Original Contributions

Hypertensive Strains and Normotensive ‘Control’ Strains
How Closely Are They Related?

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The spontaneously hypertensive rat and the Dahl salt-sensitive rat are the most widely studied genetic models of hypertension. Many investigators have attempted to study the pathogenesis of hypertension by comparing these strains with their respective normotensive “controls,” the Wistar-Kyoto rat and the Dahl salt-resistant rat. However, the genetic relation between each of these hypertensive strains and its corresponding normotensive control has never been clearly defined. Based on an analysis of DNA “fingerprint” patterns generated with six multilocus probes, we found that the spontaneously hypertensive rat (Charles River Laboratories, Inc.) is genetically quite different from its normotensive Wistar-Kyoto control: these strains only share approximately 50% of their DNA fingerprint bands in common. The inbred Dahl salt-sensitive rat (SS/Jr strain) (Harlan Sprague Dawley, Inc.) and the Dahl salt-resistant rat (SR/Jr strain) share approximately 80% of their DNA fingerprint bands in common. To the extent that the genes identified by DNA fingerprint analysis are representative of loci dispersed throughout the rodent genome, the current findings provide evidence of extensive genetic polymorphism between these commonly used hypertensive strains and their corresponding normotensive controls, particularly in the spontaneously hypertensive rat model. These findings, together with the fact that an enormous number of biochemical and physiological differences have been reported between these hypertensive and normotensive strains, suggest that continued comparison of spontaneously hypertensive rats with Wistar-Kyoto rats or Dahl salt-sensitive with salt-resistant rats will have limited value for investigating the pathogenesis of hypertension. (Hypertension 1992;19:419–424)

KEY WORDS • genetics • genetic hypertension • blood pressure • polymorphism • DNA fingerprinting • Dahl rats • spontaneously hypertensive rats • Wistar-Kyoto rats

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Rapp, many of these physiological and biochemical differences probably evolved as a consequence of random genetic drift: When these models of hypertension were developed from Wistar and Sprague-Dawley stocks, the process of selection and inbreeding not only resulted in the fixation of alleles involved in the pathogenesis of hypertension but also in the random fixation of alleles unrelated to the regulation of blood pressure. Thus, the hypertensive strains may differ from the normotensive control strains throughout multiple regions of the genome, and as a consequence, the scientific value of studies that simply compare these strains could be quite limited. Although this view has extraordinary implications for the field of hypertension research, the extent to which the hypertensive strains differ genetically from their normotensive controls has never been clearly defined. Furthermore, only limited information is available regarding the precise genealogical backgrounds of these strains.

In 1985, Jeffreys and colleagues described how Southern blot analysis of tandem repeat sequences of DNA scattered throughout the mammalian genome could be used to generate a complex pattern of restriction fragments unique to an individual. Such individual specific restriction fragment patterns have been termed DNA “fingerprints” and have been widely used to assess genetic relations between individuals and between population groups. In the current study, we used...
DNA fingerprint analysis to investigate the genetic relations of commercially available SHR and Dahl salt-sensitive strains to their corresponding WKY and Dahl salt-resistant controls.

Methods

DNA Fingerprint Analysis

We tested inbred, male Dahl salt-sensitive (SS/Jr strain) and salt-resistant (SR/Jr strain) rats from Harlan Sprague Dawley, Inc., Indianapolis, Ind., and inbred male SHR (SHR/Ncr1BR strain) and WKY (WKY/Ncr1BR strain) rats from Charles River Laboratories, Wilmington, Mass. DNA fingerprinting was performed by Southern blot analysis of six different multilocus variable number of tandem repeat (VNTR) sequences. For each VNTR sequence examined, we randomly tested at least two rats per strain to confirm that the DNA fingerprint patterns were uniform among rats within each of these four inbred strains. The multilocus VNTRs tested included the myoglobin 33.6 and 33.15 minisatellite sequences described by Jeffreys et al.5 and the CAC, CT, GTT, and GACA microsatellite sequences described by Epplen and colleagues.6,7 Genomic DNA, prepared from spleen tissue by phenol/chloroform extraction (15 µg/sample), was digested for 2 hours at 37°C with the restriction enzymes Hinfl or Alu I (Boehringer Mannheim, Indianapolis, Ind.) and electrophoresed on a 1% agarose gel at 25 V for 24 or 48 hours, depending on the probe tested.8,9 In each experiment, the DNA samples from each hypertensive strain and its corresponding normotensive strain were run on the same gel. After overnight transfer to a nylon membrane, ultraviolet fixation, and a 20-minute prehybridization, the DNA was hybridized for 20 minutes at 50°C with the probe-enzyme combination. With the Dahl strains, the DNA was hybridized for 20 minutes at 50°C with the probe-enzyme combination. With the CAC and CT microsatellite probes, filters were prehybridized for 1 hour and then hybridized at 40°C for 1 hour. With the GTT and GACA probes, the prehybridization and hybridization steps were performed at 37°C. The oligonucleotide probe sequences were as follows: 33.6 minisatellite, TGG AGG AGG GCT GTA GGA GGA C; 33.15 minisatellite, AGA GGT GGG CAG GTG GAG AGG TGG GCA GTA GC; CAC microsatellite, CAC CAC CAC CAC CAC; CT microsatellite, CTC TCT CTC CT; GTT microsatellite, GTT GTT GTT GTT GTT; and GACA microsatellite, GAC AGA CAG ACA GAC A.

An alkaline phosphatase reaction system was used to detect sequences hybridized to the probes. For the 33.6 and 33.15 minisatelites, the alkaline phosphatase was directly conjugated to the probes (Molecular Biosystems Inc., San Diego, Calif.). For the microsatellite sequences, the probes were initially end-labeled with digoxigenin-labeled deoxyuridine triphosphate by a terminal transferase reaction. The hybridized DNA was then incubated with anti-digoxigenin antibody labeled with alkaline phosphatase. After washing the membranes, DNA fingerprint patterns were developed overnight in a buffered solution containing nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrates for the alkaline phosphatase attached to the probes. In some cases, the DNA fingerprint patterns were developed by exposing the membranes to x-ray film after they had been incubated in buffer containing a chemiluminescent substrate for alkaline phosphatase (AMPPD, Tropix, Bedford, Mass.).

Estimation of Genetic Relatedness

Genetic relatedness was estimated from the band-sharing coefficient

\[
D(\%) = \frac{2N_D}{N_B + N_C} \times 100
\]

where \(N_B\) and \(N_C\) are the number of restriction fragments in the hypertensive and the control strains, respectively, and \(N_D\) is the number of restriction fragments shared by both strains.10,11 The value of \(D\) varies from 0 when no bands are shared in common to 100% when two fingerprints are identical. Fragments greater than or equal to 4 kb in size were scored on gels that ran 48 hours, and fragments greater than or equal to 2 kb were scored on gels that ran for 24 hours.

The validity of using DNA fingerprints to estimate the genetic relatedness of inbred strains depends in part on the extent to which the DNA fingerprint loci are representative of other loci dispersed throughout the genome. In a recent study in recombinant inbred strains of mice, Jeffreys et al.12 determined that many of the DNA fingerprint loci detected by the 33.15 and 33.6 minisatellite probes are dispersed and are not tightly linked. In preliminary studies in a large set of recombinant inbred strains derived from the SHR and the Brown-Norway rat, we have also found that many of the loci identified by the current minisatellite and microsatellite probes are not tightly linked.13,14 Furthermore, recent studies in the goat have demonstrated that band-sharing coefficients determined from DNA fingerprints generated with the 33.15 minisatellite probe correlate quite well with the actual genetic relations established by pedigree analysis.15 Although recent studies in cattle and in humans indicate a tendency for minisatellite sequences to be organized in clusters, it appears that DNA fingerprints are still of value for preliminary genome scanning.16

Results

Figures 1–5 show examples of the DNA fingerprint patterns of Dahl SS/Jr versus Dahl SR/Jr rats and SHR versus WKY rats for three of the six probe-enzyme combinations tested. The left half of Figure 1 shows nearly identical DNA fingerprints of a Dahl SS/Jr rat and a Dahl SR/Jr rat generated by probing Hinfl-digested genomic DNA with an oligonucleotide corresponding to the 33.15 minisatellite sequence. With this probe-enzyme combination, only one band can be observed that is not shared in common between the two Dahl strains. In contrast, the right half of Figure 1 shows the DNA fingerprint patterns of an SHR and WKY rat after they had been incubated in buffer containing a chemiluminescent substrate for alkaline phosphatase (AMPPD, Tropix, Bedford, Mass.).
FIGURE 1. Southern blot shows DNA fingerprints of inbred Dahl salt-sensitive (designated S) and inbred Dahl salt-resistant (designated R) rats and an inbred spontaneously hypertensive rat (SHR) and Wistar-Kyoto (WKY) rat generated by probing Hinfl-digested DNA with an oligonucleotide corresponding to the consensus repeat sequence of the human myoglobin 33.15 minisatellite. Arrows designate bands not shared between the hypertensive strains and their normotensive controls.

Figures 4 and 5 show examples of DNA fingerprints generated by probing Hinf1-digested DNA with the CAC microsatellite probe. Again, the Dahl strains share a higher percentage of restriction fragments (84%) than the SHR and WKY strains (58%). All DNA fingerprint patterns were identical among rats within each inbred strain.

The band-sharing values for all of the probe-enzyme combinations tested are depicted in Figure 6. In the Dahl SS/Jr versus SR/Jr comparison, the median band-sharing index of 80% was significantly greater than that in the SHR versus WKY comparison, 54% (p < 0.005 by Wilcoxon rank sum test).

Discussion

Over the past three decades, many researchers have attempted to investigate the pathogenesis of hypertension by systematically comparing SHR and Dahl salt-sensitive rats with their respective normotensive controls, i.e., WKY rats and Dahl salt-resistant rats. If the hypertensive strains were identical to the control strains at all loci except for those regulating blood pressure, such interstrain comparisons would be of great value for investigating the pathogenesis of hypertension. However, the degree of genetic similarity between the hypertensive strains and their normotensive controls has never been clearly defined, and serious questions have been raised about the usefulness of such interstrain comparisons for investigating the pathogenesis of hypertension. In fact, apart from some sketchy genealogical records, little information is available regarding the precise genetic backgrounds of these widely used animal models of hypertension.

In the current study, we used the technique of DNA fingerprint analysis to investigate the genetic relation between the SHR and Dahl SS/Jr strains and their corresponding WKY and Dahl SR/Jr controls. DNA fingerprints have been widely used to analyze genetic
SHR WKY

FIGURE 3. Southern blot shows DNA fingerprints of inbred spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats generated by probing HinfI-digested DNA with an oligonucleotide corresponding to the consensus repeat sequence of the human myoglobin 33.6 minisatellite. Arrows designate bands not shared between the SHR and WKY strains.

Based on the DNA fingerprint results generated with six multilocus probes, it appears that multiple genetic differences may exist between the Charles River SHR and its WKY control. Such extensive polymorphism raises questions about the breeding practices used to maintain the original colony of Wistar rats in Kyoto from which the SHR was derived. In the 1963 article that describes the initial development of the SHR, Okamoto and Aoki\textsuperscript{19} stated that the rats were selected from a Wistar strain that had been maintained by inbreeding since 1951. However, records from the National Institutes of Health (NIH) indicate that SHR were developed from an “outbred Wistar-Kyoto male” and that the Wistar rats from Kyoto used by the NIH to subsequently breed WKY rats were from “noninbred” stock.\textsuperscript{20,21} The NIH records, together with the results of the current studies, would suggest that the Wistar colony from which the SHR and/or WKY strains were derived may have been quite heterogeneous. Alternatively, it is possible that the strains may have diverged as a consequence of inadvertent genetic contamination of SHR or WKY breeding stock. It is also possible that recurrent mutation at certain ultravariable loci may have contributed to some of the variation in DNA fingerprint patterns observed between the SHR and WKY strains.\textsuperscript{18} However, in DNA fingerprint studies performed in a large panel of SHR recombinant inbred strains, such spontaneous mutations have only been infrequently observed (M. Pravenec and T.W. Kurtz, unpublished observations).

Given the convoluted genealogical backgrounds of these strains in the United States, multiple genetic differences presumably exist not only between SHR and WKY rats from Charles River Laboratories but also between SHR and WKY from other commercial vendors.\textsuperscript{3,8,18} Because these strains are likely to differ...
throughout multiple regions of the genome, the usefulness of studies that simply compare SHR and WKY rats would seem quite limited. Thus, Rapp’s long-held view regarding the pitfalls of interstrain comparisons for studying the pathogenesis of hypertension would seem well justified.4-17

Recently, Nabika and colleagues22 have reported that the DNA fingerprints of SHR and WKY rats from the Shimane Institute of Health in Izumo, Japan, are quite similar and that both strains carry the RTr haplotype of the major histocompatibility complex. Based on these observations, Nabika et al22 have proposed that the SHR and WKY strains from Izumo “have similar genetic backgrounds” and are thus “an appropriate set of model animals for the biochemical or biophysiological research of genetic hypertension.” Although the results of Nabika et al22 are encouraging, it should be recognized that in their study, the resolution of the DNA fingerprints was quite limited, and only two minisatellite probe-enzyme combinations were tested. Thus, it may be premature to conclude that the SHR and WKY from Izumo constitute near-isogenic strains and that restriction fragment length polymorphisms in these strains will be linked to genes that determine hypertension.

In contrast to the results in the SHR-WKY model of hypertension, DNA fingerprint analysis revealed a surprisingly high degree of apparent genetic similarity between the Dahl SS/Jr rat and the Dahl SR/Jr rat. Although the Dahl strains were originally derived from noninbred Sprague-Dawley stock,23 the high band-sharing index suggests that the progenitors of these strains were more closely related than previously recognized. In fact, the median band-sharing index of 80% between the Dahl SS/Jr and SR/Jr strains is greater than that expected between a parent and its F1 offspring (50%) or even between a parent and a first backcross generation animal (75%).

Although Dahl SS/Jr and SR/Jr rats are more closely related than SHR and WKY rats, multiple genetic differences still exist between these Dahl strains. Because most of the genetic differences between the Dahl strains are probably not related to their differences in blood pressure, interstrain comparisons in this model are also unlikely to shed much light on the pathogenesis of hypertension. Given the problem of genetic heterogeneity within noninbred Dahl rats, the scientific value of studies that use the Brookhaven Dahl model may be even more limited.24

Although current animal models of hypertension suffer from the lack of appropriate genetic controls, recent advances in molecular biology have provided important opportunities for creating improved models for high blood pressure research. By applying molecular selection techniques to backcross breeding programs, it is now possible to rapidly create congenic strains that are genetically
identical except for specific chromosome regions of interest. In preliminary studies in Dahl rats, we have used marker-assisted selection to transfer a segment of chromosome 13 that contains the renin allele of the SR/Jr strain onto the genetic background of the SS/Jr strain. In addition, Mullins et al have demonstrated the feasibility of creating rodent models of hypertension by transgenic technology. Although valuable work has been performed with the SHR and Dahl strains, the availability of new congenic and transgenic models should provide even greater opportunities for investigating the genetic determinants of hypertension.

References
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