Published in final edited form as:

Steroids. 2010 November ; 75(11): 747–753. doi:10.1016/j.steroids.2009.10.015.

Review of The Y Chromosome, Sry and Hypertension

Daniel Ely, Adam Underwood^{*}, Gail Dunphy, Shannon Boehme, Monte Turner, and Amy Milsted

Department of Biology, University of Akron, Akron, OH 44325-3908

Abstract

The following review examines the role of the SHR Y chromosome and specifically the Sry gene complex in hypertension and potential mechanisms that involve the sympathetic nervous system and renin angiotensin system. There are consistent gender differences in hypertension, with a greater proportion of males affected than females in most mammalian populations. Our earlier studies demonstrated that a portion of the gender differences in blood pressure (BP) in the SHR rat mapped to the SHR Y chromosome. In rats, males with the SHR Y chromosome have higher BP than females, or males with a different Y chromosome. Consistent with these results, several human population studies have confirmed a Y chromosome effect on BP. Our more recent studies focus on a transcription factor, Sry, as the locus involved in not only BP modulation but effects on other phenotypes. The Sry locus is an evolutionarily conserved locus on the mammalian Y chromosome responsible for testis determination and is a transcription factor. The Sry locus contains a highly conserved High Mobility Group (HMG) box region responsible for DNA binding. Mutations in the HMG box result in sex reversal. We have found multiple functional copies of Sry in SHR and WKY male rats. There is abundant evidence that testes determination may not be Sry's only function as it is expressed in the brain, kidney and adrenal gland of adult males. These findings have potential implications for gender physiology research which involve, the sympathetic nervous system, renin angiotensin system, androgen receptor regulation and prostate physiology.

Keywords

blood pressure; sympathetic nervous system; renin angiotensin system; Sry gene complex

Introduction

It has been 9 years since our group reviewed the progress on the Y chromosome and hypertension and 18 years since our original observation of a Y chromosome effect on blood pressure in SHR. This review will cover the following topics: Sry general background, potential Sry functions, Sry copies, Sry expression, Sry protein, and a comparison of Sry phenotypes. The genetic background of the Y chromosome, the development of our animal model, the effects of the Y chromosome on blood pressure phenotypes and the relationship to human studies were described in our first review [1].

Corresponding author: Daniel Ely, Department of Biology, University of Akron, Akron, OH 44325-3908, Ely1@uakron.edu.

^{*}Adam Underwood current address: Department of Biology, Walsh University, 2020 E Maple St, North Canton, OH 44720-3336

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

There are gender differences in human hypertension, with males affected at an earlier age than females, however, the prevalence is actually higher in age adjusted females and the current rise in hypertension prevalence is primarily driven by women [2,3]. Our original studies demonstrated that in the SHR rat a portion of the gender differences in blood pressure (BP) mapped to the SHR Y chromosome [4]. Males with the SHR Y chromosome have higher blood pressure than females, or males with a different Y chromosome. Consistent with our findings, several human population studies have confirmed the Y chromosome effect on blood pressure [5,6]. Our more recent studies focus on a Y chromosome specific locus, Sry, as the locus involved in not only BP modulation but effects on other phenotypes. These findings have potential implications for gender physiology research involving the sympathetic nervous system, renin angiotensin system, androgen receptor regulation and prostate physiology.

With regard to females, since hypertension prevalence in women is increasing, the mechanisms are not going to be the identical as in males with an Sry gene. The triggers for gene and physiological system activation like the RAS, SNS and oxidative stress pathways are most likely going to be regulated differently in females than in the males. Of the potential multiple genes involved in hypertension Sry is only one and females still have all the autosomal genes and potentially X-linked genes that may be involved in both protecting and facilitating hypertension.

Sry Background

The Sry locus (Sex determining region of the Y chromosome) is an evolutionarily conserved locus on the mammalian Y chromosome responsible for testis determination. Sry is a transcription factor responsible for the developmental switch in the embryonic genital ridge to testis determination in males. The Sry locus contains a highly conserved High Mobility Group (HMG) box region responsible for DNA binding, while other regions of the locus show little if any conservation [7]. Mutations in the HMG box result in sex reversal while mutations outside this region have normal sex-determination [8]. These observations have led to the assumption that the HMG box is the only functional component of a Sry locus. There is a misconception in this statement that function in this case is defined in terms of testis determination and generally identified in humans by an observation of gonadal dysgenesis. There is abundant evidence that this may not be Sry's only function as it is expressed in the brain, kidney and adrenal gland of adult males.

Potential Novel Sry Functions

Besides the initial observation of testes determination, Sry appears to have a role in regulation of many genes which are involved in behavior, androgen receptor function, sympathetic nervous system function and the renin angiotensin system (RAS). We have demonstrated the interaction of Sry with tyrosine hydroxylase and increased BP following exogenous Sry expression in normotensive WKY rats [9]. Dewing et al demonstrated that delivering Sry antisense oligonucleotides to the rat substantia nigra led to measurable motor defects in male rats [10], and suggests that the effect of Sry expression is partially responsible for sexual dimorphism in brain cells. They interpreted these results as a potential cellular sex determination in the brain not mediated by gonadal hormones. Arnold's research group has introduced a powerful model system in mice for examining the separate and interactive effects of sex chromosomes and gonadal secretions on sexually dimorphic phenotypes. They used mice in which the testis-determining gene, Sry, was moved from the Y chromosome to an autosome (by deletion of Sry from the Y and subsequent insertion of an Sry transgene onto an autosome), so that the determination of testis development occurred independently of the complement of X or Y chromosomes [11]. They compared

XX and XY mice with ovaries (females) and XX and XY mice with testes (males). These comparisons allowed an assessment of the effect of sex chromosome complement (XX vs XY) independent of gonadal status (testes vs ovaries) on sexually dimorphic neural and behavioral phenotypes. These studies are consistent with a role for sex chromosome genes in neural and behavioral sexual differentiation. For instance, using this model mouse system that allows comparison of the effects of genes or hormones on sexually dimorphic traits, De Vries et al demonstrated that XY males differed from XY^{-Sry} by several measures: latency to thrust, the number of total visits and visits to males in the social exploration tests, and tyrosine hydroxylase immunoreactive neurons of the anteroventral periventricular nucleus of the preoptic region [11]. D'Souza et al in a review of the relationship between transcriptional regulation and aggressive behavior suggest transcription factors like Sry may be important regulators of aggression [12]. Ohe et al. showed that SRY and SOX co-localize with splicing factors, implicating SRY in pre-mRNA splicing [13]. In a yeast two hybrid screen, SRY was shown to recruit KRAB as a chromatin modulator [14]. Direct interaction between androgen receptor (AR) and SRY proteins was reported [15], and SRY repressed AR transactivation. Thevenet et al. isolated a protein, SIP-1, that interacts with human and mouse Sry proteins [16]. SRY and WT1 activate transcription synergistically in an SRY binding site-dependent manner, and WT1 is recruited to SRY-binding sites in an SRY-dependent manner [17].

Consistent with additional functions, the Sry protein has potential target loci that have been identified from in vitro studies of DNA binding. SRY binding sites have been identified in a number of genes [18] including: P450 aromatase, anti-mullerian hormone (AMH), Fra-1 and Fra-2 [19]. While the P450 aromatase and AMH are known components of mammalian sex determination, the Fra-1 and Fra-2 loci are components of transcription factor AP-1 [20] and are not involved in sex determination.

The AP-1 transcription factor is not involved in testis determination, but is present and has been shown to be active in the promoters of angiotensinogen (Agt) and other RAS genes [21]. The consensus binding site for SRY co-localizes with OCT4. Promoters bound by OCT4 and SRY, but not those bound by SRY alone, were also bound by the transcription repressor KAP1 [22], consistent with our data showing that Sry can serve as either a positive or a negative regulator of promoter activity [22]. Recently Rath et al have demonstrated that SRY represses transcription of the senescence marker protein 30 in the liver [23].

In addition to these molecular, neural and behavioral effects, we have delivered Sry to kidney and adrenal gland and increased BP and other phenotypes [24,25]. These differences would not be expected if Sry were non-functional in adult males. As a transcription factor, Sry expression in any cell or tissue has the potential to modify the genes expressed in that cell. If a mutation causes a transcription factor to be expressed in a tissue, the transcriptome of that cell or tissue can change and this change could have either subtle or large affects in the phenotype of that cell or tissue.

Multiple Copies of Sry Loci in *Rattus norvegicus* (Table 1)

A unique characteristic of the rat Sry complex is that there are multiple Sry loci on a single Y chromosome. The Sry gene complex from a single *R. norvegicus* Y chromosome from the Spontaneously Hypertensive Rat strain (SHR/Akr) have been sequenced and analyzed for homology among copies [26]. The analysis of these sequences identified seven different Sry loci on the Y chromosome (Sry1, Sry2, Sry3, Sry3A, Sry3BI, Sry3B and Sry3C). These are paralogous copies consistent with a single phylogeny and the divergence between any two copies is less than 2% and there is no evidence of gene conversion. All copies have a conserved reading frame and amino acid sequence consistent with function [26]. Consistent

with Sry as the hypertensive Y chromosome locus on the SHR Y chromosome, the hypertensive Y chromosome had a copy not present on normotensive Y chromosomes (Sry3A).

Although the SHR/WKY Y chromosomes have multiple copies of Sry (WKY 6, SHR 7), the proteins of all copies are extremely similar. Most mutations between copies do not change the amino acid sequence of the protein. The open reading frame encodes a 169 amino acid protein in Sry1, Sry3, Sry3A and Sry3C, a 170 amino acid protein in Sry3B and Sry3BI, and a 156 amino acid protein in Sry2. Consistent with comparisons of Sry between species the HMG box region amino acid sequence is highly conserved. Sry1 has one difference (amino acid 38) that is unique in the HMG box region, all other amino acid differences in the HMG box region are also found in human or mouse. Amino acid differences for each copy compared to a consensus amino acid sequence; Sry1 (2 differences), Sry2 (3 differences, 13 aa deletion), Sry3 (0 differences), Sry3A (1 difference), Sry3B (1 added aa), Sry3BI (1 difference, 1 added aa), Sry3C (0 differences, Table 1).

The loci of the Sry complex are unique in that all loci contain a conserved reading frame which produces protein with transcriptional activity. All loci are expressed and different tissues have different transcripts as the most frequently expressed transcript. They seem to be controlled differently as the 5' flanking regions have diverged more than the coding regions. These do not seem to be the classic duplicate genes that are not transcribed and nonfunctional or identically expressed. The mammalian Y chromosome contains numerous pseudogenes, but these 7 loci show no characteristics of either being or becoming pseudogenes. The Sry gene complex on the SHR Y chromosome contains 7 very closely related genes that appear to have diverged to the point where the individual genes may be specialized for different subsets of functions, whereas in a species like human that may have one SRY gene, all these functions must be performed by a single gene. For the human SRY gene to have multiple functions, as few as one mutation, not SRY binding or amino acid sequence, could be enough to affect SRY expression.

Human Sry

Whether Sry and the presence of multiple copies of Sry has human relevance, remains unknown as a research question. Two recent studies reported copy number variation of the SRY genes in human males, with as many as 16 copies present in some individuals in a population exposed to background radiation [27,28]. Whether multiple human SRY copies are functional remains to be determined. The presence of multiple copies in humans lends support to our studies of the function of the multiple rat Sry loci. In our studies, Sry3, the locus unique to the hypertensive SHR/Akr Y chromosome, consistently caused the largest effects as a regulator of RAS gene transcription. If the BP effects are simply an additive effect of the contribution of each of the Sry loci, then the presence of the Sry 3 locus in hypertensive male rats but not in normotensive males may be sufficient to raise BP into the hypertensive range.

Sry Transcript Expression

Using a fragment analysis procedure with cDNA samples from SHR/y testis and SHR/y adrenal gland allow the identification of which specific Sry loci are expressed. Their proportions and expression patterns were significantly different in the testis and adrenal gland [26]. We have evidence that each locus is expressed. Some loci (Sry1, Sry2, Sry3 (3A) and Sry3C) are expressed in multiple tissues and in proportions that are greater than 25% of the total Sry transcripts in some tissues. The other two loci (Sry3B and Sry3BI) are rarely expressed at greater than 5% of the total transcripts in any tissue.

The Sry Protein

Sry (Sex-determining region of the Y chromosome), is an intronless gene that encodes a protein with a highly conserved 79–80 aa High Mobility Group (HMG) box DNA binding domain. Sry shares homology with members of the HMG-1/HMG-2 protein families [29]. In vitro Sry interacts with DNA in a sequence specific manner with a high binding affinity ($K_d \sim 10^{-9} - 10^{-10}$ nM) [30]. Sry-DNA interactions occur at the minor groove rather than the major groove, thus it is likely that this interaction would interfere sterically with protein-DNA interactions of other nearby DNA binding proteins. This is consistent with the idea that Sry interacts with DNA as a complex, with one or more proteins in vivo. Identification of a preferred high affinity response element for Sry in vitro (ACAATAG) [31], has not aided in the continued hunt for downstream targets, since this sequence is extremely common in promoter regions. The idea of Sry binding as part of a mammalian gene complex of regulatory proteins has complicated the identification of definitive direct DNA target for Sry in vivo. Evidence suggests Sry likely interacts with and regulates expression of the developmental genes: SOX 1, 3, 9; DAX 1; and Mullerian Inhibiting Substance (MIS) [32,33].

After interacting with a target DNA consensus, Sry induces a characteristic species-specific 65–80° bend [34], characteristic of an architectural transcription factor. The alteration in DNA structure is hypothesized to enhance interactions with additional DNA binding proteins both near and distant to the bend site [35–37].

As a transcription factor, Sry must localize to the nucleus. The protein has two basic aa regions (composed of primarily R and K residues) that flank the HMG box and function independently as nuclear localization sequences, the N-terminal NLS (nNLS) and C-terminal NLS (cNLS) [38]. Studies indicate that both NLSs are necessary for testis development [38], although the pathways thought to mediate Sry nuclear translocation via the nNLS or cNLS are believed to be different.

The bipartite nNLS of Sry does not appear to interact with importin α or β in vitro [39,40]. Instead, the nNLS is part of an extended aa consensus that interacts with calmodulin in an ATP- and Ca^{2+} -dependent manner, facilitating nuclear import via a Ran-GTP independent pathway [40–42]. While this type of nuclear import is not well understood, transport from the cytoplasmic compartment to the nucleus is thought to be driven by DNA concentration and binding [43]. Nuclear localization of Sry directed by interactions with Ca^{2+} activated calmodulin could link Sry nuclear translocation to many different signal transduction pathways.

Sry proteins can be divided into two groups with the HMG box located either centrally or N-terminally. In rodents, the HMG box is located near the N-terminus with 2–4 aa preceding the HMG box. In all non-rodent species, the HMG box is located at the center of the protein. The HMG box is flanked with 50–65 aa's N-terminally and about 60–65 aa's C-terminally. The significance of this region has not been determined, however serine 33 in the human flanking region has been demonstrated to act as a PKA site, which, when phosphorylated increases Sry binding affinity to DNA [44]. This is the only phosphorylation site positively identified in human SRY. In most rodent species, the C-terminal region is divided into two sections, a polyglutamine (Q-rich) activation domain and a hinge region that links the HMG box to the Q-rich region. The C-terminal flanking region in non-rodent Sry includes what is considered to be the hinge region in rodent Sry, with no Q-rich motif is conserved. This raises the question of whether the rodent polyglutamine region is essential for function.

In mouse it has been demonstrated that the Q-rich motif is required for testis development and species specific protein function. It is believed to facilitate interactions with other

transcription modulators [45–47]. Human Sry lacks a Q-rich domain. Recent reports suggest an interaction with a PDZ domain protein, termed SRY interacting protein-1 (SIP-1) [48]. This protein-protein interaction is mediated through a small aa consensus at the C-terminus of the human hinge region and is thought to facilitate interactions with transcription regulators in much the same way as the activation region in mouse. While the presence of a Q-rich region and interactions with SIP-1 suggest that Sry functions as an activator of transcription, early research using an Sry regulated reporter assay indicated that Sry can also function as a repressor [49]. Recently, support for Sry transcriptional repression come from studies that describes another protein-protein interaction via the hinge region with the protein KRAB-O [50,51]. This protein contains a Kruppel-associated box domain and unlike SIP-I, which associates with human Sry at the C-terminus of the hinge region, interacts with an aa motif near the HMG box. Interestingly this same region interacts with SIP-I in mice and most likely other rodent species [16]. KRAB-O is implicated in interactions with complexes that are linked to gene silencing. The importance of this interaction is indicated by the fact that mutations in Sry that prevent interaction with KRAB-O result in XY sex reversal [52].

Additionally, Sry is shown to directly bind to proteins such as Wilm's tumor gene 1 (WT-1) [53] and androgen receptor (AR) [54] via the HMG box. The Sry-WT-1 complex is thought to enhance Sry activity by using the activation domain encoded by WT-1, which supports the concept of Sry binding DNA with a partner or as a complex, while Sry-AR complex formation prevents AR interactions with target promoters. The direct repression of AR by Sry is implicated in reducing prostate cancer, since initiation of prostate cancer often shows reduced expression of Sry and other Y linked loci [54,55]. This direct interaction with AR also supports a role of Sry in maintaining adult male physiology.

The Sry proteins encoded on the rat Y chromosome all have the potential to express a functional protein. All are very similar. However, aa differences at regions of functional significance, such as the nNLS, C-terminal kinetic clamp and Q-rich region, indicate that in vivo each might carry out a specific function.

At the protein level, the organization and aa sequences of rat and mouse Sry are quite similar up to the Q-rich region, which in rat is 12–26 aa long compared to the 240 aa domain in some mice [47]. Excluding the Q-rich region, rat Sry2 shares greatest sequence identity with mouse proteins, particularly in the nNLS at residue 21, where both species encode an H. At this position, the remaining rat proteins encode an R, conserved in all mammalian species except for mouse. Rat Sry3-like proteins encode an aa difference at position 76, in the cNLS, a T rather than a P, that is not conserved in Sry proteins of any other mammalian species and has the potential to alter the architecture of the C-terminal extremity of the HMG domain.

Renin-Angiotensin System Genes

The renin-angiotensin system consists of both the classical form in the circulatory system and local tissue RASs. To have a significant effect on the RAS, an effective regulator of expression of RAS genes would affect more than one gene. For example, a small effect on the angiotensinogen (Agt) gene alone might not be sufficient to shift the RAS from generating predominately vasoconstrictors to vasodilators. Because of multiple overlapping compensatory systems to maintain BP, a subtle change in transcription of Agt might be masked. The more genes in the cascade that are modulated by the transcriptional regulator, the larger the overall effect can be. This is the case with Sry. Sry increases activity of the promoters of Agt, renin and ACE and it decreases ACE2 promoter activity [22]. This could favor the generation of Ang II and repress Ang-(1–7) levels.

Examination of promoter sequences of Agt, renin, ACE and ACE2 revealed the presence of consensus Sry-binding sites in each [56], consistent with the potential of Sry to bind these genes and affect their expression. Small changes in expression patterns of Agt, renin, ACE and ACE2, the major participants of the classical RAS, could serve to shift the relative amounts of production of the vasoconstriction or pro-hypertension peptide Ang II compared to the vasodilation or anti-hypertension peptide Ang-(1–7). If this change in expression occurred in response to Sry, it could provide another mechanism for gender-related differences in blood pressure.

Delivery Protocol for Exogenous Sry Electroporation to Target Tissues

In order to determine potential function of Sry loci in tissues that may have blood pressure regulation ability, experiments were designed to deliver exogenous Sry to adrenal gland and kidney. Either Sry1/pcDNA3.1(–), Sry2, Sry3 or control pcDNA3.1(–) vector without Sry sequences were electroporated into the left kidney medulla or adrenal medulla of adult male WKY rats under anesthetic [24,25]. Sry transcripts from the the plasmid, cannot be distinguished from endogenous Sry, therefore the use of females for verification. In order to verify that this procedure delivered Sry to the kidney, two WKY females were electroporated with Sry1 delivered to the kidney. Total DNA was screened for the presence of Sry1 by gel-based PCR. The females that had Sry1 electroporated in the left kidney were positive for Sry in the left kidney after 8 days, they were negative in the right kidney and positive in the liver. After 21 days the left and right kidney were negative but the liver remained positive. Histological examination of successive sections through the kidney medulla did not show any morphological differences between non-electroporated controls, control vector, or Sry1 delivered. To determine which tissues express Sry following electroporation, we delivered an expression construct containing an Sry3B1/Myc fusion protein to female rats. Figure 1 shows fusion proteins detected with a Myc antibody in tissue lysates of female rats 5 days after gene delivery. Lanes 1–5: Sry3BI/Myc was introduced hemodynamically via the saphenous vein. Lane 1, kidney, lanes 2, 3 lung, Lanes 4, 5, liver, Lanes 6 and 7: Sry3BI/Myc electroporated into the kidney (Figure 1).

Sry1 and SNS Activation: Kidney Effects

In order to explore the mechanism of the BP elevation based on our earlier observations, we measured indices of the SNS - plasma catecholamines, tissue catecholamines and markers of the renin angiotensin system-plasma renin, tissue angiotensin II, renin and ACE. There was a significant increase in telemetered BP (15–20mmHg) after Sry1 delivery compared to control vector at days 7–21 which was normalized after 28 days [25]. When Sry2 was delivered to the kidney in the same manner there was no BP effect. There was also a significant and consistent elevation in pressor response to air stress in the Sry1 group (41mmHg) compared to controls (28 mmHg). The pressor response to air stress was completely blocked in both Sry1 and control animals treated with the alpha blocker prazosin. The BP responses were not explained by heart rate. When Sry2 was delivered to the kidney in the same way there was no difference in BP response to air stress between Sry2 and controls. Renal tyrosine hydroxylase was significantly increased compared to the vector control 21 days after Sry1 delivery to the kidney, however Sry2 did not produce any significant difference compared to controls. With regard to kidney catecholamine content there was a significant increase in NE and dopamine content between animals delivered Sry1 as compared to controls or Sry2 at 35 days. Plasma NE was significantly elevated 21 days after Sry1 delivery compared to vector control.

Creatinine clearance, urinary protein, sodium and BUN were not significantly different between groups and were within normal range, suggesting that the electroporation procedure

did not cause altered renal function. Urinary Na was also measured at 7–28 days after electroporation and was not significantly different between groups. The pre-surgery baseline and weekly plasma levels of renin, aldosterone, and corticosterone for the Sry1 and control vector groups were not significantly different between treatment groups for each plasma hormone at all time periods sampled. Seven days after Sry1 delivery to the kidney urinary dopamine and NE were significantly elevated but returned to baseline levels with time.

Most likely, the elevated urine dopamine was a compensation to the elevated plasma NE. The counter balance to renal SNS-NE effects and sodium retention is the effect of renal dopamine as a natriuretic factor. Renal dopamine does not derive from the SNS or the typical neuronal NE pathway but instead proximal tubule cells use circulating or filtered L-DOPA as a source of dopamine in a paracrine fashion [57]. So the urinary excretion of dopamine reflects the renal production of the amine. With regard to differences between SHR and WKY males, SHR males excrete more dopamine and NE than WKY males across varying sodium intakes [58] even though the dopamine receptor density is not different [59]. After 7 days there was almost a doubling of urinary dopamine in Sry1 animals compared to vector controls. The elevated SNS activity induced by Sry reflected as increased plasma and urinary NE throughout this period, may have normalized the sodium excretion. It has been shown that increased renal SNS activity increases renin secretion rate and decreases urinary sodium excretion [60].

Sry3 and Renin Angiotensin System Activation: Kidney Effects (Figure 2.)

After testing the effects of Sry1 and Sry2 we studied Sry3 effects on the kidney. Sry3 significantly raised telemetered BP compared to controls about 10mmHg after 7 and 14 days and about 20mmHg after 21 days with BP normalization at 28 days [61]. Sry3 did not significantly elevate BP in response to air stress compared to controls, whereas Sry1 did [61]. The acute pressor response to air stress supports the idea that when Sry1 was exogenously delivered there was a rapid pressor response consistent with elevated SNS activity, whereas when Sry3 was delivered there was no elevation in pressor response. Plasma renin activity increased after delivery of Sry3 at 14 days compared to controls [61]. Sry3 increased renal angII content compared to controls at 14 and 21 days. SNS indices were not elevated by Sry3 at 21 days as compared to controls whereas Sry1 raised TH, dopamine and norepinephrine. The major finding with Sry on kidney physiology was the increased (40%) Na reabsorption [61]. Most likely, due to the increased plasma renin and renal angiotensin II sodium reabsorption was increased. This may have contributed to the BP rise but further studies need to be done. Further support for this idea is that Sry influences the promoters of angiotensinogen, renin and ACE and since we have observed activation of the RAS with Sry this would favor sodium reabsorption.

Sry1 and Sry3 Adrenal Effects

Similar to our kidney experiments we examined the effects of Sry electroporation into the adrenal medulla. We tested Sry1 and Sry3 but have not studied Sry2. There was a significant increase in BP (30mmHg) 3 weeks after Sry1 delivery to the adrenal medulla as compared to control vector [24]. Tyrosine hydroxylase was significantly increased compared to the vector control (48% and 57% respectively). With regard to Sry1 effects on adrenal catecholamine content there were no significant differences between groups after 3 weeks. Sry3 electroporated into the adrenal gland did not raise BP or adrenal tyrosine hydroxylase or catecholamine content (unpublished results).

Summary and Conclusions

Our earlier studies showed that males with the SHR Y chromosome had elevated indices of SNS activity and BP was elevated [62]. Focusing on Sry as the Y chromosome locus showed that specifically Sry1 given exogenously to the kidney or adrenal gland elevated BP and SNS markers [24,25]. When Sry2 was delivered to the kidney BP was not affected. However, when Sry3 was delivered to the kidney the renin angiotensin system was activated along with sodium retention and BP elevation but SNS markers were not affected [60]. The Sry3 locus in SHR may synergistically act with Sry1 to elevate BP pressure through an interaction of the SNS and RAS. The differential function of Sry1 vs. Sry3 may be due to the substitution of a glutamine for a histidine at amino acid 37 in the HMG box in Sry3.

The findings presented here confirm and extend the argument for Sry1 and Sry3 as at least two of the genes responsible for the hypertensive Y chromosome phenotype. These results are consistent with an increased SNS activity mechanism due to Sry1, and increased tissue renin angiotensin system activity due to Sry3. The functions of the other copies of the Sry complex remain to be determined.

Acknowledgments

The authors are grateful for grant support from: NIH (RO1-HL71579-01A3), Department of Biology and the Ohio Board of Regents. We appreciate the help of Emily Njus, Bob Zickelfoose, Larry Shirkey and Danny Elliott for animal care and welfare.

References

1. Ely D, Turner M, Milsted A. Review of the Y chromosome and hypertension. *Braz J Med Biol Res* 2000;33:679–91. [PubMed: 10829096]
2. Hajjar I, Kotchen TA. Trends in prevalence, awareness, treatment, and control of hypertension in the United States, 1988–2000. *JAMA* 2003;290:199–206. [PubMed: 12851274]
3. Cutler JA, Sorlie PD, Wolz M, Thom T, Fields LE, Roccella EJ. Trends in hypertension prevalence, awareness, treatment, and control rates in United States adults between 1988–1994 and 1999–2004. *Hypertension* 2008;52:818–27. [PubMed: 18852389]
4. Ely DL, Turner M. Hypertension in the spontaneously hypertensive rat is linked to the Y chromosome. *Hypertension* 1990;16:270–81.
5. Ellis JA, Stebbing M, Harrap SB. Association of the human Y chromosome with high blood pressure in the general population. *Hypertension* 2002;39:353–56. [PubMed: 11882572]
6. Charchar FJ, Tomaszewski M, Padmanabhan S, Lacka B, Upton MN, Inglis GC, et al. The Y chromosome effect on blood pressure in two European populations. *Hypertension* 2000;36:731–33. [PubMed: 11082135]
7. Nachman MW, Aquadro CF. Polymorphism and divergence at the 5' flanking region of the sex-determining locus, Sry. *Mol Bio Evol* 1994;11:539–47. [PubMed: 8015446]
8. Schafer AJ, Goodfellow PN. Sex determination in humans. *BioEssays* 1996;18:955–63. [PubMed: 8976152]
9. Milsted A, Serova L, Sabban E, Dunphy G, Turner M, Ely D. Regulation of tyrosine hydroxylase gene transcription by Sry. *Neuroscience Letters* 2004;369:203–07. [PubMed: 15464265]
10. Dewing P, Chiang CWK, Sinchak K, Sim H, Fernagut P-O, Kelly S, et al. Direct regulation of adult brain function by the male-specific factor SRY. *Current Biology* 2006;16:415–20. [PubMed: 16488877]
11. DeVries GJ, Rissman EF, Simerly RB, Yang L-Y, Scordalakes EM, Auger CJ, et al. A model system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits. *J Neurosci* 2002;22:9005–14. [PubMed: 12388607]
12. D'Souza UM, Kel A, Sluyter F. From transcriptional regulation to aggressive behavior. *Behavior Genetics* 2003;33:549–62. [PubMed: 14574132]

13. Ohe K, Lalli E, Sassone-Corsi P. A direct role of SRY and SOX proteins in premRNA splicing. *Proc Natl Acad Sci U S A* 2002;99:1146–51. [PubMed: 11818535]
14. Oh HJ, Li Y, Chris Lau Y-FC. Sry Associates with the Heterochromatin Protein 1 Complex by Interacting with a KRAB Domain Protein. *Biology of Reproduction* 2005;72:407–15. [PubMed: 15469996]
15. Yuan X, Lu ML, Li T, Balk SP. SRY interacts with and negatively regulates androgen receptor transcriptional activity. *J Biol Chem* 2001;276:46647–54. [PubMed: 11585838]
16. Thevenet L, Albrecht KH, Malki S, Berta P, Boizet-Bonhoure B, Poulat F. NHERF2/SIP-1 interacts with mouse SRY via a different mechanism than human SRY. *J Biol Chem* 2005;280:38625–30. [PubMed: 16166090]
17. Matsuzawa-Watanabe Y, Inoue J, Semba K. Transcriptional activity of testis-determining factor SRY is modulated by the Wilms' tumor 1 gene product, WT1. *Oncogene* 2003;22:7900–4. [PubMed: 12970737]
18. Cohen DR, Sinclair AH, McGovern JD. Sry protein enhances transcription of Fos-related antigen 1 promoter constructs. *Proc Natl Acad Sci USA* 1994;91:4372–76. [PubMed: 8183916]
19. Haqq CM, King CY, Donahoe PK, Weiss MA. Sry recognizes conserved DNA sites in sex-specific promoters. *Proc Natl Acad Sci USA* 1993;90:1097–01. [PubMed: 8430080]
20. Foletta VC. Transcription factor AP-1, and the role of Fra-2. *Immunol & Cell Biol* 1996;74:121–33. [PubMed: 8723999]
21. Milsted A, Underwood AC, Dunmire J, DelPuertoc HL, Martins AS, Ely DL, et al. Regulation of multiple renin–angiotensin system genes by Sry. *J Hyper*. 2009 in press.
22. Jin VX, O'Geen H, Iyengar S, Green R, Farnham PJ. Identification of an OCT4 and SRY regulatory module using integrated computational and experimental genomics approaches. *Genome Res* 2007;17:807–17. [PubMed: 17567999]
23. Rath B, Pandey RS, Debata PR, Maruyama N, Supakar PC. Molecular characterization of senescence marker protein-30 gene promoter: Identification of repressor elements and functional nuclear factor binding sites. *BMC Molecular Biology* 2008;9:43. [PubMed: 18442420]
24. Ely D, Milsted A, Bertram J, Ciotti M, Dunphy G, Turner M. Sry delivery to the adrenal medulla increases blood pressure and adrenal medullary tyrosine hydroxylase of normotensive WKY rats. *BMC Cardiovascular Disorders* 2007;7:6. [PubMed: 17324261]
25. Ely DL, Milsted A, Dunphy G, Boehme S, Dunmire J, Hart M, et al. Delivery of sry1, but not sry2, to the kidney increases blood pressure and sns indices in normotensive wky rats. *BMC Physiology* 2009;9:10. [PubMed: 19500370]
26. Turner ME, Martin C, Martin AS, Dunmire J, Farkas J, Ely DL, et al. Genomic and expression analysis of multiple Sry loci from a single Rattus norvegicus Y chromosome. *BMC Genetics* 2007;8:11. [PubMed: 17408480]
27. Premi S, Srivastava J, Chandy SP, Ahmad J, Ali S. Tandem duplication and copy number polymorphism of the SRY gene in patients with sex chromosome anomalies and males exposed to natural background radiation. *Mol Human Reprod* 2006;12:113–21.
28. Premi S, Srivastava J, Panneer G, Ali S. Startling mosaicism of the Y-chromosome and tandem duplication of the SRY and DAZ genes in patients with Turner Syndrome. *PLoS ONE* 2008;3:e3796. Epub 2008 Nov 24. [PubMed: 19030103]
29. Werner MH, Huth JR, Gronenborn AM, Clore GM. Molecular basis of human 46 X,Y sex reversal revealed from the three-dimensional solution structure of the human SRY-DNA complex. *Cell* 1995;81:705–14. [PubMed: 7774012]
30. Harley VR, Jackson DI, Hextall PJ, Hawkins JR, Berkovitz GD, et al. DNA binding activity of recombinant SRY from normal males and XY females. *Science* 1992;255:453–6. [PubMed: 1734522]
31. Harley VR, Lovell-Badge R, Goodfellow PN. Definition of a consensus DNA binding site for SRY. *Nucleic Acids Res* 1994;22:1500–1. [PubMed: 8190643]
32. Goodfellow PN, Lovell-Badge R. SRY and sex determination in mammals. *Annu Rev Genet* 1993;27:71–92. [PubMed: 8122913]
33. Graves JA. Interactions between SRY and SOX genes in mammalian sex determination. *Bioessays* 1998;20:264–9. [PubMed: 9631654]

34. Werner MH, Huth JR, Gronenborn AM, Clore GM. Molecular determinants of mammalian sex. *Trends Biochem Sci* 1996;21:302–8. [PubMed: 8772384]
35. Pontiggia A, Rimini R, Harley VR, Goodfellow PN, Lovell-Badge R, Bianchi ME. Sex-reversing mutations affect the architecture of SRY-DNA complexes. *Embo J* 1994;13:6115–24. [PubMed: 7813448]
36. Li B, Phillips NB, Jancso-Radek A, Ittah V, Singh R, et al. SRY-directed DNA bending and human sex reversal: reassessment of a clinical mutation uncovers a global coupling between the HMG box and its tail. *J Mol Biol* 2006;360:310–28. [PubMed: 16762365]
37. Phillips NB, Jancso-Radek A, Ittah V, Singh R, Chan G, et al. SRY and human sex determination: the basic tail of the HMG box functions as a kinetic clamp to augment DNA bending. *J Mol Biol* 2006;358:172–92. [PubMed: 16504207]
38. Sudbeck P, Scherer G. Two independent nuclear localization signals are present in the DNA-binding high-mobility group domains of SRY and SOX9. *J Biol Chem* 1997;272:27848–52. [PubMed: 9346931]
39. Harley VR. The molecular action of testis-determining factors SRY and SOX9. *Novartis Found Symp* 2002;244:57–66. [PubMed: 11990798]
40. Sim H, Rimmer K, Kelly S, Ludbrook LM, Clayton AH, Harley VR. Defective calmodulin-mediated nuclear transport of the sex-determining region of the Y chromosome (SRY) in XY sex reversal. *Mol Endocrinol* 2005;19:1884–92. [PubMed: 15746192]
41. Harley VR, Lovell-Badge R, Goodfellow PN, Hextall PJ. The HMG box of SRY is a calmodulin binding domain. *FEBS Lett* 1996;391:24–8. [PubMed: 8706923]
42. Sweitzer TD, Hanover JA. Calmodulin activates nuclear protein import: a link between signal transduction and nuclear transport. *Proc Natl Acad Sci U S A* 1996;93:14574–9. [PubMed: 8962094]
43. Hanover JA, Love DC, DeAngelis N, O’Kane ME, Lima-Miranda R, et al. The High Mobility Group Box Transcription Factor Nhp6Ap enters the nucleus by a calmodulin-dependent, Ran-independent pathway. *J Biol Chem* 2007;282:33743–51. [PubMed: 17878171]
44. Desclozeaux M, Poulat F, de Santa Barbara P, Capony JP, Turowski P, et al. Phosphorylation of an N-terminal motif enhances DNA-binding activity of the human SRY protein. *J Biol Chem* 1998;273:7988–95. [PubMed: 9525897]
45. Tucker PK, Lundrigan BL. Rapid evolution of the sex determining locus in Old World mice and rats. *Nature* 1993;364:715–7. [PubMed: 8355784]
46. Miller KE, Lundrigan BL, Tucker PK. Length variation of CAG repeats in Sry across populations of *Mus domesticus*. *Mamm Genome* 1995;6:206–8. [PubMed: 7749230]
47. Bowles J, Cooper L, Berkman J, Koopman P. Sry requires a CAG repeat domain for male sex determination in *Mus musculus*. *Nat Genet* 1999;22:405–8. [PubMed: 10431249]
48. Poulat F, Barbara PS, Desclozeaux M, Soullier S, Moniot B, et al. The human testis determining factor SRY binds a nuclear factor containing PDZ protein interaction domains. *J Biol Chem* 1997;272:7167–72. [PubMed: 9054412]
49. McElreavey K, Vilain E, Abbas N, Herskowitz I, Fellous M. A regulatory cascade hypothesis for mammalian sex determination: SRY represses a negative regulator of male development. *Proc Natl Acad Sci U S A* 1993;90:3368–72. [PubMed: 8475082]
50. Oh HJ, Li Y, Lau YF. Sry associates with the heterochromatin protein 1 complex by interacting with a KRAB domain protein. *Biol Reprod* 2005;72:407–15. [PubMed: 15469996]
51. Oh HJ, Lau YF. KRAB: a partner for SRY action on chromatin. *Mol Cell Endocrinol* 2006;247:47–52. [PubMed: 16414182]
52. Mitchell CL, Harley VR. Biochemical defects in eight SRY missense mutations causing XY gonadal dysgenesis. *Mol Genet Metab* 2002;77:217–25. [PubMed: 12409269]
53. Matsuzawa-Watanabe Y, Inoue J, Semba K. Transcriptional activity of testis-determining factor SRY is modulated by the Wilms’ tumor 1 gene product, WT1. *Oncogene* 2003;22:7900–4. [PubMed: 12970737]
54. Yuan X, Lu ML, Li T, Balk SP. SRY interacts with and negatively regulates androgen receptor transcriptional activity. *J Biol Chem* 2001;276:46647–54. [PubMed: 11585838]

55. Perinchery G, Sasaki M, Angan A, Kumar V, Carroll P, Dahiya R. Deletion of Y-chromosome specific genes in human prostate cancer. *J Urol* 2000;163:1339–42. [PubMed: 10737540]
56. Warner F, Lew RA, Smith AI, Lambert DW, Hooper NM, Turner AJ. Angiotensin-converting enzyme 2 (ACE2), but not ACE, is preferentially localized to the apical surface of polarized kidney cells. *J Biol Chem* 2005;280:39353–62. [PubMed: 16166094]
57. Soares-da-Silva. Source and handling of renal dopamine: Its physiological importance. *NIPS* 1994;9:128–34.
58. Hansell P, Isaksson B, Sjoquist M. Renal dopamine and noradrenaline excretion during CNS-induced natriuresis in SHR: influence of dietary sodium. *Acta Physiol Scand* 2000;168:257–66. [PubMed: 10691810]
59. Cachero S, Van-Liew JB, Feld LG. Long-term renal handling of sodium and calcium in spontaneously hypertensive rats. *Renal Physiol Biochem* 1992;15:83–88. [PubMed: 1375767]
60. Di Bona G. Neural control of the kidney: functionally specific renal sympathetic nerve fibers. *Am J Physiol Regul Integr Comp Physiol* 2000;279:R1517–24.
61. Milsted A, Underwood A, Dunphy G, Boehme S, Martins A, Turner M. Sry3 exogenously delivered to the WKY kidney increases the renal renin-angiotensin system. *The FASEB Journal* 2009;23:806. (abstract). [PubMed: 18952709]
62. Caplea A, Seachrist D, Daneshvar H, Dunphy G, Ely D. Noradrenergic content and turnover rate in kidney and heart shows gender and strain differences. *J Appl Physiol* 2002;92:567–71. [PubMed: 11796665]

Kidney Lung Lung Liver Liver Kidney Kidney

Figure 1.

Myc antibody was used to detect Sry3BI/Myc proteins in tissue lysates of female rats 5 days after gene delivery. Lanes 1–5: Sry3BI/Myc was introduced via the saphenous vein. Lane 1, kidney, lanes 2, 3 lung, Lanes 4, 5, liver, Lanes 6 and 7: Sry3BI/Myc electroporated into the kidney.

Figure 2.

Telemetered blood pressure before Sry gene delivery and through 28 days after Sry electroporation into the left kidney of WKY male rats (n=6–8/group, means, $\pm$ s.e.m., $p < 0.05$ for Sry1 and Sry3 compared to controls or Sry2, ANOVA).

sequence Comparison
sequence for each of the
is underlined.

sequence for each of the 7 copies of Sry found on the SHR Y chromosome compared to the amino acid sequence of Sry1. The HMG is underlined.

Steroids. Author manuscript; available in PMC 2011 November 1.

indicate amino acids that are deleted in Sry3B and Sry3B1. Consensus sequence is in black and differences are red.