It has recently been proposed that sequence variation in the gene coding for tissue kallikrein might be involved in the pathogenesis of hypertension. However, molecular evidence of an association between a sequence alteration in the kallikrein gene family and the transmission of increased blood pressure has never been reported. In 32 recombinant inbred (RI) strains derived from the spontaneously hypertensive rat (SHR) and the normotensive Brown Norway rat (BN), we investigated whether a restriction fragment length polymorphism (RFLP) marking the kallikrein gene family cosegregated with blood pressure. In the RI strains that inherited the kallikrein RFLP from the SHR progenitor strain, the median systolic, diastolic, and mean arterial pressures were significantly greater than in the RI strains that inherited the kallikrein RFLP from the BN progenitor strain. These findings suggest that in the rat, sequence variation in the kallikrein gene family, or in closely linked genes, may have the capacity to affect blood pressure. (Hypertension 1991;17:242–246)

In some humans with essential hypertension, urinary excretion of kallikrein has been reported to be decreased compared with that in age-matched normotensive subjects. Furthermore, in young normotensive individuals, urinary excretion of kallikrein has been found to be lower in those with hypertensive parents than in those with normotensive parents. Such findings are consistent with the suggestion that a genetically determined decrease in renal kallikrein activity might contribute to the pathogenesis of hypertension. However, molecular evidence of an association between a structural alteration in the kallikrein gene family and the transmission of increased blood pressure has never been reported.

Decreased urinary excretion of kallikrein has also been described in at least three different rat models of genetic hypertension. Although this could simply be a consequence of renal damage that precedes or accompanies hypertension, it is also possible that in the rat, genetic variation at a kallikrein locus may have the capacity to affect blood pressure. If so, in genetic studies in hypertensive rats, molecular markers for the kallikrein gene family may be associated with the transmission of an increase in blood pressure. We have identified a restriction fragment length polymorphism (RFLP) that distinguishes the kallikrein gene family of a strain of spontaneously hypertensive rats (SHR/Ola) (hereafter referred to as SHR) from that of a strain of normotensive Brown Norway rats (BN.1x/Cub) (hereafter referred to as BN). We now report that in a set of 32 recombinant inbred (RI) strains derived from these SHR and BN.
strains, the RFLP marking the kallikrein gene family of the SHR has cosegregated with an increase in blood pressure.

Methods
Development of the Recombinant Inbred Strains
Recombinant inbred strains were derived from crosses of female SHR and male BN rats (HxB strains) or female BN rats and male SHR (BxH strains) as previously described. All animals were housed under standard laboratory conditions and were fed a pelleted diet (containing 170 mmol NaCl/kg) and tap water ad libitum. The blood pressures of these RI strains, determined by averaging direct arterial pressure measurements obtained in five or six males (15±1 weeks of age) from each strain, have previously been reported. The mean arterial pressures of the strains range from approximately 110 mm Hg to 160 mm Hg.

Kallikrein Gene Analysis
To detect the RFLP distinguishing the kallikrein gene families of the SHR and BN progenitor strains, the complementary DNA (cDNA) insert of plasmid pcXF39 encoding the 3' end of the messenger RNA (mRNA) for rat pancreatic kallikrein (PS mRNA) was used to probe Southern blots of Nde I-digested genomic DNA. This probe cross-hybridizes with many closely related members within the kallikrein gene family. A random primer labeling reaction was used to nonisotopically label the probe with digoxigenin-conjugated deoxyuridine triphosphate (Genius Kit from Boehringer-Mannheim Biochemicals, Indianapolis, Ind.). Aliquots (10 μg) of genomic DNA, prepared from spleen tissue by phenol/chloroform extraction, were digested for 2 hours at 37°C with the restriction enzyme Nde I according to the conditions specified by the manufacturer (Boehringer-Mannheim). The digested DNA samples were electrophoresed on a 1% agarose gel at 35 V for 18 hours. After Southern transfer to a nylon membrane, the DNA was fixed by ultraviolet irradiation and hybridized for 20 minutes at 50°C with an alkaline phosphatase-conjugated oligonucleotide, AGA GGT GGG CAG GTG GAG AGG TGG GCA GGT GG (Molecular Biosystems Inc., San Diego, Calif.) corresponding to the consensus repeat sequence of the human myoglobin 33.15 minisatellite described by Jeffreys et al. After washing the membrane, the DNA fingerprint patterns were developed overnight in a solution containing nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrates for the alkaline phosphatase attached to the probe.

Statistical Analysis
Nonparametric statistical analysis was performed with a one-tailed Mann-Whitney rank sum test (one-tailed on the assumption that the blood pressure of the RI strains inheriting the SHR kallikrein RFLP would be greater than that of the RI strains inheriting the BN RFLP). Statistical significance was defined as p<0.05.

Results
Examples of the DNA fingerprints obtained in the parental SHR and BN strains and in two RI strains are shown in Figure 1. The DNA fingerprints of two randomly selected rats from each RI strain were identical, providing supportive evidence that the RI strains were inbred at the time of genotyping (Figure 1). All of the restriction fragments observed on DNA fingerprint analysis of the RI strains could be traced back to either the SHR or BN progenitor strain.

On Southern blots probed with the kallikrein cDNA probe, the SHR strain exhibited a unique 6.4 kb fragment, the BN strain a 2.2 kb fragment (Figure 2). Sixteen of the RI strains inherited the SHR fragment, 16 the BN fragment. None of the strains exhibited both fragments. Figure 3 depicts the blood pressures of the RI strains classified according to kallikrein genotype. In the strains that inherited the SHR kallikrein fragment, the median systolic, diastolic, and mean blood pressures were significantly greater than in the strains that inherited the BN kallikrein fragment.

Discussion
RI strains provide a useful tool for investigating the genetics of blood pressure and other quantitative traits. Because each RI strain can inherit new combinations of parental genes, a panel of RI strains can also be used for linkage analysis. In the current study in which the SHR and the normotensive BN rat served as parental strains, we found the median blood pressure of the RI strains that inherited the
FIGURE 1. DNA fingerprints of progenitor strains (one spontaneously hypertensive rat [SHR] and one Brown Norway [BN] rat) and of two recombinant inbred strains (two rats from strain 26A and two rats from strain 27A) generated by probing Alu I-digested genomic DNA with an oligonucleotide corresponding to the consensus repeat sequence of the human myoglobin 33.15 minisatellite.

SHR kallikrein RFLP to be greater than that of the RI strains that inherited the BN kallikrein RFLP. This observation constitutes the first molecular evidence suggesting that sequence variation in the kallikrein gene family or in closely linked genes may have the capacity to affect blood pressure. The specific genes detected by the Nde I RFLP remain to be identified since the probe used in this study cross-hybridizes with multiple members of the kallikrein gene family.

The median blood pressure of the RI strains that inherited the SHR kallikrein fragment was approximately 10–15 mm Hg greater than that of the RI strains that inherited the BN fragment. However, the blood pressures of the SHR and BN progenitor strains differ by 60–80 mm Hg. Thus, only a portion of the difference in blood pressure between the hypertensive and normotensive progenitor strains might be attributed to sequence variation within or near the kallikrein gene family. Given that multiple genes may be involved in the pathogenesis of spontaneous hypertension, sequence variation in a single region is not expected to account for all of the difference in blood pressure between two genetically different strains. It is also possible that under other environmental or genetic circumstances, the kallikrein alleles of the SHR and BN strains may exert much different effects on blood pressure than observed in the current study.

In the standard linkage analysis of a genetic marker and a qualitative trait, a lod score of 3 (corresponding to a critical value of approximately 0.001) is generally required for an investigator to strongly conclude that linkage is present. Because the prior probability that two randomly chosen genes will be linked is rather low, the critical threshold is set at 0.001 to yield an actual false positive rate of approximately 0.05 (assuming a reasonable power of detecting linkage when it is present). However, depending on the strength of other evidence for linkage, it may be appropriate to set the critical threshold between 0.001 and 0.05. Choice of an appropriate critical threshold in studies of recombinant inbred strains that involve a single "candidate" gene marker and a quantitative trait with multiple genetic components is problematic. In the current study in which we chose to define statistical significance as p<0.05, the analysis of the diastolic blood pressure data yielded a value of p<0.005. Nevertheless, because of the uncertainty regarding the appropriate critical threshold for linkage studies of blood pressure and a single candidate gene marker, it will be important to confirm our findings by studying other segregating populations.

Genetic studies in the SHR and in the Dahl salt-sensitive rat have provided evidence that structural alterations in the renin gene or closely linked genes have the capacity to affect blood pressure. We have also found that in the RI strains that inherited the SHR renin allele, blood pressure is significantly greater than in the RI strains that inherited the BN renin allele. Thus, in these RI strains, the preliminary results of at least one "candidate" gene study of blood pressure have been consistent with those obtained in studies of other segregating populations. In the same RI...
strains, the recent finding that blood pressure regulatory genes may be located in the region of the rat major histocompatibility complex remains to be confirmed.17

The current RFLP could be marking a blood pressure regulatory locus linked to the kallikrein gene family or within the kallikrein gene family. Sequence variation within the kallikrein gene family might affect blood pressure by affecting the activity of kallikrein, tonin, or even a kallikrein-related protein that remains to be identified. Kallikrein itself seems to be a particularly attractive candidate gene for the regulation of blood pressure.27 Decreased intrarenal activity of the kallikrein-kinin system and attendant decreases in prostaglandin synthesis might give rise to an increase in blood pressure by inducing a shift in the pressure-natriuresis curve.11,28 Furthermore, tissue kallikrein–like enzyme and tissue kallikrein mRNA have been found in rat arteries and veins and in vascular smooth muscle cells in culture.29-31 Because vascular smooth muscle cells in culture also release kininogen and kininases, it appears that all the elements necessary for localized activity of a kallikrein-kinin system may be present in arteriolar resistance vessels.30,32 Thus, in future studies of the pathogenesis of hypertension, it may be important to examine the determinants of kallikrein-kinin activity at the level of the blood vessel wall rather than just in urine or whole kidney extracts.

Acknowledgment

We thank R. MacDonald for providing the kallikrein complementary DNA probe.

References


Figure 3. Scatterplots showing systolic, diastolic, and mean arterial pressures of the recombinant inbred (RI) strains stratified according to kallikrein genotype. • Denote blood pressures of RI strains that inherited restriction fragment length polymorphism (RFLP) marking spontaneously hypertensive rat (SHR) kallikrein gene family. ▲ Denote blood pressures of RI strains that inherited RFLP marking Brown Norway (BN) kallikrein gene family. Horizontal bars denote median blood pressure of each grouping. Probability values indicate statistical significance from the Mann-Whitney rank sum analysis.


**KEY WORDS** • genetics • genetic hypertension • kallikrein • spontaneously hypertensive rats • rat studies
Cosegregation of blood pressure with a kallikrein gene family polymorphism.
M Pravenec, V Kren, J Kunes, A G Scicli, O A Carretero, L Simonet and T W Kurtz

Hypertension. 1991;17:242-246
doi: 10.1161/01.HYP.17.2.242

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 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/17/2/242

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/