Genetic Heterogeneity of the Spontaneously Hypertensive Rat

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We examined DNA fingerprints of the spontaneously hypertensive rat from Shimane Institute of Health Science, Izumo, Japan, including seven substrains that were separated in the early stages of the establishment of the stroke-prone spontaneously hypertensive rat, and compared their fingerprints with those of rats from other sources. Obtained DNA fingerprints revealed that, in both the stroke-resistant spontaneously hypertensive rat and the Wistar-Kyoto rat, there is a substantial genetic difference between the rats from the National Institutes of Health and from Shimane Institute of Health Science. By contrast, only a small genetic difference was observed either between the rats from the National Institutes of Health and Charles River Laboratories or among the substrains of the spontaneously hypertensive rat in the Shimane Institute of Health Science. Further, in the strains from the Shimane Institute of Health Science, there were fingerprinting bands that could distinguish either the Wistar-Kyoto rat from all the substrains of the spontaneously hypertensive rat or the stroke-prone from the stroke-resistant spontaneously hypertensive rat in spite of their close genetic backgrounds. From the observations above, we concluded 1) that there is substantial genetic variance of the spontaneously hypertensive rat between the two major sources in the world, the National Institutes of Health and the Shimane Institute of Health Science and 2) that by DNA fingerprinting analysis, it is possible to identify the restriction fragment length polymorphisms that are specific for the spontaneously hypertensive rat or the stroke-prone spontaneously hypertensive rat. These polymorphisms can be applied in the segregation study of the F2 generation. (Hypertension 1991;18:12-16)
that genomic loci showing RFLPs between WKY rats and SHR but not among the substrains of SHR may contribute to the development of genetic hypertension, because our seven SHR substrains (three SHRSP and four stroke-resistant SHR [SHRSR]) are expected to have the same set of "hypertensive" alleles. We thus compared DNA fingerprints between WKY rats and the substrains of SHR to see whether such RFLPs could be observed.

In this report, we show that genetic heterogeneity between different sources is found not only in WKY rats but also in SHR when examined by DNA fingerprinting analysis. In addition, we show that there are fingerprinting bands that can distinguish either WKY rats from SHR, or SHRSP from SHRSR.

Methods

Animals

Male WKY rats and SHRSR from Charles River Laboratories (WKY/CRL and SHRSR/CRL) were purchased from Charles River Laboratories, Atsugi, Japan. WKY rats, SHRSR, and SHRSP from the NIH strain (WKY/NIH, SHRSR/NIH, and SHRSP/NIH) were sent from the National Institutes of Health, Bethesda, Md., in 1987 and have been maintained at our laboratory as inbred colonies. WKY rats, four substrains of SHRSR, and three substrains of SHRSP in Shimane Institute of Health Science, Izumo, Japan (WKY/IZM, SHRSR/IZM, and SHRSP/IZM) are all direct descendants of the original Kyoto strains. They have been selected and kept as inbred colonies for over 50 generations (Figure 1).

In this report, we call SHR with a low incidence of stroke "SHRSR" and we use the name "SHR" for SHRSR and SHRSP as a whole.

DNA Analysis

Two minisatellite DNA probes called "myo" and "ins" were generously provided by Professor Ryo Kominami, University of Niigata, Niigata, Japan. These are composed of tandem repeats of the synthesized oligomers of which sequences are identical to the repetitive oligonucleotide sequences in human myoglobin (myo) and insulin (ins) genes. The repetitive sequences are 5'-GACCGAGGTCTAAAGCTGGAGGTGGGCAGGAAG-3' and 5'-ACAGGGGTGTGGGG-3' for myo and ins, respectively. These synthesized DNA (myo, 500 b; ins, 550 b) were cloned in plasmid pUC19, and these cloned plasmids were used as probes after they were labeled with phosphorus-32 by the random primer method.

Genomic DNA of the rats was extracted from the liver or the spleen by phenol/chloroform extraction. Ten micrograms of DNA was digested with 30 units of Hae III or HindIII for 3–4 hours under the conditions recommended by the manufacturer (Boehringer-Mannheim, Mannheim, FRG). Digested DNA was electrophoresed on 1% agarose gel at 40 V for 40 hours, and samples were capillary transferred onto nylon membrane. After prehybridization, performed in 4× standard saline citrate (SSC), 1% sodium dodecyl sulfate (SDS), and 5× Denhardt solution at 55°C for 24 hours, hybridization was done in 4× SSC and 1% SDS at 55°C for 16–18 hours. At the end of hybridization, the membrane was washed in 1× SSC and 0.1% SDS twice at room temperature for 90 minutes before its autoradiogram was taken.

Results

In Figure 2, we compared DNA fingerprints of the rats from three sources with each other. In both cases of WKY rats and SHRSR, it was shown 1) that the fingerprinting patterns of CRL and NIH strains were close to each other and 2) that by contrast, a substantial difference in the fingerprinting patterns were observed between NIH/CRL and IZM strains (indicated by arrowheads in Figures 2A and 2B). In addition, 3) the difference in the fingerprints was rather small among the substrains of SHR/IZM.

SHRSP of NIH and IZM strains, on the other hand, had very close fingerprinting patterns. However, variation at two fragments was seen in A4 and one litter of A3 when their fingerprints were compared with those of A1 and other litters of A3 (shown by arrows in Figure 2C). This observation indicated that we had two genetically different inbred A3 substrains, one having the same DNA fingerprinting pattern as A1 and the other having the same as A4. To distinguish them, we call the former...
Figure 2. Comparison of DNA fingerprints of Wistar-Kyoto (WKY) rats, stroke-resistant spontaneously hypertensive rats (SHRSR), and stroke-prone SHR (SHRSP) from three different sources. Genomic DNA were digested with Hae III, electrophoresed and transferred onto nylon membrane. Hybridization was performed as described in "Methods" with phosphorus-32-labeled "myo" probe. Arrowheads in panels A and B show difference of fingerprinting patterns between NIH/CRL and IZM strains. Arrows in panel C indicate two distinct bands showing variation between substrains of SHRSP. NIH, rats from National Institutes of Health colony; IZM, rats from Shimane Institute of Health Science, Izumo, Japan; CRL, rats from Charles River Laboratories. Lanes 1 and 2, WKY/NIH; 3-5, WKY/CRL; 6 and 7, WKY/IZM; 8 and 9, SHRSR/NIH; 10-12, SHRSR/CRL; 13 and 14, SHRSRb; 15 and 16, SHRSRb2; 17, SHRSRc; 18 and 19, SHRSRcL; 20 and 21, SHRSP/NIH; 22 and 23, SHRSPA1αβ; 24 and 25, SHRSPA1α; 26 and 27, SHRSPA1αβ; 28 and 29, SHRSPA1αβ.

"Aαβ" and the latter "Aαβ." Such intrastrain difference in DNA fingerprints was not found in other strains we examined.

Figure 3 shows the DNA fingerprints of WKY/IZM and the seven substrains of SHR/IZM using the two different minisatellite probes. Two panels in Figure 3 show that the fingerprinting patterns of WKY/IZM are close to those of SHR/IZM. Moreover, in addition to several differences between the substrains (indicated by arrowheads in Figure 3), Figure 3A shows that one fragment at about 3.7 kbp was commonly seen in all the substrains of SHR/IZM but not in WKY/IZM (shown by an arrow in Figure 3A). In Figure 3B, another combination of a restriction enzyme and a DNA probe revealed the SHRSP-specific (shown by arrowheads a and b) and the SHRSR-specific fingerprinting bands (indicated by arrowhead c).

Discussion

SHRSR and SHRSP have been the most commonly used model animals for the study of essential hypertension and its cerebrovascular complications. Although WKY rats have been used as the normotensive control animal for SHRSR and SHRSP in many studies, recent reports pointed out that WKY rats from different sources were not identical either in genotype or in phenotype. We confirmed this result by examining the two major sources of WKY rats, WKY/NIH and WKY/IZM, presently used in the world. Furthermore, we clearly showed that the SHR are a heterogeneous group of inbred strains; there is a substantial genetic difference between the NIH/CRL strains and the IZM strain when judged by DNA fingerprinting patterns.

SHR were sent to the NIH from the original Kyoto colony at the F13 generation, whereas SHR/CRL were separated from SHR/NIH at the F32 generation. WKY/NIH were sent from the Kyoto colony just after the inbreeding program had started, whereas WKY/CRL were separated from WKY/NIH at the F19 or F11 generation (Reference 13 and unpublished observations by Y. Yamori). These facts indicate that in both SHR and WKY rats, NIH and IZM strains had
been separated before they became fully inbred. Our fingerprinting data confirm this supposition. Because of this genetic variance in SHR as well as in WKY rats, it can be inferred that both SHR and WKY rats of different sources have phenotypic variance that results in discrepancies in experimental data between studies.\textsuperscript{13} Caution should be used when comparing physiological or biochemical data between studies in which the rats of different sources were used.

Conversely, the substrains of SHRSP, including SHRSP/NIH, had very similar fingerprinting patterns, which implies that substrains of SHRSP are genetically close to each other. However, the distinct variation at the two fragments, loss of the 8.9 kbp fragment and gain of the 4.0 kbp, were found in A\textsuperscript{a} and one litter of A\textsuperscript{b}. Because the possibility that the same changes would happen independently in two different strains are extremely low, we suspect that A\textsuperscript{b} is one litter of A\textsuperscript{a} incorrectly labeled A\textsuperscript{b}. Because all of the substrains of SHRSP develop severe hypertension and have a high incidence of stroke,\textsuperscript{1} the alleles that are different among the substrains of SHRSP seem to have no pathogenetic contribution to these abnormalities.

In the present study, we showed that WKY/IZM and the substrains of SHR/IZM had very close DNA fingerprinting patterns when the rats were examined with the two different minisatellite probes. In fact, when judged by DNA fingerprints, SHR/IZM seemed closer to WKY/IZM than to WKY/NIH (see Figures 2A and 3A). This result was supported by a recent histocompatibility study (Matsumoto et al, unpublished observations), which revealed that WKY/IZM, but not WKY/NIH or WKY/CRL, had the same RT-1 haplotype as SHR. These results imply that SHR/IZM and WKY/IZM have similar genetic backgrounds, and thus they are an appropriate set of model animals for the biochemical or biophysiological research of genetic hypertension.

We can therefore expect that it is more likely that the genetic differences observed between WKY/IZM and SHR/IZM contribute to the evolution of genetic hypertension. It is also reasonable to expect that genetic differences between the substrains of SHR are less probably associated with hypertension; they are expected to carry similar sets of "hypertensive" alleles because the substrains of SHR were separated when inbreeding was half progressed and because they do not differ very much in blood pressure. Thus, we can assume that the loci showing RFLPs between WKY/IZM and SHR/IZM but not among the substrains of SHR/IZM are more likely to contribute to the development of genetic hypertension. In the present study, we showed that such genetic difference between WKY/IZM and all of the substrains of SHR/IZM could really be found by DNA fingerprinting analysis. Since we found the SHRSP-specific fingerprinting bands, this strategy also may be useful to search the genetic loci segregating with the propensity of stroke.

Recently, Lindpaintner et al\textsuperscript{15} and Kurtz et al\textsuperscript{16} reported the segregation analysis of the F\textsubscript{2} generation between SHR (or SHRSP) and normotensive rats. Although both of them used probes that can detect

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure3.png}
\caption{Comparison of DNA fingerprinting patterns of WKY/IZM and the substrains of SHR/IZM. Genomic DNA extraction and DNA fingerprinting analysis were performed as described in "Methods." Restriction enzymes and minisatellite DNA probes used were Hae III and "myo" in panel A, and Hinf I and "ins" in panel B. Lanes 1 and 2, WKY/IZM; 3 and 4, SHRSP\textsuperscript{a}; 5 and 6, SHRSP\textsuperscript{b}; 7 and 8, SHRSP\textsuperscript{a}; 9 and 10, SHRSP\textsuperscript{b}; 11 and 12, SHRSP\textsuperscript{c}; 13 and 14, SHRSP\textsuperscript{d}; 15 and 16, SHRSP\textsuperscript{e}. WKY, Wistar-Kyoto rats; SHRSP, stroke-prone spontaneously hypertensive rats; SHRSR, stroke-resistant SHR; IZM, rats from Shimane Institute of Health Science, Izumo, Japan.}
\end{figure}
an RFLP in the renin gene, their results were inconsistent with each other. This may indicate that the apparent linkage between the renin gene and the gene responsible for hypertension is weak, and thus more extensive study of the F2 generation using other RFLP probes is essential. In this context, DNA fingerprinting analysis of WKY rats and the substrains of SHR may be useful to search the RFLP alleles associated with genetic hypertension.

References


KEY WORDS • genetics • DNA fingerprinting • spontaneously hypertensive rats • Wistar-Kyoto rats
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