Abstract—Human essential hypertension is a complex polygenic trait with underlying genetic components that remain unknown. The stroke-prone spontaneously hypertensive rat (SHRSP) is a model of human essential hypertension, and a number of reproducible blood pressure regulation quantitative trait loci have been found to map to rat chromosome 2. The SP.WKYGla2c* congenic strain was produced by introgressing a region of rat chromosome 2 from the normotensive Wistar Kyoto (WKY) strain into the genetic background of the SHRSP. Systolic and diastolic blood pressures were significantly reduced in the SP.WKYGla2c* compared with the SHRSP parental strain (198/134 ± 6.1/3.3 versus 172/120 ± 3.8/3.4 mm Hg; F = 15.8/8.1; P = 0.0009/0.013). Genome-wide microarray expression profiling was undertaken to identify differentially expressed genes among the parental SHRSP, WKY, and congenic strain. We identified a significant reduction in expression of glutathione S-transferase μ-type 2, a gene involved in the defense against oxidative stress. Quantitative reverse transcription–polymerase chain reaction relative to a β-actin standard confirmed the microarray results with SHRSP mRNA at 8.56 × 10^-4 ± 1.6 × 10^-4 compared with SP.WKYGla2c* 3.67 × 10^-3 ± 2.8 × 10^-4 (95% CI = −3.9 × 10^-3 to −1.8 × 10^-3; P = 0.0034) and WKY 4.03 × 10^-3 ± 5.1 × 10^-4; (95% CI = −5.4 × 10^-3 to −8.9 × 10^-4; P = 0.027). We also identified regions of conserved synteny, each containing the Gstm2 gene, on mouse chromosome 3 and human chromosome 1. (Hypertension. 2003;41[part 2]:847-853.)

Key Words: genetics ■ hypertension, genetic ■ gene expression ■ rats, stroke-prone SHR

Essential hypertension is a complex polygenic trait. It has been established that genetic determinants contribute between 30% and 50% of the blood pressure variation among individuals. Despite major recent advances in genome sequencing and statistical tools, the genetic dissection of essential hypertension still provides a formidable challenge. However, experimental models of genetic hypertension, which can be considered a reductionist paradigm for human disease, can be used to facilitate the identification of causative genes.

The stroke-prone spontaneously hypertensive rat (SHRSP) is a well-characterized experimental model for human essential hypertension. Similar to human disease, the genetic determination of blood pressure variation in this model is complex and due to multiple gene-gene and gene-environment interactions. The SHRSP develops a number of vascular complications, including cardiac hypertrophy, cardiac failure, and stroke. Genome-wide linkage studies have proved successful in the localization of large chromosomal regions containing quantitative trait loci (QTLs) for blood regulation in the SHRSP. In particular, previous work in our laboratory has identified at least 2 blood pressure QTLs mapping to rat chromosome 2. This region of chromosome 2 is a classic example of a common or overlapping QTL, as it has been implicated in several crosses. Moreover, the QTL contained in the telomeric region of rat chromosome 2 has been confirmed in 2 different congenic strains by introgressing the relevant region from Milan normotensive rat or Wistar Kyoto rat (WKY) into the Dahl salt-sensitive background.

Congenic strains derived from hypertensive rat models have been used to confirm the existence of several other QTLs, and 1 locus has already been fine-mapped to a 0.54-centimorgan (cM) interval. However, the requirement for the construction of minimal congenic strains may be circumvented, at least in some cases, by use of new technology such as high-throughput microarray expression profiling. Microarrays are available in 2 principle forms: complementary DNA (cDNA) and oligonucleotides. Microarray technology has been used to accelerate gene identification by Aitman et al, who used cDNA microarrays and rat chromosome 4 congenic strains to identify Cd36, a gene responsible for defective fatty acid metabolism in the spontaneously hypertensive rat (SHR).

Several mapping studies investigating genome conservation at the genetic and physical level have determined that gene order is relatively invariant showing conserved synteny between mammals. Comparative genome analysis of rat, mouse, and human can be used to identify regions of the
human genome likely to harbor genes involved in blood pressure regulation.7,16 Julier et al17 and Baima et al18 provided first successful examples of comparative mapping in which a region involved in blood pressure regulation on rat chromosome 10 indicated a susceptibility locus for human hypertension on human chromosome 17q.

Aims of the current study were to generate and phenotype a congenic strain containing a region of rat chromosome 2 from the normotensive WKY strain introgressed into the genetic background of the SHRSP. Genome-wide expression profiling was undertaken to identify differentially expressed genes among the parental SHRSP, WKY, and congenic strain. Furthermore, a comparative genome analysis was used to identify regions of conserved synteny on mouse and human chromosomes.

Methods

Production of Congenic Strains and Blood Pressure Measurements

Inbred colonies of SHRSP and WKY have been developed at the University of Glasgow since 1991, as we previously described.19 From weaning, all rats have been maintained on normal rat chow (rat and mouse No.1 maintenance diet, Special Diet Services). The congenic strain used in the present study contains a 22-cM segment transferred from WKY (donor strain) to the genetic background of SHRSP (recipient strain) using a marker-assisted “speed” congenic strategy.20 The nomenclature of the strains consists of the first abbreviation belonging to the recipient strain and the second to the donor; Gla denotes that strains originate from the Glasgow colonies, and the number 2 refers to rat chromosome 2. Thus, the congenic strain described in the current study is the SP.WKYGla2 (D2Wox9-D2 Mgh12) and is abbreviated to SP.WKYGla2c* for simplicity.

The Dataquest IV telemetry system (Data Sciences International) was used for the direct measurement of systolic and diastolic blood pressure.19,20 Briefly, rats were implanted at 12 weeks of age with a 4-mm tip from the tail of congenic animals and genotyping as we described previously.20 These studies were approved by the Home Office according to regulations regarding experiments with animals.

Genetic Linkage and Radiation Hybrid Maps

Genotyping was performed by polymerase chain reaction (PCR) amplification of microsatellite markers, and the genotypic results obtained were mapped relative to each other using the MAP-MAKER/EXP 3.0 computer package with an error-detection procedure.21 We constructed a genetic linkage map of rat chromosome 2 consisting of 74 markers polymorphic between the WKY and SHRSP strains. Genetic distances were calculated with the Haldane heterozygosity of background markers. The SP.WKYGla2c* strain incorporated only the lower blood pressure QTL on rat chromosome 2 from WKY into the recipient SHRSP strain (Figure 1). Five backcrosses were required to produce the congenic strain with no detectable heterozygosity of background markers. The SP.WKYGla2c* strain incorporated only the lower blood pressure QTL on rat chromosome 2.12 The SP.WKYGla2c* rats had an average reduction in systolic and diastolic pressures of 20 and 15 mm Hg, respectively when compared with the parental SHRSP strain (198/134±6.1±3.3 versus 172/120±3.8/3.4 mm Hg; F=15.8/8.1, P=0.0009/0.013). Weekly averaged daytime and nighttime systolic and diastolic blood pressures measured over a 3-week period are shown in Figure 2.

Results

Construction of Genetic and RH Maps

To create the new genetic linkage map, a total of 74 microsatellite markers, spanning rat chromosome 2, were genotyped in F2 animals. Of the 74 markers scored, we
positioned 55 on the RH map of rat chromosome 2. Nineteen of the markers added to the map did not position within the region of rat chromosome 2, as they were multilinked and the reduction of stringency to accommodate them would result in a less accurate map. The markers covered a distance of 1224 centiRays and were all placed with a logarithm of odds ratio (LOD) $\log_{10}(10)$. This equates to $10^{10}$ odds for linkage.

**Gene Expression Profiling**

Total numbers of differentially expressed genes between the SHRSP and SP.WKYGla2c*, as well as the SHRSP and WKY, are shown in Table 1. Of the 45 differentially expressed probe sets in the SHRSP and SP.WKYGla2c* comparison, 12 were also identified in the SHRSP and WKY comparison (Table 2). Four of these probe sets have a known rat chromosomal location, with 3 mapping to the congenic segment. All 3 probe sets that mapped to the congenic segment represented the same gene, glutathione S-transferase $\mu$-type 2 ($Gstm2$). Of these, two were gene probe sets, and one was an expressed sequence tag DNA sequence. Quantitative RT-PCR relative to the same $\beta$-actin standard curve further confirmed the microarray results (Figure 3). The LightCycler showed $Gstm2$ expression was significantly lower in SHRSP $(8.56 \times 10^{-4} \pm 1.6 \times 10^{-4})$ versus SP.WKYGla2c* $(3.67 \times 10^{-3} \pm 2.8 \times 10^{-3})$; (95% CI $-3.9 \times 10^{-3}$ to $-1.8 \times 10^{-3}$; $P=0.0034$) and versus WKY $(4.03 \times 10^{-3} \pm 5.1 \times 10^{-4})$; (95% CI $-5.4 \times 10^{-3}$ to $-8.9 \times 10^{-4}$; $P=0.027$).

**Comparative Genome Analysis**

We used publically available databases to demonstrate that the SP.WKYGla2c* congenic segment showed highly conserved synteny to mouse chromosome 3 and human chromosome 1. Orthologous genes between the 3 species are shown in Figure 4. $Gstm2$ is one of the orthologs, as it maps to the region of conserved gene synteny in all 3 species.

**Discussion**

Using a combination of congenic strategy and microarray DNA chip technology, we identified $Gstm2$ as a putative positional and physiological candidate gene within the blood
against oxidative stress. This is of physiological and pathophysiological significance in the SHRSP, as we have shown that the endothelial dysfunction in this model is owing to increased vascular oxidative stress largely accounted for by the increased generation of superoxide anion. Moreover, the induction of chronic oxidative stress by glutathione depletion has been shown to cause severe hypertension in normotensive rats. There is also some evidence for similar mechanisms in human endothelial dysfunction and essential hypertension. Although an alternative explanation may be that changes in the levels of $Gstm2$ expression between the strains are secondary to blood pressure differences already present in 15-week-old rats.

Other efforts to analyze the QTLs on rat chromosome 2 have focused on the construction of overlapping congenic strains to narrow down the region(s) under study. However, these studies have not resulted in the identification of positional candidate genes. Although theoretical considerations have suggested that it should be possible to obtain minimal congenic strains as small as 1 cM, this has been proven very difficult in practice. In the current study, we produced dense genetic and RH maps of rat chromosome 2 in an effort to narrow down the congenic region. Similar work has been performed for the remaining rat chromosomes by other groups. However, it seems unlikely that minimal congenic strains encompassing $<$3 cM will be frequently produced. Therefore, ancillary strategies such as microarray gene expression profiling might contribute to causative gene discovery. Aitman et al and Eaves et al used rat and mouse congenic strains, respectively, in combination with gene chip technology. The former study identified a strong positional and physiological candidate gene, the $Cd36$, although the congenic interval of the SHR.BN strain used was relatively large at 36 cM. However, Eaves et al failed to identify any strong candidates, despite a true minimal congenic strain at 0.35 cM. These contrasting results, together with the microarray data from the current study, confirm that although not every congenic/microarray experiment will identify causative genes, the size of congenic interval should not be seen as a limitation for a well-designed microarray experiment.

The choice of tissue for gene expression profiling has been a matter of recent debate. It seems that for gene hunting experiments in hypertension, the kidney is an ideal choice as several elegant transplantation experiments showed that hypertension always "travels with the kidney." The other important issue, already stressed by Aitman et al but also clearly seen in the current study, is the relative ease of interpretation of microarray data. Although one would expect hundreds of differentially expressed genes between the 2 parental strains, this number is significantly reduced in the congenic and parental strain comparisons. Moreover, initially one would focus on genes that are differentially expressed and mapped to the congenic region, with a possibility of later analysis of genes mapping to other chromosomes but perhaps still of functional importance through the relevant physiological pathways.

**Perspectives**

We have identified $Gstm2$ as a positional and physiological candidate gene for blood pressure regulation and oxidative stress.

---

**TABLE 1. Differentially Expressed Genes Identified by Global Error Model Filtering Technique in Paired Comparisons Between the Congenic and Parental Strains**

<table>
<thead>
<tr>
<th>Strains</th>
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<th>U34B</th>
<th>U34C</th>
<th>Total</th>
</tr>
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<td>10</td>
<td>27</td>
<td>45</td>
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<tr>
<td>SHRSP and WKY</td>
<td>119</td>
<td>339</td>
<td>326</td>
<td>784</td>
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**Figure 2.** Daytime (D, open symbols) and night-time (N, closed symbols) average systolic blood pressure (A) and diastolic blood pressure (B) recorded by radiotelemetry over a 3-week period in the SHRSP (n=9), SP.WKYGla2c* (n=11), and WKY (n=8) strains; parental SHRSP versus WKY (198/134±6.1/3.3 versus 140/102±2.7/2.0 mm Hg; $F=101.0/77.2$, $P=4.7\times10^{-9}$), SHRSP versus SP.WKYGla2c* (198/134±6.1/3.3 versus 172/120±3.8/3.4 mm Hg; $F=15.8/8.1$, $P=0.00009/0.013$).

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**Figure 3.** Depletion of glutathione in normotensive rats. There is also some evidence for similar physiological significance in the SHRSP, as we have shown that the endothelial dysfunction in this model is owing to increased vascular oxidative stress largely accounted for by the increased generation of superoxide anion. Moreover, the induction of chronic oxidative stress by glutathione depletion has been shown to cause severe hypertension in normotensive rats. There is also some evidence for similar mechanisms in human endothelial dysfunction and essential hypertension. Although an alternative explanation may be that changes in the levels of $Gstm2$ expression between the strains are secondary to blood pressure differences already present in 15-week-old rats.

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**Figure 4.** Comparative genome analysis and identification of mouse and human orthologs on chromosomes 3 and 1, respectively.

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**Figure 5.** Croarray gene expression profiling, and these results were confirmed by quantitative RT-PCR.
stress in the SHRSP. Using comparative genome analysis between rat, mouse, and human, we successfully transferred the relevant QTL across species and showed that the rat Gstm2 has orthologs on mouse chromosome 3 and human chromosome 1. Despite highly significant changes in expression between the congeneric and the SHRSP strain, we have not excluded the possibility that these could be secondary to blood pressure differences. Further studies in young animals during the development of hypertension may help elucidate primary mechanisms.

<table>
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<tr>
<th>Probe Set ID</th>
<th>SP.WKYGla2c* Normalized</th>
<th>SE</th>
<th>SHRSP Normalized</th>
<th>SE</th>
<th>Gene</th>
<th>GenBank</th>
<th>Unigene Link</th>
<th>Rat</th>
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<td>AJ005023</td>
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<td>24737</td>
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<td>H32189</td>
<td>Rn0.625</td>
<td>24424</td>
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SE indicates standard error; EST, expressed sequence tag.

Figure 3. A, Representative Affymetrix gene chip images for the 16 perfect match (upper) and mismatch (lower) probe set oligonucleotides for Gstm2 (X04229) from each of the WKY, SP.WKYGla2c*, and SHRSP strains, showing decreased levels of Gstm2 expression in SHRSP. B, Graph depicting the accumulation of fluorescence throughout the PCR for Gstm2; SHRSP (8.56×10^{-4}±1.6×10^{-4}) versus SP.WKYGla2c* (3.67×10^{-3}±2.8×10^{-4}); (95% CI −3.9×10^{-3} to −1.8×10^{-3}; P=0.0034) and versus WKY (4.03×10^{-3}±5.1×10^{-4}); (95% CI −5.4×10^{-3} to −8.9×10^{-4}; P=0.027). Inset graph indicates the β-actin standard curve.
Acknowledgments

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References


Microarray Analysis of Rat Chromosome 2 Congenic Strains

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/content/41/6/e13.full.pdf

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Correction

Change in Gene Designation

In the article “Microarray Analysis of Rat Chromosome 2 Congenic Strains” (McBride et al, Hypertension. 41[part 2]:847–853), the authors reported the differential expression of rat *Gstm2* (accession numbers X04229, J02810, and H32189) in their congenic strain. However, in the recent release of the rat annotation, the gene designation of these accession numbers has been changed to *Gstm1*.