Reciprocal Consomic Strains to Evaluate Y Chromosome Effects


Abstract—We have previously demonstrated that the SHRSP Y chromosome contains a locus that contributes to hypertension in SHRSP/WKY F2 hybrids and that SHRSP exhibit an increased vulnerability to focal cerebral ischemia after permanent middle cerebral artery occlusion (MCAO). This increased vulnerability is inherited as a codominant trait, and a putative role for the Y chromosome has been suggested in F1 hybrids. The objective of this study was to investigate further the role of Y chromosome in blood pressure (BP) regulation and in the vulnerability to cerebral ischemia. We have constructed consomic strains by selectively replacing the Y chromosome from WKY rats with that of SHRSP, and vice versa, by using a marker-assisted breeding strategy. Permanent MCAO was carried out by electrocoagulation, with infarct volume expressed as a percentage of the ipsilateral hemisphere. Systolic blood pressure was measured by radiotelemetry during a baseline period of 5 weeks followed by a 3-week period of salt loading. We observed that the transfer of the Y chromosome from WKY onto SHRSP background significantly reduced systolic BP in consomic strains, SP.WKYGlaYw (n = 6) versus SHRSP (n = 6) (209.2 ± 10.4 mm Hg versus 241.7 ± 7.7 mm Hg, F = 5.88, P = 0.038) during the salt-loading period. In the reciprocal consomic strain, WKY.SPGlaYs (n = 5), systolic BP was increased compared with WKY parental strain (n = 6) (147.6 ± 2.4 mm Hg versus 132.6 ± 5.1 mm Hg, F = 6.11, P = 0.035) during baseline. Infarct volumes in consomic strains were not significantly different from their respective parental strain: WKY.SPGlaYs (n = 7) versus WKY (n = 7), 22.8 ± 3.7% versus 22.2 ± 8.0%, 95% CI = −12.7, 4.2, P = 0.3; SP.WKYGlaYw (n = 7) versus SHRSP (n = 6), 37.7 ± 4.4% versus 33.6 ± 7.6%, 95% CI = −20.3, 12.1, P = 0.5. We conclude that the SHRSP Y chromosome harbors a locus contributing to systolic BP, whereas no contribution to vulnerability to cerebral ischemia can be detected. (Hypertension. 2001;37[part 2]:391-397.)

Key Words: hypertension ■ stroke ■ genetics ■ SHRSP ■ consomics ■ focal cerebral ischemia ■ middle cerebral artery occlusion

The stroke-prone spontaneously hypertensive rat (SHRSP) is generally regarded as a good experimental model of cerebrovascular disease and human essential hypertension.1 Spontaneous strokes and the increased vulnerability to cerebral ischemia have been well documented in the SHRSP, and several quantitative trait loci (QTLs) for these phenotypes were published.2,3 Ely and Turner4 found that the blood pressure of F2 offspring depended on the strain of the male progenitor in a WKY x SHR cross. Male offspring with an SHR male progenitor had significantly higher pressures than male offspring with a WKY progenitor. The blood pressure of F2 males was compatible with a Y-linked effect on blood pressure. Reciprocal Y-consomic strains (SHR Y chromosome on WKY background and WKY Y chromosome on SHR background) were constructed and confirmed the Y chromosome effect on blood pressure.5 Previous data from our laboratory described 143 F2 rats obtained by crossing SHRSP and WKY, which were phenotyped using a radiotelemetry system. In this study, male F2 hybrids with an SHRSP grandfather had significantly higher blood pressures compared with male F2 hybrids with the WKY grandfather, suggesting that the Y chromosome effect was also present in the SHRSP.6

The SHRSP strain exhibits an increased frequency of spontaneous strokes and an increased volume of infarction after experimentally induced focal cerebral ischemia compared with the WKY reference strain.2,3 To investigate the role of the SHRSP Y chromosome in stroke, we used permanent middle cerebral artery occlusion (MCAO) in an F1 reciprocal cross.7,8 We found that F1 males with an SHRSP male progenitor had smaller infarct than those with a WKY

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male progenitor. To examine this further, we divided male F2 rats from our previous study according to the origin of their Y chromosome. A result was in parallel with those achieved in the F1 study for which F2 males with an SHRSP grandfather had significantly smaller infarcts than those with a WKY grandfather. The aim of the present study was to assess the effect of the SHRSP and WKY Y chromosomes on blood pressure and experimentally induced focal cerebral ischemia by constructing reciprocal consomic strains using a marker-assisted strategy.

Methods

Rat Strains

Inbred colonies of SHRSP, and WKY rats have been established at the University of Glasgow since December of 1991 as previously described. All rats were housed under controlled conditions of temperature (21°C) and light (12-hour light/dark cycle; 7 AM to 7 PM) and maintained on normal rat chow (rat and mouse No. 1 maintenance diet, Special Diet Services, UK) and water ad libitum. These studies were approved by the Home Office according to regulations regarding experiments with animals in the United Kingdom.

Consomic Crosses

The development of the consomic strains used in this study involved the transfer of the Y chromosome from WKY to the genetic background of SHRSP, and in the reciprocal direction, from SHRSP to the genetic background of WKY. This required the production of an F1 generation by crossing WKY and SHRSP. Male F1 hybrids were then mated to the desired recipient strain (WKY or SHRSP). Ninety-five microsatellite markers were genotyped in the offspring from this first backcross, 83 of them were used before and were listed previously. Polymorphic markers D2Wox9, D2Wox19, D2Mit21, D2Mit14, D2Mgh12 were additionally genotyped in the current study. Selection of these markers was based on the need for a thorough coverage of the entire rat genome and location around the blood pressure and stroke QTls previously identified by ourselves. Databases used to fulfill this selection included Ratmap: The Rat Genome Database, Goteborg University, Sweden at http://ratmap.gen.gu.se/; The Whitehead Institute Center for Genome Research Rat Mapping Project, Boston, USA at http://www.genome.wi.mit.edu/rat/public/; and The Wellcome Trust Center for Human Genetics Genetic Linkage Maps of the Rat Genome, Oxford, UK at http://www.well.ox.ac.uk/~bihoreau. Males homozygous for the recipient alleles throughout the remaining genome were selected as the “best” for breeding and, thus, backcrossed again to the recipient strain to produce a second backcross generation. This procedure was repeated in all offspring after every backcross until the donor’s genetic background was eradicated as indicated by the 95 background markers.

Genotyping

To obtain DNA samples from the consomic backcrosses, the offspring were briefly anesthetized at 4 weeks of age with halothane and a 4-mm tip from their tail removed into a 1.5-mL microfuge tube. The wound was immediately sealed with an electric cauterizer (Engel-Loter 100S), and the tails were stored at −20°C. Genomic DNA was isolated as previously described. Genotyping was performed by PCR amplification of DNA around the polymorphic microsatellite markers from the total genomic DNA using the appropriate PCR primer pairs custom made by either Research Genetics or Genosys Biotechnologies as previously described.

Middle Cerebral Artery Occlusion

Anesthesia was induced (5%) and maintained (1% to 2%) by halothane in oxygen-nitrous oxide (50:50). A distal segment of the left MCA was occluded by electrocoagulation in male SHRSP, WKY, and consomic strains using the technique of Tamura et al with monitoring of physiological variables throughout MCAO and at 24 hours after MCAO as previously described. A temperature probe inserted into the temporalis muscle was used to reflect the brain temperature, which was maintained at 37°C throughout the MCAO procedure. Twenty-four hours after MCAO, tissue was processed for hematoxylin-eosin staining as previously described for the estimation of infarct volume by image analysis. Briefly, infarct volume for each brain was derived from integration of areas of damage over 8 coronal levels with end points for integration 12.5 mm anterior and 0.05 mm posterior to the interaural line. Infarct volumes were expressed as a percentage of the volume of the ipsilateral hemisphere to account for brain swelling and differences in brain size between strains.

Blood Pressure Measurement

The Dataquest IV telemetry system (Data Sciences International) was used for the direct measurement of systolic and diastolic (DBP) arterial pressure as previously described. Surgical implantation of each telemetry transmitter took place under standard sterile conditions at 12 weeks of age. Hemodynamic data were sampled every 5 minutes for 10 seconds. To allow for a full stabilization of blood pressure postoperatively, experimental observations were collected from day 7 to day 42 after surgery as “baseline hemodynamic measurements.” On day 43, rats on telemetry received 1% sodium chloride (NaCl) in their drinking water, and this treatment was continued for 2 weeks until they were killed. Data collected during this period were considered “salt-loaded hemodynamic measurements.”

Evaluation of Cardiac Hypertrophy

Immediately after exsanguination, the thorax was opened and the heart was removed, blotted with tissue paper, and weighed. The atria and right ventricle were then removed, and the left atrium and septum were weighed. Heart weight to body weight (HW:BW, mg/g) and left ventricle plus septum weight to body weight ([LV+S]:BW, mg/g) ratios were then determined.

Statistical Analysis

A total of 10,080 measurements of each blood pressure phenotype were made on each animal during the 5-week baseline phase and 6048 measurements during the 3-week salt-loaded phase. Within each phase, hemodynamic measurements were separated into daytime (7 AM to 7 PM) and nighttime (7 PM to 7 AM) periods. Summary statistics were provided for each combination of experimental phase and time of day by calculating overall means and standard errors separately by consomic strain. Comparisons of consomic strains to their corresponding background parental strains were made by repeated measures analysis of variance of daytime or nighttime means for each individual week of the two phases, reporting the F-statistics and probability value corresponding to the main effects for strain. We additionally applied the seasonal and trend decomposition procedure based on Loess (STL), because of the large number of measurements obtained by radiotlemetry and the need to correct for autocorrelation between successive time points from the same animal. The STL analysis expresses each hemodynamic series as a sum of components representing overall trend, cyclic behavior, and residual variability, as described previously. This analysis was performed to identify differential effects on trends and cyclic variation, over and above simple shifts in mean value, due to the Y chromosome in the consomic strains. Comparisons of cardiac to body weight ratios and infarct volumes were made by unpaired t test and corresponding confidence intervals, and means and standard errors were used as summary statistics.

Results

Reciprocal consomic strains (SP.WKYGl/Y and WKY.SPGl/Y) were produced through introgression of the Y chromosome from...
WKY into the recipient SHRSP strain and vice versa. In the nomenclature of the consomic strains, the first abbreviation denotes the recipient, the second denotes the donor, the letter Y refers to the Y chromosome, and the w and s subindexes indicate the origin of the Y chromosome. The number of generations of backcrossing required for each consomic strain to achieve complete homozygosity of the background genetic markers varied between BC 3 and BC 4 (in which BC indicates backcross). A total of 162 progeny were screened to produce the consomic strains, for an average of 81 animals per strain and 23 animals per backcross. Two years and 7 months was required on average to produce the consomic lines.

**Infarct Volume**
Infarct volume in SHRSP males (33.6% ± 7.6, n = 6) was greater than in WKY males (22.2% ± 8.0, n = 7) (Figure 1). Infarct volumes in SP.WKYGlaY_w males were not different from those observed in SHRSP males (Figure 1). Similarly, infarct volumes in the reciprocal WKY.SPGlaY_s consomic strain males did not differ from those observed in the parental WKY males (Figure 1).

**Blood Pressure**
Baseline and salt-loaded systolic blood pressure (SBP) recorded by radiotelemetry in the consomic strains are shown in Figure 2, and daytime and nighttime SBP and DBP means for both phases are given in Table 1. The SP.WKYGlaY_w consomic strain showed a significant reduction in SBP of approximately 30 mm Hg during the salt-loaded period compared with SHRSP parental strain (F = 5.9, P = 0.038). How-
ever, no differences in SBP were found during the baseline period between these strains. In the reciprocal consomic strain, WKY.SPGlaYs we observed a significant increase in SBP by approximately 15 mm Hg during the baseline period compared with the parental WKY strain (F = 6.1, P = 0.035).

There was also a tendency for increased SBP in the consomic strain WKY.SPGlaYs during the salt-loaded phase, but this difference did not achieve statistical significance.

The smoothed trend components for SBP for the consomic and parental strains are displayed in Figure 3. For the SP.WKYGlaYw consomic strain we observed a very significant trend divergence (F = 9.1, P = 0.001) during the salt-loaded phase compared with the SHRSP parental strain. However, no divergence between the strains was found during the baseline period (Figure 3). In the reciprocal consomic strain, WKY.SPGlaYw, there was no divergence during both recording periods compared with the parental strain (Figure 3).

Figure 4 displays estimates of the average periodic (or cyclic behavior) over the recording period of SBP for the consomic and parental strains. The average magnitude of the effect of 24-hour periodicity was between 28 and 19 mm Hg, and 25 and 15 mm Hg for consomic SP.WKYGlaYw and WKY.SPGlaYs strains, respectively. The SP.WKYGlaYw consomic strain (Figure 4) showed a very significant reduction in the amplitude of variability throughout the 24-hour period during the salt-loaded period (F = 2.96, P < 1×10^-15), compared with the SHRSP parental strain, although the two strains were similar during the baseline period.

### Blood Pressure and Cardiac Mass in Consomic and Progenitor Strains

<table>
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<th>Time</th>
<th>Mean</th>
<th>SE</th>
<th>Mean</th>
<th>SE</th>
<th>F</th>
<th>P</th>
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<td>SP.WKYGlaYw</td>
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<td>177.9</td>
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</table>

| WKY.SPGlaYs           |      |      |    |      |    |       |      |
| SBP                   |      |      |    |      |    |       |      |
| Baseline              | Day  | 141.4| 2.3| 128.8| 5.2| 4.31  | 0.068|
|                       | Night| 147.6| 2.4| 132.6| 5.1| 6.11  | 0.035|
| 1% NaCl               | Day  | 147.8| 2.4| 138.4| 5.4| 2.20  | 0.172|
|                       | Night| 159.8| 3.5| 144.9| 5.6| 4.66  | 0.059|

| WKY                   |      |      |    |      |    |       |      |
| DBP                   |      |      |    |      |    |       |      |
| Baseline              | Day  | 107.8| 1.5| 96.5 | 3.8| 4.4   | 0.065|
|                       | Night| 112.1| 1.2| 99.4 | 3.9| 6.8   | 0.028|
| 1% NaCl               | Day  | 107.6| 1.7| 100.1| 4.9| 1.8   | 0.209|
|                       | Night| 119.4| 2.8| 106.6| 4.9| 4.9   | 0.055|

Data are means and standard errors of daytime (7 AM–7 PM) or nighttime (7 PM–7 AM) period for each blood pressure over 5 weeks during baseline and 3 weeks during the salt-loaded phase (in mm Hg). F-statistics and P values are for relevant main effects from repeated measures analysis of variance, with former being compared to F (1,9) in each analysis. First consomic strain (6 males) was compared with the SHRSP parental strain (6 males); second (6 males) to the WKY strain (6 males). Heart weight to body weight ratio and left ventricular to body weight ratios are shown as means and standard errors.
Conversely, the WKY.SPGlaY s strain showed a very significant increase in the amplitude of variability (baseline, \( F = 1.24, P = 8.8 \times 10^{-3} \); salt, \( F = 1.24, P = 4.9 \times 10^{-3} \) ) compared with the WKY parental strain (Figure 4).

**Cardiac Hypertrophy**

HW:BW ratios in the two consomic strains were not different from their respective parental strain (Table 1). Similarly, LV+S:BW ratios were not different between the consomic and the parental strains (Table 1).

**Discussion**

This is the first study to construct reciprocal Y consomic strains in the rat using a marker-assisted strategy. We also assessed the role of the Y chromosome in blood pressure and vulnerability to cerebral ischemia. We could not detect any contribution of the SHRSP or WKY Y chromosomes to the vulnerability to cerebral ischemia. However, we observed a reduction in the SBP recorded by radiotelemetry in consomic strain SP.WKYGlaY, and an increase in SBP in the reciprocal strain consistent with a Y chromosome effect on blood pressure.

Results from our F1 and F2 studies\(^5,7\) suggested that the Y chromosome from the SHRSP decreased the sensitivity to cerebral ischemia compared with the WKY Y chromosome. In an F2 study, many other autosomal loci may be cosegregating in the cohort and contributing to the phenotypic difference observed. Therefore, epistatic and ecogenetic interactions between the Y chromosome and autosomal loci might govern its effect on the stroke phenotype in the F2 cohort. Consomic strains presented in this study give a unique opportunity to evaluate the individual role of the Y chromosome in stroke. Our findings are consistent with no contribution of this chromosome to the vulnerability to cerebral ischemia.

Consistent with the results from our F2 cohort,\(^6\) the current study demonstrates a Y chromosome effect on blood pressure. In the Y consomic strains produced by Ely and coworkers using the SHR/hsd and WKY/hsd, the addition (or deletion) of the SHR Y chromosome adds (or subtracts) 15 to 20 mm Hg from the parental strain SBP recorded by tail cuff.\(^5,15\) In the current study, we used radiotelemetry during the 9-week period and, therefore, the blood pressure data are

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**Figure 3.** Loess analysis of SBP for consomic and parental strains representing the estimates for overall trend in SBP (center line) with 95% pointwise confidence bands (upper and lower lines). Arrows indicate the same time points as in Figure 2.
The SHR/hsd and WKY/hsd strains for which the Y SHR chromosome is associated with increased adrenal gland nor-epinephrine (NE) and chromogranin A content, increased heart and renal NE turnover, increased plasma NE response to acute stress, and reduction in blood pressure after chemical sympathectomy or clonidine treatment.15,20

Very few genetic loci have been identified on the Y chromosome, and no appropriate microsatellite markers have been developed because the Y chromosome is not involved in recombination during meiosis apart from the small pseudo-autosomal region. Some of the Y chromosome potential candidate loci are as follows: Sry, testis determining locus; Zfy, zinc finger protein; Sts, steroid sulfatase locus; and Try, or testosterone timing locus. Ely et al17 proposed that the SHR Y chromosome causes an acceleration of testosterone release and earlier puberty, with a resulting cascade of molecular and neuroendocrine events that contribute to hypertension.

In conclusion, the SHRSP Y chromosome harbours a locus or loci that contribute to SBP. However, we found no contribution to the vulnerability to cerebral ischemia on the Y chromosome.

Acknowledgments

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