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# Genetic-Divergence Between the Wistar-Kyoto Rat and the Spontaneously Hypertensive Rat

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# Genetic Divergence Between the Wistar-Kyoto Rat and the Spontaneously Hypertensive Rat

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**A method of restriction fragment length polymorphism (RFLP) analysis was used to estimate the amount of genetic divergence between the spontaneously hypertensive rat (SHR) strain and the Wistar-Kyoto (WKY) strain. DNA from each strain was digested with eight restriction endonucleases and hybridized with six single copy gene sequences. The number of hybridization bands in each digestion was used to estimate the total number of bases analyzed and RFLPs were scored as single mutations. Divergence was then estimated by dividing the number of mutations by the number of bases analyzed. In a total of 808 bases analyzed in WKY rats, a minimum of 13 mutations were scored in SHR, which yields a nucleotide divergence of 1 change per 62 bp. This is an extremely high amount of divergence given the known origin of these two strains and is comparable to the maximum divergence possible between unrelated humans. (*Hypertension* 1992;19:425–427)**

KEY WORDS • **genetics** • **genetic divergence** • **spontaneously hypertensive rats** • **Wistar-Kyoto rats** • **genetic hypertension**

The spontaneously hypertensive rat (SHR) has been, since its release as an inbred strain in 1969, the most extensively studied of all animal models for human essential hypertension.[1] The SHR strain was the result of selection for increased blood pressure from an outbred Wistar colony at Kyoto University.[2,3] In 1970, a second strain was derived from the same outbred Wistar colony without selection for blood pressure; this strain was designated Wistar-Kyoto (WKY).[3] The WKY strain has been used extensively as a normotensive control for the hypertensive SHR strain. DNA "fingerprints" differentiate SHR and WKY rats from each other[4–6] and also identify substrains (isolates) within the SHR[4] and WKY[5,6] strains. As efforts to identify genes responsible for hypertension increase, a number of laboratories have set up crosses between WKY rats and SHR in an attempt to segregate specific genes with blood pressure.[7] The usefulness of such crosses between SHR and WKY rats has recently come under serious scrutiny.[5–8] One problem is the lack of basic genetic background knowledge concerning these two strains. How genetically similar or dissimilar are these two strains? The DNA fingerprint data demonstrate a qualitative difference but have not been used to quantify this relation. Although both strains were derived from the same colony, the selection methods applied to each strain and the times of derivation (1960 for SHR versus 1970 for WKY) were different. Therefore, it is not obvious how much genetic variation exists between the two strains. Physiological and descriptive studies have described numerous phenotypic differences between SHR and WKY (see Reference 9 for a review). Are these differences the pleiotropic effects of a few variable loci, or are there many loci that differ between the strains?

In the current study, we quantify the genetic differentiation at the nucleotide level between the SHR and WKY strains. We used a protocol that examined strain differences in the restriction fragment patterns of single copy genes with no a priori potential to affect blood pressure. From this data we have been able to calculate the nucleotide divergence between the SHR and WKY rat. A better knowledge of the genetic relatedness of the strains is necessary for designing crosses to study and identify those loci affecting blood pressure.

## Methods

### *Rat Strains*

The SHR and WKY strains used in our studies were originally obtained from Harlan Sprague Dawley, Indianapolis, Ind., who obtained their breeding stock from the National Institutes of Health (NIH). We have maintained and inbred these strains in our animal facilities for over 10 years with approximately three to four generations per year. The animals were maintained in a humane manner according to NIH guidelines.

### *Restriction Fragment Length Polymorphism Analysis*

DNA was isolated from SHR and WKY rat livers according to the method of Johnson et al.[10] The DNA was dissolved in distilled, deionized water and stored frozen at −20°C in 300-μg aliquots. Restriction enzymes (*Bam*HI, *Eco*RI, *Hin*dIII, *Msp* I, *Pst* I, *Pvu* II, *Taq* I, and *Xba* I) were purchased from BRL (Life Technologies, Inc., Grand Island, N.Y.), and digests were performed according to supplier recommendations. For each enzyme, 300 μg SHR and WKY DNA was digested

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using 4–6 units of enzyme per microgram DNA. Fifteen micrograms digested DNA was loaded in each lane of an 0.8% agarose gel. Electrophoresis was conducted for 16–18 hours in Tris-acetate buffer.[10] Twenty duplicate gels were produced. DNA size standards were included on each gel (1 kb ladder, BRL). After electrophoresis, gels were stained with ethidium bromide, photographed, transferred to Zetabind membrane (Whatman Lab Sales, Hillsboro, Ore.), and hybridized as described by the supplier. Cloned DNA probes for the RFLP analysis were obtained from ATCC (v-*abl*, No. 41035; v-*bas*, No. 41036; hypoxanthine-guanine phosphoribosyl transferase [HPRT], No. 37424) or were the generous gift of various investigators (rat L-pyruvate kinase complementary DNA [cDNA] clone G4, Dr. Axel Kahn; rat aromatase cDNA, Dr. JoAnne Richards; rat B-casein cDNA, Dr. Jeffrey Rosen). All cloned DNAs were nick translated and labeled with phosphorus-32 as described by Rodgers et al[11] to a final specific activity of $100–150\times10^6$ cpm/$\mu$g DNA. Probe concentration in the hybridizations was adjusted to give between 1 and $2\times10^6$ cpm/ml buffer. The final formamide concentration was adjusted to 40% in hybridizations involving non-rat cloned DNA probes. After hybridization, filters were washed using stringencies consistent with the hybridization conditions. Autoradiography was performed using Du Pont Cronex Lighting Plus intensifying screens (Du Pont Co., Wilmington, Del.) for 5–9 days at −70°C.

### *Nucleotide Divergence Analysis*

WKY DNA was arbitrarily chosen as the point of reference for this analysis. First, we determined the number *(N)* of hybridization bands detected for each digest that gave clear signals. To generate these $N$ bands, $N+1$ restriction sites are required. The number of restriction sites was multiplied by the number of nucleotides in the recognition sequence for that enzyme to give the number of bases screened. The sum of all bases for each enzyme for each probe gave an estimate of the total number of bases screened in the WKY DNA.

For the purpose of our analysis, a restriction fragment length polymorphism (RFLP) was defined as a difference in the hybridization banding patterns between SHR and WKY for a given enzyme. For each RFLP detected, we assumed that a single point mutation was responsible. However, in the computation of divergence only one mutation was scored for any given enzyme digest, regardless of how many RFLPs were detected. Dividing the total number of bases screened in the WKY DNA by the number of scored mutations gives an estimate of the frequency of nucleotide changes between these colonies of SHR and WKY rats. This value is a minimum estimate of nucleotide divergence based on our assumptions.

## Results

The objective of the present study was to establish the genetic relatedness, at a molecular level, of the SHR and WKY rat strains. Table 1 lists the probes tested and the results of our divergence analysis. Each of the six different probes detected at least one RFLP with our panel of eight restriction enzymes. A total of 808 bases were analyzed, and a minimum of 13 mutations were scored. This equates to an average nucleotide divergence of 1 change per 62 bp.

**Table 1. Summary of Restriction Fragment Length Polymorphism Analysis and Estimation of Divergence Estimate**

| Probe | Enzymes detecting RFLPs | Mutations scored | Bases analyzed | Divergence estimate |
|---|---|---|---|---|
| v-*abl* | *Eco*RI | 3 | 120 | 1 per 40 |
| | *Hin*dIII | | | |
| | *Xba* I | | | |
| v-*bas* | *Eco*RI | 2 | 138 | 1 per 69 |
| | *Xba* I | | | |
| L-Pyruvate kinase | *Eco*RI | 2 | 132 | 1 per 66 |
| | *Xba* I | | | |
| HPRT | *Eco*RI | 3 | 170 | 1 per 57 |
| | *Taq* I | | | |
| | *Xba* I | | | |
| B-casein | *Eco*RI | 2 | 98 | 1 per 49 |
| | *Hin*dIII | | | |
| Aromatase | *Bam*HI | 1 | 150 | 1 per 150 |
| Total | | 13 | 808 | 1 per 62 |

For each probe tested, the enzymes detecting restriction fragment length polymorphisms (RFLPs) are listed along with the number of mutations scored for each probe and the number of bases analyzed. Divergence frequency is calculated by dividing mutations scored by the number of bases analyzed. Method for calculating bases analyzed and mutations scored is described in "Methods."

The estimates of divergence for each individual probe can be used to calculate a 95% confidence interval for the overall estimate. The mean divergence calculated in this way is 1 in 61 with a 95% confidence interval of from 1/42 to 1/105.

## Discussion

A number of RFLPs have been reported in the literature with respect to genes that are postulated to play a role in hypertension (see Reference 12 for an example). To prevent bias in our analysis, a series of random, single-copy DNA probes were chosen that a priori could be predicted to have no relation to blood pressure differences reported between the SHR and WKY strains. Also, the eight restriction enzymes used recognize a total of 46 bp, of which 24 are GC and 22 are AT. All restriction enzymes do not equally identify polymorphisms. In mammalian studies, *Msp* I and *Taq* I detect an excessively high number of polymorphisms compared with other restriction endonucleases.[13] Both of these enzymes have CpG dinucleotides in their recognition sequences, and this CpG sequence is known to undergo a C to T transition at increased frequency.[14] Of the RFLPs detected in the present study, only one was identified from either *Msp* I or *Taq* I digestions. Therefore, our analysis is not inadvertently biased toward any particular subset of DNA sequences or nucleotides with increased mutation rates and is reflective of a random sample.

The divergence as estimated from individual probes was relatively constant (Table 1); the highest divergence was 1 base pair in 40 with v-*abl*, and the lowest divergence was 1

base pair in 150 with aromatase. All the other estimates were between 1 in 49 and 1 in 69. Although the absolute value of the frequency we obtained is subject to our implicit assumptions, which may not be totally valid (for instance the assumption that only one base pair change was responsible for each RFLP), the conformity of estimates from probe to probe would indicate these assumptions are at least consistent.

Studies to estimate nucleotide divergence in human populations calculate average heterozygosity.[15,16] Measures of average heterozygosity are weighted by the gene frequencies of the identified variants. In comparisons between two inbred strains such as SHR and WKY, all variants are assumed to have a gene frequency of either 1 or 0 depending on the reference strain. The value calculated between SHR and WKY rats is not an average heterozygosity but is the measured heterozygosity.

Values of average heterozygosity between unrelated humans are about 1 in 300,[15,16] which is less than that calculated for SHR and WKY rats. Another comparison with the human data would be to use polymorphic sites rather than average heterozygosity. This would measure the maximum heterozygosity in the human population. Hofker et al[16] used 40 unique or single-copy autosomal probes and assayed 2,710 bp of DNA in unrelated individuals and found 24 polymorphic sites, which is an average of 1 site in 113 bp. Murray et al[17] found eight sites in 689 bp or an average of 1 in 86. Our data suggest that WKY rats and SHR differ from each other as much as the maximum value possible for two unrelated humans.

SHR and WKY rats have been separated from the original colony and each other for at least 30 years. Some of the divergence we measured could have occurred since the strains were derived rather than having been present at their origin. Two lines of data can be used to estimate this form of divergence. From the time of divergence (30 years) and an estimate of mutation rate ($5\times10^{-9}$ substitutions per nucleotide per year),[18] the frequency of new mutations can be calculated using the equation

$$K=(\text{mutation rate})(\text{time})$$

where $K$ is the frequency of new mutations. This would predict a single base change per 6.7 million bp; therefore, given the number of bases we assayed, we would not expect to see any new mutations. As discussed previously, the first type of mutation to accumulate between the strains should be the CpG variation detected by *Msp* I and *Taq* I. Since only 1 RFLP was detected with these enzymes, most if not all, of the polymorphisms identified in the present study were probably present in the original outbred colony rather than the result of new mutations that occurred since the inbred strains were separated from the parental colony.

What does our analysis imply with respect to the search for hypertension genes in the SHR strain? First, the simple identification of an RFLP does not guarantee linkage to hypertension. In fact, our data suggest that any gene is likely to have at least one or more RFLP associated with it between SHR and WKY rats. This reinforces the admonition of Rapp that proper linkage/segregation analysis and mechanistic relevance must be demonstrated to establish a relation between a RFLP and hypertension.[7,19] Second, it raises the question of how related are SHR and WKY rats in different colonies that have been maintained throughout the world? Our data are not intended to measure the genetic differentiation within either SHR or WKY rat strains. However, given the amount of genetic variation we observed in our colonies, it would not be surprising to find substantial divergence between SHR and/or WKY rats maintained in our colony versus the same strain maintained in colonies elsewhere. Could discrepancies in results between laboratories be due to the genetic variation that probably exists? Further analysis of SHR and WKY rat isolates from other colonies will need to be performed to answer these questions.

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