Genetic Analysis of Rat Chromosome 1 and the \textit{Sa} Gene in Spontaneous Hypertension

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\textbf{Abstract}—Linkage studies in segregating populations derived from the spontaneously hypertensive rat (SHR) indicate that a blood pressure quantitative trait locus exists on rat chromosome 1 in the vicinity of the \textit{Sa} gene. On the basis of these findings and the observation of increased renal expression of the \textit{Sa} gene in SHR versus normotensive rats, the \textit{Sa} gene has been proposed as a candidate gene for spontaneous hypertension. In SHR congenic strains, we and others have found that replacement of a segment of SHR chromosome 1 that contains the \textit{Sa} gene with the corresponding chromosome segment from a normotensive Brown Norway (BN) rat or Wistar-Kyoto rat can reduce blood pressure. To test whether the \textit{Sa} gene is necessary for the effect of this region of chromosome 1 on blood pressure, we studied a new SHR congenic subline that harbors a smaller segment of BN chromosome 1 that does not include the \textit{Sa} gene. Transfer of this subregion of chromosome 1 from the BN rat onto the SHR background was associated with significant reductions in blood pressure comparable to those previously observed on transfer of a larger region of chromosome 1 that included the \textit{Sa} gene. Thus, in the SHR-BN model of hypertension, the results of these mapping studies (1) demonstrate that molecular variation in the \textit{Sa} gene is not required for the effect of this region of chromosome 1 on blood pressure and (2) should direct attention toward other candidate genes within the differential chromosome segment of the new congenic subline. (\textit{Hypertension}. 2000;35[part 2]:225-230.)

\textbf{Key Words:} hypertension, genetic \textit{n} genes \textit{n} rats, inbred SHR \textit{n} rats, inbred WKY

The \textit{Sa} gene is a proposed candidate gene for essential hypertension that was first isolated by virtue of its increased expression in the kidneys of spontaneously hypertensive rats (SHR) compared with the kidneys of normotensive Wistar-Kyoto (WKY) rats.\textsuperscript{1} In linkage studies in segregating populations derived from SHR and normotensive strains, the chromosome segment containing \textit{Sa} cosegregated with effects on blood pressure (BP).\textsuperscript{2-6} Although the \textit{Sa} gene has no known function, the results of genetic linkage studies combined with the finding of increased renal expression of the \textit{Sa} gene in SHR versus normotensive strains has generated considerable interest in the determination of whether the \textit{Sa} gene plays a role in spontaneous hypertension.\textsuperscript{7}

Recent studies in congenic strains independently derived by our group and others from SHR and Brown Norway (BN) rats\textsuperscript{8} or from SHR and WKY rats\textsuperscript{9,10} confirm that a BP quantitative trait locus (QTL) exists in an overlapping region of chromosome 1 that contains the \textit{Sa} gene. In our congenic strain (designated SHR.BN-\textit{D1Mit3/Igf2}), the transfer of a 22-cM segment of chromosome 1 that included \textit{Sa} from the BN rat into the SHR resulted in a 10 to 15 mm Hg decrease in systolic BP.\textsuperscript{8,11} To determine whether the \textit{Sa} gene is necessary for the effect of this segment of chromosome 1 on BP and to further narrow the location of the BP QTL on chromosome 1, we measured BP in a new SHR congenic subline that excludes \textit{Sa} derived from the original SHR.BN-\textit{D1Mit3/Igf2} congenic strain. Transfer of this subregion of chromosome 1 from the BN rat onto the SHR background was associated with significant reductions in BP comparable to those previously observed on transfer of the larger region of chromosome 1 that included the \textit{Sa} gene. On the basis of the study results, it appears that in the SHR-BN model of hypertension, molecular variation in the \textit{Sa} gene is not required for the effect of this region of chromosome 1 on BP.

\textbf{Methods}

\textbf{Strains}

The SHR congenic strain was derived from a progenitor strain of SHR (SHR/Ola) descended from inbred SHR originally obtained from the National Institutes of Health. This progenitor strain of SHR is commercially available in Europe and has been maintained at the Czech Academy of Sciences in Prague through brother×sister mating for >15 years.\textsuperscript{8}

The SHR.BN-\textit{D1Mit3/Igf2} congenic strain was derived as described previously through a selective breeding protocol in which a...
segment of chromosome 1 from the normotensive BN/Cr strain was transferred onto the genetic background of the progenitor SHR. The new SHR.BN-Igf2 congenic subline was derived through backcrossing of the original SHR.BN-D1Mit3/Igf2 congenic strain (N10 backcross generation) to the SHR progenitor strain. Rats from the resulting F1 generation were intercrossed to derive an F2 generation. To detect recombinants within the chromosome 1 region carried by the original congenic strain, tail DNA from F2 rats was screened with the use of chromosome 1 microsatellite markers. Two male and 2 female F2 rats heterozygous for a smaller segment of chromosome 1 defined by the markers D1Mgh21 and D1Mgh11 (a segment that excludes the Sa gene) were intercrossed, and offspring homozygous for the D1Mgh21/Igf2/D1Mgh11 segment were selected to fix the subline. The resulting subline was designated SHR.BN-Igf2 and is maintained in the homozygous state through brother x sister mating.

Genotype Analysis of the SHR.BN-Igf2 Congenic Subline
The primary markers used to screen F2 rats for recombination within the segment of chromosome 1 carried by the original congenic line were D1Arb12, D1Mit3, D1Mgh8, D1Mit4, D1Mgh9, Sa, Scnn1b, Mt1pa, D1Mgh21, Igf2 (D1Mgh22), D1Mgh10, and D1Mgh11. To further define the size of the homozygous BN segment in the SHR.BN-Igf2 subline, we used PCR analysis of the markers D1Rat68, D1Rat208, and D1Rat71 that are polymorphic between SHR and BN and that were mapped on the recently published radiation hybrid map of chromosome 1. Primers were synthesized by the University of California San Francisco Biomolecular Resource Center according to sequences obtained from the Whitehead Institute for Biomedical Research/MIT Rat genome map unless otherwise specified. The Sa gene was analyzed with the use of PCR primers polymorphic between SHR and BN as published by Gu et al. We found that the size of the homozygous BN segment transferred was a minimum of 7 cM based on the map distances of the WIBR/MIT map and Pravenec et al. The location of the markers D1Rat68, D1Rat208, and D1Rat71 are estimated based on the radiation hybrid map of Watanabe et al and the WIBR/MIT map.

Figure 1. Map showing the transferred segment of chromosome 1 in (a) the SHR.BN-D1Mit3/Igf2 congenic strain and (b) the SHR.BN-Igf2 congenic subline. The bars denote the chromosome region transferred from the BN strain. Map distances are adapted from the chromosome 1 maps of the WIBR/MIT rat genome map and Pravenec et al. The location of the markers D1Rat68, D1Rat208, and D1Rat71 are estimated based on the radiation hybrid map of Watanabe et al and the WIBR/MIT map.

Cardiovascular Phenotyping
Pulsatile arterial pressures and heart rates were measured continuously in 13 male progenitor SHR and 9 male congenic subline SHR.BN-Igf2 rats for 9 weeks beginning at 11 weeks of age. Indwelling aortic radiotelemetry transducers were implanted with the animals under ketamine/xylazine anesthesia as described previously. Systolic and diastolic BPs and heart rates were recorded in unanesthetized, unrestrained rats in 5-second bursts every 5 minutes throughout the day and night. On the basis of these data, separate daytime and nighttime 12-hour averages for systolic and diastolic BPs, mean arterial pressure, and heart rate were calculated for each rat for each day from 11 to 19 weeks of age. From weaning through 14 weeks of age, all rats were given tap water ad libitum and fed a standard pelleted laboratory diet that contained 0.58% NaCl and 1.1% K+. To test for interactions between dietary salt and the effect of the differential chromosome 1 segment on BP, 1% NaCl was added to the drinking water at age 15 weeks for 1 week. Rats were then switched back to tap water for the remainder of the study (ages 16 to 19 weeks). A baseline nighttime and daytime BP for each rat was determined by averaging the daily BP measurements obtained during the period before salt administration (11 to 14 weeks of age). The BPs of older rats (17 to 19 weeks of age) were determined by averaging the daily BP measurements beginning 1 week after administration of the supplementary dietary salt was stopped. Average BPs obtained at baseline (11 to 14 weeks of age), during salt administration (15 weeks of age), and in older animals (17 to 19 weeks of age) were analyzed with 2-way repeated measures ANOVA with the use of the Student-Newman-Keuls correction for multiple comparisons (SigmaStat; SPSS). Daytime and nighttime systolic and diastolic BPs and heart rates were analyzed separately. A value of $P<0.05$ was considered statistically significant. Differences in the BP response to NaCl between the progenitor strain and the SHR.BN-Igf2 subline were analyzed by comparing the change in BPs between week 14 (normal salt diet) and the final 3 days of week 15 (during 1% NaCl-water administration).
In addition to comparing the BPs of SHR progenitor and SHR.BN-Igf2 subline rats, we analyzed the baseline (11 to 14 weeks of age) 24-hour systolic and diastolic BPs and heart rates of SHR.BN-Igf2 subline rats \((n=9)\) versus the BPs and heart rates of the original SHR.BN-D1Mit3/Igf2 congenic rats \((n=9)\) with the use of ANOVA followed by the Student-Newman-Keuls multiple comparison method. The BPs and heart rates of the SHR.BN-D1Mit3/Igf2 rats were reported previously\(^8\) and are reanalyzed and reported here for comparison purposes.

At sacrifice, the hearts were removed and weighed. Mean differences between strains in body weight and heart weight corrected for body weight were analyzed with ANOVA. All procedures involving animals were performed in accordance with institutional guidelines.

**Sa Gene Expression**

To confirm that Sa gene expression is similar in the kidneys of SHR.BN-Igf2 subline rats and SHR rats, total RNA was extracted from the kidneys of 25-week-old SHR progenitor, BN progenitor, and SHR.BN-Igf2 subline rats and analyzed with Northern blotting.\(^{21}\) The rat Sa cDNA probe that we used was a 1.6-kb fragment synthesized through reverse transcription and PCR amplification with primers published by Samani et al.\(^2\) that correspond to positions 7 to 1923 of the Sa gene sequence.\(^1\) GAPDH expression was measured to control for sample loading.

**Results**

The minimum size of the transferred chromosome 1 segment in the SHR.BN-Igf2 subline is delineated by markers for **Igf2**. The minimum size of the transferred chromosome 1 segment is defined by the markers **D1Rat68** and **D1Rat71**. Thus, the size of the transferred chromosome segment is between 7 and 9 cM (Figure 1). **D1Mgh21**, **Igf2**, and **D1Mgh10**, which were used as the selection markers in the derivation of the congenic subline, are within the transferred chromosome segment. The BN allele of the Sa gene was not contained within the transferred chromosome segment, and therefore the new congenic subline carries the SHR allele for Sa.

Twelve-hour average daytime and nighttime systolic and diastolic BPs and mean arterial pressures determined through radiotelemetry were all significantly lower in the SHR.BN-Igf2 congenic subline rats than in the SHR progenitor rats at baseline (11 to 14 weeks of age) and during 1% NaCl-water administration and in older rats (17 to 19 weeks of age) receiving a normal salt diet (Figure 2) \((all \ P<0.0001)\). In addition, the SHR.BN-Igf2 congenic subline rats had significantly lower daytime and nighttime pulse pressures than the SHR progenitor rats \(\text{baseline nighttime pulse pressure: progenitor rats } 54\pm 1.2 \text{ mm Hg, SHR.BN-Igf2 subline rats } 45\pm 1.4 \text{ mm Hg [mean\(\pm\)SEM, } P<0.001)\). There were no significant differences in daytime or nighttime heart rates between the SHR progenitor rats and SHR.BN-Igf2 congenic subline rats.

Although there was no significant difference in mean body weight at sacrifice between the SHR progenitor and SHR.BN-Igf2 rats, heart weights corrected for body weight were significantly higher in the SHR progenitor rats \(\text{progenitor rats } 3.9\pm 0.05 \text{ mg/g body weight, SHR.BN-Igf2 subline rats } 3.7\pm 0.06 \text{ mg/g body weight [mean\(\pm\)SEM, } P<0.05)\).

Both the SHR progenitor rats and the SHR.BN-Igf2 rats showed similar increases in BP during 1% NaCl-water administration at 15 weeks of age. For example, the difference in nighttime mean arterial pressure between baseline and the final 3 days of 1% NaCl-water administration was 13\(\pm\)1.5 mm Hg for the SHR progenitor strain and 13\(\pm\)1.3 mm Hg for the SHR.BN-Igf2 subline.

We also compared the BPs of the current SHR.BN-Igf2 subline rats with the BPs of the previously reported SHR.BN-D1Mit3/Igf2 congenic rats in which we originally isolated a BP QTL on chromosome 1.\(^8\) The Table 4 shows the baseline (11 to 14 weeks of age) 24-hour average systolic and diastolic BPs and heart rates of the SHR progenitor and SHR.BN-Igf2 subline rats versus the BPs and heart rates of the original SHR.BN-D1Mit3/Igf2 congenic strain. Both the SHR.BN-Igf2 subline and the SHR.BN-D1Mit3/Igf2 strain have significantly lower diastolic and systolic BPs compared with the SHR progenitor strain \(P<0.05\). Although there was no difference in systolic BP between the original congenic strain and the SHR.BN-Igf2 subline, the systolic BPs of the SHR.BN-Igf2 rats were slightly but significantly lower than...
the systolic BPs of the original congenic rats \( (P < 0.05) \). The SHR.BN-D1Mit3/Igf2 congenic subline rats carry a larger, 22-cM segment of chromosome 1 that includes the \( Sa \) gene transferred from the BN rat, as shown in Figure 1.

With the use of PCR analysis, we confirmed that SHR.BN-Igf2 congenic subline rats are homozygous for the SHR allele of the \( Sa \) gene (not shown). Figure 3 shows that the \( Sa \) gene is expressed at much higher levels in the kidneys of 25-week-old SHR compared with BN rats, which demonstrate a level of \( Sa \) gene expression similar to that found in the kidneys of WKY rats. 2 In Figure 3, the kidneys of the SHR.BN-Igf2 congenic subline rats also show increased \( Sa \) gene expression versus BN progenitor rats, further confirming that the congenic subline carries the \( Sa \) allele of the SHR progenitor strain.

Discussion

Linkage studies in segregated populations have suggested that a BP QTL exists on rat chromosome 1. 2–6,13,22 The \( Sa \) gene was isolated through differential expression in SHR kidneys, 24 and demonstrates the key role played by the kidney in the regulation of BP. 23 and the fact that \( Sa \) is highly expressed in the proximal tubule in SHR kidneys, 24 \( Sa \) has become an important candidate gene for hypertension in the rat.

The next step after linkage studies to confirm the existence of a BP QTL in a given chromosome region is to derive congenic strains that are genetically identical except in the chromosome region of interest. 25,26 Accordingly, we constructed a congenic strain of SHR that carries a \( \approx 22 \)-cM region of chromosome 1 that includes the \( Sa \) gene transferred from the normotensive BN rat. 8 We found that the systolic BPs of male SHR.BN-D1 Mit3/Igf2 congenic rats were 10 to 15 mm Hg lower than those of age-matched progenitor SHR males. This study confirmed that a BP QTL exists on rat chromosome 1 within the transferred segment (which includes \( Sa \)) in the congenic strain. Recently, Frantz et al 9 found that mean BP was significantly lower in an SHR congenic strain (SHR.WKY-Sa) carrying a chromosome segment that contained \( Sa \) transferred from the WKY rat versus the SHR. In addition, the reciprocal WKY congenic strain (WKY.SHR-Sa) carrying the \( Sa \) gene of the SHR had significantly higher mean BP than the WKY progenitor strain. 9 Iwai et al 10 also found higher mean BPs and an increased BP response to salt-loading in WKY congenic rats carrying the \( Sa \) gene transferred from the SHR.

Taken together, the results in the SHR-BN and SHR-WKY congenic strains are convincing evidence of a BP QTL in the vicinity of \( Sa \). However, all of the congenic strains studied carry large segments (\( \approx 15 \) to 30 cM) of chromosome 1 that contain many genes in addition to \( Sa \), some of which might influence BP. Thus, whether \( Sa \) or another gene or genes linked to \( Sa \) might be responsible for the effect of this region of chromosome 1 on BP has remained uncertain.

In the present study, we derived an SHR congenic subline by backcrossing our original SHR.BN-D1Mit3/Igf2 congenic strain to the progenitor SHR strain, followed by screening for recombination using markers within the chromosome 1 segment of interest. In this fashion, we bred a congenic subline of SHR that carries a \( \approx 7 \)-cM segment of chromosome 1 transferred from the BN rat delineated by the markers \( D1Rat208, \ Igf2, \) and \( D1Mgh11 \) and that does not include the BN \( Sa \) allele. Thus, the resulting SHR.BN-Igf2 subline rats are homozygous for the SHR allele of \( Sa \) and demonstrate kidney \( Sa \) expression levels similar to those found in SHR.
Despite similar high levels of kidney Sa expression, the SHR.BN-Igf2 subline rats have significantly lower BPs than the SHR progenitor rats. The reductions in BP in 11- to 14-week-old rats were 22 mm Hg for nighttime systolic pressure and 14 mm Hg for nighttime diastolic pressure. The reductions in BP associated with transfer of this subregion of chromosome 1 from the BN rat onto the SHR background were comparable or somewhat greater than those previously observed on transfer of a larger region of chromosome 1 that included the BN Sa gene. It should be noted that the BPs of the SHR.BN-D1Mit3/Igf2 rats were not measured concurrently with the BPs of the SHR.BN-Igf2 rats but were measured and reported previously. Thus, the significance of the small difference in systolic BPs between the original congenic line and the current congenic subline is uncertain. However, the fact that the original congenic line and the current subline show similar differences compared with the progenitor SHR emphasizes that we have successfully isolated at least 1 major QTL influencing BP in the SHR.BN-Igf2 subline that is separate from Sa.

In a set of congenic sublines derived from the stroke-prone SHR (SHRSP) and the normotensive WKY rat, Hubner et al recently demonstrated that WKY rats carrying a segment of chromosome 1 (spanning the Sa gene) transferred from the SHRSP showed no difference in BP compared with progenitor WKY rats. On the basis of these results, the authors concluded that the Sa gene is not a BP-elevating candidate locus. A second overlapping WKY congenic subline did show a BP effect, and the authors conclude that a BP QTL on chromosome 1 is located centromeric to the Sa locus (versus the BP QTL isolated in the SHR.BN-Igf2 subline, which maps to the opposite side of the chromosome with respect to Sa). A comparison of the map of Hubner et al with our chromosome 1 map (Figure 1) thus indicates that the QTL identified in the SHRSP-WKY model may be distinct from the BP QTL isolated in the SHR.BN-Igf2 congenic subline. However, both studies support the conclusion that the Sa gene probably is not a major BP determinant in the rat. Further fine-mapping studies in additional congenic sublines (both in the SHR-BN and WKY-SHRSP models) will be required to determine definitively whether there are multiple BP QTL in this region of chromosome 1.

Because of the considerable interest in the Sa gene as a candidate gene for hypertension in the SHR, the focus of this report is on the genetic analysis of chromosome 1 in reference to Sa. However, other important candidate genes exist on rat chromosome 1 in this region, including the genes coding for the β and γ subunits of the epithelial sodium channel (Scnn1b and Scnn1g). Mutations in the human homologues of Scnn1b and Scnn1g have been found to cause Liddle’s syndrome in humans. Like Sa, Scnn1b and Scnn1g are not included in the differential chromosome segment isolated in the SHR.BN-Igf2 congenic subline and thus are not likely to be key determinants of hypertension in the SHR (Figure 1). Before further fine-mapping studies in the SHR.BN-Igf2 subline, it is premature to speculate about which other genes are responsible for the BP effect of the differential chromosome segment. However, this region of rat chromosome 1 is homologous to a region of mouse chromosome 7 that contains Kcnq1, one of the potassium voltage-gated channel family genes that can cause long QT syndrome in humans. We have determined that the BN Kcnq1 gene has been transferred in the SHR.BN-Igf2 subline, but preliminary sequence analysis has revealed no functionally significant sequence variation in Kcnq1 between SHR and BN rats, although it is possible that regulatory mutations exist with in the noncoding regions of Kcnq1. Further analysis of homologous regions of mouse chromosome 7 (and human chromosome 11p) may also reveal a number of other candidate genes that map to the rat chromosome 1 region isolated in the SHR.BN-Igf2 congenic subline.

In summary, the BP results in the SHR.BN-Igf2 subline indicate that at least in the SHR-BN model of hypertension, the effects of chromosome 1 on BP do not depend on molecular differences between the Sa gene of the SHR and the Sa gene of the BN rat. Thus, the marked differences in renal Sa gene expression observed between the SHR and various normotensive strains of rats may be unrelated to any strain differences in BP. The current findings underscore the limitations of studies in which comparisons are made between genetically divergent strains of hypertensive and normotensive rats. It is likely that many genes are differentially expressed between SHR and various strains of normotensive rats, and therefore, considerable caution must be exercised when interpreting such results in the absence of supporting studies in congenic sublines. With the advent of large-scale gene expression profiling, the importance of comparing near-isogenic strains rather than genetically divergent strains may become even more apparent in the near future.

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