A preliminary study of the effect of a high-salt diet on transcriptome dynamics in rat hypothalamic forebrain and brainstem cardiovascular control centers

Chitra Devi Ramachandran[1,*], Khadijeh Gholami[1,2], Sau Kuen Lam[1,3] and See Ziau Hoe[1,*]

[1] Department of Physiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Wilayah Perseketuan, Malaysia

[2] Human Biology Division, School of Medicine, International Medical University, Kuala Lumpur, Wilayah Perseketuan, Malaysia

[3] Department of Pre-Clinical Sciences, Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, Sungai Long, Selangor, Malaysia

[*] These authors contributed equally to this work.

ABSTRACT

Background. High dietary salt intake is strongly correlated with cardiovascular (CV) diseases and it is regarded as a major risk factor associated with the pathogenesis of hypertension. The CV control centres in the brainstem (the nucleus tractus solitarii (NTS) and the rostral ventrolateral medulla (RVLM)) and hypothalamic forebrain (the subfornical organ, SFO; the supraoptic nucleus, SON and the paraventricular nucleus, PVN) have critical roles in regulating CV autonomic motor outflows, and thus maintaining blood pressure (BP). Growing evidence has implicated autonomic regulatory networks in salt-sensitive HPN (SSH), but the genetic basis remains to be delineated. We hypothesized that the development and/ or maintenance of SSH is reliant on the change in the expression of genes in brain regions controlling the CV system.

Methodology. We used RNA-Sequencing (RNA-Seq) to describe the differential expression of genes in SFO, SON, PVN, NTS and RVLM of rats being chronically fed with high-salt (HS) diet. Subsequently, a selection of putatively regulated genes was validated with quantitative reverse transcription polymerase chain reaction (qRT-PCR) in both Spontaneously Hypertensive rats (SHRs) and Wistar Kyoto (WKY) rats.

Results. The findings enabled us to identify number of differentially expressed genes in SFO, SON, PVN, NTS and RVLM; that are either up-regulated in both strains of rats (SON- *Caprin2*, *Sctr*), down-regulated in both strains of rats (PVN- *Orc*, *Gkap1*), up-regulated only in SHRs (SFO- *Apopt1*, *Lin52*, *AVP*, *OXT*; SON- *AVP*, *OXT*; PVN- *Caprin2*, *Sclt*; RVLM- *A4galt*, *Slc29a4*, *Cmc1*) or down-regulated only in SHRs (SON- *Ndufaf2*, *Kcnv1*; PVN- *Pi4k2a*; NTS- *Snrpd2l*, *Ankrd29*, *St6galnac6*, *Rnf157*, *Iglon5*, *Csrnp3*, *Rprd1a*; RVLM- *Ttr*, *Faim*).

Conclusions. These findings demonstrated the adverse effects of HS diet on BP, which may be mediated via modulating the signaling systems in CV centers in the hypothalamic forebrain and brainstem.

INTRODUCTION

Hypertension (HPN) results from a complex interaction between genetic predisposition and environmental factors such as dietary salt intake. Dietary salt (i.e., sodium chloride, NaCl) intake is the most remarkable modifiable environmental factor that attracts many studies in HPN. Indeed, it has been acknowledged as an important contributing factor of the etiology and progression of HPN (*Johnson et al., 2015*). A marked elevation in BP in response to excessive dietary salt intake, known as salt-sensitive HPN (SSH), has been observed in humans as well as experimental animals (*Guyenet, 2006a*; *Iwamoto, Kita & Katsuragi, 2005*). Despite the abundant experimental, interventional and epidemiological observations demonstrating an association between dietary salt and HPN, mechanisms linking high-salt (HS) intake to the increase in BP are not well understood.

High-salt intake has been reported to influence the excitability of sympathetic regulatory networks which the brain regions controlling sympathetic tone play an important role in SSH (*Carmichael et al., 2015*; *Guo et al., 2015*; *Orlov & Mongin, 2007*). Studies have shown that blockade of sympathetic outflow; and transection of sympathetic nerves consistently lowers arterial BP, while lesions of anteroventral third ventricular regions prevent or attenuates the development and/or the severity of SSH (*Johnson et al., 2015*; *King, Osborn & Fink, 2007*; *Stocker, Madden & Sved, 2010*). Furthermore, there are now unequivocal evidences that central brain regions regulating CV autonomic motor outflows are sensitive to salt. These brain regions regulate sympathetic activity and their resultant over activity triggered by HS diet is associated with the development, maintenance and progression of hypertensive state in human patients and animal models (*Esler & Kaye, 2000*; *Guyenet, 2006a*; *Lohmeier, 2002*; *Mann, 2003*; *Petersson et al., 2002*; *Sakata et al., 2002*).

The application of newly developed neuroanatomical and functional techniques, allowed to identify neural network comprising key brain nuclei and their interconnections that controls BP (*Lohmeier et al., 2010*; *Thrasher, 2002*; *Thrasher, 2006*). Both the hypothalamic forebrain and brainstem are regarded as essential components in the regulatory neuronal network of central BP control. The regions in the hypothalamic forebrain act as an interface between the endocrine and nervous system and a wide variety of functional changes in it characterize multiple forms of HPN including SSH (*Carmichael & Wainford, 2015*). The brainstem, on the other hand, plays an important role in the control of cardiac and vascular baroreceptor reflexes to regulate arterial BP (*Sved, Ito & Sved, 2003*).

In the present study, one of the circumventricular organs (CVOs)—the subfornical organ (SFO) which is known to be important in regulating fluid homeostasis and drinking behaviors in SSH; two hypothalamic forebrain regions that are known for regulating osmotic stability in SSH—the supraoptic nucleus (SON) and the paraventricular nucleus (PVN); and two important medullary structures—the nucleus tractus solitarii (NTS) and

the rostral ventrolateral medulla (RVLM), which both play important roles in the control of cardiac and vascular baroreceptor reflexes in SSH have been focused.

The SFO which is situated on the midline wall of the third ventricle in the dorsal region of lamina teminalis is endowed with numbers of functional receptors for signals vital in the regulation of fluid homeostasis and drinking behaviors (*Ahmed et al., 2014*). In addition, the neurons of the SFO exert their effects by direct and indirect efferent projections to other important hypothalamic nuclei that are involved in controlling autonomic functions or neuroendocrine actions including PVN and the SON (*Cottrell & Ferguson, 2004*; *Rowland et al., 1996*; *Smith & Ferguson, 2010*); as well as to key CV regulatory brainstem sites, NTS and the RVLM (*Moellenhoff et al., 1998*; *Sakai et al., 2007*). Meanwhile, both PVN and SON at the hypothalamic forebrain are known as important integrative structures that regulate coordinated responses to perturbations in CV homeostasis through endocrine projections especially in the regulation of osmotic stability (*Hindmarch et al., 2006*). The PVN, in addition, has been reported to reciprocally projects to autonomic nuclei in brainstem (NTS and RVLM) and spinal cord which are responsible in activating sympathetic nervous system (SNS) including CV regulation (*Pyner, 2009*). Here, they have the potential to change sympathetic nerves activity by virtue of direct descending projections that terminate on or near to sympathetic pre-ganglionic neurones found in intermediolateral cell column (IML) of the spinal cord (*Motawei et al., 1999*; *Pyner, 2009*; *Ranson et al., 1998*).

The NTS, on the other hand, plays a pivotal role in the regulation of both the set-point and the gain of baroreflex for homeostatic control of BP (*Waki et al., 2010*; *Waki, Takagishi & Gouraud, 2014*). It is the principal site of termination of baroreceptor afferent fibers and as such mediate inhibitory action of baroreceptor on sympathetic discharge (*Frigero, Bonagamba & Machado, 2000*; *Machado, 2001*; *Waki, Takagishi & Gouraud, 2014*). This area contains many neurotransmitters or neuromodulators that are important in CV control, and the intermediate portion of NTS is richly innervated by fibers arising from different brain nuclei that are also known to have an important role in CV control (*Colombari et al., 2001*; *Duale et al., 2007*; *Kasparov & Teschemacher, 2008*; *Zoccal et al., 2014*). The NTS neurons send excitatory amino acid projections to the caudal ventrolateral medulla (CVLM) which, in turn, inhibits RVLM neurons via GABAergic inhibitory pathway (*Guyenet, 2006b*; *Sapru, 1996*; *Yao et al., 2008*). Meanwhile, RVLM lies ventral to the rostral part of the nucleus ambiguous, caudal to the facial nucleus and ventral to Bötzinger complex (*Dampney, 1994*; *Geraldes et al., 2014b*). The RVLM is the region where the sympathetic pre-motor neurons controlling vasomotor sympathetic nerve activity are located. The RVLM neurons project to the sympathetic preganglionic neurons in the IML cell column of the spinal cord and receive a direct glutamatergic projection from NTS (*Card et al., 2006*; *Dampney, 1994*; *Geraldes et al., 2014b*; *Leman, Viltart & Sequeira, 2000*). These neurons also receive excitatory and inhibitory inputs from other brain areas such as hypothalamus and other part of medulla oblongata (*Koga et al., 2008*; *Sved, Ito & Yajima, 2002*).

Hence, the brain mechanisms in both initiation and maintenance of BP are explored as increasing evidence as a key contributor to the development and maintenance of SSH (*Carmichael & Wainford, 2015*; *DiBona, 2013*).

In the present study, we hypothesized that elevated dietary salt intake might alter gene expression in the SFO, SON, PVN, NTS and RVLM which may affect the functional activity of these nuclei, resulting in altered excitability and increases the gain of central sympathetic neurons. Hence, we have used Next Generation Sequencing (RNA-Seq) to describe the transcriptional profile of the hypothalamic forebrain regions of SFO, SON and PVN; and the medullary NTS and RVLM of SHRs under condition of regular- and high-salt consumptions. Comparison of these datasets has allowed us to identify genes that are putatively differentially expressed as a result of a chronic HS diet. We have further validated these data using quantitative reverse transcription PCR (qRT-PCR) in the SHR, and in the WKY rats.

MATERIALS AND METHODS

Animals and animal care

The male WKY rats and SHRs used in this study were bred at the University of Malaya Animal Experimental Unit from stock obtained from BioLASCO (Taiwan). After being weaned at five weeks of age, rats were housed in groups of five to six under controlled laboratory conditions (temperature 23 ± 5 °C, 12:12-hour light/ dark cycle and humidity 50% to 60%) with food and water *ad libitum* for at least a week prior to the onset of experimentation. All the experimental protocols involving animals and housing thereof were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Malaya (Reference: 2014-01-07/Physio/R/HSZ) which maintains a full Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accreditation.

Grouping and experimental design

Six-week-old WKY rats and SHRs were randomly assigned to receive food with either a regular-salt (RS) content (0.2% w/v NaCl) or high-salt (HS) content (4% w/v NaCl; Harlan Teklad, Germany) with free access of water. Four groups were thus studied:
  Group 1: WKY rats receiving RS diet (WRS)
  Group 2: WKY rats receiving HS diet (WHS)
  Group 3: SHRs receiving RS diet (SRS)
  Group 4: SHRs receiving HS diet (SHS)
  The treatment period continued for six weeks.

Tissue collection and preparation

At the end of diet treatment, rats were euthanized (between the hours of 0800 to 1100) and tissue was isolated and processed as described below. Rats were humanely killed by stunning, followed immediately by decapitation with an animal guillotine (Harvard Apparatus, Holliston, MMA, USA). Brains were quickly removed from the cranium, washed in PBS, then cut into two parts to separate the forebrain and the brainstem which were then snap-frozen in dry ice (within 3 min after stunning) before being stored at −80 °C. The SFO, SON, PVN, NTS and RVLM were accurately localised based on rat brain atlas (George *Paxinos & Watson, 2014*). Frozen forebrain and brainstem were mounted on the

cryostat stage set at −20 °C, equilibrated for 10 min and sectioned at 60 μm using a Thermo Scientific Shandon Cryotome FE and FSEE Cryostat. The sections were mounted on glass slides and stained with Toluidine blue (Sigma Aldrich; 1% w/v in 70% v/v ethanol) then visualised on a light microscope to identify the SFO, SON, PVN (at forebrain) and NTS and RVLM (at brainstem). Once localised, punches of SFO, SON, PVN, NTS and RVLM were then taken with one mm and 0.5 mm micro punches (Fine Scientific Tools) from the unstained tissue. The SFO was collected upon identification of anterior commissure followed by choroid plexus meets the third ventricle to form interventricular foremen. Six to eight consecutive punches were made to collect SFO region. Meanwhile, the supraoptic chiasm which was observance by 'naked' eye was used as reference point to collect SON and 12 to 14 serial punches were made to collect this region as defined by the rat brain atlas. Meanwhile, about eight consecutive punches were made upon opening of third ventricle to collect PVN. On the other hand, hypoglossal nucleus and facial nucleus were used as references for dissecting NTS (caudal, intermediate and rostral) and RVLM, respectively. As defined by the rat brain atlas, 20 to 23 consecutive sections were made for NTS, with the first six slices being central punches and the rest bilateral punches. For the RVLM, eight consecutive sections were punched after the disappearance of the facial nucleus. Each of the SFO, SON, PVN, NTS and RVLM punches then dispensed into 1.5ml tubes, suspended QIAzol reagent then stored at −80 °C prior to RNA extraction.

RNA extraction and quality assessment

Total RNA was extracted using Qiagen RNeasy kit protocols (Qiagen, Hilden, Germany). The frozen punched samples were allowed to thaw to ambient temperature and QIAzol phase later was separated with 350 μl chloroform (15 min, 12,000 g, 4 °C). The upper aqueous phase was removed then mixed with 70% (v/v) ethanol to precipitate the total RNA, which was resuspended and applied to RNeasy columns in accordance with the manufacturer's instruction. For RNAseq experiments, punched samples at QIAzol phase were pooled (5 per group), whereas individual samples were used in qRT-PCR ($n = 6$). The RNA concentration was measured using a Nanodrop spectrophotometer (ND-2000, Thermo Scientific, Wilmington, DE) and Qubit Fluorometer 2.0 (Life Technologies). The RNA samples were also analysed using 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA 95051) to obtain RNA integrity numbers (RIN numbers) as a measure of their quality (*Schroeder et al., 2006*). All RNA samples met the RIN quality criterion of >8.5.

RNA-Sequencing analysis

Amplified cDNA libraries were prepared from isolated SFO, SON, PVN, NTS and RVLM RNA samples from SRS and SHS groups ($n = 3$) and sequenced using Illumina HiSeq 2500 sequencer (Illumina Inc., USA). Briefly, total RNA samples were enriched by hybridization to bead-bound rRNA probes using Ribo Zero kit to obtain rRNA-depleted samples. This was followed by the construction of Illumina libraries using ScriptSeq v2 (Illumina Inc., USA) that applied unique barcode adapters. The libraries were assessed for their quality using Qubit dsDNA High Sensitivity DNA kit and Agilent 2100 Bioanalyzer (Agilent Technologies, A, USA; Agilent High Sensitivity DNA kit). This was followed with further

enrichment and amplification of the libraries by qRT-PCR using KAPA Biosystems Library Quantification kit, and normalisation to 2nM. Equal volumes of individual libraries were pooled and run on a MiSeq using MiSeq Reagent kit v2 (Illumina) to validate the library clustering efficiency. The libraries were then re-pooled based on the MiSeq demultiplexing results and sequenced on a HiSeq 2500 sequencing platform (Illumina, San Diego, California, USA) and cBot with v3 flow cells and sequencing reagents. The library reads of greater than 30 to 35 million were generated for each individual library. The data were then processed using RTA and CASAVA thus providing four sets of compressed FASTQ files per library. All raw reads were pre-processed for quality assessment, adaptor removal, quality trimming and size selection using the FASTQC toolkit to generate quality plots for all read libraries. The RNA-Seq alignment and data analysis were all performed in-house using a high-performance computer; "Hydra". The pipeline made use of Bash and Python scripting to accept RNA-Seq post-trimmed data as input, before ultimately producing output tables of differentially expressed transcripts. Paired-end (2X100bp) raw input data are initially aligned with Tophat to the sixth iteration of the *Rattus norvegicus* reference genome (Rn6) (*Trapnell, Pachter & Salzberg, 2009*). HTseq was used to generate read counts, using the ENSEMBL GRCh37 annotation for reference (*Anders, Pyl & Huber, 2015*). In the present study the pipeline used EdgeR statistical method from the R Bioconductor package to call differential gene expression (DGE) (*Risso et al., 2014*; *Robinson, McCarthy & Smyth, 2010*). This allowed us to predict low *p*-values ($p < 0.05$) and rank from highest to lowest fold changes (FC) which were utilised in downstream validation. Raw FASTQ files can be found at https://www.ebi.ac.uk/ena/data/view/PRJEB35016. A gene catalogues that described putative changes in gene expression as a consequence of being fed with HS diet in SHRs SFO, SON, PVN, NTS and RVLM were generated. All significantly expressed gene data then filtered to obtain genes expressed at a level of at least 20 read counts in one of the SHS versus SRS comparisons. This followed with sorting the genes according to FC from highest to lowest which later allowed us to select 10 genes (5 in each high and low FC) in each brain regions for subsequent validation with qRT-PCR. As well as validating the SHRs RNA-Seq data, the effect of the HS diet in WKY rats was also included.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

The cDNA synthesis was performed using QuantiTect Reverse Transcription kit (Qiagen) using total input RNA of 75ng for SFO, 200 ng for SON and PVN; 300 ng for NTS and 100 ng for RVLM, in accordance with manufacturer's instructions. Primers for qRT-PCR were designed from NCBI official website (http://www.ncbi.nlm.nih.gov). All primers for target and endogenous control genes were obtained from Integrated DNA Technologies and the primer sequences are provided in Table S1. The qRT-PCR reactions were carried out in duplicate in 96-well plates and each PCR sample consisted of 6 μl 2X SYBR green master mix buffer (Roche), 0.024 μl of both forward and reverse primers 25nmole and 3.953 μl of RNase-free water. The reactions were performed using Applied Biosystems StepOne Plus Real-time PCR system and FCs were assessed by establishing delta-delta cycle threshold (C_T) between *Rpl19*, a 60-s ribosomal protein L19 as a calibrator gene
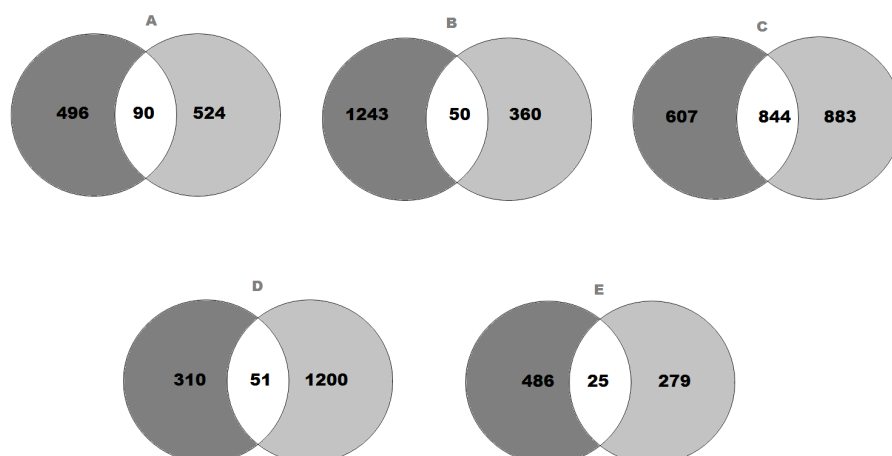
Figure 1 Venn diagram representing the number of genes that expressed significant changes in (A) SFO; (B) SON; (C) PVN; (D) NTS and (E) RVLM. The light grey area indicates (A) 586 genes in SFO; (B) 1,293 genes in SON; (C) 1,451 genes in PVN; (D) 361 genes in NTS and (E) 511 genes in RVLM of SHRs consuming high-salt (SHS) when compared with SHRs on regular-salt (SRS) diet; whilst the dark grey area represents (A) 614 genes in SFO; (B) 410 genes in SON; (C) 1,727 genes in PVN; (D) 1,251 genes in NTS and (E) 304 genes in RVLM of WKY rats on HS (WHS) when compared with WKY rats on RS (WRS) diet. Meanwhile, the (A) 90 genes in SFO; (B) 50 genes in SON; (C) 844 genes in PVN; (D) 51 genes in NTS and (E) 25 genes in RVLM are unique genes found in both strains of rats to intersect.

Full-size 🖼 DOI: 10.7717/peerj.8528/fig-1

and target genes. The following temperature profile was used: two minutes at 95 °C for reverse transcription according to the manufacturer's instruction, followed by 40 cycles at 95 °C for five seconds and 60 °C for 10 s. The average C_T values of target and calibrator genes obtained from qRT-PCR instrumentation were imported into a Microsoft Excel spreadsheet and the $\Delta\Delta C_T$ was calculated using $(C)_{TTarget}$ - C_{TRpl19} as described by *Livak & Schmittgen (2001)*.

Statistical analysis

Statistical analysis was performed using GraphPad Prism (GraphPad Software, La Jolla, CA, USA). All data are expressed as the mean ± standard error of means (SEM) of 6 rats. Comparisons between groups i.e., HS and RS of each individual strains were performed by independent unpaired Student's *t*-test. The differences were considered statistically significant at *p* values <0.05.

RESULTS

RNA-Seq analyses of SFO, SON, PVN, NTS and RVLM of WKY rats and SHRs fed with RS and HS diets

The transcriptomic data composed of gene catalogues with a wide variety of genes of diverse functions for all brain regions that compared the effect of high-salt (HS, 4% NaCl) diet with regular-salt (RS, 0.2% NaCl) diet in SHRs of SFO (Table S2), SON (Table S3), PVN (Table S4), NTS (Table S5) and RVLM (Table S6). All the significantly expressed genes of all brain regions are summarized in a Venn diagram (Figs. 1A to 1E). The Venn diagram explains the change in brain regions of SHRs fed with HS compared with SHRs

fed with RS diet and similarly for WKY rats. In addition, the diagram also demonstrates the intersection between WKY rats and SHRs indicating the presence of genes in both strains of rats as consequence of HS diet intake.

In SFO, there were 586 genes found to be significantly changed (edgeR; $p < 0.05$) in SHRs fed with HS (4% NaCl) diet when compared with SHRs on RS (0.2% NaCl) diet. Meanwhile, there were 614 genes significantly changed in WKY rats receiving HS diet as compared to WKY rats on RS diet. As shown in Fig. 1A, 90 genes observed at the intersection, indicating expression changes of identical genes in SFO in SHRs (SHS compared to SRS) and WKY rats (WHS compared to WRS).

On the other hand, the expression levels of 1,293 genes were significantly (edgeR; $p < 0.05$) changed in SHRs fed with HS (4% NaCl) diet when compared with SHRs on RS (0.2% NaCl) diet. Meanwhile, 410 genes were found to be significantly changed in WKY rats receiving HS diet as compared to WKY rats on RS diet. As shown in Fig. 1B, 50 genes were observed in the intersection, indicating expression changes of identical genes in SFO in SHRs (SHS compared to SRS) and WKY rats (WHS compared to WRS).

Meanwhile, there were 1,451 genes showed significant changes (edgeR; $p < 0.05$) in their expression levels in SHRs fed with HS (4% NaCl) diet when compared with SHRs on RS (0.2% NaCl) diet in PVN. A comparison of WKY rats receiving HS diet with WKY rats fed with RS diet showed that the expression levels of 1,727 genes were significantly changed, as displayed in Fig. 1C. The same figure shows an intersection of 844 genes, indicating expression changes of identical genes in PVN in SHRs (SHS compared to SRS) and WKY rats (WHS compared to WRS).

In the NTS, 361 genes were found to be significantly changed (edgeR; $p < 0.05$) in SHRs fed with HS (4% NaCl) diet when compared with SHRs on RS (0.2% NaCl) diet. Meanwhile, 1,251 genes were significantly changed in WKY rats receiving HS diet as compared to WKY rats on RS diet. As shown in Fig. 1D, 51 genes observed at the intersection, indicating expression changes of identical genes in NTS in SHRs (SHS compared to SRS) and WKY rats (WHS compared to WRS).

Meanwhile, there were 511 genes showed significant changes (edgeR; $p < 0.05$) in their expression levels in SHRs fed with HS (4% NaCl) diet when compared with SHRs on RS (0.2% NaCl) diet in RVLM. A comparison of the WKY rats receiving HS diet with WKY rats fed with RS diet, showed that the expression levels of 304 genes were significantly changed, as displayed in Fig. 1E. The same figure shows an intersection of 25 genes, indicating expression changes of identical genes in RVLM in SHRs (SHS compared to SRS) and WKY rats (WHS compared to WRS).

qRT-PCR validations of selected genes

The validation analyses for all target genes were compared relative to *Rpl19* as in all brain regions, *Rpl19* expression showed no significant differences in the expression when compared with that of another two well-known calibrator genes, *Gapdh* and *β-actin*, between the four experimental groups (WRS, WHS, SRS and SHS). For each tissue, based on the qRT-PCR analysis, validated genes were categorised as follows:

(A) Genes upregulated in both strains of rat.

Table 1 Summary of validation of putative SFO differentially expressed genes.

Gene	Gene name	RNA-Seq analysis				qRT-PCR validation
		SHS. AvgCount	SRS. AvgCount	EdgeR.FC	EdgeR *P*-value	Category
Rmrp	RNA component of mitochondrial processing endoribonuclease	13,845.20	5,339.07	2.58	2.02E–12	
Crip1	Cysteine-rich protein 1	226.25	149.41	2.53	6.15E–03	
Hbb	Homoglobin subunit β-1	5,450.44	2,198.55	2.47	2.90E–04	
Lin52	Protein Lin52	179.77	72.05	2.46	1.42E–03	C
Apopt1	Apoptogenic protein 1	258.84	105.15	2.45	1.32E–07	C
AVP	Vasopressin	127.89	12,256.18	0.01	1.20E–04	C
Sim1	Protein Sim 1	110.85	1,308.32	0.04	7.40E–04	
Pdyn	Proenkephalin-B	141.82	1,791.44	0.05	9.30E–04	
OXT	Oxytocin	219.56	3,452.25	0.06	1.00E–02	C
Th	Tyrosine 3-monooxygenase	57.55	504.04	0.07	2.39E–03	

Notes.

Category C: upregulated genes only in SHRs when analyzed with qRT-PCR.

Abbreviation: Avg count, average count of the genes' number; FC, fold change; *p*-value, the sum of significance; SHS, SHRs fed with HS diet; SRS, SHRs fed with RS diet.

(B) Genes downregulated in both strains of rat.

(C) Genes that showed significant upregulation only in SHRs.

(D) Genes that showed significant downregulation only in SHRs.

SFO: Among the 10 genes of the SFO chosen for validation (Table 1), 4 (*Apopt1*, *Lin52*, *AVP*, and *OXT*) were significantly upregulated only in SHRs when analyzed by qRT-PCR as evidenced in Figs. 2A to 2D.

SON: Among the 10 genes of the SON chosen for validation (Table 2), 2 (*Caprin2* and *Sctr*) were significantly upregulated in both strains of rats (Figs. 3A and 3B); 2 (*AVP* and *OXT*) were significantly upregulated only in SHRs (Figs. 3C and 3D) and 2 (*Kcnv1* and *Ndufaf2*) were significantly downregulated only in SHRs (Figs. 3E and 3F) when analyzed by qRT-PCR.

PVN. Among the 10 genes of the PVN chosen for validation (Table 3), 2 (*Orc6* and *Gkap*) were significantly downregulated in both strains of rats (Figs. 4A and 4B); 2 (*Caprin2* and *Sclt1*; Figs. 4C and 4D) were significantly upregulated only in SHRs and 1 (*Pi4k2a*) was significantly downregulated only in SHRs (Fig. 4E) when analyzed by qRT-PCR.

NTS. Among the 10 genes of the NTS chosen for validation (Table 4), 7 (*Rprd1a*, *Csrnp3*, *Snrpd2l*, *Iglon5*, *Rnf157*, *St6galnac6* and *Ankrd*) were significantly downregulated only in SHRs when analyzed by qRT-PCR as evidenced in Figs. 5A to 5G.

RVLM: Among the 10 genes of the RVLM chosen for validation (Table 5), 3 (*A4galt*, *Slc29a4* and *Cmc1*) were significantly upregulated only in SHRs (Figs. 6A–6C) whilst 2 (*Ttr* and *Faim*) were significantly downregulated only in SHRs (Figs. 6D to 6E) when analyzed by qRT-PCR.
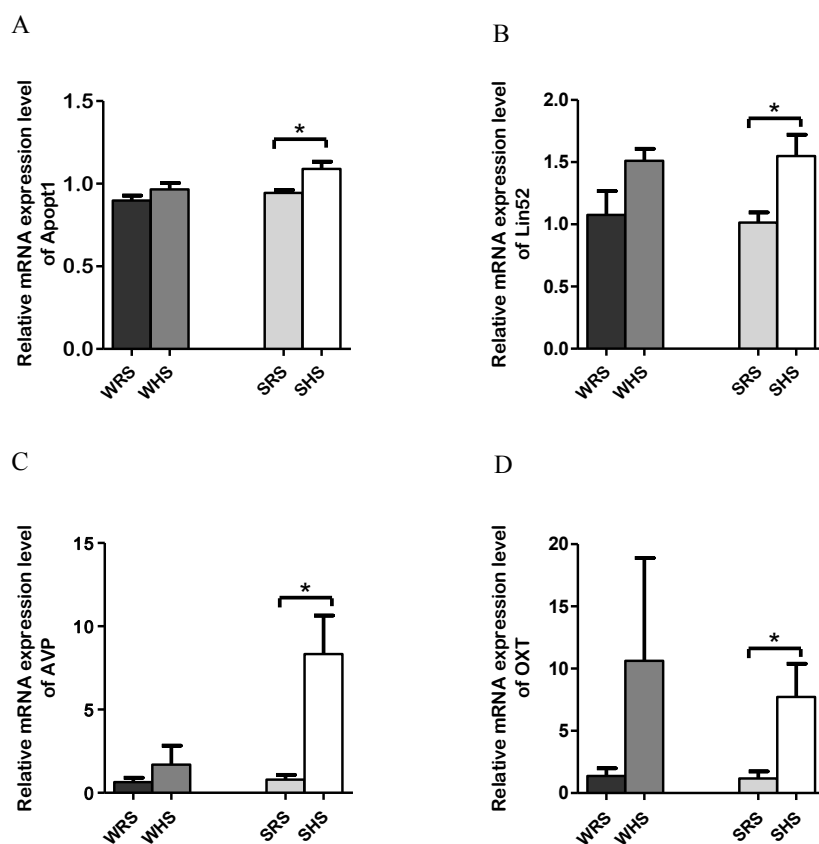
A

B

C

D

Figure 2 **Relative mRNA expression levels of up-regulated genes i.e., *Apopt1*, *Lin52*, *AVP* and *OXT* only in SHRs of SFO.** Data are presented as mean ± SEM; $n = 6$ rats. *$p < 0.05$ compared between SHS with SRS using Student's t-test.

Full-size ⤓ DOI: 10.7717/peerj.8528/fig-2

DISCUSSION

General consideration

In the present study, RNA-Seq was used to document changes in gene expression profile in SFO, SON, PVN, the hypothalamic forebrain region for maintaining homeostasis; and NTS and RVLM brainstem CV control centres in SHRs following the consumption of an HS diet. The SHR is a well-documented animal experimental model in functional genetic and physiological studies as it has been attributed to the similarity of its pathophysiology with essential HPN in human (*Doggrell & Brown, 1998*; *Korner, 2010*; *Leong, Ng & Jaarin, 2015*). Several expert panels have reported that SHRs are an excellent model of experimental HPN that could serve as a counterpart for clinical essential HPN as well as model for complications of HPN (*Badyal, Lata & Dadhich, 2003*). Moreover, it has been proposed that the pathogenesis of HPN in the SHR is heterogeneous with the underpinning of CNS, neurohumoral, renal, and cellular abnormalities (*DePasquale et al., 1992*; *Fortepiani et al., 2003*; *Sarikonda et al., 2009*). Our previous findings showed significant augmentation in mean arterial pressure (MAP) of SHRs fed with HS diet when compared with SHRs on

Table 2 Summary of validation of putative SON differentially expressed genes.

Gene	Gene name	RNA-Seq analysis				qRT-PCR validation
		SHS. AvgCount	SRS. AvgCount	EdgeR.FC	EdgeR P-value	Category
OXT	Oxytocin	35,909.30	62,162.60	1.73	1.13E–05	C
Sctr	Secretin receptor	159.86	137.71	1.58	4.24E–02	A
Ndufaf2	NADH: Ubiquinone oxidoreductase complex assembly factor 2	737.97	540.03	1.37	3.20E–04	D
AVP	Vasopressin	17,611.80	23,547.60	1.34	6.07E–03	C
Caprin2	Caprin family member 2	4,400.93	3,478.99	1.27	5.54E–03	A
Kl	Klotho	146.45	1,578.87	0.08	3.37E–04	
Clic6	Chloride intracellular channel 6	177.25	625.57	0.28	6.71E–03	
Lrp1b	LDL receptor related protein 1B	703.17	748.28	0.43	5.38E–01	
Rgs7bp	Regulator of G-protein signalling 7 binding protein	210.56	371.92	0.57	2.97E–03	
Kcnv1	Potassium-voltage gated channel modifier subfamily V member	94.46	138.54	0.68	1.77E–02	D

Notes.

Category A: upregulated genes in SHRs and WKY rats; Category C: upregulated genes only in SHRs and Category D: downregulated genes only in SHRs when analyzed with qRT-PCR.

Abbreviation: Avg count, average count of the genes' number; FC, fold change; *p*-value, the sum of significance; SHS, SHRs fed with HS diet; SRS, SHRs fed with RS diet.

a regular-salt diet. However, there was no significant change in the MAP of WKY rats as a consequence of the consumption of HS-diet. Thus, further confirming that SHRs developed a salt-sensitive component to the established HPN and are a model for studying the central mechanisms of salt-sensitive HPN.

RNA-Seq analysis and qRT-PCR validation

Using the SHR model, catalogues of genes that are differentially expressed in SFO, SON, PVN, NTS and RVLM as a consequence of consuming the HS diet were generated using RNA-Seq analysis which was then validated with qRT-PCR. We also asked if the HS differentially expressed genes validated in the SHRs were also altered in expression by HS in normotensive WKY rats' SFO, SON, PVN, NTS and RVLM. The comparisons were made between HS and RS diets in both the rat strains that revealed four categories of genes: genes up-regulated (A) or down-regulated (B), in both SHRs and WKY rats; genes up-regulated only in SHRs (C); genes down-regulated only in SHRs (D). Here, one might assume that it is the latter genes (categories C and D) that might have importance in the elevated BP seen in this strain as a consequence of HS. However, it may well be that genes commonly regulated in both strains have different effects due to WKY rats- or SHRs-specific interactions dependent upon genetic background. We also concede that we have revealed a relatively very small number of HS-responsive genes and many more remain to be mined from our RNAseq datasets.

Subfornical organ (SFO)

The SFO is known to be an important CV control region regulating fluid homeostasis by initiating volumetrically controlled thirst and drinking responses (*Denton et al., 1999*; *Freece*
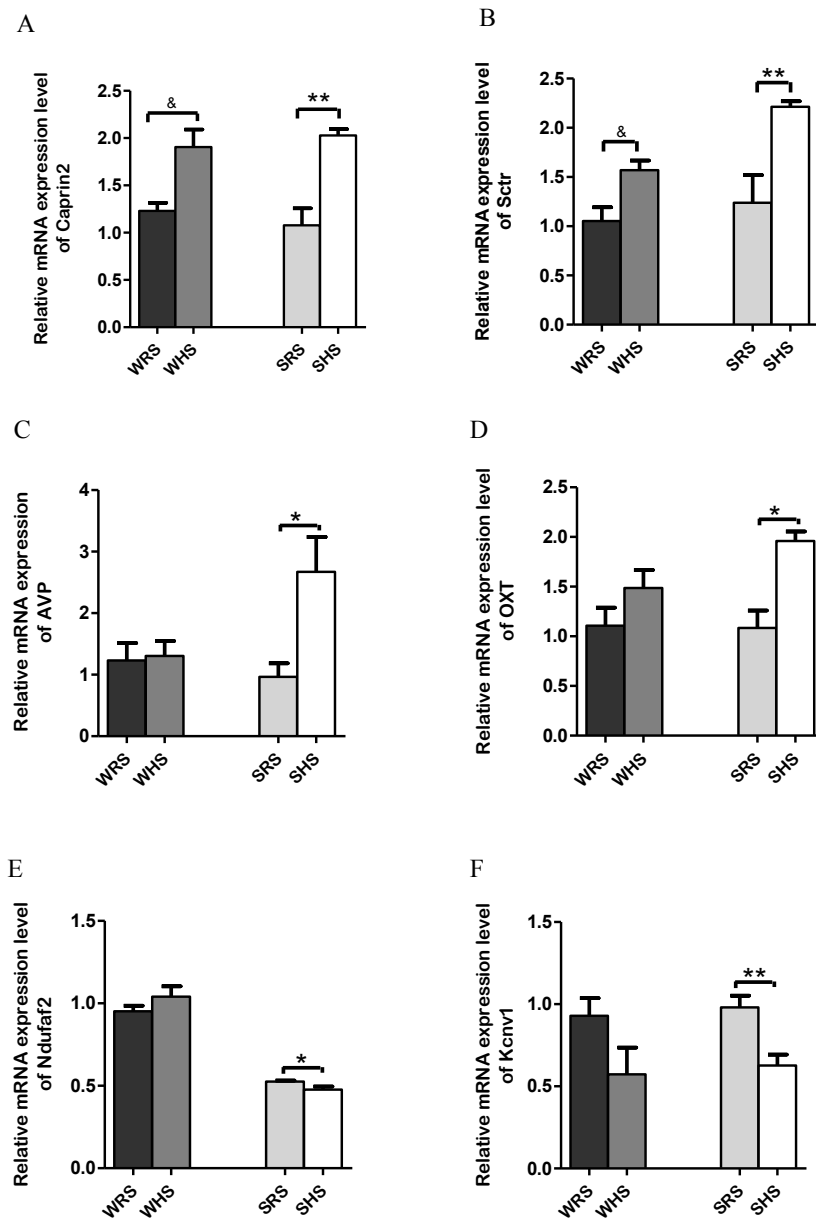
Figure 3 **Relative mRNA expression levels of up-regulated genes i.e., (A) *Caprin2* and (B) *Sctr* in both SHRs and WKY rats; up-regulated genes i.e., (C) *AVP* and (D) *OXT* only in SHRs; and down-regulated genes i.e., (E) *Kcnv1* and (F) *Ndufaf2* only in SHRs.** Data are presented as mean ± SEM; $n = 6$ rats. $*p < 0.05$ and $**p < 0.01$ compared between SHS with SRS and $\& p < 0.05$ compared between WHS with WRS using Student's t-test.

Full-size 🖼 DOI: 10.7717/peerj.8528/fig-3

et al., 2005; *Smith, Beninger & Ferguson, 1995*; *Stachenfeld, 2008*). The osmotic information through osmoreceptors from OVLT is transmitted neurally to the hypothalamus and ultimately results in thirst sensation, drinking behaviour and release of AVP; hence, retain water in the body. Thus, the up-regulation of *AVP* in the present study (Fig. 2C) is in accordance to the claim that the rat's SFO to have high-affinity binding sites for AVP as well

Table 3 Summary of validation of putative PVN differentially expressed genes.

| Gene | Gene name | RNA-Seq analysis | | | | qRT-PCR validation |
		SHS. AvgCount	SRS. AvgCount	EdgeR.FC	EdgeR P-value	Category
Pi4k2a	Phosphatidylinositol 4-kinase Type 2 alpha	232.15	108.97	2.13	4.27E–06	D
Prrc2a	Proline rich coiled-coil 2A	960.03	509.31	1.89	1.38E–06	
AVP	Vasopressin	9,331.38	5,415.72	1.73	8.45E–09	
Caprin2	Caprin family member 2	2,337.86	1,551.92	1.51	4.57E–04	C
OXT	Oxytocin	37,526.70	28,121.00	1.23	1.86E–03	
Sclt1	Sodium Channel and Clathrin Linker 1	1,663.35	1,707.38	0.97	8.46E–01	C
Gkap1	G Kinase Anchoring Protein 1	1,417.12	2,419.13	0.59	2.80E–05	B
Orc6	Origin Recognition Complex Subunit 6	97.98	173.50	0.57	3.29E–02	B
Isy1	SY1 Splicing Factor Homolog	498.23	915.38	0.55	1.67E–05	
Nsmce1	NSE1 Homolog, SMC5-SMC6 Complex Component	125.59	235.92	0.54	2.77E–03	

Notes.
Category B: downregulated genes in SHRs and WKY rats; Category C: upregulated genes only in SHRs and Category D: downregulated genes only in SHRs when analyzed with qRT-PCR.
Abbreviation: Avg count, average count of the genes' number; FC, fold change; *p*-value, the sum of significance; SHS, SHRs fed with HS diet; SRS, SHRs fed with RS diet.

as its degradation product of AVP (*Anthes et al., 1997*; *Jurzak, Fahrenholz & Gerstberger, 1993*). Furthermore, the mammalian SFO has been reported to contain vasopressinergic fibre endings from magnocellular or parvocellular portions, and the presence of *AVP* mRNA in SFO implies that AVP is formed and released there (*Anthes et al., 1997*). On the other hand, *OXT* was shown to be expressed in a similar pattern as *AVP*. Both AVP and OXT have been reported to be secreted simultaneously in response to hyperosmolality and hypovolemia (*Haanwinckel et al., 1995*; *Ventura et al., 2002*), and they have synergistic effect on the inner medullary collecting ducts (*Ventura et al., 2002*). The oxytocinergic fibres have also been reported to be found in SFO suggesting that OXT may affect salt and water intake in rats (*Hosono et al., 1999*). As such, the up-regulation of *OXT* level in SFO of SHRs in the present study is defensible.

In addition, both RNA-Seq and qRT-PCR data also revealed significant up-regulation of *Lin52* and *Apopt1* (Table 1 and Figs. 2A and 2B) in SHRs being fed with HS diet. The *Lin52* (protein Lin52) which was significantly up-regulated in SHS has been associated with clinical CV events among African Americans from atherosclerosis risk communities' study. A genome-wide analysis of meta-analysis study by *Shendre et al. (2017)* showed the presence of *Lin52* gene in African-American of CV events such as stroke, atherosclerosis and myocardial infarction incidents. However, the study did not further evaluate the functional role of this gene with the occurring of these diseases and suggested further studies. Hence, our finding may propose that HS intake could be the initial triggering factor leading to CV diseases. However, its certainty needs further exploration. Meanwhile, the *Apopt1*, an apoptogenic protein 1, has been shown to be localised within mitochondria and related with mitochondrial disorders characterised by cyclooxygenase (COX) deficiency (*Melchionda et al., 2014*). The COX deficiency is one of the most common biochemical abnormalities found in mitochondrial disorders with half of all cases remain genetically undefined. Moreover,
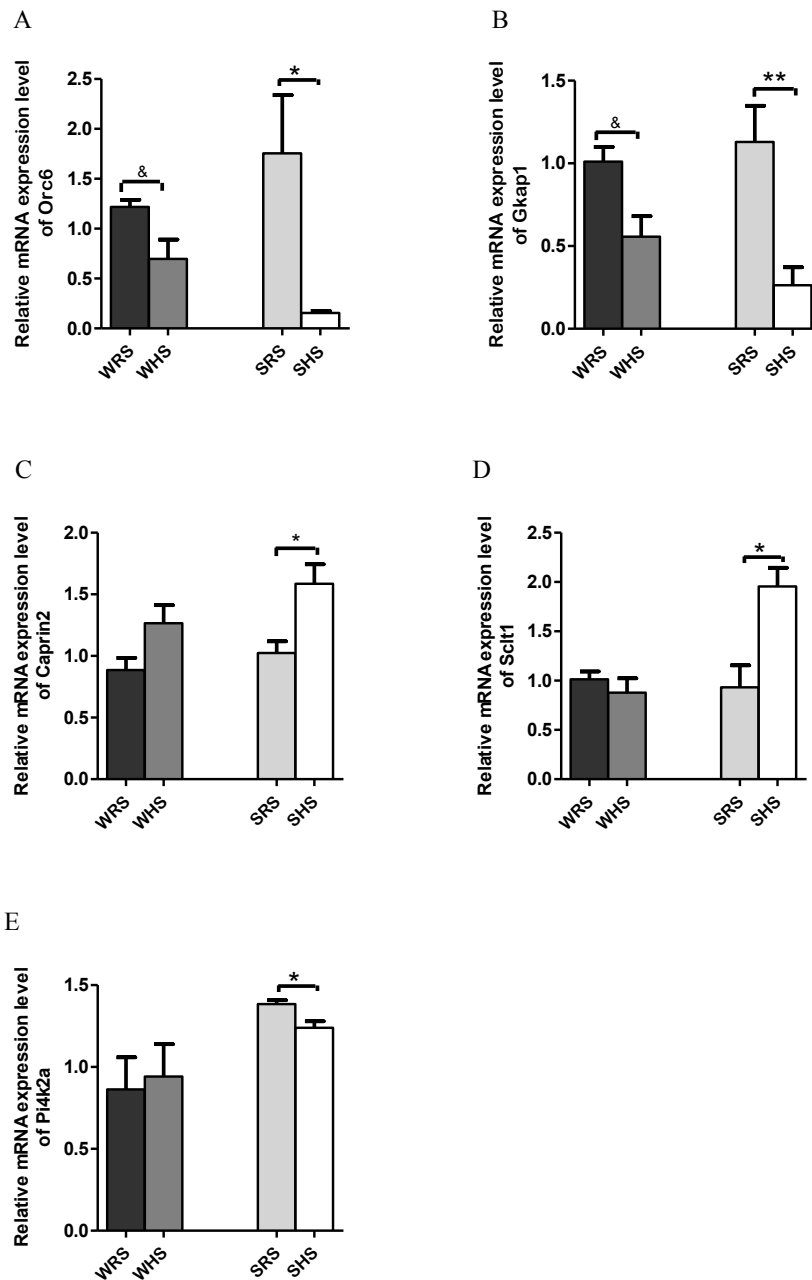
Figure 4 **Relative mRNA expression levels of down-regulated genes i.e., (A)** *Orc* **and (B)** *Gkap1* **in both SHRs and WKY rats; up-regulated genes i.e., (C)** *Caprin2* **and (D)** *Sclt* **only in SHRs and down-regulated gene i.e., (E)** *Pi4k2a* **only in SHRs.** Data are presented as mean $\pm$ SEM; $n = 6$ rats. $^*p < 0.05$ and $^{**}p < 0.01$ compared between SHS with SRS. $^*p < 0.05$ and $^{**}p < 0.01$ compared between SHS with SRS; and & $p < 0.05$ compared between WHS with WRS using Student's t-test.

Table 4 Summary of validation of putative NTS differentially expressed genes.

Gene	Gene name	RNA-Seq analysis				qRT-PCR validation
		SHS. AvgCount	SRS. AvgCount	EdgeR.FC	EdgeR P-value	Category
Nkain4	Sodium/Potassium Transporting ATPase Interacting 4	165.64	110.68	1.50	1.38E–02	
St6galnac6	ST6 N-Acetylgalactosaminide Alpha-2,6-sialyltransferase 6	638.48	436.19	1.47	2.83E–03	D
Ankrd29	Ankyrin Repeat Domain 29	161.05	142.31	1.50	4.96E–02	D
Rnf157	Ring Finger Protein 157	912.69	673.41	1.36	3.67E–02	D
Rprd1a	Regulation of Nuclear Pre-mRNA Domain Containing 1A	695.73	899.43	0.77	3.70E–02	D
Csrnp3	Cysteine and Serine Rich Nuclear Protein 3	980.12	1,302.83	0.75	2.56E–02	D
Pdyn	Prodynorphin	488.66	753.66	0.65	5.09E–03	
Snrpd2l	Small Nuclear Ribonucleoprotein D2-like	199.17	377.78	0.53	5.68E–03	D
Romo1	Reactive Oxygen Species Modulator 1	159.40	119.00	3.06	2.43E–06	
Iglon5	IgLON Family Member 4	128.26	155.44	0.51	4.71E–03	D

Notes.
Category D: downregulated genes only in SHRs when analyzed with qRT-PCR.
Abbreviation: Avg count, average count of the genes' number; FC, fold change; p-value, the sum of significance; SHS, SHRs fed with HS diet; SRS, SHRs fed with RS diet.

a connection between apoptosis and increased reactive oxygen species (ROS) production has been suggested to play an important role in the pathophysiology of mitochondrial diseases associated with COX deficiency (*Di Giovanni et al., 2001*; *Kadenbach et al., 2004*; *Melchionda et al., 2014*). Therefore, the expression of *Apopt1* in SFO needs further work to elucidate its functional significance in salt-induced HPN.

Supraoptic nucleus (SON)

The SON is known as a specialised nucleus in the hypothalamus that contains magnocellular neurons that secrete only AVP and OXT (*Armstrong, 1995*). A large amount of AVP and OXT are released into bloodstream to play a critical role in the regulation of salt and water balance as well as acting as vasoconstrictor, antidiuretic, CV regulator, lactation and affiliative behaviour when the normal physiological osmolality and volume are challanged (*Hatton, 1990*; *Hatton, 1997*; *Jansen, Van der Zee & Gerkema, 2007*; *Mlynarik et al., 2007*; *Young, 1999*). Thus, the high rates of neuropeptide synthesis, transport, and release in AVP and OXT have made SON as an important experimental model for the study peptidergic neuronal cell biology (*Burbach et al., 2001*; *Hatton, 1990*). The present RNA-Seq and qRT-PCR analyses of SON showed up-regulation of *AVP* and *OXT* (Table 2 and Figs. 3C and 3D) genes in SHRs as a result of consuming HS. These findings are in accordance with many other previous studies that have shown large increases in expression of the principal neuropeptide genes, *AVP* and *OXT* in magnocellular neurons (MCNs) in SON during salt-loading (*Burbach et al., 2001*; *Greenwood et al., 2015b*; *Johnson et al., 2015c*). It has been claimed that chronic salt loading produces large increase in volumes of MCNs which are recognized to be due to global increase in transcription and protein synthesis in the MCNs under hyperosmotic stimulations (*Johnson et al., 2015c*; *Miyata, Nakashima & Kiyohara,*
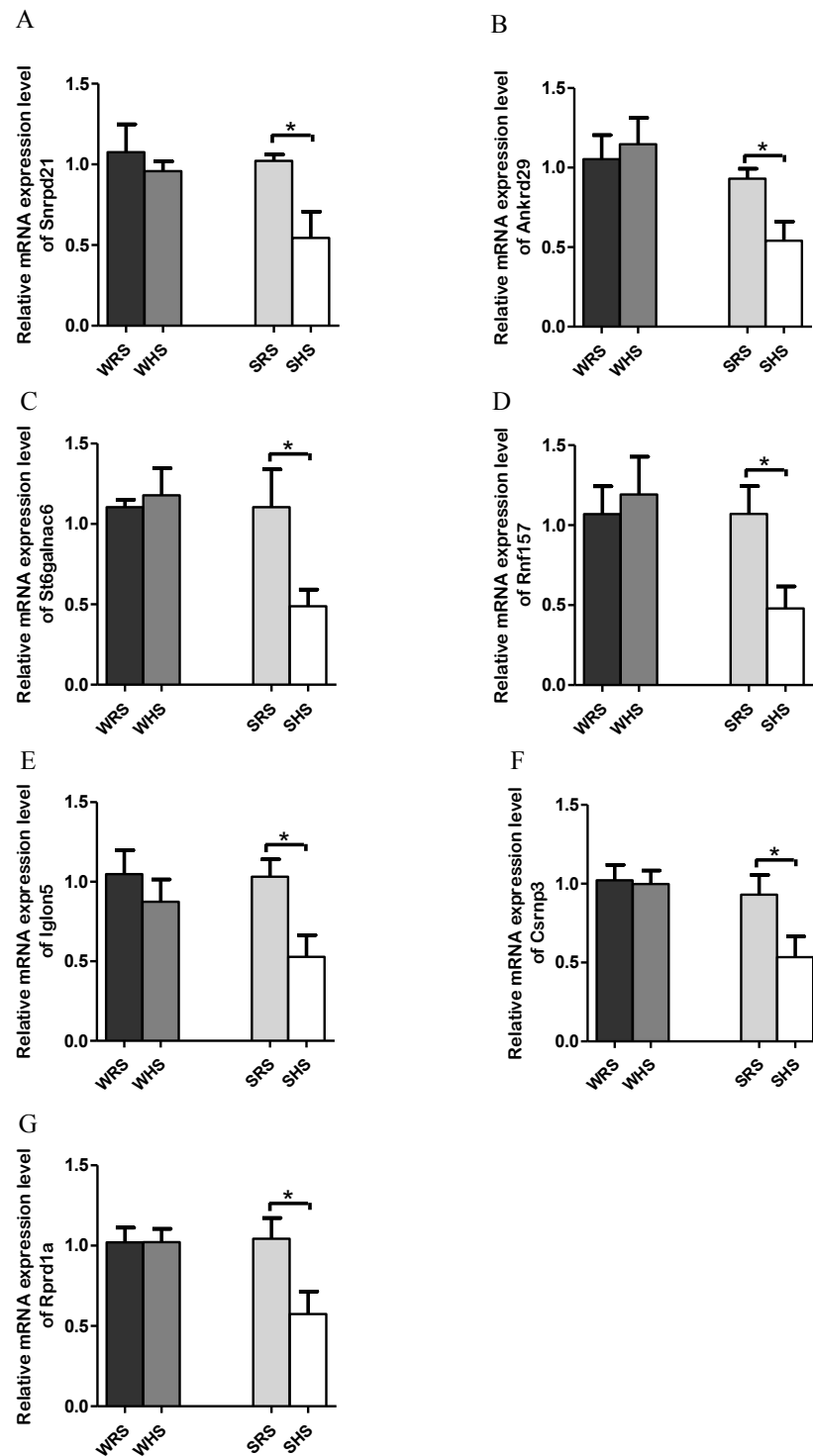
Figure 5 **Relative mRNA expression levels of down-regulated genes i.e., (A) *Snrpd2l*, (B) *Ankrd*, (C) *St6galnac6*, (D) *Csrnp3*, (E) *Iglon5*, (F) *Rnf157*, and (G) *Rprd1a* only in SHRs.** Data are presented as mean ± SEM; $n = D6$ rats. $^*p < 0.05$ compared between SHS with SRS using Student's t-test.

Full-size 🖼 DOI: 10.7717/peerj.8528/fig-5

Table 5 Summary of validation of putative RVLM differentially expressed genes.

Gene	Gene name	RNA-Seq analysis				qRT-PCR validation
		SHS. AvgCount	SRS. AvgCount	EdgeR.FC	EdgeR P-value	Category
A4galt	Alpha 1,4-galactosyltransferase	150.17	122.08	2.32	2.62E–03	C
Pnmt	Phenylethanolamine-N-methyltransferase	159.04	71.17	2.27	2.51E–03	
Snrpd2l	Small Nuclear Ribonucleoprotein D2-like	426.26	214.49	1.99	4.60E–02	
Slc29a4	Solute Carrier Family 29 Member 4	425.29	242.05	1.77	2.75E–03	C
Gmfb	Glia Maturation Factor, Beta	1,271.81	735.76	1.75	7.62E–03	
Ttr	Transthyretin	237.20	1,103.01	0.22	1.35E–02	D
Gmfg	Glia Maturation Factor, gamma	125.93	178.24	0.34	1.68E–03	
Cmc1	C-x(9)-C Motif containing 1	82.97	206.42	0.41	1.65E–07	C
S100a6	S100 Calcium Binding Protein A6	130.77	262.70	0.50	3.29E–03	
Faim	Fas Apoptotic Inhibitory Molecule	278.34	386.77	0.73	3.80E–02	D

Notes.
Category C: upregulated genes only in SHRs and Category D: downregulated genes only in SHRs when analyzed with qRT-PCR.
Abbreviation: Avg count, average count of the genes' number; FC, fold change; *p*-value, the sum of significance; SHS, SHRs fed with HS diet; SRS, SHRs fed with RS diet.

1994; *Zhang et al., 2001*). In addition, the increased *AVP* level in SHRs is in accordance with the hypothesis that SHRs have high AVP release i.e., high transcription of *AVP* as increasing levels of mineralocorticoid receptor and mineralocorticoids have been regarded to be involved in central regulation of BP, and they are known to drive the release of AVP to maintain hypertension (*Carmichael & Wainford, 2015*; *Pietranera et al., 2012*). Hence, it is not a surprise to observe a drastic increase of *AVP* in SHRs fed HS but there remains a need to elucidate the mechanism of how AVP related to salt-sensitive HPN. The increased expression of *AVP* is associated with an increased expression of *Caprin2*. The *Caprin2* is a gene that encodes RNA binding protein that plays a role in central osmotic defense receptor (*Konopacka et al., 2015*; *Loh et al., 2017*). It was found to directly bind to mRNA that encodes *AVP* and responsible to increase the length of poly-A tail (structures added to the end of all newly-made mRNAs as more *AVP* mRNAs molecules needed to produce during dehydration) (*Konopacka et al., 2015*). The team also concluded that *Caprin2* plays a critical role in mediating brain responses to osmotic stress as it is expressed in MCNs in SON and PVN, and that *Caprin2* expression in these neurones increases during osmotic stress. The study further demonstrated that *Caprin2* knockdown in SON and PVN disrupts physiological osmoregulatory mechanisms. Therefore, the up-regulation of *Caprin2* in the present study (Fig. 3A) further strengthen the findings of studies by *Konopacka et al. (2015)*. On the other hand, the OXT which is known to be co-secreted with AVP in response to osmotic and blood volume changes also been reported to induce a decrease in MAP and total peripheral resistance (*Dutil et al., 2001*; *Petersson et al., 1996*). This further suggest that OXT may play an important role in the control of body fluids homeostasis, causing natriuresis and vasodilatation, and reducing cardiac contractility and heart rate. Thus, the upregulation of *OXT* in SHRs fed with HS (Fig. 3D) suggesting that *OXT* along with *AVP* is transcribed more than normal levels induced by HS diet in SHRs in order to maintain BP.
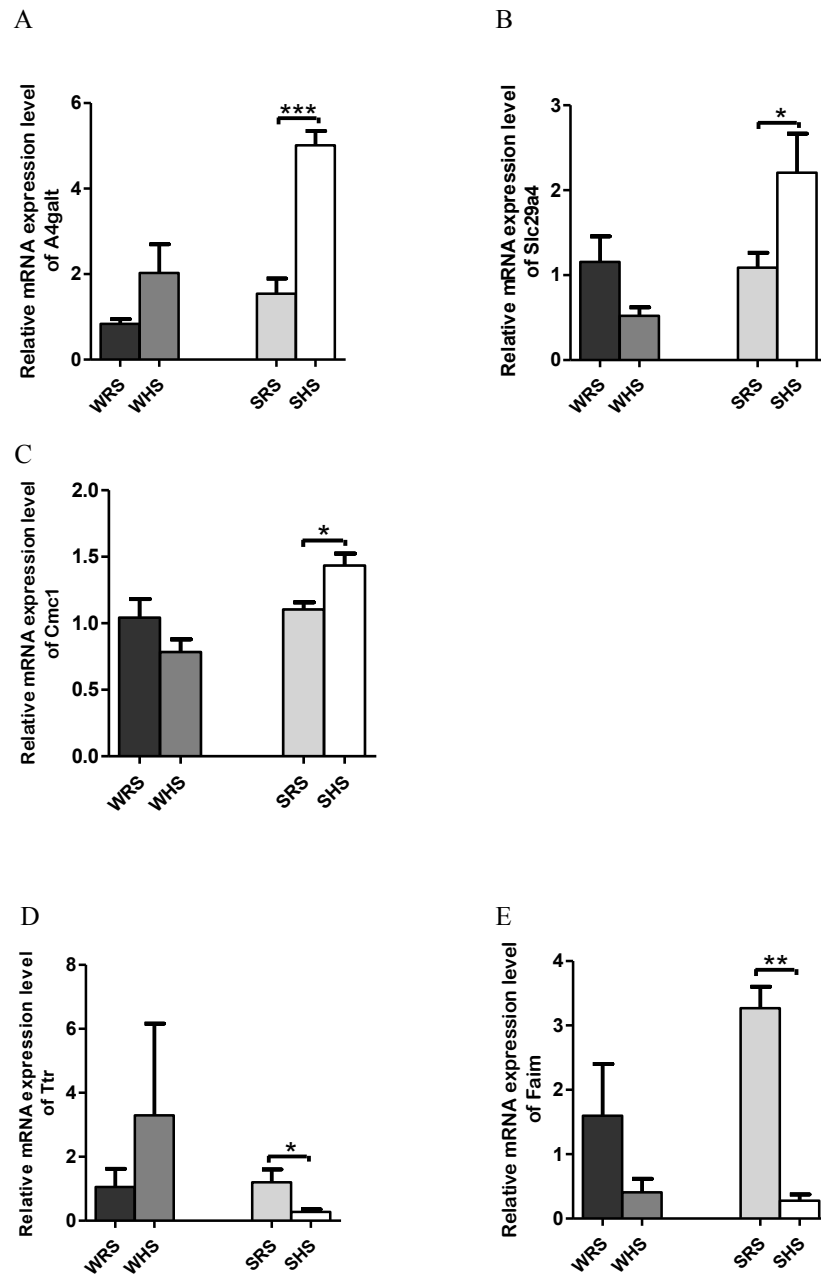
Figure 6 Relative mRNA expression levels of up-regulated genes i.e., (A) *A4galt*, (B) *Slc29a4* and (C) *Cmc1* only in SHRs; and down-regulated genes i.e., (D) *Ttr* and (E) *Faim* only in SHRs. Data are presented as mean ± SEM; $n = 6$ rats. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ compared between SHS with SRS using Student's t-test.

Full-size ☑ DOI: 10.7717/peerj.8528/fig-6

In addition, the *Sctr*, a secretin receptor is known as a potent regulator of pancreatic bicarbonate, electrolyte and volume secretions (*Baiocchi et al., 1999*; *Chow et al., 2004*; *Chu et al., 2007*) was found to up-regulated in both SHRs and WKY rats fed with HS diet (Fig. 3Aii). The secretin though originally isolated from upper intestinal mucosal

Ramachandran et al. (2020), *PeerJ*, DOI 10.7717/peerj.8528

18/35

extract, has been associated with translocation of water channels such as translocation of aquaporin 2 triggered by AVP and OXT to and from plasma membrane in renal tubules, a critical role for renal water absorption (*Chu et al., 2007*; *Jeon et al., 2003*; *Noda & Sasaki, 2005*). Since AVP has been proved in regulating renal water reabsorption and some of these mechanisms have been associated with cyclic-AMP-protein kinase A-mediated phosphorylation of water channels, it has been hypothesized that secretin could modulate renal water permeability by inducing AVP-independent translocation of functional aquaporin 2 (*Chu et al., 2007*; *Jeon et al., 2003*; *Li et al., 2006*; *Lorenz et al., 2003*; *Wilke et al., 2005*) which the water homeostasis is very much related to salt-sensitive HPN.

On the other hand, *Ndufaf2* and *Kcnv1* (Figs. 3E and 3F) were found to be down-regulated in SHRs fed with HS diet. Mutation of *Ndufaf2* has been reported in mitochondrial related diseases in humans (*Anderson et al., 2013*; *Chinnery & Hudson, 2013*; *Koene et al., 2012*); however, its exact role pertaining to HPN has not been documented. Meanwhile, the *Kcnv1*, voltage-gated channels have been reported as one of the gene expressed in preeclampsia, a hypertensive condition in pregnant women (*Chang et al., 2011*). To the best of our knowledge, the information of this gene associating with SSH is still lacking and not well documented in animal models.

Paraventricular nucleus (PVN)

The PVN is an important central sympathoexcitatory region which may become more active in hypertensive conditions (*Allen, 2002*; *Geraldes et al., 2014a*). It has been also referred to as a command nucleus providing feedforward excitatory synaptic drives to coordinate lower brainstem CV and respiratory motor activity (*Dampney et al., 2005*). The activation of PVN promotes an increase in sympathetic output and a pressor effect mediated via direct and indirect projections via RVLM to the spinal cord thereby accessing sympathetic neurons to modulate BP (*Badoer, 2001*; *Coote, 2005*; *Hardy, 2001*; *Motawei et al., 1999*). Extensive studies have been conducted even at the molecular level which all indicated PVN as an important hypothalamus region in BP modulation. The present study is also to serve added knowledge to the existing findings on PVN.

Similar, to SON, the PVN also accounts for greater secretion of AVP with clear cardinal physiological function i.e., to control renal excretion of water, to regulate hemodynamic parameters dependent of effective blood volume (vasopressor activity) and to regulate secretion of ACTH (from PVN's subdivision of parvocellular neurons) (*Sherman et al., 1986*). It is also well documented that the hypothalamic levels of *AVP* mRNA increase following chronic salt-loading and/or dehydration (*Greenwood et al., 2015a*; *Hayashi et al., 2006*; *Holbein, Bardgett & Toney, 2014*; *Holbein & Toney, 2015*), and this is clearly indicated in the present study which the mRNA expression level of *AVP* in PVN was increased in the expression insignificantly in SHRs fed with HS diet (Table 3). The SHRs is known for overactivated sympathetic activity even before hypertension development (*Simms et al., 2009*); thus, several increased in FC in *AVP* mRNA expression in chronic exposure to salt diet is considered as an estimated outcome. On the other hand, the increase in the expression of *OXT* in SHRs (Table 3) as a consequence of HS diet further strengthen the importance of OXT in salt appetite. In addition, it is known that OXT is released along

with AVP upon hypertonic stimulation. Meanwhile, the *Caprin2* (Fig. 4C) was also found to express in a similar manner as SON for the reason explained earlier. In addition to *AVP* and *OXT*, the PVN also expressed other genes with multiple functions. The *Sclt1* (sodium channel and clathrin linker 1) has been found to be associated with nephronophthisis, the most frequent genetic cause of chronic renal failure in children (*Failler et al., 2014*). This gene was found to be up-regulated in SHRs fed with HS diet; however, the relative contribution of this gene to salt-sensitive HPN is not able to explain at this moment.

On the other hand, *Pi4k2a* (Fig. 4E), *Gkap1* and *Orc6* (Figs. 4A and 4B) were found to be down-regulated in SHRs fed with HS diet. The *Pi4k2a*, mRNA encoding phosphatidylinositol 4-kinase type 2 isoform A is found in synaptic vesicles and involved in exocytosis which is important in the release of neurotransmitter from synaptosomes (*Solich et al., 2015*). Pharmacological inhibition of *Pi4k2a* causes significant decrease in norepinephrine release but does not affect the release of GABA or glutamate, suggesting an association of *Pi4k2a* with norepinephrine neurons (*Khvotchev & Sudhof, 1998*; *Solich et al., 2015*). A study conducted by Solich and team (*2016*) showed that *Pi4k2a* was down-regulated in the frontal cortex of norepinephrine transporter (NET) knock-out mice. The NET has been claimed to serve as the main target of antidepressant drugs and many diseases, and HPN has been linked with NET dysfunction (*Bonisch & Bruss, 2006*). Furthermore, the activity of NET is dependent on the concentration of sodium/chloride ions (*Shafqat et al., 1993*) and it present has been detected in brain regions rich with norepinephrine terminals i.e. thalamus, hypothalamus and amygdala (*Bonisch & Bruss, 2006*; *Lorang, Amara & Simerly, 1994*; *Schroeter et al., 2000*). Hence, the downregulation of this gene in SHRs being fed with HS diet seem to corelate with the development salt-sensitive HPN. Furthermore, this gene was also found in kidney transcriptome as well as haemoglobin where both studies were related to HPN (*Marques et al., 2017*; *Zhang et al., 2014*), and it has also been reported that *Pi4k2a* act via WNT-signaling pathway (*Kuzmanov et al., 2016*).

Nucleus tractus solitarii (NTS)

The NTS plays a pivotal role in the regulation of both the set-point of BP (*Waki et al., 2010*; *Waki, Takagishi & Gouraud, 2014*) and the gain of baroreflex for homeostatic control of BP (*Waki et al., 2010*). It is the principal site of termination of baroreceptor afferent fibres and as such mediates inhibitory action of baroreceptor on sympathetic discharge (*Frigero, Bonagamba & Machado, 2000*; *Machado, 2001*; *Talman, Perrone & Reis, 1981*; *Waki, Takagishi & Gouraud, 2014*). This area contains many neurotransmitters or neuromodulators that are important in CV control, and the intermediate portion of NTS is richly innervated by fibres arising from different brain nuclei that are also known to have an important role in CV control (*Colombari et al., 2001*; *Duale et al., 2007*; *Kasparov & Teschemacher, 2008*; *Zoccal et al., 2014*). The NTS neuron sends excitatory amino acid projections to the CVLM which in turn, inhibits RVLM neuron via GABAergic inhibitory pathway (*Guyenet, 2006b*; *Sapru, 1996*; *Yao et al., 2008*).

The present qRT-PCR data revealed that HS diet altered the expression level of genes that are functionally associated with transporters, transmembrane, signalling pathways, transcription factors and binding protein in both NTS. Genes such as *St6galnac6*, *Akrd29*,

Rnf157, *Rprd1a*, *Csrnp3*, *Snrpd1a,* and *Iglon5* were found significantly down-regulated only in SHRs fed with HS diet (Figs. 5A and 5G). The downregulation of *Snrpd2l*, small nuclear ribonucleoprotein D2-like suggests that aspects of RNA processing are changed in NTS due to HS diet. Meanwhile, the *Csrnp3* has been reported as one of the targeted genes in causing HPN (*Miao et al., 2017*; *Yamamoto et al., 2013*); however, the functional role of it was not discussed. The *Rnf157*, a ring finger protein 157, has been described to predominantly be expressed in brain and is implicated in the regulation of neuronal survival (*Matz et al., 2015*). Thus, its downregulation in the present study remains to be elucidated. Similarly, to *St6galnac6*, ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 6 which was identified as an enzyme in the synthesis of disialyl monosialyl Lewis (*Tsuchida et al., 2003*). Meanwhile, the *Ankrd2a*, ankyrin repeat domain 2a has been identified to belongs to the family of stress-inducible protein that expressed in striated muscle and its mutation has been recognised in cardiomyopathy patients (*Bang et al., 2014*). Finding by *Jasnic-Savovic et al. (2015)* has suggested *Ankrd2a* silencing to alter hypertrophic and dilated cardiomyopathy pathways and demonstrated the localisation of this protein in the intercalated disk of human cardiomyocytes. As there were not many reports on the presence of this gene in animal models, it hints for detail exploration in them. Meanwhile, *Rprd1a* has been associated with Wnt/β-catenin signalling and considered as an inhibitor for cell proliferation (*Liu et al., 2015*; *Zhang et al., 2012*). In addition, a study by *Arvind et al. (2015)* has reported up-regulation of *Rprd1a* in coronary artery disease in human. However, the correlation of this gene with SSH is yet to be documented and our study may be considered to be the first to report of its existence induced by HS diet. Similarly, the *Iglon5* which its functional role remains to be elucidated.

Rostral ventrolateral medulla (RVLM)

The RVLM is the region where the sympathetic pre-motor neurons controlling vasomotor sympathetic nerve activity are located. Thus, it is important in determining the basal sympathetic tone and receives inputs from various autonomic areas within the central nervous system (*Kumagai et al., 2012*; *Thomas et al., 2013*). The RVLM neurons project to the sympathetic preganglionic neurons in the IML cell column of the spinal cord and receive a direct glutamatergic projection from NTS (*Card et al., 2006*; *Dampney, 1994*; *Geraldes et al., 2014b*; *Leman, Viltart & Sequeira, 2000*). These neurons also receive excitatory and inhibitory inputs from other brain areas, such as the hypothalamus and other part of the medulla oblongata (*Koga et al., 2008*; *Sved, Ito & Yajima, 2002*). The RVLM has been found to have enhanced angiotensin-II-dependent superoxide accumulation under the influence of dietary salt (*Braga, 2010*).

In the present qRT-PCR analysis, *A4galt*, *Slc29a4*, and *Cmc1* were found to be up-regulated in SHRs being fed with HS diet. The *Slc29a4*, a nucleosides transporter, which is known for its presence in renal epithelia (*Duan & Wang, 2013*) with minimal functional property has been reported to be catalyse the reuptake of monoamines into presynaptic neurons, thus determining the intensity and duration of monoamine neural signalling. In addition, it has also been shown to transport several compounds, including serotonin, dopamine and neurotoxin 1-methyl-4 phenylpyridinium. If this is the case, the upregulation

of this gene in SHRs (Fig. 6B) may have been partially resolved; however, further studies are still require to ascertain the functional properties pertaining to HS diet intake. Meanwhile, *A4galt* has been reported to be up-regulated in clinical trials of primary aldosteronism (*Chenlong et al., 2017*), the most common form of endocrine hypertension which has been characterized by excessive and autonomous aldosterone secretion causing increased sodium retention, potassium excretion, hypervolemia, suppressed renin activity and HPN (*Rossi, 2011*). Therefore, the upregulation of *A4galt* in the present study (Fig. 6A) may suggest a direct or indirect involvement in increasing MAP in SHRs being fed with the HS diet.

Meanwhile, *Ttr* and *Faim* were found to be significantly down-regulated in SHRs (Figs. 6D and 6E), where *Ttr* mutation was strongly associated in amyloidosis, a genetic disorder characterized in many forms such as amyloid cardiomyopathy amyloid, familial amyloid polyneuropathy, leptomeningeal amyloidosis (*Faria et al., 2015*; *Gertz et al., 2015*; *Gonzalez-Lopez et al., 2015*; *Lobato et al., 2003*; *Palaninathan, 2012*) and senile amyloidosis (typical pulmonary lesions) (*Kruczak et al., 2013*). The amyloidosis has been linked with the occurrence of HPN as reported in case of studies by *Eder et al. (2007)*, *Culafic et al. (2007)* and *Cirulis et al. (2016)*; however, all these reports failed to mention on the mechanism of *Ttr* in regulating HPN.

CONCLUSIONS

In summary, high dietary sodium intake is thought to be one of the most prevalent risk factors for hypertension in modern societies, and there is a need to better understand the mechanisms involved. We found that chronic ingestion of a HS diet altered gene expression profiles at the level of SFO, SON, PVN, NTS and RVLM in both SHRs and WKY rats. These findings are suggestive of changes in hypothalamic forebrain and brainstem signalling systems that might participate in SSH. We suggest that, irrespective of the primary cause of hypertension, the genes involved in generating and regulating CV autonomic outflow from the hypothalamic forebrain and brainstem are potential targets for effective new therapies for SSH.

ACKNOWLEDGEMENTS

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

Author Contributions

- Chitra Devi Ramachandran conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Khadijeh Gholami and See Ziau Hoe conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Sau Kuen Lam conceived and designed the experiments, authored or reviewed drafts of the paper, review & suggestion, and approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

All the experimental protocols involving animals and housing thereof were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Malaya (Reference: 2014-01-07/Physio/R/HSZ)

Data Availability

The following information was supplied regarding data availability:

The raw data is available at EBI: PRJEB35016.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.8528#supplemental-information.

REFERENCES

Ahmed AS, Dai L, Ho W, Ferguson AV, Sharkey KA. 2014. The subfornical organ: a novel site of action of cholecystokinin. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **306(5)**:R363–R373 DOI 10.1152/ajpregu.00462.2013.

Allen AM. 2002. Inhibition of the hypothalamic paraventricular nucleus in spontaneously hypertensive rats dramatically reduces sympathetic vasomotor tone. *Hypertension* **39(2)**:275–280 DOI 10.1161/hy0202.104272.

Anders S, Pyl PT, Huber W. 2015. HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics* **31(2)**:166–169 DOI 10.1093/bioinformatics/btu638.

Anderson CD, Biffi A, Nalls MA, Devan WJ, Schwab K, Ayres AM, Valant V, Ross OA, Rost NS, Saxena R, Viswanathan A, Worrall BB, Brott TG, Goldstein JN, Brown D, Broderick JP, Norrving B, Greenberg SM, Silliman SL, Hansen BM, Tirschwell DL, Lindgren A, Slowik A, Schmidt R, Selim M, Roquer J, Montaner J, Singleton AB, Kidwell CS, Woo D, Furie KL, Meschia JF, Rosand J, International Stroke Genetics Consortium. 2013. Common variants within oxidative phosphorylation genes influence risk of ischemic stroke and intracerebral hemorrhage. *Stroke* **44(3)**:612–619 DOI 10.1161/STROKEAHA.112.672089.

Anthes N, Schmid HA, Hashimoto M, Riediger T, Simon E. 1997. Heterogeneous actions of vasopressin on ANG II-sensitive neurons in the subfornical organ of rats. *American Journal of Physiology* **273(6 Pt 2)**:R2105–R2111.

Armstrong WE. 1995. Morphological and electrophysiological classification of hypothalamic supraoptic neurons. *Progress in Neurobiology* **47(4–5)**:291–339 DOI 10.1016/0301-0082(95)80005-S.

Arvind P, Jayashree S, Jambunathan S, Nair J, Kakkar VV. 2015. Understanding gene expression in coronary artery disease through global profiling, network analysis and independent validation of key candidate genes. *Journal of Genetics* **94(4)**:601–610 DOI 10.1007/s12041-015-0548-3.

Badoer E. 2001. Hypothalamic paraventricular nucleus and cardiovascular regulation. *Clinical and Experimental Pharmacology and Physiology* **28(1–2)**:95–99 DOI 10.1046/j.1440-1681.2001.03413.x.

Badyal DK, Lata H, Dadhich A. 2003. Animal models of hyertension and effect of drugs. *Indian Journal of Pharmacology* **35**:349–362.

Baiocchi L, LeSage G, Glaser S, Alpini G. 1999. Regulation of cholangiocyte bile secretion. *Journal of Hepatology* **31(1)**:179–191 DOI 10.1016/S0168-8278(99)80180-9.

Bang ML, Gu Y, Dalton ND, Peterson KL, Chien KR, Chen J. 2014. The muscle ankyrin repeat proteins CARP, Ankrd2, and DARP are not essential for normal cardiac development and function at basal conditions and in response to pressure overload. *PLOS ONE* **9(4)**:e93638 DOI 10.1371/journal.pone.0093638.

Bonisch H, Bruss M. 2006. The norepinephrine transporter in physiology and disease. *Handbook of Experimental Pharmacology* **(175)**:485–524.

Braga VA. 2010. Dietary salt enhances angiotensin-II-induced superoxide formation in the rostral ventrolateral medulla. *Autonomic Neuroscience* **155(1–2)**:14–18 DOI 10.1016/j.autneu.2009.12.007.

Burbach JP, Luckman SM, Murphy D, Gainer H. 2001. Gene regulation in the magnocellular hypothalamo-neurohypophysial system. *Physiological Reviews* **81(3)**:1197–1267 DOI 10.1152/physrev.2001.81.3.1197.

Card JP, Sved JC, Craig B, Raizada M, Vazquez J, Sved AF. 2006. Efferent projections of rat rostroventrolateral medulla C1 catecholamine neurons: implications for the central control of cardiovascular regulation. *Journal of Comparative Neurology* **499(5)**:840–859 DOI 10.1002/cne.21140.

Carmichael CY, Carmichael AC, Kuwabara JT, Cunningham JT, Wainford RD. 2015. Impaired sodium-evoked paraventricular nucleus neuronal activation and blood

pressure regulation in conscious Sprague-Dawley rats lacking central Galphai proteins. *Acta Physiologica* DOI 10.1111/apha.12610.

Carmichael CY, Wainford RD. 2015. Hypothalamic signaling mechanisms in hypertension. *Current Hypertension Reports* **17(5)**:39–46 DOI 10.1007/s11906-015-0550-4.

Chang SD, Chao AS, Peng HH, Chang YL, Wang CN, Cheng PJ, Lee YS, Chao A, Wang TH. 2011. Analyses of placental gene expression in pregnancy-related hypertensive disorders. *Taiwanese Journal of Obstetrics and Gynecology* **50(3)**:283–291 DOI 10.1016/j.tjog.2011.07.005.

Chenlong C, Chenhui Z, Zhiwei Z, Mingwei W, Zhaohui Z, Anqing Y, Wenlong Z, Chu C, Zhao C, Zhang Z, Wang M, Zhang Z, Yang A, Ma B, Gu M, Cui R, Xin Z, Huang T, Zhou W. 2017. Transcriptome analysis of primary aldosteronism in adrenal glands and controls. *International Journal of Clinical and Experimental Pathology* **10(9)**:10009–10018.

Chinnery PF, Hudson G. 2013. Mitochondrial genetics. *British Medical Bulletin* **106**:135–159 DOI 10.1093/bmb/ldt017.

Chow BK, Cheung KH, Tsang EM, Leung MC, Lee SM, Wong PY. 2004. Secretin controls anion secretion in the rat epididymis in an autocrine/paracrine fashion. *Biology of Reproduction* **70(6)**:1594–1599 DOI 10.1095/biolreprod.103.024257.

Chu JY, Chung SC, Lam AK, Tam S, Chung SK, Chow BK. 2007. Phenotypes developed in secretin receptor-null mice indicated a role for secretin in regulating renal water reabsorption. *Molecular and Cellular Biology* **27(7)**:2499–2511 DOI 10.1128/MCB.01088-06.

Cirulis MM, Emerson LL, Bull DA, Hatton N, Nativi-Nicolai J, Hildebrandt GC, Ryan JJ. 2016. Pulmonary arterial hypertension in primary amyloidosis. *Pulm Circ* **6(2)**:244–248 DOI 10.1086/686172.

Colombari E, Sato MA, Cravo SL, Bergamaschi CT, Campos Jr RR, Lopes OU. 2001. Role of the medulla oblongata in hypertension. *Hypertension* **38(3 Pt 2)**:549–554 DOI 10.1161/01.HYP.38.3.549.

Coote JH. 2005. A role for the paraventricular nucleus of the hypothalamus in the autonomic control of heart and kidney. *Experimental Physiology* **90(2)**:169–173 DOI 10.1113/expphysiol.2004.029041.

Cottrell GT, Ferguson AV. 2004. Sensory circumventricular organs: central roles in integrated autonomic regulation. *Regulatory Peptides* **117(1)**:11–23 DOI 10.1016/j.regpep.2003.09.004.

Culafic D, Perisic M, Boricic I, Culafic-Vojinovic V, Vukcevic M. 2007. Primary amyloidosis presenting with cholestasis and hyperkinetic portal hypertension. *Journal of gastrointestinal and liver diseases: JGLD* **16(2)**:201–204.

Dampney RA. 1994. Functional organization of central pathways regulating the cardiovascular system. *Physiological Reviews* **74(2)**:323–364 DOI 10.1152/physrev.1994.74.2.323.

Dampney RA, Horiuchi J, Killinger S, Sheriff MJ, Tan PS, McDowall LM. 2005. Long-term regulation of arterial blood pressure by hypothalamic nuclei: some critical

questions. *Clinical and Experimental Pharmacology and Physiology* **32(5–6)**:419–425 DOI 10.1111/j.1440-1681.2005.04205.x.

Denton D, Shade R, Zamarippa F, Egan G, Blair-West J, McKinley M, Fox P. 1999. Correlation of regional cerebral blood flow and change of plasma sodium concentration during genesis and satiation of thirst. *Proceedings of the National Academy of Sciences of the United States of America* **96(5)**:2532–2537 DOI 10.1073/pnas.96.5.2532.

DePasquale MJ, Fossa AA, Holt WF, Mangiapane ML. 1992. Central DuP 753 does not lower blood pressure in spontaneously hypertensive rats. *Hypertension* **19(6 Pt 2)**:668–671 DOI 10.1161/01.HYP.19.6.668.

Di Giovanni S, Mirabella M, Papacci M, Odoardi F, Silvestri G, Servidei S. 2001. Apoptosis and ROS detoxification enzymes correlate with cytochrome c oxidase deficiency in mitochondrial encephalomyopathies. *Molecular and Cellular Neuroscience* **17(4)**:696–705 DOI 10.1006/mcne.2001.0970.

DiBona GF. 2013. Sympathetic nervous system and hypertension. *Hypertension* **61(3)**:556–560 DOI 10.1161/HYPERTENSIONAHA.111.00633.

Doggrell SA, Brown L. 1998. Rat models of hypertension, cardiac hypertrophy and failure. *Cardiovascular Research* **39(1)**:89–105 DOI 10.1016/S0008-6363(98)00076-5.

Duale H, Waki H, Howorth P, Kasparov S, Teschemacher AG, Paton JF. 2007. Restraining influence of A2 neurons in chronic control of arterial pressure in spontaneously hypertensive rats. *Cardiovascular Research* **76(1)**:184–193 DOI 10.1016/j.cardiores.2007.06.018.

Duan H, Wang J. 2013. Impaired monoamine and organic cation uptake in choroid plexus in mice with targeted disruption of the plasma membrane monoamine transporter (Slc29a4) gene. *Journal of Biological Chemistry* **288(5)**:3535–3544 DOI 10.1074/jbc.M112.436972.

Dutil J, Moujahidine M, Lemieux C, Jankowski M, Gutkowska J, Deng AY. 2001. Chromosomal and comparative mapping of rat oxytocin, oxytocin receptor and vasopressin genes. *Cytogenetics and Cell Genetics* **93(1–2)**:57–59 DOI 10.1159/000056949.

Eder L, Zisman D, Wolf R, Bitterman H. 2007. Pulmonary hypertension and amyloidosis—an uncommon association: a case report and review of the literature. *Journal of General Internal Medicine* **22(3)**:416–419 DOI 10.1007/s11606-006-0052-9.

Esler M, Kaye D. 2000. Sympathetic nervous system activation in essential hypertension, cardiac failure and psychosomatic heart disease. *Journal of Cardiovascular Pharmacology* **35(7 Suppl 4)**:S1–S7.

Failler M, Gee HY, Krug P, Joo K, Halbritter J, Belkacem L, Filhol E, Porath JD, Braun DA, Schueler M, Frigo A, Alibeu O, Masson C, Brochard K, Hurault de Ligny B, Novo R, Pietrement C, Kayserili H, Salomon R, Gubler MC, Otto EA, Antignac C, Kim J, Benmerah A, Hildebrandt F, Saunier S. 2014. Mutations of CEP83 cause infantile nephronophthisis and intellectual disability. *American Journal of Human Genetics* **94(6)**:905–914 DOI 10.1016/j.ajhg.2014.05.002.

Faria TQ, Almeida ZL, Cruz PF, Jesus CS, Castanheira P, Brito RM. 2015. A look into amyloid formation by transthyretin: aggregation pathway and a novel kinetic model. *Physical Chemistry Chemical Physics* **17(11)**:7255–7263 DOI 10.1039/c4cp04549a.

Fortepiani LA, Yanes L, Zhang H, Racusen LC, Reckelhoff JF. 2003. Role of androgens in mediating renal injury in aging SHR. *Hypertension* **42(5)**:952–955 DOI 10.1161/01.HYP.0000099241.53121.7F.

Freece JA, Van Bebber JE, Zierath DK, Fitts DA. 2005. Subfornical organ disconnection alters Fos expression in the lamina terminalis, supraoptic nucleus, and area postrema after intragastric hypertonic NaCl. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **288(4)**:R947–R955 DOI 10.1152/ajpregu.00570.2004.

Frigero M, Bonagamba LG, Machado BH. 2000. The gain of the baroreflex bradycardia is reduced by microinjection of NMDA receptor antagonists into the nucleus tractus solitarii of awake rats. *Journal of the Autonomic Nervous System* **79(1)**:28–33 DOI 10.1016/S0165-1838(99)00089-2.

Geraldes V, Goncalves-Rosa N, Liu B, Paton JF, Rocha I. 2014a. Chronic depression of hypothalamic paraventricular neuronal activity produces sustained hypotension in hypertensive rats. *Experimental Physiology* **99(1)**:89–100 DOI 10.1113/expphysiol.2013.074823.

Geraldes V, Goncalves-Rosa N, Liu B, Paton JF, Rocha I. 2014b. Essential role of RVL medullary neuronal activity in the long term maintenance of hypertension in conscious SHR. *Autonomic Neuroscience* **186**:22–31 DOI 10.1016/j.autneu.2014.09.002.

Gertz MA, Benson MD, Dyck PJ, Grogan M, Coelho T, Cruz M, Merlini G, Berk JL, Plante-Bordeneuve V, Schmidt HHJ, Merlini G. 2015. Diagnosis, prognosis, and therapy of transthyretin amyloidosis. *Journal of the American College of Cardiology* **66(21)**:2451–2466 DOI 10.1016/j.jacc.2015.09.075.

Gonzalez-Lopez E, Gallego-Delgado M, Guzzo-Merello G, De Haro-Del Moral FJ, Cobo-Marcos M, Robles C, Bornstein B, Salas C, Lara-Pezzi E, Alonso-Pulpon L, Garcia-Pavia P. 2015. Wild-type transthyretin amyloidosis as a cause of heart failure with preserved ejection fraction. *European Heart Journal* **36(38)**:2585–2594 DOI 10.1093/eurheartj/ehv338.

Greenwood M, Greenwood MP, Paton JF, Murphy D. 2015a. Transcription factor CREB3L1 regulates endoplasmic reticulum stress response genes in the osmotically challenged rat hypothalamus. *PLOS ONE* **10(4)**:e0124956 DOI 10.1371/journal.pone.0124956.

Greenwood MP, Mecawi AS, Hoe SZ, Mustafa MR, Johnson KR, Al-Mahmoud GA, Elias LL, Paton JF, Antunes-Rodrigues J, Gainer H, Murphy D, Hindmarch CC. 2015b. A comparison of physiological and transcriptome responses to water deprivation and salt loading in the rat supraoptic nucleus. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **308(7)**:R559–R568 DOI 10.1152/ajpregu.00444.2014.

Guo L, Meng J, Xuan C, Ge J, Sun W, O'Rourke ST, Sun C. 2015. High salt-diet reduces SLC14A1 gene expression in the choroid plexus of Dahl salt sensitive rats. *Biochemical and Biophysical Research Communications* **461(2)**:254–259 DOI 10.1016/j.bbrc.2015.04.010.

Guyenet PG. 2006a. The sympathetic control of blood pressure. *Nature Reviews Neuroscience* **7(5)**:335–346 DOI 10.1038/nrn1902.

Guyenet PG. 2006b. The sympathetic control of blood pressure. *Nature Reviews Neuroscience* **28**:990–999.

Haanwinckel MA, Elias LK, Favaretto AL, Gutkowska J, McCann SM, Antunes-Rodrigues J. 1995. Oxytocin mediates atrial natriuretic peptide release and natriuresis after volume expansion in the rat. *Proceedings of the National Academy of Sciences of the United States of America* **92(17)**:7902–7906 DOI 10.1073/pnas.92.17.7902.

Hardy SG. 2001. Hypothalamic projections to cardiovascular centers of the medulla. *Brain Research* **894(2)**:233–240 DOI 10.1016/S0006-8993(01)02053-4.

Hatton GI. 1990. Emerging concepts of structure-function dynamics in adult brain: the hypothalamo-neurohypophysial system. *Progress in Neurobiology* **34(6)**:437–504 DOI 10.1016/0301-0082(90)90017-B.

Hatton GI. 1997. Function-related plasticity in hypothalamus. *Annual Review of Neuroscience* **20**:375–397 DOI 10.1146/annurev.neuro.20.1.375.

Hayashi M, Arima H, Goto M, Banno R, Watanabe M, Sato I, Nagasaki H, Oiso Y. 2006. Vasopressin gene transcription increases in response to decreases in plasma volume, but not to increases in plasma osmolality, in chronically dehydrated rats. *American Journal of Physiology, Endocrinology and Metabolism* **290(2)**:E213–E217 DOI 10.1152/ajpendo.00158.2005.

Hindmarch C, Yao S, Beighton G, Paton J, Murphy D. 2006. A comprehensive description of the transcriptome of the hypothalamoneurohypophyseal system in euhydrated and dehydrated rats. *Proceedings of the National Academy of Sciences of the United States of America* **103(5)**:1609–1614 DOI 10.1073/pnas.0507450103.

Holbein WW, Bardgett ME, Toney GM. 2014. Blood pressure is maintained during dehydration by hypothalamic paraventricular nucleus-driven tonic sympathetic nerve activity. *Journal de Physiologie* **592(17)**:3783–3799 DOI 10.1113/jphysiol.2014.276261.

Holbein WW, Toney GM. 2015. Activation of the hypothalamic paraventricular nucleus by forebrain hypertonicity selectively increases tonic vasomotor sympathetic nerve activity. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **308(5)**:R351–R359 DOI 10.1152/ajpregu.00460.2014.

Hosono T, Schmid HA, Kanosue K, Simon E. 1999. Neuronal actions of oxytocin on the subfornical organ of male rats. *American Journal of Physiology* **276(6 Pt 1)**:e1004–1008.

Iwamoto T, Kita S, Katsuragi T. 2005. Salt-sensitive hypertension, Na+/Ca2+ exchanger, and vascular smooth muscle. *Trends in Cardiovascular Medicine* **15(8)**:273–277 DOI 10.1016/j.tcm.2005.08.004.

Jansen K, Van der Zee EA, Gerkema M. 2007. Vasopressin immunoreactivity, but not vasoactive intestinal polypeptide, correlates with expression of circadian rhythmicity in the suprachiasmatic nucleus of voles. *Neuropeptides* **41(4)**:207–216 DOI 10.1016/j.npep.2007.04.003.

Jasnic-Savovic J, Nestorovic A, Savic S, Karasek S, Vitulo N, Valle G, Faulkner G, Radojkovic D, Kojic S. 2015. Profiling of skeletal muscle Ankrd2 protein in human

cardiac tissue and neonatal rat cardiomyocytes. *Histochemistry and Cell Biology* **143(6)**:583–597 DOI 10.1007/s00418-015-1307-5.

Jeon US, Joo KW, Na KY, Kim YS, Lee JS, Kim J, Kim GH, Nielsen S, Knepper MA, Han JS. 2003. Oxytocin induces apical and basolateral redistribution of aquaporin-2 in rat kidney. *Nephron Experimental Nephrology* **93(1)**:e36–e45.

Johnson AK, Zhang Z, Clayton SC, Beltz TG, Hurley SW, Thunhorst RL, Xue B. 2015. The roles of sensitization and neuroplasticity in the long-term regulation of blood pressure and hypertension. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **309(11)**:R1309–R1325 DOI 10.1152/ajpregu.00037.2015.

Johnson KR, Hindmarch CC, Salinas YD, Shi Y, Greenwood M, Hoe SZ, Murphy D, Gainer H. 2015c. A RNA-Seq analysis of the rat supraoptic nucleus transcriptome: effects of salt loading on gene expression. *PLOS ONE* **10(4)**:e0124523 DOI 10.1371/journal.pone.0124523.

Jurzak M, Fahrenholz F, Gerstberger R. 1993. Vasopressin anti-idiotypic antibody staining in the rat brain: colocalization with [35S] [pGlu4, Cyt6]AVP(4-9) binding sites. *Journal of Neuroendocrinology* **5(5)**:523–531 DOI 10.1111/j.1365-2826.1993.tb00517.x.

Kadenbach B, Arnold S, Lee I, Huttemann M. 2004. The possible role of cytochrome c oxidase in stress-induced apoptosis and degenerative diseases. *Biochimica et Biophysica Acta/General Subjects* **1655(1–3)**:400–408 DOI 10.1016/j.bbabio.2003.06.005.

Kasparov S, Teschemacher AG. 2008. Altered central catecholaminergic transmission and cardiovascular disease. *Experimental Physiology* **93(6)**:725–740 DOI 10.1113/expphysiol.2007.041814.

Khvotchev M, Sudhof TC. 1998. Newly synthesized phosphatidylinositol phosphates are required for synaptic norepinephrine but not glutamate or gamma-aminobutyric acid (GABA) release. *Journal of Biological Chemistry* **273(34)**:21451–21454 DOI 10.1074/jbc.273.34.21451.

King AJ, Osborn JW, Fink GD. 2007. Splanchnic circulation is a critical neural target in angiotensin II salt hypertension in rats. *Hypertension* **50**:547–556 DOI 10.1161/HYPERTENSIONAHA.107.090696.

Koene S, Rodenburg RJ, Van der Knaap MS, Willemsen MA, Sperl W, Laugel V, Ostergaard E, Tarnopolsky M, Martin MA, Nesbitt V, Fletcher J, Edvardson S, Procaccio V, Slama A, Van den Heuvel LP, Smeitink JA. 2012. Natural disease course and genotype-phenotype correlations in Complex I deficiency caused by nuclear gene defects: what we learned from 130 cases. *Journal of Inherited Metabolic Disease* **35(5)**:737–747 DOI 10.1007/s10545-012-9492-z.

Koga Y, Hirooka Y, Araki S, Nozoe M, Kishi T, Sunagawa K. 2008. High salt intake enhances blood pressure increase during development of hypertension via oxidative stress in rostral ventrolateral medulla of spontaneously hypertensive rats. *Hypertension Research* **31(11)**:2075–2083 DOI 10.1291/hypres.31.2075.

Konopacka A, Greenwood M, Loh SY, Paton J, Murphy D. 2015. RNA binding protein Caprin-2 is a pivotal regulator of the central osmotic defense response. *Elife* **4** DOI 10.7554/eLife.09656.

Korner PI. 2010. The phenotypic patterns of essential hypertension are the key to identifying high blood pressure genes. *Physiological Research* **59(6)**:841–857.

Kruczak K, Duplaga M, Sanak M, Papla B, Soja J, Nizankowska-Mogilnicka E, Sladek K. 2013. Transthyretin amyloidosis with pulmonary involvement in a patient with monoclonal gammapathy. *Pneumonologia i Alergologia Polska* **81(6)**:537–541.

Kumagai H, Oshima N, Matsuura T, Iigaya K, Imai M, Onimaru H, Sakata K, Osaka M, Onami T, Takimoto C, Kamayachi T, Itoh H, Saruta T. 2012. Importance of rostral ventrolateral medulla neurons in determining efferent sympathetic nerve activity and blood pressure. *Hypertension Research* **35(2)**:132–141 DOI 10.1038/hr.2011.208.

Kuzmanov U, Guo H, Buchsbaum D, Cosme J, Abbasi C, Isserlin R, Sharma P, Gramolini AO, Emili A. 2016. Global phosphoproteomic profiling reveals perturbed signaling in a mouse model of dilated cardiomyopathy. *Proceedings of the National Academy of Sciences of the United States of America* **113(44)**:12592–12597 DOI 10.1073/pnas.1606444113.

Leman S, Viltart O, Sequeira H. 2000. Expression of Fos protein in adrenal preganglionic neurons following chemical stimulation of the rostral ventrolateral medulla of the rat. *Brain Research* **854(1–2)**:189–196 DOI 10.1016/S0006-8993(99)02343-4.

Leong XF, Ng CY, Jaarin K. 2015. Animal models in cardiovascular research: hypertension and atherosclerosis. *BioMed Research International* **2015**:528757 DOI 10.1155/2015/528757.

Li C, Wang W, Summer SN, Cadnapaphornchai MA, Falk S, Umenishi F, Schrier RW. 2006. Hyperosmolality in vivo upregulates aquaporin 2 water channel and Na-K-2Cl co-transporter in Brattleboro rats. *Journal of the American Society of Nephrology* **17(6)**:1657–1664 DOI 10.1681/ASN.2005121381.

Liu C, Zhang Y, Li J, Wang Y, Ren F, Zhou Y, Su F, Jia B, Wang D, Chang Z. 2015. p15RS/RPRD1A (p15INK4b-related sequence/regulation of nuclear pre-mRNA domain-containing protein 1A) interacts with HDAC2 in inhibition of the Wnt/beta-catenin signaling pathway. *Journal of Biological Chemistry* **290(15)**:9701–9713 DOI 10.1074/jbc.M114.620872.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* **25(4)**:402–408 DOI 10.1006/meth.2001.1262.

Lobato L, Beirao I, Silva M, Bravo F, Silvestre F, Guimaraes S, Sousa A, Noël LH, Sequeiros J. 2003. Familial ATTR amyloidosis: microalbuminuria as a predictor of symptomatic disease and clinical nephropathy. *Nephrology, Dialysis, Transplantation* **18(3)**:532–538 DOI 10.1093/ndt/18.3.532.

Loh SY, Jahans-Price T, Greenwood MP, Greenwood M, Hoe SZ, Konopacka A, Campbell C, Murphy D, Hindmarch CCT. 2017. Unsupervised network analysis of the plastic supraoptic nucleus transcriptome predicts Caprin2 regulatory interactions. *eNeuro* **4(6)** DOI 10.1523/ENEURO.0243-17.2017.

Lohmeier TE. 2002. The sympathetic nervous system and long-term blood pressure regulation. *American Journal of Hypertension* **14**:147S–154S.

Lohmeier TE, Iliescu R, Dwyer TM, Irwin ED, Cates AW, Rossing MA. 2010. Sustained suppression of sympathetic activity and arterial pressure during chronic activation of the carotid baroreflex. *American Journal of Physiology-Heart and Circulatory Physiology* **299(2)**:H402–H409 DOI 10.1152/ajpheart.00372.2010.

Lorang D, Amara SG, Simerly RB. 1994. Cell-type-specific expression of catecholamine transporters in the rat brain. *Journal of Neuroscience* **14(8)**:4903–4914 DOI 10.1523/JNEUROSCI.14-08-04903.1994.

Lorenz D, Krylov A, Hahm D, Hagen V, Rosenthal W, Pohl P, Maric K. 2003. Cyclic AMP is sufficient for triggering the exocytic recruitment of aquaporin-2 in renal epithelial cells. *EMBO Reports* **4(1)**:88–93 DOI 10.1038/sj.embor.embor711.

Machado BH. 2001. Neurotransmission of the cardiovascular reflexes in the nucleus tractus solitarii of awake rats. *Annals of the New York Academy of Sciences* **940**:179–196.

Mann SJ. 2003. Neurogenic essential hypertension revisited: the case for increased clinical and research attention. *American Journal of Hypertension* **16(10)**:881–888 DOI 10.1016/S0895-7061(03)00978-6.

Marques FZ, Nelson E, Chu PY, Horlock D, Fiedler A, Ziemann M, Tan JK, Kuruppu S, Rajapakse NW, El-Osta A, Mackay CR, Kaye DM. 2017. High-fiber diet and acetate supplementation change the gut microbiota and prevent the development of hypertension and heart failure in hypertensive mice. *Circulation* **135(10)**:964–977 DOI 10.1161/CIRCULATIONAHA.116.024545.

Matz A, Lee SJ, Schwedhelm-Domeyer N, Zanini D, Holubowska A, Kannan M, Farnworth M, Jahn O, Göpfert MC, Stegmuller J. 2015. Regulation of neuronal survival and morphology by the E3 ubiquitin ligase RNF157. *Cell Death and Differentiation* **22(4)**:626–642 DOI 10.1038/cdd.2014.163.

Melchionda L, Haack TB, Hardy S, Abbink TE, Fernandez-Vizarra E, Lamantea E, Marchet S, Morandi L, Moggio M, Carrozzo R, Torraco A, Diodato D, Strom TM, Meitinger T, Tekturk P, Yapici Z, Al-Murshedi F, Stevens R, Rodenburg RJ, Lamperti C, Ardissone A, Moroni I, Uziel G, Prokisch H, Taylor RW, Bertini E, Van der Knaap MS, Ghezzi D, Zeviani M. 2014. Mutations in APOPT1, encoding a mitochondrial protein, cause cavitating leukoencephalopathy with cytochrome c oxidase deficiency. *American Journal of Human Genetics* **95(3)**:315–325 DOI 10.1016/j.ajhg.2014.08.003.

Miao R, Wang Y, Wan J, Leng D, Gong J, Li J, Zhang Y, Pang W, Zhai Z, Yang Y. 2017. Microarray analysis and detection of MicroRNAs associated with chronic thromboembolic pulmonary hypertension. *BioMed Research International* **2017**:8529796 DOI 10.1155/2017/8529796.

Miyata S, Nakashima T, Kiyohara T. 1994. Structural dynamics of neural plasticity in the supraoptic nucleus of the rat hypothalamus during dehydration and rehydration. *Brain Research Bulletin* **34(3)**:169–175 DOI 10.1016/0361-9230(94)90057-4.

Mlynarik M, Zelena D, Bagdy G, Makara GB, Jezova D. 2007. Signs of attenuated depression-like behavior in vasopressin deficient Brattleboro rats. *Hormones and Behavior* **51(3)**:395–405 DOI 10.1016/j.yhbeh.2006.12.007.

Moellenhoff E, Lebrun CJ, Blume A, Culman J, Herdegen T, Unger T. 1998. Central angiotensin AT1 and muscarinic receptors in ITF expression on intracerebroventricular NaCl. *American Journal of Physiology* **275(1 Pt 2)**:R234–R244.

Motawei K, Pyner S, Ranson RN, Kamel M, Coote JH. 1999. Terminals of paraventricular spinal neurones are closely associated with adrenal medullary sympathetic preganglionic neurones: immunocytochemical evidence for vasopressin as a possible neurotransmitter in this pathway. *Experimental Brain Research* **126(1)**:68–76 DOI 10.1007/s002210050717.

Noda Y, Sasaki S. 2005. Trafficking mechanism of water channel aquaporin-2. *Biology of the Cell* **97(12)**:885–892 DOI 10.1042/BC20040120.

Orlov SN, Mongin AA. 2007. Salt-sensing mechanisms in blood pressure regulation and hypertension. *American Journal of Physiology-Heart and Circulatory Physiology* **293(4)**:H2039–H2053 DOI 10.1152/ajpheart.00325.2007.

Palaninathan SK. 2012. Nearly 200 X-ray crystal structures of transthyretin: what do they tell us about this protein and the design of drugs for TTR amyloidoses? *Current Medicinal Chemistry* **19(15)**:2324–2342 DOI 10.2174/092986712800269335.

Paxinos G, Watson C. 2014. The rat brain in stereotaxic coordinates 7th ed. In: *Stereotaxic coordinates.* London: Elsevier.

Petersson M, Alster P, Lundeberg T, Uvnas-Moberg K. 1996. Oxytocin causes a long-term decrease of blood pressure in female and male rats. *Physiology and Behavior* **60(5)**:1311–1315 DOI 10.1016/S0031-9384(96)00261-2.

Petersson MJ, Rundqvist B, Johansson M, Eisenhofer G, Lambert G, Herlitz H, Jensen G, Friberg P. 2002. Increased cardiac sympathetic drive in renovascular hypertension. *Journal of Hypertension* **20(6)**:1181–1187 DOI 10.1097/00004872-200206000-00031.

Pietranera L, Brocca ME, Cymeryng C, Gomez-Sanchez E, Gomez-Sanchez CE, Roig P, Lima A, De Nicola AF. 2012. Increased expression of the mineralocorticoid receptor in the brain of spontaneously hypertensive rats. *Journal of Neuroendocrinology* **24(9)**:1249–1258 DOI 10.1111/j.1365-2826.2012.02332.x.

Pyner S. 2009. Neurochemistry of the paraventricular nucleus of the hypothalamus: implications for cardiovascular regulation. *Journal of Chemical Neuroanatomy* **38(3)**:197–208 DOI 10.1016/j.jchemneu.2009.03.005.

Ranson RN, Motawei K, Pyner S, Coote JH. 1998. The paraventricular nucleus of the hypothalamus sends efferents to the spinal cord of the rat that closely appose sympathetic preganglionic neurones projecting to the stellate ganglion. *Experimental Brain Research* **120(2)**:164–172 DOI 10.1007/s002210050390.

Risso D, Ngai J, Speed TP, Dudoit S. 2014. Normalization of RNA-seq data using factor analysis of control genes or samples. *Nature Biotechnology* **32(9)**:896–902 DOI 10.1038/nbt.2931.

Robinson MD, McCarthy DJ, Smyth GK. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26(1)**:139–140 DOI 10.1093/bioinformatics/btp616.

Rossi G. 2011. Diagnosis and treatment of primary aldosteronism. *Endocrinology and Metabolism Clinics of North America* **40(2)**:313–332 DOI 10.1016/j.ecl.2011.01.005.

Rowland NE, Fregly MJ, Li BH, Han L. 1996. Angiotensin-related induction of immediate early genes in rat brain. *Regulatory Peptides* **66(1–2)**:25–29 DOI 10.1016/0167-0115(96)00054-7.

Sakai K, Agassandian K, Morimoto S, Sinnayah P, Cassell MD, Davisson RL, Sigmund CD. 2007. Local production of angiotensin II in the subfornical organ causes elevated drinking. *Journal of Clinical Investigation* **117(4)**:1088–1095 DOI 10.1172/JCI31242.

Sakata K, Yoshida H, Obayashi K, Ishikawa J, Tamekiyo H, Nawada R, Doi O. 2002. Effects of losartan and its combination with quinapril on the cardiac sympathetic nervous system and neurohormonal status in essential hypertension. *Journal of Hypertension* **20(1)**:103–110 DOI 10.1097/00004872-200201000-00015.

Sapru HN. 1996. Carotid chemoreflex. Neural pathways and transmitters. *Advances in Experimental Medicine and Biology* **410**:357–364 DOI 10.1007/978-1-4615-5891-0_55.

Sarikonda KV, Watson RE, Opara OC, Dipette DJ. 2009. Experimental animal models of hypertension. *Journal of the American Society of Hypertension* **3(3)**:158–165 DOI 10.1016/j.jash.2009.02.003.

Schroeder A, Mueller O, Stocker S, Salowsky R, Leiber M, Gassmann M, Lightfoot S, Menzel W, Granzow M, Ragg T. 2006. The RIN: an RNA integrity number for assigning integrity values to RNA measurements. *BMC Molecular Biology* **7**:3 DOI 10.1186/1471-2199-7-3.

Schroeter S, Apparsundaram S, Wiley RG, Miner LH, Sesack SR, Blakely RD. 2000. Immunolocalization of the cocaine- and antidepressant-sensitive l-norepinephrine transporter. *Journal of Comparative Neurology* **420(2)**:211–232 DOI 10.1002/(SICI)1096-9861(20000501)420:2<211::AID-CNE5>3.0.CO;2-3.

Shafqat S, Velaz-Faircloth M, Guadano-Ferraz A, Fremeau Jr RT. 1993. Molecular characterization of neurotransmitter transporters. *Molecular Endocrinology* **7(12)**:1517–1529 DOI 10.1210/mend.7.12.7908408.

Shendre A, Irvin MR, Wiener H, Zhi D, Limdi NA, Overton ET, Shrestha S. 2017. Local ancestry and clinical cardiovascular events among African Americans from the atherosclerosis risk in communities study. *Journal of the American Heart Association* **6(4)**:e004739 DOI 10.1161/JAHA.116.004739.

Sherman TG, Civelli O, Douglass J, Herbert E, Watson SJ. 1986. Coordinate expression of hypothalamic pro-dynorphin and pro-vasopressin mRNAs with osmotic stimulation. *Neuroendocrinology* **44(2)**:222–228 DOI 10.1159/000124649.

Simms AE, Paton JF, Pickering AE, Allen AM. 2009. Amplified respiratory-sympathetic coupling in the spontaneously hypertensive rat: does it contribute to hypertension? *Journal de Physiologie* **587(3)**:597–610 DOI 10.1113/jphysiol.2008.165902.

Smith PM, Beninger RJ, Ferguson AV. 1995. Subfornical organ stimulation elicits drinking. *Brain Research Bulletin* **38**:209–213 DOI 10.1016/0361-9230(95)00088-V.

Smith PM, Ferguson AV. 2010. Circulating signals as critical regulators of autonomic state–central roles for the subfornical organ. *American Journal of*

Physiology-Regulatory, Integrative and Comparative Physiology **299**(2):R405–R415 DOI 10.1152/ajpregu.00103.2010.

Solich J, Kolasa M, Kusmider M, Pabian P, Faron-Gorecka A, Zurawek D, Szafran-Pilch K, Kedracka-Krok S, Jankowska U, Swiderska B, Dziedzicka-Wasylewska M. 2015. Life-long norepinephrine transporter (NET) knock-out leads to the increase in the NET mRNA in brain regions rich in norepinephrine terminals. *European Neuropsychopharmacology* **25**(8):1099–1108 DOI 10.1016/j.euroneuro.2015.04.019.

Stachenfeld NS. 2008. Acute effects of sodium ingestion on thirst and cardiovascular function. *Current Sports Medicine Reports* **7**(4 Suppl):S7–S13 DOI 10.1249/JSR.0b013e31817f23fc.

Stocker SD, Madden CJ, Sved AF. 2010. Excess dietary salt intake alters the excitability of central sympathetic networks. *Physiology & Behavior* **100**:519–524 DOI 10.1016/j.physbeh.2010.04.024.

Sved AF, Ito S, Sved JC. 2003. Brainstem mechanisms of hypertension: role of the rostral ventrolateral medulla. *Current Hypertension Reports* **5**(3):262–268 DOI 10.1007/s11906-003-0030-0.

Sved AF, Ito S, Yajima Y. 2002. Role of excitatory amino acid inputs to the rostral ventrolateral medulla in cardiovascular regulation. *Clinical and Experimental Pharmacology and Physiology* **29**(5–6):503–506 DOI 10.1046/j.1440-1681.2002.03663.x.

Talman WT, Perrone MH, Reis DJ. 1981. Acute hypertension after the local injection of kainic acid into the nucleus tractus solitarii of rats. *Circulation Research* **48**(2):292–298 DOI 10.1161/01.RES.48.2.292.

Thomas AJ, Gross BA, Jacob A, Easwer E. 2013. Essential hypertension as a result of neurochemical changes at the rostral ventrolateral medulla. *Journal of Clinical Neuroscience* **20**(12):1682–1687 DOI 10.1016/j.jocn.2013.02.040.

Thrasher TN. 2002. Unloading arterial baroreceptors causes neurogenic hypertension. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **282**(4):R1044–R1053 DOI 10.1152/ajpregu.00431.2001.

Thrasher TN. 2006. Arterial baroreceptor input contributes to long-term control of blood pressure. *Current Hypertension Reports* **8**(3):249–254 DOI 10.1007/s11906-006-0058-z.

Trapnell C, Pachter L, Salzberg SL. 2009. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics* **25**(9):1105–1111 DOI 10.1093/bioinformatics/btp120.

Tsuchida A, Okajima T, Furukawa K, Ando T, Ishida H, Yoshida A, Nakamura Y, Kannagi R, Kiso M, Furukawa K. 2003. Synthesis of disialyl Lewis a (Le(a)) structure in colon cancer cell lines by a sialyltransferase, ST6GalNAc VI, responsible for the synthesis of alpha-series gangliosides. *Journal of Biological Chemistry* **278**(25):22787–22794 DOI 10.1074/jbc.M211034200.

Ventura RR, Gomes DA, Reis WL, Elias LL, Castro M, Valenca MM, Carnio EC, Rettori V, McCann SM, Antunes-Rodrigues J. 2002. Nitrergic modulation of vasopressin, oxytocin and atrial natriuretic peptide secretion in response to sodium intake and hypertonic blood volume expansion. *Brazilian Journal of Medical and Biological Research* **35**(9):1101–1109 DOI 10.1590/S0100-879X2002000900011.

Ramachandran et al. (2020), *PeerJ*, DOI 10.7717/peerj.8528

34/35

Waki H, Gouraud SS, Maeda M, Paton JFR. 2010. Evidence of specific inflammatory condition in nucleus tractus solitarii of spontaneously hypertensive rats. *Experimental Physiology* **95**:595–600 DOI 10.1113/expphysiol.2009.047324.

Waki H, Takagishi M, Gouraud SS. 2014. Central mechanisms underlying antihypertensive effects of exercise training. *The Journal of Physical Fitness and Sports Medicine* **3(3)**:317–325 DOI 10.7600/jpfsm.3.317.

Wilke C, Sheriff S, Soleimani M, Amlal H. 2005. Vasopressin-independent regulation of collecting duct aquaporin-2 in food deprivation. *Kidney International* **67(1)**:201–216 DOI 10.1111/j.1523-1755.2005.00071.x.

Yamamoto H, Okuzaki D, Yamanishi K, Xu Y, Watanabe Y, Yoshida M, Yamashita A, Goto N, Nishiguchi S, Shimada K, Nojima H, Yasunaga T, Okamura H, Matsunaga H, Yamanishi H. 2013. Genetic analysis of genes causing hypertension and stroke in spontaneously hypertensive rats. *International Journal of Molecular Medicine* **31(5)**:1057–1065 DOI 10.3892/ijmm.2013.1304.

Yao F, Sumners C, O'Rourke ST, Sun C. 2008. Angiotensin II increases GABAB receptor expression in nucleus tractus solitarii of rats. *American Journal of Physiology-Heart and Circulatory Physiology* **294(6)**:H2712–H2720 DOI 10.1152/ajpheart.00729.2007.

Young LJ. 1999. Oxytocin and vasopressin receptors and species-typical social behaviors. *Hormones and Behavior* **36**:212–221 DOI 10.1006/hbeh.1999.1548.

Zhang B, Glasgow E, Murase T, Verbalis JG, Gainer H. 2001. Chronic hypoosmolality induces a selective decrease in magnocellular neurone soma and nuclear size in the rat hypothalamic supraoptic nucleus. *Journal of Neuroendocrinology* **13(1)**:29–36 DOI 10.1046/j.1365-2826.2001.00593.x.

Zhang X, Cao Q, Liu X, Liu S, Wang J, Sun S, Wang O, Tian Z, Liu H, Kuang J, Zhang W. 2012. Cellular and molecular evidence for malignancy-inhibitory functions of p15RS. *Cell Cycle* **11(10)**:1988–1998 DOI 10.4161/cc.20400.

Zhang X, Zhang W, Ma SF, Desai AA, Saraf S, Miasniakova G, Sergueeva A, Ammosova T, Xu M, Nekhai S, Abbasi T, Casanova NG, Steinberg MH, Baldwin CT, Sebastiani P, Prchal JT, Kittles R, Garcia JG, Machado RF, Gordeuk VR. 2014. Hypoxic response contributes to altered gene expression and precapillary pulmonary hypertension in patients with sickle cell disease. *Circulation* **129(16)**:1650–1658 DOI 10.1161/CIRCULATIONAHA.113.005296.

Zoccal DB, Furuya WI, Bassi M, Colombari DS, Colombari E. 2014. The nucleus of the solitary tract and the coordination of respiratory and sympathetic activities. *Frontiers in Physiology* **5**:238–249 DOI 10.3389/fphys.2014.00238.