Successful Isolation of a Rat Chromosome 1 Blood Pressure Quantitative Trait Locus in Reciprocal Congenic Strains

Simon A. Frantz, Michael Kaiser, Sheila M. Gardiner, Dominique Gauguier, Madeleine Vincent, John R. Thompson, Terence Bennett, Nilesh J. Samani

Abstract—Linkage analyses in experimental crosses of hypertensive and normotensive rats have strongly suggested the presence of a quantitative trait locus (QTL) influencing blood pressure on rat chromosome 1, at or near the Sa gene. To confirm the presence of such a locus and move toward identification of the causative gene, we have developed, through targeted breeding over 10 generations using an Sa gene polymorphism to select breeders at each generation, 2 congenic strains, 1 containing a segment of spontaneously hypertensive rat (SHR) chromosome 1 in a Wistar-Kyoto rat (WKY) genetic background (WKY.SHR-Sa), and the other a segment of WKY chromosome 1 in an SHR background (SHR.WKY-Sa). WKY.SHR-Sa contains at least 26 cM of SHR chromosome 1, between markers mD7mit206 and D1Mit2 (and including the SHR allele of the Sa gene), and SHR.WKY-Sa carries at least 15 cM of WKY chromosome 1, between mD7mit206 and D1Wox34 (and including the WKY allele of the Sa gene). Blood pressure of WKY.SHR-Sa rats measured at 16, 20, and 25 weeks of age was significantly higher than that of WKY, whereas blood pressure of SHR.WKY-Sa rats was significantly lower than that of SHR. At 25 weeks, the mean differences in systolic and diastolic blood pressure between WKY.SHR-Sa and WKY were +11.5 mm Hg (P=0.001) and +11.6 mm Hg mm Hg (P<0.001), respectively. The corresponding differences between SHR.WKY-Sa and SHR were −11.3 mm Hg (P=0.002) and −9.1 mm Hg (P=0.005), respectively. The differences represent about one fifth of the blood pressure difference between SHR and WKY. Renal Sa mRNA levels in the congenic strains reflected their Sa allele with a high level in WKY.SHR-Sa and a low level in SHR.WKY-Sa, consistent with previous data suggesting that the level of Sa expression is primarily determined by cis-acting elements in or near the Sa gene. Our results show that we have successfully isolated a major rat chromosome 1 blood pressure QTL located in the vicinity of the Sa gene in reciprocal congenic strains derived from SHR and WKY. The strains can now be used to further define the region containing the QTL and also to characterize intermediary mechanisms through which the QTL influences blood pressure. In addition, comparison of the regions introgressed in our congenic strains with the location of the peak LOD score for chromosome 1 blood pressure QTL in second filial generation progeny derived from our SHR×WKY cross suggests that there may be at least 1 further QTL influencing blood pressure on this rat chromosome. (Hypertension. 1998;32:639-646.)

Key Words: hypertension, genetic ♦ congenic strains ♦ quantitative trait locus ♦ genes ♦ rats, inbred SHR

Using experimental crosses derived from genetically hypertensive and control rat strains, or by analyzing recombinant inbred strains, quantitative trait loci (QTL) influencing blood pressure have been mapped to several rat chromosomes.1-10 Such mapping represents the first step in identifying the causative genes. The precision of localization at each site depends on several factors, including the size of the cross studied, the magnitude of the locus effect, and the density of the local linkage map. For most of the loci mapped, this is currently in the region of 20 to 50 cM (20 to 50 million bp). Such large segments clearly can contain several potential candidate genes. To identify the causative gene, 2 approaches are possible. First is analysis of candidate genes known to be located within the chromosomal segment of interest. This approach is intuitively attractive but has the major drawback that it can be applied only to known genes whose position has been mapped. The second approach is based on isolation of the chromosomal segment containing the QTL in a congenic strain, followed by systematic narrowing of the segment to a size (1 to 2 cM) at which positional cloning becomes realistic and candidate gene analysis more focused.

A congenic strain is a strain in which a segment of chromosome from 1 strain (the donor strain, strain A) is introgressed into the genetic background of a recipient strain (strain B).11 This is achieved by an initial mating of strain A
with strain B, followed by multiple rounds of back-crossing of the progeny to strain B, maintaining heterozygosity for the region of interest by genotyping the progeny at each generation with markers flanking the segment to be transferred and only using heterozygote progeny for the next round of breeding.13 On the other hand, at each back-cross, the rest of the genome should become increasingly that of strain B (by a factor of 0.5 of the residual heterozygosity at each generation). Thus, after 10 rounds of back-crossing, >99.9% of the background genome should be of strain B. At this stage, the animals are intercrossed to obtain a new (congenic) strain of animals homozygous for strain A for the segment of interest but with the rest of the genome of strain B. Once transfer of the QTL into the congenic strain has been confirmed, further targeted dissection (narrowing) of the segment can be achieved more expeditiously by again cross-breeding with strain B and developing congenic substrains from animals that show meiotic recombination within the introgressed region. As before, each substrain is analyzed to see whether it demonstrates the phenotype of interest. This process can be repeated until the QTL has been isolated into the smallest possible segment.

In previous studies, we12 and others5,7,13–17 have shown the presence of a major blood pressure QTL on rat chromosome 1. The locus was initially identified through studies investigating the role of the Sa gene in hypertension.12–17 The Sa gene codes for a 580 amino acid protein, of as-yet undetermined function, which is expressed at a markedly higher level in the proximal renal tubule of the spontaneously hypertensive rat (SHR) compared with the Wistar-Kyoto rat (WKY).18 In second filial generation (F2) progeny derived from a cross of the SHR with the WKY, we found that inheritance of the Sa gene allele from the SHR was associated with an increase in blood pressure in the F2 progeny in a codominant fashion, accounting for 28% and 21%, respectively, of the genetic variance of systolic (SBP) and diastolic (DBP) blood pressure in these animals.12 Although the Sa gene remains a candidate to explain the effect of the locus on blood pressure, other potential candidate genes have been mapped to this region of chromosome 1, including the genes coding for the β and γ subunits of the epithelial sodium channel,9,20 where mutations have been shown to cause a rare form of genetic hypertension in humans, Liddle’s syndrome.21,22 The purpose of this study was to isolate a chromosomal segment containing the chromosome 1 blood pressure QTL located at or near the Sa gene to facilitate detailed genetic dissection. We report the successful bilateral transfer of this QTL into congenic strains derived from SHR and WKY strains.

Methods

Animals

The SHR and WKY used for the construction of congenic strains were obtained from the same colonies as used in our previous F2 study,12 which identified the chromosome 1 QTL. The colonies were initially started from SHR and WKY breeding pairs derived from Charles River Laboratories (Margate, UK) in 1988 and maintained by strict brother×sister mating in the Biomedical Services Unit, University of Leicester. All animals (parental and congenic) were housed under identical controlled conditions (temperature, 21±1°C; humidity, 60±10%; 12-hour day/night cycle), fed standard rat chow.

Characterization of Introgressed Segments in WKY.SHR-Sa and SHR.WKY-Sa

Microsatellite markers on rat chromosome 1 were identified from published maps7,25–27 and tested for polymorphism between our SHR and WKY strains by polymerase chain reaction amplification as previously described.12 For those that were polymorphic, the congenic strains (n=6 animals from each strain) were then genotyped to determine which allele they carried. An identical protocol was used to test the status of the background genome of both congenic strains.
using 60 additional microsatellite markers scattered throughout the genome (at least 2 per chromosome) and known to be polymorphic between our SHR and WKY strains (References 10 and 24; and D. Gauguier, unpublished data, 1998).

Measurement of Blood Pressure in Parental and Congenic Strains

Indirect blood pressure was measured at 16 and 20 weeks of age in conscious, male animals by tail plethysmography (Narco Biosysm physiograph and transducer). Animals were pretrained to 34°C for 20 minutes before measurements were taken, which were always carried out in the morning between 9 AM through 1 PM. At each age, readings were taken on 2 separate days with 3 measurements on each occasion. The average of all readings was taken as the value for that age.

Direct blood pressure measurements were made at 25 weeks of age. This was carried out in conscious, unrestrained animals as previously described.26 Briefly, with rats under sodium methohexital anesthesia (40 to 60 mg/kg IP, supplemented as required), an arterial catheter was connected to a pressure transducer (Bell & Howell, type 4-422) via a low-volume displacement dome. Recordings of SBP and DBP were obtained in the undisturbed state from conscious, freely moving animals. Measurements were made over a period of at least 45 minutes, and the mean values for SBP and DBP were calculated from the whole period of recording. Measurements in each congenic strain and its respective parental strain were carried out concurrently, and the person performing the measurement was blinded to the parental or congenic status of the animals.

Mapping of Chromosome 1 BP QTL in SHR×WKY F2 Rats

DNAs from the F2 progeny of our SHR×WKY cross12 were also genotyped for selected polymorphic chromosome 1 markers to construct a linkage map for the chromosome and to map the blood pressure QTL in more detail. The characteristics of the cross and its phenotyping have been described in detail previously.12 Briefly, blood pressure was measured directly in these rats (n=193 males) at 6 and 25 weeks of age. This was carried out in conscious, unrestrained animals as previously described.28 The SHR×WKY F2 progeny of our SHR×WKY cross was shown to be highly genetically heterogeneous, with 15 cM introgressed from WKY.SHR- and the segment of WKY chromosome 1 present in SHR.WKY-Sa and the segment of WKY chromosome 1 present in SHR.WKY-Sa are illustrated in Figure 3. The segment of SHR chromosome 1 present in WKY.SHR-Sa and the segment of WKY chromosome 1 present in SHR.WKY-Sa contains a minimum of ∼26 cM introgressed segment (between mD7mit206 and D1Mit2), whereas SHR.WKY-Sa contains a minimum of ∼15 cM introgressed segment (between mD7mit206 and D1Wox34), with both, as expected from our selection strategy (see Methods), containing the donor allele of the Sa gene. In both strains, extensive analysis of the rest of the genome using 60 polymorphic

RNA Analysis

Total cellular RNA was extracted from kidneys of 6- and 25-week-old male rats as previously described.25 RNA concentrations were determined by spectrophotometry at 260 nm. Northern blotting and hybridization were performed using standard protocols.26 Briefly, 60 µg of RNA per sample was electrophoresed through 1.2% (wt/vol) agarose gels containing 2.2 mol/L formaldehyde and transferred to Hybond N membrane (Amsersham Ltd) as recommended by the manufacturer. Membranes were initially probed for Sa mRNA with an Sa complementary DNA probe and then for mRNA for GAPDH (loading control) using a 29-mer complementary oligonucleotide.29 The Sa probe (20 ng per reaction) was radioactively labeled with deoxy(α-32P)CTP by the random-priming method.26 The GAPDH probe (50 ng per reaction) was end-labeled using terminal deoxynucleotidyl transferase.26

Statistical Analysis

Construction of the chromosome 1 linkage map and QTL localization were done with the MAPMAKER programs11,12 kindly provided by Dr. Lande (Whitehead Institute, Cambridge, Mass). Differences in blood pressures between parental and congenic strains were evaluated by ANOVA using MINITAB (Minitab Inc).

Results

Of 84 chromosome 1 markers tested, 38 (45%) were polymorphic between our SHR and WKY strains. The linkage map derived from genotype analysis of 23 of these markers in F2 progeny of our SHR×WKY cross is shown in Table 1. The map spanning 184 cM closely agrees with linkage maps derived in other crosses.23,24 The QTL maps for SBP and DBP for chromosome 1 derived using these markers in our F2 cross are shown in Figure 2. As expected on the basis of our previous analysis with the Sa gene polymorphism,12 both showed peaks with highly significant LOD values. The peak LOD scores were 7.5 and 6.6 for SBP and DBP, respectively. Interestingly, the peaks lay ∼20 cM away from Sa in the interval between the marker D1Mit2 and D1Wox29. SBP and DBP of 25-week-old F2 rats of different genotypes at the D1Mit2 locus are shown in Table 2.

The segment of SHR chromosome 1 present in WKY.SHR-Sa and the segment of WKY chromosome 1 present in SHR.WKY-Sa are illustrated in Figure 3. The segment of SHR chromosome 1 present in WKY.SHR-Sa and the segment of WKY chromosome 1 present in SHR.WKY-Sa contains a minimum of ∼26 cM introgressed segment (between mD7mit206 and D1Mit2), whereas SHR.WKY-Sa contains a minimum of ∼15 cM introgressed segment (between mD7mit206 and D1Wox34), with both, as expected from our selection strategy (see Methods), containing the donor allele of the Sa gene. In both strains, extensive analysis of the rest of the genome using 60 polymorphic

**TABLE 1. Markers Analyzed on Chromosome 1**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Locus</th>
<th>Distance, cM</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1Smu2</td>
<td>Adrenergic receptor α2a (Adra2a)</td>
<td>13.0</td>
</tr>
<tr>
<td>D2Smu1</td>
<td>Adrenergic receptor β1 (Adrb1)</td>
<td>3.9</td>
</tr>
<tr>
<td>D1Mit14</td>
<td></td>
<td>2.6</td>
</tr>
<tr>
<td>D1Mgh15</td>
<td></td>
<td>8.9</td>
</tr>
<tr>
<td>D1Mgh13</td>
<td>Internexin α (Inexa)</td>
<td>5.5</td>
</tr>
<tr>
<td>D1Wox25</td>
<td>Cytochrome P450 15-β gene (Cyp2c12)</td>
<td>6.6</td>
</tr>
<tr>
<td>D1Mit7</td>
<td></td>
<td>14.1</td>
</tr>
<tr>
<td>D1Wox10</td>
<td></td>
<td>17.3</td>
</tr>
<tr>
<td>mD7mit206</td>
<td></td>
<td>2.2</td>
</tr>
<tr>
<td>D1Wox19</td>
<td>Metallothionein 1, pseudogene A (Mt1pa)</td>
<td>1.3</td>
</tr>
<tr>
<td>D1Smu6</td>
<td>Interleukin 4 receptor (Il4r)</td>
<td>4.1</td>
</tr>
<tr>
<td>D1Smu8</td>
<td>Epithelial sodium channel β subunit (Scnn1b)</td>
<td>1.9</td>
</tr>
<tr>
<td>Sa</td>
<td>Sa gene</td>
<td>5.6</td>
</tr>
<tr>
<td>D1Wox34</td>
<td>Adrenomedullin precursor (Ap)</td>
<td>2.3</td>
</tr>
<tr>
<td>D1Wox33</td>
<td>N-ras1.2ep pseudogene (N12ep)</td>
<td>6.2</td>
</tr>
<tr>
<td>D1Mit3</td>
<td></td>
<td>2.8</td>
</tr>
<tr>
<td>D1Mit2</td>
<td></td>
<td>10.8</td>
</tr>
<tr>
<td>D1Wox29</td>
<td></td>
<td>4.6</td>
</tr>
<tr>
<td>D1Wox5</td>
<td></td>
<td>10.5</td>
</tr>
<tr>
<td>D1Wox18</td>
<td>Kallikrein 1, renal/pancreas/salivary (Kai)</td>
<td>16.3</td>
</tr>
<tr>
<td>D1Wox11</td>
<td>Protein kinase C, type 1 (Pkc)</td>
<td>25.1</td>
</tr>
<tr>
<td>DIN40</td>
<td></td>
<td>15.0</td>
</tr>
<tr>
<td>D1Wox26</td>
<td></td>
<td>…</td>
</tr>
</tbody>
</table>

Marker names are those assigned by Jacob et al25 (Mit/Mgh), Bihoreau et al24 (Wox), or Nabika et al.29

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markers showed no evidence of introgression of donor DNA elsewhere (data not shown).

The blood pressures of the congenic strains compared with their respective parental strains at 16, 20, and 25 weeks of age are shown in Table 3. At all ages, the blood pressure of WKY.SHR-Sa was significantly higher than that of WKY rats, whereas that of SHR.WKY-Sa rats was significantly lower than that of SHR. At 25 weeks, the mean differences in SBP and DBP between WKY.SHR-Sa and WKY were +11.5 mm Hg and +11.6 mm Hg, respectively. The corresponding differences between SHR.WKY-Sa and SHR were −11.3 mm Hg and −9.1 mm Hg, respectively.

The levels of Sa mRNA in the kidneys of parental and congenic strains at 6 weeks of age are shown in Figure 4. As previously reported,12,18,33 Sa mRNA level was markedly elevated in the SHR kidney compared with the WKY. Sa mRNA level was also markedly elevated in WKY.SHR-Sa compared with SHR and similar to the level in the SHR. Conversely, Sa mRNA level was markedly lower in SHR.WKY-Sa compared with SHR and similar to the level in the WKY. Similar results were obtained in 25-week-old rats (data not shown).

TABLE 2. Direct Blood Pressures in 25-Week-Old F2 Rats Derived From SHR×WKY Cross With Different Genotypes at D1Mit2 Marker

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Mean±SD SBP, mm Hg</th>
<th>Mean±SD DBP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>WW</td>
<td>49</td>
<td>178.1±13.0</td>
<td>115.4±10.7</td>
</tr>
<tr>
<td>WS</td>
<td>89</td>
<td>184.5±13.0</td>
<td>120.2±9.8</td>
</tr>
<tr>
<td>SS</td>
<td>47</td>
<td>193.4±14.1</td>
<td>126.1±9.2</td>
</tr>
<tr>
<td>F (ANOVA)</td>
<td></td>
<td>16.7</td>
<td>14.3</td>
</tr>
<tr>
<td>P (ANOVA)</td>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 2. Direct Blood Pressures in 25-Week-Old F2 Rats Derived From SHR×WKY Cross With Different Genotypes at D1Mit2 Marker

Characteristics of the F2 cross and its phenotyping have been described in detail previously.12 Blood pressure was measured directly in conscious, unrestrained rats through a lower aortic cannula introduced through the femoral artery. WW indicates homozygous for WKY allele; WS, heterozygous; SS, homozygous for SHR allele; and n, number of rats. Genotypes for the D1Mit2 marker were not available on 8 animals.

Figure 2. QTL LOD plots for SBP and DBP in 25-week-old F2 rats derived from SHR×WKY cross. See Table 1 for details of markers and intermarker distances and Methods for details of cross. The horizontal dotted line indicates the threshold for significance of the LOD score (3.0).

Discussion

In this study we have successfully isolated a segment of rat chromosome 1 containing a major blood pressure QTL in congenic strains. The findings confirm data from cosegregation analysis5,7,12–17 that strongly indicated, but did not by themselves prove, the presence of such a QTL on this rat chromosome. Furthermore, we demonstrate bilateral transfer of the blood pressure QTL leading to both a lowering of blood pressure in a hypertensive recipient strain and an elevation of blood pressure in a normotensive recipient strain. The availability of the reciprocal congenic strains should now facilitate investigations into the mechanisms through which the QTL influences blood pressure, as well as permit progress toward identification of the causative gene.
The ideal way of proceeding to isolate a QTL in a congenic strain is to first define the boundary of the region most likely to contain the QTL (usually taken as 2 LOD score distances to either side of the peak), by constructing a QTL map in a segregating population, and then use flanking markers to control the transfer of the whole region during the backcrossing.11 This would ensure that the QTL is captured in the congenic strain with a high probability. However, such a strategy requires (1) a sufficiently detailed linkage map for the region being studied and (2) markers that are informative between the strains used. With the recent advances in the development of the rat linkage map23,24 and the availability of an increasing number of rat microsatellite markers, this is now feasible for most rat chromosomes. However, when our congenic breeding program started more than 4 years ago, this information was not available; therefore, our selection was based on a marker in a single candidate gene, Sa. This explains (1) why different-sized regions were captured in WKY.SHR-Sa and SHR.WKY-Sa and (2) the discrepancy between the regions introgressed in both strains (Figure 3) and the apparent location of the QTL based on the additional analysis performed in our SHR × WKY F2 cross when more markers became available (Figure 2). Indeed, Sa lies just over 2 LOD scores away from the QTL peak. These observations raise some interesting questions about the chromosome 1 blood pressure QTL. If there is a single QTL, our congenic strains and especially SHR.WKY-Sa define the region in which such a QTL occurs. When this is considered together with the data from the QTL map (Figure 2), the most likely location of the QTL would seem to be in the segment between Sa and D1Wox33 (the overlap region between the introgressed fragment in SHR.WKY-Sa and the interval defining the 2 LOD score distance from the peak on the QTL map). However, an alternate possibility is that the QTL map reflects the composite effect of 2 (or possibly even more) loci affecting blood pressure in this region of chromosome 1, and that we have captured 1 of these in our congenic strains. This would require at least 1 further QTL to be present downstream of D1Wox33 to explain the location of the observed peak. There is some evidence to support this possibility. In F2...
rats from a cross of the Dahl salt-sensitive rat (Dahl S) with the Lewis rat, Gu et al. were able to map 2 loci on chromosome 1 influencing blood pressure. One region was centered around the Sa locus, while the other region was 55 cM downstream centered around a cluster of cytochrome 450 genes. Such a distance is large and should have been resolvable in our cross if accurate. However, based on the 95% intervals in which the 2 QTLs identified by Gu et al. could lie in relation to each other, it is possible that the actual distance between them is much closer and therefore compatible with the composite peak observed in our cross (Figure 2). The only way of resolving this issue would be to see whether a region of chromosome 1 downstream of D1Wox33 also influences blood pressure when isolated in a congenic strain.

Previous attempts at isolating blood pressure QTLs in congenic strains have mostly, apart from a few exceptions, involved transferring the region in a single direction. In the majority of cases, this has been from the normotensive strain into the hypertensive strain, on the assumption that demonstrating a decrease in blood pressure would be easier than an increase. Here we show that the SHR chromosome 1 segment around the Sa gene locus is capable of raising blood pressure in the WKY. The reciprocal changes seen in WKY.SHR-Sa and SHR.WKY-Sa strongly suggest that the effects seen are directly due to the QTL on chromosome 1 rather than due to a QTL present on another chromosome that by chance was also transferred together with the chromosome 1 region. Although 10 generations of back-crossing should result in >99.9% of the background genome being recipient (supported by our analysis of microsatellite polymorphisms on other chromosomes), the possibility of a donor region elsewhere having an effect cannot be completely excluded with a single strain. However, the finding of complementary changes in 2 strains, which diverged at a very early stage (Figure 1), makes it very much more likely that the effect is due to the region selected for.

The magnitude of the increase in blood pressure observed in WKY.SHR-Sa compared with WKY was similar to the magnitude of the fall in blood pressure in SHR.WKY-Sa compared with SHR (Table 3). Furthermore, both effects were similar in magnitude to the difference in blood pressure that we previously found between F_2 animals homozygous for the SHR and WKY alleles of the Sa gene (SBP and DBP at 25 weeks: −13.6 mm Hg and −9.7 mm Hg, respectively) and indeed for the marker closest to the peak, D1Mit2 (Table 2). These findings therefore suggest that we have captured the same blood pressure locus around the Sa gene previously identified in F_2 crosses and further that this locus does not require the epistatic interaction of other loci for its full expression. Given an average difference in 25-week SBP and DBP between SHR and WKY of 65 mm Hg and 47 mm Hg, respectively (Table 3), the locus accounts for between 17% and 25% of the hypertension in the SHR versus WKY.

Recently, St. Lezin et al. reported significantly lower 24-hour radiotelemetric blood pressure in a congenic strain in which an ≈22- to ≈33-cM region of chromosome 1 in the SHR (defined by the markers D1Mit3 and Igf2) was replaced with the corresponding segment from the normotensive Brown-Norway strain. The reductions in blood pressure in SHR.BN-D1Mit3/Igf2 were 10 to 15 mm Hg for SBP and 6 to 10 mm Hg for DBP, mirroring the amounts seen in our strains. Furthermore, the regions introgressed in SHR.BN-D1Mit3/Igf2 and our congenic strains overlap considerably (Figure 3). Thus, our findings strongly corroborate those of St. Lezin et al. Because the introgressed region present in SHR.WKY-Sa is contained within that present in SHR.BN-D1Mit3/Igf2, comparison of the 2 strains does not allow the location of the QTL to be significantly better defined at this stage. However, as substrains are developed from each and the number of precisely mapped markers available for the region increases, such comparisons may prove very helpful.

There is approximately a 4- to 6-fold higher steady-state level of Sa mRNA in the kidney of the SHR compared with the WKY. Previously we have shown that the difference in Sa mRNA levels between SHR and WKY cosegregated with the Sa genotype in 6- and 25-week-old F_2 rats, suggesting that cis-acting elements in or around the Sa gene that differ between the 2 strains influence the level of Sa gene expression. The cotransfer of the level of renal Sa expression with the Sa allele in the congenic strains (Figure 4) confirms

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**TABLE 3. Blood Pressures of WKY.SHR-Sa and SHR.WKY-Sa Compared With Respective Parental Strains at 16, 20, and 25 Weeks of Age**

<table>
<thead>
<tr>
<th>Strain</th>
<th>16 Weeks</th>
<th>20 Weeks</th>
<th>25 Weeks, SBP</th>
<th>25 Weeks, DBP</th>
<th>P</th>
<th>&lt;0.001</th>
<th>0.001</th>
<th>0.002</th>
<th>0.005</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY (n=28)</td>
<td>120.6±7.1</td>
<td>126.0±7.3</td>
<td>167.3±11.6</td>
<td>102.1±12.1</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.002</td>
<td>0.005</td>
</tr>
<tr>
<td>WKY.SHR-Sa</td>
<td>127.3±8.6</td>
<td>133.4±7.5</td>
<td>178.8±12.5</td>
<td>113.7±9.4</td>
<td>0.012</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>WKY.SHR-Sa</td>
<td>186.5±14.3</td>
<td>195.3±10.9</td>
<td>220.9±14.3</td>
<td>139.2±12.5</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>SHR (n=28)</td>
<td>195.7±13.1</td>
<td>206.8±12.5</td>
<td>232.2±12.1</td>
<td>148.3±11.2</td>
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<td></td>
</tr>
<tr>
<td>SHR.WKY-Sa</td>
<td>186.5±14.3</td>
<td>195.3±10.9</td>
<td>220.9±14.3</td>
<td>139.2±12.5</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Blood pressures at 16 and 20 weeks are indirect tail-cuff in conscious, unrestrained rats; 25-week blood pressures are direct arterial pressures in conscious, unrestrained rats.

*For 25-week blood pressure, n=24.

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**Figure 4.** Sa mRNA levels in kidneys of 6-week-old SHR, WKY, and congenic strains. Northern blots of kidney RNAs prepared from 6-week-old SHR, WKY, SHR.WKY-Sa, and WKY.SHR-Sa probed for Sa mRNA (upper panel) and then reprobed for GAPDH mRNA (lower panels) are shown. Note the very low level of expression of Sa mRNA in the kidneys of WKY and SHR.WKY-Sa.
that basal levels, at least in the SHR and WKY, are primarily determined by DNA elements within or in the proximity of the Su gene. The function of the Su gene remains unknown, and therefore any involvement in hypertension uncertain, with conflicting data for both animal models and humans. The development of SHR.WKY-Sa and WKY.SHR-Sa now makes it possible to investigate formally the continued candidacy of the Sa gene as the QTL (and indeed other candidate genes) by making substrains (see introduction of article) and examining whether it lies within or outside the minimum resolvable region that influences blood pressure; this work, as well as the physiological characterization of the congenic strains and the definition of intermediary phenotypes through which the QTL acts, is currently in progress.

Acknowledgments

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References


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