Transfer of the *Rf-1* region from FHH onto the ACI background increases susceptibility to renal impairment

ABRAHAM P. PROVOOST,1,* MASAHIDE SHIOZAWA,2* RICHARD P. E. VAN DOKKUM,1 AND HOWARD J. JACOB2

1Department of Pediatric Surgery, Erasmus University, 3000 DR Rotterdam, The Netherlands; and 2Laboratory for Genetics Research, Department of Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Received 26 May 2000; accepted in final form 20 December 2001

Provoost, Abraham P., Masahide Shiozawa, Richard P. E. Van Dokkum, and Howard J. Jacob. Transfer of the *Rf-1* region from FHH onto the ACI background increases susceptibility to renal impairment. *Physiol Genomics* 8: 123–129, 2002. First published January 22, 2002; 10.1152/physiolgenomics.00030.2000.—The genetically hypertensive fawn-hooded (FHH/Eur) rat is characterized by the early presence of systolic and glomerular hypertension, progressive proteinuria (UPV), and albuminuria (UAV), and focal glomerulosclerosis, resulting in premature death from renal failure. Previous studies showed that at least five genetic loci (*Rf-1* to *Rf-5*) were linked to the development of renal impairment. Of these five, *Rf-1* appears to play a major role. To study the impact of *Rf-1* in the absence of the other loci, we transferred the *Rf-1* region of chromosome 1, between the markers D1Mit34 and D1Rat156, *Rf-1B* for short, onto the genomic background of the normotensive August × Copenhagen Irish (ACI) rat. In this congenic strain, named ACI.FHH-D1Mit34/Rat156 or ACI.FHH-ACI, we challenged the renal hemodynamic function of these animals by studying the effects of unilateral nephrectomy (UNX) alone, or combined with *N*^6^-nitro-L-arginine methyl ester (L-NNAME)-induced hypertension. Following UNX, the congenic strain developed significantly more UPV and UAV than the ACI progenitor. The differences were even more pronounced when UNX was combined with an L-NNAME-induced rise in systolic blood pressure to about 150 mmHg, i.e., the level of hypertension present in the parental FHH strain. These findings indicate that the *Rf-1B* region of the FHH rat contains at least one gene affecting the susceptibility to progressive renal failure, especially in the presence of an increase in blood pressure.

proteinuria; albuminuria; glomerulosclerosis; renal failure; hypertension; congenic rat; fawn-hooded hypertensive rat

**GENETIC FACTORS** are thought to play a role in progressive renal failure in humans (18), with direct evidence recently obtained for familial focal segmental glomerulosclerosis (8). Initially, the involvement of genes in renal disease came from various animal models (11, 13, 22). Our studies using the hypertensive fawn-hooded (FHH/Eur) rat demonstrated the presence of various quantitative trait loci (QTLs) influencing susceptibility to chronic renal damage in this strain (1). The inbred FHH rat develops a moderately elevated level of systolic blood pressure (SBP), progressive proteinuria (UPV), mainly albuminuria (UAV), and focal glomerulosclerosis (FGS) at a relatively young age, leading to premature death due to end-stage renal failure (ESRF) (14, 16, 21, 27, 30). In a follow-up study with an (FHH × ACI) F2 intercross, we identified five QTLs, named *Rf-1* to *Rf-5*, responsible for the high susceptibility to develop UPV and structural renal damage following unilateral nephrectomy (UNX) (19). Remarkably, all loci except the *Rf-2* locus appeared to be genetically independent of blood pressure.

Localizing and confirming the presence of a QTL is only the beginning of a long road to gene identification via a positional cloning strategy (7). Once a QTL is identified, the next step is to generate a congenic strain in which the chromosomal region carrying the QTL is being introgressed from a donor strain into a recipient. In recent years, over 20 congenic strains and substrains, carrying various blood pressure QTLs, have been generated and studied (for review see Refs. 3 and 17).

Congenic animals are not only useful for positional cloning, but offer a new tool for physiological studies. Prior to congenics, physiologists using rats were restricted to making comparisons between strains. This strategy was hindered by the fact that any physiological difference observed could be 1) the result of a component involved in a disease pathway; 2) the result of physiological changes induced by the disease, or 3) a strain difference due to genetic drift that has nothing to do with a causative disease pathway. Unfortunately, the latter case was the most likely scenario. Congenic animals offer the physiologist the opportunity to compare two strains that differ at a single genomic region. Thus the experimental and control strains differ at a limited number (although the numbers could be in the hundreds) of genes. Finally, positional cloning of these genes will very likely require detailed physiological characterization. Therefore, we are building a series of overlapping congenic animals for the *Rf-1* region,
which could be used to narrow the size of interval (by taking advantage of crossing over of the susceptible and resistant chromosomes) that contains the gene(s) and for physiological characterization. For our studies, we chose to introgress the chromosomal region containing \( Rf-I \) from the susceptible FHH donor rat into the normotensive August \( \times \) Copenhagen Irish (ACI) recipient rat.

Here, we report the first of the congenic strain (ACLFHH-Rf1B) that was tested for renal susceptibility and compared with the ACI progenitor strain. This congenic strain covers 15 cM of the \( Rf-I \) region of rat chromosome 1 and shows a significant increase in UPV after UNX that is not observed in the ACI progenitor strain. Combining UNX with N\(^N\)-nitro-L-arginine methyl ester (L-NAME)-induced hypertension markedly accelerates the development of UPV in the congenic rats but not the ACI control (26–28). These studies indicate that the \( Rf-I \) chromosomal region contains one or more genes influencing the susceptibility to renal damage, validating the QTL mapping reported previously and setting the stage for the eventual positional cloning, while enabling us to conduct various physiological studies.

**MATERIAL AND METHODS**

**Animals.** All breeding was done at the Animal Research Center of the Erasmus University, Rotterdam, The Netherlands. Animals were housed in standard rat cages with lights on from 8:00 AM to 8:00 PM. Standard commercial rat chow containing 56% carbohydrates, 26% digestible protein, 7% fat, 4% fiber, and 5% minerals (AM II; Hope Farms, Woerden, The Netherlands) and drinking fluid (tap water, acidified to pH 3) were provided ad libitum.

**Congenics.** To generate the ACLFHH-Rf1B congenic, we used the speed congenic strategy (10, 12), which involves a series of backcrosses to the ACI and selection of the breeders of the next generation based on genotyping results. As the introgressed region is carried as a heterozygote in the backcrosses, the congenic rat strain is then established by a final intercross of a male and a female heterozygous for the \( Rf-I \) region and homozygous for ACI on the rest of the genome. We started a breeding procedure using a single F2 rat derived from an intercross of the FHH/Eur and ACI/Eur grandparental strains. The F2 rat selected was used as part of the linkage analysis (18), developed marked UPV following UNX, and was found to be homozygous for the FHH alleles in the region of chromosome 1 covering the complete \( Rf-I \) QTL, as identified in our previous backcross study (1). The F2 rat was backcrossed with three ACI females. Six female offspring from this N1 generation were again backcrossed with ACI males to obtain the N2 generation and to introduce the Y chromosome of the ACI into the genome. This N2 generation was genotyped for 15 genetic markers within the \( Rf-I \) region and 138 markers distributed with an average interval size of ~10 cM (Table 1). Genotyping was used to identify male breeders that were heterozygous for the \( Rf-I \) QTL or a subset of the \( Rf-I \) region, and had the largest number of homozygous ACI alleles on the remaining part of the genome. The best male breeders were then crossed with three ACI females to obtain the N3 generation. The breeder selection and backcross procedure was repeated twice by crossing selected male breeder with ACI females for the N4 and N5 generation.

The N5 generation resulted in several males and females that were heterozygous for the \( Rf-I \) QTL overlapping congenic, while carrying all other chromosomal markers homozygous (validated by genotyping) from the ACI strain. The \( Rf-1B \Delta1Mit34 \text{ and } \Delta1Rat156, \text{ Fig. 1}) congenic was selected for study first, because it had one of the largest segments of the \( Rf-I \) region. The official designation for this congenic strain is ACLFHH-D1Mit34/Rat156, although we will refer to it as the ACLFHH-Rf1B congenic strain.

**Genotyping.** Genomic DNA was extracted from the tail tips of the backcross and intercross progeny by standard methods (9). The samples collected in Rotterdam were sent to Milwaukee, WI, for further analysis. The DNA was diluted to 4 ng/\( \mu \)l.

**Table 1. Chromosomal markers used during the generation of the congenic strain**

<table>
<thead>
<tr>
<th>Chr</th>
<th>N</th>
<th>Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23</td>
<td>Mgh12, Mgh13, Mgh14, Mgh20, Mgh25, Mgh33, Mit2, Mit3, Mit8, Mit14, Mit15, Mit18, Mit28, Mit30, Mit34, Rat75, Rat86, Rat118, Rat119, Rat142, Rat156, Rat194, Rat235</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>Mgh1, Mgh3, Mgh11, Mgh13, Mgh29, Mit1, Mit5, Mit25, Mit30, Rat1</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>Mgh1, Mgh4, Mgh21, Mgh25, Mit3, Mit4, Mit10, Mit13, Mit17, Rat44, Rat50</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>Mgh13, Mgh22, Mgh24, Mgh26, Mit18, Mit22</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>Mgh1, Mgh5, Mgh16, Mgh20, Mit2, Mit11, Mit13, Mit15</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>Mgh4, Mgh9, Mgh11, Mit1, Mit9, Mit10, Mit12, Mit16</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>Mgh8, Mgh9, Mit4, Mit9, Mit13, Mit16, Mit28, Rat29</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>Mgh4, Mit17, Mit2, Mit13, Mit15, Mit12</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>Mgh2, Mgh3, Mgh8, Mit1, Mit2, Mit3, Mit7, Mit9, Rat1</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>Mgh10, Mit11, Mit13, Mit14, Mit16, Rat58</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>Mgh2, Mgh4, Mit1, Rat20</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>Mgh10, Mit2, Mit4, Rat5</td>
</tr>
<tr>
<td>13</td>
<td>6</td>
<td>Mgh2, Mgh6, Mgh18, Mit3, Mit6, Mit7</td>
</tr>
<tr>
<td>14</td>
<td>7</td>
<td>Mgh2, Mgh7, Mit1, Mit2, Mit4, Mit11, Rat29</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>Mgh2, Mit12, Mit2, Mit4, Mit6</td>
</tr>
<tr>
<td>16</td>
<td>5</td>
<td>Mgh3, Mgh5, Mgh9, Mit2, Rat15</td>
</tr>
<tr>
<td>17</td>
<td>8</td>
<td>Mgh5, Mit1, Mit3, Mit7, Mit8, Pas1, Rat8, Rat46</td>
</tr>
<tr>
<td>18</td>
<td>6</td>
<td>Mgh4, Mgh7, Mit6, Mit12, Mit16, Mit17</td>
</tr>
<tr>
<td>19</td>
<td>5</td>
<td>Mgh2, Mgh8, Mit4, Rat18, Rat21</td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>Mgh2, Mit1, Mit4, Uw1</td>
</tr>
<tr>
<td>X</td>
<td>4</td>
<td>Mgh1, Mit4, Mit5, Mit7</td>
</tr>
</tbody>
</table>

Chr, chromosome; N, no. of animals.
RENAL FAILURE SUSCEPTIBILITY IN CONGENIC RATS

ACI.FHH-Rf1B adrenal gland and associated connective tissue. with ethyl ether, and the right kidney was removed after exposure of rats to UNX is a frequently used procedure to accelerate the susceptibility, we used the changes in level of UPV and UAV following UNX during a 24-wk follow-up period as an index. To determine the renal susceptibility, we used the changes in level of UPV and UAV following UNX during a 24-wk follow-up period as an index. In rats, UNX is a frequently used procedure to accelerate the development or progression of renal damage. We and others have reported that large genetic differences in renal damage exist among inbred rat strains (15, 32). Furthermore, UNX was used in our second linkage analysis study to markedly accelerate the development of UPV and to confirm the importance of RF-1 in influencing renal susceptibility.

In this first protocol, a total of 12 male ACI and 11 male ACFHHRf1B congenic rats were used. All rats were subjected to UNX at about 5–6 wk of age. The animals were anesthetized with ethyl ether, and the right kidney was removed after exposure through a midline incision and careful separation from the adrenal gland and associated connective tissue.

However, since the ACI.FHH-Rf1B congenic strain was anticipated to have a low systemic blood pressure similar to ACI and only one of the five susceptibility genes, we expected to see rather little renal damage within a limited 24-wk period after UNX. Previously, we reported that lowering blood pressure with an angiotensin converting enzyme inhibitor was very effective in preventing the development of UPV and FGS following UNX in FHH rats (20). Thus we decided to also test the effect of combining UNX with an increase in systemic blood pressure. Previously, we have shown that L-NAME-induced hypertension is a very efficient way to unravel differences in renal susceptibility in inbred rat strains (26–28).

In this second protocol, we used 10 male ACI and 10 male ACI.FHH-Rf1B congenic rats. As in the first protocol, the rats were subjected to UNX at 5–6 wk of age. After recovery from surgery (about 3–4 days), a chronic treatment with L-NAME (150 mg/l) in their drinking water was started and continued during a 24-wk follow-up period. The dose of L-NAME was chosen because it was shown previously to cause an increase in SBP to a level of about 150–160 mmHg in ACI rats with UNX (28). This level is similar to the SBP level normally present in the young adult FHHR (21, 27, 30).

Testing renal susceptibility. To determine the renal susceptibility, we used the changes in level of UPV and UAV following UNX during a 24-wk follow-up period as an index. In rats, UNX is a frequently used procedure to accelerate the development or progression of renal damage. We and others have reported that large genetic differences in renal damage exist among inbred rat strains (15, 32). Furthermore, UNX was used in our second linkage analysis study to markedly accelerate the development of UPV and to confirm the importance of RF-1 in influencing renal susceptibility.

In this first protocol, a total of 12 male ACI and 11 male ACFHHRf1B congenic rats were used. All rats were subjected to UNX at about 5–6 wk of age. The animals were anesthetized with ethyl ether, and the right kidney was removed after exposure through a midline incision and careful separation from the adrenal gland and associated connective tissue.

However, since the ACI.FHH-Rf1B congenic strain was anticipated to have a low systemic blood pressure similar to ACI and only one of the five susceptibility genes, we expected to see rather little renal damage within a limited 24-wk period after UNX. Previously, we reported that lowering blood pressure with an angiotensin converting enzyme inhibitor was very effective in preventing the development of UPV and FGS following UNX in FHH rats (20). Thus we decided to also test the effect of combining UNX with an increase in systemic blood pressure. Previously, we have shown that L-NAME-induced hypertension is a very efficient way to unravel differences in renal susceptibility in inbred rat strains (26–28).

In this second protocol, we used 10 male ACI and 10 male ACI.FHH-Rf1B congenic rats. As in the first protocol, the rats were subjected to UNX at 5–6 wk of age. After recovery from surgery (about 3–4 days), a chronic treatment with L-NAME (150 mg/l) in their drinking water was started and continued during a 24-wk follow-up period. The dose of L-NAME was chosen because it was shown previously to cause an increase in SBP to a level of about 150–160 mmHg in ACI rats with UNX (28). This level is similar to the SBP level normally present in the young adult FHHR (21, 27, 30).

Phenotypes. In both protocols, at 8, 16, and 24 wk after UNX, we determined the levels of UPV and UAV and SBP. To collect urine, animals were kept in metabolic cages (Tecniplast Gazzada, Buguggiate, Italy) and allowed to adapt to the new situation over the weekend. Urine was then collected during two consecutive 24-h periods. Urinary protein and albumin concentrations were determined colorimetrically using pyrogallol red/molybdate complex (31) and bromocresol green (4), respectively. Plasma and urine creatinine levels were measured with the Jaffe method without deproteinization. All assays were carried out using an automatic analyzer (ELAN; Eppendorf/Merck, Darmstadt, Germany).

The SBP was measured by the indirect tail-cuff plethysmography in awake, but restrained animals, using a semi-automatic system (model 1279; ITLC Life Science, Woodland Hills, CA). Animals were trained by exposing them to the restraint during the week prior to taking the measurements. For blood pressure determinations, at least three measurements were taken on each of three consecutive days. The mean of these three values was used for analysis.

After completion of the last urine collections and the blood pressure measurements, the rats were killed. A blood sample was obtained, and the kidney and heart were removed and weighed. The kidney was fixed and embedded in paraffin. Sections (3 μm thick) were stained with hematoxylin and eosin and with periodic acid-Schiff reagent. The incidence of FGS was scored in at least 50 glomeruli and expressed as the percentage of glomeruli with sclerotic lesions, as described earlier (21, 26).

Statistical analysis. Data are presented as means ± SE unless stated otherwise. Comparison of the differences between the various parameters in the different strains in the two protocols was done by Student’s t-test. P < 0.05 was used as an indication of statistical significance.

RESULTS

We used UNX to accelerate the onset of UPV and the progression of renal disease in our F2 intercross (19). We used the same stimulus here. How UNX accelerates the renal disease is not known, but it is not simply a result of increasing SBP, as the congenics only had a 7 mmHg increase over that of the ACI (Fig. 2A). However, the effect on UPV was more pronounced. At 8 wk following UNX, normotensive ACFHH-Rf1B congenic rats already showed a small, but significant increase in UPV and UAV compared with normotensive ACI rats. The difference between the congenics and the ACI rats increased with time. At the end of the follow-up, the levels of UPV were 23.8 ± 3.2 and 36.6 ± 3.2 μg/day for ACI and the congenics, respectively (P = 0.002). The values for UAV amounted to 10.4 ± 1.5 and 26.6 ± 3.2 μg/day (P < 0.001) (Fig. 2, B and C). The increases in UPV over time is not likely to be the result of the small increase in blood pressure, and although this is significant, it is far lower than the ≈400 mg/day we would see in the FHHR after UNX and at this age. For positional cloning studies
and other physiological studies, we would like a greater level of UPV. Therefore, we set out to determine the impact of introducing a moderate level of hypertension (to the level of the FHH) in the presence of UNX.

To increase SBP, we used chronic treatment with L-NAME. This treatment increased SBP to a level of about 150–160 mmHg. All untreated rats as well as the L-NAME-treated ACI rats survived the complete 24-wk follow-up period. In contrast, 4 of 10 L-NAME-treated ACI.FHH-Rf1B congenic rats died prematurely due to renal and/or cardiac failure, indicating that Rf-I region introgressed is having a major impact on the renal phenotype and its impact is markedly influenced by increasing SBP. At 8 and 16 wk the SBP of the congenics was about 12 mmHg higher compared with ACI. This difference had disappeared at the time of the final measurement, after 24 wk of follow-up (Fig. 3A).

Combining UNX with L-NAME-induced mild systolic hypertension unveils marked differences between the congenics and the ACI rats in UPV. Already after 8 wk, the ACI.FHH-Rf1B congenics developed marked increases in UPV and UAV, to a level of 60 and 46 mg/day, respectively. The L-NAME-treated congenics showed a threefold increase above the congenics in both UPV and UAV, to 182 ± 11 and 150 ± 9 mg/day, respectively. In contrast, the final UPV and UAV levels of the L-NAME-treated ACI rats were 47.8 ± 4.0 and 30.6 ± 3.4 mg/day, respectively, both being significantly different (P < 0.001) from the levels of L-NAME-treated congenic rats. Collectively, these data indicate that there is some type of interaction between the Rf-I region and blood pressure that will be of interest to physiologists, as well as a more robust phenotype for the positional cloning project.

In addition to protein and SBP, we also assessed morphometric traits and indices of glomerular function. Kidney and heart weight, the incidence of FGS,
the plasma creatinine levels and calculated creatinine clearances for both protocols are presented in Table 2. Heart weight of the L-NAME-treated ACI and congenic rats was significantly higher compared with the non-hypertensive rats. Wet kidney weight did not differ significantly between the four groups. In contrast, significant differences were present in the incidence of FGS. The incidence of FGS of normotensive congenics was significantly higher than that of the normotensive ACI rats. The increase in FGS within a normotensive level of SBP demonstrates that the Rf-1 gene is inducing FGS via some other mechanism. When SBP was increased by L-NAME, there was a small increase in the incidence of FGS in the ACI control. However, the incidence of FGS in the hypertensive congenics was significantly higher, amounting to over 50% of affected glomeruli, which points to the interaction between the SBP and the Rf-1 gene.

Plasma creatinine level was found to be increased significantly in the L-NAME-treated congenics. This increase in plasma creatinine was also reflected in a reduced creatinine clearance, which was statistically different in the L-NAME-treated congenics, when compared with the L-NAME-treated ACI rats. These data provide an estimate for glomerular filtration rate and indicate that the kidneys of the congenic animals are profoundly altered by introgressing a relatively small (~1%) of the genome onto the ACI background that is relatively protected from renal disease (26–28). Thus all renal parameters taken together indicate that renal function in the L-NAME-treated congenics was most adversely affected.

**DISCUSSION**

The primary finding of the present study, using a newly generated congenic rat strain (ACIFHH-D1Mit34/Rat156), is the increase in susceptibility to develop renal damage after transferring the Rf-1 QTL from the susceptible FHH strain into the renal resistant ACI rat genomic background. The Rf-1 region is one of the five QTLs, named Rf-1 to Rf-5, that we have previously identified by linkage analysis to markedly influence the development of renal disease in the FHH rat (1, 19). The transfer of the Rf-1 region from FHH to ACI has a slight effect on blood pressure. The increase in pressure observed in the congenic may be due to a closely linked gene, as our linkage data had a suggestive "logarithm of the odds ratio" (LOD) score of 2.7 for SBP. The increase in UPV and UAV was smaller, albeit significant, than we expected from the linkage analysis, suggesting that the gene interactions predicted by Shiozawa et al. (19) are likely to be true.

Based on our previous studies, we anticipated that raising the SBP to the mildly systolic hypertensive level normally present in FHH may reveal the increased renal susceptibility of the congenic strain, as indicated by the development of marked UPV. Although not tested in the present study, previous findings indicate that the development of UPV in the FHH rat (carrying all five Rf-QTLs) is more pronounced. At 24 wk after UNX, the level of UPV will be in the order of 400–500 mg/day, which is well above the 180 mg/day found in the ACI.FHH-Rf1B congenic. Thus about 35–40% of the UPV found in the FHH appears to be explained by the Rf-1 gene(s) from FHH. This is more than indicated by the linkage analysis. Following UNX, about 20% of the genetic variation in UPV was explained by the Rf-1 QTL (19). As the mean SBP of the F2 animals was significantly lower (133 ± 11 mmHg) than the congenics treated with L-NAME, it may be that this ~20 mmHg increase in blood pressure in the congenics accounts for the difference in UPV found.

Our study provides the first direct evidence that the presence of gene(s) that influence renal susceptibility can be markedly influenced by mild to moderate systolic hypertension. Differences in genetic susceptibility to hypertension-induced renal damage have been studied by Churchill et al. (2). They performed elegant studies transplanting BN rat kidneys into SHR-RT1.N recipients, i.e., spontaneously hypertensive rats (SHR) carrying the major histocompatibility complex from the Brown Norway (BN) strain. This permits renal transplantation free of rejection damage, allowing two genetically different kidneys to be studied under identical conditions. At an SBP level of 200–220 mmHg, induced by deoxycorticosterone acetate (DOCA)-salt treatment on top of the spontaneous hypertension present in SHR, the BN kidneys were shown to develop more severe damage than those from SHR. This indicates that BN kidneys are more susceptible to renal damage than kidneys from SHR. The same group re-

**Table 2. Body, heart, and kidney weights, incidence of FGS, plasma creatinine levels, and calculated creatinine clearances**

<table>
<thead>
<tr>
<th></th>
<th>ACI (n = 12)</th>
<th>ACI-N (n = 10)</th>
<th>Rf-1B (n = 11)</th>
<th>Rf-1B-N (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>303 ± 12</td>
<td>301 ± 17</td>
<td>281 ± 15†</td>
<td>296 ± 27</td>
</tr>
<tr>
<td>Heart wt, mg</td>
<td>768 ± 25</td>
<td>905 ± 85*§</td>
<td>740 ± 40†</td>
<td>976 ± 92†§</td>
</tr>
<tr>
<td>Kidney wt, mg</td>
<td>1,596 ± 96</td>
<td>1,577 ± 89</td>
<td>1,558 ± 142</td>
<td>1,689 ± 174</td>
</tr>
<tr>
<td>FGS, % glomeruli</td>
<td>3.8 ± 2.5</td>
<td>17.0 ± 9.2§</td>
<td>12.9 ± 3.9§</td>
<td>52.0 ± 7.2†§</td>
</tr>
<tr>
<td>Plasma creatinine, umol/l</td>
<td>64 ± 6</td>
<td>61 ± 4</td>
<td>65 ± 6</td>
<td>81 ± 7*§</td>
</tr>
<tr>
<td>Creatinine clearance, ml/min</td>
<td>0.92 ± 0.13</td>
<td>1.04 ± 0.19</td>
<td>0.88 ± 0.13†</td>
<td>0.82 ± 0.13†</td>
</tr>
</tbody>
</table>

Values are means ± SD in the four experimental groups obtained at the end of the 24-wk follow-up period after unilateral nephrectomy at 5–6 wk of age; n = no. of rats. ACI, ACI rat; ACI-N, ACI treated with N’-nitro-l-arginine methyl ester (l-NAME); Rf1B, ACI.FHH-D1Mit34/Rat156 congenic strain; Rf1B-N, congenic treated with l-NAME; FGS, incidence of focal glomerulosclerosis. †P < 0.05 vs. ACI, †P < 0.05 vs. ACI-N, and §P < 0.05 vs. Rf1B (ANOVA followed by the Student-Newman-Keuls test for multiple comparisons).
ported further evidence obtained in SHR.BN-D1Mit3/1gfl congenic rats (23). These SHR rats are congenic for a 22-cM segment of the BN rat, between the markers D1Mit3 and 1gfl on chromosome 1, which most probably includes the Rf-2 region. Previously, it was shown that this region affected blood pressure (24). After 33 days of severe hypertension (SBP, 200–220 mmHg), UPV was higher in the congenics than in the SHR progenitor strain, with structural renal damage characteristic of malignant nephrosclerosis, which was primarily vascular damage. Thus the region between D1Mit3 and 1gfl2 may also carry a gene that influences the development of hypertension-associated renal damage.

The present study does not give any indication which gene(s) within the Rf-1 region is responsible for the increased renal susceptibility. However, it does validate the region and provides a good rationale for us to develop and examine the congenics carrying smaller intervals, thereby reducing the number of genes within the QTL that must be investigated.

For the field at large, our data demonstrate for the first time that in the presence of a renal susceptibility gene, such as Rf-1, even mild levels of systolic hypertension (~150 mmHg) can lead to progressive renal impairment (Fig. 3). Detailed studies of the renal physiology of the congenic strains can help to unravel potential pathways that may explain an enhanced susceptibility and may thus facilitate the identification of candidate genes. In this regard, it may be of interest that in the Rf-1 region, a QTL influencing renal hemodynamics has been identified in a F2 cross of SHR and Wistar-Kyoto (WKY) rats (5). The QTL appears to be responsible for the differences in renal resistance between SHR and WKY rats. In FHH, impaired renal autoregulation underlies the high renal susceptibility to renal damage (25, 29). Future studies in ACI.FHH-Rf-1B congenics will determine whether impaired renal autoregulation is also involved in the renal susceptibility in this strain.

In conclusion, by studying the effects of UNX alone or in combination with L-NAME-induced hypertension in a newly derived congenic strain, we found direct evidence for the presence of one or more genes in the Rf-1 region with a prominent effect on the susceptibility to develop progressive renal damage. The interaction between this gene and blood pressure is likely to increase our understanding of how even a mild hypertension can lead to end-stage renal failure in some patients.

We thank M. van Aken, A. P. Boijmans, I. M. Hekking-Weyma, C. Luhrman-Scholmski, J. Mahabier, and C. Peekstok at Erasmus University, Rotterdam, for excellent technical assistance.

Studies were performed with financial support from National Institutes of Health Grant RO1 HL-56284 (to H. J. Jacob and A. P. Provoost) and the Dutch Kidney Foundation Grant C 98:1775 (to A. P. Provoost), and this work is part of EURHPYGEN II Concerted Action within the Biomed 2 program of the European Union.

REFERENCES


Physiol Genomics • vol 8 • www.physiolgenomics.org