Isolation of a Chromosome 1 Region That Contributes to High Blood Pressure and Salt Sensitivity

Naoharu Iwai, Yasuyuki Tsujita, Masahiko Kinoshita

Abstract—Linkage analyses in the spontaneously hypertensive rat (SHR) suggest that a gene involved in blood pressure regulation may be located on rat chromosome 1, in the Sa region. To confirm this possibility, we replaced a region of chromosome 1 in the Wistar-Kyoto rat (WKY) defined by the markers D1Mit3 and MTPA with the corresponding chromosome segment from SHR. Genotyping using 65 polymorphic microsatellite markers throughout the entire genome confirmed the congenic status of this new strain designated WKY.SHR-D1Mit3/Rat57. In male WKY.SHR-D1Mit3/Rat57, mean blood pressures in the daytime and in the nighttime assessed by radiotelemetry were significantly higher than those in male progenitor WKY. Moreover, salt loading significantly increased the mean blood pressure in male WKY.SHR-D1Mit3/Rat57 but not in male progenitor WKY. The present study confirmed the existence of a gene that contributes to high blood pressure and salt sensitivity in this chromosomal segment. This congenic strain represents a new animal model for fine mapping and characterization of the gene in this region involved in salt-sensitive hypertension. (Hypertension. 1998;32:636-638.)

Key Words: blood pressure ■ rats, inbred SHR ■ genetics ■ congenic strain ■ chromosome 1

The spontaneously hypertensive rat (SHR) is the most widely used animal model of human essential hypertension. Although recent developments in molecular genetics have revealed several candidate loci that influence blood pressure (BP) in SHR, the distinct genes that contribute to hypertension have not been identified.1,2 Using this congenic strain, we confirmed the existence of a gene MTPA with the corresponding chromosome region from SHR. Strains

Methods

Strains

The WKY congenic strain was derived by a selective breeding protocol in which a segment of chromosome 1 from SHR was transferred onto the genetic background of the progenitor WKY. The progenitor SHR and WKY were obtained from Charles River Laboratories (Atsugi, Japan), and the inbred status of these strains was confirmed with 65 microsatellite markers throughout the genome. After 10 generations of selective back-crossing to the WKY progenitor strain, during which the presence of the SHR allele of the Sa gene in the heterozygous state and absence of the SHR allele in the D1Mit18 and D1Mit17 loci were confirmed, we selected a litter that had the WW genotype in D1Mit17, D1Rat89, MTPA, and RCA0120 markers and SW genotype in D1Mit3, Sa, and D1Rat57. By brother × sister mating and selective breeding of the offspring, the chromosomal segment around Sa was fixed and maintained. Animals of the N10F3 generation were used in the present study.

Genotype Analysis

To determine the length of the differential chromosome 1 segment, we genotyped the congenic strain using the following genetic markers that were polymorphic between the SHR and WKY progenitor strains: D1Mgh14, D1Mgh13, D1Mit7, D1Mit18, RCA0120, MTPA, D1Rat139, D1Rat57, Sa, D1Mit3, D1Rat89, and D1Mit17. The map positions of these markers were determined with Mapmanager by genotyping 68 male F2 rats derived from the SHR and WKY progenitor strains.

The congenic status of the congenic strain established in the present study was confirmed by genotyping the following markers that were polymorphic between the SHR and WKY progenitor strains: D2Mgh6, D2Mgh14, D2Mit3, D2Mit21, D3Mit10, D4Mgh4, D4Mgh8, D4Mit2, D4IL6, D5Mgh2, D5Mit5, D6Mit4, D6Mit6, D6IGHE, D7Mgh10, D7Mgh11, D7Mit4, D7Mit12, D8Mgh8, D8Mit3, D8Mit7, D9Mit9, D9Mit6, D10Mgh6, D10Mgh8, D11Mgh4, D11Mgh6, D12Mgh2, D12Mgh3, D12Mgh5, D13Mgh1, D13Mgh2, D13Mit3, Renin, D14Mit2, D15Mit2, ETB, D16Mit11, D16Mit2, D17Mgh5, D17Mit3, PRLb, D17Mgh8, D18Mgh2, D18Mit7, D19Mit5, D19Mit7, D20Mgh2, UW1, and D21Mgh5.
Cardiovascular Phenotyping

Pulsatile arterial pressures and heart rates were measured in unanesthetized, unrestrained male rats at 16 and 18 weeks of age. Indwelling radiotelemetry transducers, connected to catheters implanted in the lower abdominal aorta, were implanted in rats at 12 weeks of age. Pulsatile pressures and heart rates were recorded in 5-second bursts every 5 minutes for 24 hours in 16-week-old rats on a standard diet (NaCl; 0.35%) and in 18-week-old rats after 2 weeks of salt loading with a high salt diet (NaCl; 8.0%). Rats were kept at a controlled room temperature with light from 7 AM to 7 PM (daytime) and were given tap water ad libitum. The BP of 8 male progenitor WKY and 8 male congenic rats was measured in the present study. This study was conducted in accordance with the current guidelines for the care and use of experimental animals of Shiga University of Medical Science.

Statistical Analysis

Two-way ANOVA was used to delineate the effects of strain and salt loading, as well as the interaction between these parameters, on BP.

Results

Genotype analysis of markers on chromosome 1 confirmed the successful transfer of a defined segment of chromosome from the SHR strain onto the WKY genetic background. The maximum size of the transferred segment was defined by the markers D1Rat89 and MTPA, and the minimum size was defined by the markers D1Mit3 and D1Rat57 (Figure). Genotype analysis using 65 genetic markers throughout the genome confirmed the congenic status of the new strain, designated WKY.SHR-D1Mit3/Rat57.

Mean BP was significantly higher in the WKY.SHR-D1Mit3/Rat57 congenic strain than in the WKY progenitor strain during both the day (7 AM to 7 PM; \( P = 0.0182 \), t test; Table) and the night (7 PM to 7 AM; \( P = 0.0105 \)) at 16 weeks of age under a standard diet. The difference in BP levels was greater during the night.

Two weeks of salt loading with a high salt diet increased the mean BP more in the WKY.SHR-D1Mit3/Rat57 strain than in the WKY progenitor strain, especially at night. Thus, mean BP was markedly higher in the WKY.SHR-D1Mit3/Rat57 strain than in the WKY progenitor strain at 18 weeks of age on a high salt diet during both the day (\( P = 0.0005 \)) and the night (\( P < 0.0001 \)).

Two-way ANOVA showed a significant difference in BP between the 2 strains and a significant difference in the BP response to salt loading between the 2 strains (Table).

Discussion

The kidney plays a crucial role in the pathogenesis and maintenance of hypertension in the SHR. Renal transplantation experiments have suggested that the kidneys of SHR may have primary genetic defects.\(^{12,13}\) On the basis of the hypothesis that a gene that contributes to hypertension in SHR might be differentially expressed in the kidneys of SHR and its control strain WKY, we previously identified a gene, designated \( Sa \), by differential screening. The expression levels of the \( Sa \) gene transcript were about 10 times higher in the kidneys of SHR than in those of WKY at 4 weeks of age.\(^{3}\) In situ hybridization analysis has indicated that the transcript of the \( Sa \) gene is present in proximal tubules,\(^ {14} \) which suggests that a product of the \( Sa \) gene may be involved in body fluid homeostasis. However, differential expression and/or tubular expression do not necessarily mean that the gene is involved in the pathogenesis of hypertension. Subsequently, cosegregation analyses of the genotype of the \( Sa \) gene with BP were carried out in several \( F_2 \) populations and confirmed that the \( Sa \) gene was in the region of a QTL for BP. However, it remains to be determined whether the \( Sa \) gene itself is the gene responsible for SHR hypertension.

To confirm that the \( Sa \) gene locus is a QTL for BP and to perform more fine mapping of a QTL for BP in this region, we constructed a congenic strain by transferring the SHR chromosome 1 segment around the \( Sa \) gene onto the genetic background of the WKY strain. The present study confirmed that transfer of a chromosome 1 segment defined by the markers D1Mit3 and D1Rat57 from SHR to WKY led to a significant increase in BP. Moreover, salt loading increased BP more in the WKY.SHR-D1Mit3/Rat57 strain than in the WKY progenitor strain.
The present findings are consistent with the results of previous segregation studies. Polymorphism at the Sa and/or Acnn1b loci cosegregated with basal BP in F2 populations derived from SHR×WKY\(^6,9\) and SHR×Brown-Norway (BN) rats.\(^7\) In an F2 population derived from SHR-SP×WKY\(^3\) and Dahl salt-sensitive×Lewis\(^8\) rats, polymorphism at the Sa locus cosegregated with BP after salt loading.

St. Lezin et al\(^9\) recently reported that transfer of chromosome 1 segment defined by the markers D1Mit3 and Igf2 from normotensive BN onto the SHR genetic background was sufficient to induce a significant reduction in arterial BP. Our present study further confirmed the existence of a QTL for BP in this chromosome 1 region and more precisely mapped the QTL for BP between markers D1Mit3 and D1Rat57. Moreover, we found one of the features of this QTL for BP, ie, salt sensitivity.

This chromosome region contains the Sa, Scnn1b, and Scnn1g genes as candidate genes for hypertension. Although the expression of the Sa gene was observed in proximal tubular cells\(^6\) and marked differences in the expression levels were observed between SHR and WKY strains,\(^1\) the functions of the Sa gene remain to be determined. No sequence variations in Scnn1b and Scnn1g genes have been reported between SHR and normotensive BN.\(^5\) Thus, we have not yet identified a gene or genes responsible for salt-sensitive hypertension in this chromosomal region. Many other genes exist on this chromosomal region, which is homologous to human chromosomes 16p and 11p and mouse chromosome 7.\(^10\) Recent development in human and mouse genome projects may reveal a number of other candidate genes in this chromosomal region in the near future.

The WKY.SHR-D1Mit3/Rat57 strain is a new model for salt-sensitive hypertension. Analyses of physiological parameters, including renal hemodynamic and tubular functions, and hormonal features will help to identify a gene that contributes to salt-sensitive hypertension. Moreover, the establishment of congenic sublines will refine the map position of a QTL for BP.

**References**


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<th>Mean Blood Pressure Levels Determined by Radiotelemetry</th>
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<td><strong>BP, mm Hg/basal</strong></td>
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<td><strong>Nighttime (7 PM to 7 AM)</strong></td>
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Values are expressed as mean±SD. Two-way ANOVA (repeated-measures) was used to assess the effects of strain and salt loading on blood pressure. Blood pressure levels during the day (7 AM to 7 PM) and night (7 PM to 7 AM) were analyzed separately. WKY indicates progenitor Wistar-Kyoto rat strain; congenic, WKY.SHR-D1Mit3/Rat57.
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Hypertension. 1998;32:636-638
doi: 10.1161/01.HYP.32.4.636

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
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:
http://hyper.ahajournals.org/content/32/4/636

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