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Monte E. Turner

*University of Akron Main Campus, met1@uakron.edu*

Joel Farkas

Jeff Dunmire

Daniel Ely

*University of Akron Main Campus*

Amy Milsted

*University of Akron Main Campus, milsted@uakron.edu*

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## Which Sry Locus Is the Hypertensive Y Chromosome Locus?

Monte E. Turner, Joel Farkas, Jeff Dunmire, Daniel Ely, Amy Milsted

**Abstract**—The Y chromosome of the spontaneously hypertensive rat (SHR) contains a genetic component that raises blood pressure compared with the Wistar-Kyoto (WKY) Y chromosome. This research tests the Sry gene complex as the hypertensive component of the SHR Y chromosome. The Sry loci were sequenced in 1 strain with a hypertensive Y chromosome (SHR/Akr) and 2 strains with a normotensive Y chromosome (SHR/Crl and WKY/Akr). Both SHR strains have 7 Sry loci, whereas the WKY strain has 6. The 6 loci in common between SHR and WKY strains were identical in the sequence compared (coding region, 392-bp 5' prime flanking, 1200-bp 3' flanking). Both SHR strains have a locus (Sry3) not found in WKY rats, but this locus is different between SHR/Akr and SHR/Crl rats. Six mutations have accumulated in Sry3 between the SHR strains, whereas the other 6 Sry loci are identical. This pattern of an SHR-specific locus and mutation in this locus in SHR/Crl coinciding with the loss of Y chromosome hypertension is an expected pattern if Sry3 is the Y chromosome–hypertensive component. The SHR/y strain showed a significant increase in total Sry expression in the kidney between 4 and 15 weeks of age. There are significant differences in Sry expression between adrenal glands and the kidney (15 to 30 times higher in kidneys) but no significant differences between strains. These results, along with previous studies demonstrating an interaction of Sry with the tyrosine hydroxylase promoter and increased blood pressure with exogenous Sry expression, suggest the Sry loci as the hypertensive component of the SHR Y chromosome. (*Hypertension*. 2009;53[part 2]:430-435.)

**Key Words:** hypertension Y chromosome rats ■ inbred SHR genes ■ Sry gene copy number

Crosses between the spontaneously hypertensive rat strain (SHR) and the normotensive Wistar-Kyoto strain (WKY) demonstrated the hypertensive effect of the SHR Y chromosome.<sup>1</sup> The identification of the SHR Y chromosome influencing hypertension is analogous to the identification of a QTL increasing blood pressure. Most techniques to identify genes responsible for an individual QTL involve the principles of linkage and recombination.<sup>2</sup> The unique genetics of the mammalian Y chromosome eliminate any technique that relies on genetic linkage and recombination to identify the locus or loci responsible for the hypertensive phenotype, because the mammalian Y chromosome is present in a single copy in males, and the majority of the chromosome does not undergo recombination. The properties elucidated originally by Rapp<sup>3</sup> for the determination of genetic determinants of hypertension can be used to identify and test a locus without the necessity of recombination or linkage.

Physiological analysis of the SHR Y chromosome blood pressure component is consistent with an increase in sympathetic nervous system activity and stress responsiveness associated with the increased blood pressure. We have evaluated the Sry locus, the testis determining gene, on the Y chromosome as a hypertension candidate gene.<sup>4</sup> Our studies demonstrating the interaction of Sry with the tyrosine hydrox-

ylase promoter are indicative of potential sympathetic nervous system and blood pressure function for Sry in adult males.<sup>5</sup> In a test of this hypothesis, we electroporated an Sry expression vector into a WKY adrenal gland and measured blood pressure and stress responsiveness. Individuals with exogenous Sry electroporated had significantly elevated blood pressure and stress responsiveness 21 days after electroporation.<sup>4</sup> These results are consistent with Sry as a hypertensive locus and a pathway of increased sympathetic nervous system activity through increased expression of tyrosine hydroxylase. Although these studies demonstrate a hypertensive potential for Sry, they do not prove that Sry is responsible for the SHR Y chromosome hypertensive phenotype.

The *Rattus norvegicus* Y chromosome contains multiple Sry loci, although theoretically only 1 copy should be required for testis determination. We have sequenced the Sry gene complex from a single Y chromosome from the Akron SHR strain (SHR/Akr).<sup>6</sup> The analysis of these sequences identified 6 different Sry loci on the SHR Y chromosome (Sry1, Sry2, Sry3, Sry3BI, Sry3B, and Sry3C). These are paralogous copies consistent with a single phylogeny. Divergence between any 2 copies is <2%. All 6 of the loci have a conserved reading frame and amino acid sequence consistent with function.

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From the Department of Biology, University of Akron, Ohio.

Correspondence to Monte E. Turner, Department of Biology, University of Akron, Akron, OH 44325-3908. E-mail meturner@uakron.edu

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The Sry hypertensive locus (loci) should have a pattern of mutation and/or expression consistent with the blood pressure phenotype in comparisons between hypertensive and normotensive Y chromosomes.<sup>3</sup> Vincent et al<sup>7</sup> did reciprocal crosses between SHR and WKY rats from Charles River Laboratories (SHR/Crl and WKY/Crl) and failed to find a hypertensive Y chromosome effect. The original hypertensive Y chromosome crosses and confirmations used SHR strains originally obtained from Harlan Sprague-Dawley (SHR/Hsd and WKY/Hsd). Thus, in a test of hypertensive Sry loci,  $\geq 1$  of the Sry loci in SHR/Crl will have a mutation that causes loss of the hypertensive Y chromosome phenotype. In this study, we compared the DNA sequences of Sry loci from SHR/Akr, SHR/Crl, and WKY/Akr rats and expression patterns from SHR/y and WKY/Akr rats. If  $\geq 1$  of the Sry loci are the hypertensive locus, then mutation and expression patterns should match the strain of origin and the hypertensive phenotype.

Comparison of these strains demonstrates a Sry locus unique to the SHR strains (Sry3) and not found in normotensive strains. This locus has mutations in the SHR/Crl strain that could change its pattern of expression, losing the hypertensive potential of the SHR/Crl Y chromosome. These results, along with the previous physiological results, are suggestive that the Y chromosome hypertensive phenotype is the result of the Sry loci on the SHR Y chromosome. This gene complex fits all of the necessary criteria set forth by Rapp<sup>3</sup> as the genetic determinant responsible for the Y chromosome hypertension phenotype.

## Materials and Methods

### Strains and Nomenclature

SHR, SHR/y, and WKY males were obtained from the University of Akron breeding colonies (SHR/y, SHR/Akr, and WKY/Akr). This colony was established over 20 years ago with animals purchased from Harlan Sprague-Dawley (SHR/Hsd and WKY/Hsd). The SHR/y strain is a Y chromosome consomic strain developed from SHR/Akr and WKY/Akr rats. The SHR/y strain has the SHR/Akr Y chromosome and WKY/Akr autosomes and X chromosome. SHR males were purchased from Charles River Laboratories USA, Wilmington, Mass (SHR/Crl). Loci from each strain are identified with superscript strain identification. For instance, Sry1 from SHR/Akr is designated Sry1<sup>SHR/Akr</sup>, and Sry1 from WKY/Akr is designated Sry1<sup>WKY/Akr</sup>.

### Amplification and Cloning of Sry From Genomic DNA

Primers JF-7L (TGCAGTCTCTACATTTACAATCCA) and JF-3R (CAGAAATGTGACTTCCTGTGCAT) were used to amplify genomic DNA from each strain. Sry2 specific amplification used JF(Sry2)-1L (CCAATGTTACCTTCTCACGGTA) and JF-3R. Sry3A was also confirmed in strains using specific primers JF(Sry3A)-1R (GAATGTTCCGGGAACCATTTG) and JF-7L. Reactions were set up using Platinum PCR SuperMix High Fidelity (Invitrogen). The thermal cycling process consisted of 40 cycles, each with primer annealing at 57°C and 4.5-minute extension at 72°C. PCR products were separated by electrophoresis on a 1.5% agarose gel. On verification of the expected amplicon size, the Sry band was cut from the gel for DNA extraction. DNA was extracted from gel slices using the Zymoclean Gel DNA Recovery kit (Zymo Research). To ensure efficient cloning, Sry amplicons were further treated with *Taq* polymerase and 2'-deoxynucleoside 5'-triphosphate mix to ensure 3' overhanging adenylation. DNA was purified using

the Zymo DNA Clean and Concentrator kit (Zymo Research). Purified PCR product was cloned into the pCR 4-TOPO vector using the TOPO TA Cloning Kit for Sequencing (Invitrogen). Plasmid DNA from transformants was purified using the Zyppy Plasmid Miniprep kit (Zymo Research).

For each strain, the process of PCR amplification, cloning, and sequencing was carried out in multiple, independent replicates. To avoid excessive sequencing of multiple clones of the same Sry copy identity, clones were screened by fragment analysis to determine copy identity.<sup>6</sup> At least 2 clones of each copy identity, from each PCR replicate, were selected for sequencing. Clone sequences were assigned a copy identity by comparison with SHR/Akr reference sequence. Clones of like copy were assembled together using Sequencher software (GeneCodes), and the consensus sequence was determined to be the sequence for that Sry copy. The convention was adapted that, for a base difference to be scored, it must be seen from multiple clones from different amplification reactions.<sup>6</sup>

Sry TOPO clones were screened by fragment analysis, and clones were selected for sequencing. Cycle sequencing reactions were set up in 0.2-mL 96-well PCR plates, in a total volume of 10  $\mu$ L, using the BigDye Terminator 3.1 Cycle Sequencing kit (Applied Biosystems). The thermal cycling process consisted of 50 cycles. Samples were loaded onto the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) and separated by capillary electrophoresis. Sequence data were produced using Sequencing Analysis 5.2 software (Applied Biosystems).

### Real-Time PCR

Tissue collection and RNA isolation were as described previously<sup>8</sup> from 4- and 15-week-old SHR/Y and WKY males ( $n=4$  to 5 per group). One microgram of RNA was DNAsed and reverse transcribed to cDNA using Ambion Array Script reverse transcriptase and random nonamers for the primers. Each well of a 96-well sample plate was set up for individual PCRs with 100 ng of cDNA, 12.5  $\mu$ L of ABI SYBR Green *Taq* Polymerase Master Mix, and male-specific primer sets for Sry (5'-TGGGATTCTG TTGAGCCAAC-3' and 5'-GCGCCCCATGAATGCAT-3') and the invariant reference transcript, S26 (5'-CGATTCTGACAACCTTGCTATG-3' and 5'-CGTGCTTCCCAAGCTCTATG-3'). The Sry primers detect all of the Sry transcripts and do not discriminate among them. Controls included no reverse transcription or reagent blanks (no template) from each tissue sample. All of the samples were run in triplicate. The reactions were run in the ABI Prism 7700 Real-Time PCR System using standard parameters as specified by ABI. Transcript levels in different samples were compared using the relative  $C_T$  method.  $C_T$  values for the S26 normalizer were subtracted from  $C_T$  values for experimental samples to obtain  $\Delta C_T$  values. Significance of differences between groups was determined by ANOVA followed by *t* tests, using the individual  $C_T$  values.

### GenBank

All of the sequences have been submitted to GenBank as follows. SHR/Akr sequences include the following: Sry1<sup>SHR/Akr</sup> (GenBank EU984075), Sry2<sup>SHR/Akr</sup> (FJ168057), Sry3<sup>SHR/Akr</sup> (EU984077), Sry3A<sup>SHR/Akr</sup> (EU984078), Sry3B<sup>SHR/Akr</sup> (FJ168058), Sry3BI<sup>SHR/Akr</sup> (FJ168059), and Sry3C<sup>SHR/Akr</sup> (EU984076). SHR/Crl sequences include the following: Sry1<sup>SHR/Crl</sup> (FJ168060), Sry2<sup>SHR/Crl</sup> (FJ168061), Sry3<sup>SHR/Crl</sup> (FJ168062), Sry3A<sup>SHR/Crl</sup> (FJ168063), Sry3B<sup>SHR/Crl</sup> (FJ168064), Sry3BI<sup>SHR/Crl</sup> (FJ168065), and Sry3C<sup>SHR/Crl</sup> (FJ168066). WKY/Akr sequences include the following: Sry1<sup>WKY/Akr</sup> (FJ168067), Sry2<sup>WKY/Akr</sup> (FJ168068), Sry3A<sup>WKY/Akr</sup> (FJ168069), Sry3B<sup>WKY/Akr</sup> (FJ168070), Sry3BI<sup>WKY/Akr</sup> (FJ168071), and Sry3C<sup>WKY/Akr</sup> (FJ168072).

### Results

Primers used spanned the coding region and included  $\geq 392$  bases of 5' flanking, the entire coding region, and  $\approx 1200$  bases of 3' flanking. Approximately 2100 bases of contin-

**Table. Number of DNA Sequence Mutations Between the Sry Loci From SHR/Akr**

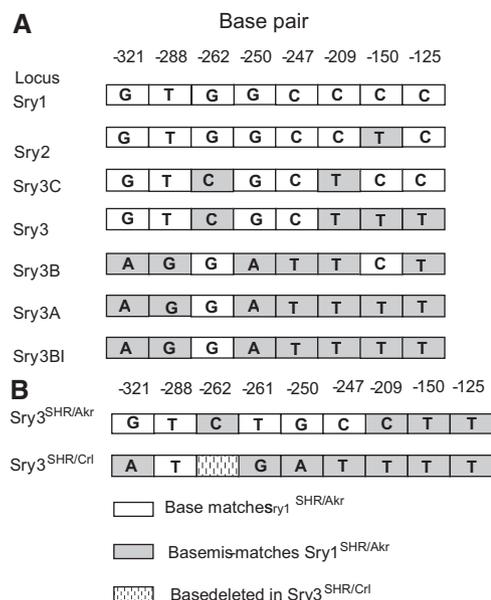
Locus	Sry1	Sry2	Sry3	Sry3A	Sry3B	Sry3BI	Sry3C
Sry1		18	8	13	13	13	10
Sry2	8		19	22	23	23	21
Sry3	3	9		7	7	7	4
Sry3A	5	9	2		6	6	11
Sry3B	4	9	1	3		4	9
Sry3BI	5	10	2	4	1		11
Sry3C	3	9	0	2	1	2	

The total numbers of differences (base pairs) between loci are above the diagonal, and differences (base pairs) only in the 5' flanking region are below the diagonal. Insertion/deletion/repeat differences are counted as a single mutation. The total number of base pairs in each comparison is different because of insertion/deletion/repeat mutations between the loci.

uous sequence were obtained for each copy from each strain or isolate (−392 through +1751); exact numbers differ because of small insertion/deletion/repeat differences between copies. Amplification products were only obtained from males; no amplification was seen in female DNA, confirming a Y chromosome location for all of the copies identified. Sry2 copies were found in the amplification products from primers JF-7L and JF-3R but not in any of the clones from any strain examined. Specific primers unique to Sry2 and Sry3A were developed and used to amplify genomic DNA and to determine Sry2 and confirm Sry3A sequences. All of the strains amplified Sry2 and Sry3A with these primers. Both SHR/Akr and SHR/Crl contain 7 Sry loci (Sry1, Sry2, Sry3, Sry3A, Sry3B, Sry3BI, and Sry3C; Table), whereas the normotensive WKY/Akr has only 6 Sry loci (Sry1, Sry2, Sry3A, Sry3B, Sry3BI, and Sry3C). The Table shows the total divergence between copies and divergence in the 5' flanking region. Although the 5' flanking region comparisons have only 18% to 19% of the sequence compared, in some comparisons, such as Sry2 compared with Sry3BI, 50% of the differences occurred in this region. The 3' flanking region was highly conserved, and there were only 2 differences seen in >1200 bp of flanking between any of the SHR/Akr Sry loci, and both of these differences were in Sry1.

The Sry1, Sry2, Sry3A, Sry3B, Sry3BI, and Sry3C loci were identical across WKY/Akr, SHR/Akr, and SHR/Crl. The only differences found between strains were in Sry3. Sry3 was found only in the SHR strains not in WKY rats. The Sry3 sequences for SHR/Akr and SHR/Crl had 6 differences between the 2 copies, and all of these differences were in the 5' flanking region. Shared differences between copies showed that the 5' flanking region of Sry3<sup>SHR/Crl</sup> matched the flanking region of Sry3A<sup>SHR/Crl</sup> and Sry3BI<sup>SHR/Crl</sup> better than the Sry3<sup>SHR/Akr</sup> flanking region (Figure 1).

Quantitative real-time PCR values are summarized in Figure 2. In the WKY kidney, Sry expression was the same at 4 weeks and 15 weeks. In the SHR/y kidney, there was a 7.25-fold higher Sry expression in the 15-week adult kidney than in the 4-week kidney ( $P<0.03$ ; Figure 2). At 4 weeks and 15 weeks, the WKY and SHR/y kidney expressions were



**Figure 1.** Shared differences between Sry loci in the 5' flanking region. Single base-pair differences are indicated as like Sry1 (white) or different from Sry1 (gray). Mutations unique to an individual locus are not illustrated in A. Base-pair numbering is in relation to the first base of coding region as bp 1, and negative base-pair numbers indicate 5' flanking the coding region. A, Sry loci from SHR/Akr. B, Comparison of Sry3 from SHR/Akr and SHR/Crl.

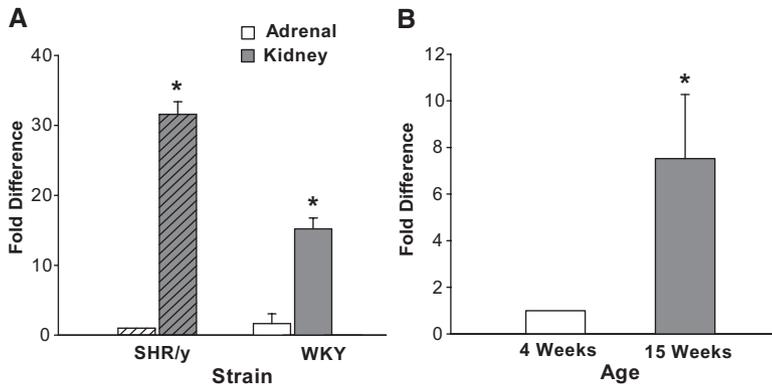
not significantly different (4 week:  $P=0.22$ ; 15 week:  $P=0.17$ ).

In the WKY adrenal gland, Sry expression as decreased from 4 weeks to 15 weeks but did not reach statistical significance ( $P=0.059$ ). In SHR/y, adrenal gland expression of Sry was the same at 4 weeks and 15 weeks (not significant:  $P=0.88$ ). At 4 weeks or 15 weeks, WKY and SHR/y adrenal expressions were not different (4 weeks:  $P=0.25$ ; 15 weeks:  $P=0.08$ ).

Sry expression varied in a tissue-specific manner. Kidneys expressed significantly more Sry than adrenal glands at 15 weeks of age. In the WKY strain, Sry kidney expression was 15.2 times the adrenal gland expression ( $P<0.001$ ), and in the SHR/y strain, the kidney expression was 31.6 times higher than adrenal gland expression ( $P<0.001$ ).

## Discussion

The Sry locus is an evolutionarily conserved locus on the mammalian Y chromosome responsible for testis determination, although there is abundant evidence that this is not its only function, because it is expressed in the brain, kidney, and adrenal gland. Dewing et al<sup>9</sup> showed that Sry is expressed in substantia nigra and in T-helper-expressing neurons and that downregulation of Sry with antisense oligos decreased T-helper expression in the neurons, producing motor deficits (asymmetrical limb use). Ohe et al<sup>10</sup> showed that SRY and SOX colocalize with splicing factors, implicating SRY in pre-mRNA splicing. In vitro DNA binding studies have identified potential Sry target loci. Sry binding sites and in vitro binding have been identified in a



**Figure 2.** A, Total Sry expression varies with tissue; 15-week SHR/y kidneys express  $31.60 \pm 1.78$  times more Sry than SHR/y adrenal glands. In WKY kidneys, Sry levels are  $15.20 \pm 1.55$  times higher than in WKY adrenal glands. \* $P < 0.001$ , kidney vs adrenal gland. Strain differences are not significant. Values for SHR/y adrenal gland were set equal to 1, and all other values compared with them. B, Total Sry expression in the SHR/y kidney increases with age. Sry levels are  $7.25 \pm 2.75$  times higher in the 15-week samples. \* $P < 0.03$ . Values for 4 weeks were set equal to 1, and values for 15 weeks were compared with them.

number of genes: P450 aromatase, antimüllerian hormone (*AMH*), *Fra-1*, and *Fra-2*.<sup>11,12</sup> Although the P450 aromatase and AMH are known components of mammalian sex determination, the *Fra-1* and *Fra-2* loci are components of transcription factor activator protein-1.<sup>13</sup> We have demonstrated that Sry1 expression increases activity of the tyrosine hydroxylase promoter consistent with a potential sympathetic nervous system blood pressure affect.<sup>4</sup> These results indicate that Sry has the potential to be more than just the testis-determining factor. Any cell or tissue expressing Sry expressed could demonstrate altered transcriptional regulation of Sry/SOX-controlled loci, because Sry and SOX loci have the same consensus-binding site.<sup>14</sup>

All of the Sry loci sequenced from these strains have a full-length, potentially active coding region, based on conserved regions identified from previous sequencing results.<sup>6</sup> We had previously identified 6 Sry loci in the SHR/Akr strain,<sup>6</sup> and these results show 7 loci from the same strain. The Sry3 and Sry3A loci identified in the current data were cryptic, and only by using primers that amplified a larger region than previous results could these loci be differentiated, changing the number present from 6 to 7. The sequence differences between the different Sry loci within the SHR/Akr strain are  $\leq 1\%$ , although the 5' flanking region has much higher divergence in some comparisons (Table). Sry2 is the most divergent copy and the most common transcript in the testis and adrenal.<sup>6</sup> The DNA sequence differences in any particular copy between the strains are virtually nonexistent; the only differences seen in any copy between strains are in Sry3 between SHR/Akr and SHR/Crl.

The Rapp paradigm<sup>3</sup> requires 4 criteria to identify genes that cause differences in blood pressure: (1) a difference between strains; (2) a mendelian inheritance pattern; (3) a cosegregation of genes with blood pressure increment; and (4) a logical physiological link to blood pressure. Crosses between the SHR strain and the normotensive WKY strain demonstrated the hypertensive effect of the SHR Y chromosome.<sup>1</sup> Other crosses with SHR substrains and other normotensive strains confirmed this conclusion.<sup>15</sup> The development of strains consomic for the SHR and WKY Y chromosomes demonstrates the expected blood pressure for a Y chromosome hypertensive locus.<sup>16</sup>

The Sry loci have been examined as candidate hypertensive loci from the Y chromosome. Experiments in cell culture

demonstrated that Sry expression could increase transcriptional activity of the tyrosine hydroxylase promoter consistent with the sympathetic nervous system activation seen associated with Y chromosome hypertension.<sup>5</sup> Electroporation of Sry expression vectors into the adrenal gland transiently increased blood pressure in normotensive animals.<sup>4</sup> These results indicated that the Sry loci could be responsible for increased blood pressure, and Sry could affect a known physiological pathway of blood pressure control in appropriate tissues (tyrosine hydroxylase in the sympathetic nervous system). A mutation analysis in hypertensive and normotensive strains is needed to confirm the Sry hypertensive locus hypothesis.

The current data demonstrate a Sry copy number variation between the SHR and WKY strains. This is consistent with the Sry gene complex as the hypertensive component of the SHR Y chromosome. The 6 Sry loci that are found in both SHR and WKY strains have no differences between the strains in a total of  $>13$  kb compared. The comparison between Sry loci in SHR/Akr and SHR/Crl is important in confirming Sry as the hypertensive locus. If the Sry gene complex were identical in SHR/Akr and SHR/Crl strains, the Sry gene complex would be eliminated as the Y chromosome hypertensive component. The SHR/Crl isolate has the same copy number variation as the SHR/Akr strain but does not have a Y chromosome that increases blood pressure. Thus, the presence of the additional copy alone cannot be responsible for the hypertensive phenotype. Consistent with the SHR-to-WKY comparison, for the 6 loci found in all of the SHR and WKY strains, there are no differences between SHR/Akr and SHR/Crl. The Sry3 locus, which is the unique copy in the SHR strains, has 6 differences between SHR/Akr and SHR/Crl (Figure 1). The loss of hypertensive potential is associated with a mutant copy of the SHR-specific Sry locus, whereas all of the other Sry loci are identical. The loss of the hypertensive potential is most likely the result of expression differences between SHR/Akr and SHR/Crl, because all of the identified differences are in the area 5' to the coding region of the gene. This pattern of Sry differences, first copy number variation between SHR and WKY and second mutations between SHR/Akr and SHR/Crl, is a predicted pattern if the Sry gene complex is responsible for the hypertensive Y chromosome. The chance of this pattern of mutation occurring between SHR/Akr and SHR/Crl

occurring randomly would be extremely small, because there are no other differences observed between the other Sry loci.

A hypertensive Sry would need to be expressed in appropriate tissues, such as the kidney and adrenal gland, and potentially differs between SHR and WKY strains. The quantitative real-time PCR values for Sry expression use primers that amplify all of the copies. Thus, the measured values are total Sry expression without regard to copy identity. Expression levels are significantly different between the kidney and adrenal gland, with significantly higher levels in the kidney than in the adrenal gland (Figure 2). Although there are no significant differences between the strains, there is a significant difference between 4-week and 15-week SHR/y. There is >7 times more expression in the 15-week SHR/y kidney than in the 4-week SHR/y kidney. In SHR/y, Sry expression is increasing at the same time that blood pressure is increasing. We do not see the same significance in the WKY rats. This may be because of the timing of a developmental change in expression and the exact timing of the switch. Consistent with this idea is the observation that the developmental timing of testosterone production during puberty is slightly but significantly earlier in SHRs than WKY rats.<sup>17</sup> The expression results are not as conclusive as the mutation analysis but do not exclude Sry as a hypertensive locus. We do not have a complete copy-specific expression profile that may clarify the hypertensive expression pattern and mechanism. We also have not completely excluded the possibility of additional Y chromosome components contributing to Y chromosome hypertension.

What is the genetic mechanism of the Sry gene complex and hypertension? The Sry1 locus has been demonstrated to have hypertensive potential. The Sry1 and Sry3 predicted amino acid sequences have only 2 amino acids different and only 1 in the 3-hydroxy-3-methylglutaryl box (amino acids 5 to 73) responsible for binding DNA. The Sry3 locus has a glutamine at amino acid 37 and Sry1, a histidine.<sup>6</sup> The Sry3 locus has a mutational pattern consistent with the phenotype of hypertensive and normotensive Y chromosomes. The expression data of Sry are at only 2 time points and not copy specific. It is possible that the expression of multiple Sry loci is able to increase blood pressure in a quantitative threshold model, where the expression of multiple Sry loci has an additive effect, and blood pressure is elevated only after a certain threshold level of expression is reached. The extra Sry3 locus on the hypertensive Y chromosomes increases the Sry activity over some threshold value. The mutant flanking region of the Sry3<sup>SHR/Crl</sup> locus changes its expression pattern, reducing the Sry activity in tissues responsible for hypertension.

Sry is a transcription factor, and, as such, the Sry protein does not directly increase blood pressure but changes the expression pattern of target loci, which then cause an increase in blood pressure. Regardless of the exact mechanism for the differences observed, it is extremely improbable that the pattern seen for the hypertension phenotypes and Sry DNA sequence differences are unrelated. In conclusion, the Sry loci fit the criteria elucidated by Rapp<sup>3</sup> for determining a hypertensive locus. Although the exact mechanism or mechanisms for the increase is not known, the data are consistent with Sry

as the loci responsible and the SHR/Akr Sry3 locus as the difference between the hypertensive and nonhypertensive Y chromosomes.

### Perspectives

In human populations, different Y chromosomes have been demonstrated to be significantly different in hypertensive and normotensive comparisons.<sup>18,19</sup> The human Y chromosome has only a single copy of the SRY locus, although mutant human Y chromosomes have been identified with multiple copies of SRY.<sup>20</sup> The 3-hydroxy-3-methylglutaryl box of all mammalian Sry loci is highly conserved, and in vitro Sry and the related Sox loci all bind to the same DNA response sequences.<sup>14</sup> The rat Sry loci can probably control many of the same loci as the human SRY locus. Any proposed mechanism for the hypertensive potential of human SRY does not rely only on multiple copies, but the expression of SRY in tissues of hypertensive potential turning on loci, which increases blood pressure. A mutation in human SRY that would cause or increase expression in an appropriate tissue (adrenal or kidney) could result in increased blood pressure. In a human hypertensive Y chromosome, the SRY expression mutation must not eliminate wild-type SRY expression but add to the normal expression pattern. Information on mutant human SRY loci is limited to individuals without normal testis determination capabilities. There is no study of normal variation for functional human SRY loci. Having multiple copies of Sry, as in *R norvegicus*, may increase the opportunity for Sry expression differences causing hypertension, because other loci can maintain wild-type Sry expression.

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### Disclosures

None.

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