ABSTRACT

The Dahl salt-sensitive hypertensive (S) rat develops albuminuria early in life even on a low-salt diet. In contrast, the spontaneously hypertensive rat (SHR) is highly resistant to developing albuminuria despite elevated BP. An F1 hybrid of S and SHR showed a low urinary albumin excretion (UAE) and low urinary protein excretion (UPE) similar to SHR, i.e., SHR was dominant. A genetic analysis was carried out on a large recessive S strain; the population was fed a low-salt diet. Genome scans done at 8, 12, and 16 wk of age yielded ten quantitative trait loci (QTL) for UAE and/or UPE with variable time-course patterns on nine rat chromosomes (RNO), i.e., RNO1, RNO2, RNO6, RNO8, RNO9, RNO10, RNO11, RNO13, and RNO19. There were two UPE QTL on RNO6. At most of the UAE and/or UPE QTL, the S allele was associated with increased excretion, except for one of the QTL on RNO6 and the QTL on RNO11, where the S allele caused decreased excretion. Only the UAE and UPE QTL on RNO10 co-localized with a BP QTL. The S allele on RNO10 caused higher BP and higher UAE. Two additional BP QTL were detected on RNO1 and RNO6. Most of the UAE and UPE QTL co-localized with QTL for kidney lesions characteristic of S rats. Multiple interactions were observed for UAE, many of which involved RNO2. In summary, UAE is highly polygenic and the majority of the QTL altering UAE do not co-localize with QTL for BP as evaluated by tail-cuff measurements of BP.

End-stage renal disease (ESRD) is an important medical problem. Clinical observations suggest that malignant hypertension leads to renal failure and that less severe hypertension will accelerate the progression of renal disease (1–4). Moreover, the clinical impression is that there are factors other than hypertension also influencing the progression of renal disease. The best example being the high incidence of ESRD in hypertensive black patients compared with hypertensive white patients (5–12), leading to the idea that genetic factors independent of BP influence the progression of renal disease. Also, the relatives of people with ESRD have an increased risk of ESRD in both black (13) and white (14) Americans.

In the 1960s, Dahl selectively bred rats for sensitivity (S rats) to the hypertensive effect of high-salt diet (15). Besides fulminant hypertension, S rats on high-salt diet develop progressive proteinuria (16,17) and severe renal vascular and glomerular lesions and associated renal tubular lesions (18–21). In a careful longitudinal study, Sterzel et al. (17) showed that 5 to 6-wk-old S rats have proteinuria associated with segmental retraction of podocyte foot process. The early phase of hypertension was not associated with an overall loss of renal function, lower number of glomeruli, or glomerular hypertrophy. Of course, as hypertension progressed, vascular and glomerular lesions and loss of renal function became marked (17). BP of S rats has undergone extensive genetic analysis as has the spontaneously hypertensive rat (SHR). Despite the hypertension in SHR, the progression of renal lesions appears slow (24) compared with the development of such lesions in S rats. Karlsen et al. (25) have directly compared S and SHR fed a high-salt diet for 4 wk. Histologically, the SHR kidneys were essentially normal, whereas the S kidneys showed significant lesions. As will be seen such differences are dramatically reflected by differences in urinary albumin excretion (UAE) between the two strains.

To perform a genetic analysis, it is often useful to produce a segregating population by crossing S rats to a contrasting strain. There is an interesting contrast between the Dahl S rat and the spontaneously hypertensive rat (SHR). Despite the hypertension in SHR, the progression of renal lesions appears slow (24) compared with the development of such lesions in S rats. Karlsen et al. (25) have directly compared S and SHR fed a high-salt diet for 4 wk. Histologically, the SHR kidneys were essentially normal, whereas the S kidneys showed significant lesions. As will be seen such differences are dramatically reflected by differences in urinary albumin excretion (UAE) between the two strains.

Our experimental design here was to produce a segregating population derived from S and SHR and to follow UAE longitudinally. In this initial work, the rats were fed a low-salt diet to minimize changes in BP. A genetic analysis at each time point yielded several strong quantitative trait loci (QTL) controlling UAE, most of which were independent of known QTL for BP.

MATERIALS AND METHODS

Animals

Inbred Dahl salt-sensitive (SS/Jr) rats were from our colony at the Medical College of Ohio, Toledo, and will be referred to as S. Spontaneously hypertensive rats (SHR/NHsd) were from Harlan
Sprague-Dawley (Indianapolis, IN) and will be referred to as SHR. To determine UAE in F1 animals, S females were crossed with SHR males to produce F1(SxSHR) animals. S, F1(SxSHR), and SHR (n = 6 in each group) males (all age-matched) were studied for UAE, urinary protein excretion (UPE), and BP at 8, 12, 20, and 26 wk of age.

A backcross population, F1(SxSHR)xS, of 276 male rats was produced by crossing F1 females to S males. Rats were weaned at 30 d of age and placed on a low-salt (0.3% NaCl) diet (diet TD 7034; Harlan Teklad, Madison, WI) for the duration of the experiment. The backcross population was studied for the progression of UAE, UPE, and BP at 8, 12, and 16 wk of age.

The 276 rats were handled in four blocks of 69 animals. Of the 69 rats in each block, a subset of 48 was selected at random for BP determination, but urine was collected on all 69 rats in each block. Rats in each block were closely age-matched, but between blocks the average ages differed by a few days. This allowed BP and urine collections to proceed on all rats at approximately the same ages. Block effects, if any, were removed statistically before further analysis.

Rats were killed by CO2 inhalation. Liver and kidneys were harvested and weighed. Pieces of liver were archived at -80°C for subsequent DNA extraction. The right kidney was fixed and embedded in paraffin for subsequent sectioning and staining. Body, heart, and kidney weights were measured. Adjusted heart weight or adjusted kidney weight was calculated by adjusting organ weight for differences in body weight by regression analysis using programs from SPSS (Chicago, IL).

**Blood Pressure**

A subset consisting of 192 backcross animals were selected (as noted above) to measure systolic BP by the tail-cuff method on conscious restrained rats warmed to 28°C. BP measurements were made between 7 a.m. and 12 p.m. Starting at 8 wk of age, BP was measured on two consecutive days by two operators at two workstations. Each rat had one session with each operator. Three to four consistent BP measurements were taken at each session and averaged for that day’s BP reading. The final BP of the rat was the average of both sessions. BP measurements were again taken at 12 and 16 wk of age for all 192 animals.

**UAE and UPE Determination**

Urine was collected for all 276 backcross animals. To collect urine, animals were kept in metabolism cages (Lab Products, Seafood, Delaware) for 24 h. Sodium azide was added to the collection vials to provide about 0.1% in the urine. Food was withheld, but the animals had free access to water. UAE was determined colorimetrically using pyrogallol red/molybdate complex (Quantimetrix, Redondo Beach, CA). UPE was expressed as mg protein/24 h. At 8 wk of age, UAE was determined by a rat albumin EIA kit (SPL-bio, France) and also by SDS-PAGE. The correlation between the albumin determined using the EIA kit and SDS-PAGE was r = 0.89. Subsequent UAE determinations (weeks 12 and 16) were done using SDS-PAGE because it was more efficient and cost-effective than the EIA. All albumin measurements reported here were from SDS-PAGE and were done as follows. Urine samples were loaded on 10% Criterion Tris-HCL precast gels (Biorad, Hercules, CA) along with albumin standards. The gels were stained with Bio-Safe Coomassie Stain (Biorad, Hercules, CA), destained in water, and then scanned using a HP scanjet 5300C scanner (Hewlett Packard, Palo Alto, CA). The gels were analyzed using Scion Image software (Scion Corp, Frederick, MD). UAE is expressed as mg albumin/24 h.

**Histology**

Kidney sections were stained with hematoxylin and eosin and graded in a blinded fashion on an arbitrary semiquantitative scale from 0 to 4 for kidney lesions. One central longitudinal section was made through the right kidney, and the entire section was examined. The lesions observed were characteristic of S rats and apparently start as glomerular sclerosis in isolated glomeruli. These glomeruli leak protein, leading to tubular casts of protein, tubular cell degenerative changes, and areas of pronounced tubular regeneration. Vascular lesions include arterial wall thickening, cell proliferation, and necrosis. The glomerular and tubular changes occur in individual nephrons with adjacent glomeruli and their tubules intact. Thus the cortex becomes streaked with areas of damage interspersed with relatively normal tissue. Foci of lymphocytic infiltration are sometimes present. The kidney lesion grades (KLG) were 0 = normal, 1 = mild, 2 = moderate, 3 = marked, 4 = severe. It was possible to assign half grades so the grades were 0, 0.5, 1, 1.5, etc. The grades represent a visually integrated assessment of the severity and extent of the lesions. No attempt was made to grade the individual components (e.g., glomeruli, tubules, vasculature) separately.

**Genotyping**

Genomic DNA was prepared from liver samples using DNeasy 96 tissue kits (Qiagen, Valencia, CA). Genotyping was done using microsatellite markers amplified by the PCR and evaluated by electrophoresis as previously reported (26). Markers polymorphic between S and SHR were selected from the following sources: (1) The Whitehead Institute for Biomedical Research (www.genome.wi.mit.edu); (2) Wellcome Trust Center for Human Genomics (Oxford, UK; www.well.ox.ac.uk); (3) Medical College of Ohio, Department of Physiology (www.mco.edu/depts/physiology/research); and (4) from references 27 and 28. A total of 174 markers approximately evenly spaced throughout the genome were used.

**Linkage and Statistical Analysis**

Linkage analysis and QTL localization were performed using Mapmaker/EXP and Map program (29–31) and Map Manager QTX (32). The Map Manager QTX program offers an easier method than Mapmaker for conducting QTL analysis on multiple traits. Once a preliminary QTL analysis was done using Map Manager and potential QTL were identified, Mapmaker/QTL was used to generate an LOD plot. For a backcross population, an LOD score of at least 1.9 is considered evidence for suggestive linkage and a LOD score of 3.3 or above indicates significant linkage between phenotype and genotype (33). Determination of the “phenotypic effect” for phenotypes with suggestive or significant linkage to a chromosome was done by selecting one index marker at or near the QTL peak. The phenotypic effect was calculated as the average phenotype of rats with the S/S genotype minus the average phenotype of rats with the S/SHR genotype at the selected marker.

**Interactions**

Interactions throughout the genome were examined using a computer program provided by Dr. Gary Churchill (www.jax.org/research/churchill). The method examines all pairs of marker loci for an interaction with a given phenotype, in this case, UAE. The program calculates an F_{adj} statistic from a full regression model, which assumes that the marker pair represents two QTL interacting to affect UAE.
versus the null hypothesis of no effect at either locus. The significance threshold for \( F_{\text{all}} \) was determined by permutation analysis. For marker pairs that had an \( F_{\text{all}} \) statistic above the significance threshold, a second \( F \) statistic (\( F_{\text{int}} \)) was computed to compare a model wherein the marker pair represent two interacting QTL with a model wherein the marker pair represent two QTL acting additively to affect UAE. Interactions between two markers were accepted when both the \( F_{\text{all}} \) statistic and \( F_{\text{int}} \) were above the significance threshold as determined by permutation analysis.

**Results**

Table 1 gives data for BP and body weights for S, SHR, and their \( F_1 \) cross, i.e., \( F_1(S\times SHR) \), for an 18-wk period starting at 8 wk of age. There was little difference in BP between S and SHR except that S were higher at week 8, and the development of hypertension in \( F_1(S\times SHR) \) rats lagged behind the parental strains at week 12 and 20. Body weight was always larger in the \( F_1 \) hybrids than either parental strain. At the end of the 18-wk period, there were no meaningful differences in heart or kidney weight adjusted for differences in body weight. The data demonstrate that, like the SHR, the inbred Dahl S rats from our colony slowly develop hypertension even on a low-salt diet.

In contrast to the similarity of BP among S, SHR, and their \( F_1 \) hybrid, there were dramatic differences in UAE and UPE among groups. S rats have markedly higher UAE and UPE than SHR at all time points (Figure 1) regardless of only minor strain differences in BP (Table 1). The \( F_1(S\times SHR) \) were indistinguishable from SHR with regard to UAE or UPE, that is, the SHR phenotype was strongly dominant to the S phenotype at all time points. In this situation, a backcross to the recessive (S) strain is appropriate for a genetic analysis.

A large \( F_1(S\times SHR)\times S \) population (\( n = 276 \)) was produced; population data for BP, UAE, UPE, body weight, and KLG are given in Table 2. The distributions of UAE and UPE at all time points were markedly skewed to the right for this population. Thus all analyses of UAE and UPE for linkage and interactions were carried out on the natural logarithm (ln) of these data to approximate normal distributions. Representative frequency distributions are shown for UAE (Figure 2A) and lnUAE (Figure 2B) at week 12. The ln-transformed data are also suggestive of a bimodal distribution with three fourths of the rats in the lower mode and one fourth in the higher mode. This bimodality was seen only with the UAE distributions and was not present in the UPE distributions.

Genome scans for BP, lnUAE, and lnUPE were done at weeks 8, 12, and 16 on the \( F_1(S\times SHR)\times S \) population maintained on low-salt (0.3% NaCl) diet. Kidney lesion grade (KLG) was also studied at week 16 after killing the animals. The data are presented as LOD plots in Figure 3 for all rat chromosomes (designated RNO for *Rattus norvegicus*) on which a quantitative trait locus (QTL) was observed for any measurement. Weak signals for BP QTL (dark blue lines in Figure 3) were seen on RNO1 at all time points, weakly on RNO2 only at week 16, and on RNO6 becoming progressively stronger with time. The BP QTL on RNO10 was particularly interesting because it was undetectable at week 8, became highly significant at week 12, and then attenuated at week 16.

In general, QTL for UAE and UPE (red and orange lines, respectively, in Figure 3) were detected together, although higher LOD scores were usually seen with UAE. The strongest UAE QTL was on RNO2, which gave LOD scores from 10 to 13 from week 8 persisting through week 16. Significant UAE and/or UPE QTL were also seen on RNO1, RNO6, RNO8, RNO9, RNO10, RNO11, RNO13, and RNO19. Most of these

<table>
<thead>
<tr>
<th>Week 8</th>
<th>S</th>
<th>( F_1(S\times SHR) )</th>
<th>SHR</th>
<th>One-Way ANOVA, ( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP (mmHg)</td>
<td>168 ± 5.1</td>
<td>152 ± 5.4</td>
<td>148 ± 4.7</td>
<td>0.039</td>
</tr>
<tr>
<td>body weight (g)</td>
<td>293 ± 9.8</td>
<td>304 ± 6.4</td>
<td>203 ± 6.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Week 12</td>
<td>BP (mmHg)</td>
<td>182 ± 4.1</td>
<td>163 ± 5.6</td>
<td>0.009</td>
</tr>
<tr>
<td>body weight (g)</td>
<td>384 ± 8.9</td>
<td>408 ± 6.1</td>
<td>285 ± 6.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Week 20</td>
<td>BP (mmHg)</td>
<td>193 ± 10.5</td>
<td>179 ± 7.5</td>
<td>0.49</td>
</tr>
<tr>
<td>body weight (g)</td>
<td>451 ± 18.5</td>
<td>503 ± 7.3</td>
<td>375 ± 13.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Week 26</td>
<td>BP (mmHg)</td>
<td>208 ± 4.6</td>
<td>192 ± 6.2</td>
<td>0.28</td>
</tr>
<tr>
<td>body weight (g)</td>
<td>485 ± 11.9</td>
<td>519 ± 7.9</td>
<td>392 ± 7.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>adjusted heart weight (g)</td>
<td>1.675 ± 0.018</td>
<td>1.599 ± 0.023</td>
<td>1.626 ± 0.012</td>
<td>0.042</td>
</tr>
<tr>
<td>adjusted kidney weight (g)</td>
<td>3.306 ± 0.140</td>
<td>3.130 ± 0.030</td>
<td>3.173 ± 0.027</td>
<td>0.273</td>
</tr>
</tbody>
</table>

\( n = 6 \) rats per group. Data are mean ± SEM. Data were analyzed by a one-way analysis of variance (ANOVA). Data are from the same rats used in Figure 1. Weeks given are the ages of the rats. S, salt-sensitive hypertensive rat(s); SHR, spontaneously hypertensive rat; \( F_1(S\times SHR) \), backcross population.
QTL were either present at week 8 and persisted through week 16 or became progressively more prominent with time. Two interesting exceptions were RNO10 and RNO13. On RNO10, the UAE and/or UPE QTL appeared strong early and were weaker at week 16. The UAE QTL on RNO13 was similarly very strong at week 8 but much reduced after that. RNO6 was unique in showing two UPE QTL on the same chromosome. The percentage of the total population variance explained by all the QTL was 25% for UPE and 68% for UAE at week 8 and varied from 57% to 70% for UPE and UAE at weeks 12 and 16.

At 16 wk of age the backcross rats were killed and kidneys were studied histologically. The population parameters for KLG are given in Table 2, and KLG was also analyzed for QTL at week 16 (light blue in Figure 3). RNO2 had the strongest KLG QTL corresponding to the UAE and UPE QTL. KLG QTL were observed at suggestive levels of significance concomitant with most other UAE or UPE QTL, i.e., those on RNO6, RNO8, RNO9, RNO11, RNO13, and RNO19. The exceptions were RNO1 and RNO10, where KLG QTL were not observed in the presence of the UAE and UPE QTL.

Besides the LOD plots, Figure 3 also gives the magnitude and direction of the observed effects for a microsatellite marker at or near the LOD peak. “Phenotypic effect” values are given only if the LOD plot was at least of suggestive significance. The phenotypic effect was defined at a given marker as the average phenotypic value for all rats homozygous for the S allele (S/S genotype) minus the average phenotypic value for all rats that were heterozygous, i.e., carried one allele from S and the other from SHR (S/SHR genotype). Because this was a backcross to S, these are the only genotypes present at each locus. A positive value for phenotypic effect means that S/S rats were higher than S/SHR; a negative value means that S/S were lower than S/SHR.

Table 2. Descriptive statistics for BP, UAE, UPE, body weight, and KLG for the F₁(SxSHR)xS population of 276 rats raised on 0.3% NaCl diet

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>week 8</td>
<td>147</td>
<td>15.2</td>
<td>106</td>
<td>187</td>
</tr>
<tr>
<td>week 12</td>
<td>156</td>
<td>16.4</td>
<td>113</td>
<td>206</td>
</tr>
<tr>
<td>week 16</td>
<td>159</td>
<td>16.1</td>
<td>129</td>
<td>212</td>
</tr>
<tr>
<td>UAE (mg/24 h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>week 8</td>
<td>3.6</td>
<td>5.37</td>
<td>0.09</td>
<td>46.8</td>
</tr>
<tr>
<td>week 12</td>
<td>4.0</td>
<td>5.78</td>
<td>0.0</td>
<td>37.7</td>
</tr>
<tr>
<td>week 16</td>
<td>4.9</td>
<td>7.04</td>
<td>0.05</td>
<td>40.1</td>
</tr>
<tr>
<td>UPE (mg/24 h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>week 8</td>
<td>9.4</td>
<td>6.39</td>
<td>2.0</td>
<td>58.9</td>
</tr>
<tr>
<td>week 12</td>
<td>21.9</td>
<td>9.83</td>
<td>7.8</td>
<td>62.1</td>
</tr>
<tr>
<td>week 16</td>
<td>23.5</td>
<td>12.78</td>
<td>9.2</td>
<td>110.3</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>week 8</td>
<td>250</td>
<td>20</td>
<td>205</td>
<td>296</td>
</tr>
<tr>
<td>week 12</td>
<td>371</td>
<td>17</td>
<td>319</td>
<td>427</td>
</tr>
<tr>
<td>week 16</td>
<td>428</td>
<td>22</td>
<td>362</td>
<td>490</td>
</tr>
<tr>
<td>KLG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>week 16</td>
<td>0.70</td>
<td>0.59</td>
<td>0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* UAE, urinary albumin excretion; UPE, urinary protein excretion; KLG, kidney lesion grade.
In general, BP effects of each QTL were modest (6 to 10 mmHg), and the S/SHR genotype was associated with increased BP on RNO1 and RNO6, and the S/S genotype increased BP on RNO2 and RNO10. Only the BP QTL on RNO10 was aligned with the UAE and UPE QTL and the S/S genotype increased BP, UAE, and UPE.

In evaluating magnitude and direction of effects of UAE or UPE QTL the mean of the ln-transformed values for each genotype was converted back to mg/24 h by taking the antilog. This yields the geometric mean value for each genotype. This was done to avoid giving results in ln units, which are difficult to envisage in physiologic terms. Thus the phenotypic effect values in Figure 3 for UAE or UPE are the difference between the geometric means for the two genotypes for a marker at or near the LOD peak. Most of the effects are modest in the range of 1 to 5 mg/24 h. As one might expect, the S/S genotype was associated with increased UAE or UPE at most QTL, i.e., on RNO1, RNO2, QTL 2 on RNO6, RNO8, RNO9, RNO10, RNO13, and RNO19. Only on RNO11 and QTL 1 on RNO6, was the S/SHR genotype associated with increased UAE and/or UPE. RNO6 was interesting in that for the two UAE QTL on RNO6, the S/S genotype decreased UAE at QTL 1 and the S/S genotype increased UPE at QTL 2 (Figure 3). In general, one does not expect to resolve such offsetting effects on the same chromosome unless, as in this case, the QTL are spaced far apart. For all UAE and/or UPE QTL, the direction of the phenotypic effect was always concordant with the direction of the phenotypic effect for the KLG QTL (Figure 3).

Figure 4 summarizes the pair-wise interactions observed in the F1(SxSHR)xS population for UAE. The figure plots a symbol in a position on a two-dimensional chromosomal grid corresponding to the two chromosomes involved in the interaction. Different symbols indicate interactions seen at week 8, 12, or 16. The microsatellite markers involved on each chromosome are indicated. Several pair-wise interactions involved markers of which both were at or near UAE QTL found by the initial linkage analysis. There were also several interactions involving only one marker near a QTL, the other marker of the pair being on a chromosome where no QTL was observable except through its interaction. RNO2 was highly interactive with several other chromosomes.

Figure 5 summarizes LOD plots for heart weight and kidney weight adjusted for differences in body weight. Of the six heart weight QTL observed, only one at D1Rat36 corresponded to a BP QTL. Of the three kidney weight QTL observed, two of them (at D1Rat86 and D19Uia1) corresponded reasonably well with UAE and/or UPE QTL. In both cases, increased kidney weight was associated with increased UAE. Where a heart weight or a kidney weight QTL fell on a chromosome shown in Figure 3, the position of the organ weight QTL is also indicated in Figure 3.

QTL for body weight were also observed in the backcross population. The following is a list of markers at the LOD peak if a body weight QTL was observed at any of the three time points studied: D2Rat82, D3Rat160, D10Mco57, D12Mgh3, D15Rat5, D17Mit7, D18Rat57. Markers D2Rat82 and D10Mco15 fall within the UAE and UPE QTL (Figure 3) and had their maximum phenotypic effect of -10 g at week 16 on RNO2 and 7.5 g at week 4 on RNO10.

**Discussion**

Our purpose in designing this work was to take advantage of the marked strain difference in UAE between S and SHR and to look early in the development of the rat for initial genetic...
causes of proteinuria. Which QTL and how many QTL are observed for any trait of course depends on the strains that are studied (22,34). Ten QTL for UAE and UPE were found, all with modest but significant effects. In general, a large number of QTL influencing a quantitative trait is not surprising. BP in S rats is influenced by as many as 16 QTL as summarized recently (23), and diabetes in the non-obese diabetic mouse, for example, is controlled by 17 QTL (35). As expected at the majority of QTL, the S/S genotype was associated with increased UAE and UPE. Considering the modest quantitative effects of each QTL and the young age of the rats, the correspondence of KLG QTL with the UAE and UPE QTL was striking. Our KLG grading system did not dissect lesions into individual components (e.g., glomerular versus tubular lesions) but rather was a semiquantitative composite of all lesions. As such, the main usefulness of the KLG was to show that the modest degrees of change in UAE and UPE associated with each QTL were sufficient to be reflected in histologically observable secondary renal damage.

UPE has been studied in several other rat strains. The Milan normotensive strain (MNS) develops glomerular sclerosis and severely increased UPE with aging in the absence of hyper-

![Image](image_url)
tension (36,37). Similarly aged BUF/Mna rats develop glomerular sclerosis and markedly increased UAE in the absence of hypertension. A genetic cross indicated that high UAE was strongly recessive (38), similar to the present work. A genome scan involving a backcross of \(F_1\) (BUFxWKY) to BUF yielded significant linkage only to a broad region of RNO13 (39), which is roughly consistent with our linkage result for UAE on RNO13.

The Fawn-Hooded hypertensive rat (FHH) develops progressive glomerular sclerosis and hypertension with aging. Again, proteinuria appeared recessive in a cross with ACI rats, and a genome scan for QTL was done on a backcross of \(F_1\) (FHHxACI) to FHH (40) as well as on an \(F_2\) (FHHxACI) population that had undergone unilateral nephrectomy (41). Both populations yielded strong evidence for two UPE QTL on RNO1.

The QTL were named Rf-1 and Rf-2 for renal failure 1 and 2. Rf-1 gave the strongest signal and was not associated with a QTL for BP. A congenic strain for the FHH QTL allele on the ACI background at Rf-1 was subsequently made and was shown to increase proteinuria after unilateral nephrectomy.
or induction of hypertension by inhibition of nitric oxide synthase (42).

Our results for the UAE and UPE QTL on RNO1 agree exceptionally well with Rf-1 data as to the location of the QTL and the absence of a co-located BP QTL. Rf-2 in contrast was co-localized with a QTL for BP (40,41). The BP QTL we observed on RNO1 is in the same location as Rf-2. Although we did not observe a second UAE or UPE QTL in the Rf-2 location, it is likely that such an effect would be observable if our rats were comparable to those in the FHH studies, i.e., older and/or unilaterally nephrectomized. In the more recent study on FHH (41), where the population studied was an F₂(FHHxACI) cross and the rats had been unilaterally nephrectomized, additional QTL for UAE and UPE were seen on RNO3, RNO14, and RNO17. No comparable QTL were seen in our work.

There are two strains of Sabra rats, SBH and SBN selectively bred for susceptibility or resistance, respectively, to the hypertensive effect of deoxycorticosterone plus increased dietary NaCl treatment. SBH rats develop proteinuria as well as salt-induced hypertension. Although a genome scan for UPE QTL was not done on these strains, consomic strains placing either RNO1 or RNO17 from SBH onto the SBN background were constructed on the basis of the location of previously described BP QTL in Sabra rats and on the basis of work with the FHH rats noted above. Under the condition of unilateral nephrectomy and aging, the consomic rats showed increased proteinuria compared
with the SBN controls, confirming the presence of UPE QTL on RNO1 and RNO17 (43).

The susceptibility of the kidney to damage by hypertension has been compared by kidney cross-transplantation studies between histocompatible SHR and Brown Norway (BN) rats. It was concluded that the BN kidney was inherently more susceptible to damage resulting from hypertension than the SHR kidney (44). A congenic strain with a segment of RNO1 that probably includes the Rf-2 region from BN was introgressed into SHR. This congenic strain was more susceptible to hypertension-induced renal damage than SHR (45).

In all of the above work quoted from the literature, the rat models are (a) studied with advanced age, (b) unilaterally nephrectomized, or (c) treated to exacerbate hypertension. This has its merits to stimulate phenotypic expression of UAE and UPE. In our experiment, the genetic background of 75% S and 25% SHR in the backcross population is obviously permissive for hypertension, but we kept the salt-sensitive component of hypertension to a minimum by using a low-salt diet and by initiating studies relatively early in the life of the rat. It was, therefore, interesting in our study to observe many QTL for UAE and UPE with strong statistical support for their existence without additional exacerbating factors. Similarly, a recent study using Munich Wistar Frömter (MWF) rats also looked for UAE QTL over time starting at 8 wk of age without exacerbating factors (46). The MWF strain has a reduced number of glomeruli and develops proteinuria and mild hypertension with aging.
In a backcross study involving MWF and Lewis rats, high UAE was strongly recessive as in the present work, and QTL for UAE were found on RNO1, RNO6, RNO12, and RNO17. None of these co-localized with BP QTL. Schulz et al. (46) state that the RNO1 QTL co-localizes with Rf-2 noted above in work with the FHH strain and the RNO17 QTL co-localizes with a QTL also described in FHH. The RNO6 QTL for UAE in the MWF rat study apparently falls between the two UPE QTL seen here on RNO6.

In the present study, the high UAE of the S rat was
strongly recessive to the low UAE of SHR. This pattern of high UAE being recessive is strongly and consistently seen in all other proteinuria rat models where $F_2$ rats were studied, i.e., in BUF (38), FHH (41), MWF (46), and MNS (47). The frequency distribution in Figure 2B is suggestive of the segregation of two recessive genes in the backcross population. Matsuyama et al. (38) interpreted their data on BUF rats to indicate two recessive genes, but they were ultimately able to locate only one QTL on RNO13 (39). Schulz et al. (46) suggested that if any three of the four UAE QTL described in MWF rats were homozygous recessive, then high UAE was achieved. In the present work, it was not possible to explain the bimodal distribution in Figure 2B by any two particular QTL of the ten observed, and the origin of the bimodality is obscure.

It is well known that hypertension can cause/exacerbate glomerular damage leading to proteinuria. It has, however, also been established in the work with the FHH rat that the Rf-1 QTL for proteinuria on RNO1 is not associated with a BP QTL (40,41). In the recent study on the MWF rat, none of the UAE QTL co-localized with BP QTL (46). Similar BP QTL (40,41). In the recent study on the MWF rat, none of the UAE QTL co-localized with BP QTL (46). Similar results were seen here. In fact, under the conditions of our experiment, of the ten QTL observed for UAE and UPE, only the QTL on RNO10 obviously co-localized with a BP QTL (Figure 3). We have, however, studied an $F_2$(SxSHR) population fed an 8% NaCl diet for BP QTL (UAE was not available from this population). BP QTL were seen on RNO3, RNO8, and RNO9 (28). The BP QTL on RNO3 was not co-localized with the UAE or UPE QTL described here. The BP QTL on RNO8 seen on high-salt diet was, however, exactly co-localized with the UAE and UPE QTL on RNO8, and the BP QTL on RNO9 overlaps with the UAE and UPE QTL on RNO9. Thus, on the basis of existing data, it appears that only a minority of UAE and UPE QTL are also linked to BP. It is emphasized, however, that in all of these studies the tail-cuff method was used to measure BP. This has the limitation of providing information on only systolic BP measured during one part of the diurnal cycle (between 7 a.m. and 12 p.m. in our case).

The resistance of the SHR kidney to the development of glomerular sclerosis and subsequent proteinuria despite sustained systemic hypertension is striking when compared with normotensive WKY (24) or hypertensive S rats (25). Similar results are also seen in the present data, in which systemic hypertension in SHR (Table 1) is not translated into albuminuria (Figure 1). This phenomenon has been attributed to increased renal vascular resistance in SHR due largely to preglomerular vasoconstriction in response to systemic hypertension (48). Subsequent work supported this concept (49–52). Such a preglomerular constriction would protect the SHR from glomerular hypertension and consequent glomerular damage. The S/SHR genotype was associated with lower UAE or UPE at most of the QTL described here. Which QTL may involve preglomerular vasoconstriction (if any) cannot be determined from the present data.

It is also interesting that the most significant heart weight QTL, which was on RNO9 (Figure 5), was not associated in the present work with a BP QTL. In our previous study (28) of an $F_2$(SxSHR) population raised on 8% NaCl diet, there was a prominent BP QTL on RNO9 overlapping with the heart weight QTL observed here. Thus it appears possible that on RNO9 genetically controlled differences in heart weight seen here on low-salt diet preceded the BP differences induced by a high-salt diet.

A description of QTL for a trait is incomplete without considering the interactions among the QTL. The multiple interactions observed here serve to stress the ultimate complexity of the genetic control of a quantitative trait (53). This complexity includes chromosomal regions harboring QTL that are observable only through their interactions, which here were on RNO5, RNO7, RNO18, RNO20, and RNOX (Figure 4).

In summary of the present work and the other rat strains showing albuminuria, it is consistently observed that (a) albuminuria, although highly polygenic, is strongly recessive and (b) most, but not all, of the QTL regulating albuminuria are not co-localized with QTL influencing BP.

Acknowledgments

This work was supported by a grant from the National Institute of Health to J. Rapp and by the Helen and Harold McMaster Endowed Chair in Biochemistry and Molecular Medicine to J. Rapp.

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