Isolation of a Chromosome 1 Region Affecting Blood Pressure and Vascular Disease Traits in the Stroke-Prone Rat Model

Norihiro Kato, Toru Nabika, Yi-Qiang Liang, Tomoji Mashimo, Hyoe Inomata, Takehiro Watanabe, Kazuyuki Yanai, Yukio Yamori, Yoshio Yazaki, Takehiko Sasazuki

Abstract—Recently, a genome-wide screen has shown a major quantitative trait locus (QTL) for a stroke-associated phenotype on rat chromosome 1 (RNO1) independent of QTL for blood pressure (BP) in the stroke-prone spontaneously hypertensive rat (SHRSP) of a Heidelberg colony. However, it remains to be elucidated whether these observations reflect the existence of different genes predisposing to each of the disorders. To address this issue, we performed comprehensive approaches in a Japanese colony, Izm, as follows. First, we undertook genome-wide searches in F1 (SHRSP/Izm×WKY/Izm)×SHRSP/Izm back-cross (n=63) to pursue a causal relation between hypertension and stroke. Although the strongest linkage to BP (LOD score of 3.4) was identified on RNO1, its relevance to stroke was not supported in the F1 back-cross studied. Second, we also investigated linkage to BP in F2 progeny (n=175) involving the stroke-resistant (or normal) spontaneously hypertensive rat (SHR). In F2 studies of SHR/Izm, this locus did not appear to constitute a principal BP QTL. Third, we constructed congenic animals with detailed phenotype characterization. Transfer of a chromosomal fragment between markers Klkl and D1Rat116 from WKY/Izm onto the SHRSP/Izm background lowered systolic BP by 20 to 80 mm Hg, prevented development of apparent stroke, and exaggerated impaired glucose tolerance. In conclusion, we have successfully isolated an RNO1 region affecting BP, stroke, and glucose tolerance in SHRSP/Izm-derived congenic rats. The size of the introgressed region is large, but our novel congenic strain should help delineate complex, genetic impairments underlying BP and associated vascular disease phenotypes. (Hypertension. 2003;42:1191-1197.)

Key Words: genetics ■ hypertension, genetic ■ stroke ■ rats, inbred SHR ■ vascular diseases

Hypertension does not always lead to apparent clinical symptoms, but it represents a major health burden because of its association with an increased risk of certain vascular disorders, such as stroke. Although considerable efforts have been made in studies of molecular genetics of human hypertension, the inherently complex nature has hampered progress in the elucidation of the genes involved. Studies of animal models of hypertension, especially inbred hypertensive rats, circumvent many of the problems encountered in human studies. For instance, blood pressure (BP) measurement can be performed repeatedly under more controlled conditions and may be more reproducible than in humans. Genetic heterogeneity should be small, and environmental “noise” can be reduced considerably, since animals can be raised in the same environmental conditions. Among a number of hypertensive rat strains developed to date, the stroke-prone spontaneously hypertensive rat (SHRSP), characterized by severe hypertension and the propensity for stroke, is considered to be a unique model organism.1 The genealogy of SHRSP has been described elsewhere.1 Briefly, selective breeding was originally made for stroke-proneness to separate SHRSP from the A subline of the spontaneously hypertensive rat (SHR) during the F35–36 generations (in 1973), by which time 3 distinct sublines of SHR (the A, B, and C sublines) had been maintained in Kyoto (and later transferred to Izumo), Japan. Stroke-resistant or normal SHR, SHR-SR, was thereafter derived from the B and C sublines, and a total of 7 substrains of SHR (3 SHRSP and 4 SHR-SR substrains) have been kept at Shimane Medical University, all direct descendants of the original colony. Whereas SHR(SR)/NIH was separated from our colony as early as F13 generation and established as an inbred strain at the National Institutes of Health (NIH, Bethesda, Md), 3 colonies of SHRSP—Izumo, Japan (Izm), Glasgow (Gg), and Heidelberg (Bbb)—were separated at the F35–36 generations of inbreeding in Japan. We previously performed genome-wide searches of quantitative trait loci (QTLs) for BP but not for stroke phenotypes.
in F2 progeny derived from SHRSP and its normotensive control, Wistar Kyoto rat (WKY) of our colony, Izm.2 We observed significant evidence of linkage in the broad region on rat chromosome 1 (RNO1), where QTL contributing to a stroke-associated phenotype—latency until the manifestation of stroke—was also reported in SHRSP of a Heidelberg colony, SHRSP/Bbb.3 Among 3 QTLs identified for stroke latency in SHRSP/Bbb, QTL with the highest LOD score appears to overlap with RNO1 BP QTL in SHRSP/Izm.2 As far as linkage to BP is concerned, this locus has not been consistently documented among different colonies of SHRSP.4,5 In SHRSP/Bbb, despite the significant linkage to stroke latency on RNO1, linkage to BP was not detectable until a gene-gene interaction (or epistasis) was taken into consideration between chromosome 1 and 10 regions.5 In a Glasgow colony of SHRSP, SHRSP/Gg, on the other hand, linkage to BP has not been reported on RNO1 at all.6 Here, it should be noted that WKY strains derived from respective colonies have been used as controls and that some degree of genetic heterogeneity is assumed to exist between them.

Under these circumstances, we performed comprehensive approaches as follows: (1) genome-wide searches were undertaken in F2(SHRSP/Izm×WKY/Izm)×SHRSP/Izm back-cross to confirm linkage to BP on RNO1 in our colony and to pursue a pathophysiological relation between hypertension and the propensity for stroke; (2) because BP QTLs had been reported on RNO1 in both stroke-prone and stroke-resistant SHR substrains, F2 study involving stroke-resistant SHR/Izm was conducted and detailed SHR substrain comparison was made at the genotype levels as part of investigating the disease relevance of RNO1 QTL; and (3) congenic animals were constructed from SHRSP/Izm and WKY/Izm to verify functional significance of RNO1 QTL.

Methods

Animal Procedures

We use the name of SHR for SHRSP and rats with a low incidence of spontaneous stroke (“SHRSR”) as a whole. The SHRSP, SHRSR, and WKY have been maintained at Shimane Medical University with brother-sister mating from the initiation of inbreeding.3 All SHR substrains that we used in the present study had originated from a single colony of Shimane Medical University, Izumo, Japan. Accordingly, they were designated with a suffix, Izm, to be distinguished from Heidelberg and Glasgow colonies.

Male SHRSP/Izm (A3 strain) and female WKY/Izm were mated to produce F1 rats, and female F1 rats were back-crossed to male SHRSP/Izm. A total of 63 male back-crossed rats were produced and analyzed in the present study. Separately from the SHRSP/Izm back-cross, male SHRSP/Izm (B1 strain) was crossed with female WKY/Izm, and the F1 offspring were inter-crossed to produce 175 male F2 rats. Rats were weaned at 4 weeks after birth and were placed on a normal rat chow (SP diet, Funabashi Shibayagi). Serum total cholesterol, triglycerides, and free fatty acids concentration was determined with an ELISA kit (Insulin-rat U type, Shibayagi). Serum total cholesterol, triglycerides, and free fatty acids were measured by an enzymatic method (Kyowa and Wako, Inc).

The rats were killed under pentobarbital anesthesia, and pieces of the liver were frozen at −70°C for subsequent DNA extraction. All rats were laboratory animals and were treated in compliance with institutional regulations.

Genotype Characterization

Genotyping was done with the use of microsatellite markers amplified by PCR and evaluated by electrophoresis as previously described.8 A total of 154 markers were selected for a genome screen amplified in the F2(SHRSP/Izm×WKY/Izm)×SHRSP/Izm back-cross to avoid genotyping closely linked markers of the same chromosomal region. In this selection, markers were spaced as evenly as possible to provide a marker every 10 to 20 cm. Also, a total of 17 markers were selected to investigate linkage to BP on RNO1 in the F2(SHRSP/Izm×WKY/Izm) population.

Substrain Comparison of Marker Alleles in the Chromosome 1 Region

In the region that was assumed to encompass QTLs for stroke latency1 and BP on RNO1, we examined allelic distribution patterns of microsatellite markers among 7 substrains of SHR and WKY of our colony. Substrain comparison was made by scoring a total of 17 markers, where the allele size of PCR products for each marker was determined in base pair with GeneScan and Genotyper software on ABI 377 DNA Sequencer (Applied Biosystems). Two microsatellite markers were typed for the Sa gene, as reported by Gu et al.9

Gene Expression Studies

Total RNA was extracted from the kidney of SHRSP/Izm and SHRSR/Izm at 8 to 12 weeks of age. A semi-quantitative PCR assay of the Sa gene, which had been reported to be differentially expressed in the kidney between SHR(Crl)/Crl and WKY(Crl),10 was performed by using the full-length cDNAs synthesized from reverse-transcribed mRNA of the kidney. The PCR primers were 5′-CTA-ACCACAAAATCCTAGC3′ (forward) and 5′-GGATCTCTGG-GCGAATACAC3′ (reverse), which amplified a 464-bp fragment. PCR was terminated during the exponential phase, and the products were electrophoresed on a 1.5% agarose gel. Semi-quantitative analysis was performed by densitometric scanning and normalized by GAPDH levels.

Construction of Congenic Strains

A speed congenic strategy11 was used to transfer a segment of RNO1 from WKY/Izm onto the genetic background of SHRSP/Izm (A3 strain). The breeding paradigm was as follows: male F1 rats obtained by crossing SHRSP/Izm and WKY/Izm were back-crossed...
A genome screen was undertaken in the F1(SHRSP/WKY/Izm)×SHRSP/Izm back-cross, using a total of 154 microsatellite markers. Test for the proportion showed that no markers were associated with the incidence of stroke (Table 1). These 2 markers were not linked to BP at any measurement periods, whereas markers from the BP-linked regions on 3 chromosomes—chromosomes 1, 3, and 20—were not associated with the incidence of stroke (Table 1). The mean BP (at 6 months of age) in the group that had stroke was significantly different from that in the group that did not (241.8±17.7 mm Hg versus 226.9±2.6 mm Hg, P<0.003).

Since several studies had reported BP QTLs on RNO1 in normal SHR of NIH-derived colonies, we further evaluated linkage to BP on RNO1 in the F1(SHRSP/WKY/Izm). In contrast with the LOD score plot depicted in the F1(SHRSP/Izm×WKY/Izm)×SHRSP/Izm back-cross, there was no significant evidence of linkage in the F1(SHRSP/Izm×WKY/Izm) population studied (data not shown).

Results

Linkage Analysis in F1 Back-Cross and F2(SHRSR/Izm×WKY/Izm)

In the F1(SHRSP/Izm×WKY/Izm)×SHRSP/Izm back-cross, the most significant linkage to BP was observed on RNO1, and suggestive linkage to BP was also seen on rat chromosome 3 (at 4 and 5 months of age) and chromosome 20 (at 6 months of age alone) (Table 1). Among 63 back-crossed rats, 19 rats eventually had a stroke between 11 and 18 months of age under a normal rat chow diet. Test for the proportion showed that no markers were associated with the incidence of stroke according to genome-wide significance levels, whereas 2 markers—D6Mit9 and D10Rat47—attained a nominal significance level of P<0.05 (data not shown). These 2 markers were not linked to BP at any measurement periods, whereas markers from the BP-linked regions on 3 chromosomes—chromosomes 1, 3, and 20—were not associated with the incidence of stroke (Table 1). The mean BP (at 6 months of age) in the group that had stroke was significantly different from that in the group that did not (241.8±17.7 mm Hg versus 226.9±2.6 mm Hg, P<0.003).

Suggestive (LOD ≥1.9) or significant (LOD ≥3.3) linkage was found in 3 chromosomal regions (on chromosomes 1, 3, and 20), where the degree of linkage was shown in a maximal LOD score at the most closely linked markers from the individual regions. On the other hand, marker association with stroke incidence was evaluated with the χ2 test. Among the 154 typed markers, none reached genome-wide significance levels; 2 markers—D6Mit9 and D10Rat47—showed modest departure from the null hypothesis at the level of P<0.05, when genotype frequencies were compared between rats that had eventually developed stroke (n=19) and those without stroke (n=44).

TABLE 1. Linkage to BP and Association With Stroke Incident in F1(SHRSP×WKY)×SHRSP Backcross

<table>
<thead>
<tr>
<th>Chr</th>
<th>Marker</th>
<th>Linkage to BP (Maximal LOD Score)</th>
<th>Association With Stroke Incident</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3 Months</td>
<td>4 Months</td>
</tr>
<tr>
<td>1</td>
<td>Arix</td>
<td>2.0</td>
<td>2.8</td>
</tr>
<tr>
<td>2</td>
<td>D3Mit9</td>
<td>0.5</td>
<td>2.2</td>
</tr>
<tr>
<td>20</td>
<td>D20Mgh1</td>
<td>1.0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

A genome screen was undertaken in the F1(SHRSP×WKY)×SHRSP backcross using a total of 154 microsatellite markers. Systolic BP was measured at 4 points—3, 4, 5, and 6 months of age—and the incidence of stroke was monitored until 18 months of age.

Suggestive (LOD ≥1.9) or significant (LOD ≥3.3) linkage was found in 3 chromosomal regions (on chromosomes 1, 3, and 20), where the degree of linkage was shown in a maximal LOD score at the most closely linked markers from the individual regions. On the other hand, marker association with stroke incidence was evaluated with the χ2 test. Among the 154 typed markers, none reached genome-wide significance levels; 2 markers—D6Mit9 and D10Rat47—showed modest departure from the null hypothesis at the level of P<0.05, when genotype frequencies were compared between rats that had eventually developed stroke (n=19) and those without stroke (n=44).
Substrain Comparison of RNO1 Markers and Sa Gene Expression

Next, to explore the relevance of substrain differences to BP QTL on RNO1, we determined allele distribution patterns of 17 microsatellite markers among 7 substrains of SHR and WKY of our colony (Figure 2). An interval <25 cM in size, between D1Wox18 (Klk1) and D1Mit2, was identical among the 7 SHR substrains. Allele distribution patterns differed between the C subline (CH and CL) and the others in an interval between Calca and D1Mgh21. Of note is the fact that the Sa gene was located in this interval, and its expression pattern was same as the allele distribution patterns. A semi-quantitative assay by reverse transcription PCR demonstrated that the Sa gene expression was 8 to 16 times lower in the C subline than in the A and B sublines and WKY/Izm. Also, the Sa gene expression was decreased in WKY of a Charles River Japan colony (WKY/Crj), contrary to WKY/Izm, whereas the Sa gene was abundantly expressed in SHR/Crj. These findings in the Charles River colony reflect original findings of the differential Sa gene expression reported by Iwai et al. 10

Genetic and Phenotypic Characterization of RNO1 Congenic Animals

An RNO1 congenic strain was obtained by crossing a male and a female rat in which all visible background heterozygosity had been removed after the 6th back-cross. This strain inherited a chromosomal fragment between markers Cyp2a3 and D1Wox10 derived from WKY/Izm (Figure 2), where the most proximal and the most distal markers demonstrated to be in the fragment were Klk1 and D1Rat116. Thus, the congenic strain was designated as SHRSP.WKY-(Klk1-D1Rat116)/IzmTkoyo.

Transfer of this fragment, ~70 cM in size, from WKY/Izm onto the genetic background of SHRSP/Izm significantly lowered systolic BP by 20 to 80 mm Hg, as determined through continuous and direct recording with radiotelemetry. BP differences between a congenic strain and SHRSP/Izm were more prominent in male than in female rats and increased with aging (Figures 3 and 4). When exposed to NaCl loading, all male SHRSP/Izm and 60% of female SHRSP/Izm had a stroke within 1 month. By contrast, none of SHRSP.WKY-(Klk1-D1Rat116)/IzmTkoyo rats (13 male and 9 female rats) had an apparent stroke (Figure 4).

Physiological and biochemical phenotypes were compared between SHRSP.WKY-(Klk1-D1Rat116)/IzmTkoyo and 2 progenitor strains, as shown in Table 2. The SHRSP.WKY-(Klk1-D1Rat116)/IzmTkoyo was as lean as the SHRSP/Izm in both sexes. Nevertheless, fasting plasma glucose levels were significantly higher in female SHRSP.WKY-(Klk1-D1Rat116)/IzmTkoyo than in female SHRSP/Izm (P<0.01). To further characterize this metabolic dysfunction, the
This is the first report to evaluate a relation between hyper-

tension and the propensity for stroke by combining genetic

linkage analysis with congenic experimentation in SHRSP, a
widely used model organism of severe hypertension and
stroke. A causal link between these 2 phenotypes has been
debrated,15 and hence 3 features of the present study are
notable. First, our genome-wide searches have shown BP
QTL on RNO1 as the strongest genetic determinant in
SHRSP/Izm, but its relevance to stroke has not been sup-
ported by the test of association. Second, in stroke-resistant
SHR (or SHRSR)/Izm, this locus has not been shown to
constitute a principal BP QTL. Third, subsequent develop-
ment of congenic animals has allowed the verification of this
RNO1 QTL: not only marked BP reduction but also complete
prevention of apparent stroke has been attained in SHRSR.
WKY-(Klk1-D1Rat116)/IzmTkyo with WKY/Izm-derived
alleles transferred onto the genetic background of
SHRSP/Izm.

First of all, in SHRSP/Izm, we attempted to test colocal-
ization of genetic susceptibility for hypertension and stroke in
the F1 (SHRSP/Izm × WKY/Izm) × SHRSP/Izm back-cross.
Only a modest number of rats (n = 19) eventually had a stroke,
and so the observed tendency of association (at D6Mit19 and
D10Rat47, P < 0.05) is far from conclusive. More noticeably,
deepth strong evidence of BP linkage, the lack of significant
association between RNO1 markers and the incidence of
stroke suggests that principal genetic susceptibility for stroke
after a long-term exposure to high BP would be independent of
that for hypertension on RNO1. Here, it must be noted that
study design and outcome phenotypes are different between
the previous study by Rubattu et al1 and ours. To remove BP
as a confounding factor of stroke, Rubattu et al1 elaborately
performed a cosegregation analysis in F2 progeny involving
SHRSP/Bbb and SHRSR/Bbb, since BP levels were almost
comparable between the 2 strains in a Heidelberg colony.
Also, the authors considered latency until the manifestation of
stroke as a quantitative phenotype with the rats exposed to
1% NaCl drinking water. In our colony, however, there is a
substantial difference in BP levels between SHRSP/Izm and
SHRSR/Izm,1 and a similar approach cannot be practical. To
mimic physiological situations in humans, we did not conduct

### TABLE 2. Physiological and Biochemical Parameters Measured in SHRSP, Chromosome-1 Congenic, and WKY Rats

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHRSP</td>
<td>Chr-1 Congenic</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>219.6±6.8</td>
<td>181.8±5.1*</td>
</tr>
<tr>
<td>Body weight (BW), g</td>
<td>245.4±5.9</td>
<td>226.0±2.8†</td>
</tr>
<tr>
<td>Heart weight per BW, %</td>
<td>0.407±0.012</td>
<td>0.383±0.005</td>
</tr>
<tr>
<td>Retropertioneal fat pad per BW, %</td>
<td>0.761±0.055</td>
<td>0.780±0.046</td>
</tr>
<tr>
<td>Peri-testis fat pad per BW, %</td>
<td>1.108±0.063</td>
<td>1.123±0.038</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>1.42±0.05</td>
<td>1.28±0.10</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.65±0.12</td>
<td>0.48±0.09</td>
</tr>
<tr>
<td>Free fatty acid, mmol/L</td>
<td>0.61±0.08</td>
<td>0.43±0.03</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>7.88±0.24</td>
<td>8.28±0.31</td>
</tr>
</tbody>
</table>

Values are mean±SE, calculated by using 5 rats in each group.
Systolic BP was measured by tail-cuff method at 13 weeks of age. After a 16-hour fast, the rats underwent IVGTT, when blood samples were taken from the penial dorsal vein (males) or the cervical vein (females) for the measurement of plasma glucose and lipid levels. The rats were then euthanized under pentobarbital anesthesia, and body and organ weight was measured. The percentage of organ weight against body weight is shown.

*P < 0.01, †P < 0.05; the values were significantly different between SHRSP and chromosome-1 congenic rats by 1-way ANOVA.
dietary intervention of salt loading in the present study. Nevertheless, a mean of systolic BP in the entire F1 backcrossed population exceeded 230 mm Hg at 6 months of age and appeared to produce sufficient hemodynamic stress in the cerebral arteries. Thus, although they do not refute the existence of stroke-associated gene(s) independent of BP on RNO1, our data provide insights supplementary to the previous study3 regarding the complex nature of stroke susceptibility even in the animal model.

From the genealogical perspective, SHRSP is assumed to possess “additional” genetic factors to promote the propensity for stroke in comparison with SHRSR. Two lines of evidence, though circumstantial, are in favor of the notion that RNO1 QTL is one of such genetic factors. First, no significant linkage to BP was found on RNO1 in the F2 (SHRSR/Izm×WKY/Izm) population, implying that genetic variation(s) in the relevant region may be specific for SHRSP/Izm. Second, certain chromosomal fragments were proven to differ between SHRSP/Izm and SHRSR/Izm in allele distribution patterns (Figure 2). In the introgressed regions of the congenic strain, some chromosomal fragments that differ between SHRSP/Izm and SHRSR/Izm also differ from the normotensive WKY/Izm (eg, Igf2), whereas some chromosomal fragments differ strictly between the normotensive and hypertensive strains (eg, D1Wox29 and Pace). Additional 6-microsatellite markers have been typed in the interval between D1Wox29 and Pace, ~10 cM in size, and have verified that this region is actually conserved (data not shown). In this line, contrary to the original report,10 the Sa gene expression turned out to be heterogeneous among substrains of SHRSR and WKY. Differential expression is solely dependent on the combination of substrains, and for that reason, the Sa gene itself does not appear to be a principal genetic determinant of BP in SHR, as previously argued elsewhere.16 Thus, certain molecular variants responsible for differential gene expression may have been incidentally coinherited with true nearby causative gene alleles. Genetic heterogeneity further reflects the complex nature of diseases such as hypertension and stroke, even in animal models, which should be less complex than human beings. Since the presence of substantial heterogeneity is even found in substrains of SHRSP, SHRSR, and WKY derived from the same progenitors, it is possible that a mixed background effect, for example, coexistence of different susceptibility alleles in the QTL regions, has masked a potential linkage to stroke on RNO1 in our F1 back-crossed population. To resolve these issues, we will study subcongenic lines by using the conserved/different regions between the various substrains.

The hypothesis that RNO1 QTL contains a gene or genes that influence BP is supported by the isolation of this QTL in congenic animals. Introgressing a region of RNO1, ~70 cM in size, from WKY/Izm into SHRSP/Izm resulted in a significant decrease in BP and complete prevention of apparent stroke (Figure 4). Significant changes were also observed for several other metabolic phenotypes in congenic rats. Above all, a WKY-derived “diabetic” gene allele was trapped in the relevant chromosomal fragment, which accounted for most of the phenotypic differences in IVGTT patterns between the 2 progenitor strains. In general, insulin resistance is known to be characteristic of SHR,17,18 but some confusion seems to prevail about this issue. In SHRSP/Izm, obesity and impaired glucose tolerance are not prominent, but elevated BP, cardiac hypertrophy, low total cholesterol, and high triglyceride levels are exaggerated as compared with WKY/Izm (Table 2). With reference to a so-called insulin resistance syndrome in humans, it is tempting to speculate that some common genetic defects underlie the clustering of several metabolic traits in SHR, but this should be carefully evalu-
ated. Although the results for the glucose tolerance tests presented appear unexpected and contrary to what has been occasionally seen with genetically hypertensive rat strains, some investigators have already reported observations similar to ours, that is, more exaggerated diabetic IVGTT patterns in WKY than in SHR.7,19 Several confounding factors such as the manner (or the usage) of anesthesia have been shown to influence the glucose tolerance20 and need to be further evaluated. In the case of our congenic rats derived from SHRSR/Izm and WKY/Izm, because hypertension and impaired glucose tolerance are attributed to the alternate progenitor alleles, it is plausible that different genes rather than identical genes with pleiotropic effects underlie each of the metabolic traits. Thus far, several QTLs have been reported for metabolic traits and insulin resistance-associated phenotypes in SHR(SR) of NIH-derived colonies,21,22 whereas a few of them have not been replicated in SHRSR/Izm.23 The discrepancies may result from potential interstrain diversity that is assumed to exist between SHRSR and SHRSR and/or between SHR(SR) of different colonies. Also, the discrepancies may be partly due to differences in the nutritional state of the animals at the time of the study and in the technique used for the insulin suppression test, for example, intravenous versus intraperitoneal glucose challenge.24

**Perspectives**

An RNO1 region has been proven to harbor gene(s) predisposing to BP, the incidence of stroke, and some metabolic traits in SHR(SR) of NIH-derived colonies,21,22 whereas a few of them have not been replicated in SHRSR/Izm.23 The discrepancies may result from potential interstrain diversity that is assumed to exist between SHRSR and SHRSR and/or between SHR(SR) of different colonies. Also, the discrepancies may be partly due to differences in the nutritional state of the animals at the time of the study and in the technique used for the insulin suppression test, for example, intravenous versus intraperitoneal glucose challenge.24

**Acknowledgments**

This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (No. 13204095), Novartis Foundation for the Promotion of Science, and Takeda Science Foundation.

**References**

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Hypertension. 2003;42:1191-1197; originally published online November 17, 2003;
doi: 10.1161/01.HYP.0000103161.27190.67

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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