Original Article

Introgressed chromosome 2 quantitative trait loci restores aldosterone regulation and reduces response to salt in the stroke-prone spontaneously hypertensive rat

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Background: The genetic contribution to salt-sensitivity in hypertension remains unclear. We have previously identified a quantitative trait locus on chromosome 2 in stroke-prone spontaneously hypertensive rats (SHRSPs) responsible for an increase in SBP in response to a salt challenge. This response is blunted in the congenic SHRSP strain with the Wistar–Kyoto (WKY) chromosome 2 region (10 cM) introgressed (SP.WKY$_{Gla}$2k). We aimed to discover the mechanisms that underlie the effects of this region on salt-handling in the SHRSP strain.

Method: Renal and adreno-cortical function were compared in the WKY, SHRSP and the congenic SP.WKY$_{Gla}$2k strains.

Results: In response to the salt challenge, all strains excreted more sodium, but the SHRSP strain excreted more protein and a greater amount of sodium compared with either the WKY or the SP.WKY$_{Gla}$2k strain (0.19 ± 0.02 vs. 0.12 ± 0.01 g/24 h and 0.09 ± 0.02 g/24 h, respectively). Glomerular filtration was not affected by diet or genotype, but renal plasma flow was decreased in the SP.WKY$_{Gla}$2k and SHRSP strains. The SHRSP strain had higher plasma aldosterone in association with greater adrenal CYP11B2 (aldosterone synthase) and 3β hydroxysteroid dehydrogenase mRNA gene expression when compared to the WKY strain. Strikingly, introgression of the WKY chromosome 2 region into the SHRSP strain corrected the proteinuria and reduced sodium excretion, plasma aldosterone levels and 3β hydroxysteroid dehydrogenase mRNA gene expression in response to the salt challenge when compared to the SHRSP strain. Glucocorticoid levels and markers of glucocorticoid synthesis were unaffected.

Conclusion: Our findings suggest that introgression of the chromosome 2 congenic interval from the WKY into the SHRSP strain is associated with restored aldosterone regulation sufficient to reduce salt-sensitive hypertension and proteinuria.

Keywords: aldosterone, cardiovascular disease – salt intake, gene expression/regulation, genetics – animal models

Abbreviations: ANOVA, analysis of variance; cDNA, complimentary DNA; eRPF, effective renal plasma flow; GFR, glomerular filtration rate; PAH, para-aminohippuric acid; QTL, quantitative trait locus; SHRSP, stroke-prone spontaneously hypertensive rat; SP.WKY$_{Gla}$2k, chromosome 2 congenic rat strain, comprising the stroke-prone spontaneously hypertensive rat background and a small region on chromosome 2 from the Wistar–Kyoto strain; WKY, Wistar–Kyoto normotensive rat

INTRODUCTION

Despite recent advances, it is predicted that 90% of normotensive individuals between 55 and 65 years of age will develop hypertension in their lifetime [1]. It is well recognized that increased dietary salt intake results in marked elevations in arterial pressure [2–12] and that some individuals are more sensitive to changes in dietary sodium intake than others. These individuals are classified as salt-sensitive [13,14]. Whilst there is a large body of evidence describing this phenomenon, the mechanism underlying salt-sensitivity remains elusive. We and others have implicated several genes that influence arterial pressure regulation [15–18] and, in particular, those that affect the development of hypertension. Indeed, glutathione S-transferase Mu 1, endothelial differentiation gene 1 and vascular cell adhesion protein 1 are located within this introgressed chromosome 2 quantitative trait locus (QTL), and influence renal oxidative stress, vascular inflammation and leukocyte recruitment [15,17,19]. Many of the

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genes that are associated with salt-sensitive hypertension are known, directly or indirectly, to alter the production and function of aldosterone [20–28]. Given that aldosterone is the main mineralocorticoid hormone which controls sodium retention and blood volume, this is not surprising.

The stroke-prone spontaneously hypertensive rat (SHRSP) is one of the best models for the study of essential hypertension. This model develops severe hypertension at approximately 12 weeks of age with cardiac hypertrophy [29], stroke [30–36], insulin resistance [37–40] and endothelial dysfunction due to oxidative stress [29,41–45], which is similar to the human condition. The SHRSP strain also shows impaired aldosterone regulation in response to a high salt challenge when compared to both the normotensive Wistar–Kyoto (WKY) strain and the spontaneously hypertensive rat (SHR) strain [46]. Previously, we have identified a QTL on chromosome 2 associated with arterial pressure regulation [15]. Introgression of this QTL from a normotensive rat strain into a hypertensive rat strain results in a reduction in arterial pressure [15]. Furthermore, we observed a chromosome 2-dependent increase in SBP in response to the 1% salt challenge; increases of: 141 ± 3 to 147 ± 3 mmHg in the WKY strain, 179 ± 7 to 201 ± 4 mmHg in the SP.WKY$_{Gla}$2k strain and 194 ± 5 to 235 ± 6 mmHg in the SHRSP strain ($P_{strain} < 0.01$) [15]. This congenic interval contains a number of genes, including the 3β hydroxysteroid dehydrogenase (3βHSD) gene family, which are involved in corticosteroid synthesis. Recent studies have demonstrated that distinct members of the 3βHSD gene family are required for the glucocorticoid synthesis. Over-expression of adrenal 3βHSD6 in mice specifically increases aldosterone synthesis, leading to salt-sensitive hypertension [47]. The aldosterone-specific 3βHSD2 gene in rats has not been confirmed, but in humans, it is thought to be 3βHSD1 [47]. The current study was designed to investigate whether altered renal and adreno-cortical function are responsible for the salt-sensitive hypertension that is associated with the identified chromosome 2 QTL in the SHRSP strain. We hypothesized that introgression of this congenic region from the WKY strain would improve salt-handling in the SHRSP strain by decreasing the expression of genes responsible for aldosterone synthesis.

METHODS

Animals

All studies were conducted in accordance with UK Home Office regulations. Inbred colonies of WKY and SHRSP have been maintained at the University of Glasgow since 1991, as described previously [42,48]. From weaning, the rats were maintained on normal rat chow (rat and mouse No. 1 maintenance diet: 0.5% NaCl; Special Diet Services, UK). A congenic strain was generated using a marker-assisted 'speed' congenic strategy [49] in which WKY (donor strain) segments of chromosome 2 were introgressed into the SHRSP (recipient strain) genetic background as previously described [15]. The congenic strain used in the present study is described by the nomenclature SP.WKY$_{Gla}$2k, where SHRSP represents the recipient strain and WKY refers to the donor strain, *Gla* signifies that the strains originate from the Glasgow colony, and 2k refers to chromosome 2 region 'k' which was introgressed [15]. The congenic interval of the SP.WKY$_{Gla}$2k is D2Mit21-D2Rat157, which is approximately 10 cM [15].

Experimental protocol

At 18 weeks of age, groups of male rats were given either water or 1% NaCl to drink for 3 weeks. In week 3, the animals were trained in the metabolic cage, and at the end of week 3, 24-h measurements of consumption and excretion parameters were made. In addition, in-vivo renal function tests were performed using standard inulin and para-aminohippuric acid (PAH) clearance techniques [50]. Briefly, the left jugular vein and the left carotid artery were cannulated for intravenous infusions and arterial pressure measurements (Biopac MP100 data-acquisition system), respectively. The bladder was catheterized for urine collections. The animals were infused with saline containing bovine serum albumin (BSA 2%; Sigma, St Louis, Missouri, USA), inulin (7.5%, INUTEST, Fresenius Kabi, Germany) and PAH (1.5%, Sigma). After a 45-min equilibration, urine was collected for 30 min with a carotid blood sample taken at 15 min. Urine volume was measured gravimetrically. Hearts, kidneys and adrenal glands were collected, weighed and snap-frozen for gene expression analysis.

Renal function calculations

Blood and urine samples collected during the renal function protocol were analysed for inulin, PAH and plasma steroid concentrations. Urine samples collected during the 24-h metabolic cage measurements were analysed for urinary electrolyte excretion, protein and steroid excretion. Inulin and PAH concentrations were determined by spectrophotometry. Renal function was calculated as previously described [50], with glomerular filtration rate (GFR) normalized per gram of kidney weight. Urinary electrolytes were measured by flame photometry. Plasma and urinary aldosterone and corticosterone were estimated using highly specific and sensitive in-house ELISA methods at the Steroid Research Laboratory, Queen Margaret University, Edinburgh [51,52].

Adrenal gene expression

Total RNA was extracted from whole adrenal homogenates using RNeasy kits (Qiagen, Venlo, The Netherlands) as described previously [15,17]. Normalization was confirmed by performing real-time PCR (TaqMan; Applied Biosystems, Life Technologies, Madrid, Spain) of *Actb* (ß-actin; Promega, Madison, WI, USA) with comparable threshold cycles. TaqMan probes for expression of mineralocorticoid receptor (assay number Rn00565562_m1), 3βHSD1 (assay number Rn01774741_m1), 3βHSD2 (assay number Rn01789220_m1), 3βHSD5 (assay number Rn00680494_m1), 3βHSD6 (assay number Rn01533661_m1), 3βHSD7 (assay number Rn01506641_g1), 11βhydroxylase (CYP11B1, assay number Rn02607234_g1) and aldosterone synthase (CYP11B2, assay number, Rn02396730_g1) were multiplexed with *Actb* (4352340E-labeled VIC). 3βHSD1, 3βHSD2, 3βHSD5, 3βHSD6 genes are located within the 2k region on chromosome 2, whereas 3βHSD7 is located on chromosome 1, outside of the 2k region. Gene expression was calculated relative to *Actb* using the comparative

TABLE 1. Body and organ weights at 21 weeks of age

Strains		BW (g)	KW (g)	KW/BW (g/kg)	HW (g)	HW/BW (g/kg)
WKY	Water ($n=8$)	359 ± 13	2.01 ± 0.05	5.65 ± 0.23	1.17 ± 0.02	3.27 ± 0.09
	1% NaCl ($n=8$)	361 ± 12	2.08 ± 0.05	5.79 ± 0.19	1.25 ± 0.04	3.48 ± 0.12
SP.WKY$_{Gla}$2k	Water ($n=7$)	$284 \pm 5^{**}$	$2.43 \pm 0.10^{**}$	$8.29 \pm 0.20^{**}$	1.13 ± 0.05	$3.97 \pm 0.12^{**}$
	1% NaCl ($n=8$)	$292 \pm 9^{**}$	$2.62 \pm 0.06^{**}$	$9.03 \pm 0.29^{**}$	1.23 ± 0.02	$4.37 \pm 0.15^{**}$
SHRSP	Water ($n=7$)	$282 \pm 9^{**}$	$2.43 \pm 0.08^{**}$	$8.45 \pm 0.14^{**}$	1.13 ± 0.11	$4.33 \pm 0.07^{**}$
	1% NaCl ($n=8$)	$275 \pm 3^{**}$	$2.30 \pm 0.04^{*}$	$8.28 \pm 0.21^{**}$	1.28 ± 0.03	$4.61 \pm 0.15^{**}$

Body weight (BW, g), kidney weight (KW, g) and heart weight (HW, g) adjusted for body weight (KW/BW and HW/BW) in the WKY, SHRSP and the congenic SP.WKY$_{Gla}$2k strains treated with water or 1% NaCl for 3 weeks. All data are presented as mean ± SEM.
$^{*}P < 0.05$.
$^{**}P < 0.005$ compared to the WKY-water-treated strain.

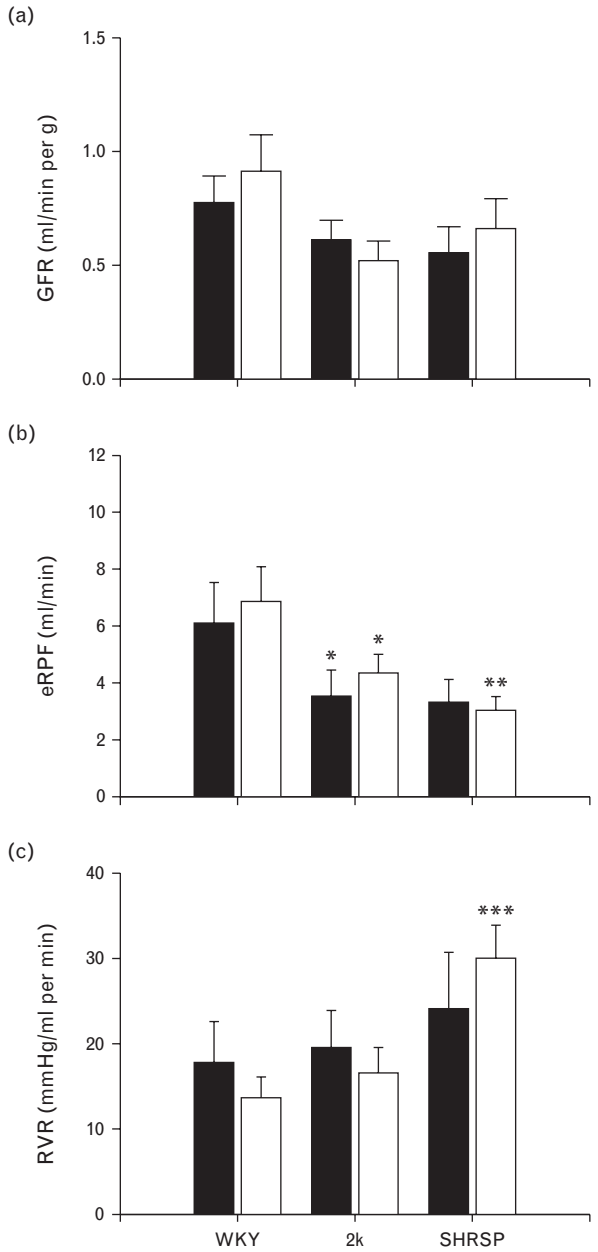

FIGURE 1 Renal hemodynamics. (a) Glomerular filtration rate (GFR). (b) Effective renal plasma flow (eRPF). (c) Renal vascular resistance (RVR) in WKY, SP.WKY$_{Gla}$2k (2k) and SHRSP strains treated with water (closed bars) and 1% NaCl (open bars) ($n \geq 6$). Data are presented as mean ± SEM. (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.005$ compared to the WKY-no salt strain.

(ΔΔ threshold change) method. Briefly, a mean ΔCT value was obtained from triplicate analyses of each sample. The average of ΔCT values for the WKY water-treated group was subtracted from each sample to generate a ΔΔCT which was then substituted into the equation $2^{-\Delta\Delta CT}$. In this way, gene expression in each sample is expressed as a fold change relative to the control WKY water-treated group.

Statistical analysis

All of the data are presented as mean ± SEM. Body weights, tissue weights, renal haemodynamics, steroid concentrations and gene expression were analysed using a two-way analysis of variance (ANOVA) with post-hoc Tukey using the factors strain (P_{strain}: WKY, SP.WKY$_{Gla}$2k or SHRSP) and treatment (P_{treat}: 3 weeks of water or 1% NaCl treatment) and the interaction between strain and treatment (P_{S*T}, SYSTAT version 9.0). Statistical significance was accepted at a P value less than 0.05.

RESULTS

All the three strains were age-matched, but this resulted in a weight difference as previously documented [15]; WKY animals were significantly heavier than the SP.WKY$_{Gla}$2k and the SHRSP strains, regardless of treatment (WKY: 360 ± 12 g vs. SP.WKY$_{Gla}$2k: 289 ± 8 g, $P < 0.001$; and SHRSP: 278 ± 8 g, $P < 0.001$; Table 1). Kidney weight in the SP.WKY$_{Gla}$2k and the SHRSP strains was greater than the WKY animals at the time of sacrifice – both wet tissue weight and when corrected for body weight (Table 1). Heart weight was not different between the three strains; however, when corrected for body weight, it was significantly greater in the SP.WKY$_{Gla}$2k and the SHRSP strain compared to the WKY strain (Table 1).

Renal haemodynamics

There were no differences in GFR between strains or between treatments (Fig. 1). Effective renal plasma flow (eRPF) was greatest in the WKY strain irrespective of treatment (water-treated: 6.1 ± 1.4 ml/min, 1% NaCl-treated: 6.9 ± 1.2 ml/min, respectively) when compared to water and 1% NaCl-treated SP.WKY$_{Gla}$2k (3.5 ± 1.4 ml/min, $P = 0.041$ and 4.4 ± 0.7 ml/min, $P = 0.036$, respectively, compared to WKY-water) and SHRSP strains (3.3 ± 0.8 ml/min, $P = 0.027$ and 3.0 ± 0.5 ml/min, $P = 0.006$, respectively, compared to WKY-water; Fig. 1). Renal vascular resistance in the WKY-water-treated animals (17.8 ± 4.8 mmHg/ml per min)

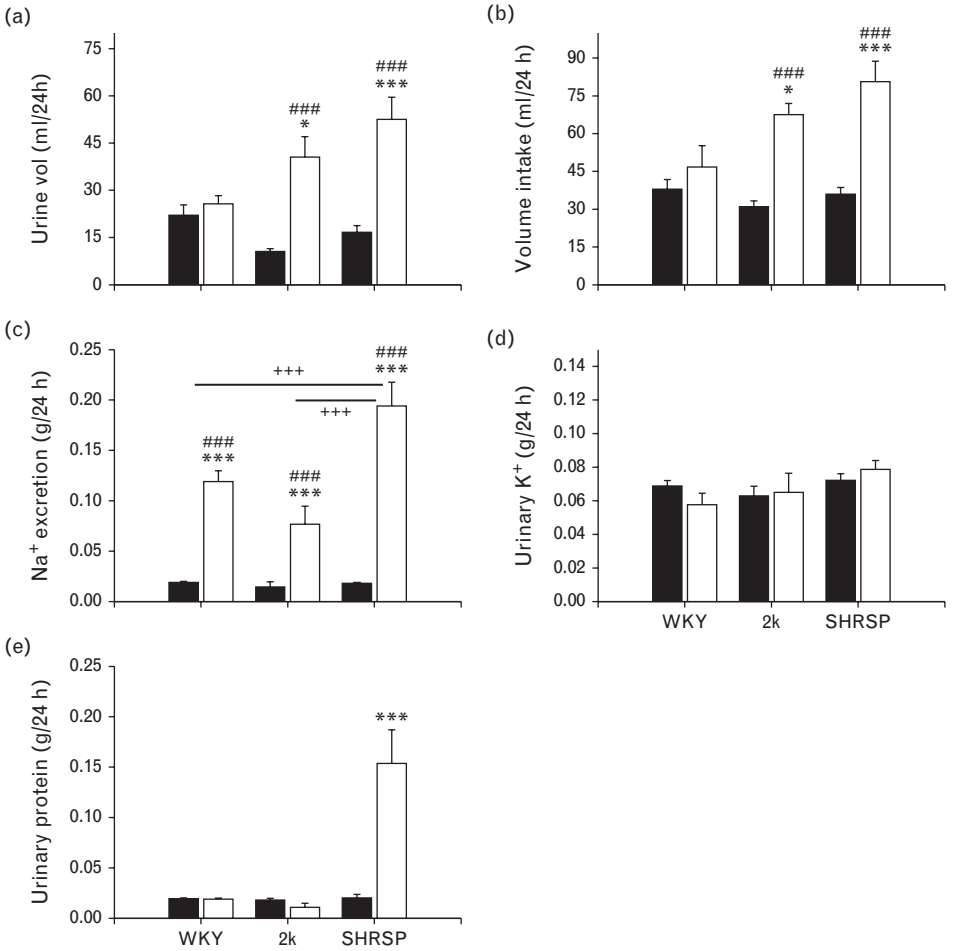

FIGURE 2 Metabolic consumption and excretion parameters. (a) Urine volume excreted in 24 h (ml/24 h). (b) Volume consumed in 24 h (ml/24 h). (c) Sodium excreted in 24 h (Na+, g/24 h). (d) Potassium excreted in 24 h (K+, g/24 h). (e) Protein excreted in 24 h (g/24 h) in the WKY, SP.WKY$_{Gla}$2k (2k) and SHRSP strains treated with water (closed bars) and 1% NaCl (open bars) ($n \geq 6$). Data are presented as mean ± SEM. (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.005$ compared to the WKY-no salt strain. (###) $P < 0.005$ compared with the water-treated counterpart. (+++) $P < 0.005$ compared to the SHRSP-1% NaCl group.

was not significantly different to all other treatment groups except for the 1% NaCl-treated SHRSP strain, which was significantly greater (30.0 ± 3.8 mmHg/ml per min, $P = 0.004$ compared to WKY-water group; Fig. 1).

We observed no strain difference in the urine volume or water consumption in animals treated with water (Fig. 2a and b). In the 1% NaCl-treated SP.WKY$_{Gla}$2k and SHRSP strains, we observed a greater urine volume over 24 h (41 ± 6 ml, $P = 0.03$ and 53 ± 7 ml, $P = 0.001$, respectively, compared to WKY-water 22 ± 3 ml; Fig. 2a) and water consumption (68 ± 4 ml, $P = 0.017$ and 81 ± 8 ml, $P = 0.004$, respectively, compared to WKY-water 38 ± 4 ml; Fig. 2b). Sodium excretion reflected salt consumption and was higher in all strains treated with 1% NaCl compared with those given water. 1% NaCl intake and urinary sodium was higher in the SHRSP strain, showing greater sodium excretion than the WKY or the SP.WKY$_{Gla}$2k strain (Fig. 2c). Urinary potassium excretion was not affected by strain or 1% NaCl intake (Fig. 2d). Genotype did not affect protein excretion in animals that were given water to drink. However, SHRSP animals given 1% NaCl to drink were markedly proteinuric compared to the WKY and the SP.WKY$_{Gla}$2k animals ($P < 0.001$; Fig. 2e), perhaps reflecting very high blood pressure in this group.

Plasma aldosterone concentration in the SHRSP strain was greater than in both the WKY and SP.WKY$_{Gla}$2k strains, regardless of treatment ($P < 0.05$; Fig. 3a). We observed no effect of 1% NaCl on plasma aldosterone, irrespective of genotype (Fig. 3a), and there was no effect of 1% NaCl consumption or genotype on urinary aldosterone or corticosterone concentration, or plasma corticosterone concentration (Fig. 3b–d).

Gene expression

Overall, adrenal mRNA expression of 3βHSD2 was greater in the SHRSP strain regardless of the treatment compared to the WKY strain ($P < 0.05$), with no difference in expression between the WKY and the SP.WKY$_{Gla}$2k strains (Fig. 4a). In addition, we observed no significant difference in the expression of 3βHSD1 or 3βHSD7 mRNA between groups (Fig. 4b, d). The pattern of expression of 3βHSD6 was similar to that of 3βHSD2 gene expression, with higher expression in the SHRSP vs. the WKY strain, regardless of treatment. There was no difference in adrenal 3βHSD6

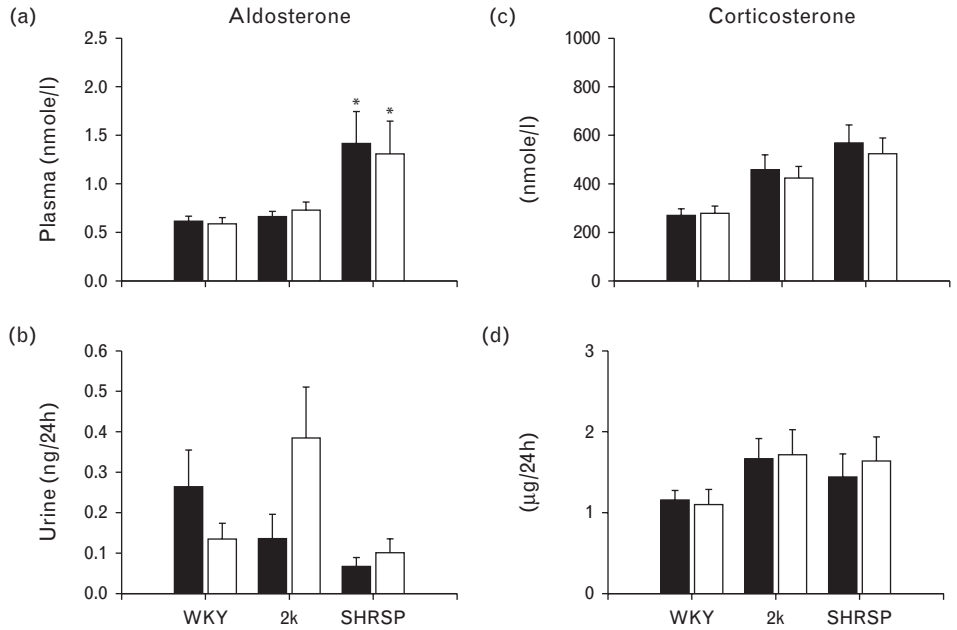

FIGURE 3 Urinary and plasma steroid concentrations. (a) Plasma aldosterone, (b) urinary aldosterone, (c) plasma corticosterone, and (d) urinary corticosterone in the WKY strain treated with water (black, $n=7$) or 1% NaCl (white, $n=8$), SP.WKY$_{Gla}$2k strain (2k) treated with water (black, $n=6$) or 1% NaCl (white, $n=7$) and SHRSP strain treated with water (black, $n=6$) or 1% NaCl (white, $n=7$). (*) $P<0.05$ compared to the WKY-water-treated strain.

gene expression between the SP.WKY$_{Gla}$2k and the WKY strains (Fig. 4c). We were unable to detect 3βHSD5. In terms of relative expression of these isoforms, both 3βHSD2 and 3βHSD6 are more highly expressed than 3βHSD1 or 3βHSD7, with a difference in ΔCT value of greater than eight cycles; with each cycle number increase indicative of a two-fold decrease in the expression levels. Adrenal 11β hydroxylase (CYP11B1) expression was not affected by 1% NaCl consumption. Although WKY and SHRSP CYP11B1 were not different, expression in the SP.WKY$_{Gla}$2k-water-treated animals was lower than in the WKY-water-treated animals (Fig. 4e). Aldosterone synthase (CYP11B2) gene expression was generally lower in 1% NaCl-treated animals and was higher in SHRSP-water-treated rats. However, expression in the SP.WKY$_{Gla}$2k rats was not different to that in the WKY controls (Fig. 4f). There were no differences in adrenal mineralocorticoid receptor mRNA gene expression (Fig. 4g).

DISCUSSION

The search for genes responsible for the salt-sensitive hypertension trait on the chromosome 2 of the SHRSP strain has been narrowed to a 10-cM region [15]. Congenic SHRSP rats in which the 2k region of chromosome 2 has been introgressed have a significantly reduced response to salt and have lower blood pressures [15]. This 2k region is characterized as the location of many members of the 3βHSD gene family which encode enzymes responsible for the conversion of pregnenelone to progesterone, which is a key step in the biosynthesis of adrenal and gonadal steroid hormones [53]. In mice and humans, one of these 3βHSD gene family members has been shown to be expressed specifically in the zona glomerulosa of the adrenal cortex, a region responsible for aldosterone synthesis [47]. Recent studies have shown that dysregulation of this gene in mice causes hyperaldosteronism and salt-sensitive hypertension [47]. In rats, a number of 3βHSD genes have been identified [54], but, as yet, the 3βHSD gene family member that is specifically required for aldosterone synthesis has not been confirmed. Nevertheless, the possibility that the phenotypic effects on blood pressure of region 2k on chromosome 2 might involve altered patterns of steroidogenesis merits further investigation.

In the current study, we quantified mRNA levels of the four 3βHSD gene family members that were expressed at significant levels in the adrenal glands. Gene expression was assessed due to the high nucleotide and protein sequence homology between the 3βHSD gene family members encoded by the 2k region (80–97% homology). 3βHSD2 and 3βHSD6 were up-regulated in the SHRSP rats, but 3βHSD1 and 3βHSD7 were not. This pattern of expression is interesting for several reasons. Firstly, increased expression of 3βHSD2 and 3βHSD6 corresponded to increased plasma aldosterone levels. Secondly, both the raised aldosterone levels and increased adrenal 3βHSD2 and 3βHSD6 expression of SHRSP rats were corrected in the congenic SP.WKY$_{Gla}$2k strain. Thirdly, 3βHSD2 and 3βHSD6 were not down-regulated by the 1% NaCl challenge. This is consistent with the idea that zona glomerulosa 3βHSD activity is regulated by clock genes rather than the rennin–angiotensin system [47]. In contrast, it is notable that the adrenal glands of the SHRSP strains expressed higher levels of CYP11B2 which encodes aldosterosterone synthase. Changes in the aldosterone synthesis in response to sodium intake are well known to be mediated by expression of CYP11B2, and it is significant that irrespective

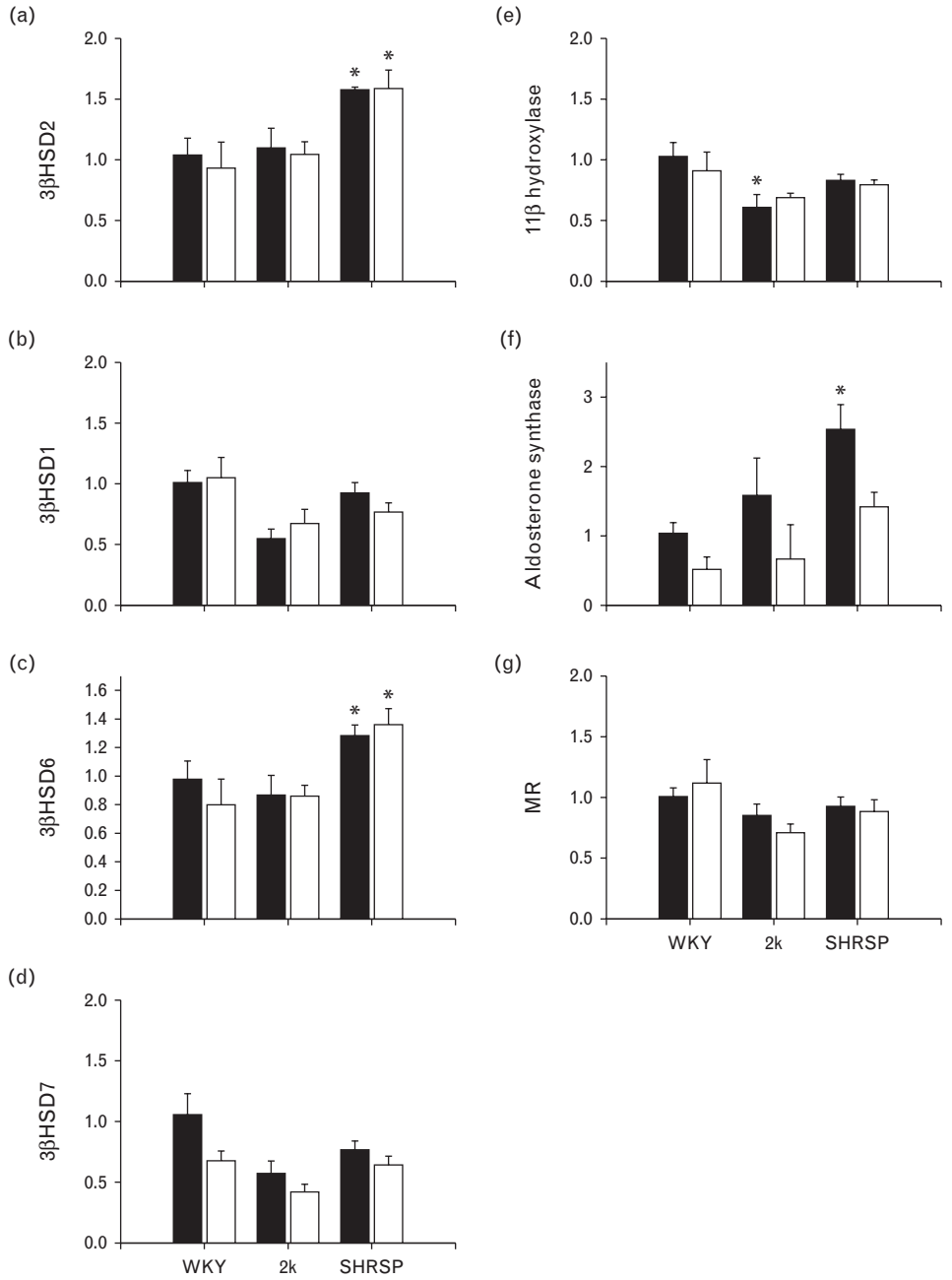

FIGURE 4 Relative adrenal mRNA gene expression of (a) 3βHSD2, (b) 3βHSD1, (c) 3βHSD6, (d) 3βHSD7, (e) 11βhydroxylase (CYP11B1), (f) aldosterone synthase (CYP11B2), and (g) mineralocorticoid receptor (MR) in the WKY, SP.WKY$_{Gla}$2k (2k) and SHRSP strains treated with water (closed bars, $n = 5$/group) and 1% NaCl (open bars, $n = 5$/group). Data are presented as mean ± SEM. (*) $P < 0.05$ compared to the WKY-no salt strain.

of genotype, CYP11B2 was suppressed in rats given 1% NaCl to drink.

Glucocorticoid hormones also have cardiovascular effects and their synthesis also involves 3βHSD activity. However, the 3βHSD isoenzyme in mouse and human adrenal gland in glucocorticoid synthesis are encoded by 3βHSD1 and 3βHSD2, respectively, and are regulated by the hypothalamo-pituitary axis rather than clock genes or the renin–angiotensin system. It is significant, therefore, that neither corticosterone synthesis nor adrenal 3βHSD1 or 3βHSD7 mRNA level was affected by diet or genotype, and that CYP11B1, the homologue of CYP11B2 that catalyses the final step in glucocorticoid biosynthesis, was also unchanged.

Taken together, the adrenal expression data support the hypothesis that SHRSP strains synthesize excess progesterone and aldosterone, which are the main mineralocorticoid hormones in rats. Introgression of the 2k region from the WKY strain (SP.WKY$_{Gla}$2k) resulted in reduced activity of 3βHSD2 and 3βHSD6, resulting in a reduction in both progesterone and aldosterone production. Consequently, with no increase in progesterone synthesis, the activity and expression of aldosterone synthase would remain unchanged, as observed in the WKY strain. The increase

in plasma aldosterone in the SHRSP strain is consistent with previous work, demonstrating impaired aldosterone regulation in the SHRSP strain [46]. The SHRSP strains are reported to maintain plasma aldosterone levels in response to a salt challenge which is suggested to be mediated by an increase in adrenal aldosterone synthase expression [46]. We observed no difference in plasma aldosterone levels with the high salt challenge in any of our strains, which is possibly explained by the lack of reduction in aldosterone synthase expression. Given that aldosterone is the main anti-natriuretic hormone, it appears paradoxical that urinary sodium excretion by the SHRSP strain is greatly increased when given 1% NaCl to drink. However, in rat models of mineralocorticoid-induced hypertension, there is generally a requirement for increased dietary sodium availability. This is associated with a mineralocorticoid-stimulated sodium appetite, and in the present study, there is a genotype-dependent increase in saline consumption. It is therefore tempting to speculate that aldosterone generated by the adrenal in the SHRSP strain increases salt appetite, which in turn leads to greater overall urinary sodium excretion, but also a proportional renal retention of sodium and a net increase in blood pressure. In support of this hypothesis, there are many studies showing activation of mineralocorticoid receptor in specific brain regions directly stimulates salt appetite and can cause hypertension without affecting peripheral levels of the mineralocorticoid hormone [55]. The caveat in the present experiments is that the penetrance of aldosterone through the blood–brain barrier is poor so that aldosterone generated peripherally may not reach the central mineralocorticoid receptor targets. Nevertheless, it is worth noting that aldosterone may be synthesized extra-adrenally in the brain [55,56]. It is also significant that the brain is well known to express 3βHSD. Although the precise 3βHSD gene family member is not known, it could be that the 2k region of chromosome 2 that determines corticosteroid synthesis in the brain as well as in the adrenal gland. It is highly significant, therefore, that the salt-sensitive hypertension of Dahl rats is blocked by the intracerebroventrocular infusion of trilostane, an inhibitor of 3βHSD activity [57].

Despite the reported differences in blood pressure between the SHRSP, SP.WKY$_{Gla}$2k and WKY strains [15], there was no difference in GFR between any strains, consistent with the literature [58,59]. Furthermore, eRPF was significantly lower in the SHRSP strain as compared to the WKY strain, as reported previously [59]. Importantly, eRPF in the SP.WKY$_{Gla}$2k strain was not different to that in the SHRSP strain, suggesting that the reduction in eRPF is not mediated by the chromosome 2 QTL examined. Consistent with the previous studies, we observed increased proteinuria in the salt-challenged SHRSP strain compared to all other strains [11,58]. Strikingly, introgression of this normotensive chromosome 2 region was sufficient to restore sodium handling and urinary protein excretion in the SP.WKY$_{Gla}$2k to WKY levels, despite the significant difference in SBP between the two strains, suggesting the genes within the 2k region influence sodium handling in the SHRSP strain.

Extensive DNA analysis was undertaken to identify single-nucleotide polymorphisms or other genetic differences between the 3βHSD gene family members implicated in this study. However, this locus is complex and it was not possible to disentangle the promoter sequences between closely related gene members even with the availability of the genome sequence [60]. The complexity and relatedness is consistent with poor assembly of the rat genome within this region on chromosome 2, with an overrepresentation of sequencing reads to this locus accounting for distinct 3βHSD cDNAs (Supplementary Fig. 1, http://links.lww.com/HJH/A373).

The current study demonstrates that the introgression of the WKY chromosome 2k region is sufficient to reduce salt responsiveness and restore sodium handling, plasma aldosterone levels, and adrenal 3βHSD2 and 3βHSD6 gene expression in the SHRSP strain. Despite advancements in understanding the genetic contribution to disease progression, the genes involved in salt-sensitivity remain unclear. This study provides novel evidence that the excessive aldosterone synthesis in the SHRSP strain is regulated by genes located on chromosome 2 within the 2k region.

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Conflicts of interest

There are no conflicts of interest.

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Reviewer's Summary Evaluation

Referee 1

The present work assessed the mechanism by which the introgressed chromosome 2 Quantitative Trait Loci (QLT) fragment from Wistar–Kyoto (WKY) rats into stroke-prone spontaneously hypertensive rats (SHRSP) restores the salt sensitivity of aldosterone synthesis in SHRSP. The authors conclude that the introgression of the chromosome 2 regions in SHRSP corrected the proteinuria, reduced sodium excretion, plasma aldosterone levels and 3βHSD expression. The study is of interest and suggests that chromosome 2 regions restored aldosterone regulation in SHRSP reduce salt-sensitive hypertension. The major weakness, however, is that most of the studied enzymes were by RT-PCR and not by activity or western blot, which is impossible due to similarity among them.