Deficiency in Six2 during prenatal development is associated with reduced nephron number, chronic renal failure, and hypertension in Br/+ adult mice

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Abstract

Using a mouse model carrying a targeted mutation in Six2 (Br), we characterized the development and renal morphology and function in the adult. Adult Br/+ mice suffer from CRF associated with reduced nephron number and renal hypoplasia, as well as glomerulosclerosis. Electron microscopy of the Br/+ glomerulus revealed pathological alterations such as hypercellularity, extracellular matrix accumulation, and a thick irregular glomerular basement membrane. These results indicate that adult Br/+ mice suffer from CRF associated with reduced nephron number and renal hypoplasia, as well as glomerulosclerosis. Defects are associated with embryological deficiencies of Six2, suggesting that proper levels of this protein during nephrogenesis are critical for normal glomerular development and adult renal function.

Chronic renal failure (CRF) is a disease affecting an estimated 4.5% of adults in the United States with a high associated mortality, largely due to the subsequent development of cardiovascular disease (1, 78, 81). A leading cause of CRF in childhood is bilateral renal hypoplasia, which is characterized by abnormally small and dysplastic kidneys resulting from abnormal morphogenesis (37, 80). Typically, the hypoplastic kidney is associated with CRF since the nephrons do not differentiate properly and the renal tubules progressively dis- tend while the interstitial architecture becomes disrupted. The kidney eventually fails since it becomes unable to excrete wastes, retain electrolytes, and concentrate urine. During this process, alterations in sodium and water reabsorption can cause the formation of renal cysts with secondary effects on the cardiovascular system resulting in systemic hypertension. Although numerous molecules are involved in the development of CRF, specific cellular and genetic defects causing renal hypoplasia remain poorly understood.

Reduced renal mass models, usually produced by surgical removal of one kidney and two-thirds of the remaining kidney, have long been used for studying the consequences of CRF. Typically performed in rats due to their larger size, relatively few reduced renal mass mice models have been developed to analyze renal failure as a consequence of decreased numbers of nephrons (79, 92, 100). The identification of a mouse model with heritable renal hypoplasia and low numbers of nephrons would be useful to identify specific morphogenetic pathways responsible for the progression of CRF and the cardiovascular complications.

We described a mouse mutant, called Brachyrrhine (Br), which arose from X-ray irradiation of the 3H1 strain and carries a semidominant mutation that results in renal hypoplasia and frontonasal dysplasia (35, 59–63, 68). The homozygous mutation is embryonic lethal, whereas the heterozygote mutant survives to adulthood and shows small kidneys and severe midfacial micrognathia. Analysis of Br/Br developing kidneys demonstrated the successful induction of the ureteric bud and initial metanephric mesenchymal condensation at embryonic (E) day 11.5, but showed severe disruption of nephrogenesis shortly afterward, with generalized glomerulopathy, large renal cysts, and the absence of a discernable renal cortex (60). We successfully mapped the Br mutation to a critical region containing only one gene, the Six2 homeobox transcription factor (35). During embryonic development of the Br/Br mouse, Six2 expression was shown to be almost completely absent from where it is normally expressed (35), which is primarily in the mesenchymal cells of the kidney and midface (75). In the same developing tissues of the heterozygous mutant mouse (Br+/+), Six2 expression was measured at ~50% of the levels measured in wild-type tissues (35). In a previous study, we noted that embryonic Br/+ kidneys were significantly smaller than wild-type, with a smaller nephrogenic zone and abnormal surface arteries (62). In addition, we observed distended collecting tubules in neonatal Br/+ kidneys, indicating possible cyst development. Recently, a Six2 knockout mouse was created and demonstrated a similar phe-
notype, with the homozygous null kidney showing severely disrupted nephrogenesis due to excessive mesenchimal-to-epithelial differentiation and subsequent depletion of the metanephric mesenchymal progenitor cells (87); however, the heterozygous Six2-null mouse was not described.

In this study, we analyze the morphology and physiological functioning of the hypoplastic Br/ + adult kidney and test the hypothesis that adult Br/ + mice display physiological features consistent with CRF. We collected large numbers of adult kidneys to compare gross, histological, and ultrastructural characteristics of Br/ + mutant kidneys with normal kidneys, and also undertook a stereological approach to measure total kidney volumes and quantify the number of glomeruli. Cardiorenal and metabolic physiological parameters were measured, and immunostaining of proteins critical to renal function was performed on Br/ + and normal kidneys. Real-time quantitative PCR confirmed Six2 is not normally expressed in the adult kidney, but only in the kidney during fetal development. Data from this study support the hypothesis that Six2 plays a critical role in the development of the kidney and that renal function is greatly compromised in the adult mouse when Six2 expression is downregulated prenatally.

**MATERIALS AND METHODS**

**Animal breeding.** All experiments were conducted using inbred 3H1 (C3H/He × 101/H) wild-type (+/+) and heterozygous mutant (Br/+) mice. All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee in accordance with local and federal standards. Breeding of 3H1 Br/ + and +/+ mice was carried out in the Animal Laboratory Service facility at University of Hawaii. Adult mice were housed under standard conditions with 12-h light cycle and they were supplied with tap water and food pellets (Agway ProLab Feed, Waverly, NY) ad libitum.

**Real-time quantitative PCR.** Kidneys from embryos at E13.5, newborn, and adult mice were dissected out, minced, and immediately submerged in RNA-later (Sigma, St. Louis, MO) and stored at 4°C for up to 1 wk before processing. Kidneys were collected from three mice of each age and genotype. Total RNA was extracted from the tissues with a RNeasy kit (Qiagen) using a mortar and pestle to disrupt the tissue. Total RNA was reverse transcribed with iScript cDNA synthesis kit (Bio-Rad, Hercules, CA), and 200 ng of the resulting cDNA samples were used as template for quantitative (q)PCR by using the iQ SYBR Green Supermix reaction procedure with the MxyQ iCycler thermal cycler and single-color real-time PCR detection system (Bio-Rad). All reactions were run in triplicate to minimize experimental error. Primer sets specific for Six2 (forward) 5’-GCC TGC GAG CAC CTC CAC AAG AAT-3’, (reverse) 5’-CAC CGA CTT GCC ACT GCC ATT GAG-3’ and actin (forward) 5’-CAA TGA GCT GCG TGT GG-3’, (reverse) 5’-CAA CAC AGC CTG GAT GG-3’ were used in qPCR reactions. The PCR condition consisted of an initial 5-min denaturation at 94°C, followed by reactions cycled through denaturation for 15 s at 94°C, annealing for 20 s at 60°C, and extension for 40 s at 72°C. After 50 cycles of amplification, the PCR products were resolved on an agarose gel to confirm that a single band of predicted size was amplified. The expression levels of Six2 in each kidney sample were calibrated against actin expression, with the average relative ratio set to 1, as described previously (2, 35, 66). The amount of Six2 mRNA from the newborn and adult kidneys was compared with the normalized amounts from the E13.5 kidneys, and Six2 mRNA levels from Br/ + mutant kidneys were compared with wild-type levels in E13.5 kidneys.

**Verification of renal hypoplasia in adult Br/ + mice.** Mean renal mass was determined in three groups of randomly selected adult animals: 3H1 stock mice, 3H1 +/+ mice, and 3H1 Br/ + mutant mice. The 3H1 +/+ mice were siblings of Br/ + mice and raised in the same litters. Mice were identified based on morphological phenotype (62) and weighed using precision standard TS 1205 electronic top loading balance (Ohaus, Pinebrook, NJ). The mice were killed by cervical dislocation following anesthesia with isoflurane, and both kidneys were resected. After the surrounding connective tissue was excised, wet weights were recorded. Additionally, left kidneys were dried in a 55°C oven for 48 h and the dry weights were recorded.

**Stereological analysis of adult kidneys.** A total of 29 kidneys was used in this study, 15 3H1 +/+ and 14 Br/ + adult mice of both genders. The specimens were fixed and embedded in paraffin blocks, sectioned in a transverse plane at a thickness of 10 μm, and stained with hematoxylin and eosin (H&E). The sections were viewed ×200 magnification using a Olympus BX41 microscope and photographed. Initially, 17 kidneys were exhaustively sectioned and preliminary experiments were conducted to establish and validate the stereological sampling system. Subsequently, 9 specimens were represented by ~20% of the kidney while ~10% of the kidney was retained for the remaining 6 animals.

For stereological estimations, a systematic random sampling procedure was used (43). Specimen volume of the reference tissue (Vref) was determined using the Cavalieri method. Stereological estimation of the number of glomeruli per unit volume was obtained using the disector method (42, 70). At least 50 glomeruli from each mouse were measured for volume and surface area, and the mean values from each mouse were used for analysis.

A C++ computer program was written to automate data collection. Initial experiments were conducted utilizing the exhaustively sectioned specimens (7 3H1 +/+ and 7 Br/ + specimens). Convergence of glomerular number was used as a primary indicator of accuracy and this was achieved with a disector size of 800 μm² and a Q of ~50. System validation was achieved by generating stereological estimators for a square lattice of spheres 20 μm in diameter and comprising 33% of the test volume. The lattice was sectioned electronically generating 10 sample fields of circles. The system achieved a percentage error of 3.7% for average object volume and 7.1% for average object surface area.

**Physiological measurements of cardiorenal function.** Mean arterial pressure (MAP) measurement, heart rate (HR) recording, and blood sampling were performed on conscious animals after carotid artery catheter implantation, based on the procedure we previously described for rats (15, 53). Briefly, mice were anesthetized with isoflurane (1–3% in oxygen) administered via a gas mask Plexiglas tube modified for mice. Surgery was performed under aseptic conditions and catheters were sterilized before implantation. The carotid artery was catheterized with polyethylene tubing (PE-10) that was threaded through larger flared polyethylene tubing (PE-50) used to secure the catheter to the carotid artery with silk (6–0) ties. The catheter was threaded subcutaneously to exit at the back of the neck and the skin incision was closed. The catheter was connected to a physiological recorder (Gould Instruments, Valley View, OH). The animal was removed from isoflurane exposure and allowed to recover from anesthesia in a commercial plastic experimental chamber (Kent Scientific, Torrington, CT).

The animal was continuously monitored for 60 to 120 min after recovery from isoflurane, to obtain steady baseline blood pressure and...
HR. Blood (0.2 ml) was sampled from the catheter and plasma was separated for osmolality and protein assays using a CX3 Delta Chemistry Analyzer (Beckman, Brea, CA). The mouse was euthanized by cervical dislocation following anesthesia with isoflurane (inhalant) and additional blood was obtained via cardiac puncture. For urine analysis, mice were placed individually into metabolic cages and urine was collected under mineral oil and pooled for a 24-h period to assess kidney function. Urine volume was determined gravimetrically and samples were analyzed for osmolality, creatinine, protein, potassium, and sodium using a CX3 Delta Chemistry Analyzer.

**Physiological measurements of cholesterol and glucose metabolism.** Animals were fasted overnight and blood samples were obtained as described above. Plasma levels of glucose, triglycerides, cholesterol, and high-density lipoproteins were determined on a clinical chemistry analyzer (VITROS DT60, Ortho-Chemical Diagnostics, Rochester, NY). Plasma insulin and leptin levels were determined by radioimmunoassay (Linco Research, St. Louis, MO).

**Histological and immunohistochemical analysis.** Normal and mutant mice were identified according to phenotype (62), euthanized with an overdose of isoflurane, and a midtransverse section of the left kidney from each mouse was fixed for 72 h at 4°C in 10% neutral buffered formaldehyde. The kidneys were dehydrated in an ascending concentration of ethanol, cleared in xylene, embedded in paraffin wax, sectioned at 6 μm, and stained with H&E.

For immunohistochemistry, the kidneys were excised and fixed in 4% paraformaldehyde (0.1 M PBS, pH 7.4) at room temperature for 4 h. Kidneys were transferred to 30% sucrose and stored at 4°C (24 h), and then snap-frozen in liquid nitrogen and embedded in OCT (Miles, Naperville, IL). Cryostat sections (10 μm) were air-dried and stored at −70°C. For ET-1, rabbit anti-ET-1 serum (Peninsula Laboratories) was used as primary antibody at 1:400. The secondary antibody was goat anti-rabbit Ig conjugated with FITC (Sigma) at 1:400. For ETα, a sheep anti-ETα (Maine Biotechnology Services) was used at 10 μg/ml and for ETβ, the sheep anti-ETβ (Maine Biotechnology Services) was used at 20 μg/ml. The secondary antibody was rabbit anti-sheep conjugated with FITC at 1:200 dilution. For Na-K-ATPase, mouse monoclonal antibodies (anti-Na-K-ATPase α-1 subunit, Upstate Biotechnology) were used at 5 μg/ml, and FITC-conjugated goat anti-mouse IgG was used for secondary antibodies (Sigma). For immunostaining, the sections were deilesued for 5 min) at room temperature, blocked using normal goat or rabbit serum (30 min), and then incubated with primary antibodies overnight at 4°C. After being washed, sections were incubated with secondary antibodies (1 h) at room temperature. All incubations were performed in a moist chamber. Negative controls were performed by either omitting the primary antibodies or replacing them with the same nonimmune serum routinely used for the experimental tissues. Images were taken on an Olympus BX41 fluorescent microscope.

**Ultrastructural analysis.** For light and transmission electron microscopy (TEM), three pairs of age-matched normal and mutant mice (20–24 wk old) were deeply anesthetized with intraperitoneal injection of 0.6 ml avertin (30 mg/ml; 4–6 mg/10 g), following which the thoracic cavity of each mouse was quickly opened for perfusion. A 20-gauge needle was inserted into the left ventricle, saline was infused, and the right atrium was immediately transected. Gravity perfusion of 50 ml of 0.9% saline washout solution continued until the fluid was clear and was followed by infusion of 120 ml of cold (4°C) PIPES fixative (1% paraformaldehyde with 1% gluteraldehyde and 5% dextran in 0.1 M piperazine-N,N'-bis 2-ethane sulphonylic acid) buffered with NaOH (pH 7.6) (8). The peritoneal cavity was opened and kidneys were harvested, weighed, and placed into cold PIPES fixative. Renal capsules and associated perirenal fat were removed and transverse slices were made. The cortex was dissected away from each slice and further minced into ~1-mm³ pieces which were placed into small glass vials containing cold PIPES fixative for 48 h (4°C) and then washed three times (30 min each) in PIPES buffer with 2% sucrose. Renal cortical tissue samples were postfixed in a solution of one part 2% osmium tetroxide (OsO₄) and 3% potassium ferricyanide K₃Fe(CN)₆ in PIPES buffer, and one part 6% sodium iodate and 4% sucrose in PIPES buffer overnight at 4°C (8). Tissue samples were then dehydrated in graded ethanol and propylene oxide at room temperature. Embedding was carried out in a 1:1 Epon/Araldite as previously described (24). This mixture was replaced by pure Epon/Araldite overnight and cured at 55°C for ~72 h.

Thick sections (250 nm) were cut from the epoxy blocks on a RMC MTX ultramicrotome equipped with a Diatome diamond knife and stained with 1% toluidine blue (in 1% sodium borate). These sections were examined by bright field microscopy for presence and location of renal glomeruli. Thin sections (~70 nm in thickness and silver-gray interference color) of the desired block region were cut and mounted on 200-μm-mesh naked copper grids. These were stained with 2% uranyl acetate, rinsed vigorously, counterstained with lead citrate (95), and rinsed again. Thin sections were examined and photographed in a Hitachi model H-7500 TEM at an accelerating voltage of 80 kV.

**Glomerular basement membrane measurement.** Morphometry of glomerular basement membrane (GBM) was carried out as previously described (23). The method was a modification (29) of the "orthogonal intercept" procedure of Jensen and co-workers (48). Twenty sets of five identical magnification (37,500 diameters) transmission electron micrographs of GBMs from at least three glomeruli from each of two control (+/+) and three mutant (Br/+) animals were used in this study. Randomized measurements of peripheral GBMs (mesangial and stalk GBM regions were excluded) were made with a measuring ruler with a logarithmic scale utilizing a digitizing tablet and appropriate software (Biovant, R&M Biometrics). Approximately 40 measurements were made from each micrograph set. “True” GBM thickness in each set of micrographs was generated from the measurements according to the orthogonal intercept technique.

**Statistical analysis.** One-way ANOVA was performed to compare body and kidney weights using Prism3 statistical software (Graphpad, San Diego, CA). Physiological and stereological values were expressed as means ± SE or means ± SD. Differences between means for any parameter measured in two groups of mice were evaluated using Student’s t-test (P < 0.05) following a test of normality of data. A correlation analysis was performed comparing MAP and plasma creatinine (mg/dl) of individual mice.

**RESULTS**

**Adult f mice have severe renal hypoplasia with distended tubules, which is associated with the reduced expression of Six2 during embryonic development.** The newborn Br/+ heterozygote mutant mice have been previously described as displaying kidney hypoplasia (60). However, a detailed analysis of the microscopic morphology and physiological functioning of the adult Br/+ mutant kidney has not been previously undertaken. Comparison of the gross morphology of a kidney from a representative adult Br/+ mouse with one from its normal sibling littermate demonstrates that the Br/+ kidneys are much smaller in size with a rough external surface resulting from enlarged and distended renal tubules (Fig. 1, A and B). Histological sections confirmed that the adult Br/+ kidneys display distended tubules and renal cysts with increased interstitial tissue in contrast to normal kidney tissue (Fig. 1, C and D). The distended tubules in the mutant kidney varied in size and typically were lined by a single layer of epithelial cells surrounding an expanded fluid-filled lumen. In addition, some Br/+ mutant tubules contained casts while glomerular capsules appeared enlarged and filled with fluid, which suggested impaired renal function. Renal blood vessels also appeared distended with thicker vascular walls in the Br/+
adult female \( Br/+ \) mice weighed an average of 7.30 g less than \(+/+\) females (Table 1). The wet weights of right and left kidneys from both male and female \( Br/+ \) mutant mice were significantly reduced, ranging from 43 to 52% less than normal (Table 1). There were no statistically significant differences between the 3H1 stock and 3H1 \(+/+\) body weights or wet kidney weights. After drying, the left kidney weights also showed dramatic differences between \(+/+\) and \( Br/+ \) mutant mice, decreased 51% in males and 53% in females. There was no significant difference between female 3H1 stock and 3H1 \(+/+\) dry left kidney weights, but there was a difference between male 3H1 stock and 3H1 \(+/+\) dry left kidney weights (\( P < 0.01 \)). It is unclear whether this represents a real biological difference. In addition, the percentage of body weight represented by the total kidney weight was calculated and compared (Table 1). In \( Br/+ \) males, the kidneys made up 0.98% of body weight, while in \(+/+\) males, the kidneys made up 1.49% of body weight. In \( Br/+ \) females, the kidneys made up 0.90% of body weight, while in \(+/+\) females, the kidneys made up 1.20%. For both male and female mice, there was no significant difference between 3H1 stock and 3H1 \(+/+\) total kidney weight of body weight percentage. Overall, these results statistically confirmed gross observations that in adult \( Br/+ \) mice, there was a greatly reduced renal mass, which was highly suggestive of defective renal physiologic function.

We previously showed that misexpression of the Six2 transcription factor during embryonic development is associated with \( Br/Br \) neonatal lethality and the \( Br/+ \) mutant phenotype (35). Six2 is hypothesized to inhibit the mesenchymal condensation and conversion to epithelial cells that is required for nephrogenesis, and thus maintain a mesenchymal progenitor cell population in the nephrogenic zone. Although hypothesized that Six2 is only expressed in the kidney during embryonic development, no studies have measured Six2 expression in adult mouse kidneys. Real-time qPCR performed on wild-type kidney samples showed that Six2 expression is high during embryonic development (E13.5), reduced 75.8% from E13.5 levels in newborns, and is undetectable in adult kidneys (Fig. 2A). In addition, we confirmed by qPCR that Six2 is decreased by 50% in isolated \( Br/+ \) E13.5 kidneys (Fig. 2B). Together, these data support the hypothesis that it is an embryonic Six2 deficiency that leads to the \( Br/+ \) mutant renal phenotype.

*Fig. 1. Photomicrographs of representative kidneys from sibling littermate \(+/+\) (A, C) and \( Br/+ \) (B, D) mice. Note the larger size of the \(+/+\) kidney compared with the smaller, cystic \( Br/+ \) kidney. Normal glomeruli (C, arrowheads) display cuboidal epithelia in \(+/+\) animals compared with the 3H1 \( Br/+ \) kidney with enlarged glomeruli (D, arrowheads), enlarged tubules (t), and increased cellularity between nephrons (*). Bar = 3.0 mm in A and B; 200 \( \mu \)m in C and D.*

mutants, and there was also an increase in cellularity between the tubules (Fig. 1D).

To quantitatively compare characteristics such as average body weight, wet and dry kidney weight, and male vs. female kidney weight, we collected kidneys from a large number of 3H1 stock, 3H1 \(+/+\), and 3H1 \( Br/+ \) mutant mice. The \(+/+\) mice were siblings of \( Br/+\) mice and raised in the same litters, so by comparing 3H1 stock and \(+/+\), potential \( Br \) maternal/paternal effects could be identified. Overall, we collected 245 pairs of right and left adult kidneys for analysis: 15 from 3H1 stock males, 25 from 3H1 stock females, 34 from \(+/+\) males, 48 from \(+/+\) females, 66 from \( Br/+ \) male, and 57 from \( Br/+ \) females. Immediately after dissection, we weighed both the left and right kidneys (wet weight), and then fixed the right kidney for histology and oven-dried the left kidney to assess the dry weight. Since the mutant kidneys’ tubules and glomeruli were distended with apparent higher proportional volumes of fluid, we measured the dry weight to compare the tissue mass. The collected data were subjected to one-way ANOVA, followed by a post hoc Tukey multiple comparison test to determine whether the differences between any two groups were statistically significant.

The results showed that adult male \( Br/+ \) mice weighed an average of 6.27 g less than their \(+/+\) counterparts, and the
which was 88% lower than the mean total number of glomeruli of 18,880 ± 927 for +/+ kidneys (Fig. 3C). So although the gross weight of the Br+/+ kidney is 43–52% of that of +/+ controls (Table 1), and the average volume of the Br+/+ kidneys is 64% less than that of +/+ controls, the Br+/+ kidneys only have ~12% of the number of glomeruli measured in control +/+ kidneys. 

The mean average glomeruli volume of adult Br+/+ mice was 163,400 ± 17,820 µm³, which was 180% higher than the mean average glomeruli volume of 89,590 ± 4,800 µm³ for adult +/+ mice (P = 0.0002). This suggests that the reduced number of glomeruli in Br+/+ kidneys has become hypertrophic or distended in an attempt to handle the increased physiologic renal burden, and it further verifies what has been observed in routine histological analysis of Br+/+ kidneys. The mean average surface area of glomeruli in adult Br+/+ was 14,600 ± 1,479 µm², which was significantly higher than the mean average glomeruli surface area of 11,310 ± 788 µm² for +/+ mice (P < 0.05). Expressing each mouse’s Vm relative to its SAM confirms that there is a significant increase in the size of the mutant glomeruli (P < 0.01; Fig. 3D). In total, these stereological findings suggested that the Br+/+ glomeruli are extremely overburdened, which indicated that Br+/+ kidneys are unlikely to maintain an adequate glomerular filtration rate (GFR) or to concentrate urine as normal.

**Br+/+ mice have impaired renal function, hypertension, and low levels of metabolic indicators.** Since all morphological and stereological data suggested impaired renal physiology, we measured various aspects of cardiorenal physiological markers, as well as indicators of cholesterol and glucose metabolism. Plasma analysis revealed a small but statistically significant increase in plasma osmolality of Br+/+ mutant mice compared with +/+ mice (Table 2). However, the average plasma creatinine concentration was greatly increased (233%; P < 0.01) in Br+/+ mutant mice compared with normal, which indicated a steep decline in steady-state GFR. The average concentration of plasma proteins was not significantly different between Br+/+ and +/+ mice (Table 2).

Urinalysis showed numerous features consistent with CRF in the Br+/+ mutant mouse (Table 2). Polyuria was indicated by a significantly larger volume of urine output (over a 24-h period) for mutant mice compared with normal. In addition, the average urine osmolality of the Br+/+ mutant mice was 69% lower than what was measured in +/+ mice, and the average urine creatinine concentration was 74% lower in mutant mice (Table 2). This decrease in urine osmolality and creatinine supports the elevated plasma creatinine as a strong indication of impaired creatinine clearance and depressed GFR. Accordingly, Na⁺ and K⁺ ions were present in significantly decreased concentrations in the urine of Br+/+ animals compared with 

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**Table 1. Comparison of gross renal characteristics between 3H1 +/+ and Br+/+ mice**

<table>
<thead>
<tr>
<th></th>
<th>3H1 Stock</th>
<th>+/+</th>
<th>Br+/+</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean BW, g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>28.11±7.13 (15)</td>
<td>25.66±8.91 (34)</td>
<td>19.39±5.86 (67)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>26.36±6.12 (25)</td>
<td>24.95±9.21 (48)</td>
<td>17.65±5.81 (57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Right kidney, g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.225±0.043 (15)</td>
<td>0.198±0.061 (34)</td>
<td>0.108±0.050 (66)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>0.158±0.036 (25)</td>
<td>0.148±0.042 (48)</td>
<td>0.084±0.036 (57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Left kidney, g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.207±0.038 (15)</td>
<td>0.180±0.057 (34)</td>
<td>0.087±0.042 (66)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>0.147±0.029 (25)</td>
<td>0.139±0.040 (48)</td>
<td>0.073±0.032 (57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Left kidney dry, g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.056±0.012 (15)</td>
<td>0.043±0.017 (24)</td>
<td>0.021±0.011 (65)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>0.038±0.007 (25)</td>
<td>0.034±0.011 (38)</td>
<td>0.016±0.008 (57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Total KW/BW, %</strong></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>1.563±0.157 (15)</td>
<td>1.492±0.272 (34)</td>
<td>0.983±0.217 (66)</td>
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<tr>
<td>Female</td>
<td>1.167±0.116 (25)</td>
<td>1.201±0.218 (48)</td>
<td>0.898±0.229 (57)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = mice). BW, body weight; KW, kidney weight. P values are for +/+ vs. Br+/+ comparisons.

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Fig. 2. Real-time quantitative (q)PCR shows Six2 expression in the kidney is highest during embryonic (E) development, which is greatly decreased in Br mutant kidneys. Expression of Six2 in normal newborn and adult kidneys is shown relative to expression of Six2 in E day 13.5 (E13.5) kidneys after being normalized against the measured levels of actin (A). Expression of Six2 in Br+/+ and Br+/Br E13.5 kidneys is shown relative to expression of Six2 in +/+ E13.5 kidneys after being normalized against the measured levels of actin (B).
normal animals, but urinary protein concentrations were not significantly different between groups (Table 2).

Hypertension is a common secondary effect of reduced numbers of glomeruli and CRF, both in animal models (7, 12, 13, 27, 30, 41, 79) and humans (25, 76, 78, 81, 86). The MAP of Br+/H11001 mutant mice averaged 156 ± 8 mmHg, which was significantly higher than the average MAP of +/+ mice, 126 ± 5 mmHg (P < 0.01; Table 2). In addition, the average resting HR of Br+/ mice was 621 ± 34, which was significantly higher than the average resting HR of +/+ mice, 525 ± 29 (P < 0.05; Table 2). Furthermore, in 19 mice, the MAP and plasma creatinine concentrations were measured and showed a positive correlation (r = 0.59, P < 0.01; Fig. 4), suggesting that the impaired renal function in the Br/+ mutant mice likely contributed to the observed hypertension.

Physiological parameters of glucose and cholesterol metabolism were measured and compared between Br/+ and +/+ mice. The average plasma concentration of insulin was 58% lower in Br/+, and the average leptin and triglycerides concentrations were 70 and 42% lower, respectively (Table 3).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>+/+</th>
<th>Br/+</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>POsm, mosmol/kgH₂O</td>
<td>333 ± 3 (8)</td>
<td>349 ± 5 (9)</td>
<td>0.02</td>
</tr>
<tr>
<td>PCre, mg/dl</td>
<td>0.18 ± 0.02 (11)</td>
<td>0.42 ± 0.06 (11)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PPro, mg/ml</td>
<td>45.2 ± 2.0 (8)</td>
<td>51.6 ± 2.5 (9)</td>
<td>0.07, ns</td>
</tr>
<tr>
<td>Urine volume, ml</td>
<td>0.787 ± 0.161 (12)</td>
<td>1.310 ± 0.280 (13)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>UOsm, mosmol/kgH₂O</td>
<td>2,240 ± 157 (13)</td>
<td>693 ± 142 (13)</td>
<td>0.01</td>
</tr>
<tr>
<td>Ucre, mg/dl</td>
<td>35.4 ± 1.9 (13)</td>
<td>9.3 ± 1.8 (13)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ucre, mg/ml</td>
<td>12.8 ± 1.2 (8)</td>
<td>12.2 ± 1.8 (9)</td>
<td>0.78, ns</td>
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<tr>
<td>Ucre, mmol/l</td>
<td>199.7 ± 8.7 (7)</td>
<td>112.9 ± 18.0 (9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>UNa, mmol/l</td>
<td>271.55 ± 19.55 (7)</td>
<td>147.90 ± 24.64 (9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>UK, mmol/l</td>
<td>126.5 ± 12 (12)</td>
<td>156 ± 8 (11)</td>
<td>&lt;0.01</td>
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<tr>
<td>MAP, mmHg</td>
<td>525 ± 29 (12)</td>
<td>621 ± 34 (11)</td>
<td>0.04</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>23.2 ± 3.4 (13)</td>
<td>17.8 ± 2.4 (13)</td>
<td>0.21, ns</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = mice). MAP, mean arterial pressure; HR, heart rate; POsm, plasma osmolality; PCre, plasma creatinine; PPro, plasma protein; UOsm, urine osmolality; Ucre, urine creatinine; Ucre, urine protein; UNa, urine sodium; UK, urine potassium; ns, not significant.
average plasma glucose level in Br/+ animals was lower than controls, but did not reach statistical significance. No statistically significant differences were observed in average plasma levels of cholesterol, HDL, or glucose (Table 3).

Kidney tubules of the Br/+ mice have increased protein levels of ET-1, ET_A, ET_B, and Na-K-ATPase with altered subcellular localization. ET-1 is a multifunctional peptide that exerts potent effects on the cardiovascular and renal systems by signaling through endothelin receptors A and B (ET_A and ET_B) (65, 67). In the kidney, ET-1 acts in the renal vasculature to regulate renal hemodynamics and the GFR, while in nephron tubules, ET-1 regulates water and sodium reabsorption (38, 56). Immunofluorescent staining for ET-1 showed low-intensity staining in normal mouse kidney tubules, with staining confined to the basolateral surface of the renal tubules (Fig. 5A). However, enlarged Br mutant tubules showed intense staining throughout the epithelia, with staining also appearing along the apical surface (Fig. 5B). Immunostaining for ET_A in the distended Br/+ mutant tubules was also much more intense than seen in normal tubules (Fig. 5, C and D). The subcellular localization of the ET_A receptor also appeared altered in the Br/+ tubule epithelia, showing a greater apical localization than that seen in normal tubules. Immunostaining for the ET_B receptor demonstrated a similar pattern, with faint basolateral staining in normal tubules (Fig. 5E), and highly elevated levels in the Br/+ enlarged tubules with greater apical localization (Fig. 5F). Although the mean plasma and urine concentrations of the ET-1 peptide were not significantly different between Br/+ and +/- mice (Table 4), due to the increased average urine output of the Br/+ mice (Table 2), we can conclude that the overall urinary excretion of ET-1 is greater in the mutant mice. In addition, there was a much greater variability in urine levels of ET-1 in the Br/+ mice with a nearly fivefold higher standard deviation than in the +/- mice.

The sodium-potassium-activated adenosine triphosphatase (Na-K-ATPase) membrane transporter is critical to renal physiology by driving tubular reabsorption of sodium, and thus reabsorption of water (40, 91). In +/- kidneys, immunostaining for Na-K-ATPase showed characteristic basolateral staining in normal renal tubules (Fig. 6A). However, in Br/+ mutant kidney tubules, dramatically increased levels of Na-K-ATPase immunostaining were detected, with much of the staining located on the apical surface of the tubule epithelial cells (Fig. 6B). It is unclear whether the Na-K-ATPase molecules ob-

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Table 3. Comparison of cholesterol and glucose metabolism physiological parameters between 3H1 +/- and Br/+ mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>+/-</th>
<th>Br/+</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>181±11 (16)</td>
<td>152±11 (12)</td>
<td>0.08, ns</td>
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<tr>
<td>Triglycerides, mg/dl</td>
<td>93±4 (16)</td>
<td>54±9 (12)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>111±6 (16)</td>
<td>125±4 (12)</td>
<td>0.12, ns</td>
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<tr>
<td>Insulin, ng/ml</td>
<td>1.25±0.24 (14)</td>
<td>0.53±0.07 (11)</td>
<td>0.02</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>11.05±1.38 (5)</td>
<td>3.31±0.48 (7)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>133±6 (16)</td>
<td>140±6 (12)</td>
<td>0.39, ns</td>
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Values are means ± SE (n = mice).

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Fig. 5. Immunofluorescent staining of endothelin-1 (ET-1) in kidneys from +/- (A, C, E) and Br/+ (B, D, F) adult mice. Renal tubules in +/- mice showed low level background staining for ET-1 (A) compared with intense staining for ET-1 around enlarged mutant tubules (B, arrows). Staining for ET_A (C, arrow) and ET_B (E, arrow) revealed localization in the basolateral epithelia of normal tubules, but in the distended tubules of Br/+ mice, extensive staining was observed throughout the epithelium, particularly along the apical surfaces (D, F, respectively). Bar = 100 μm.
served in locations other than the basolateral surface are functional. Since the concentration of Na\textsuperscript{+} in the urine was significantly decreased in the Br\textsuperscript{+/} mice (Table 2) and yet the amount of excreted water was significantly increased, it is difficult to conclude the physiological consequences of this overexpression and disregulation of the Na-K-ATPase in the renal tubules.

**Ultrastructural analysis of Br\textsuperscript{+/} mutant glomeruli revealed hypercellularity, increased mesangial matrix, and thickened irregular GBMs.** TEM analysis was carried out on renal tissues from three adult +/+ and Br\textsuperscript{+/} mice pairs primarily to provide qualitative ultrastructural data of renal glomeruli and to provide comparative views of the blood-urine barrier in both animal types. Light microscopic (LM) sections were made in an effort to identify glomerular position and relative number and to prepare the tissues for TEM thin sectioning. As seen in Fig. 7A, renal cortical tissues from control animals showed glomeruli with patent capillaries empty of blood cells and normal histoarchitectures. Contrariwise, many but not all mutants demonstrated substantial glomerulopathic alterations including hypercellularity and extracellular matrix overexpression (Fig. 7B).

Ultrastructural studies of mutant/control renal tissues using TEM confirmed our LM observations. Figure 7C shows that glomeruli from +/+ mice appeared normal in all respects and demonstrated excellent perfusion fixation. Normal numbers and morphological features of podocytes, endothelial cells, and mesangial cells were found on the intervening GBM. At higher magnifications glomeruli from wild-type animals (Fig. 7E) exhibited classic ultrastructural features including a well-defined blood-urine barrier consisting of a highly fenestrated endothelium lining the capillary lumen, a relatively thin, moderately electron dense and regular GBM, and small podocytic foot processes connected by slit membranes.

As expected, glomeruli from Br\textsuperscript{+/} mice were more difficult to locate and characterize by TEM. As in the LM specimens, some but not all mutant glomeruli were indistinguishable from controls. Nevertheless, most showed clusters of mesangial cells with expanded areas of extracellular matrix (Fig. 7D), and many glomerular capillaries were highly occluded with nodular hypercellularity and areas of increased mesangial matrix. A dominant feature of mutant glomerulopathy was a thick irregular GBM, with substantial extracellular matrix accumulation.

At higher magnifications (Fig. 7F), the increased thickness of the blood-urine barrier in Br\textsuperscript{+/} mice was clearly evident. Capillary lumina were lined by poorly fenestrated endothelial cells subtended by a decidedly thickened and often highly irregular GBMs often associated with broad areas of extracellular matrix. Using an orthogonal intercept method, the thickness of GBMs was measured in age- and gender-matched +/+ and Br\textsuperscript{+/} mice and found to be significantly thicker in mutant kidneys (P < 0.0001; Table 5). In addition, many podocyte foot processes in the mutant mice were decidedly broadened and “club-like” (podocytic “effacement”).

**DISCUSSION**

The Br mutant mice have been previously described as having semidominant hereditary kidney hypoplasia, along with frontonasal dysplasia (59, 60, 62, 68). In the present study, we characterize the kidney malformation and impaired renal functioning of the adult Br\textsuperscript{+/} heterozygote mutant mouse. Although the specific chromosomal aberration that causes the Br phenotype has not yet been identified, it appears likely to directly inhibit the transcription of the homeobox transcription factor Six2 during embryonic development (35). We mapped the Br mutation to the genomic region surrounding the Six2 gene, and Six2 mRNA and protein are nearly absent in Br/Br homozygote mutant embryos and ~50% in the Br\textsuperscript{+/} heterozygote mutant embryos. This hypothesis is supported by the recent characterization of the Six2-null homozygote embryos, which appear very similar to Br/Br embryos, although analysis of the Six2-null heterozygotes was not performed (87). The Six2 transcription factor is expressed in the metanephric mesenchymal cells surrounding the branching uretic bud (20, 74, 75) and is believed to function as an inhibitor of nephrogenic differentiation in this cell population (35, 54, 87). When Six2 expression is lost in these cells, it appears that nephrogenesis proceeds too quickly and the mesenchymal progenitor cell population is lost, resulting in premature cessation of nephrogenesis (35, 54, 60, 87). The members of the DNA-binding Six family of proteins, including Six2, bind to the EYA family of transcriptional activators to initiate transcription of downstream targets (21, 50), although not much is known about which genes Six2 directly regulates.

Haploinsufficiency of critical transcription factors during mammalian embryonic development can often cause abnormal morphogenesis that may manifest with pathological conse-
quences in adults. For example, haploinsufficiency of the transcription factor GATA3 during embryonic development causes human hypoparathyroidism, deafness, and renal syndrome, with hyperparathyroidism, deafness, and renal malformations (94). Embryonic gene dosage is also important for the forkhead transcription factor FOXC1 which, if missing one allele, causes defects of the anterior eye, leading to early glaucoma (71, 101). Having previously demonstrated haploinsufficiency of Six2 in the Br/Br/H11001 embryos at E13.5 (35), we show here that Six2 expression in normal kidneys was greatly decreased by birth and was undetectable in adult kidneys. Therefore, the renal dysplasia and pathophysiology seen in the adult mice are likely solely due to the abnormal embryonic kidney development. We observed that the embryonic Br/Br kidney initially differentiates normally, with the ureteric bud forming at gestational day 11 and inducing condensation of the metanephric mesenchyme (60). However, we observed that at day 13, the nephrogenic zone remains poorly developed and Pax-2 expression is absent in the peripheral area of the kidney. Although some nephrons develop in the Br/Br and can be observed by gestational day 15, they appear greatly reduced in number (60). The decrease in glomerular number in the Br/H11001 adult kidney measured by stereology confirms that proper levels of Six2 during embryonic development are critical for the development of an adequate number of nephrons.

Fig. 7. Light (A, B) and transmission electron micrographs (TEM; C, D, E, F) of renal cortical tissues from age-matched adult +/- (A, C, E) and Br/+ (B, D, E) mice. Control (+/-) mice (A) exhibit normal glomerular histarchitectures, whereas massive increases in extracellular matrix (ECM) are seen in the mutants (B). TEM of normal (C) and mutant (D) tissues illustrate the increase in specific cellularity and ECM in the mutants. Relatively high-magnification TEM of normal (E) and mutant (F) mice show a substantial increase in the blood-urine barrier thickness (double-ended arrows) in the mutants. CL, capillary lumen; P, podocyte. A and B, ×600; C, ×3,000; D, ×2,800; E, ×9,200; F, ×9,100.

Table 5. GBM thickness in 3H1 +/- and Br/+ mice measured from TEM

<table>
<thead>
<tr>
<th></th>
<th>+/-</th>
<th>Br/+</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBM thickness, nm</td>
<td>115.9±5.2 (8)</td>
<td>150.4±4.3 (12)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = glomeruli). For each glomerulus analyzed, 30–50 glomerular basement membrane (GBM) measurements were made and the mean was used as the data point for the t-test.
disease, bone loss, and renal fibrosis and has provided a useful model for studying complications and treatments of these diseases (3, 7, 13, 14, 18, 26, 32, 41, 69, 89, 99). We hypothesize that the reduced renal mass in the Br/+/ mouse, as in the nephrectomized rat, leads to an increased vascular resistance which may promote some of these same pathologies. In this respect, the Br/+/ mouse may serve as a useful new genetic model to study the pathological changes due to heritable reduced renal mass and reduced nephron number.

The glomerular changes in Br/+/ kidneys revealed by ultrastructural analysis could represent a wide variety of hyperplastic and hypertrophic glomerulopathies. However, it was clear that the mutant glomeruli did not directly resemble the characteristic nodular glomerulosclerosis of chronic diabetic nephropathy (52). Rather they showed clusters of hypertrophic endothelial cells and mesangial cells surrounded by redundant extracellular (mesangial) matrix with evidence of hypovascularity. So, although low levels of insulin were measured in the Br/+/ mice, it appears the primary kidney damage is not due to a diabetic condition. The thick irregular GBM and podocyte effacement observed in Br/+/ glomeruli are typically associated with proteinuria, although the urinary concentration of protein in mutant mice was not significantly different from +/+ mice. However, given that the mutant mice showed roughly double the normal daily production of urine, the Br/+/ mutant mice excreted roughly double the normal amount of protein. Further research needs to be done to determine whether the glomerular filtration in these mutant mice allows increased amounts of serum proteins into the ultrafiltrate or whether an impaired reabsorption is the reason for this increase.

Histopathology confirmed gross observations of distented glomeruli and tubules with renal cysts in adult Br/+/ kidneys, and stereology showed that average Br/+/ glomeruli volume was nearly double compared with +/+ glomeruli. Shortly after birth, Br/+/ kidneys become polycystic, presumably due to increased filtration demands (62). This cystic development appears secondary in Br/+ and is a characteristic feature of acquired cystic kidney disease and a common result of CRF (4, 5, 33, 58). The mechanism by which renal cysts form is not completely understood, but it is generally accepted that the process includes fluid accumulation and epithelial proliferation. Fluid accumulation occurs as a result of aqueous secretion throughout all segments of the nephron while mislocation of Na-K-ATPase to the apical epithelial surface causes secretion of sodium with concomitant water loss (39). It is well-documented that Na-K-ATPase polarity is affected during tubular distension in the genesis of renal cystic lesions (73, 96, 97). Immunolocalization of Na-K-ATPase in wild-type adult mice showed normal distribution along the tubular basolateral epithelium; however, distended tubules in Br/+/ mutants showed intense staining throughout the epithelium. Therefore, the physiological defects in fluid reabsorption seen in Br/+/ mutant mice were associated with a dramatic increase of Na-K-ATPase expression, as would be expected. Experimental evidence suggests that Na-K-ATPase activity is enhanced along nephronic segments in hypertensive rats displaying CRF (16, 34, 36). In Milan hypertensive rats, increased Na-K-ATPase activity is linked to a higher number of pump sites with a significant increase in pretranslational α and β isomer mRNA levels before the onset of hypertension (34). Thus, an increase in Na-K-ATPase activity in the Br/+/ mice could lead to an increased pressor effect, contributing to the development of hypertension with progressively detrimental renal functional effects.

The Br/+/ kidney tubule epithelia showed an increase in immunostaining intensity and apical distribution of ET-1, as well as ET_A and ET_B receptors. Br/+/ mice also show an increased overall urinary excretion of ET-1, the secretion of which likely accounts for the apical distribution of ET-1 in the renal tubules. ET-1 is actively synthesized by renal tubular cells and appears to be upregulated during kidney disease (9, 11, 28, 57). It has been shown that renal ET-1 gene expression and urinary ET-1 excretion occur in a time-dependent fashion among rats with surgically reduced renal mass (10, 77), and it has also been shown that the synthesis of ET-1 and its receptors also increases in renal epithelial cells as cysts develop (45). In addition, CRF, renal cysts, and hypertension have all been observed in transgenic mice overexpressing ET-1 (44, 90, 93). ET-1 may contribute to the progression of CRF by promoting the renal inflammatory process (31) as well as the subsequent tissue repair processes (49, 55, 77, 82). As CRF progresses, production of vasoactive and inflammatory molecules correlates with increasing expression of ET-1 in renal tubular cells (22, 77). The findings in the Br/+/ mice suggest that in individuals with renal hypoplasia and reduced nephron number, activated renal ET-1 may play an important role in the pathogenesis of CRF and systemic hypertension.

Rodents with CRF are characterized by an inability to concentrate urine and conserve fluid. Adult Br/+/ mice showed polyuria, with almost double the daily output of urine as +/+ siblings, and the osmolality of urine in Br/+/ mice was 31% of what was measured in +/+ mice. In addition, average urine concentrations of sodium and potassium were both significantly lower in Br/+/ mice (43 and 46%, respectively). These urinalysis data indicate a decrease in the ability to reabsorb water in the nephron and collecting ducts. The overall diuresis in the Br/+/ mice should presumably lead to loss of extracellular fluid volume, which was supported by our findings of a significant increase in plasma osmolality in Br/+/ mice. Plasma creatinine values were 233% higher in Br/+/ mice than in +/+ , whereas urine creatinine was 74% lower, which together indicated a decreased GFR in the mutant mice. However, we hypothesize that an increased vascular resistance in Br/+/ mice, due to fewer nephrons, will cause a compensatory rise in filtration rate of individual glomeruli, but since there are much less glomeruli, the overall GFR is decreased in these animals. The stereological data showed increased glomerular volume in the Br/+/ kidneys, which is a morphological change likely resulting in increased filtration of individual glomeruli.

In addition to the decreased body weights, the Br/+/ mutant mice were found to have significantly reduced levels of plasma insulin, triglycerides, and leptin. It is possible that Br/+/ mice exhibit signs of type I diabetes, which is characterized by an insulin deficiency due to the loss of pancreatic beta cells. Expression of mouse Six2 was briefly noted near the embryonic pancreas at E9.5 (75), and Northern blotting of human Six2 revealed expression in the adult pancreas (17), but the role in Six2 in pancreatic development or function has not been further investigated. It will be important to analyze the pancreas in the Br/+/ mice to determine whether morphology or function is abnormal, as well as to ascertain the diabetic condition of these adult mice. The leptin and triglyceride levels may be lower in...
were oxide synthase gene, which displayed blood pressures that models have been produced either by mutant screening or in these mice.

The renin-angiotensin, erythropoietin, and vasopressin systems and hypertension as the haploinsufficiency of kidneys at autopsy (51). Although many genes critical to humans by using stereological analysis to count glomeruli in such data, a recent study was able to show a high correlation and also CRF (19, 25, 64). Despite the difficulty in collecting correlates with their risk of developing essential hypertension.

Where the number of nephrons in an individual inversely provided evidence for a theory of “nephron endowment,” even higher levels of variation in humans (46, 47). This finding 300,000 to over 1 million (72), and subsequent studies showed the normal human kidney was highly variable, ranging from the number of nephrons in an individual inversely correlates with their risk of developing essential hypertension. In a landmark study, hypertension typically develops with progression of CRF, causing dramatically reduced nephron number and kidney hypoplasia, with the development of renal cysts. Gene dosage, and perhaps genetic polymorphisms which may influence expression or activity of Six2, should be investigated in human populations with familial kidney hypoplasia, reduced nephron numbers, or essential hypertension.

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