Influence of genetic background on albuminuria and kidney injury in Ins2+/C96Y (Akita) mice

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Gurley SB, Mach CL, Stegbauer J, Yang J, Snow KP, Hu A, Meyer TW, Coffman TM. Influence of genetic background on albuminuria and kidney injury in Ins2+/C96Y (Akita) mice. Am J Physiol Renal Physiol 298: F788–F795, 2010. First published December 30, 2009; doi:10.1152/ajprenal.90515.2008.—Previous studies have shown that Akita mice bearing the Ins2+/C96Y mutation have significant advantages as a type I diabetes platform for developing models of diabetic nephropathy (DN; Gurley SB, Clare SE, Snow KP, Hu A, Meyer TW, Coffman TM, Am J Physiol Renal Physiol 290: F214–F222, 2006). In view of the critical role for genetic factors in determining susceptibility to DN in humans, we investigated the role of genetic background on kidney injury in Akita mice. To generate a series of inbred Akita mouse lines, we back-crossed the Ins2+/C96Y mutation more than six generations onto the 129/SvEv and DBA/2 backgrounds and compared the extent of hyperglycemia and renal disease with the standard C57BL/6-Ins2+/C96Y line. Male mice from all three Akita strains developed marked and equivalent hyperglycemia. However, there were significant differences in the level of albuminuria among the lines with a hierarchy of DBA/2 > 129/SvEv > C57BL/6. Renal and glomerular hypertrophy was seen in all of the lines, but significant increases in mesangial matrix compared with baseline nondiabetic controls were observed only in the 129 and C57BL/6 backgrounds. In Fl(DBA/2 x C57BL/6)-Ins2+/C96Y mice, the extent of albuminuria was similar to the parental DBA/2-Ins2+/C96Y line; they also developed marked hyperfiltration. These studies identify strong effects of genetic background to modify the renal phenotype associated with the Ins2+/C96Y mutation. Identification of these naturally occurring strain differences should prove useful for nephropathy modeling and may be exploited to allow identification of novel susceptibility alleles for albuminuria in diabetes.

DIABETIC NEPHROPATHY is the most common cause of end-stage renal disease (ESRD) in the United States (26) with >40% of incident ESRD patients having diabetes (26). Animal models of diabetic nephropathy that more closely recapitulate the features of human disease would be useful tools in understanding the pathogenesis and developing new treatment strategies for this devastating disorder. Because of the potential for genetic manipulation, a model of diabetic nephropathy in the mouse would be particularly attractive. Yet, while progress has been made, there are still no mouse models that reproduce the pathological and functional features of human diabetic nephropathy (2b). As a platform for developing models of diabetic complications, the Akita mouse line shows promise (5). These mice have a single nucleotide substitution in the Ins2 gene (Ins2+/C96Y), originally identified as a spontaneous mutation in a colony of C57BL/6 mice in Akita, Japan (30). The mutation leads to improper folding of the insulin protein, causing proteotoxicity to the pancreatic β cell. Heterozygosity for the mutation in male mice is associated with marked hyperglycemia, and homozygosity leads to perinatal lethality.

In previous studies, we compared the characteristics of C57BL/6-Ins2+/C96Y mice with C57BL/6 mice treated with streptozotocin (STZ) (5). Relative to STZ-treated animals, the C57BL/6-Ins2+/C96Y mice developed more marked hyperglycemia and elevated blood pressure. C57BL/6-Ins2+/C96Y mice also had higher levels of albuminuria and more consistent renal pathological changes. Thus the C57BL/6-Ins2Akita model appeared to have some advantages over the STZ model of chemically induced diabetes as a platform for developing models of diabetic nephropathy (5). Nonetheless, even the C57BL/6-Ins2+/C96Y mice develop only modest levels of proteinuria, and renal pathological changes are limited to mesangial expansion.

A key contribution of genetic factors to the development of diabetic nephropathy in humans is well established. For example, within the population of patients with diabetes followed longitudinally for as long as 30 years, only a subset develops nephropathy (12). Within this group, the incidence of clinical nephropathy peaks between 10 and 20 years of diabetes and then declines with time, consistent with a defined population of susceptible subjects. Furthermore, the risk of overt nephropathy is significantly greater in certain ethnic groups including African-Americans and Native Americans and in patients with siblings or parents with diabetic nephropathy (17, 23, 25). Thus it seems very likely that genetic factors play a major role in determining susceptibility, as well as resistance, to kidney disease in diabetes. Specific genes determining susceptibility to diabetic nephropathy in humans have not been identified, but several large-scale human studies have been established with the goal of identifying genes associated with the development of diabetic nephropathy (16, 31). Genetic determinants also seem to modulate the susceptibility to kidney injury from diabetes in mice. For example, we and others have identified significant strain differences affecting the severity of proteinuria and renal pathological changes in mice with chemically induced (STZ) diabetes (5, 18). Accordingly, we reasoned that genetic background might also influence the development of kidney injury in Akita mice. Identification of strains with enhanced susceptibility to kidney injury in diabetes should be useful for developing better mouse models of nephropathy. Moreover, these strains could be used to identify specific gene variants accelerating proteinuria and kidney injury in this setting.

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Here, we provide evidence for genetic modifiers of albuminuria and other renal manifestations of diabetes in Akita mice. The C57BL/6 background confers resistance whereas the 129/SvEv and DBA/2 backgrounds are associated with enhanced susceptibility to kidney injury. Animals from an F1 intercross generated between the resistant strain and one of the susceptible strains (DBA/2) predominantly expressed the susceptible phenotype, consistent with dominant inheritance of genetic modifiers that increase the propensity for albuminuria in this model of type I diabetes.

MATERIALS AND METHODS

Animals. C57BL/6-Ins2+/C96Y (Akita) and wild-type DBA/2(J) mice were purchased from The Jackson Laboratory (Bar Harbor, ME), and wild-type 129/SvEv mice from Taconic Farms (Germantown, NY). Inbred DBA/2-Ins2+/Ins2 and 129SvEv-Ins2+/Ins2 mice were generated by successive backcrossing of the Akita mutation onto wild-type DBA/2 and 129/SvEv mice, respectively. For each of the strains, experiments were performed using mice generated after six successive backcrosses. Transmission of the C96Y point mutation was determined by PCR analysis of genomic DNA obtained from weaning mice. Mice were housed in an Association for the Assessment and Accreditation of Laboratory Animal Care-accredited animal facility at the Durham Veterans Affairs Medical Center under National Institutes of Health guidelines. Throughout the period of study, animals were provided free access to water and standard rodent chow (Lab Diet, Purina Mills, St. Louis, MO). Because of the limited hyperglycemia observed in diabetic, female mice, only male mice were studied.

Blood glucose measurements. Beginning at 10 wk of age, blood glucose was measured at monthly intervals using a Glucometer Elite testing system (Ascensia Bayer). After the tarsal area of the left leg was shaved, blood samples were obtained by puncturing the left lateral saphenous vein with a 25-gauge needle. Approximately 2 μl of blood was collected directly onto the testing strip for measurement.

Blood pressure measurements. During the last month of the study, systolic blood pressures were measured in conscious mice using a computerized tail-cuff system (Hatteras Instruments, Cary, NC) that determines systolic blood pressure using a photoelectric sensor as described (10, 11). This system allows measurements in four mice simultaneously and minimizes the potential for observer bias. Before measurements were initiated, mice were adapted to the apparatus for at least 10 days. Blood pressures were then measured for at least 15 days. The validity of this system has been established previously (10, 11).

Urinary albumin measurements. At the end of the study, 24-h urine collections were obtained from mice housed in individual metabolic cages with free access to water and food. Water intake, urine volume, and body weight were monitored to ensure basal conditions. Urinary albumin excretion was measured using an indirect competitive ELISA according to the manufacturer’s instructions (Albuwell M, Exocell). Urinary albumin excretion was measured using an indirect competitive ELISA according to the manufacturer’s instructions (Albuwell M, Exocell).

Measurement of glomerular filtration rate. FITC-inulin clearance was measured in conscious mice as previously described (19). FITC-inulin (5%) was injected into the penile vein of mice that were briefly anesthetized with isoflurane. At 3, 7, 10, 15, 35, 55, and 75 min after the FITC-inulin injection, blood samples were obtained from the lateral saphenous vein (8). Fluorescence intensity was measured at 485 excitation/520 emission using a FLUOstar Omega plate reader (BMG Labtech). Plasma fluorescence was fitted to a two-phase exponential decay using nonlinear regression (GraphPad Prism), and glomerular filtration rate (GFR) was calculated as using standard formulas (18) and is reported as microliters per minute per gram body weight (7).

Renal pathological examination. At 6 mo of age, kidneys were harvested for pathological examination. Before harvest, a cannula was placed in the left ventricle and the kidneys were perfused in situ with freshly prepared 4% paraformaldehyde (PFA) using a peristaltic pump (Peristaltic Pump P-1, Amersham Biosciences) at a rate of 7 ml/min. The right kidney was then removed, decapsulated, and weighed. Perfusion-fixed tissue was embedded in paraffin, and sections were stained with periodic acid-Schiff for examination by light microscopy. Glomerular volume was calculated from the mean cross-sectional area of 20 glomerular profiles of each animal using the method of Weibel (29). Glomerular mesangial area was scored from 0 to 3 on 25–40 glomerular profiles from a single section, and the mean was entered as the score for the animal.

Statistical analysis. The values for each parameter within a group are expressed as means ± SE. For comparisons between two experimental groups, an unpaired t-test was used to assess statistical significance, and a paired t-test was used for comparisons within a group. For comparisons among multiple groups, ANOVA with Tukey’s multiple comparisons test was used.

RESULTS

Equivalent hyperglycemia in Akita mouse lines. As shown in Fig. 1, blood glucose levels were significantly elevated in Ins2+/C96Y mice on all three genetic backgrounds compared with their respective wild-type (Ins2+/+) controls. The marked levels of hyperglycemia were sustained from the beginning of the experiment when the animals were 8 wk old, through the entire 16-wk observation period. During the study period, there were no statistically significant differences in blood glucose levels among the inbred diabetic strains (P = 0.59 ANOVA + Tukey’s).

Throughout the entire study period, both C57BL/6-Ins2+/C96Y and 129/SvEv-Ins2+/C96Y mice appeared healthy and gained weight normally such that their body weights remained similar to nondiabetic wild-type littermate controls. In contrast, the DBA/2-Ins2+/C96Y animals failed to gain weight and their mean body weights at the end of the study (22.9 ± 1.5 g) were not significantly different from the values at 2 mo of age when the study was initiated (23.9 ± 0.5 g; P = 0.8). At the end of the study, body weights in the DBA/2-Ins2+/C96Y mice (22.9 ± 1.5 g) were significantly lower than DBA/2-Ins2+/+ nondiabetic controls (30.1 ± 0.8 g; P = 0.0003). As the study progressed, many of the DBA/2-Ins2+/C96Y animals appeared ill and >50%
had to be euthanized before completion of the study because of failure to thrive.

**Blood pressures in inbred Akita mice.** Since hypertension is a typical clinical feature associated with diabetic nephropathy in humans, we monitored systolic blood pressure in all of the groups of mice in this study. In the nondiabetic controls, we found significant strain differences in baseline blood pressures (Table 1). As shown in Fig. 2, systolic blood pressures were higher in both the nondiabetic 129/SvEv-Ins2<sup>+/−</sup> (130 ± 4 mmHg) and DBA/2-Ins2<sup>+/−</sup> animals (134 ± 2 mmHg) compared with the C57BL/6-Ins2<sup>+/−</sup> group (103 ± 2 mmHg; P < 0.01, ANOVA). The blood pressure response to diabetes also varied among strains. Systolic blood pressures were significantly higher in the C57BL/6-Ins2<sup>+/C96Y</sup> (112 ± 4 vs. 103 ± 2 mmHg; P = 0.04) and 129/SvEv-Ins2<sup>+/C96Y</sup> groups (143 ± 3 vs. 130 ± 4 mmHg; P = 0.03) compared with their respective Ins2<sup>+/−</sup> wild-type littersmates. In contrast, blood pressures were virtually identical in 6-mo-old DBA/2-Ins2<sup>+/C96Y</sup> and DBA/2-Ins2<sup>+/−</sup> wild-type controls (134 ± 3 vs. 134 ± 2 mmHg; P = 0.89).

**Differences in albuminuria among strains of inbred Akita mice.** We measured 24-h albumin excretion and albumin-to-creatinine ratio as a marker of renal injury associated with diabetes. Among the nondiabetic wild-type (Ins2<sup>+/−</sup>) mice, there were strain differences in albumin excretion. Wild-type DBA/2 mice had significantly higher levels of albumin excretion (116 ± 28 μg/day) than wild-type C57BL/6 animals (14 ± 2 μg/day; P = 0.009) (Fig. 3), whereas albumin excretion rates in wild-type C57BL/6 and 129/SvEv mice (33 ± 20 μg/day; P = NS) were not significantly different. On each of the three genetic backgrounds, the presence of the Ins2<sup>+/C96Y</sup> mutation was associated with significant increases in albumin excretion. Compared with their respective nondiabetic controls, albuminuria was increased by 2.8-fold in C57BL/6-Ins2<sup>+/C96Y</sup> mice (P < 0.0001), 3-fold the DBA/2-Ins2<sup>+/C96Y</sup> group (P = 0.048), and ~5-fold in 129/SvEv-Ins2<sup>+/C96Y</sup> animals (P = 0.03). Among the three Akita strains, absolute levels of albumin excretion were highest in the DBA/2-Ins2<sup>+/C96Y</sup> mice (345 ± 110 μg/day), intermediate in 129/SvEv-Ins2<sup>+/C96Y</sup> animals (151 ± 77 μg/day), and lowest in the C57BL/6 and Ins2<sup>+/C96Y</sup> group (40 ± 3 μg/day). These general patterns were also observed with albumin-to-creatinine ratios (Fig. 3B). Thus, while the presence of the Akita mutation was associated with increased albumin excretion in each of the lines, strain-specific genetic modifiers influence the magnitude of albuminuria in this model of diabetes.

**GFR in Akita strains.** To determine whether there were strain differences in the effect of diabetes on renal function, we measured GFR in the C57BL/6 and 129/SvEv strains as described (19). Compared with the nondiabetic, wild-type mice, GFR was significantly higher in the 129/SvEv-Ins2<sup>+/−</sup>-Ins2<sup>+/+</sup> (18.2 ± 1.7 ml/min−1·g body wt−1) than the C57BL/6 mice (11.3 ± 1.4 ml/min−1·g body wt−1; P = 0.04). The levels of GFR increased significantly in Akita mice on both backgrounds compared with their respective nondiabetic, wild-type controls (Fig. 4, Table 2). Between the two strains of Akita mice, GFR was significantly higher in the 129-Ins2<sup>+/−</sup>/C96Y compared with the C57BL/6-Ins2<sup>+/−</sup> mice (P = 0.0004). Thus, at similar levels of hyperglycemia, the magnitude of hyperfiltration differs between strains of Akita mice and higher levels of GFR are associated with more severe albuminuria.

**Effects of Ins2<sup>+/C96Y</sup> mutation on kidney size and pathology.** Renal hypertrophy is a characteristic feature of human diabetics. Thus we compared the kidney sizes among the different strains of Akita mice. Interestingly, there were strain differences in kidney size among the 6-mo-old nondiabetic mice. The mean kidney weight-to-body weight ratio in the DBA/2-Ins2<sup>+/−</sup> mice (9.63 ± 0.16) was significantly greater than that of the C57BL/6-Ins2<sup>+/−</sup> (5.95 ± 0.1) or 129/SvEv animals (6.06 ± 0.1; P < 0.001 ANOVA). Compared with the kidney sizes in their respective wild-type control strains, all of the Ins2<sup>+/C96Y</sup> lines manifested significant renal hypertrophy by 6 mo of age, and among the diabetic animals, the kidney weight-to-body weight ratio was also highest in the DBA/2-Ins2<sup>+/C96Y</sup> group (P < 0.001, ANOVA, Table 3).

Along with the generalized increased kidney size seen with diabetes, increased glomerular volume is also typical of humans with diabetic nephropathy (3, 13). Among the Ins2<sup>+/−</sup> controls, the glomerular volumes were significantly increased in the DBA/2 line compared with the other two strains (P < 0.001, ANOVA), and glomerular volumes were significantly higher in C57BL/6 wild-type mice compared with 129/SvEv (P < 0.001, ANOVA). The glomerular volumes were significantly higher in both the C57BL/6- and 129/SvEv-Ins2<sup>+/C96Y</sup> groups with diabetes compared with their respective nondiabetic controls (P < 0.0002). However, there was no difference in the glomerular volume between the DBA/2-Ins2<sup>+/−</sup>/C96Y and DBA/2-Ins2<sup>+/−</sup> groups and, thus, despite the strain differences at baseline, glomerular volumes were very similar among the three diabetic lines at 6 mo of age (Table 4).

In general, the Ins2<sup>+/C96Y</sup> mutation caused only modest abnormalities of renal structure, largely confined to expansion

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**Table 1. Systolic blood pressure in inbred mouse lines**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Control</th>
<th>Diabetic</th>
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<tbody>
<tr>
<td>C57BL/6</td>
<td>103 ± 2 (n = 7)</td>
<td>112 ± 4* (n = 6)</td>
</tr>
<tr>
<td>129/SvEv</td>
<td>130 ± 4 (n = 7)</td>
<td>143 ± 3* (n = 5)</td>
</tr>
<tr>
<td>DBA/2</td>
<td>134 ± 2 (n = 10)</td>
<td>134 ± 3 (n = 6)</td>
</tr>
<tr>
<td>F1 (D2:B6)</td>
<td>127 ± 34 (n = 20)</td>
<td>138 ± 5* (n = 20)</td>
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</table>

Values are means ± SE, n. No. of mice. *P < 0.04 between diabetic and wild-type. †P = 0.06 between diabetic and wild-type. ‡P < 0.01 compared with 129/SvEv and DBA/2 wild-type.
of mesangial matrix, and the patterns of mesangial pathology were similar between the groups. As shown in Fig. 5, pathological changes were largely confined to the glomerulus in diabetic animals with preservation of normal morphology in the tubular and interstitial regions. To compare the severity of glomerular pathology between the Akita lines, the extent of mesangial expansion was scored on a scale of 0–3 as described previously (5). Representative glomeruli with severity scores of 0 (normal), 1 (mild), and 2 (moderate) are depicted in Fig. 5. Severely affected glomeruli (score of 3) were rarely observed. There were no significant differences in the mesangial pathology scores among the wild-type Ins2+/+ animals from the three strains (Table 4, Fig. 5, A and C). However, mesangial pathology scores were significantly higher in diabetic C57BL/6-Ins2+/+ mice compared with their respective nondiabetic littermate controls (Table 4). The magnitude of increase was very similar between these two strains (2- to 2.5-fold). On the other hand, mesangial scores were virtually identical in 6-mo-old wild-type DBA/2 (Ins2+/+) and DBA/2-Ins2+/+ mice. Accordingly, Akita mice on the C57BL/6 and 129/SvEv backgrounds had more severe mesangial expansion than the DBA/2-Ins2+/+ mice (P < 0.001, ANOVA). Of note, several of the DBA/2-Ins2+/+ animals developed focal area interstitial inflammation consisting of substantial numbers of polymorphonuclear neutrophils along with other mononuclear inflammatory cells with an overall appearance suggestive of pyelonephritis and were excluded from analysis.

**Fig. 4.** Glomerular filtration rate (GFR) in 6-mo-old male mice. GFR was measured in C57BL/6, 129/SvEv, and F1(DBA/2 x C57BL/6) wild-type and Akita (Ins2+/+ mice). GFR was higher in wild-type 129/SvEv group compared with wild-type C57BL/6 and wild-type F1(DBA/2 x C57BL/6) mice (†P < 0.04). Also, diabetic 129/SvEv-Ins2+/+ mice had higher GFR levels compared with C57BL/6-Ins2+/+ mice (‡P = 0.0004). The levels of GFR were significantly higher in all groups of diabetic Akita mice compared with their respective wild-type controls (⁎P < 0.05).
Like the other \(\text{Ins}^{2+/\text{C589}}\) strains, \(\text{F}1(\text{DBA}/2 \times \text{C57BL}/6)\text{Ins}^{2+/\text{C589}}\) mice also developed significant hyperfiltration with diabetes and the absolute levels of GFR were similar to the \(129\text{-Ins}^{2+/\text{C589}}\) group and higher than the \(\text{C57BL}/6\text{-Ins}^{2+/\text{C589}}\) mice. Moreover, the relative change in GFR in the \(\text{F}1(\text{DBA}/2 \times \text{C57BL}/6)\text{Ins}^{2+/\text{C589}}\) mice compared with their wild-type controls (250% was greater than the 129 (130%) and \(\text{C57BL}/6\) (140%) groups. Among the three strains, the relative increases in GFR parallel their susceptibilities to proteinuria (\(\text{F}1 > 129 > \text{C57BL}/6\)).

Similar to the other \(\text{Ins}^{2+/\text{C589}}\) strains, \(\text{F}1(\text{DBA}/2 \times \text{C57BL}/6)\text{Ins}^{2+/\text{C589}}\) mice developed significant renal hypertrophy compared with nondiabetic \(\text{F}1(\text{DBA}/2 \times \text{C57BL}/6)\text{Ins}^{2+/+}\) controls (kidney weight-to-body weight ratio: 12.4 ± 0.3 vs. 6.9 ± 0.2; \(P < 0.0001\)). The extent of their hypertrophy was similar to the \(\text{DBA}/2\text{-Ins}^{2+/+}\) parental line and greater than the \(\text{C57BL}/6\text{-Ins}^{2+/+}\) line (Table 3). Among the nondiabetic \(\text{Ins}^{2+/+}\) lines, glomerular volumes in the \(\text{F}1\text{-Ins}^{2+/+}\) group were similar to the levels seen in the two parental lines, \(\text{C57BL}/6\) and \(\text{DBA}/2\) (Table 4, Fig. 6C). With diabetes, the glomerular volumes increased further in the \(\text{F}1(\text{DBA}/2 \times \text{C57BL}/6)\text{-Ins}^{2+/+}\) animals to 0.38 ± 0.01 (Table 4), also similar to increases in the two parental lines.

Mesangial scores in the nondiabetic \(\text{F}1(\text{DBA}/2 \times \text{C57BL}/6)\text{-Ins}^{2+/+}\) animals (Table 4) were virtually identical to the relatively high basal levels seen in wild-type \(\text{DBA}/2\) mice (0.51 ± 0.05 vs. 0.56 ± 0.09; \(P = 0.142\), ANOVA). As discussed above, there was no further increase in the severity of mesangial pathology in the \(\text{DBA}/2\text{-Ins}^{2+/+}\) line compared with the baseline levels seen in the \(\text{DBA}/2\text{-Ins}^{2+/+}\) controls. By contrast, mesangial scores were significantly higher in the \(\text{F}1(\text{DBA}/2 \times \text{C57BL}/6)\text{-Ins}^{2+/+}\) mice than their nondiabetic littermate controls (0.91 ± 0.06 vs. 0.51 ± 0.05; \(P < 0.001\)), such that the extent of mesangial pathology in the \(\text{F}1\) mice was very similar to that seen in the parental \(\text{C57BL}/6\text{-Ins}^{2+/+}\) line (0.94 ± 0.06) (Fig. 6D, Table 4). As with the other lines, this corresponds to a relatively modest degree of mesangial expansion. Interestingly, several of the \(\text{F}1(\text{DBA}/2 \times \text{C57BL}/6)\text{-Ins}^{2+/+}\) mice developed the pathological picture of pyelonephritis that was observed in the parental \(\text{DBA}/2\text{-Ins}^{2+/+}\) line.

**DISCUSSION**

In humans with diabetic nephropathy, control of blood pressure and blockade of the renin-angiotensin system can slow progression of renal disease. However, these maneuvers only delay inexorable progression of kidney injury, and new approaches to therapy are needed (6). Development of animal models recapitulating the features of diabetic nephropathy in humans could be very useful for identifying new therapeutic targets in this area. Such models might also have value for understanding the early phases of diabetic kidney disease where relatively modest increases in albuminuria predict poor cardiovascular outcomes (1, 28). The mouse is particularly attractive for this sort of human disease modeling because of its tractability for genetic manipulation. While current mouse models of diabetes recapitulate features of the early phases of diabetic kidney disease including microalbuminuria and mesangial expansion, progress toward mouse models with pathological and functional characteristics of later stages of human diabetic nephropathy has been limited (2).

In previous studies comparing type 1 diabetes models, we found that the Akita (\(\text{Ins}^{2+/\text{C589}}\)) mouse has some advantages as a platform for complication modeling compared with chemically induced diabetes with STZ (5). These included sustained and robust hyperglycemia, enhanced levels of albuminuria, more consistent mesangial pathology, and simple genetics wherein a single copy of the mutant allele generates profound diabetes. However, in our studies carried out with the original line of \(\text{C57BL}/6\text{-Ins}^{2+/\text{C589}}\) mice from Jackson Laboratories, the extent of proteinuria and mesangial pathology was relatively modest (5). As we and others have shown that genetic background has a strong influence on the characteristics of kidney injury associated with STZ-induced diabetes (5, 18), we considered the possibility that genetic background might have

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**Table 2. Urine studies and glomerular filtration rate measurements**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Albumin Excretion, µg/day</th>
<th>Glomerular Filtration Rate, µl/min/g body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Diabetic</td>
</tr>
<tr>
<td>C57BL/6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>129/SvEv</td>
<td>33.1 ± 20.4</td>
<td>151.3 ± 77.9*</td>
</tr>
<tr>
<td>DBA/2</td>
<td>116.2 ± 37</td>
<td>344.7 ± 109*</td>
</tr>
<tr>
<td>F1(D2:B6)</td>
<td>43.1 ± 9.7</td>
<td>239.7 ± 54.0*</td>
</tr>
<tr>
<td>Values are means ± SE. *P &lt; 0.05 between diabetic and wild-type. †P &lt; 0.03 between 129 wild-type vs. C57BL/6 wild-type and F1(D2:B6) wild-type. ‡P &lt; 0.0001 ANOVA between 129/SvEv-Ins2+/C589 and C57BL/6-Ins2+/C589.</td>
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**Table 3. Kidney weights**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Left Kidney Weight, mg</th>
<th>Kidney Weight-to-Body Weight Ratio, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Diabetic</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>162.2 ± 3.5 (n = 7)</td>
<td>217.4 ± 4.3* (n = 6)</td>
</tr>
<tr>
<td>129/SvEv</td>
<td>150.7 ± 5.1 (n = 7)</td>
<td>258.4 ± 12.7* (n = 5)</td>
</tr>
<tr>
<td>DBA/2</td>
<td>292.5 ± 9.9† (n = 10)</td>
<td>297.8 ± 13.2Δ (n = 6)</td>
</tr>
<tr>
<td>F1(D2:B6)</td>
<td>267 ± 9.8 (n = 20)</td>
<td>378 ± 12.9* (n = 22)</td>
</tr>
<tr>
<td>Values are means ± SE. n, No. of mice. *P &lt; 0.001 between diabetic and wild-type. †P &lt; 0.001 vs. other strains. ‡P = 0.002 vs. C57BL/6</td>
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</tbody>
</table>
similar effects in the \textit{Ins2}/\textit{C96Y} model. Accordingly, we generated novel lines of inbred \textit{Ins2}/\textit{C96Y} mice by successive generations of back-crossing. Since our objective was to produce Akita mouse lines with enhanced renal injury, we chose the DBA/2 strain, based on its enhanced susceptibility for albuminuria in the STZ model (5, 18), and the 129/SvEv strain, which has been demonstrated to have enhanced propensity for kidney injury in nondiabetic models (9, 21).

We carried out extensive phenotyping of the inbred \textit{Ins2}/\textit{C96Y} lines with a primary focus on albuminuria, an early clinical indicator of renal involvement in humans with diabetes (15) where it is also a potent marker of increased cardiovascular risk (4, 22). Even among the nondiabetic wild-type control groups, we found significant strain differences in albuminuria, with the highest levels observed in the DBA/2 strain. This is consistent with a recent study that also found increased albuminuria in wild-type DBA/2 mice and mapped several, interacting quantitative trait loci (QTL) influencing this trait to mouse chromosome 2 (24). One of these loci overlaps a QTL for albuminuria previously identified in rats (33) and humans (34). We find that exaggerated albuminuria in the wild-type, nondiabetic DBA/2 mice was associated with increased kidney size and higher glomerular volumes, but no evidence of structural changes in the kidney. Because of the loss of some the DBA/2 Akita cohort before the study was completed, it is possible that our data might reflect selection against more severely affected diabetic mice. However, we were able to collect urine for albumin measurements in all but one mouse in this group. Furthermore, even with this possible skewing, the levels of albuminuria in the DBA/2-\textit{Ins2}/\textit{C96Y} animals were significantly greater than in the C57BL/6 or 129 strains. Moreover, a similar phenotype was observed in the F1(DBA/2 x C57BL/6)-\textit{Ins2}/\textit{C96Y} animals, which were harder and survived the entire study period.

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The \textit{Ins2}/\textit{C96Y} mutation caused robust and equivalent levels of hyperglycemia in each of the strains. However, the manifestations of diabetes in the kidney were quite variable, suggesting differences in genetic susceptibilities to diabetes-induced renal injury. While albumin excretion was increased in each of the three \textit{Ins2}/\textit{C96Y} strains compared with their respective wild-type controls, levels of albuminuria were significantly higher in the 129/SvEv-\textit{Ins2}/\textit{C96Y} and DBA/2-\textit{Ins2}/\textit{C96Y} lines compared with the C57BL/6 \textit{Ins2}/\textit{C96Y} animals. Similar to our previous study using the STZ model of diabetes, the highest levels of albumin excretion were observed with \textit{Ins2}/\textit{C96Y} mutation on the DBA/2 background. However, because of the high

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mesangial Score</th>
<th>Glomerular Volume</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Diabetic</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>0.37 ± 0.04</td>
<td>0.94 ± 0.06*</td>
</tr>
<tr>
<td>129/SvEv</td>
<td>0.48 ± 0.07</td>
<td>1.05 ± 0.06*</td>
</tr>
<tr>
<td>DBA/2</td>
<td>0.56 ± 0.09</td>
<td>0.56 ± 0.06</td>
</tr>
<tr>
<td>F1(D2:B6)</td>
<td>0.51 ± 0.05</td>
<td>0.91 ± 0.06*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.0002 between diabetic and wild-type.
baseline level of albuminuria in the DBA/2-Ins2+/− controls, the relative change in albuminuria was only about threefold in the DBA/2-Ins2+/−/C96Y line compared with almost fivefold in the 129/SvEv-Ins2+/−/C96Y group.

We also observed strain-related differences in GFR, both in the wild-type animals and with diabetes. Wild-type 129 mice had a higher GFR than wild-type C57BL/6 male mice of the same age (Fig. 4). In the presence of diabetes, hyperfiltration had a higher GFR than wild-type C57BL/6 male mice of the parental DBA/2 line but modestly increased compared with the C57BL/6 line (†P = 0.003, ANOVA). F1 animals developed a >6-fold increase in albuminuria compared with their littermate Ins2+/− controls. This level of albuminuria was significantly higher than in the C57BL/6-Ins2+/−/C96Y animals (†P = 0.02), but was not different from the DBA/2-Ins2+/−/C96Y line. C: F1(DBA/2 x C57BL/6)-Ins2+/−/C96Y mice developed significant glomerular hypertrophy compared with nondiabetic F1 controls. The extent of their hypertrophy was similar to the DBA/2-Ins2+/−/C96Y parental line and greater than the C57BL/6-Ins2+/−/C96Y line. D: like the C57BL/6-Ins2+/−/C96Y line, F1(DBA/2 x C57BL/6)-Ins2+/−/C96Y mice also developed significant mesangial expansion compared with wild-type littermates whereas the parental DBA/2-Ins2+/−/H11001 mice did not. *P < 0.05 between Akita (black bars) and wild-type (gray bars).

In summary, we have generated novel lines of inbred mice and have demonstrated strong genetic modifiers influencing albuminuria and renal injury. These strains could be used to map these naturally occurring modifiers of renal disease in diabetes.
We suggest that the 129/SvEv-Ins2+/C96Y line may be particularly useful for further model development, especially in combination with other genetic manipulations carried out in embryonic stem cells derived from 129 substrains that may accelerate or exaggerate the development of glomerulosclerosis and renal failure in diabetes.

GRANTS
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DISCLOSURES
No conflicts of interest are declared by the authors.

REFERENCES