Physiol. Res. 52: 285-289, 2003

Genetic Isolation of Quantitative Trait Loci for Blood Pressure Development and Renal Mass on Chromosome 5 in the Spontaneously Hypertensive Rat

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Received February 6, 2002
Accepted July 11, 2002

Summary

Total genome scans of genetically segregating populations derived from spontaneously hypertensive rats (SHR) and other rat models of essential hypertension suggested a presence of quantitative trait loci (QTL) regulating blood pressure on multiple chromosomes, including chromosome 5. The objective of the current study was to test directly a hypothesis that chromosome 5 of the SHR carries a blood pressure regulatory QTL. A new congenic strain was derived by replacing a segment of chromosome 5 in the SHR/Ola between the D5Wox20 and D5Rat63 markers with the corresponding chromosome segment from the normotensive Brown Norway (BN/Crl) rat. Arterial pressures were directly monitored in conscious, unrestrained rats by radiotelemetry. The transfer of a segment of chromosome 5 from the BN strain onto the SHR genetic background was associated with a significant decrease of systolic blood pressure, that was accompanied by amelioration of renal hypertrophy. The heart rates were not significantly different in the SHR compared to SHR chromosome 5 congenic strain. The findings of the current study demonstrate that gene(s) with major effects on blood pressure and renal mass exist in the differential segment of chromosome 5 trapped within the new SHR.BN congenic strain.

Key words

SHR • Congenic strain • Chromosome 5 • Blood pressure • Renal mass

Introduction

In previous linkage studies in genetically segregating populations derived from the SS/Jr (salt sensitive Dahl rat), SHR (spontaneously hypertensive rat), and SHRSP (stroke prone SHR) strains and normotensive control strains, quantitative trait loci (QTL) for blood pressure were found on multiple rat chromosomes, including chromosome 5 (Deng *et al*. 1994, Garrett *et al*. 1998, Kloting *et al*. 2001, Stec *et al*. 1996, Zhang *et al*. 1996, 1997). The putative QTL on chromosome 5 in the SS/Jr strain was genetically isolated within the SS.LEW congenic strain, which was derived by a transfer of chromosome 5 segment from the LEW strain onto the genetic background of the Dahl salt-sensitive (SS/Jr) strain (Garrett *et al*. 1998). To test a

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hypothesis that a blood pressure regulatory QTL(s) exist on chromosome 5 also in the SHR strain, we developed a new congenic strain by transferring a segment of chromosome 5 from the normotensive Brown Norway (BN/Crl) rat onto the genetic background of the SHR/Ola strain. We found that the SHR chromosome 5 congenic strain (SHR-5) exhibited a significant decrease in blood pressure and amelioration of renal hypertrophy when compared to the SHR progenitor strain.

Methods

Derivation and genetic characterization of the SHR chromosome 5 congenic strain (SHR-5)

The SHR-5 congenic strain was derived by selective backcross breeding to the progenitor SHR strain (SHR/OlaIpcv) that descends from the inbred SHR, originally obtained from NIH. The SHR strain was beyond the F90 generation when the congenic breeding was initiated. A differential segment of chromosome 5 was transferred onto the genetic background of the SHR/OlaIpcv progenitor from a normotensive Brown Norway (BN/Crl) strain (Charles River Laboratories). The D5Wox12 (Cyp4a2) and D5Wox20 markers were used for selection of heterozygotes in each backcross generation. After 10 generations of selective backcrossing to the SHR progenitor strain, the differential segment was fixed by intercrossing heterozygotes and selecting for offspring inheriting the homozygous BN chromosome segment. The size of the differential segment was estimated by genotyping selected microsatellite markers (Bihoreau *et al.* 1997, Steen *et al.* 1999). Congenic status of the SHR-5 strain was checked by genotyping SHR-5 rats with 60 gene markers widely dispersed throughout the genome.

Hemodynamic studies

Arterial blood pressures and heart rates were measured continuously in unanesthetized, unrestrained male SHR progenitor rats (n=10) and SHR-5 congenic rats (n=8) between 80 and 100 days of age using radiotelemetry. All rats were allowed to recover for at least 10 days after implantation of radiotelemetry transducers before the blood pressure recordings were started. Pulsatile pressures and heart rates were recorded in 5-second bursts every 5 min during the day (06:00 to 18:00) and during the night (18:00 to 06:00). From these data, individual means for daytime and night-time blood pressures and heart rates were calculated for each rat in each day of the study. The results from the rats were then averaged to obtain group means. Rats were fed standard lab chow and tap water *ad libitum* throughout the study. At the end of the study, the rats were sacrificed and their body weight, heart and kidney weights were determined.

Statistical analysis

All data are expressed as means ± S.E.M. Day-time and night-time blood pressures and heart rates over the course of the study were separately evaluated by the analysis of variance (ANOVA) with repeated measures. Individual means of body weight, heart and kidney weights were compared by the t-test. Statistical significance was defined as $p<0.05$.

Results

Genotyping results

The differential segment of chromosome 5 was fixed between the D5Wox20 and D5Rat63 markers. The size of the transferred BN chromosome segment is approximately 23 cM as estimated by testing with the microsatellite markers (Fig. 1). Genotyping results obtained with 60 widely dispersed polymorphic microsatellite markers confirmed that the SHR progenitor strain and the new SHR-5 congenic strain were genetically identical except for the chromosome 5 differential segment transferred from the BN donor strain.

Hemodynamic analysis

Night-time and daytime systolic blood pressures were significantly lower in the SHR-5 congenic strain than in the SHR progenitor strain with no significant interaction between group and repeated measurement effects ($p<0.05$) (Fig. 2a,b). The interactions in ANOVA design between factors GROUP and TIME are insignificant, but a linear increase of systolic blood pressure in the SHR-5 animals is significantly greater than in the SHR controls at the 5 % level. Thus the difference between strains at the last 10 days computed in separate ANOVA analysis was reduced and significant only at the 5 % level. In the last 5 days the difference was not statistically significant.

Night-time and day-time diastolic blood pressures tended to be lower in the SHR-5 congenic rats, however, the observed difference did not attain statistical significance (Fig. 2b,c,d). Heart rate was similar in the SHR progenitor and congenic strains despite the clear differences in blood pressure (data not shown). The attenuation of hypertension in the SHR-5 congenic strain was accompanied by amelioration of renal hypertrophy: the average relative kidney weight of the SHR-5 congenic strain (0.690±0.024 g/100 g) was significantly lower

($p<0.05$) when compared to the SHR progenitor (0.807± 0.036 g/100 g). Transfer of the chromosome 5 segment was associated with a significant increase of body weight in the SHR-5 congenic strain versus the SHR progenitor (325.5±8.8 g vs. 291.9±11.4 g, $p<0.05$). Average relative cardiac weights were similar in both strains. To distinguish whether the difference in renal mass is an *a priori* difference in renal hypertrophy and/or hyperplasia or a secondary decrease due to a lower blood pressure, we determined relative renal mass in males at the age of 4 weeks. At this prehypertensive stage, the average relative renal mass in the SHR strain (0.473g± 0.010 g, n=5) was similar to that in the SHR-5 congenic strain (0.471±0.010 g, n=5).

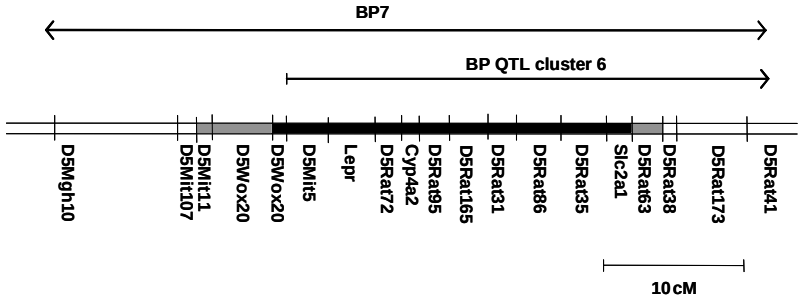

Fig. 1. *Linkage map showing the transferred segment of chromosome 5 in the SHR-5 congenic strain. The solid bar denotes the chromosome region transferred from the BN strain, and the open region denotes the flanking segment of SHR chromosome. The segments with residual heterozygosity are depicted by the shaded regions. Arrows depict positions of QTL for blood pressure that overlap with the QTL trapped within the SHR-5 congenic strains, QTL the BP cluster 6 (Stoll et al. 2000) and BP7 (Garrett et al. 1998) described previously.*

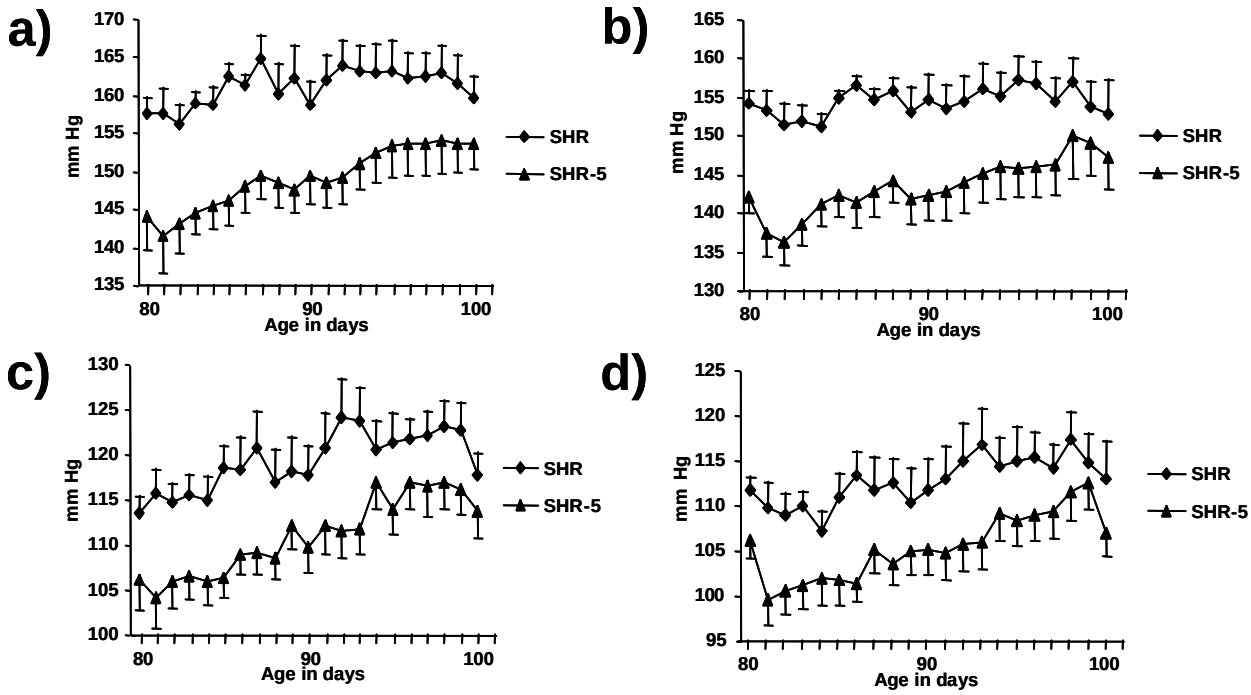

Fig. 2. *Average blood pressures determined by radiotelemetry over a period of 20 days in the SHR progenitor and the SHR-5 congenic strains. a) systolic night-time blood pressures, b) systolic day-time blood pressures: the average night-time and day-time systolic blood pressures of the SHR-5 congenic strain were significantly lower than those of the SHR progenitor strain ($p<0.001$) throughout the study; a linear increase of systolic blood pressure in the SHR-5 animals is significantly greater than in the SHR controls at 5% level, c) diastolic night-time blood pressures, d) diastolic day-time blood pressures: the average night-time and day-time diastolic blood pressures of the SHR-5 congenic strain tended to be lower than in the SHR strain, but the difference did not attain statistical significance.*

Discussion

The results of the current study show that the transfer of a segment of chromosome 5 from the BN strain onto the genetic background of the SHR strain is associated with a significant decrease in blood pressure and amelioration of renal hypertrophy. Blood pressure regulatory QTL on chromosome 5 were reported in crosses of both the SHR and SS/Jr strains. The differential segment trapped within the SHR-5 congenic strain partially overlaps with previously reported QTL regions designated BP7 (Garrett *et al.* 1997) and BP QTL cluster 6 (Stoll *et al.* 2000, Rat Genome Database) (Fig. 1). It is therefore possible that the chromosome 5 blood pressure effects are associated with the same gene(s). On the other hand, QTL for blood pressure described by Zhang *et al.* (1996, 1997) was located outside the differential chromosome segment. A QTL for blood pressure was also found in a homologous region of mouse chromosome 4 (Sugiyama *et al.* 2001).

As can be seen in Figures 2a and 2b, the slope of blood pressure development is significantly different in the SHR-5 congenic strain versus the SHR control which suggests that the development of hypertension may be only delayed in the congenic. Thus the transfer of chromosome 5 differential segment is associated with the development of blood pressure rather than with its lowering.

The transfer of chromosome 5 segment from the BN strain onto the SHR background was associated with a significant amelioration of renal hypertrophy. Hamet *et al.* (1998) reported a suggestive QTL on chromosome 5 that was associated with newborn and adult kidney weights in the HXB/BXH sets of recombinant inbred (RI) strains. This QTL, however, does not overlap with the differential segment of chromosome 5 isolated within the SHR-5 congenic strain. It remains to be determined whether the observed decrease of renal mass in the congenic strain is secondary to amelioration of hypertension or whether it is independent of blood pressure. The former possibility seems to be more likely since no significant differences in relative renal mass were observed in a prehypertensive stage, in 4-week-old SHR versus SHR-5 males.

The differential segment of chromosome 5 trapped within the SHR/Ola congenic strain carry several positional candidate genes as discussed in previous reports (Deng *et al.* 1994, Deng and Rapp 1995, Garrett *et al.* 1998, Truett *et al.* 1995). However, since the differential segment is relatively large, it is premature to implicate any of these genes in the regulation of blood pressure and renal mass associated with the chromosome 5 segment. In conclusion, the results of the current study provide definitive evidence that rat chromosome 5 harbors QTL with major effects on blood pressure and renal mass. Accordingly, the new SHR congenic strain can now be used for subline derivation and detailed mapping studies of the responsible QTL in the differential chromosome segment. Identification of these QTL at the molecular level could shed important light on the pathogenesis of hypertension.

Acknowledgements

This work has been supported by grants 305/00/1646 and 204/98/K015 from the Grant Agency of the Czech Republic to VK and MP, by grant HL56028 from the National Institutes of Health, and NIH Fogarty International Research Collaboration Award RO3TW001236 to MP and TWK; MP is an International Research Scholar of the Howard Hughes Medical Institute.

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