Kidney Specificity of Rat Chromosome 1 Blood Pressure Quantitative Trait Locus Region

Jenny-Rebecca Clemitson, Julian R. Pratt, Simon Frantz, Steven Sacks, Nilesh J. Samani

Abstract—Rat chromosome 1 has a region containing loci that influence blood pressure. In the present study, we investigated whether these loci mediate their effect via the kidney. Taking advantage of the histocompatibility between a congenic strain (WKY.SHР-Sa, which contains the relevant chromosomal region from the spontaneously hypertensive rat) and its parental strain, the Wistar-Kyoto rat (WKY), we compared the effect of transplanting a kidney at 5 to 6 weeks of age from either congenic rats or WKY into bilaterally nephrectomized WKY. WKY.SHР-Sa animals and WKY with intact kidneys and with unilateral nephrectomy were studied as controls. Blood pressure was measured at 12, 16, 20, and 25 weeks of age. At all time points, blood pressure was significantly higher (by between 8 to 22 mm Hg, \( P<0.001 \)) in 2-kidney WKY.SHР-Sa animals compared with WKY. This genotype-related difference was maintained in unilaterally nephrectomized rats. Most importantly, WKY that received transplants from WKY.SHР-Sa rats had significantly higher blood pressure (\( P<0.001 \) at all time points) compared with those that received transplants from other WKY. At any age, this difference was between 70\%-100\% of the difference observed between the 1-kidney groups. There was no difference in plasma urea or creatinine between groups or evidence of chronic rejection in the cross-transplant group. The findings indicate that the major proportion of the blood pressure effect of loci on rat chromosome 1 is mediated through the kidney, and provide a rational basis for investigating genes located in the relevant chromosomal region and expressed in the kidney as likely candidates. (Hypertension. 2002;40:292-297.)

Key Words: hypertension, experimental ■ genetics ■ rats, spontaneously hypertensive ■ genes ■ transplantation, renal

In the past decade, quantitative trait loci (QTLs) affecting blood pressure (BP) have been mapped to regions on several rat chromosomes by use of linkage analysis in segregating progeny from crosses of inbred hypertensive and normotensive rat strains.\(^1\) In many instances, the presence of the QTLs has been confirmed by their capture in congenic strains.\(^1\) These are strains in which a chromosomal region from one strain (eg, a hypertensive strain) is introgressed into another (eg, normotensive strain) by marker-selected backcrossing.\(^2\) If the QTL is present in the introgressed segment, the congenic strain should have a different BP (higher in the above example) than that of the recipient parental strain.

In previous studies, we\(^3\) and others\(^1\) have shown that a region in the midportion of rat chromosome 1 (RNO1) has \(\geq1\) QTLs affecting BP. In second filial generation (F\(_2\)) progeny from a cross of the spontaneously hypertensive rat (SHR) and the Wistar-Kyoto rat (WKY) strains, the QTL region accounted for \(\approx25\%\) of the variance in BP.\(^3\) This segment of RNO1 is particularly interesting because it also harbors QTLs for stroke latency,\(^4\) renal damage,\(^5\) and diabetes-related phenotypes.\(^6\) More recently, we have captured the region containing the BP QTL(s) in reciprocal congenic strains derived from SHR and WKY,\(^7\) and have begun the process of dissecting the region to narrow the intervals containing the BP QTLs.\(^8\)

Apart from allowing positional cloning of QTLs, congenic strains have other advantages. Their genetic identity (apart from the region of interest) to the corresponding parental strain means that they provide a powerful tool for investigating the physiological basis of the QTL effect. Any differences between a congenic line and its parental strain is likely to be either an intermediary phenotype or a consequence of the QTL effect. In turn, such findings may provide important clues as to the nature of the QTL. Several lines of evidence\(^9,10\) suggest that the kidney plays an important primary role in the pathogenesis of hypertension in SHR. Using our congenic resource\(^7\) and a transplant approach, the aim of the present study was to determine whether the BP QTL region on RNO1 mediates its effect via the kidney.

Methods

Strains and Study Design
The strains used were WKY and WKY.SHР-Sa, a congenic strain that harbors the RNO1 BP QTL region from the SHR.\(^7\) The minimal

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pedicle, and microvascular clips were placed to leave aorta and inferior vena cava were exposed caudal to the left renal The circulation to the adrenal gland was left intact. The recipient removed by placing a ligature around the renal vessels and ureter.

Anticoagulation was provided by 250 U heparin intravenously to the patch of aorta and the entire length of the renal vein and ureter. (1% halothane delivered via a Fluotec vaporizer and mixed with

ing signs of distress (5%) were culled. One week later, the surgical

mosed end-to-side to the inferior vena cava. In this way, blood flow was reestablished to the donor kidney with a minimal chance of any

mosed end-to-end with the recipient ureter. Total ischemic time was generally <20 minutes to minimize the effect of ischemia/reperfu-

sion injury. Postoperatively, animals were carefully monitored, and any showing signs of distress (<5%) were culled. One week later, the surgical

wound was reopened, and the transplant kidney directly inspected to confirm that all the anastomoses were sound and that the kidney was functioning normally. The native right kidney was then removed. Briefly, after mobilization of the native kidney from fat and connective tissue, the kidney capsule was peeled off, and a single tie (4/0 silk suture) was placed to grossly ligate the renal artery, vein, and ureter. The kidney was then cut out, and the animal closed. In the 1K animals, the right kidney was removed using a similar procedure at 6 weeks of age.

BP Measurements

Indirect tail-cuff BPs were measured at 12, 16, 20, and 25 weeks (Figure 1) by use of the same methods as those used in our initial study to map the RNO1 BP QTL. Briefly, BP was measured in conscious but restrained animals, prewarmed to 34°C for 20 minutes by use of a photoelectric signal (Linton’s Instruments). For each time point, BP was measured 3 times on 2 separate days, and the mean value of all readings was taken as the average for the animal. Animals were individually numbered, and the person recording the BP measurements was blinded to the group assignment of the transplanted animals or the strain for the 1K and 2K animals. BP was also measured at 6 weeks of age in a separate group of male WKY and WKY.SHR-Sa rats.

Biochemical Measurements

At euthanasia, a blood sample was collected from the aorta in anticoagulant (lithium heparin) and was plasma-separated by spin-

ning at 15 000 rpm for 10 minutes. The plasma were stored at −20°C and batch-analyzed for urea and creatinine by use of an automated hospital analyser (Abbott Aeroset Analyser, Abbott Laboratories Ltd).

Histological Studies

The transplanted kidneys were excised at euthanasia, cut longitudi-

nally, and fixed in 10% formaldehyde. After paraffin embedding, 2-µm sections were cut, stained with hematoxylin and eosin, and examined for signs of chronic rejection and damage. This was performed for 5 WKY-WKY transplant kidneys and 5 CON-WKY transplant kidneys.

Statistical Analysis

BP and plasma variables were compared between specified groups using ANOVA. In addition to analysis at individual time points, analysis was also performed, when indicated, to assess an overall group or procedure effect by combining data across the 4 time points by use of repeated-measures ANOVA in Stata (Stata Statistical Software, version 7.0; Stata Corp).

Results

WKY and WKY.SHR-Sa animals did not differ in their BP at 6 weeks of age (WKY, 93.9±2.9 mm Hg [n=16]; WKY.SHR-Sa, 94.1±2.3 mm Hg [n=19]; P=0.849). There was no evidence of hyperacute or acute rejection of WKY-SHR-Sa kidneys transplanted into WKY. Both groups of transplanted animals grew normally, and there was no weight difference between these animals and either 1K animals or 2K animals (Table 1). However, the kidney weights of both transplanted groups and the 1K animals were significantly greater (P<0.01) than those of their respective 2K groups (Table 1), indicating compensatory hypertrophy caused by the reduction in renal mass. There were no differences in kidney weights of the transplant and IK groups (Table 1).

The BPs of the experimental groups of animals at 12, 16, 20, and 25 weeks are plotted in Figure 2. At each age (and across the 4 time points), CON2K animals had significantly higher BP (P<0.001) compared with that of WKY2K ani-
mals, reconfirming the previously observed difference between the strains. Unilaterally nephrectomized animals (WKY1K and CON1K) tended to have higher BPs (5 to 10 mm Hg, \( P < 0.001 \), when averaged over weeks and strains) than that of their 2K counterparts (Figure 2), presumably reflecting the hemodynamic effect of a reduction in renal mass. However, the genetically mediated difference in BP between the congenic animals (CON1K) and the WKY (WKY1K) was maintained at each time point and across the time points (\( P < 0.001 \)) (Figure 2). Most importantly, at each age and across the time points, WKY that had received a transplant from a congenic rat (CON-WKY) also had significantly higher BP (\( P < 0.001 \)) than those that had received a transplant from another WKY (WKY-WKY) (Figure 2). However, it should be noted that the transplanted animals did not have significantly higher BP compared with that of their corresponding 1K counterparts, indicating that the transplantation procedure itself had not materially affected BP or led to hypertension (Figure 2). Indeed averaged over the 4 time points, CON-WKY animals had significantly lower BP compared with that of CON1K animals (\( P = 0.003 \)). This was owing to significantly lower BP in the CON-WKY group at 20 and 25 weeks (Figure 2). There was no significant difference between WKY-WKY and WKY1K animals, either averaged over the 4 time points (\( P = 0.664 \)) or at any individual time point.

The differences in BP at each age between corresponding groups are shown in Table 2. The difference in BP between CON2K and WKY2K animals increased from 8.4 mm Hg at 12 weeks to 20.2 mm Hg at 25 weeks. A similar pattern was observed between CON1K and WKY1K animals. The BP difference between the 2 transplant groups also increased with age. The proportion of the BP difference between CON1K and WKY1K rats that was present between the CON-WKY and WKY-WKY rats varied between 70% and 100%, depending on age (Table 2). In a formal analysis across the 4 time points, the increase in BP difference with

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, gm</th>
<th>Kidney Weight, gm</th>
<th>Plasma Urea, mmol/L</th>
<th>Plasma Creatinine, ( \mu )mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY2K</td>
<td>329.3 ± 24.5</td>
<td>1.21 ± 0.16*</td>
<td>5.20 ± 1.39</td>
<td>57.6 ± 10.4</td>
</tr>
<tr>
<td>CON2K</td>
<td>343.1 ± 26.9</td>
<td>1.25 ± 0.25*</td>
<td>5.16 ± 0.55</td>
<td>53.9 ± 9.9</td>
</tr>
<tr>
<td>WKY1K</td>
<td>347.3 ± 35.5</td>
<td>1.56 ± 0.25†</td>
<td>6.28 ± 1.03</td>
<td>53.7 ± 9.7</td>
</tr>
<tr>
<td>CON1K</td>
<td>341.3 ± 29.7</td>
<td>1.62 ± 0.29†</td>
<td>5.87 ± 0.85</td>
<td>52.2 ± 5.4</td>
</tr>
<tr>
<td>WKY-WKY</td>
<td>335.8 ± 31.4</td>
<td>1.54 ± 0.23†</td>
<td>5.68 ± 1.07</td>
<td>46.0 ± 9.6</td>
</tr>
<tr>
<td>CON-WKY</td>
<td>338.8 ± 26.6</td>
<td>1.49 ± 0.24†</td>
<td>6.59 ± 0.89</td>
<td>54.1 ± 8.7</td>
</tr>
</tbody>
</table>

Values are mean ± SD. WKY2K indicates WKY rats with 2 kidneys (n = 19); CON2K, WKY.SHR-Sa congenic rats with 2 kidneys (n = 20); WKY1K, unilaterally nephrectomised WKY rats (n = 20); CON1K, unilaterally nephrectomised WKY.SHR-Sa rats (n = 19); WKY-WKY, WKY rats with a transplant from another WKY rat (n = 17); and CON-WKY, WKY rats with a transplant from a WKY.SHR-Sa congenic rat (n = 20).

*For the 2K animals, the weight is the weight of the left kidney.
†\( P < 0.01 \) vs corresponding 2K group.
relevant tissue.14 For most of the BP QTLs, little data exist as Cd36, prior studies had established adipose tissue as the appropriate RNA samples can be compared. In the case of tissue site through which the QTL exerts its effect so that the gene expression profiling requires knowledge of the likely

time was not significantly different between the 2 1K groups and the 2 transplant groups (P=0.334).

The plasma urea (WKY, 5.12±1.22 mmol/L; WKY.SHR-Sa, 4.38±0.73 mmol/L; P=0.121) and creatinine (WKY, 40.2±6.9 μmol/L; WKY.SHR-Sa, 40.2±7.3 μmol/L; P=0.999) levels in the 2 donor groups at 5 weeks of age were not significantly different. Likewise, there was no significant difference in urea and creatinine levels between the various experimental groups at 28 weeks of age (Table 1). Plasma urea tended to be higher by ≈1 mmol/L in the 1K groups compared with the 2K rats, but this did not reach significance and was not reflected in the creatinine levels (Table 1).

Kiddies taken at euthanasia from several animals of both transplant groups were examined histologically in a blinded fashion, by an experienced renal histopathologist (J.P.), for evidence of chronic rejection and damage. Tests included microscopic assessment of glomerular and tubular structure, vascular morphology, and evidence of cellular infiltration. Kidneys derived from both WKY and WKY.SHR-Sa looked healthy, and specifically, there was no evidence of leukocytic infiltrate, tubulitis, or arteritis, which would indicate an ongoing immune response or transplant rejection in kidneys from the latter strain (data not shown).

**Discussion**

Although many QTLs for BP and related phenotypes have been mapped in rodent models,1 few12,13 have actually been identified. Attempts at positional cloning via congenic strains are being widely pursued, although this approach becomes increasingly difficult to apply once the interval has been narrowed to <1 cM. Clearly, complementary strategies are necessary. One possibility is to identify genes mapping to the interval that are differentially expressed between the parental and congenic strain. Indeed, such an approach led to identification of Cd36 (fat) as a QTL-causing defective fatty acid and glucose metabolism in hypertensive rats.13 However, gene expression profiling requires knowledge of the likely tissue site through which the QTL exerts its effect so that the appropriate RNA samples can be compared. In the case of Cd36, prior studies had established adipose tissue as the relevant tissue.14 For most of the BP QTLs, little data exist as to the tissue specificity of the BP effect. Although previous findings from transplantation studies involving SHR and WKY suggest that the kidney plays a primary role in hypertension,15–17 its involvement in individual QTLs needs to be shown. In the present study, we take an important step forward with regard to the BP QTL region on RNO1, by providing direct evidence that a significant proportion of the phenotypic effect of the responsible BP genes in this region is mediated via the kidney.

An important concern in transplantation studies of this type is whether any differential effect on BP is owing to the transfer of a kidney from a strain that has already been damaged by hypertension.9,17 However, this is unlikely to be the case here. First, transplants were performed at a very young age when there was no significant BP differences observed between WKY and WKY.SHR-Sa. Furthermore, we compared 2 strains whose BP is not markedly different (unlike, eg, that of SHR and WKY), even when older, and most probably is not sufficient to directly cause renal damage. This is supported by the lack of either biochemical or histological evidence of more significant damage in kidneys derived from WKY.SHR-Sa at euthanasia.

We studied 2 control groups for specific reasons. First, despite our previous data7 showing capture of the RNO1 BP QTL region in WKY.SHR-Sa, we felt it essential to perform repeat measurements in contemporaneous animals, in case any unrecognized changes in the ‘environment’ that the animals were housed in or the way BP was measured affected the phenotype and, hence, the interpretation of the findings in the transplant animals. As it turned out, we were able to confirm our previous observation in intact 2K animals.7 The 1K controls had 2 purposes. First, they provided an assessment of any impact on BP from the reduction in renal mass that was inevitable in the transplanted groups and, in particular, any differential effect between strains. Compared with 2K animals, we found that 1K animals had BP values that were 5 to 10 mm Hg higher, but most importantly, the genotype-related difference was maintained between WKY1K and CON1K animals. The second purpose of the 1K control groups was to help evaluate any impact of the transplant procedure itself on BP. In particular, any ischemic or physical damage to the kidneys, or the superimposition of secondary renovascular hypertension, could have resulted in elevated BP in the transplant groups and could have ameliorated any strain-specific differences. However, comparison of

**TABLE 2. Differences in Blood Pressure Between Paired Groups of Rats at Different Time Points**

<table>
<thead>
<tr>
<th>Age</th>
<th>ΔBP CON2K to WKY2K</th>
<th>ΔBP CON1K to WKY1K</th>
<th>ΔBP CON-WKY to WKY-WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 weeks</td>
<td>8.4</td>
<td>9.9</td>
<td>7.3</td>
</tr>
<tr>
<td>16 weeks</td>
<td>14.1</td>
<td>9.1</td>
<td>10.1</td>
</tr>
<tr>
<td>20 weeks</td>
<td>19.0</td>
<td>15.4</td>
<td>10.1</td>
</tr>
<tr>
<td>25 weeks</td>
<td>20.2</td>
<td>21.9</td>
<td>15.4</td>
</tr>
</tbody>
</table>

Column 2 shows the difference between WKY.SHR-Sa congenic (CON2K) and WKY (WKY2K) rats with both kidneys intact; column 3, the difference between unilaterally nephectomized congenic (CON1K) and WKY (WKY1K) rats; and column 4, the difference between WKY animals that have received a transplant from a congenic animal (CON-WKY) and those with a transplant from another WKY rat (WKY-WKY). The last column shows the proportion of the difference between the 2 1K groups (column 3) that is also seen between the 2 transplant groups (column 4).
the BPs between each 1K and the corresponding transplant group indicates that the transplant procedure itself did not have a major effect on BP and that the steps taken (see Methods) to avoid ischemic damage or renal artery stenosis had been successful.

Because of the genetic similarity of WKY.SHR-Sa with WKY, apart from the RNO1 BP QTL region (as previously shown using multiple genome-wide microsatellite markers7) and, in particular, the sharing of the same major histocompatibility complex on chromosome 20, we did not anticipate major rejection problems. Nonetheless, there was the theoretical possibility that other immunomodulatory genes could be located in the differential segment of RNO1 and promote chronic immune-mediated renal damage in the CON-WKY group, with consequent effect on BP. However, the similar renal biochemistry between the WKY-WKY and CON-WKY groups at euthanasia and the lack of any cellular infiltration that is suggestive of immune damage in the latter group exclude this possibility.

An interesting observation was that at 3 of the 4 time points, the proportion of the difference between the CON1K and WKY1K animals that was also present between the CON-WKY and WKY-WKY transplant animals was only \( \approx 70\% \) (Table 2). Given the high variability of BP, it is possible that this is simply a reflection of the imprecision of BP measurement and that there is no genuine difference. However, the consistency of the finding suggests that other possibilities need to be considered. An important difference between the 1K animals and the transplant animals is that the residual kidney in the former remains innervated. Previous studies have shown that renal denervation delays the onset and progression of hypertension, but not its eventual expression, in the SHR.18,19 In WKY, the direct effect on BP of denervation seems minimal, as we did not find any difference in BP between WKY1K and WKY-WKY rats. However, it is conceivable that this lack of renal neural input and its interaction with the RNO1 QTL(s) is responsible for a smaller proportion of the effect being expressed in the CON-WKY group. This is supported by the finding that BP was significantly lower in the CON-WKY animals compared with the CON1K animals at 20 and 25 weeks (Figure 2). Another possibility is suggested by our recent findings from genetic dissection of the RNO1 BP QTL region.8 Nonoverlapping subcongenic strains spanning the region introgressed in WKY.SHR-Sa were found to carry BP effects, indicating that there are at least 2 QTLs that influence BP in this region.8 Therefore, it is possible that the difference seen between the transplant groups represent the phenotypic expression of only one of these QTLs. One way of confirming this possibility would be by doing the reverse transplant experiment (ie, WKY or WKY.SHR-Sa kidney into a WKY.SHR-Sa background) and see if, in this instance, all the difference between the 1K animals is consistently seen between the 2 transplant groups. This would be expected, as the WKY.SHR-Sa animal receiving the isogenic transplant should express the effects of QTLs expressed both intrarenally and extrarenally. Irrespective of which of the above possibilities is correct, our data clearly show that the major proportion of the BP effect of QTLs in this region of chromosome 1 is mediated via the kidney.

In this study, we were unable to directly investigate the physiological basis of the renal effect. However, in a reciprocal congenic strain to that studied here (in which we transferred the QTL region from the WKY to the SHR), we have recently demonstrated an increased glomerular filtration rate, an enhanced pressure-natriuresis associated with a lower tubular sodium reabsorption, and a markedly reduced BP response to salt load compared with those of SHR.20 Although, it is difficult to prove primary causality for alterations in pressure natriuresis, because of its intimate relationship with the level of BP, the congruent findings between the 2 studies suggest that changes in this important function of the kidney may underlie the effect of QTL on BP.

Finally, our findings need to be analyzed in relation to those reported recently by Churchill et al21 on a congenic strain (CSHR) carrying a 22-cM segment of RNO1 (overlapping with ours7) from the normotensive Brown-Norway strain in an SHR background, which was previously shown to have a lower BP compared with that of SHR.22 In an elegant series of experiments, they compared the BP effects of a 4-way transplantation protocol: SHR kidney into SHR recipient (SHRsh), CSHR kidney into SHR recipient (CSHRsh), SHR kidney into CSHR recipient (CSHRsh), and CSHR kidney into CSHR recipient (CSHRsh). The transplants were carried out at 6 to 8 weeks of age, and BP development was monitored by continuous radiotelemetry for 8 weeks. As expected and consistent with our findings, SHRsh had lower BP than did SHRsh animals (by 17 mm Hg), and was similar to CSHRsh animals. Surprisingly, transplantation of an SHR kidney into a CSHR rat did not raise BP, and the CSHRsh animals had a similar BP to SHRsh and CSHRsh animals. The investigators interpreted this as reflecting a contribution of the Brown-Norway genotype in extrarenal tissues in the CSHRsh group. By itself, this is also not inconsistent with our findings (see above). However, if this deduction was correct, the observation that BP was similar but not lower in the CSHRsh group than in the 2 cross-transplant groups would suggest that the effects of combined renal and extrarenal Brown-Norway genotype are not additive. Looked at another way, it suggests that to increase BP (compared with that in the congenic strain), expression of the SHR RNO1 genotype is essential both inside and outside the kidney. The mechanism underlying such a requirement is obscure. It is also inconsistent with our data in which transfer of the kidney alone from a strain carrying the RNO1 region from the SHR was sufficient to raise BP compared with a WKY kidney. The reasons behind this discrepant finding require further investigation.

**Perspectives**

Using a transplant approach and a congenic resource, we provide direct evidence that a significant proportion of the phenotypic effect of the RNO1 BP QTL region is mediated through the kidney. The findings provide a rational basis for investigating genes located in the relevant chromosomal region and expressed in the kidney as likely candidates underlying the BP effect.
Acknowledgments
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References
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