6.3</td>
<td>Diffuse</td>
</tr>
</tbody>
</table>
Gene
to GAPDH expression. No difference was observed between the expression levels of nondiabetic subjects (1.94 ± 2.83) and diabetic subjects (2.99 ± 2.36) (P = 0.75).

Renal tissue AT1 mRNA of nondiabetic and diabetic subjects
AT1 expression was measured at 10^−3 order to GAPDH expression. No difference was observed between the expression levels of nondiabetic subjects (2.75 ± 4.12) and diabetic subjects (2.50 ± 3.42) (P = 0.34).

CONCLUSIONS — The results of the study suggest the upregulation of the ACE gene in renal tissue of human diabetic nephropathy. For animal models, a considerable number of data have been accumulated, especially for the streptozotocin diabetes model. First, renal tissue angiotensin II concentration has been variously reported to be increased (19,20), to be comparable (21), and to be decreased (22) compared with nondiabetic kidney. With respect to the gene expressions of RAS in the animal model kidney, renin expression is reportedly increased at the beginning of the disease (19,23,24) but decreased at the late stage (20,23). Renal tissue AGT expression was reported to be comparable (20,23,25). Renal tissue ACE was reported to be comparable (20,23,25) and to be decreased (26). Renal tissue ACE2 was reported to be decreased (26). With regard to receptors, it was reported that nonglycosylated AT1 receptor protein expression was increased in isolated glomeruli in streptozotocin-induced diabetic rats with no change in mRNA (27), while reduced expression of the AT1 receptor in diabetic spontaneously hypertensive rats and no such reduction in AT1 expression was observed.

Table 3—Renal tissue mRNA levels of RAS

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nondiabetic subjects</th>
<th>Diabetic subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>REN (10^−3)</td>
<td>0.89 ± 2.12</td>
<td>0.60 ± 0.56</td>
</tr>
<tr>
<td>RER (10^−3)</td>
<td>2.32 ± 2.53</td>
<td>2.07 ± 2.42</td>
</tr>
<tr>
<td>AGT (10^−2)</td>
<td>6.00 ± 10.7</td>
<td>2.82 ± 2.57</td>
</tr>
<tr>
<td>ACE (10^−3)</td>
<td>2.66 ± 5.44</td>
<td>8.98 ± 14.7*</td>
</tr>
<tr>
<td>ACE2 (10^−2)</td>
<td>1.94 ± 2.83</td>
<td>2.99 ± 2.36</td>
</tr>
<tr>
<td>AT1 (10^−2)</td>
<td>3.54 ± 4.03</td>
<td>2.50 ± 2.11</td>
</tr>
<tr>
<td>AT2 (10^−4)</td>
<td>2.75 ± 4.12</td>
<td>2.50 ± 3.42</td>
</tr>
</tbody>
</table>

Data are means ± SD. *P < 0.05.
in diabetic Wistar Kyoto rats (28). Because the streptozotocin-induced diabetic animal is a model of type 1 diabetes, it is possible that the expression of genes differ from that in type 2 diabetes.

Compared with animal data, only a small number of studies have been conducted about the expression of renal tissue RAS on human specimens. At first, elevated angiotensin II immunohisto-staining was observed in tubular and infiltrating cells in diabetic human kidney (29). With regard to ACE, the immunostain was elevated in tubular cells and appeared in interstitial cells (29). Another immunohistochemical study indicated that ACE staining was significantly enhanced in glomeruli in diabetic patients (30). The former study also reported a downregulation of AT1 and upregulation of AT2 receptors (29). These assessments were based on non- or semiquantitative histochemical methods, making precise comparisons difficult. Only one quantitative assay was made for AT1 expression with competitive RT-PCR method, and the authors reported that AT1 receptor mRNA levels were significantly lower in eight samples from patients with diabetic nephropathy (31).

As described above, systematic quantitative assessment of gene expression of RAS in human diabetic nephropathy has not been performed. Therefore, we examined this issue for the first time and revealed the upregulation of the ACE gene in renal tissue of human diabetic nephropathy (i.e., in spite of the hyporeninemic state of the circulatory system, tissue RAS is activated). Accordingly, ACE inhibitors and ARBs might counteract this activation, thereby contributing to the favorable effects described in large-scale prospective studies (5–11). Alternatively, in the view of personal oriented medicine, our assessment might provide a new therapeutic approach based on renal tissue gene expression on renal diseases.

In summary, the gene expression of RAS, i.e., renin, renin receptor, AGT, ACE, ACE2, AT1, and AT2, was assayed with a very small quantity of human renal tissues of nondiabetic and diabetic subjects by quantitative methods. The results suggest that renal tissue RAS might be activated in the respect that ACE gene expression is upregulated in spite of a tendency to low renin expression in type 2 diabetic nephropathy. Further investigations including assessment of disease stage and severity might provide further insight into the role of RAS in human diabetic nephropathy.

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References


