Chromosomal Mapping of Quantitative Trait Loci That Influence Renal Hemodynamic Functions

Naoharu Iwai, MD, PhD; Masahiko Kinoshita, MD, PhD; Hitoshi Shimoike, MD, PhD

Background—Impaired renal hemodynamic function has been suspected to be responsible for hypertension in the spontaneously hypertensive rat (SHR).

Methods and Results—We measured renal hemodynamic functions, including the glomerular filtration rate, renal plasma flow, and renal vascular resistance, in an F2 rat population derived from spontaneously hypertensive and Wistar-Kyoto (WKY) rats and performed a genome-wide screening to map quantitative trait loci that influence these functions to gain insight into the relationship between renal hemodynamic functions and blood pressure control. The D1 Mit7 locus was identified as a major locus that influenced renal hemodynamic functions, and we transferred the SHR chromosomal segment around the D1 Mit7 locus into the WKY strain. The congenic rats exhibited impaired renal hemodynamic functions. The systolic blood pressure of the congenic rats was significantly higher than that of age-matched WKY rats, but only at nighttime. No significant differences in systolic blood pressure during daytime or diastolic blood pressure were observed between the 2 strains.

Conclusions—We have identified a chromosome segment that influences renal hemodynamic function. The SHR chromosome segment around the D1 Mit7 locus had significant, but not dramatic, effects in increasing blood pressure in the WKY genetic background. However, further studies will be necessary to determine the significance of this locus in SHR hypertension. (Circulation. 1999;100:1923-1929.)

Key Words: hypertension ■ genetics ■ kidney

Essential hypertension is a heterogeneous disease that involves multiple genetic and environmental factors. The multifactorial nature of essential hypertension makes it very difficult to directly identify contributing genes in human studies. One solution to this problem is to use inbred animal models of genetic hypertension. Investigations of genetic factors in such animal models may provide insight into the pathogenesis of hypertension that can then be applied to studies in humans.

The spontaneously hypertensive rat (SHR) line was originally established by Okamoto and Aoki and is now the most commonly used animal model of genetic hypertension. An increasing body of evidence supports the notion that the kidney plays a critical role in the pathogenesis of hypertension in SHR. Renal transplantation experiments have suggested that primary genetic defects reside in the kidneys of SHR. SHR retain excessive amounts of salt and water between 4 and 8 weeks of age. During this developmental phase of hypertension, a reduced glomerular filtration rate (GFR), reduced renal blood flow, and reduced fluid flow in the distal convolution have been reported. Blood pressure then increases to normalize GFR and renal blood flow.

However, phenotypic differences between SHR and Wistar-Kyoto rats (WKY) are not necessarily the cause of the genetically determined difference in blood pressure between the 2 strains. To establish a cause-and-effect relationship between renal hemodynamic impairment and hypertension in SHR, it is necessary to show the cosegregation of such impairment with high blood pressure in F2 populations. Harrap and Doyle showed that renal blood flow and the GFR were inversely correlated with blood pressure in F2 rats prepared from SHR and WKY when hypertension was just beginning to develop. Norrelund et al. showed that a narrowed lumen diameter in distal afferent arterioles at 7 weeks of age was associated with high blood pressure at 23 weeks of age. These observations suggest that the impairment of renal hemodynamic functions may indeed contribute to hypertension in SHR. However, in the fawn-hooded rat, a susceptibility locus (Rf-1) for renal impairment had no significant effects on blood pressure, and hypertension was ascribed to a locus other than Rf-1.

The purpose of the present study was to determine whether renal hemodynamic impairment is indeed responsible for hypertension in SHR. For this purpose, we measured renal hemodynamic functions in an F2 population derived from SHR and WKY and identified a major locus and a few minor loci that influence renal hemodynamic functions. We then...
TABLE 1. Markers Used in the Present Study

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</table>

Markers used in the present study are listed according to the chromosome number. Genotype of each marker was determined by PCR. The genotypes of SA and Renin were determined as previously reported.12,22

established a congenic strain that possessed the major locus from SHR on a WKY genetic background. We measured the blood pressure of the congenic rats by telemetry. Our results suggest that the impairment of renal hemodynamic functions plays a small but significant role in the development of hypertension in SHR.

Methods

Animals

F2-generation rats were produced by crossbreeding F1 rats bred from WKY female (n = 3) and SHR male (n = 3) crosses. SHR and WKY rats were purchased from Charles River Laboratories (Atsugi, Japan). The rats were housed in a temperature-controlled room with lights on from 7 AM to 7 PM and were fed normal pelleted rat chow (0.39% NaCl) and tap water ad libitum.

Because the D1 Mit7 locus was identified as a major locus that influenced renal hemodynamic functions in the F2 population, we transferred the SHR D1 Mit7 locus into the WKY strain by backcross breeding and selection using the D1 Mit7 marker. After 5 generations of selective backcrossing to the WKY progenitor strain, during which the presence of the SHR allele of D1 Mit7 in the heterozygous state and the absence of the SHR allele in D1 Mit18 and 10 to 20 markers on other chromosomes randomly selected from the list in Table 1 were confirmed, the chromosomal segment around D1 Mit7 was fixed and maintained by brother×sister mating and selective breeding of the offspring. Animals of the NSF5 or NSF5 generation were used in the present study.

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: D1Rat90, Mgh14, D1 Mgh13, D1 Mit3, D1Rat77, D1 Mit18, RCA0120, MTPA, D1Rat119, D1Rat57, SA, D1 Mit4, D1 Mit3, D1Rat89, D1 Mit17. The map positions of these markers were determined with Mapmanager by genotyping 68 male F2 rats derived from the SHR and WKY progenitor strains.

Rat genomic DNA was isolated from the liver as previously reported.12 The congenic status of the congenic strain established in the present study was confirmed by genotyping the following markers: D1Rat90, D1 Mit18, RCA0120, MTPA, D1Rat139, D1Rat57, SA, D1 Mit4, D1 Mit3, D1Rat99, D1 Mit17, D2 Mit6, D2 Mgh11, D2 Mit21, D3 Mit10, D4 Mgh18, D4/N02, D4/L6, D5 Mgh2, D5 Mit5, D5 Mit10, D6 Mit4, D6/IGHE, D7 Mit12, D8 Mgh4, D8 Mgh7, D8 Mit7, D9 Mit2, D10 Mit6, D10 Mit10, D10/NGRF, D11 Mit4, D11 Mgh6, D12 Mgh2, D13 Mit4, D13/Renin, D13 Mgh5, D14 Mit2, D15 Mit2, D15 Mit3, D15 Mit2, D15 Mit6, D16 Mit2, D17 Mit3, D17/PRL, D17 Mgh8, D17 Mit5, D17/Rat12, D17/Rat19, D17/Rat25, D18 Mit2, D18 Mgh4, D19 Mit5, D19 Mit7, D20 Mit2, and DXRat19. The rat genetic markers used in the present study were based on a previous report13 and on information from Research Genetics Inc. The inbred status of the progenitor strains was confirmed by the markers listed in Table 1.

Phenotypes

In the present study, we measured renal hemodynamic functions in 33 male F2 rats (6 to 7 weeks old), 8 male congenic rats (7 weeks old), and 8 male WKY rats (7 weeks old). Rats were anesthetized by an injection of sodium pentobarbital (50 mg/kg IP) and placed on a temperature-regulated table to maintain body temperature at 37°C. A catheter was inserted into the left common carotid artery for continuous monitoring of blood pressure and blood sampling. Another catheter was inserted into the left jugular vein for infusion of inulin and para-aminohippurate (PAH) solution. The urinary bladder was catheterized to collect a urine sample. Inulin (1%) and PAH (2%) in NaCl 154 mmol/L solution maintained at 37°C were infused at a rate of 1.6 mL·h⁻¹·100 g body wt⁻¹ for 20 minutes as a priming load and then infused continuously with a syringe pump at a rate of 0.6 mL·h⁻¹·100 g body wt⁻¹. After a 60-minute equilibration period, a 30-minute clearance study was performed. A blood sample (0.2 mL) was collected at the midpoint of the clearance study for determination of plasma inulin, PAH, and creatinine concentrations, and the blood was immediately replaced with the same volume of 154 mmol/L NaCl solution. The inulin concentration was measured with a cysteine-tryptophan reaction,14 the creatinine concentration was measured with a commercial kit based on an enzyme method (Mizuho Medy), and the PAH concentration was measured by the method of Brun.15 Inulin, creatinine, and PAH clearance were corrected by the sum of the weights of both kidneys.

Heritability of inulin and PAH clearance and renal vascular resistance (RVR) were estimated to be 56%, 37%, and 42%, respectively. Heritability was roughly estimated as 100%×(Vp-V)/V, where Vp is the variance of the F2 population and V is the average of the variance of WKY and congenic WKY.SHR.D1Rat90Mit18.

Blood pressure was assessed in 8 congenic and 8 WKY unanesthetized and unrestrained male rats at 16 and 18 weeks of age. Indwelling radiotelemetry devices (Data Sciences International) were implanted into 12-week-old rats and connected to catheters implanted in the lower abdominal aorta. Pulsatile pressures and heart rates were recorded in 5-second bursts every 5 minutes for 24 hours at 16 weeks of age under a standard diet (NaCl, 0.35%). After the assessment of blood pressure at 16 weeks of age, rats were fed a high-salt diet (8% NaCl) for 2 weeks, and blood pressure was then reassessed at 18 weeks of age. Because the blood pressure level of 1 WKY rat (104.6/68.5 mm Hg daytime and 110.0/74.4 mm Hg nighttime at 16 weeks of age) was more than 15 mm Hg lower than those of other WKY rats, the blood pressure data of this WKY rat were excluded. This study was conducted in accordance with current guidelines for the care and use of experimental animals of Shiga University of Medical Science.
Loci That Influenced Renal Hemodynamics

Tables 2 through 4 summarize the analysis of the chromosomal mapping of quantitative trait loci (QTL) that influence renal plasma flow (RPF), the GFR, and RVR. Multiple regression analyses revealed that the D1 Mit7 (codominant) and D12 Mgh2 (dominant) loci had significant effects on GFR (Table 2) and that the D1 Mit7 (codominant), D17 Mit3 (recessive), and D12 Mgh2 (dominant) loci had significant effects on RPF (Table 3). The SHR genotype of D1 Mit7 was associated with lower GFR and lower RPF, and D17 Mit3 was associated with higher RPF. Multiple regression analyses indicated that the D1 Mit7 (codominant) and D17 Mit3 (recessive) loci had significant effects on RVR (Table 4). The SHR allele of D1 Mit7 was associated with higher RVR, and the SHR genotype of D17 Mit3 was associated with lower RVR. Figure 1 shows the relationships between the phenotype analyzed and the genotype of the D1 Mit7 locus of F2 rats.

Renal hemodynamic functions are influenced by blood pressure. Indeed, GFR tended to be correlated with blood pressure by a single regression model (P=0.0663). However, stepwise multiple regression excluded blood pressure as a predictor of GFR, RPF, or RVR.

MQM mapping confirmed that QTLs for GFR, RPF, and RVR were located around the D1 Mit7 locus (Figure 2). The QTL for GFR was calculated with D12 Mgh2 as a cofactor. QTLs for RPF and RVR were calculated with D17 Mit3 as a cofactor.

Because the D1 Mit7 locus was shown to strongly affect renal hemodynamic functions, we transferred the SHR chromosomal segment around the D1 Mit7 marker into the WKY strain by backcrossing. 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 D1 Rat90 and D1 Mit18, and the minimum size was defined by the markers D1 Mgh14.
and D1Rat77 (Figure 3). Genotype analysis using 56 genetic markers throughout the genome confirmed the congenic status of the new strain, designated WKY.SHR-D1 Mgh14/Rat77.

Characteristics of the Congenic Strain

Figure 4 shows renal hemodynamic functions of the congenic rats (n=8) compared with those of WKY rats (n=8) at 6 to 7 weeks of age. The GFR (P<0.0001) and RPF (P=0.0005) of congenic rats were lower than those of WKY rats, and the RVR (P=0.0027) of congenic rats was higher than that of WKY rats. This confirms that a gene in the D1 Mit7 locus significantly affects renal hemodynamic functions.

The systolic and diastolic blood pressures of congenic rats (n=8) at 16 weeks of age were not significantly different from those of age-matched WKY rats (n=7) during daytime (Table 5). However, the systolic blood pressure of congenic rats during nighttime after 2 weeks of salt loading was significantly higher (P=0.0018, t test) than that of age-matched WKY rats during nighttime (Table 5). Two-way ANOVA (repeated measures) confirmed that the systolic blood pressure of congenic rats was significantly higher (P=0.031) than that of the progenitor WKY strain only at nighttime (Table 5). The diastolic blood pressure of the congenic rats during nighttime tended to be higher (P=0.092) than that of the progenitor WKY strain.

Discussion

In the present study, we identified some loci that influenced renal hemodynamic functions. In particular, the D1 Mit7 locus was identified as the major locus that influenced renal hemodynamic functions in the present F2 rat population. Considering the hypothesis that renal hemodynamic impair-
ment may contribute to hypertension in SHR, we expected that the D1 Mit7 locus would affect blood pressure. Our previous study using an F2 rat population derived from SHR and WKY17 and other segregation studies in the same or other progenitor strains18–21 did not indicate the presence of a QTL for blood pressure around the D1 Mit7 locus. However, any subtle effects of a locus on blood pressure might be overlooked if a study design with F2 rats and incomplete methods for blood pressure assessment were used, such as the tail-cuff and direct catheter methods. Therefore, in the present study we established a congenic line and assessed the blood pressure of the congenic rats and the progenitor strain WKY rats by telemetry.

The congenic WKY.SHR-D1 Mgh14/Rat77 rats, whose renal hemodynamic functions were impaired compared with those of age-matched WKY rats, had a higher systolic blood pressure than age-matched WKY rats only at night. No significant differences were observed between the 2 strains with regard to diastolic blood pressure or systolic blood pressure during the daytime. Moreover, the difference in systolic blood pressure at night, while statistically significant (P = 0.031, Table 2), was <5 mm Hg.

It is well known that 5 ⁄6 renal ablation combined with salt loading, but not uninephrectomy alone, is sufficient to increase blood pressure. Thus, it is possible that the degree of impairment conferred by the SHR chromosomal segment around the D1 Mit7 locus is not sufficient to dramatically increase blood pressure even under salt loading.

Another possibility is that the impairment of renal hemodynamic functions is necessary but not sufficient to increase blood pressure. A combination of several independent factors might be necessary to increase blood pressure, with each factor alone being insufficient. This situation exists in DOCA-salt hypertension, in which the combination of both DOCA and salt loading is necessary to increase blood pressure in uninephrectomized rats. In the presence of other SHR genes that contribute to SHR hypertension, the significance of the D1 Mit7 locus might be better clarified. Potentially important epistatic interactions between genes on the chromosome 1 locus and genes on chromosomes 12 and 17 may be lost in the congenic strain. In this sense, a reciprocal test of the effects of the homologous WKY locus against the SHR genetic background might be necessary to confirm the effects of this locus on blood pressure.

A third explanation of the failure to link renal hemodynamic characteristics with blood pressure is that renal hemodynamic parameters measured under anesthetized conditions may not reflect those under conscious conditions. To exclude this possibility, we determined creatinine clearance (Ccr) measured from 24-hour urine and the serum creatinine concentration in conscious congenic rats housed in a metabolic cage. Because creatinine may be secreted from or absorbed by tubular cells, Ccr is not a precise reflection of C inulin. However, Ccr was significantly correlated with C inulin (R² = 0.487, P < 0.0001) in F2 rats (measured under anesthesia) (data not shown). The Ccr of the congenic rats at 6 weeks of age (n = 5) was significantly lower than that of age-matched WKY rats (n = 5) (P = 0.0015, data not shown). Although these experimental results suggest that the D1 Mit7 locus also significantly affected renal hemodynamic functions in the conscious state, a precise assessment of renal

Figure 3. Linkage map showing transferred segment of chromosome 1 in WKY.SHR-D1 Mgh14/Rat77. Solid bar indicates chromosome region transferred from SHR strain, and open regions indicate flanking segments of WKY chromosomes. Shaded portions indicate boundaries of transferred chromosomes.

Figure 4. Renal hemodynamic functions of congenic rats. GFR, RPF, and RVR were assessed in congenic rats (n = 8) and WKY rats (n = 8) at 7 weeks of age. Congenic rats had impaired renal hemodynamic functions compared with WKY rats.
hemodynamic functions and blood pressure in the conscious state with no anesthesia will be necessary.

We have not yet determined which gene in the D1 Mit7 locus influences renal hemodynamic functions. Interestingly, in the fawn-hooded rat, which is a model for genetic hypertension and renal impairment, the locus that is responsible for renal impairment has been reported to be near the D1 Mit7 locus.\(^{11}\) Our preliminary data (unpublished observations) indicate that the SHR genotype of CYP2C6, which has steroid 21-hydroxylase activity and is located near the D1 Mit7 locus, might be a gene that influences renal hemodynamic functions. Interestingly, the SHR genotype of CYP2C6, which has steroid 21-hydroxylase activity and is located near the D1 Mit7 locus, might be a gene in this locus that influences renal hemodynamic functions.

### Study Limitations

As described above, the lack of a dramatic correlation between blood pressure and the D1 Mit7 locus might be a result of our experimental design, because hemodynamic parameters measured under anesthesia may not reflect those in the conscious state. In future studies, hemodynamic functions should be assessed in the conscious state, and this may require technical innovations.

Moreover, because potential epistatic interactions between SHR genes are lost in the congenic strain with the WKY genetic background, a reciprocal test of the effects of the WKY D1 Mit7 locus against the SHR genetic background might be necessary to observe dramatic effects of the D1 Mit7 locus on blood pressure.

### References


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_Circulation._ 1999;100:1923-1929
doi: 10.1161/01.CIR.100.18.1923

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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