Brown Norway Chromosome 13 Confers Protection From High Salt to Consomic Dahl S Rat

Allen W. Cowley, Jr, Richard J. Roman, Mary L. Kaldunski, Pierre Dumas, Jeffrey G. Dickhout, Andrew S. Greene, Howard J. Jacob

Abstract—Consomic rats (SS.BN13), in which chromosome 13 from normotensive inbred Brown Norway rats from a colony maintained at the Medical College of Wisconsin (BN/Mcw) was introgressed into the background of Dahl salt-sensitive (SS/Mcw) rats, also maintained in a colony at the Medical College of Wisconsin, were bred. The present studies determined the mean arterial pressure (MAP) responses to salt and renal and peripheral vascular responses to norepinephrine and angiotensin II; 24-hour protein excretion and histological analyses were used to assess renal pathology in rats that received a high salt (4% NaCl) diet for 4 weeks. MAP of rats measured daily during the fourth week averaged 170±3.3 mm Hg in SS/Mcw rats, 119±2.1 mm Hg in SS.BN13 rats, and 103±1.3 mm Hg in BN/Mcw rats. After salt depletion, MAP fell an average of 27±4.5 mm Hg in SS/Mcw rats, 9±2.6 mm Hg in SS.BN13 rats, and 11±3.0 mm Hg in BN/Mcw rats. Protein excretion of SS/Mcw rats on a high salt diet averaged 189±30 mg/24 h, 63±18 mg/24 h in SS.BN13 rats, and 40±6.4 mg/24 h in BN/Mcw rats. Compared with SS.BN13 and BN/Mcw rats, SS/Mcw rats exhibited significantly greater increases of renal vascular resistance in response to intravenous norepinephrine and angiotensin II. Severe medullary interstitial fibrosis and tubular necrosis after a high salt diet were found consistently in SS/Mcw rat kidneys but were largely absent in the SS.BN13 and BN/Mcw rat kidneys. A similar degree of glomerular sclerosis was found in both SS/Mcw and SS.BN13 rats. In rats fed a 0.4% salt diet, the glomerular filtration rate of SS/Mcw rats was significantly less than that of BN/Mcw and SS.BN13 rats. These results reveal a powerful gene, or set of genes, within chromosome 13 of BN/Mcw rats that confers protection from the detrimental effects of high salt to the SS/Mcw rats. (Hypertension. 2001;37[part 2]:456-461.)

Key Words: hypertension, sodium dependent ■ rats ■ sodium ■ chromosome 13 ■ blood pressure ■ consomic

Dahl salt-sensitive (SS) rats exhibit many of the abnormalities that occur with hypertension in African Americans, including blood pressure salt sensitivity, insulin resistance, and hyperlipidemia. They have a low renin form of hypertension that is refractory to treatment with converting enzyme inhibitors and is effectively treated with diuretics. Moreover, these rats rapidly develop severe progressive hypertensive glomerulosclerosis that leads to end-stage renal disease, as is commonly also seen in African Americans with hypertension. For these reasons, insights and findings of the studies in SS rats may provide valuable clues to the genetic basis of hypertension and related traits in African Americans.

The explosion of genomic resources in the rat has led to remarkable advances in identifying the regions of the rat genome that contain blood pressure quantitative trait loci (QTLs), as reviewed by Hamet et al, Zicha and Kunes, and Rapp. We have recently completed a linkage analysis based on an intercross of SS and Brown Norway (BN) salt-insensitive rats in which total genome scans using 238 polymorphic markers, evenly distributed throughout the genome, were scored. All F2 rats (113 males and 99 females) were extensively phenotyped for 239 measured or derived traits. This linkage analysis indicated the existence of a broad range of traits related to pathways of functional importance in hypertension that mapped to 19 chromosomes. The development of congenic strains has been used by a number of laboratories, including our own, to confirm and narrow QTL regions of interest. Despite the usefulness of congenic rat models in the deconstruction of complex traits and the identification of candidate genes, this work has been hampered by the time and expense involved in producing these informative recombinant rats. Even with the use of marker-assisted selection to identify the rats best suited for backcrossing in generations, we have found that the process of developing an inbred congenic strain requires nearly 2 years and 5 to 7 generations of backcrosses to achieve rats that are sufficiently isogenic to make meaningful comparisons.

To overcome these limitations, we have been developing a panel of 44 reciprocal consomic inbred rat strains. A consomic strain is developed by introgression of an individual chromosome into the genomic background of the recipient.
strain. Our consomic panels consist of inbred Brown Norway rats maintained at the Medical College of Wisconsin (BN/Mcw), whose chromosomes have been systematically transferred into the genomic background of SS/Mcw rats, and vice versa. Nadeau et al.\(^1\) have reported the generation of consomics or chromosomal substitution strains in mice. Once a consomic line has been developed, which requires 7 to 8 backcrossed generations, congenic inbred strains can be obtained for any region in 2 generations. A simple F2 cross between the consomic and the parental (recipient) strain would then produce ∼25% homozygous congenic rats that can be inbred. A congenic rat can thereby be used to generate congenic inbred strains within 6 months. The present study presents the phenotyping results from our first completed congenic inbred strains within 6 months. The present study would then produce 25% homozygous congenic rats that can be inbred. A congenic rat can thereby be used to generate congenic inbred strains within 6 months.

Methods

Generation of Consomic Rats

The consomic rat line was derived by using inbred normotensive BN/SSNShd/Mcw rats and salt-sensitive hypertensive Dahl SS/JrHsd/Mcw rats, referred to herein as BN/Mcw and SS/Mcw strains, whose origin has been described previously.\(^1\) \(^4\) Residual heterozygosity and genetic contamination were eliminated by using a set of 182 microsatellite markers (Research Genetics) for genotyping that provided even coverage of the 21 chromosomes (10-cM intervals). The progenitor rats used for the present study were homozygous for all regions tested, and each of these parental strains has since undergone a period total genome scan to ensure allelic homogeneity.

The generation of a panel of reciprocal consomic rats with the use of SS/Mcw and BN/Mcw rats was initiated by using a single F1 male, originating from a male SS/Mcw and a female BN/Mcw, backcrossed to 2 or 3 SS/Mcw and BN/Mcw females. For subsequent backcrosses, each selected male breeder was backcrossed to 3 to 6 parental females. DNA was extracted from tail tips of males and genotyped with markers spaced every 10 cM for the introgressed parent strain.

The rats carrying full-length heterozygous target chromosomes were crossed to fix the donor chromosome, and a total genome scan was performed to verify that the line was isogenic. The consomic line was then maintained by brother-sister matings.

Salt Diet and Surgical Preparation

Breeding animals were maintained on a 0.4% NaCl rat chow diet (Dyets, Inc) because a lower salt diet impairs fertility. Study rats were maintained on a 0.1% NaCl diet until 10 weeks of age (until postnatal development of the kidney was complete) and then switched to a high salt (4.0% NaCl) diet for 3 weeks before the study. A femoral catheter was implanted during the third week of high salt intake as described previously,\(^1\)\(^4\) followed by a 5- to 7-day recovery period.

Arterial Pressure Measurements and Urine Collection in Conscious Rats

All rats were housed individually in metabolic cages, and arterial pressure was measured daily for 3 hours as previously described.\(^1\)\(^4\) Urine was collected during the final 2 days of high salt intake to determine daily urine volume, protein, and creatinine excretion, and a 500-μL blood sample was collected for measurement of plasma creatinine concentration and plasma renin activity (PRA). After 3 days of blood pressure measurements during the “inactive” light period with the rats on the high salt diet, an intraperitoneal injection of furosemide (Lasix, 10 mg/kg) was administered, and rats were returned to a 0.4% salt intake. Arterial pressure was measured again for 3 hours after 36 hours of salt depletion, and urine and blood samples were collected again.

Arterial Pressure and RBF Responses to Intravenous Infusion of Ang II and NE in Anesthetized Rats

Rats that had undergone sodium depletion in the above protocol were anesthetized with ketamine (30 mg/kg IP), which was followed by 5-sec-butyl-5-ethyl-2-thioobarbituric acid (Inactin, 30 mg/kg IP), and prepared for measurement of renal blood flow (RBF) by using electromagnetic flowmetry as described previously.\(^2\)\(^0\) A 1% albumin solution in isotonic saline was infused at a rate in of 50 μL/min throughout the study. After a 30-minute equilibration period, control measurements were made during a 15-minute control period. Then, RBF and blood pressure responses to graded, 5-minute, intravenous infusions of angiotensin II (Ang II) were measured (20, 100, and 200 ng/kg per minute). After a 10-minute reequilibration period, the response to intravenous infusions of norepinephrine (NE) at 0.5, 1.0, and 3.0 μg/kg per minute were measured. Pressure and flow data reported in the present study represent the average of the final 2 minutes of each 5-minute infusion.

Assessment of Glomerular Injury

At the end of the study, the kidneys were removed and immersion-fixed in 10% neutral buffered formalin and paraffin-embedded, and sections were prepared and stained with PAS and Masson’s trichrome stain, which highlights the fibrotic tissue. Glomeruli (20 to 25 per slide) were evaluated (scored from 0 to 4) on the basis of the degree of glomerulosclerosis and mesangial matrix expansion as previously described by Raij et al.\(^2\)\(^1\) The renal medullae were also assessed for interstitial fibrosis and tubular necrosis. The percentage of the outer medullary tissue that was PAS positive in 20 randomly chosen frames per rat kidney was determined by using Metamorph Image Analysis software (version 4.0, Universal Imaging Systems Corp). This percentage largely reflects outer medullary tissue occupied by protein casts in necrotic thick ascending limbs of Henle.

Measurement of GFR and Filtration Fraction

Separate groups of the SS/Mcw, SS.BN13, and BN/Mcw rats were used to determine glomerular filtration rate (GFR), RBF (Transonic Systems Inc), and filtration fraction (GFR/calculated renal plasma flow). The rats were maintained from birth on a 0.4% salt intake and studied at 14±0.4 weeks of age. Rats were anesthetized (30 mg/kg IP ketamine and 30 mg/kg IP Inactin) and surgically prepared as described above for measurements. GFR was determined by the clearance of \(^{[3}\text{H}]\text{inulin as previously described.}\(^2\)\(^8\) Two 20-minute urine collections were performed while arterial pressure and RBF were continuously recorded. Urine flow rate was determined gravimetrically. Sodium and potassium concentrations of urine and plasma samples were measured by using a flame photometer (Corning 480, Bayer Corp). Urinary excretion data, RBF, and GFR were factored per gram kidney weight.

Statistical Analysis

Data are presented as mean±SE, with significance determined by ANOVA followed by a least significant difference multiple comparison test. The significance of differences between the parental strain representing the genomic background (SS/Mcw) and the consomic background (SS.BN13) was further tested by an unpaired t test. Ang II and NE dose responses were analyzed by 2-way repeated-measures ANOVA, with the Tukey multiple comparison test. A probability of \(P<0.05\) for a 2-tailed test was considered significant (ANOVA, SigmaStat Inc, version 2.03).
Results

Genotypic Validation of Consomic Rats

The consomic SS.BN13 rats that produced the progeny used in the present study were genotyped with the use of a total of 182 markers, spaced every 10 cM, giving an average of 10 to 15 markers on the longer chromosomes (chromosomes 1 to 10) and 6 to 10 markers on the shorter chromosomes (chromosomes 11 to 20 and X). Chromosome 13 was found to be homozygous BN at all marker sites. The remainder of the SS/Mcw genome exhibited, on average, 9.6% heterozygosity. None of these heterozygous markers fell into any of the 115 suggestive QTL regions that were mapped in the SS/Mcw X BN/Mcw F2 intercross study.

Blood Pressure and Renin Responses Before and After Sodium Depletion in Unanesthetized Rats

As seen in Figure 1, top, the transfer of BN/Mcw chromosome 13 into the SS/Mcw genomic background had a profound effect on the mean arterial pressure (MAP) as recorded during the fourth week of the high salt diet. MAP averaged 170±3.3 mm Hg in SS/Mcw rats, 119±2.1 mm Hg in SS.BN13 rats, and 103±1.3 mm Hg in BN/Mcw rats.

Figure 1, bottom, shows that the average reduction of MAP with salt depletion was significantly less in consomic SS.BN13 rats (9±2.6 mm Hg) than in SS/Mcw rats (27±4.5 mm Hg). MAP fell 11±3.0 mm Hg in BN/Mcw rats. Not shown are the PRA responses to sodium depletion, which only increased from 0.4±0.1 to 1.0±0.2 ng angiotensin I (Ang I)/mL per hour in SS/Mcw rats. A significantly greater increase was seen in SS.BN13 rats, with PRA increasing from 0.6±0.1 to 2.4±0.3 ng Ang I/mL per hour. The greatest increase was found in BN/Mcw rats, with PRA increasing from 1.5±0.2 to 7.0±0.8 ng Ang I/mL per hour.

Renal Morphology and Urine Protein and Creatinine Excretion in High Salt–Fed Rats

Protein excretion of SS.BN13 rats on the 4% salt diet (63±18 mg/24 h) was significantly less than that of the SS/Mcw rats (190±30 mg/24 h). BN/Mcw rats averaged 40±6 mg/24 h, a level not significantly different from that seen in SS.BN13 rats. Plasma creatinine concentration was nearly identical in the 3 strains (0.4±0.04 mg/dL), and creatinine clearances did not differ significantly, averaging 1.8±0.3, 1.4±0.2, and 1.2±0.2 mL/min in SS/Mcw, SS.BN13, and BN/Mcw rats, respectively.

Glomerular injury and tubular interstitial disease as assessed morphologically are illustrated in Figure 2. SS/Mcw rats exhibited considerable tubular interstitial disease, averaging 23.8±2% PAS positive in the outer medulla of rats fed a high salt diet, in accord with data we have previously reported.25 Tubulointerstitial fibrosis was seen in the thick ascending limbs of Henle (protein casts), leading to fibrotic elimination of the vasa recta in the SS/Mcw rats. In contrast, this region of the kidney in consomic SS.BN13 rats appeared to be well protected from the damage of the high salt diet (Figure 2), averaging significantly less PAS-positive damage (7.9±2%) than that found in the SS/Mcw rats. BN/Mcw rats averaged 14.7±2% PAS-positive damage, a value significantly less than that found in SS/Mcw rats but greater (P<0.05) than that in SS.BN13 rats.

There was marked expansion of the mesangial matrix in the glomeruli and loss of capillaries in the SS/Mcw rat, yielding a mean glomerular injury index of 2.2±0.1. This was similar to that measured in SS.BN13 rats (2.1±0.5). Both were significantly greater than that seen in the BN/Mcw rats, which averaged 1.1±0.2. These observations indicate that the SS.BN13 rats were protected from the salt-induced interstitial nephritis but not from glomerular disease.

Responsiveness to Ang II and NE

The average MAP of SS/Mcw rats on a high (4%) salt diet remained higher even under anesthesia (124±4 mm Hg) compared with that of the BN/Mcw rats (111±3 mm Hg) and the consomic SS.BN13 rats (114±3 mm Hg). The arterial pressure Ang II and NE dose-response relationships did not significantly differ in these 3 strains of rats (data not shown). In contrast, major differences were observed in the renal vascular responses to these compounds, with SS/Mcw rats exhibiting significantly greater increases of renal vascular resistance in response to both Ang II and NE infusions than those found in the BN/Mcw or SS.BN13 rats (Figure 3).

GFR and Filtration Fractions in Rats Maintained on a 0.4% Salt Diet

As summarized in the Table, GFR values, normalized per gram kidney weight, and filtration fractions of SS.BN13 rats...
were significantly higher than those of SS/Mcw rats and did not differ from those of BN/Mcw rats. RBF values of SS/Mcw and SS.BN13 rats were significantly different from each other and from those of BN/Mcw rats. The average left kidney weights of SS/Mcw and SS.BN13 rats did not differ from each other, and both were significantly greater than those of BN/Mcw rats. The fractional excretion of Na\(^+\) did not differ significantly among the 3 strains of rats; however, fractional excretion of K\(^+\) was significantly higher in SS/Mcw rats than in SS.BN13 rats.

**Discussion**

**Influence of Chromosome 13 on Blood Pressure**

The results demonstrate that substitution of BN/Mcw chromosome 13 on the isogenic background of the inbred SS/Mcw rat nearly abolished the rise of arterial pressure and associated proteinuria observed with a high salt diet in the parental SS/Mcw rats. These data suggest that a gene, or set of genes, on BN/Mcw chromosome 13 confers protection from salt-induced hypertension in the SS/Mcw rat. These studies provide a strong rationale to begin narrowing and searching for candidate genes of hypertension and/or renal disease on chromosome 13.

**Influence of Chromosome 13 on Renal Function**

Kidney morphology of the consomic SS.BN13 rats after 4 weeks of a high salt diet was not entirely normal compared with that of the BN/Mcw rats, inasmuch as the consomic rats did not appear to be protected from the development of glomerular sclerosis. However, despite the similar glomerular injury scores seen in the SS/Mcw and SS.BN13 rats, SS.BN13 rats did not exhibit the severe proteinuria seen in SS/Mcw rats. It appears that the semiquantitative glomerular injury index does not reflect functional differences in glomeruli that could importantly influence glomerular protein sieving and/or proximal tubular reabsorption of filtered proteins. Preliminary morphological studies in our laboratory (J.G.D.) indicate that the glomerular sclerosis seen in SS.BN13 rats was a result of the high salt diet, because when maintained on a low salt diet (0.4%), they exhibit no apparent injury.

The outer medulla of the consomic SS.BN13 rats had normal fibrotic deposition, a remarkable observation because it was this region of the SS/Mcw kidneys that exhibited severe fibrosis of capillaries and tubular interstitial disease after 2 to 4 weeks of the high salt feeding seen in a previous study.\(^{22}\) It remains to be determined whether the renal protective effects, conferred to SS/Mcw rats by BN/Mcw chromosome 13, were secondary to the antihypertensive effect of chromosomal transfer or a direct protective effect.

**Chromosome 13 and the SS/Mcw Rat**

Blood pressure responsiveness to Ang II and NE among the 3 strains of rats did not differ, as assessed by the pressure dose-response relationships. In contrast, SS/Mcw rats exhib-
parental SS rats. Consistent with this view, St Lezin et al. found that transfer of this region from SS rats reduced PRA and blood pressure in Dahl R rats. On the other hand, DiPaolo et al. found that transfer of a larger region from Dahl R rats lowered blood pressure by 25 mm Hg and had no effect on PRA in congenic SS rats. More recently, Zhang et al. localized this antihypertensive gene to the interval between Syt 2 and D13 Mit108, which excluded the renin gene. In the present study, transferring the entire chromosome 13 from the BN/Mcw into the Dahl S genetic background increased PRA. Despite the increased PRA, the net effect of the genes on BN/Mcw chromosome 13 was to prevent salt-induced hypertension. Thus, a powerful antihypertensive gene(s) must lie on chromosome 13 that can overcome the prohypertensive effect of transferring a normal renin gene and elevating PRA in SS/Mcw rats.

### Power of Chromosomal Substitution Studies

The results of the present study demonstrate the power of the consomic design for mapping important functional traits and the ability of these chromosomal substitution studies to guide us toward genomic regions important in hypertension and other complex traits. The present results were unexpected because our genetic linkage study between the SS/Mcw and BN/Mcw rats failed to reveal significant blood pressure QTLs on chromosome 13, although chromosome 13 has been implicated in several other studies using F2 crosses and congenic rat studies as described above. On the basis of statistical modeling of data (not presented in the present article), it appears that the heterogeneous genetic background of the F2 masked the effects of the powerful antihypertensive alleles contained on chromosome 13. The power of the consomic approach is that it enables one to avoid the confounding effects of heterogeneous genomic backgrounds in which the phenotypic noise may make the detection of weak or complex QTLs difficult. These observations support the conclusions of others that chromosome-substitution approaches provide greater statistical power to detect linkage than do standard intercross linkage studies. Consomic rats also provide a renewable source of animals for subsequent genetic, physiological, and/or longitudinal studies, which would narrow and examine a QTL on the chromosome by facilitating the development of congenic substrains on an identical genomic background.

### Acknowledgments

This work was supported by grants HL-54998, HL-29587, U10 HL-154508, and U10 HL-66579 and a grant from the Merck.

### Table: Renal Parameters of Rats Maintained on a Low Salt (0.4% NaCl) Intake

<table>
<thead>
<tr>
<th></th>
<th>MAP, mm Hg</th>
<th>RBF, ml·min⁻¹·kwt⁻¹</th>
<th>GFR, ml·min⁻¹·kwt⁻¹</th>
<th>LKW, g</th>
<th>Filtration Fraction, %</th>
<th>fNa Excr, %</th>
<th>fK Excr, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS/Mcw (n=13)</td>
<td>124±4*</td>
<td>5.2±0.6*</td>
<td>0.78±0.07*</td>
<td>1.9±0.1</td>
<td>27±1.7*</td>
<td>3.1±0.3</td>
<td>22.3±3*</td>
</tr>
<tr>
<td>SS.BN13 (n=10)</td>
<td>112±3†</td>
<td>6.9±0.3†</td>
<td>0.92±0.05†</td>
<td>1.8±0.1</td>
<td>20.8±0.6†</td>
<td>3.3±0.2</td>
<td>13.8±2†</td>
</tr>
<tr>
<td>BN/Mcw (n=10)</td>
<td>109±3†</td>
<td>8.6±0.6†</td>
<td>1.1±0.08†</td>
<td>1.2±0.1†</td>
<td>21.8±1.7†</td>
<td>3.8±1.0</td>
<td>20.1±2†</td>
</tr>
</tbody>
</table>

Values are mean±SE. LKW indicates left kidney weight; fNa Excr and fK Excr, fractional excretion of sodium and potassium, respectively.

*P<0.05 vs SS.BN13; †P<0.05 vs SS/Mcw.
Genome Research Institute. The authors wish to thank Kate Senkbeil, Sheri Jene, Paulo Soares, Annette Dahly, and Terry Kurth for their excellent technical assistance in the animal studies, Camille Torres and Luann Kelly for the analytical measurements, and Christine Kendziorski for statistical modeling.

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Hypertension. 2001;37:456-461
doi: 10.1161/01.HYP.37.2.456

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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