Characterization of diabetic nephropathy in a transgenic model of hypoinsulinemic diabetes

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Am J Physiol Renal Physiol 291: F1315–F1322, 2006. First published May 16, 2006; doi:10.1152/ajprenal.00379.2005.—Genetic mouse models provide a unique opportunity to investigate gene function in the natural course of the disease. Although diabetic nephropathy (DN) in models of type II diabetes has been well characterized, diabetic renal disease in hypoinsulinemic diabetic mice is still incompletely understood. Here, we characterized renal changes in the pdx1/HNF6 transgenic mouse that exhibits β-cell dysfunction and nonobese hypoinsulinemic diabetes. Male transgenic mice developed hyperglycemia by the age of 7 wk and survived for over 1 yr without insulin treatment. Diabetes ensued earlier and progressed more severely in the HNF6 males than the females. The HNF6 males exhibited albuminuria as early as 10 wk of age, and the urinary albumin excretion increased with age, exceeding 150 μg/24 h at 11 mo of age. Diabetic males developed renal hypertrophy after 7 wk of age, whereas glomerular hypertrophy was not observed in the mice. Hypertension and hyperlipidemia were not observed in the diabetic mice. Histological analysis of the HNF6 kidneys displayed diabetic glomerular changes, including glomerular enlargement, diffuse mesangial proliferation and matrix expansion, thickened glomerular basement membrane, and arteriolar hyalinosis. Mesangial matrix accumulation increased with age, resulting in nodular lesions by 44 wk of age. Immunohistochemistry showed accumulation of type IV collagen and TGF-β1 in the mesangial area. No significant immune complex deposition was observed in the HNF6 glomeruli. Thus the HNF6 mouse exhibits diabetic renal changes that parallel the early phase of human DN. The model should facilitate studies of genetic and environmental factors that may affect DN in hypoinsulinemic diabetes.

With the development of unique genetic resources and the availability of technology to generate knockout and transgenic animals, the mouse has become a desirable species in medical research. In particular, a genetic mouse model of diabetes provides a unique opportunity to investigate gene function in the natural course of the disease. To date, DN has been characterized in several genetic models of murine diabetes (4, 5). These include the NOD mouse (a model of type I diabetes) and db/db and KKAy mice (models of type 2 diabetes). The well-studied db/db and KKAy mice exhibit albuminuria and substantial glomerular pathology, including mesangial matrix expansion and thickening of the glomerular capillary basement membrane. These two models of type 2 diabetes are widely used in DN research (6, 27). In contrast, very little work has been done to study diabetic complications in NOD mice due to the late age of onset of diabetes, requirement of insulin treatment, and the complex genetics (4). Thus diabetic renal disease in genetic mouse models of hypoinsulinemic diabetes is poorly characterized.

The pdx1/HNF6 mouse is a transgenic model of nonobese hypoinsulinemic diabetes (11). In this model, persistent expression of hepatocyte nuclear factor-factor-6 (HNF6) specifically in pancreatic islets causes disassembly of islet cells and β-cell dysfunction. The mouse shows diabetic symptoms soon after weaning due to impaired glucose-stimulated insulin secretion, resulting, in part, from downregulation of the glucose transporter GLUT2. There is no peripheral insulin resistance in the diabetic mouse. The diabetic phenotype most closely resembles maturity-onset diabetes of the young in humans, and the model provides a system for studying diabetic complications of prolonged and untreated hypoinsulinemic diabetes.

In the present study, we characterized functional and structural renal changes in diabetic pdx1/HNF6 mice. Our results demonstrate that pdx1/HNF6 male mice exhibit modest albuminuria and glomerular pathology that parallel the early phase of human DN, including glomerular hypertrophy, mesangial cell proliferation and matrix expansion, and thickening of the glomerular basement membrane (GBM). These mice represent a useful model for the study of genetic and environmental factors that may affect DN in hypoinsulinemic diabetes.

MATERIALS AND METHODS

Animals. pdx1/HNF6 transgenic mice on a B6D2F1 background (C57BL6 × DBA2 F1 hybrid) and their nontransgenic littermates were used (11). Mice were housed in microisolator cages in a natural course of the disease and the development of new diagnostic and therapeutic interventions.

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pathogen-free barrier facility and fed standard chow (laboratory diet no. 5015, PMI Nutrition International, Richmond, IN) and given water ad libitum. All animal experiments were conducted in accordance with the guidelines of Vanderbilt University Institutional Animal Care and Use Committee.

Blood parameter measurements. Blood samples were obtained by saphenous vein puncture under anesthesia or via cardiac puncture at death. The blood glucose level was measured using Accu-Chek test strips (Roche Applied Science, Indianapolis, IN). Blood urea nitrogen (BUN) was determined using the i-stat system (HESKA, Fort Collins, CO). Plasma creatinine level was determined by an HPLC-based method as previously described (8). For assessment of serum lipid levels, the mice were fasted for 6 h, and total cholesterol and triglycerides were measured by standard enzymatic assays using reagents from Raichem (San Diego, CA).

Urine albumin and creatinine measurement. Urine (24-h period) was collected from individually caged mice using polycarbonate metabolic cages (Tecniplast, Buguggiate, Italy). Spot urine was collected in the morning, and the albumin-to-creatinine ratio (ACR) was determined. Urinary albumin excretion was determined using an Albunet M Murine Microalbuminuria ELISA kit (Exocell, Philadelphia, PA). Urine creatinine was measured using a Creatinine Companion kit (Exocell).

Blood pressure measurement. Systolic blood pressure was measured in conscious, trained mice at room temperature using a tail-cuff monitor (model 65–12, IITC life Science).

Measurement of glomerular filtration rate. Glomerular filtration rate (GFR) was measured by the single-bolus FITC-inulin injection method as previously described (25). Briefly, 5% FITC-inulin (Sigma, St. Louis, MO) was dialyzed and injected (3.74 μl/g body wt) retroorbitally to mice under light anesthesia. Approximately 20 μl of blood were collected via the saphenous vein at 3, 7, 10, 15, 35, 55, and 75 min after injection, and the plasma concentrations of FITC-inulin were determined using a Fluoroscan Ascent FL (FIN-0081, Labsystems, Helsinki, Finland) at 485-nm excitation and 538-nm emission. GFR was calculated using the equation $GFR = \frac{I}{l(A/\alpha + B/\beta)}$, where $l$ is the amount of injected FITC-inulin, $A$ and $B$ are y-intercept values of the two decay rates, and $\alpha$ and $\beta$ are decay constants for the distribution and elimination phases (25).

Histological analysis. Renal histology was assessed in the $pdx1^{PB-}$ HNF6 males and nontransgenic littermates at 3, 5, 7, 10, 15, 20, and 44 wk of age. The mice were anesthetized, and the kidneys were perfused with PBS via the left ventricle of the heart, followed by incision of the renal vein. The perfused kidneys were removed, weighed, and fixed overnight in 4% paraformaldehyde at 4°C. The kidney tissues were embedded in paraffin using standard techniques, and 4-μm-thick sections were stained with periodic acid-Schiff (PAS). The sections were examined by light microscopy (Carl Zeiss, Axioskop) for renal histology. The glomerular cross-sectional area was measured using image-analysis software (Bioquant, Nashville, TN). A total of 50 glomeruli from the outer cortex/mouse were analyzed, and an average was calculated for each animal. Glomerular volume was calculated with the formula $GV = \beta/2(GA)^{3/2}$, where $\beta = 1.38$, the shape coefficient for a sphere, and $\kappa = 1.1$, the size distribution coefficient, and $GA$ is glomerular cross-sectional area (19, 24). The mesangial area in the glomerular tuft was defined as the area that stained positively for PAS, and the mesangial fraction in the glomerulus was calculated by point-counting as previously described (26). At least 4 mice/group were analyzed, and 30 glomeruli were measured for each animal.

For electron microscopy, kidneys were removed, cut into small tissue blocks (1 mm$^3$), and fixed in 2.5% glutaraldehyde fixative with 0.1 mol/l cacodylate buffer (pH 7.4) overnight at 4°C. After postfixation with 1% osmium tetroxide, tissues were dehydrated in a series of graded ethanol preparations and embedded in epoxy resin (Poly/Bed 812 Embedding Media, Polysciences, Warrington, PA). Ultrathin sections were stained with uranyl acetate and lead citrate. Sections were observed by transmission electron microscope (TEM; H-7000, Hitachi, Tokyo, Japan) at 75 kV.

![Fig. 1](http://ajprenal.physiology.org/) Development of diabetes in $pdx1^{PB-}$ HNF6 mice. (A) and body weight (B) were plotted against the number of weeks after birth. $\bullet$, $pdx1^{PB-}$ HNF6 males; $\bigcirc$, nontransgenic males; $\blacksquare$, $pdx1^{PB-}$ HNF6 females; $\triangle$, nontransgenic females. Values are means ± SE of at least 8 mice. Statistical analyses were performed by unpaired t-tests. *$P < 0.05$, **$P < 0.01$ vs. nontransgenic counterparts.
Table 1. Physiological parameters of 20-wk-old pdx1PB-HNF6 and nontransgenic males

<table>
<thead>
<tr>
<th></th>
<th>pdx1PB-HNF6</th>
<th>Nontransgenic</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>28.70±0.55</td>
<td>37.82±1.99</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Blood glucose, mg/dl</td>
<td>486.4±44.0</td>
<td>166.4±11.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>117.8±2.6</td>
<td>119.7±2.7</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma creatinine, mg/dl</td>
<td>0.068±0.006</td>
<td>0.068±0.003</td>
<td>NS</td>
</tr>
<tr>
<td>BUN, mg/dl</td>
<td>19.6±1.6</td>
<td>19.0±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>107.4±11.2</td>
<td>131.3±15.5</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>110.6±7.7</td>
<td>122.3±8.2</td>
<td>NS</td>
</tr>
<tr>
<td>Urine volume, ml/day</td>
<td>5.91±1.95</td>
<td>1.06±0.10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Urinary albumin/creatinine, μg/mg</td>
<td>83±6</td>
<td>50±7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>GFR, ml/min/g body</td>
<td>6.68±0.59</td>
<td>5.88±0.26</td>
<td>NS</td>
</tr>
<tr>
<td>Kidney wt, g</td>
<td>0.525±0.020</td>
<td>0.516±0.019</td>
<td>NS</td>
</tr>
<tr>
<td>Kidney wt-to-body wt ratio, g/g</td>
<td>1.86±0.07</td>
<td>1.32±0.05</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers of mice are in parentheses. GFR, glomerular filtration rate; BUN, blood urea nitrogen. P value was determined by unpaired t-test.

Immunohistochemistry. Immunohistochemistry of TGF-β1 or type IV collagen was performed as previously described (29). In brief, cryostat sections of 5-μm thickness were fixed in cold acetone for 10 min, washed with PBS, and endogenous avidin binding sites were blocked using a avidin/biotin blocking kit (Vector Laboratories, Burlingame, CA). After blocking with 5% normal goat serum, the sections were incubated with rabbit anti-TGF-β1 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) or rabbit anti-mouse type IV collagen antibody (Biodesign, Saco, ME) overnight at 4°C. Endogenous peroxidase was quenched with 3% hydrogen peroxide for 10 min, and sections were incubated with biotinylated goat anti-rabbit IgG antibody (Vector Laboratories) for 1 h at room temperature. Immunoreactivity was detected by the avidin/biotinylated enzyme complex method (Vector Laboratories) and visualized using 3,3-diaminobenzidine (Fast DAB tablets, Sigma). The sections were photographed by light microscopy (Carl Zeiss, Axioskop).

Statistics. Data are presented as means ± SE. An unpaired Student’s t-test was used to assess statistical significance. A P value <0.05 was considered to be significant.

RESULTS

Development of diabetes in pdx1PB-HNF6 mice. The β-cell dysfunction in pdx1PB-HNF6 mice has been previously described (11). In the present study, we first investigated the timing of development of diabetes in this model. As shown in Fig. 1A, the nonfasting blood glucose level in pdx1PB-HNF6 males was significantly higher than that in nontransgenic littermates at 3 wk of age, when the mice were weaned. The incidence of hyperglycemia (>300 mg/dl) in male mice was 10% (n = 20) at 3 wk of age, 50% (n = 20) at 5 wk of age, and 100% of pdx1PB-HNF6 males (n = 20) developed hyperglycemia by 7 wk of age. Although blood glucose levels continued to rise in male transgenics, exceeding 400 mg/dl at 15 wk of age, the mice were able to survive for over 1 yr without insulin treatment (n = 17). Diabetic male mice exhibited glycosuria (>250 mg/dl) and profound polydipsia/polyuria after 7 wk of age. In contrast to male transgenic mice, the elevation of blood glucose levels was transient in female transgenic mice and diabetic symptoms were relatively mild (Fig. 1A). The non-

Fig. 2. Renal changes in male pdx1PB-HNF6 mice. A: urinary albumin-to-creatinine ratio (ACR; left, n = 8/group) and urinary albumin excretion (UAE; right) were examined in pdx1PB-HNF6 and control males. B: glomerular filtration rate (GFR) in unanesthetized mice (n = 8/group) was determined by the clearance of FITC-inulin as described in MATERIALS AND METHODS. BW, body wt. C: kidney weight-to-body weight ratios were examined in pdx1PB-HNF6 control males (n = 8/group) at 3–20 wk of age. Values are means ± SE. Statistical analyses were performed by unpaired t-tests. *P < 0.05, **P < 0.01 vs. nontransgenic counterparts.
transgenic littermates exhibited normal glucose levels during the entire study period (from 3 to 44 wk of age). No hypertension or hyperlipidemia was observed in pdx1\(^{PB}\)-HNF6 males at 20 and 44 wk (data not shown) of age (Table 1). The lack of hyperlipidemia could be explained by preserved basal insulin levels and the absence of obesity in this model (11).

Since pdx1\(^{PB}\)-HNF6 males exhibited early hyperglycemia, we next examined the growth rate of diabetic mice. As shown in Fig. 1B, despite the early onset of diabetes, the growth rate of diabetic males was comparable to that of nontransgenic controls up to 10 wk of age. After this age, diabetic male mice showed no increase in body weight and the mean value of the body weight of the mice was 76% of nontransgenic males at 20 wk of age. Since an obvious loss of fat tissue was observed in 20- and 44-wk-old diabetic males, we assume that fat loss caused by catabolic effects of insulin deficiency may result in the weight loss in the mice. In contrast to transgenic males, pdx1\(^{PB}\)-HNF6 females showed slightly increased body weight after 10 wk of age compared with their nontransgenic littermates (Fig. 1B).

Renal changes in pdx1\(^{PB}\)-HNF6 male mice. The early-onset and overt diabetes in pdx1\(^{PB}\)-HNF6 males led us to investigate diabetic renal changes in these mice. To determine the onset of nephropathy, we first monitored urinary ACR. As shown in Fig. 2A, our data revealed an increase in spot ACR in transgenic males at as early as 10 wk of age. Consistent with these findings, the transgenic males showed significantly increased urinary albumin excretion (UAE) at 12 wk of age (data not shown), and the level increased as the mice aged, exceeding 150 μg/day at 44 wk of age (Fig. 2A). Because early diabetic renal change is characterized by hyperfiltration and hypertrophy in experimental models of diabetes (9), we next investigated GFR and renal hypertrophy in diabetic male mice. As shown in Fig. 2C, the kidney weight-to-body weight ratios in pdx1\(^{PB}\)-HNF6 males revealed significantly higher values after 7 wk of age compared with nontransgenic controls, whereas no

![Fig. 3. Histology of pdx1\(^{PB}\)-HNF6 glomeruli. A: representative light microscopic features of periodic acid-Schiff (PAS)-stained kidney sections from pdx1\(^{PB}\)-HNF6 (a–e) and nontransgenic (f) males. a: 5 wk of age. b: 10 wk of age. c: 20 wk of age. d–f: 44 wk of age. Note nodular mesangial expansion (arrow) in pdx1\(^{PB}\)-HNF6 glomerulus at 44 wk of age. Original magnification: ×400. B: glomerular volume (left) and mesangial matrix fraction (right) were measured in PAS-stained kidney sections from pdx1\(^{PB}\)-HNF6 and nontransgenic males as described in MATERIALS AND METHODS. Values are means ± SE of at least 5 mice/group. Statistical analyses were performed by unpaired t-tests. *P < 0.05, **P < 0.01 vs. nontransgenic counterparts.](http://ajprenal.physiology.org/)

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significant difference was observed in GFR between those two groups (Fig. 2B). The transgenic males showed no decline in GFR up to 44 wk of age (data not shown) and there was no significant difference in plasma creatinine levels between pdx1PB-HNF6 males and nontransgenic controls at 20 and 44 wk of age (Table 1 and data not shown). Although Ins2Akita and OVE26 diabetic mice have been shown to develop hydronephrosis with high frequency (14, 36), this occurred in <10% of pdx1PB-HNF6 diabetic mice.

**Histological changes in the pdx1PB-HNF6 kidney.** The glomeruli of diabetic males were assessed by light and electron microscopy (Fig. 3). As shown in Fig. 3A, moderate mesangial cell proliferation and matrix expansion, as evidenced by increased accumulation of PAS-positive material in the mesangial area, were observed in glomeruli of pdx1PB-HNF6 mice at 5 mo of age; mesangial expansion progressed as the mice aged. Nodular mesangial expansion (Fig. 3A, e) and arteriolar hyalinosis (not shown) were noted in pdx1PB-HNF6 glomeruli at 44 wk of age (Fig. 3A). Tubulointerstitial fibrosis was not observed in pdx1PB-HNF6 kidneys.

Quantitative examination of PAS-stained sections revealed significantly increased glomerular volume (after 10 wk of age) and mesangial matrix fraction (after 20 wk of age) in pdx1PB-HNF6 glomeruli compared with nontransgenic counterparts (Fig. 3B). There were no significant differences in glomerular number between pdx1PB-HNF6 males and nontransgenic controls (data not shown). In agreement with the observations by light microscopy, electron microscopic examination of pdx1PB-HNF6 glomeruli at 20 (data not shown) and 44 wk of age displayed expanded mesangial area accompanied by mesangial matrix accumulation and irregularly thickened GBM (20% increase in the thickness at 20 wk of age, 35% at 44 wk of age compared with nontransgenic controls) (Fig. 4). No electron-dense deposits were observed in pdx1PB-HNF6 glomeruli.

Last, immunohistochemical examination revealed accumulation of type IV collagen, TGF-β1, and laminin (data not shown) in the mesangial area of the pdx1PB-HNF6 mice, histological changes commonly observed in glomeruli in diabetes (Fig. 5). Control slides treated with nonspecific antisera showed no staining. There were no significant immunoglobulin deposits in the pdx1PB-HNF6 glomeruli, including IgA, IgG, and IgM compared with nontransgenic controls (data not shown).

**DISCUSSION**

In the present study, we characterized renal changes in the pdx1PB-HNF6 diabetic mouse. Male pdx1PB-HNF6 mice showed elevated blood glucose levels soon after weaning, and the mice became diabetic by 7 wk of age. Hyperglycemia was modest in female pdx1PB-HNF6 mice, and the blood glucose level remitted to a normal level by sexual maturation. Such a gender difference in susceptibility to diabetes has been observed in other rodent diabetic models (13, 35). It is known that estrogen and prolatin regulate ß-cell function and proliferation and have antidiabetic effects (17, 20). Furthermore, it has been suggested that androgens decrease insulin sensitivity by reducing adiponectin production (21, 33). Therefore, we assume that these ovarian hormones may block development of diabetes in pdx1PB-HNF6 females.

After the onset of diabetes, pdx1PB-HNF6 males developed albuminuria as early as 10 wk of age, and urinary albumin excretion increased as the mice aged. This was accompanied by renal/glomerular hypertrophy, mesangial matrix expansion, and GBM thickening. Human DN is known to progress through several pathophysiological stages, including early glomerular hyperfiltration and renal hypertrophy followed by dipstick-positive albuminuria and mesangial expansion, with a progressive decline in GFR and development of glomerulosclerosis (3, 32). The pdx1PB-HNF6 mouse displayed renal hypertrophy at 7 wk of age, before the development of albuminuria, and the glomerular lesion progressed to mesangial
matrix expansion and thickening of GBM without evident immune-complex deposition. Thus we suggest that renal disease in \( \texttt{pdx1}^{\text{PB}} \)-HNF6 males parallels the early phase of human DN. Although glomerular hyperfiltration is known to occur early after the onset of diabetes in rodents as well as in humans (22, 27, 31), preceding glomerular morphological changes, evident glomerular hyperfiltration was not observed in \( \texttt{pdx1}^{\text{PB}} \)-HNF6 diabetic mice. Diabetic hyperfiltration has been proposed to be caused by an increase in sodium reabsorption in proximal tubules and subsequent tubuloglomerular feedback through the macula densa as well as by extracellular volume expansion (30). Furthermore, many humoral and vascular mediators have been implicated in diabetic hyperfiltration, including insulin-like growth factor, prostaglandins and kinins, nitric oxide, angiotensin II, and atrial natriuretic peptide (30). Since diabetes develops at an early age in the \( \texttt{pdx1}^{\text{PB}} \)-HNF6 mouse, it is probable that developmental alterations of these factors and rapid tissue growth may antagonize or mask diabetic hyperfiltration in this mouse. In fact, the \( \texttt{pdx1}^{\text{PB}} \)-HNF6 mice exhibited higher values of GFR than nontransgenic controls after 10 wk of age, and similar results were demonstrated for the OVE26 insulinopenic diabetic mice (36).

As summarized in Table 2, several mouse models of hypoinsulinemic diabetes have been recently reported. Although the onset and the severity of diabetes in these models are similar, the models demonstrate distinct renal phenotypes. For instance, the \( \texttt{Ins2Akita} \) diabetic mouse develops hyperglycemia at \( \approx 7 \) wk of age; however, an increase in urinary albumin excretion is not observed in the mice at \( 20 \) wk of age (10). Similarly, albuminuria is indistinguishable in iNOS-Tg mice at \( 4 \) mo of age, whereas the mice develop overt diabetes at \( \approx 4 \) wk of age (23, 34). In contrast, \( \texttt{pdx1}^{\text{PB}} \)-HNF6 and ICER I \( \texttt{I}^{\text{C57BL6}} \) mice at \( 20 \) wk of age exhibited definitely higher ACR than those models (15). Among the reported models, the OVE26 model showed the most severe renal phenotype, including decline in GFR and glomerulosclerosis (36). The occurrence of hydronephrosis was noted in this model; however, its contribution to renal dysfunction has not been determined. It is noteworthy that the kidney size prominently increases in OVE26 mice from 2 to 3 mo of age, and the glomerular volume is twice that of nontransgenic controls, exceeding \( 600 \times 10^3 \times \)

<table>
<thead>
<tr>
<th>Mouse Model (Strain)</th>
<th>Gene</th>
<th>Onset of DM</th>
<th>Renal Disorders</th>
<th>Renal Pathology</th>
<th>Reference No (s).</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \texttt{pdx1}^{\text{PB}} )-HNF6 (C57BL6 \times DBA2)</td>
<td>HNF6</td>
<td>5–7w ( (M&gt;F) )</td>
<td>Albuminuria ( (10w) )</td>
<td>Mesangial expansion ( (15–20w) )</td>
<td>11</td>
</tr>
<tr>
<td>( \texttt{Ins2 Akita} ) (C57BL6, C3H)</td>
<td>Islet overexpression</td>
<td>7–10w ( (M&gt;F) )</td>
<td>( \text{Cr}_{\text{T}} \downarrow ) ( (20–40w) )</td>
<td>GFR ( \downarrow ) (9M)</td>
<td>10, 14, 35</td>
</tr>
<tr>
<td>ICER I ( \texttt{I}^{\text{C57BL6}} )</td>
<td>Inducible cAMP early repressor ( \beta\text{-cell overexpression} )</td>
<td>2w</td>
<td>Albuminuria ( (20w) )</td>
<td>Mesangial expansion ( (20w) )</td>
<td>15</td>
</tr>
<tr>
<td>OVE26 (FVB)</td>
<td>Calmodulin ( \beta\text{-cell overexpression} )</td>
<td>2–3w</td>
<td>Albuminuria ( (2M) )</td>
<td>Mesangial expansion ( (2.5–10M) )</td>
<td>36</td>
</tr>
<tr>
<td>iNOS-Tg (CD1)</td>
<td>iNOS ( \beta\text{-cell overexpression} )</td>
<td>1–3w</td>
<td>Albuminuria ?</td>
<td>Diffuse/nodular lesion</td>
<td>23, 34</td>
</tr>
</tbody>
</table>

DM, diabetes mellitus; M, males; F, females; w, weeks; M, months; GBM, glomerular basement membrane; CCr, creatinine clearance; GFR, glomerular filtration rate; iNOS, inducible nitric oxide synthase.
μm³, at 6 mo of age. Such a rapid and marked renal enlargement is not observed in other models, including the pdx1\(^{PB}\)-HNF6 mouse. Growing evidence suggests a scenario in which the added susceptibility to progressive diabetic renal disease is due to genetic factors independent of diabetes in mice as well as in humans (4, 5, 12). Since the above mouse models were generated using different mouse strains, distinct genetic backgrounds may explain the diversity of renal phenotype among the models. Indeed, backcrossing the pdx1\(^{PB}\)-HNF6 mouse onto the C57BL/6 strain reduced urinary albumin excretion to the level of Ins2\(^{Akita}\) mice (data not shown). Comparison of the models with the same genetic background and standardized protocols would be required to characterize the renal phenotype in each model.

Mice provide an experimental platform of unparalleled power to investigate the molecular pathways underlying mammalian diseases. To date streptozotocin (STZ) treatment has been widely used for the study of DN in hypoinsulinemic diabetes. Although STZ-treated animals develop DN on various backgrounds, chemical induction causes some diversity among individual animals in terms of the severity and the onset of diabetes (36). In addition, nonspecific tissue toxicity of STZ complicates the interpretation of the results (16). In this context, a genetic mouse model provides a desirable system for investigating the pathogenesis of DN. The pdx1\(^{PB}\)-HNF6 model is considered to be a suitable model of DN in several aspects. First, the renal changes in the diabetic mice closely mirror the early phase of human DN. The complication of IgA nephropathy, as reported for Ins2\(^{Akita}\) diabetic mice (14) and NOD mice (7), is not observed in pdx1\(^{PB}\)-HNF6 diabetic mice. In addition, hydrenephrosis is less prominent in pdx1\(^{PB}\)-HNF6 mice compared with Akita and OVE26 mice (35, 36). Second, the pdx1\(^{PB}\)-HNF6 mouse survives over 1 yr without insulin treatment. Its survival rate (94% at 44 wk of age, n = 17) is definitely higher than that of Akita diabetic mice (40% at 45 wk of age) (35), although the exact reason behind this difference remains unknown. Since the pdx1\(^{PB}\)-HNF6 mouse showed a similar survival rate (95% at 44 wk of age, n = 20) on the C57BL/6 background, it is unlikely that the higher survival rate of the pdx1\(^{PB}\)-HNF6 mouse is due to the C57BL/6xDBA2 F1 hybrid strain. Finally, stable induction of diabetes and DN on C57BL/6xDBA2 F1 and C57BL6 (data not shown) backgrounds may provide a suitable system for studying gene function in DN using knockout or transgenic mice. Numerous knockout and transgenic mice are currently available on these backgrounds. Thus the pdx1\(^{PB}\)-HNF6 model provides a system for studying diabetic complications of prolonged and untreated hypoinsulinemic diabetes. The model should facilitate studies on the genetic and environmental factors that affect development and progression of DN in hypoinsulinemic diabetes.

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