Identification of Quantitative Trait Loci for Serum Cholesterol Levels in Stroke-Prone Spontaneously Hypertensive Rats

Norihiro Kato, Tomoko Tamada, Toru Nabika, Keita Ueno, Takanari Gotoda, Chiho Matsumoto, Tomoji Mashimo, Makoto Sawamura, Katsumi Ikeda, Yasuo Nara, Yukio Yamori

Abstract—The stroke-prone spontaneously hypertensive rat (SHRSP) has been reported to show significantly lower levels of serum total cholesterol than the normotensive control strain Wistar-Kyoto rat (WKY). Because selective inbreeding was conducted for stroke proneness, this concomitantly inherited characteristic of SHRSP may play some pathophysiological role in stroke. We evaluated the genetic determinants of the cholesterol trait by estimating heritability and subsequently undertaking a genome-wide screen with 161 genetic markers in \( F_2 \) progeny involving SHRSP and WKY (104 male and 106 female rats). Three quantitative trait loci (QTLs) were detected on rat chromosomes 5, 7, and 15. Markers from the linked region on chromosome 15 indicated significant evidence of linkage with a maximal log of the odds (LOD) score of 7.7, whereas those on chromosomes 5 and 7 cosegregated with the trait in a sex-specific manner (the QTL close to genetic marker \( D5 Mit5 \) reached an LOD score of 7.3 in males, and that close to \( D7 Mit10 \) reached an LOD score of 3.2 in females). The male-specific QTL on chromosome 5 appeared to overlap with previously reported QTLs for stroke-associated phenotypes, but an identical gene (or genes) appeared unlikely to control these and the cholesterol traits simultaneously. In the present study, serum cholesterol levels were shown to be highly genetically determined in SHRSP (the heritability estimates are 76% in males and 83% in females), and 3 QTLs with substantial effects were identified. Further work, however, is required to clarify whether the cholesterol trait is related to the etiology of stroke or has been retained by chance through the inbreeding process in SHRSP. (Arterioscler Thromb Vasc Biol. 2000;20:223-229.)

Key Words: stroke ■ susceptibility ■ lipids ■ linkage ■ sex specificity

Stroke has become a major public health issue because it is a leading cause of death in industrialized societies and because it also often leaves a formidable burden of physical disability on stroke survivors and their community. Therefore, stroke prevention would be more desirable than treating survivors after the incident. Several risk factors for stroke have been identified, including age, hypertension, diabetes, and cigarette smoking. For example, it has been shown that the incidence of stroke increases in parallel with the degree of blood pressure elevation and that antihypertensive treatment is useful for prevention. However, the pathophysiological relevance of dyslipidemia, especially hypercholesterolemia, to stroke remains controversial despite considerable efforts to determine whether cholesterol-lowering interventions reduce stroke risk. Some epidemiological studies have shown that the incidence of thromboembolic stroke increases with elevated serum cholesterol levels, but a J-shaped association is also seen because of an increased risk for hemorrhagic stroke at low cholesterol levels. On the other hand, large, prospective cohort studies do not necessarily support the association between risk for overall stroke and cholesterol levels. With respect to these arguments for humans, of great interest is the fact that low levels of serum total cholesterol are characteristic of the stroke-prone spontaneously hypertensive rat (SHRSP), a principal animal model of stroke.

The SHRSP was originally established from the A substrain of the SHR in Kyoto, Japan, by selective inbreeding for stroke proneness, whereas 2 other substrains of SHR, the B and C substrains, are stroke resistant (SR). Through extensive searches of differences in lipid metabolism between these substrains of SHR and normotensive control, Wistar-Kyoto rats (WKY), several lines of evidence have emerged for the speculation that some impaired processes of lipid metabolism may play a role in the etiology or exacerbation of stroke in SHRSP. It is known that SHRSP (or its A substrain) shows significantly low serum total cholesterol levels and high serum triglyceride levels compared with WKY when both are maintained on a normal rat chow.
diet.\textsuperscript{13,14} When fed a high-fat, high-cholesterol diet, the SHRSP not only demonstrates a highly responsive increase in serum cholesterol levels but also develops acute fat deposition in small arteries.\textsuperscript{14,15,20} Moreover, supplementation with dietary cholesterol for a 2-month period has been shown to decrease the incidence of cerebral lesions in SHRSP.\textsuperscript{13,19} These findings motivated us to investigate the genetic bases of impaired lipid metabolism in SHRSP.

In the present study, we evaluated the heritability of serum total cholesterol levels between SHRSP and WKY and subsequently performed a genome-wide screen in the F\textsubscript{2} population. As part of our study, microsatellite markers were developed for 2 candidate genes for investigation of serum cholesterol levels, and the genes were localized by linkage mapping. These are the rat 3-hydroxy-3-methylglutaryl coenzyme A reductase (Hmgcr) gene and the mevalonate pyrophosphate decarboxylase (Mvpd) gene.

### Methods

#### Animal Procedures

The SHRSP (A3 substrain) and WKY rat colonies had been maintained at our institute with brother-sister mating from the initiation of inbreeding and as such, are genetically homogeneous.\textsuperscript{16} For the estimation of heritability, 2 reciprocal F\textsubscript{1} crosses were produced: 12 rats (6 males and 6 females) were produced from male SHRSP and female WKY, and 19 rats (12 males and 7 females) were produced from female SHRSP and male WKY. To produce the F\textsubscript{2} population, 4 possible combinations of F\textsubscript{1} rats were mated, resulting in 4 distinct F\textsubscript{2} cohorts as shown in Figure 1. A total of 210 F\textsubscript{2} progeny (104 males and 106 females) were used in a genome-wide screen. Rats were weaned at 4 weeks after birth and were placed on an SP diet (Funabashi Farm) containing 5% fat, 0.4% sodium, and 0.75% potassium and were given free access to tap water. All rats were laboratory animals and treated humanely in compliance with local regulations.

#### Genotype Characterization

Microsatellite markers were either purchased from Research Genetics Inc or synthesized according to primer sequences previously published.\textsuperscript{21} To find informative markers between the 2 parental strains, we initially screened 600 markers, one third of which proved to be polymorphic. Markers used for the genome scan were chosen on the basis of their location in published genetic maps\textsuperscript{22,23} to avoid genotyping closely linked markers in the same chromosome region. Polymerase chain reaction (PCR) was run on a PHC-3 thermal cycler (Techne) with a solution of the following composition: 45 mmol/L Tris-HCl (pH 8.8), 50 mmol/L KCl, 1.5 mmol/L or 2.0 mmol/L MgCl\textsubscript{2}, 11 mmol/L (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, 6.7 mmol/L \(\beta\)-mercaptoethanol, 4.5 mmol/L dNTPs, 33 ng of each primer, 50 ng of genomic DNA, and 0.4 U of Ampli Taq DNA polymerase (Perkin Elmer) in a reaction volume of 15 \(\mu\)L. Initial denaturation occurred at 96°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C or 60°C for 1 minute, and extension at 72°C for 20 seconds; final extension was at 72°C for 7 minutes. The concentration of magnesium and the annealing temperature were dependent on the primer set. PCR products were electrophoresed in 7.5% (or 10%) polyacrylamide gels on the mini vertical-gel system (Wako Inc) followed by ethidium bromide staining. When necessary, markers with product size differences <0.8 bp were analyzed with \(^{3}P\)-labeled primers in which case, the product was separated in 6% polyacrylamide/7 mol/L urea gels on the model S2 sequencing apparatus (Life Technologies Inc).

### Developing Polymorphic Markers for the Hmgcr and Mvpd Genes and 3 Other Genes on Rat Chromosome 15

Genetic markers were developed for 2 candidate genes in cholesterol biosynthesis, Hmgcr, which is known to be a rate-limiting step, and Mvpd, whose enzyme activity was shown to be reduced in the liver soluble fractions of SHRSP.\textsuperscript{24} In addition, microsatellite markers for rat cardiac \(\alpha\)-myosin heavy chain (Myhca), clusterin (Clu), and endothelin receptor type B (Ednr\textsuperscript{b}) were developed in the chromosome region with significant linkage to the trait, which corresponded to a conserved synteny between rat chromosome 15 and mouse chromosome 14. Although rat homologues of Clu (also known as Trpm-2\textsuperscript{25} and Ednr\textsuperscript{b}\textsuperscript{26} had been previously mapped to chromosome 15, we developed new polymorphic markers for these loci to complete our consensus map of rat chromosome 15 and to facilitate comparison of maps between rats and other species. PCR primer sets were designed to flank repetitive motifs either in the published sequences (Clu)\textsuperscript{27} or in newly determined intron sequences (Hmgcr, Mvpd, Myhca, and Ednr\textsuperscript{b}). Rat primer sets used in the present study are listed in Table 1. Map locations for the 3 markers on rat chromosome 15 (Myhca, Clu, and Ednr\textsuperscript{b}) are shown in Figure 2, and those for the other 2 (Hmgcr and Mvpd) are discussed in Results.

#### Substrain Comparison of Marker Alleles in the Linked Region of Rat Chromosome 15

In the region encompassing the cholesterol quantitative trait locus (QTL) on rat chromosome 15, we examined allele distribution patterns of microsatellite markers among WKY and substrains of SHR that included 3 SHRSP substrains (A1-sh, A3, and A4) and 4 SHRSR substrains (B1, B2, CH, and CL).\textsuperscript{16,28} Markers other than those characterized above were taken from the genetic maps of the rat genome, release 4 (http://www.genome.wi.mit.edu/rat/public/). Allele sizes of PCR products for each marker were determined with GeneScan and Genotyper software on an ABI 377 DNA sequencer (Applied Biosystems) according to the manufacturer’s protocol. A genetic linkage map of the selected region involving all markers that were polymorphic between the A3 substrain of SHRSP and WKY was constructed by genotyping 110 male F\textsubscript{2} rats, which were also used in the genome scan, and 6 additional rats (Figure 2).
Haldane’s mapping function. Multipoint linkage analysis was performed by using the MAPMAKER/QTL 1.1 program. The map constructed by using the MAPMAKER/EXP 3.0 program with an error threshold recommended by Lander and Kruglyak was used to estimate the map location of the Hmgcr locus. Allele sizes of all available markers that exhibited polymorphism between SHRSP (A3 substrain) and WKY substrains were derived from a single pair of progenitors, in which the strongest evidence of linkage was found in the chromosome 15 region spanning 252/254 centimorgans (cM) of the genome in sex-averaged genetic length, with an average spacing of 12.3 cM. The strongest evidence of linkage was found in the region on rat chromosome 15 (P<10^-6 for the entire progeny), whereas 2 other regions on rat chromosomes 5 and 7 also showed significant evidence of linkage in either of the sex subgroups. SHRSP-derived alleles revealed a dominant mode of cholesterol-lowering effects in male rats (P<10^-4) alone for the chromosome 5 QTL and in female rats (P=0.001) alone for the chromosome 7 QTL (Table 4). LOD score plots by multipoint linkage analysis are shown in Figure 2 (chromosome 15) and Figure 3 (chromosomes 5 and 7). Percentages of variance (R^2) attributed to individual QTLs were 28% for the chromosome 5 QTL and 16% for the chromosome 15 QTL in male rats and 14% for the chromosome 7 QTL and 19% for the chromosome 15 QTL in female rats; these values were calculated at the most closely linked markers from each chromosome region. When considered simultaneously, QTLs thus detected accounted for 40% and 29% of the phenotypic variance in male and female rats, respectively. There was no significant epistatic interaction between the cholesterol QTLs in either sex.

To pursue the relevance of the cholesterol QTLs to stroke, we performed a strain comparison of marker alleles in the region spanning D15Mgh7 to Ednrb on rat chromosome 15, in which the strongest evidence of linkage was found in the whole group of F2 animals (Figure 2). Because all SHR substrains were derived from a single pair of progenitors, in theory as many as 4 alleles can be observed for any marker locus. Allele sizes of all available markers that exhibited polymorphism between SHRSP (A3 substrain) and WKY were determined in base pairs for 7 SHR substrains and WKY (see Methods) and were then categorized into 3 groups: the SHRSP-type (A3 substrain) allele, the WKY-type allele, and.

### Table 1. List of PCR Primer Sets Developed in the Present Study

<table>
<thead>
<tr>
<th>Locus</th>
<th>GenBank Accession No.</th>
<th>Sense Primer</th>
<th>Antisense Primer</th>
<th>PCR Product Size, bp in SHRSP/WKY</th>
<th>Position of Polymorphism</th>
<th>Repeat Motif</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hmgcr</td>
<td>AF074961</td>
<td>5'-GGCGACGCTGCTGTGAGAAG-3'</td>
<td>5'-GGAGGCAACTGGAACAACT-3'</td>
<td>149/146</td>
<td>3'-UTR</td>
<td>GGC</td>
</tr>
<tr>
<td>Mvpd</td>
<td>AF036709</td>
<td>5'-GACCGATCTGCGTAA-3'</td>
<td>5'-GGCTGGGGAGAAGTTGTTG-3'</td>
<td>515/532</td>
<td>Intron 1</td>
<td>17-bp Repeat</td>
</tr>
<tr>
<td>Myhca</td>
<td>AF074962</td>
<td>5'-AGGAGGTCTGCTGAC-3'</td>
<td>5'-GCACAAACCCCAACT-3'</td>
<td>180/192</td>
<td>Intron 1</td>
<td>Poly(A)</td>
</tr>
<tr>
<td>Clu</td>
<td>U02891</td>
<td>5'-TCTGGGCAGGTTAGAGATG-3'</td>
<td>5'-GAACGGTATTGTTGCTA-3'</td>
<td>87/107</td>
<td>Intron 1</td>
<td>CA</td>
</tr>
<tr>
<td>Ednrb</td>
<td>AF074963</td>
<td>5'-GCTCTGACTCCCAATGAT-3'</td>
<td>5'-GATGCCCCACTGCAACC-3'</td>
<td>252/254</td>
<td>Intron 5</td>
<td>CA</td>
</tr>
</tbody>
</table>

Hmgcr indicates 3-hydroxy-3-methylglutaryl coenzyme A reductase rat gene; Mvpd, mevalonate pyrophosphate decarboxylase gene; Myhca, myosin heavy-chain, cardiac α- gene; Clu, clusterin gene; Ednrb, endothelin-B receptor gene; and 3'-UTR, 3'-untranslated region. The exact number of introns for Mvpd remains unknown because it was randomly chosen from the cDNA sequences.

### Results

In the first step of the present study, we evaluated heritable aspects of serum total cholesterol levels in segregating crosses between SHRSP and WKY. Tables 2 and 3 display the summary of trait values in each generation. Because phenotype distributions were significantly different between males and females in progenitor (P=0.001 for SHRSP and P=0.024 for WKY) and F2 (P<0.0001) generations, the broad sense heritability was calculated separately for each sex: 76% in male rats and 83% in female rats. However, the relatively small number of animals used in progenitor and F2 generations may have influenced the accuracy of the estimates to some extent. Based on the data in F2 and F3 crosses, there was no evidence that loci on the X or Y chromosome accounted for the observed variation in cholesterol levels between sexes.

Subsequently, we conducted a genome-wide search for QTLs controlling serum cholesterol levels in the F2 population. A total of 161 microsatellite markers distributed across 20 rat autosomes and the rat X chromosome were characterized in the whole F2 progeny (104 males and 106 females). The typed markers spanned ~1720 centimorgans (cM) of the genome in sex-averaged genetic length, with an average spacing of 12.3 cM. The strongest evidence of linkage was found in the region on rat chromosome 15 (P<10^-6 for the entire progeny), whereas 2 other regions on rat chromosomes 5 and 7 also showed significant evidence of linkage in either of the sex subgroups. SHRSP-derived alleles revealed a dominant mode of cholesterol-lowering effects in male rats (P<10^-4) alone for the chromosome 5 QTL and in female rats (P=0.001) alone for the chromosome 7 QTL (Table 4). LOD score plots by multipoint linkage analysis are shown in Figure 2 (chromosome 15) and Figure 3 (chromosomes 5 and 7). Percentages of variance (R^2) attributed to individual QTLs were 28% for the chromosome 5 QTL and 16% for the chromosome 15 QTL in male rats and 14% for the chromosome 7 QTL and 19% for the chromosome 15 QTL in female rats; these values were calculated at the most closely linked markers from each chromosome region. When considered simultaneously, QTLs thus detected accounted for 40% and 29% of the phenotypic variance in male and female rats, respectively. There was no significant epistatic interaction between the cholesterol QTLs in either sex.

To pursue the relevance of the cholesterol QTLs to stroke, we performed a strain comparison of marker alleles in the region spanning D15Mgh7 to Ednrb on rat chromosome 15, in which the strongest evidence of linkage was found in the whole group of F2 animals (Figure 2). Because all SHR substrains were derived from a single pair of progenitors, in theory as many as 4 alleles can be observed for any marker locus. Allele sizes of all available markers that exhibited polymorphism between SHRSP (A3 substrain) and WKY were determined in base pairs for 7 SHR substrains and WKY (see Methods) and were then categorized into 3 groups: the SHRSP-type (A3 substrain) allele, the WKY-type allele, and.
alleles different from both types. On the basis of the constructed genetic linkage map and allele distribution pattern, the B2 strain of SHRSP and SHRSR substrains shared a large chromosome fragment identical by descent in the region of chromosome 19 (presumably in the interval between D19Mit7 and D19Wox4 of the above linkage map), and it could be a potential candidate gene as well. We were unable to exactly assign this locus in our linkage map, as the CA-repeat sequences, which we identified in the last intron of the Lcat gene (GenBank accession No. AF 074964), turned out not to be polymorphic between the 2 parental strains. There was no significant cosegregation of the cholesterol trait with any of the 5 markers typed on rat chromosome 19, suggesting that Lcat was unlikely to be linked to the trait in the F2 population studied.

**Discussion**

This is the first report of a comprehensive evaluation of QTLs influencing serum total cholesterol levels between SHRSP, a principal animal model of stroke, and WKY and provides new insights into the genetics of SHRSP. In the present study, we have shown that serum total cholesterol levels are a highly heritable trait and are possibly regulated in a sex-specific manner in part. Sex differences in trait means and distribution between male and female F2 rats can be explained by several hypotheses, such as sex chromosome effects, a sex hormone difference, and gametic imprinting. According to our results, sex chromosome effects do not appear to play a major role in the observed sex difference. A genome-wide search successfully identified 3 chromosome regions linked to the trait, 1 each on rat chromosomes 5, 7, and 15, among which sex specificity was implied for the first 2 QTLs.

### Table 2. Serum Total Cholesterol Levels (mg/dL) in Parental Strains and F1 Crosses

<table>
<thead>
<tr>
<th>Strain</th>
<th>Males</th>
<th>Females</th>
<th>F1 (W♂ × SP♀)</th>
<th>F1 (SP♂ × W♀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHRSP</td>
<td>50.22±2.02 (5)</td>
<td>55.72±1.38 (5)</td>
<td>65.57±4.26 (12)</td>
<td>69.72±7.03 (6)</td>
</tr>
<tr>
<td>WKY</td>
<td>72.54±3.45 (5)</td>
<td>80.60±5.48 (5)</td>
<td>65.60±3.70 (7)</td>
<td>66.72±6.86 (6)</td>
</tr>
</tbody>
</table>

Sex difference P = 0.001, P = 0.024, NS, NS

Values are mean±SD, with the number of animals in each group expressed in parentheses. In each sex, the trait values were significantly different between parental SHRSP and WKY strains by 1-way ANOVA (P < 0.0001) but were not significantly different between reciprocal F1 crosses, i.e., between F1 (W♂ × SP♀) and F1 (SP♂ × W♀).
Strong evidence of linkage was found on rat chromosome 5 in male rats alone ($P<10^{-5}$). Of note is the fact that this region on rat chromosome 5 apparently overlaps with a QTL for infarct volumes after middle cerebral artery occlusion (so-called ischemic vulnerability) reported by Jeffs et al.\(^3\) However, ischemic vulnerability has been shown to be inherited as a dominant trait in both sexes,\(^3\) and the QTL in question did not indicate any sex specificity in the F\(_2\) progeny. Therefore, it is unlikely that the identical susceptibility gene (or genes) would affect these 2 distinct phenotypes in SHRSP. Similar arguments can be proposed for the stroke-latency QTL, although only the linkage data analyzed for both sexes together were presented in the relevant report.\(^3\) For the region on rat chromosome 7, linkage was found in female rats alone ($P=0.001$). It is of interest that a cluster of QTLs for lipoprotein metabolism has been reported on mouse chromosome 15, which appears to be homologous to the region harboring the rat chromosome 7 QTL in our study.

Gametic imprinting could be a potential mechanism for quantitative traits to be differentially expressed by sex.\(^4\) Although the F\(_2\) population used in the present study was produced by reciprocal crossbreeding for the investigation of sex chromosome effects, it does not provide sufficient statistical power in analyzing the data cohort by cohort, because each F\(_2\) cohort comprised a relatively small, unequal number of rats. Further work is thus required to explore the imprinting hypothesis by increasing the number of animals in each cohort.

Two genome screens were previously undertaken for lipid traits in experimental crosses derived from SHR (a substrain of SHRSR in our nomenclature): 1 is between SHR and Brown Norway (BN) rats,\(^3\) and the other is between SHR and spontaneously diabetic rats (BB/OK).\(^4\) In both cases, SHR showed lower cholesterol levels than did the control strains BN and BB/OK. The former study reported a QTL for the HDL2 cholesterol fraction on rat chromosome 19 but none for total cholesterol, whereas the latter indicated a QTL for total cholesterol on rat chromosome 18. Neither of the corresponding chromosome regions was linked to serum total cholesterol levels of SHRSP and WKY in our study.

Different from humans, rodents have plasma cholesterol mainly in the HDL fraction rather than the LDL and VLDL fractions.\(^4\) It is therefore possible that defects in the SHRSP occur not in the processes of cholesterol synthesis but rather in reverse cholesterol transport from tissues to plasma, which governs maturation of HDL particles. This may be supported by observations that the SHRSP has the most prominent cholesterol reduction in the apo E–rich HDL fraction\(^1\) and that cholesterol levels in both LDL and VLDL fractions can be markedly increased in SHRSP when fed a high-fat, high-cholesterol diet.\(^1\) Because reverse cholesterol transport is mediated by Lcat,\(^4\) we investigated the Lcat gene locus as well as the 2 candidate genes (Hmgcr and Mvd) involved in cholesterol biosynthesis, in all cases resulting in the absence of a linkage with total cholesterol levels. Caution is needed in extrapolating findings from rats to humans, because lipoprotein metabolism and the composition of serum cholesterol are known to be considerably different between the 2 species.\(^4\)

Now that the cholesterol trait in SHRSP has been proved to be substantially determined by genetic factors, one may raise the question of whether this trait is associated with stroke proneness, a unique characteristic of SHRSP, at all. Only circumstantial evidence has so far been obtained regarding this speculation.\(^1,13-15,18,19\) It is, however, not practicable to examine the speculation directly in the F\(_2\) population involv-

### Table 3. Serum Total Cholesterol Levels (mg/dL) in Each Cohort of F\(_2\) Rats

<table>
<thead>
<tr>
<th>Chr</th>
<th>Total Panel</th>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Cohort 3</th>
<th>Cohort 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>3</td>
<td>62.04 ± 0.29 (104)</td>
<td>73.61 ± 0.11 (106)</td>
<td>60.67 ± 0.79 (12)</td>
<td>71.57 ± 0.11 (31)</td>
<td>60.63 ± 0.11 (23)</td>
</tr>
<tr>
<td>7</td>
<td>56.04 ± 0.29 (104)</td>
<td>73.61 ± 0.11 (106)</td>
<td>60.67 ± 0.79 (12)</td>
<td>71.57 ± 0.11 (31)</td>
<td>60.63 ± 0.11 (23)</td>
</tr>
</tbody>
</table>

Categorization into individual cohorts is schematically described in Figure 1. No statistically significant differences (at the level of $P<0.05$) were observed between any 2 cohorts of each sex by the unpaired t test. However, the comparison between female cohorts 1 and 4 revealed a borderline difference ($P=0.089$).

### Table 4. Estimate of Trait Means (Serum Cholesterol Levels) According to Genotypes for Individual QTLs

<table>
<thead>
<tr>
<th>Chr</th>
<th>Males (n=104)</th>
<th>Females (n=106)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S/S</td>
<td>56.4 ± 8.4</td>
<td>65.0 ± 13.1</td>
</tr>
<tr>
<td>S/W</td>
<td>63.8 ± 9.1</td>
<td>73.3 ± 10.0</td>
</tr>
<tr>
<td>W/W</td>
<td>64.5 ± 8.3</td>
<td>79.3 ± 11.5</td>
</tr>
<tr>
<td>P</td>
<td>0.0008</td>
<td>0.0001</td>
</tr>
<tr>
<td>S/S</td>
<td>58.4 ± 6.0</td>
<td>73.4 ± 9.8</td>
</tr>
<tr>
<td>S/W</td>
<td>59.8 ± 8.8</td>
<td>73.9 ± 14.7</td>
</tr>
<tr>
<td>W/W</td>
<td>70.8 ± 7.5</td>
<td>73.3 ± 8.7</td>
</tr>
<tr>
<td>P</td>
<td>&lt;10^-4</td>
<td>0.97</td>
</tr>
<tr>
<td>S/S</td>
<td>61.3 ± 9.6</td>
<td>69.8 ± 11.9</td>
</tr>
<tr>
<td>S/W</td>
<td>62.4 ± 9.5</td>
<td>71.9 ± 9.4</td>
</tr>
<tr>
<td>W/W</td>
<td>62.2 ± 8.6</td>
<td>79.8 ± 13.1</td>
</tr>
<tr>
<td>P</td>
<td>0.88</td>
<td>0.001</td>
</tr>
</tbody>
</table>

S indicates SHRSR allele; W, WKY allele. Serum total cholesterol levels are expressed in mg/dL (mean ± SD). The means were obtained by transformation to the original trait scale. The number of rats in each genotype class is shown in parentheses. In all instances, the nominal $P$ values are from the ANOVA test with the marker from the region showing the strongest evidence of linkage, ie, D15Mit2 on chromosome (chr) 5, D5Mit5 on chr 5, and D7Mit10 on chr 7.
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ing SHRSP and WKY because only a small proportion of F2 animals (8.4%) eventually suffered from stroke due to a milder blood pressure elevation than did the parental SHRSP.45 As an alternative approach, we took notice of strain differences in marker alleles from the linked region on rat chromosome 15. So far as the genealogy of SHR is concerned, substrains of SHRSP (A1-sb, A3, and A4) were separated from substrains of SHRSR (B1, B2, CH, and CL) at the F1 generation, and thereafter selective inbreeding was conducted for stroke proneness.16 Consequently, a striking contrast in stroke proneness was observed between SHRSP and SHRSR. The mutations promoting stroke should therefore clearly differentiate SHRSP from SHRSR substrains. In the investigated region, we found a small interval (<7 cM) that satisfied the above criterion (Figure 2). These findings can be further applied to the following strategies: (1) development of a congenic line by transferring the WKY gap fragment to the genetic background of SHRSP to see whether the small interval harbors susceptibility for both traits and (2) linkage analysis in an F2 population involving SHRSP and any of the SHRSR substrains that would be useful to explore the relationship between the region of interest and stroke. All F2 rats in such a cross would be expected to develop severe hypertension from an early age and hence, would be more suitable for monitoring the incidence of stroke than are F2 progenies with WKY, as Rubattu et al37 actually performed in an F2 cross bred from SHRSP and SHR from a German colony. In the molecular genetics of stroke, 2 previous studies have undertaken genome-wide linkage analysis with “stroke-associated” phenotypes in F2 populations derived from SHRSP and have detected QTLs for each of the phenotypic traits tested.36,37 It should be stressed that these traits do not exclusively represent stroke proneness in SHRSP. Given the likely polygenic nature of stroke proneness, there may exist a number of susceptibility loci for different physiological pathways effecting stroke. The choice of an appropriate phenotype, the so-called intermediate phenotype, would be crucial to dissect complex genetic determinants. However, the paucity of feasibility and reproducibly measurable phenotypes that can reflect the degree of stroke proneness has hampered studies of the molecular genetics of stroke in SHRSP. The cholesterol trait, as examined in our study, or other impairments of lipoprotein metabolism, eg, an exaggerated increase in serum cholesterol levels after ingestion of a high-fat diet, may constitute such a panel of intermediate phenotypes. Although the possibility should be borne in mind that the cholesterol trait is concomitantly inherited in SHRSP simply by chance, further studies are warranted toward the identification of relevant susceptibility genes to clarify this ambiguity.

References


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doi: 10.1161/01.ATV.20.1.223
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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