Association of Glomerulopathy With the 5′-End Polymorphism of the Aldose Reductase Gene and Renal Insufficiency in Type 2 Diabetic Patients

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The expression of nephropathy in type 2 diabetes has several levels of abnormalities. To define the primary abnormalities of diabetic nephropathy, we conducted an autopsy study of 186 consecutive patients with type 2 diabetes to determine correlations among the aldose reductase gene, renal histopathologies, extracellular matrix, glomerular function, and clinical characteristics. Compared with cases of near-normal renal structure (n = 51) and atypical diabetic glomerulopathy (n = 75), patients with classic diabetic glomerulopathy (n = 60) had advanced glomerular disease, as reflected by elevated plasma creatinine levels (133.2 ± 59.8 vs. 166.0 ± 65.7 vs. 243.8 ± 82.6 μmol/l; P < 0.001), glomerular matrix fractions (20.8 ± 6.7 vs. 33.5 ± 16.8 vs. 39.2 ± 14.3%; P < 0.001), and risk of renal failure (odds ratio [OR] 1 vs. 3.5 vs. 21.4; P < 0.001). Compared with noncarriers of the aldose reductase z-2 allele (n = 92) and z-2 heterozygotes (n = 77), z-2 homozygotes (n = 17) had elevated plasma creatinine (164.1 ± 73.7 vs. 190.6 ± 60.9 vs. 241.1 ± 86.2 μmol/l; P < 0.001) and an increased risk of classic diabetic glomerulopathy (OR 1 vs. 0.9 vs. 3.3; P = 0.026). Overexpression of transforming growth factor-β1, mesangial cell transdiffentiation by expression of α-smooth muscle actin, and aberrant deposition of collagen type IV, fibronectin, and laminin were found in classic diabetic glomerulopathy. These data suggest genetic, biochemical, pathophysiological, and clinical correlations among the aldose reductase gene, extracellular matrix, classic diabetic glomerulopathy, and renal insufficiency. Gene mutation, cellular transdifferentiation, growth factor upregulation, extracellular matrix expansion, and glomerular filtration impairment are the primary abnormalities in type 2 diabetic patients with nephropathy. Diabetes 53:2984–2991, 2004

Diabetes, mostly type 2 diabetes, is the most common cause in developed countries of end-stage renal disease (ESRD) requiring either dialysis or renal transplantation (1). In Hong Kong, up to 50% of type 2 diabetic patients attending hospital medical clinics have nephropathy (2). Moreover, the incidence of type 2 diabetes and renal failure caused by type 2 diabetes is escalating worldwide (1,3). Compounding the global epidemic of renal failure in type 2 diabetes, the survival rate of patients with renal failure caused by type 2 diabetes is much worse than that of patients with renal failure resulting from type 1 diabetes or other causes (4).

Nephropathy and renal failure in type 2 diabetes have primary abnormalities at several levels. The functional abnormalities such as reduced glomerular filtration rate (GFR), elevated serum creatinine levels, and proteinuria are similar in type 1 and type 2 diabetes (5,6). However, the renal histopathologies in type 2 diabetic patients with nephropathy are heterogeneous (7–10). Classic diabetic glomerulopathy, characterized by a balanced severity of extracellular matrix expansion in glomerular, arteriolar, and tubulointerstitial compartments, has been found in ~33–66% of type 2 diabetic patients with microalbuminuria (7) or overt clinical nephropathy (9). A limited number of renal structure-function studies have shown poor correlation of glomerulopathy with serum creatinine and urinary protein excretion (7–9). Only type 2 diabetic patients with advanced classic glomerulopathy have structural-functional relations similar to that of type 1 diabetic patients (11).

The clinical and pathologic heterogeneity of nephropathy in type 2 diabetes may reflect the polygenic defects and associated heterogeneous cellular and growth pathways (12–14). Overexpression of transforming growth factor-β1 (TGF-β1) (15–18), immunophenotypic transformation of mesangial cells by expression of α-smooth muscle actin (α-SMA) (19–21), and subsequent abnormal deposition of extracellular matrix have been implicated in the signal transduction cascades of high glucose and angiotensin II in the pathogenesis of diabetic nephropathy in experimental studies (22,23); however, data from human studies are scarce (24–26). Furthermore, the heterogeneity of glomerulopathy in type 2 diabetes may also account for the conflicting data from gene marker studies. Some (27–29), but not all (30–34), studies have suggested that the 5′-
DNA extraction. Formalin-fixed, paraffin-embedded blocks of spleen tissue were cut at 4 μm, and four to six tissue sections were collected in an autoclaved plastic microtube (1.5 ml). Genomic DNA was extracted as previously described (30). After samples were incubated with 800 μl lysis buffer (protease K 20 mg/ml, 1 ml Tris-HCl solution 10 μl, 0.5 mg/ml EDTA 2 ml), overnight, two changes of 700 μl of freshly prepared phenol:chloroform at L1 were added to the supernatant fluid, after which further extraction and purification were performed using chlorormisonamyl alcohol (241). The upper aqueous supernatant was pipetted to a fresh microtube, mixed with 0.1 vol 3 mol/l sodium acetate (pH 5.2) and 2.5 vol 100% ethanol by vortexing, and incubated at −20°C for at least 30 min. The precipitated DNA was centrifuged at 12,000 rpm at 4°C for 20 min, then further centrifuged. The final extracted DNA was dissolved in Tris-HCL-EDTA buffer (pH 8.0) after being allowed to dry completely at room temperature.

Aldose reductase genotyping. The 5′-(CA)n dinucleotide repeat sequence −2.1 kb upstream of the transcription start site of the aldose reductase gene was genotyped using a LICOR DNA Analyzer (LI-COR, Lincoln, NE), as described by Wang et al. (29). The region containing the CA dinucleotide repeat was amplified with a pair of amplification primers that flanked a 128-bp region (sense strand sequence 5′-GAAGTCTTAACTGCTCTGAC-3′ and antisense strand sequence Arp2 5′-GCCGAGCCCTATACCAGT-3′). All alleles were sized by comparing them with a plasmid DNA containing a (CA)n repeat of the aldose reductase gene (29). Genotypes for the z-2 allele (136 bp) included z-2/z-2, z-2/z (x representing non−z−z allele), and x/x.

Stratifications and definitions. Patients were stratified into three groups based on aldose reductase genotypes (z-2/z-2, z-2/x, or x/x) or histopathologic patterns. The three histopathologic patterns included classic diabetic glomerulopathy, atypical diabetic glomerulopathy, and near-normal renal structure (Fig. 1) (7,10). Classic diabetic glomerulopathy included glomerular hypertrophy, diffuse and nodular glomerulosclerosis, and exudative lesions (arteriolar hyalinization, fibrinoid caps, and capsular drops) (8,10). All the lesions in classic diabetic glomerulopathy were PAS positive (Fig. 1A and B) (40). Notably, classic diabetic glomerulopathy was characterized by a roughly balanced severity of glomerular, tubulointerstitial, and arterial changes. In contrast, atypical diabetic glomerulopathy was characterized by relatively mild diabetic glomerulopathy but disproportionately severe ischemic glomerulosclerosis and chronic tubulointerstitial damages associated with marked arteriosclerosis (Fig. 1C) (8,10). Diffuse or nodular glomerulosclerosis was not evident in patients with atypical diabetic glomerulopathy (8,10). In this series, all the specimens were analyzed by two pathologists (H.-L.Z. and F.M.M.L.) who were blinded to the clinical characteristics and genetic analyses of the patients. The concordance on histopathologic classification was 96.3%. Based on the pathologic analysis, ~50% of the patients exhibited mild tubulointerstitial nephritis (41). No cases of other nondiabetic renal disease as defined by clinical characteristics, biochemical parameters, and nephropathology were found in this series.

Patients were considered to have been hypertensive if their blood pressure was ≥140/90 mmHg or they had received antihypertensive medications. Renal insufficiency was said to be present if plasma creatinine was ≥150−500 μmol/l. Renal failure was defined as the need for dialysis or if plasma creatinine was >500 μmol/l. In this study, ESRD was identical to "end stage kidney," as defined by microscopic histopathologies, including diffuse fibrotic cortex and interstitium, sclerotic glomeruli and arteries, atrophic tubules with pink casts, and scattered chronic inflammatory infiltrates (Fig. 1).

Statistics. Data are expressed as means ± SD or as percent. One-way ANOVA with Bonferroni’s post-test correction was used to compare means in three groups, and the association between sets of parametric data was evaluated using the Pearson correlation coefficient (SPSS 10.07 for Windows 2000; SPSS, Chicago, IL). Differences in frequencies and odds ratios (ORs) were assessed using the χ² test (GraphPad InStat version 3.00 for Windows; Graphpad Software, San Diego, CA). A two-tailed P value <0.05 was considered significant.

RESULTS
Clinical-pathologic correlation. Table 1 shows the clinical and histopathologic characteristics of the 186 Chinese patients with type 2 diabetes. Myocardial infarction and cerebral infarction/hemorrhage were confirmed in 51.6% and 28.0% of cases, respectively, by autopsy examination. Furthermore, classic diabetic glomerulopathy, ESRD, renal insufficiency, and clinical renal failure were found in 32.5 (60 of 186), 27.4 (51 of 186), 45.7 (85 of 186), and 6.5% (12 of 186) of cases. Patients with classic diabetic glomerulopathy had advanced renal disease and prevalent...
hypertension (Table 1). Patients with atypical diabetic glomerulopathy were older than those with near-normal renal structure \((P < 0.01)\).

Table 2 shows the association of renal histopathologies with plasma creatinine levels. Patients with classic diabetic glomerulopathy had increased risks of renal insufficiency \((OR 2.8, P = 0.0037)\), renal failure \((OR 12.4, P = 0.0003)\), and ESRD \((OR 4.4, P < 0.0001)\) compared with those without glomerulopathy. The risks of renal insufficiency and renal failure were increased in patients with atypical diabetic glomerulopathy compared with those with near-normal renal structure.

**Aldose reductase gene polymorphism.** We identified eight alleles \((z-6, z-4, z-2, z, z + 2, z + 4, z + 6, z + 8)\) and 23 genotypes of aldose reductase gene polymorphism. The frequencies of alleles \(z-2, z, z + 2\) were 29.6, 30.6, and 25.3% and of the genotypes \(z-2/z-2, z/z, z + 2/z + 2, 9.1, 9.1, 7.5\)%, respectively. The clinical characteristics of aldose reductase genotypes \(z-2/z-2\) (frequency 9.1%), \(z-2/x\) (40.9%), and \(x/x\) (50%) are shown in Table 3. Among the three groups of patients, carriers of the \(z-2/z-2\) genotype had the shortest known duration of diabetes and worst glomerular function (Table 3).

The frequency of the \(z-2\) allele was not significantly different in patients with plasma creatinine levels <100, 100–149, 150–500, and >500 \(\mu\text{mol/l}\) (25 vs. 30.9 vs. 30.8 vs. 37.5%; all \(P > 0.25)\). Renal failure occurred 1.5 times more often in patients carrying the \(z-2\) allele \((95\% \text{ confidence interval 0.6219–3.462, } P = 0.3652)\). Table 4 shows the correlation of the aldose reductase genotypes for the \(z-2\) allele with plasma creatinine levels. Patients homozygous for the \(z-2\) allele had an increased risk of renal insufficiency \((OR 3.2, P = 0.0405)\) and renal failure \((OR 3.8, P = 0.0833)\).

The frequency of the \(z-2\) allele was not significantly different among patients with classic diabetic glomerulopathy, atypical diabetic glomerulopathy, and near-normal renal structure \((35 \text{ vs. } 25.3 \text{ vs. } 30.4; \text{ all } P > 0.1)\). Carriers of the \(z-2\) allele had a moderately increased risk of classic diabetic glomerulopathy \((OR 1.4, P = 0.1465)\) and ESRD \((OR 1.3, P = 0.3760)\). Table 5 shows the correlation of the aldose genotypes for the \(z-2\) allele with glomerulopathy. Patients homozygous for the \(z-2\) allele had a significantly increased risk of classic diabetic glomerulopathy \((OR 3.4, P = 0.0261)\) and ESRD \((OR 4.5, P = 0.0075)\).

**Renal sclerosis.** Figure 2 illustrates the glomerular and renal matrix fractions relating to histopathologic patterns.
Data are percent (OR).

**Creatinine (µmol/l)**

<table>
<thead>
<tr>
<th>Creatinine (µmol/l)</th>
<th>Near-normal renal structure (n = 51)</th>
<th>Atypical diabetic glomerulopathy (n = 75)</th>
<th>Classic diabetic glomerulopathy (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;100 (n = 54)</td>
<td>70.6 (1)</td>
<td>21.3 (0.3)</td>
<td>3.3 (0.01)</td>
</tr>
<tr>
<td>100–149 (n = 47)</td>
<td>21.6 (1)</td>
<td>24 (3.7)</td>
<td>30 (29.5)</td>
</tr>
<tr>
<td>150–500 (n = 73)</td>
<td>7.8 (1)</td>
<td>52 (13.5)</td>
<td>50 (17.6)</td>
</tr>
<tr>
<td>&gt;500 (n = 12)</td>
<td>0 (1)</td>
<td>2.7 (3.5)</td>
<td>16.7 (21.4)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Data are percent (OR).
TABLE 3
Correlation of aldose reductase genotypes for the z-2 allele with clinical characteristics

<table>
<thead>
<tr>
<th>n</th>
<th>z-2/z-2</th>
<th>z-2/x</th>
<th>x/x</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>77</td>
<td></td>
<td></td>
<td></td>
<td>0.196</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67.8 ± 10.1</td>
<td>72.2 ± 10.2</td>
<td>69.8 ± 11.6</td>
<td></td>
</tr>
<tr>
<td>Male (%)</td>
<td>52.9</td>
<td>50.6</td>
<td>51.1</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.1 ± 4.4</td>
<td>23.9 ± 5.9</td>
<td>22.7 ± 3.6</td>
<td></td>
</tr>
<tr>
<td>Duration of diabetes (months)</td>
<td>96.0 ± 36.7 ±</td>
<td>171.1 ± 21.0 ±</td>
<td>127.9 ± 20.2</td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>11.0 ± 4.0</td>
<td>11.9 ± 5.0</td>
<td>11.6 ± 5.7</td>
<td></td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>8.3 ± 1.4</td>
<td>8.4 ± 1.8</td>
<td>9.1 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>144.7 ± 45.5</td>
<td>149.0 ± 27.2</td>
<td>141.4 ± 25.2</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79.0 ± 18.2</td>
<td>79.3 ± 12.3</td>
<td>77.1 ± 7.6</td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>58.8</td>
<td>63.6</td>
<td>56.5</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.1 ± 1.1</td>
<td>4.8 ± 0.9</td>
<td>4.8 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.8 ± 1.1</td>
<td>1.7 ± 0.6</td>
<td>1.8 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>20.5 ± 20.0</td>
<td>14.0 ± 10.8</td>
<td>14.0 ± 10.2</td>
<td></td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>241.1 ± 86.2 ±</td>
<td>190.6 ± 60.9 ±</td>
<td>164.1 ± 73.7</td>
<td></td>
</tr>
<tr>
<td>Creatinine clearance (ml·min⁻¹·1.73 m²⁻²)</td>
<td>34.6 ± 26.8</td>
<td>48.6 ± 17.6</td>
<td>57.9 ± 27.2</td>
<td></td>
</tr>
<tr>
<td>Glomerular filtration rate (ml·min⁻¹·1.73 m²⁻²)</td>
<td>27.2 ± 24.1</td>
<td>39.0 ± 15.2</td>
<td>41.5 ± 21.2</td>
<td></td>
</tr>
</tbody>
</table>

Data are percent or means ± SD. *P < 0.001, †P < 0.05, ‡P < 0.01 vs. x/x; †P < 0.01, ‡P < 0.05 vs. z-2/x.

However, there are limitations related to the use of serum creatinine as a marker of renal function (37,46). In addition to glomerular filtration, the level of serum creatinine is influenced by changes in muscle mass and dietary intake of protein, tubular secretion, and extrarenal elimination (47). Accordingly, patients with a reduced GFR may have normal serum creatinine (47). Thus, an elevation in serum creatinine is not a sensitive measurement of a decrease in glomerular function. Alternatively, the Cockcroft-Gault and Levey MDRD formulas have been recommended for calculating creatinine clearance and GFR (36,37). We observed that type 2 diabetic patients with classic diabetic glomerulopathy and the aldose reductase z-2/z-2 genotype had the worst calculated glomerular functions, whereas those with near-normal renal structure and carriers of the x/x genotype had well-preserved glomerular function (Tables 1 and 3). The calculated creatinine clearance and GFR were lower in diabetic patients with classic glomerulopathy compared with those with atypical glomerulopathy. However, the Cockcroft-Gault and Levey MDRD formulas have not been validated in the Chinese population. Moreover, the Cockcroft-Gault formula significantly underestimates GFR in type 2 diabetic patients with normal renal function (48). Therefore, a more accurate and precise method for assessment of GFR is warranted.

In this study of Chinese patients with type 2 diabetes, the aldose reductase z-2/z-2 genotype highly correlated with classic diabetic glomerulopathy, ESRD, an excess in

TABLE 5
Correlation of aldose reductase genotypes for the z-2 allele with diabetic glomerulopathy

<table>
<thead>
<tr>
<th>n</th>
<th>z-2/z-2</th>
<th>z-2/x</th>
<th>x/x</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>58.8 (3.3)</td>
<td>28.6 (0.9)</td>
<td>30.4 (1)</td>
</tr>
<tr>
<td>75</td>
<td>23.5 (0.7)</td>
<td>39.0 (0.7)</td>
<td>44.6 (1)</td>
</tr>
<tr>
<td>51</td>
<td>17.6 (0.6)</td>
<td>32.5 (1.4)</td>
<td>25 (1)</td>
</tr>
<tr>
<td>186</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Data are percent (mean OR relating to x/x genotype).

LAGEN type IV, fibronectin, and laminin was increased at the periphery of large nodular lesions and in the glomeruli with small nodular lesions, diffuse sclerosis, or hypertension. In contrast, the central region of large mesangial nodules in classic glomerulopathy and ischemic sclerosis in atypical glomerulopathy exhibited a diminished immunostaining for these three matrix components. In patients with atypical diabetic glomerulopathy and near-normal renal structure, immunoreactivity was predominantly localized in arterial walls and basement membranes.

**DISCUSSION**

We investigated the genetic, biochemical, pathophysiological, and clinical abnormalities of nephropathy in 186 consecutive autopsy cases with type 2 diabetes. We used serum creatinine, rather than the urinary albumin excretion rate, as the renal functional measurement. Serum creatinine is the most widely used measurement of the GFR in clinical practice. In a 10-year follow-up study of 416 Caucasian patients with type 2 diabetes and microalbuminuria (incipient nephropathy), serum creatinine significantly correlated with urinary albumin (43). In type 2 diabetic patients with proteinuria (overt nephropathy), serum creatinine, the urinary protein extraction rate, and the global score of tissue injury are the three independent predictors of renal disease progression (44). Moreover, an elevated serum creatinine is very specific for a reduced GFR (37). Therefore, an elevated serum creatinine and a doubling of the baseline serum creatinine have been used as end points in clinical trials (45).
renal matrix expansion, and elevated plasma creatinine levels. Patients carrying the aldose reductase z-2/z-2 genotype had a marked increase in risk for ESRD and classic diabetic glomerulopathy. Classic diabetic glomerulopathy, characterized by a balanced severity of glomerular, arteriolar, and tubulointerstitial damages (8–10), was associated with an excess of matrix deposition, adverse glomerular functions, and an advanced renal disease. In

![FIG. 2. Glomerular (□) and renal (■) matrix fractions relating to histopathologic patterns (A), ESRD (B), plasma creatinine (C), and aldose reductase genotypes for the z-2 allele (D).](image)

![FIG. 3. Immunoreactivity of TGF-β1 and α-SMA in classic diabetic glomerulopathy, ESRD, atypical diabetic glomerulopathy, and normal renal structure. *Arteriolar lumen; n, mesangial nodules; t, tubules. Immunostain, ×400.](image)
type 2 diabetes, only 33% of patients with microalbuminuria have classic diabetic glomerulopathy (7,8). The histopathologic heterogeneity of nephropathy in type 2 diabetes may account for the controversial findings regarding the 5′- (CA)_n polymerism of the aldose reductase gene (28,29,33). In type 1 diabetes, the structural basis for nephropathy is usually the classic diabetic glomerulopathy, as reflected by mesangial expansion and basement membrane thickening (49). Homozygosity for the z-2 allele in type 1 diabetes is associated with an increased expression of the aldose reductase gene and nephropathy (28). In type 2 diabetes, the z-2 allele is also associated with an increased aldose reductase activity and nephroretinopathy (50). In cultured mesangial cells, inhibition of aldose reductase has been shown to prevent glucose-induced increase in TGF-β activity and extracellular matrix accumulation (51,52). The present study showed that the excess of extracellular matrix accumulation in classic diabetic glomerulopathy is accompanied by overexpression of TGF-β1 and α-SMA. These results suggest major gene effects on cellular and growth pathways in diabetic nephropathy.

Diabetic nephropathy is a multifactorial and polygenic disease (12). Because of the genetic heterogeneity and multifactorial pathogenesis, no mutation has been identified that can explain the development of nephropathy in the majority of diabetic patients (13). In the present study, major gene effects were found in the z-2 homozygotes (n = 17), but not the z-2 heterozygotes (n = 77). On average, the z-2 heterozygotes had elevated plasma creatinine levels and an adverse calculated creatinine clearance. Despite having an advanced age and longer duration of known diabetes, patients heterozygous for the z-2 allele had a similar risk of elevated plasma creatinine compared with the noncarriers. This might be explained by interactions of gene-to-gene, gene-to-aging, and gene-to-environment factors that modulate the renal outcomes in diabetic patients (28).

In summary, classic diabetic glomerulopathy, extracellular matrix fractions, and the aldose reductase z-2/z-2 genotype are the primary abnormalities correlated with elevated plasma creatinine levels in Chinese patients with type 2 diabetes. Homozygosity for the z-2 allele may be an important risk factor for the development of classic diabetic glomerulopathy. Overexpression of TGF-β1 and α-SMA in renal cells may contribute to the aberrant deposition of extracellular matrix and consequently glomerulopathy in type 2 diabetes.

ACKNOWLEDGMENTS

This study was partly funded by the Graduate School and the Hong Kong Foundation for Research and Development of Diabetes, under the auspices of the Chinese University of Hong Kong.

We gratefully acknowledge the technical assistance of Dr. Xiao-Man Zou, Hai-Jing Song, Vincent K.L. Lam, and all the staff in the Lee Hysan Clinical Research Laboratories. We thank Dr. Ying Wang, Dr. Maggie C.Y. Ng, Prof. Ho-Keung Ng, and the late Prof. Julian A.J.H. Critchley for their support and advice.

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