Genetic Isolation of a Chromosome 1 Region Affecting Susceptibility to Hypertension-Induced Renal Damage in the Spontaneously Hypertensive Rat

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Abstract—Linkage studies in the fawn-hooded hypertensive rat have suggested that genes influencing susceptibility to hypertension-associated renal failure may exist on rat chromosome 1q. To investigate this possibility in a widely used model of hypertension, the spontaneously hypertensive rat (SHR), we compared susceptibility to hypertension-induced renal damage between an SHR progenitor strain and an SHR congenic strain that is genetically identical except for a defined region of chromosome 1q. Backcross breeding with selection for the markers D1Mit3 and Igf2 on chromosome 1 was used to create the congenic strain (designated SHR.BN-D1Mit3/Igf2) that carries a 22 cM segment of chromosome 1 transferred from the normotensive Brown Norway rat onto the SHR background. Systolic blood pressure (by radiotelemetry) and urine protein excretion were measured in the SHR progenitor and congenic strains before and after the induction of accelerated hypertension by administration of DOCA-salt. At the same level of DOCA-salt hypertension, the SHR.BN-D1Mit3/Igf2 congenic strain showed significantly greater proteinuria and histologically assessed renal vascular and glomerular injury than the SHR progenitor strain. These findings demonstrate that a gene or genes that influence susceptibility to hypertension-induced renal damage have been trapped in the differential chromosome segment of the SHR.BN-D1Mit3/Igf2 congenic strain. This congenic strain represents an important new model for the fine mapping of gene(s) on chromosome 1 that affect susceptibility to hypertension-induced renal injury in the rat. (Hypertension. 1999;34:187-191.)

Key Words: genetics ■ chromosome, rat, 1 ■ hypertension, renal

The incidence of end-stage renal disease in the United States, including the fraction attributed to hypertension, has continued to increase despite improvements in the detection and treatment of high blood pressure (BP) in the population.1 Although the link between malignant hypertension and renal failure is clear, studies have focused only recently on the extent to which mild to moderate hypertension can induce or affect the progression of renal damage.2-5 Moreover, it is unknown why just a small percentage of patients with mild to moderate hypertension develop renal failure.6 In humans, ethnic differences in the rate of hypertension-associated end-stage renal disease suggest that genetic factors might affect susceptibility to hypertension-induced renal damage.7-9 For example, the risk of hypertension-associated end-stage renal failure is much higher in blacks than in whites, even after correcting for severity of hypertension or differences in treatment.7,10,11 In addition, the risk of developing end-stage renal disease in siblings of blacks with hypertension-associated end-stage renal disease is far greater than that in the general population.12 In inbred animal models of hypertension, susceptibility to hypertension-induced renal damage has also been attributed to genetic factors.13,14 In genetic crosses derived from the fawn-hooded hypertensive rat (FHH), an inbred strain that develops both hypertension and renal failure, Brown et al14 mapped 2 quantitative trait loci (QTL) that influence susceptibility to renal failure to chromosome 1q. At least 1 of the QTL described (Rf-1) appears to affect the risk of renal impairment independently of an effect on BP. In contrast to the FHH, which spontaneously develops severe renal damage with relatively mild hypertension, the SHR exhibits minimal hypertensive damage, despite its having higher BPs than the FHH. In renal cross transplant studies in which susceptibility...
to hypertension-induced renal disease could be studied in genetically different kidneys that were simultaneously maintained in the same hemodynamic and metabolic environment, Churchill et al.\textsuperscript{15} recently found that the kidney of the SHR was much less susceptible to hypertension-induced damage than the kidney of the normotensive Brown Norway rat (BN).

To investigate whether genes on chromosome 1 contribute to the difference in susceptibility to hypertension-induced renal damage between the SHR and BN strains, we compared the extent of renal damage, after we had induced accelerated hypertension, in an SHR progenitor strain and in an SHR congenic strain that carried a defined segment of chromosome 1 from BN. After the induction of a controlled degree of deoxycorticosterone acetate (DOCA)-salt–accelerated hypertension, the SHR congenic strain exhibited significantly greater renal damage than the SHR progenitor strain. These findings demonstrate that a gene or genes that influence susceptibility to hypertension-induced renal damage exist on rat chromosome 1 in the differential chromosome segment trapped in the SHR.BN-D1Mit3/Igf2 congenic strain.

Methods

Strains

The SHR congenic strain (designated SHR.BN-D1Mit3/Igf2) was derived from a progenitor strain of SHR (SHR/Ola) as previously described.\textsuperscript{16} Briefly, the SHR congenic strain was derived by a selective breeding protocol in which a segment of chromosome 1 from the normotensive BN strain (BN/Cr) was transferred onto the genetic background of the progenitor SHR. After 10 generations of backcross breeding to the SHR progenitor strain, the SHR congenic strain was fixed using the markers D1Mit3 and Igf2, and maintained by brother×sister mating. Previous analysis of DNA microsatellite markers showed that the SHR.BN-D1Mit3/Igf2 congenic strain is genetically identical to the progenitor SHR except for a 22 cM segment of chromosome 1 transferred from the BN/Cr strain (Figure 1).\textsuperscript{16}

Experimental Protocol

Continuous measurements of pulsatile arterial BP were obtained in 7 male progenitor SHR and 7 male SHR.BN-D1Mit3/Igf2 beginning at 14 to 16 weeks of age by use of radiotelemetry transducers as previously described.\textsuperscript{15–19} In brief, systolic BP (SBP) was determined every heart beat for a 10-second interval every 10 minutes, 24 hours a day. The baseline period before DOCA-salt lasted for 1 week, during which there were about 60 480 determinations of SBP in each rat. After baseline BP measurements, both groups of rats were given 100 mg/kg body weight DOCA by subcutaneous pellet implantation. Before DOCA-salt administration, SBPs and mean arterial pressures (MAPs) determined by radiotelemetry were slightly determined by radiotelemetry transducers as previously described.\textsuperscript{15–19} In brief, systolic BP (SBP) was determined every heart beat for a 10-second interval every 10 minutes, 24 hours a day. The baseline period before DOCA-salt lasted for 1 week, during which there were about 60 480 determinations of SBP in each rat. After baseline BP measurements, both groups of rats were given 100 mg/kg body weight DOCA by subcutaneous pellet implantation. Before DOCA-salt administration, SBPs and mean arterial pressures (MAPs) determined by radiotelemetry were slightly elevated compared with baseline levels.

Histological Studies

After the final 24 hours urine protein collection, the kidneys were perfusion fixed at the ambient pressure by use of 150 mmol/L NaCl at 38°C followed by modified Karnovsky’s fixative (2% weight/volume paraformaldehyde and 2.5% glutaeraldehyde in 0.1 mol/L cacodylate buffer, pH 7.4), as described previously.\textsuperscript{15,21} Two transverse sections of the kidney through the papilla were postfixed in buffered formalin and embedded in paraffin. Sections (3 to 4 μm) were stained with hematoxylin and eosin and periodic acid Schiff. Glomerular and vascular injury were measured separately in both of the sections from each kidney, as previously described.\textsuperscript{15, 21} All of the glomeruli in each section were counted and classified as normal or abnormal. Abnormal glomeruli were separated into those that exhibit (a) acute hypertensive injury (necrosis, thrombosis, microembolisms, and capillary wall disruption), (b) segmental glomerular sclerosis (collapsed capillary loops with mesangial matrix expansion), and (c) ischemic injury (globally shrunken glomeruli with collapsed capillary loops). The percentages of glomeruli exhibiting each of these 3 lesions were recorded. The total number of vascular profiles exhibiting evidence of acute hypertensive injury (fibroinoid necrosis, myointimal proliferation, fragmentation of internal elastic lamellae, and aneurysmal dilatation) were counted in each section. The number of such vascular profiles with injury was expressed per 100 glomeruli as a vascular injury score to correct for any differences in the amount of renal parenchyma present in sections from individual kidneys.

Analysis

All results (SBP, proteinuria, and renal damage scores) are expressed as mean±SEMs. There were 2 SBP values for each rat: (1) baseline (the average of about 60 480 determinations during 7 days) and (2) DOCA-salt (the average of about 285 120 determinations during 33 days). The statistical significance of differences in mean values between the SHR progenitor and the SHR.BN-D1Mit3/Igf2 congenic strains was analyzed by ANOVA and t tests. A P<0.05 was considered significant.

Results

Before DOCA-salt administration, SBPs and mean arterial pressures (MAPs) determined by radiotelemetry were slightly
but significantly lower in the SHR.BN-D1Mit3/Igf2 congenic strain versus the SHR progenitor strain (mean SBP±SEM of SHR congenic versus progenitor rats, 161±2.2 mm Hg versus 177±2.9 mm Hg, P<0.001; MAP±SEM of SHR congenic versus progenitor rats, 146±2.4 mm Hg versus 136±1.9 mm Hg, P<0.05). However, during the period of DOCA-salt administration, the average SBPs of the congenic and progenitor rats were not significantly different and, for both strains, averaged between 200 and 220 mm Hg (Figure 2). MAPs were similarly elevated after DOCA-salt administration and showed no significant difference between progenitor and congenic strains. Although the baseline BPs of the SHR.BN-D1Mit3/Igf2 congenic rats were lower than the BPs of the SHR progenitors, no strain differences in urinary excretion of protein before administration of DOCA-salt were seen. After 33 days of DOCA-salt administration, however, proteinuria was significantly greater in the SHR.BN-D1Mit3/Igf2 congenic rats versus the progenitor SHR rats (Figure 2).

Histologic examination of perfusion fixed kidneys demonstrated that the SHR congenic rats carrying the chromosome 1 segment transferred from the BN strain had significantly greater renal vascular and total glomerular damage scores than the SHR progenitor rats, despite exposure to the same level of DOCA-salt accelerated hypertension (Figure 3). The kidneys of the SHR.BN-D1Mit3/Igf2 congenic strain showed a significantly greater percentage of glomeruli with nerosis and ischemia (both P<0.05) and a tendency toward greater glomerulosclerosis (P=0.07) than did the kidneys of the SHR progenitor rats (Figure 3). Overall, ~7% of glomeruli in the SHR congenic kidneys showed histological evidence of glomerular damage. Figure 4 illustrates the typical and contrasting histology observed between the kidneys of the SHR progenitor rats (Figure 4A) and the kidneys of the congenic rats (Figure 4B). Focal but severe hypertensive damage in a pattern characteristic of malignant nephrosclerosis was observed in the SHR.BN-D1Mit3/Igf2 kidneys. Affected vessels showed lesions of fibrinoid necrosis, myointimal proliferation, aneurysmal dilatation, and thrombosis. Similarly, glomerular pathology, which is indicative of severe hypertensive damage, was observed. The pathology included lesions of fibrinoid necrosis and mesangiolysis, segmental sclerosis, and ischemia. In contrast, SHR progenitor kidneys showed minimal histological injury, despite being exposed to similar BPs.

**Discussion**

In renal cross transplant studies between histocompatible strains of SHR and BN, Churchill et al15 demonstrated that the kidney of the BN is inherently more susceptible to hypertension-induced damage than the kidney of the SHR. The magnitude of this increase in susceptibility to hypertension-induced renal damage in BN kidneys versus SHR kidneys was ~10-fold. The genes responsible for this strain difference in susceptibility to hypertension-induced renal damage are unknown, but previous linkage studies in the FHH suggest that the QTL(s) that influence susceptibility to renal failure may exist on chromosome 1q.14 In the present study, we tested this possibility in the SHR and found that transfer of a 22 cM segment of chromosome 1 defined by the markers D1Mit3 and Igf2 from the BN onto the genetic background of the SHR is sufficient to induce a significant increase in susceptibility to hypertension-accelerated renal damage in the SHR. After elevating SBPs in both the SHR congenic and progenitor rats to a range of 200 to 220 mm Hg
by administration of DOCA-salt, the congenic SHR, which carried the transferred segment of chromosome 1 from BN, showed significantly greater proteinuria than the progenitor SHR. In addition, the kidneys of congenic SHR showed a 3- to 4-fold increase in histological evidence of both glomerular and vascular damage versus the kidneys of progenitor SHR. Thus, our results suggest that at least 1 gene that influences susceptibility to hypertension-induced renal damage exists on rat chromosome 1 in the vicinity of the markers $D1Mit3$ and $Igf2$. On the basis of the difference in hypertension-induced renal damage found between the SHR congenic and progenitor strains in the present study, we estimate that gene(s) on the transferred segment of chromosome 1 may account for 20% to 30% of the difference in susceptibility to hypertension-induced renal damage observed between SHR and BN kidneys in the previous crosstransplant studies.

In our initial characterization of the SHR.BN-$D1Mit3/Igf2$ congenic line, we found that the SBPs of male congenic SHR were 10 to 15 mm Hg lower than those of age-matched male progenitor SHR. We estimated that this BP difference accounted for $\approx 15\%$ to 20% of the BP difference between the SHR and BN parental strains. In the present study, we observed a similar difference in SBP between the congenic and progenitor strains at 14 to 16 weeks of age, before administration of DOCA-salt. The increased BP in the SHR progenitor strain versus the SHR congenic strain may allow for protective adaptation of the SHR kidney to hypertension and promote resistance to renal damage induced by DOCA-salt–accelerated hypertension. However, administration of DOCA-salt for 33 days allowed induction of a controlled, equivalent degree of accelerated hypertension in both the SHR progenitor and congenic strains, despite the initial 10 mm Hg difference in BP at baseline. In addition, the previous crosstransplant experiments by Churchill et al indicate that protective adaptation alone is unlikely to explain the relative resistance to hypertension-induced renal damage in the SHR progenitor strain. In their studies, BN kidneys were transplanted into the histocompatible SHR.1N strain rendered normotensive by pharmacological treatment before transplantation. After transplantation, both the SHR.1N and BN kidneys were exposed to similar levels of elevated BP. The kidneys transplanted from the BN rats still showed increased renal damage versus the SHR.1N kidneys, although both the SHR.1N and BN kidneys were exposed only to normal BPs before transplantation.

The results of the present study are consistent with those in the FHH in which Brown et al found linkage between renal damage (as evidenced by proteinuria and renal sclerosis) and a region of chromosome 1 defined by the markers $D1Mgh12$ and $D1Mit6$. This locus was designated $Rf-1$ and mapped $\approx 45$ to 60 cM away from another locus, $Bphh-1$, that was linked to an effect on BP. A second locus linked to occurrence of renal failure, $Rf-2$, mapped in the vicinity of $D1Mit3$ $\approx 17$ cM away from $Bphh-1$. Our present studies in the SHR.BN-$D1Mit3/Igf2$ congenic strain suggest that we have successfully isolated at least 1 gene that influences susceptibility to hypertension-induced renal damage in the SHR model. Based on comparison of the region of chromosome 1
transferred in our congenic strain\textsuperscript{16} and the mapping results of Brown et al.\textsuperscript{14} it appears that the differential chromosome segment trapped in the SHR.BN-D1Mit3/Igf2 congenic strain overlaps with the region containing Rf-2 and Bpf1-I and, possibly, with the region containing Rf-I. In linkage studies in the stroke-prone SHR, a similar region of chromosome 1 in the vicinity of D1Mit3 and Igf2 has been reported to contain a gene, STRI, which influences susceptibility to stroke.\textsuperscript{20} The current studies in the SHR, together with previous linkage studies in the FHH and stroke-prone SHR, raise the possibility that chromosome 1q in the rat contains QTLs that promote susceptibility to hypertension-induced damage in a variety of organ vascular beds.

In humans, mutations in the nephrin gene on chromosome 19q have been found to cause congenital nephrotic syndrome.\textsuperscript{23} In addition, a locus responsible for inherited focal segmental glomerulosclerosis has been linked to chromosome 19q.\textsuperscript{24} Although rat chromosome 1 is homologous to segments of human chromosomes 19q and 16p (and to mouse chromosome 7), the segment of rat chromosome 1 transferred to includes the chromosome region implicated in human renal failure. Nevertheless, the SHR.BN-D1Mit3/Igf2 congenic strain represents a potentially important model for mapping other genes on chromosome 1 that influence susceptibility to hypertension-induced renal damage in the rat. Sublines of the SHR.BN-D1Mit3/Igf2 congenic strain are now being derived for exclusion mapping and for localizing QTL(s) with effects on BP and/or susceptibility to hypertension-induced renal damage.

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\section*{References}
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