Original Research

A custom rat and baboon hypertension gene array to compare experimental models

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Abstract

One challenge in understanding the polygenic disease of hypertension is elucidating the genes involved and defining responses to environmental factors. Many studies focus on animal models of hypertension; however, this does not necessarily extrapolate to humans. Current technology and cost limitations are prohibitive in fully evaluating hypertension within humans. Thus, we have designed a single-array platform that allows direct comparison of genes relevant to hypertension in animal models and non-human primates/human hypertension. The custom array is targeted to 328 genes known to be potentially related to blood pressure control. Studies compared gene expression in the kidney from normotensive rats and baboons. We found 74 genes expressed in both the rat and baboon kidney, 41 genes expressed in the rat kidney that were not detected in the baboon kidney and 34 genes expressed in the baboon kidney that were not detected in the rat kidney. To begin the evaluation of the array in a pathological condition, kidney gene expression was compared between the salt-sensitive deoxycorticosterone acetate (DOCA) rat model of hypertension and sham animals. Gene expression in the renal cortex and medulla from hypertensive DOCA compared with sham rats revealed three genes differentially expressed in the renal cortex: annexin A1 (up-regulated; relative intensity: 1.316 ± 0.321 versus 2.312 ± 0.283), glutamate-cysteine ligase (down-regulated; relative intensity: 3.738 ± 0.174 versus 2.645 ± 0.364) and glutathione-*S* transferase (down-regulated; relative intensity: 5.572 ± 0.246 versus 4.215 ± 0.411) and 21 genes differentially expressed in the renal medulla. Interestingly, few genes were differentially expressed in the kidney in the DOCA-salt model of hypertension; this may suggest that the complexity of hypertension may be the result of only a few gene-by-environment responsive events.

Keywords: hypertension, gene array, kidney, rat, baboon

Experimental Biology and Medicine 2012; **237**: 99–110. DOI: 10.1258/ebm.2011.011188

Introduction

A less-than-optimal blood pressure is the leading risk factor for cardiovascular disease throughout the world, particularly increases in blood pressure. If left untreated, hypertension can contribute to heart disease, stroke, myocardial infarction and end-stage renal disease.[1] Hypertension is a multifaceted disease that is composed of both genetic and environmental influences, and thus challenges in hypertension research include defining genetic factors and elucidating gene-by-environment interactions for the repertoire of genes involved in the initiation of this polygenic disease. The kidney plays a vital role in blood pressure maintenance. Due to the influence of the kidney on body fluid homeostasis, sympathetic and cardiovascular functions, it makes the kidney a logical candidate organ to evaluate hypertension-specific changes. Clinical and experimental renal transplant studies have demonstrated that the genetic background of the kidney plays a major role in long-term blood pressure regulation.[2] In addition, renal cross-transplantation experiments have further emphasized that the kidney plays a key role in primary hypertension in that renal transplant experiments revealed that blood pressure of the rats was determined largely by the donor kidney rather than the genotype of the recipient.[3,4]

There are many species of animals used as models of hypertension including mice, rats, sheep, pigs and baboons. Deoxycorticosterone acetate (DOCA) salt-induced hypertension is a form of pharmacologically induced hypertension that has been used to mimic excess secretion of mineralocorticoids that was initially described 15 years prior to the isolation of aldosterone in rats.[5] Salt-sensitive hypertension studies have been performed in rodents, sheep, pigs and baboon. While studies in rodents have certain advantages, they do not address hypertension in primates/humans. Differences are known to exist in some genetic mechanisms of salt-sensitive hypertension between primates and other mammals. For example, sodium lithium countertransport (SLC) activity, a quantitative trait for salt-sensitive hypertension, is heritable in primates but not in other mammals.[6] Therefore, we developed a custom hypertension gene array that could be utilized to evaluate salt-sensitive hypertension in both rats and non-human primates, the baboon, models of salt-sensitive hypertension.

Development of this cross-species array allows for studies that can compare genetic mechanisms of salt-sensitive hypertension between primates and rodents on the same platform; this currently is not feasible with commercially available gene arrays. We hypothesized that the custom gene array would identify key hypertension-related genes/pathways in the kidney that were similar across species, therefore identifying key areas on which future studies could focus; moreover, examination of the DOCA-salt model would further narrow the candidates to study. The goal of this study was to define gene expression common to, and different between, rat and baboon renal cortex and medulla, as well as to define underlying genetic responses occurring in the kidney from hypertensive DOCA-salt rats.

Materials and methods

DOCA-salt treatment

All animal procedures were performed in accordance with the Michigan State University Institutional Animal Care and Use Committee guidelines. Male Sprague-Dawley rats (250–300 g) were purchased from Charles River Laboratories (Portage, MI, USA), housed in clear plastic boxes and allowed *ad libitum* access to standard rat chow (Harlan/Tekland 8640 rodent diet; Harlan Teklad, Madison, WI, USA) and tap water. Animals, under isoflurane gas anesthesia, were unilaterally nephrectomized. DOCA-salt rats received a subcutaneous silicone implant impregnated with 150 mg/kg DOCA and postoperatively were given a solution of 1% NaCl and 0.2% KCl for drinking. A single injection of butorphanol tartrate (0.5 mg/kg, subcutaneous injection; Abbott Laboratories, Abbott Park, IL, USA) was given postoperatively as analgesia. Sham rats did not receive a DOCA implant and drank normal tap water. Systolic blood pressure measurements were taken using the tail cuff method (pneumatic transducer; Narco Biosystems, Houston, TX, USA) four weeks after surgical intervention.

Tissue isolation

Kidney collection from rats

Rats were deeply anesthetized with an overdose of sodium pentobarbital (100 mg/kg) (eyelid reflex loss and lack of withdrawal from painful stimuli [foot pinch] was used as the indicator of anesthetization). A bilateral pneumothorax was performed and the remaining kidney was removed. The cortex and medulla were dissected apart based on visual examination and quick-frozen in liquid nitrogen. Samples were stored at -80°C until RNA isolation.

Kidney collection from baboons

All procedures were approved by the Texas Biomedical Research Institutional Animal Care and Use Committee and conducted in Association for Assessment and Accreditation of Laboratory Animal Care-approved facilities. Baboon kidneys ($n = 3$) were collected opportunistically from baboons euthanized for reasons unrelated to kidney function (colony management). Necropsies were performed by a Texas Biomedical Research Institute staff veterinary pathologist. Kidneys were separated into the cortex and medulla and aliquots of each were quick-frozen in liquid nitrogen and stored at -80°C.

RNA extraction

Trizol Reagent was used to isolate total RNA from tissue according to the manufacturer's instructions. Briefly, tissues were homogenized in 1 mL of Trizol Reagent per 50–100 mg of tissue using a power homogenizer (PowerGen, Fisher Scientific, Pittsburgh, PA, USA). Homogenized samples were incubated for five minutes at 25°C to permit complete dissociation of nucleoprotein complexes. Chloroform (0.2 mL per 1 mL of Trizol Reagent) was added to each sample, shaken vigorously for 15 s and incubated at 25°C for three minutes; samples were centrifuged at 12,000 $\times$ *g* for 15 min at 4°C and the aqueous phase transferred to a fresh tube. RNA was precipitated in 0.5 mL of isopropyl alcohol per 1 mL of Trizol Reagent and incubated at 25°C for 10 min followed by centrifugation at 12,000 $\times$ *g* for 10 min at 4°C. The supernatant was decanted and the pellet washed with 75% ethanol, adding at least 1 mL of 75% ethanol per 1 mL of Trizol Reagent. After air-drying, the RNA pellet was dissolved in diethylpyrocarbonate (DEPC)-treated ddH_2O and quantified spectrophotometrically. RNA was separated into single-use portions and stored at -140°C.

Design of a cross-species oligonucleotide array

A list of genes relevant to hypertension and/or sodium management was developed based on review of current literature and published databases (PubMed, OMIM and Rat Genome databases) (Supplemental Table 1; for all supplemental tables, please see http://ebm.rsmjournals.com/lookup/suppl/doi:10.1258/ebm.2011.011188/-/DC1). In addition, gene expression profiling results for kidney biopsies from two baboons before and after a six-week high-salt dietary challenge with known genetically heritable linkages with high blood pressure (sodium lithium transport) were used to augment the list of genes by interrogating a human

Affymetrix Gene Chip (Affymetrix, Santa Clara, CA, USA) for each RNA sample and determining genes with expression that differed by two-fold or more.[7] After gene identification, the coding sequence of each human gene (USCS Browser, NCBI Search Nucleotide) and the rat homolog (USCS Genome Browser, NCBI-Search Nucleotide, GeneLynx, Rat Genome database) were imported into Sequencher (Gene Codes, Inc, Ann Arbor, MI, USA). Human and rat gene homologs were aligned and an oligonucleotide was designed based on conserved coding regions using Oligo Primer Analysis Software (Molecular Biology Insights, Inc, Cascade, CO, USA).

Before designing gene-specific oligonucleotides, we tested oligonucleotide-target gene specificity by analyzing oligonucleotide length and array wash conditions. We found a better signal with 66-nt oligonucleotides compared with 50-nt oligonucleotides. In addition, we observed slightly different results for target oligonucleotides carrying mono- and dinucleotide substitutions. Filters washed at a final temperature of 45°C showed poor hybridization of probe to target oligonucleotides carrying dispersed mononucleotide sequence differences up to 91%, but good hybridization to targets of >94% similarity. These same conditions led to positive hybridization of probes to targets carrying dinucleotide differences down to 91% similarity, but negative hybridization to targets at 88% similarity. Inasmuch as naturally occurring differences between baboon and human target sequences are likely to include a mixture of mono- and dinucleotide substitutions and are likely to have >91% sequence identity, the 45°C wash condition provides the greatest specificity of hybridization while still allowing for species sequence differences. We also tested differing salt concentrations during washing and did not find any significant difference in resolution (data not shown).

Oligonucleotide design constraints included: (1) oligonucleotide ≥65 nucleotides long; (2) less than eight mismatches between species; (3) 45–55% GC content; (4) no tetranucleotide repeats; (5) no significant hairpin loops (less than 7 bonds in a hairpin); and (6) optimal probe with highest T_m and the highest negative ΔG value for GC clamp. If a single oligonucleotide could not be designed for a gene for both species that met these constraints, then separate rat and human gene-specific oligonucleotides were designed. After oligonucleotide design, sequence specificity was confirmed by performing an NCBI-BLAST search and uniqueness of the oligonucleotide was confirmed allowing less than 90% maximum identity with non-target sequences. After the gene orientation was confirmed, oligonucleotides were synthesized and nylon-based arrays were printed (Sigma-Genosys, Woodlands, TX, USA). The hypertension array included oligonucleotides representing 393 genes: 328 genes were represented by one oligonucleotide (18 of these were positive controls based on baboon kidney biopsy RNA interrogation of commercial genechips [Affymetrix U133A 2.0]); 49 genes were represented by separate oligonucleotides for human and rat gene homologs; six genes were represented by two oligonucleotides to ensure detection of splice variants without common exons; and 10 *arabidopis* genes were included as negative controls. All oligonucleotides were pooled to serve as positive controls and grid anchors at each corner of the array. Moreover, all oligonucleotides were spotted in duplicate (MIAME Accession Number: GPL8396; see Supplemental Table 1 for a list of genes on the array). Designations for rat gene identification in text are in title case, whereas upper-case designation has been used for baboon-specific genes or genes expressed in both the rat and the baboon.

Gene array interrogation

Complementary RNA probes were synthesized from total RNA for each individual RNA sample by synthesizing cDNA and using the cDNA to synthesize radioactively labeled cRNA by including α^{32}P-UTP in the *in vitro* transcription reaction according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). For first-strand cDNA synthesis, primers were annealed to the mRNA templates by incubating 1 μL total RNA (1 μg) with 1 μL 5 μmol/L T7-Oligo (dT) (Ambion, Austin, TX, USA) for six minutes at 70°C and then cooling the sample to 4°C for two minutes. The mRNA was then reverse transcribed by adding 1 μL 5× first-strand buffer (Invitrogen), 0.5 μL 100 mmol/L dithiothreitol, 0.375 μL 10 mmol/L dNTP mix, 0.25 μL RNase inhibitor (40 Units/μL, Invitrogen), 0.5 μL SuperScript II (200 Units/μL, Invitrogen) and 0.375 μL DEPC-treated water to the RNA-primer mixture. The reaction was incubated for one hour at 42°C and the SuperScript II heat-inactivated for 10 min at 70°C. The cDNA second strand was synthesized by adding 7.5 μL 5× second-strand buffer (Invitrogen), 0.75 μL 10 mmol/L dNTP mix, 0.25 μL DNA ligase I (10 Units/μL, Invitrogen), 1 μL DNA polymerase I (10 Units/μL, Invitrogen) and 0.25 μL RNase H (2 Units/μL, Invitrogen) to the first-strand reaction and incubated for two hours at 16°C. T4 DNA polymerase (1 μL; 5 Units/μL, Invitrogen) was then added to the reaction and incubated for 10 min at 16°C. The cDNA was precipitated by first adding 2 μL glycogen (5 mg/mL) as a carrier and then 80 μL DEPC-treated water followed by 0.6 volumes 5 mol/L ammonium acetate and 2.5 volumes cold absolute ethanol. After precipitating the cDNA overnight at −20°C, samples were centrifuged at 17,000 × *g* for 30 min at 4°C. DNA pellets were washed in 70% ethanol (with DEPC-treated dH_2O) and air-dried.

Complementary RNA synthesis by *in vitro* transcription was performed using the MAXIscript Kit (Ambion) and cleaned using a Sephadex G-50 column (Roche mini Quick Spin RNA columns; Roche Applied Science, Indianapolis, IN, USA) according to the manufacturer's instructions. An aliquot of cRNA was counted in a scintillation counter to determine synthesis efficiency. Purified cRNA was then fragmented using fragmentation buffer (Ambion) according to the manufacturer's instructions.

For hybridization, the arrays were prehybridized for two hours at 42°C with Ultrahyb Buffer (Ambion). Denatured cRNA probe synthesized from each individual RNA sample was added to the membrane in prehybridization buffer and hybridized for 42 h at 42°C. The nylon membrane arrays were washed: once in 2× sodium chloride-sodium citrate buffer (SSC)/0.1% sodium dodecyl sulfate (SDS) for five minutes, twice in 2× SSC/0.1% SDS for five minutes at 35°C, and twice in 0.2× SSC/0.1% SDS for

10 min at 35°C. After washing, nylon membrane arrays were air-dried and placed in phosphorimager cassettes for image capture.

Gene array analysis

Each gene array image was acquired by exposing nylon filters to phosphorimager cassettes and capturing the image with a Phosphorimager (Storm 840; GE Healthcare, Piscataway, NJ, USA) using ImageQuantTL Image Analysis Software (GE Healthcare). Each image was loaded into ImaGene 5.6 Microarray Image Analysis software (Biodiscovery, Inc, El Segundo, CA, USA) and the template containing the annotated grid applied to the image. Pooled targets were used as reference points to properly align the grid over the image. Data were quantified by compiling numerical intensity values, quality measurements and spot location of each spot. Background for each spot was measured in a rectangular region around the spot and a circular buffer region. The median of background values within 5×5 spots was subtracted from the signal value of the center spot for background correction (Local Group Median option). A manual quality control check was then performed on the data to remove miscalled spots (due to background or slight membrane defects) and to flag quality discrepancies for evaluation. Manual spot editing was extremely limited and was recorded in the saved data file for later review. Each data-set was then refined by verifying positive and negative controls. In addition, data for empty, poor quality and absent spots (including spots that did not have an acceptable signal from its duplicate) were removed. Intensities and quality values were averaged for replicate spots. The data reported from the oligonucleotide arrays are the relative intensity (median normalized for all genes on all arrays).

Statistical analysis of array data

After data cleaning, array data were uploaded into GeneSifter (GeneSifter.net; VizXLabs, Seattle, WA, USA), all-median normalized and $\log_2$ transformed. Box plots were inspected to ensure that the median for each group was zero and the variance among groups was similar. Data were filtered by spot quality. All genes that passed the quality filter were subjected to pair-wise analysis by *t*-test and for group analysis by ANOVA (analysis of variance) assuming unequal variance.

Realtime reverse transcription polymerase chain reaction

RNA that was isolated for gene array experiments was also used in the reverse transcription polymerase chain reaction (QRT-PCR). For QRT-PCR of rat RNA samples, the Reaction Ready TM First Strand cDNA Synthesis kit (SuperArray Bioscience Corporation, Fredrick, MD, USA) and RT^2 Real-Time^{TM} Gene Expression Assay PCR kit and SuperArray RT^2 PCR Primer sets (SuperArray Bioscience Corporation; *Fxyd2*, Catalog number: PPR43671A; *LpL*, Catalog number: PPR43250A; *Agt*, Catalog number: PPR43580A; *Nos3*, Catalog number: PPR49724A; *Accn1*, Catalog number: PPR48988A; and *GAPDH* [glyceraldehyde 3-phosphate dehydrogenase], Catalog number: PPR06557A) were used. For QRT-PCR of baboon RNA samples, the Assays-on-Demand system (Applied Biosystems Inc, Foster City, CA, USA) was used. Although no baboon-specific primers and probes are available, we have successfully used the Assays-on-Demand system for more than 50 different baboon genes. We quantified mRNA according to the manufacturer's instructions (Applied Biosystems Inc; *ACE1*, Hs00174179_m1; *GAL*, Hs00544355_m1; *IGF1*, Hs00153126_m1). *GAPDH* was used as the endogenous control and no-template controls were run in parallel. Quantification was performed using the comparative threshold cycle (CT) method to derive a ΔCT values.

Results

Rat and baboon kidney gene expression profiling using a custom oligonucleotide array

Interrogation of a custom gene array using RNA-generated probes from rat and baboon kidney showed similar hybridization efficiencies for baboon and rat cRNA probes based on pooled oligonucleotide signals. Analysis of array data showed an expression of 41 genes in rat kidney that were not expressed in baboon; 11 of these were not expressed in the cortex and five were not expressed in the medulla. Gene expression detected in rat kidney but not baboon included: annexin A1 (*ANXA1*), endothelin-converting enzyme 1 (*ECE1*), glutamate-cysteine ligase, catalytic subunit (*GCLC*), glucagon (*GCG*) and sodium channel, voltage-gated, type I, beta (*SCN1B*) (Table 1). Gene array analysis showed expression of 34 genes in baboon kidney that were not expressed in rat kidney; six of these were not expressed in the cortex and 10 of these were not expressed in the medulla. Gene expression detected in baboon kidney but not in rat included: alpha-1A-adrenergic receptor (*ADRA1A*), renin-binding protein (*RENBP*) and two predicted genes (Table 2). Seventy-four genes were expressed in both rat and baboon kidneys; 49 of these genes were expressed in the cortex and medulla in both species. Gene expression common to rat and baboon renal cortex and medulla included: aquaporins 1–3 (*AQP1*, *AQP2* and *AQP3*), arginine vasopressin receptor 2 (*AVPR2*), insulin-like growth factor 1 (*IGF1*), uromodulin (*UMOD*) and superoxide dismutase 1–2 (*SOD1* and *SOD2*) (Table 3).

Baboon gene expression validation

Gene expression results were validated by QRT-PCR in baboon RNA samples for a few genes randomly selected from the list of expressed genes. In baboon renal cortex, QRT-PCR was used to analyze the expression of angiotensin I-converting enzyme 1 (*ACE1*), galanin (*GAL*) and insulin-like growth factor 1 (*IGF1*) (Table 4).

Rat blood pressure data

Systolic blood pressure measurements were taken using the tail cuff method four weeks after surgery. Systolic blood pressures were significantly greater in the DOCA-salt

Table 1 Genes expressed in the rat renal cortex and medulla

Genes detected in rat cortex and/or medulla	Gene ID	Rat cortex	Rat cortex SEM	Rat medulla	Rat medulla SEM
Acyl-CoA synthetase medium-chain family member 3	ACSM3	21.91	3.42	14.19	3.95
Annexin A1	ANXA1	10.41	3.51	19.92	2.52
Aspartyl-tRNA synthetase	DARS	2.40	2.19	0.00	0.00
ATPase, H+ transporting, lysosomal 14 kDa, V1 subunit F	ATP6V1F	14.56	4.08	22.37	2.41
Cbp/p300-interacting transactivator	Cited2	0.00	0.00	21.35	2.42
Cyclin-dependent kinase-like 1	CDKL1	25.28	16.25	15.31	2.97
Cysteine rich transmembrane BMP regulator 1	CRIM1	0.00	0.00	3.98	3.03
Cytochrome P450, family 11, subfamily B, polypeptide 2	CYP11B2	1.39	1.27	4.23	3.22
Endothelin converting enzyme 1	ECE1	0.00	0.00	4.19	3.19
Eukaryotic translation initiation factor 4E	Eif4e	10.20	4.47	11.02	2.17
Four-and-a-half LIM domains 1	FHL1	1.29	1.18	11.96	4.07
G protein-coupled receptor kinase 4	Gprk2l	0.00	0.00	6.07	2.99
Glucagon	Gcg	1.38	0.157	0	0
Glutamate-cysteine ligase, catalytic subunit	Gclc	32.95	9.30	83.95	6.97
Glutathione *S*-transferase A3	Gsta3	97.43	36.70	94.44	10.87
Glutathione *S*-transferase A4	Gsta4	13.12	6.35	10.73	3.14
Glutathione *S*-transferase A5	Gsta5	13.58	5.99	5.12	1.95
Glutathione *S*-transferase, theta 2	GSTT2	12.22	5.00	11.10	3.16
Haptoglobin	HP	0.00	0.00	6.45	2.54
Hemoglobin, gamma A	HBG1	14.40	6.83	36.00	2.78
Hydroxysteroid (11-beta) dehydrogenase 1	Hsd11b1	47.26	9.71	37.40	2.16
Hydroxysteroid (11-beta) dehydrogenase 2	Hsd11b2	4.11	3.76	0.00	0.00
Leucine-rich repeat-containing G protein-coupled receptor 4	LGR4	4.94	2.94	0.00	0.00
Lipoprotein lipase	LPL	0.00	0.00	21.54	2.74
Neural precursor cell expressed, developmentally down-regulated 4	NEDD4	8.96	2.96	8.49	2.31
Nuclear factor-like 1	Nfe2l1	0.00	0.00	4.82	3.67
Phosphatase and tensin homolog pseudogene 1	PTENP1	9.90	3.26	5.87	3.22
Potassium inwardly rectifying channel, subfamily J, member 1	KCNJ1	11.37	3.87	31.46	3.58
Protein phosphatase 1, catalytic subunit, gamma isoform	PPP1CC	14.33	3.86	18.98	3.19
Rho-associated, coiled-coil-containing protein kinase 1	ROCK1	20.14	8.73	11.00	3.75
Serum/glucocorticoid regulated kinase 1	SGK1	0.00	0.00	5.01	2.11
SMAD family member 1	SMAD1	0.00	0.00	0.00	0.00
Sodium channel, voltage-gated, type I, beta	Scn1b	0.00	0.00	12.78	3.48
Sodium channel, voltage-gated, type V, alpha	Scn5a	3.69	3.37	0.00	0.00
Sodium channel, voltage-gated, type X, alpha	SCN10A	26.17	11.43	12.24	2.35
Solute carrier family 12 member 3	SLC12A3	23.80	8.57	1.18	0.90
Solute carrier family 17 member 8	SLC17A8	0.00	0.00	5.85	2.83
Solute carrier family 5 member 11	SLC5A11	20.91	7.48	13.32	1.91
Superoxide dismutase 3, extracellular	SOD3	28.26	5.38	15.35	4.44
Tumor protein p53	TP53	3.29	3.01	7.28	3.11
WW domain containing E3 ubiquitin protein ligase 2	WWP2	1.79	1.63	7.23	3.94

Genes were detected in the rat cortex and/or medulla and were not detected in the baboon cortex and/or medulla
SEM, standard error of the mean

animals compared with sham ($n = 6$; sham: 143 ± 6 mmHg versus DOCA-salt: 210 ± 20 mmHg, $P \leq 0.05$).

Rat renal cortex and medulla gene expression profiling

Results from gene expression profiling of the 393 sodium- and hypertension-related genes showed detectable expression of 61 genes from the renal cortex RNA and 72 genes from renal medulla RNA. Of these genes, 41 were expressed in both the renal cortex and medulla. Ten genes were expressed solely in the cortex and 21 genes were solely expressed in the medulla (Table 1).

Rat renal cortex differential gene expression – DOCA-salt

Expression analysis showed three genes differentially expressed in renal cortex RNA samples. Annexin A1 (*Anxa1*) was significantly up-regulated in renal cortex RNA samples from DOCA-salt compared with sham animals. Two genes, glutamate-cysteine ligase (*Gclc*) and glutathione transferase YA (*Gsta5*), were down-regulated in rat renal cortex RNA samples from DOCA-salt rats as compared with sham (Table 5; expression values for all genes on the array are shown in Supplemental Table 2).

Rat renal medulla differential gene expression – DOCA-salt

Expression analysis showed 22 genes differentially expressed in renal medulla RNA samples (Table 6; expression values for all genes on the array are shown in Supplemental Table 3). Four genes – annexin A1 (*Anxa1*), apolipoprotein E (*Apoe*), tropomyosin 1 (alpha) (*Tpm1*) and tumor protein p53 (*Tp53*) – were up-regulated in renal medulla RNA samples from DOCA-salt as compared with sham rats. However, 18 genes were down-regulated in DOCA-salt rat renal medulla RNA samples compared with sham, including *Gclc*, which was down-regulated in both the medulla and cortex (Tables 5 and 6).

Table 2 Genes expressed in the baboon renal cortex and medulla

Genes detected in baboon cortex and/or medulla	Gene ID	Baboon cortex	Baboon cortex SEM	Baboon medulla	Baboon medulla SEM
Actinin, alpha 3	ACTN3	24.02	4.38	2.84	2.32
Adducin 1, alpha	ADD1	5.71	4.66	0.00	0.00
Adrenergic, alpha-1A-, receptor	ADRA1A	0.00	0.00	10.68	8.72
Alanine-glyoxylate aminotransferase	AGXT	21.69	5.14	6.73	5.49
Albumin	ALB	28.45	3.67	5.42	4.43
Amiloride-binding protein 1	ABP1	89.16	17.24	23.29	10.82
Apolipoprotein H	APOH	6.72	5.49	0.00	0.00
Arginine vasopressin	AVP	5.52	4.51	11.79	4.91
Chloride channel Kb	CLCNKB	5.48	4.47	0.00	0.00
Collagen, type I, alpha 1	COL1A1	0.00	0.00	4.62	3.77
Galanin prepropeptide	GAL	3.07	2.51	17.03	7.06
Immunoglobulin heavy constant gamma 3	IGHG3	29.23	1.72	37.26	9.13
Immunoglobulin J polypeptide	IGJ	12.97	5.29	6.04	2.48
Insulin-like growth factor 2	IGF2	0.00	0.00	4.70	3.83
kininogen 1	KNG1	16.33	6.67	28.39	2.72
Lens intrinsic membrane protein 1	LIM1	13.63	5.65	16.85	2.17
Phosphoserine aminotransferase 1	PSAT1	5.93	4.84	0.00	0.00
Predicted Gene	M87790	11.98	1.48	8.43	6.88
Predicted Gene	FLJ12365	83.61	19.42	168.46	54.63
Prolylcarboxypeptidase (angiotensinase C)	PRCP	19.57	3.68	0.00	0.00
Receptor activity modifying protein 2	RAMP2	19.70	2.80	19.41	2.78
Renin binding protein	RENBP	19.33	2.36	5.48	4.48
Rho GTPase-activating protein 29	ARHGAP29	0.00	0.00	8.61	7.03
Sarcolipin	SLN	20.66	9.54	0.00	0.00
Similar to BETA-GLUCURONIDASE PRECURSOR	FLJ32313	23.68	3.65	25.38	4.13
SMAD family member 2	SMAD2	9.80	4.02	0.00	0.00
Sodium channel, non-voltage-gated 1 alpha	SCNN1A	0.00	0.00	6.99	2.96
Solute carrier family 14 (urea transporter), member 2	SLC14A2	7.13	5.82	0.00	0.00
Solute carrier family 17 member 3	SLC17A3	35.68	4.85	16.65	7.39
Solute carrier family 4 member 1	SLC4A1	0.00	0.00	3.87	3.16
Solute carrier family 4 member 2	SLC4A2	10.47	5.16	0.00	0.00
Thyrotropin-releasing hormone	TRH	7.58	6.19	0.00	0.00
WD repeat and SOCS box-containing 1	WSB1	11.55	4.77	12.43	0.96
WNK lysine-deficient protein kinase 4	WNK4	12.56	5.13	18.10	1.21

Genes were detected in the baboon cortex and/or medulla that were not detected in the rat cortex and/or medulla
SEM, standard error of the mean

QRT-PCR validation of rat gene expression profiles

Small subsets of genes were chosen to verify the gene expression profiling. Expression profiles for three genes, two of which were differentially expressed, Fxyd domain containing ion transport regulator 2 (*Fxyd2*) and lipoprotein lipase (*Lpl*) and nitric oxide synthase 3 (*Nos3*), which was expressed but not different, were validated by QRT-PCR. Both *Fxyd2* and *Ldl* were significantly different between RNA samples ($P = 0.008$ and $P = 0.005$, respectively), and no differences were found in *Nos3* expression, consistent with the oligonucleotide array data (Table 7).

Discussion

The kidney plays a key role in sodium and water balance, and in turn the maintenance of blood pressure. Consequently, defining changes in gene expression associated with blood pressure requires investigation of the kidney. A dilemma in using rodent models to evaluate the underlying genetic component of hypertension is the lack of translation of these findings to primates. One mechanism to address this is to develop an experimental platform that tests both species within a single methodology. This would allow scientists to translate their research findings from rodents to primates. An additional impediment in examining multiple species is the cost involved to develop necessary technology. Therefore, in order to address genetic variation that influences hypertension in both rodents and primates, and provide a cost-effective cross-species platform, we developed a nylon-based array that allows investigators to perform gene expression profiling for hundreds of genes in a single experiment without the need for specialized equipment, such as glass-based array readers, dramatically reducing the cost of gene array experiments. This enables the evaluation of both species within the same experimental platform in a cost-effective and truly comparative manner. Including all genes within the species would enlarge the array to such a size to limit its effectiveness as well as increase the cost of such an array, making it not as feasible to use. Therefore, to limit the size of the gene array while maintaining the goal of it being a cross-species array with a focus on hypertension, genes included on the array were chosen based on an extensive review of current published literature with an emphasis on genes implicated in sodium management and/or hypertension in rodents and/or primates and by evaluating baboon renal differential gene expression in response to

Table 3 Genes expressed in the baboon and rat renal cortex and medulla

Genes detected in baboon and rat cortex and/or medulla	Gene ID	Baboon cortex	Baboon cortex SEM	Baboon medulla	Baboon medulla SEM	Rat cortex	Rat cortex SEM	Rat medulla	Rat medulla SEM
Actin, alpha 1	ACTA1	21.90	2.60	22.72	3.79	9.12	3.89	28.26	4.93
Angiotensin I-converting enzyme 1	ACE1	17.56	4.03	13.45	3.21	1.08	0.98	5.39	2.94
Angiotensinogen	AGT	10.75	4.43	7.63	3.50	2.73	2.50	8.03	3.08
Apolipoprotein E	APOE	37.67	4.98	31.56	3.62	16.29	3.96	11.52	2.92
Aquaporin 1	AQP1	19.20	2.01	5.06	4.13	14.90	3.64	34.30	2.30
Aquaporin 2 (collecting duct)	AQP2	5.74	4.68	12.78	5.33	7.72	3.46	31.28	2.33
Aquaporin 3	AQP3	4.31	3.52	11.16	4.60	2.84	2.59	26.84	4.55
Arginine vasopressin receptor 2	AVPR2	5.87	4.80	4.83	3.94	10.68	4.43	19.38	2.47
ATPase, H+ transporting, lysosomal 70 kDa, V1 subunit A	ATP6V1A	36.04	6.11	22.37	4.31	2.45	1.47	7.20	2.87
ATPase, Na+ /K+ transporting, alpha 1 polypeptide	ATP1A1	139.46	24.14	129.67	7.65	56.84	21.69	172.12	20.43
ATPase, Na+/K+ transporting, beta 1 polypeptide	ATP1B1	39.74	6.80	40.18	6.73	49.54	13.88	69.87	6.66
BCL2-like 1	BCL2L1	17.96	2.11	2.29	1.87	0.00	0.00	3.89	2.05
Calreticulin	CALR	21.23	3.01	18.09	2.05	0.00	0.00	3.10	2.36
Catalase	CAT	41.19	7.97	18.97	8.46	53.37	10.65	59.79	5.50
Connexin 40	CX40	3.63	2.96	0.00	0.00	10.15	3.15	1.60	1.22
Cytochrome P450, family 4A, polypeptide 11	CYP4A11	37.97	8.33	9.21	7.52	23.15	8.68	20.19	3.96
Enhancer of yellow 2	DC6	25.55	2.49	21.79	1.32	18.08	5.64	24.35	2.07
Eukaryotic translation initiation factor 4 gamma, 2	EIF4G2	3.81	3.11	0.00	0.00	40.44	12.69	42.25	4.81
FXYD domain containing ion transport regulator 2	FXYD2	79.56	11.00	59.74	3.51	77.98	33.00	227.82	24.70
Gamma-glutamyltransferase 1	GGT1	26.75	4.72	8.03	6.55	42.69	12.36	57.54	5.26
Glucagon receptor	GCGR	5.92	4.83	20.47	1.50	10.83	4.85	20.03	3.48
Glucagon-like peptide 1 receptor	GLP1R	4.22	3.45	0.00	0.00	18.36	8.30	21.94	1.27
Glucosamine (*N*-acetyl)-6-sulfatase	GNS	5.46	4.46	0.00	0.00	20.34	7.73	15.29	2.71
Glutathione *S*-transferase theta 1	GSTT1	18.11	2.84	0.00	0.00	11.48	4.82	15.42	2.76
Glutathione transferase zeta 1	GSTZ1	31.30	5.39	12.17	5.02	2.45	1.42	4.13	3.14
GNAS complex locus	GNAS	11.81	5.15	12.38	5.10	20.84	4.99	37.48	4.54
Golgi SNAP receptor complex member 1	GOS2	13.48	5.54	12.26	5.43	20.74	12.83	1.91	1.46
H2A histone family, member Z	H2AFZ	15.77	6.52	23.51	2.33	7.78	1.80	26.90	1.53
Heat shock 60 kDa protein 1	HSPD1	20.17	2.10	14.52	5.95	19.95	2.82	23.97	2.07
Heme oxygenase (decycling) 2	HMOX2	4.97	4.06	0.00	0.00	8.36	3.59	15.31	3.79
Hemoglobin, alpha 1	HBA1	31.80	2.59	40.66	8.09	40.40	6.24	105.21	12.33
Hypoxia-inducible factor 1, alpha subunit	HIF1A	4.58	3.74	0.00	0.00	0.97	0.88	13.64	3.61
Insulin-like growth factor 1	IGF1	20.17	2.1	14.52	5.95	0.00	0.00	19.43	4.28
Integral membrane protein 2B	ITM2B	122.12	24.35	105.18	9.63	62.48	14.46	132.01	17.92
Kallikrein 1	KLK1	26.48	2.92	6.09	4.97	26.92	8.51	7.53	2.79
Lectin, galactoside-binding, soluble, 1 (galectin 1)	LGALS1	14.75	6.08	31.48	8.21	2.04	1.19	17.31	3.10
Lysosomal protein transmembrane 4 alpha	LAPTM4A	39.63	6.20	35.18	1.72	0.00	0.00	2.73	1.32
Malate dehydrogenase 1, NAD (soluble)	MDH1	58.53	10.29	46.17	2.73	23.87	6.38	41.01	2.76
MYST histone acetyltransferase 1	PRSS8	6.93	5.66	4.23	3.45	23.17	5.74	22.09	5.35
Parathyroid hormone receptor 1	PTHR1	24.55	4.32	5.25	4.29	24.34	4.79	19.74	2.96
Peroxiredoxin 3	PRDX3	31.29	4.65	32.57	7.25	14.70	4.60	20.85	2.32
Phosphoglycerate dehydrogenase	PHGDH	5.59	4.56	0.00	0.00	26.22	6.26	5.37	1.84
Potassium channel modulatory factor 1	KCMF1	10.40	4.25	0.00	0.00	16.82	7.02	5.97	3.16
Prostaglandin E receptor 3	PTGER3	9.15	4.12	17.90	1.26	0.00	0.00	19.42	4.90
Protein phosphatase 1, catalytic subunit, alpha isoform	PPP1CA	11.03	4.87	15.58	4.01	17.54	3.93	29.77	3.44
RAB11B, member RAS oncogene family	RAB11B	17.33	2.81	6.09	4.97	0.00	0.00	5.11	2.81
Ras homolog gene family, member A	RHOA	10.35	4.23	3.91	3.19	21.61	9.85	27.45	1.39
Ras-related C3 botulinum toxin substrate 1	RAC1	18.09	1.89	4.63	3.78	9.30	2.97	11.82	3.29

(Continued)

Table 3 Continued

Genes detected in baboon and rat cortex and/or medulla	Gene ID	Baboon cortex	Baboon cortex SEM	Baboon medulla	Baboon medulla SEM	Rat cortex	Rat cortex SEM	Rat medulla	Rat medulla SEM
Ribosomal protein S16	RPS16	31.25	3.16	33.86	2.02	5.71	5.21	0.00	0.00
SH3-domain GRB2-like endophilin B2	SH3GLB2	12.05	4.92	16.67	0.89	0.00	0.00	5.00	2.56
Solute carrier family 12 member 1	SLC12A1	0.00	0.00	4.14	3.38	0.00	0.00	28.09	2.59
Solute carrier family 13 member 3	SLC13A3	35.68	7.83	13.48	5.75	19.16	4.94	14.06	2.61
Solute carrier family 17 member 1	SLC17A1	31.34	10.04	11.72	4.81	23.11	5.78	10.61	2.80
Solute carrier family 17 member 2	SLC17A2	37.01	5.18	15.48	6.32	75.29	14.80	59.36	5.76
Solute carrier family 34 member 1	SLC34A1	60.39	9.89	23.19	9.47	36.66	22.45	28.33	7.36
Solute carrier family 9 member 3 regulator 1	SLC9A3R1	24.23	3.31	6.25	5.10	21.80	8.21	13.83	2.57
Solute carrier family 9 member 3 regulator 2	SLC9A3R2	13.05	5.38	0.00	0.00	9.62	4.22	23.32	5.46
Spermidine/spermine N1-acetyltransferase	SAT	25.53	3.79	23.08	0.27	33.14	9.40	30.62	1.64
Stromal cell derived factor receptor 1	SDFR1	56.13	10.33	51.64	4.90	40.41	7.70	62.21	4.96
Superoxide dismutase 1, soluble	SOD1	48.65	6.81	32.31	2.60	39.89	10.76	49.12	3.36
Superoxide dismutase 2, mitochondrial	SOD2	17.57	2.19	9.16	7.48	19.03	6.25	35.16	4.29
Thioredoxin 2	TXN2	16.21	1.29	4.78	3.90	15.82	5.16	10.54	2.89
TIMP metallopeptidase inhibitor 1	TIMP1	6.66	5.44	17.93	8.24	0.00	0.00	3.47	2.64
Transforming growth factor, beta 1	TGFB1	6.17	5.04	7.77	3.76	1.38	1.26	10.46	4.25
Transmembrane and coiled-coil domains 1	TMCO1	14.10	5.78	10.30	4.21	0.00	0.00	4.81	1.67
Transmembrane protein 27	TMEM27	28.95	7.11	16.90	7.53	32.91	6.34	21.75	2.70
Tropomyosin 1	TPM1	17.99	2.69	19.55	1.66	0.00	0.00	14.66	0.88
Tropomyosin 2	TPM2	9.68	4.28	21.97	4.70	0.00	0.00	2.71	2.06
Tropomyosin 3	TPM3	10.13	4.42	0.00	0.00	0.00	0.00	1.35	1.03
Uromodulin	UMOD	184.58	32.68	423.34	115.41	27.59	4.93	110.43	5.93
Vascular endothelial growth factor	VEGF	5.74	4.69	0.00	0.00	47.54	17.25	19.44	3.48
Vascular endothelial growth factor B	VEGFB	22.54	3.14	19.02	2.38	15.18	2.98	13.23	3.52
V-maf musculoaponeurotic fibrosarcoma oncogene homolog	MAF	21.78	2.44	5.98	4.88	12.00	4.19	10.10	3.70
WW domain containing E3 ubiquitin protein ligase 1	WWP1	10.93	4.47	8.22	3.44	12.79	3.02	21.93	2.54

SEM, standard error of the mean

dietary salt. We realize that there is an ever-evolving list of genes suggested to be involved in the etiology of hypertension; moreover, we realize the current array does not reflect all genes of interest at this time. The array was not intended to be an exhaustive listing of genes; therefore, the array was limited to 394 genes.

In this study, we used the array to compare gene expression among rat kidney cortex and medulla and baboon kidney cortex and medulla. Results indicated 74 genes were expressed in both rat and baboon, 41 genes were unique to rat and 34 genes were unique to baboon. Genes common to both rat and baboon include: angiotensinogen (*AGT*), angiotensin I-converting enzyme 1 (*ACE1*), potassium channel modulatory factor 1 (*KCMF1*), vascular endothelial growth factor (*VEGF*) and vascular endothelial growth factor B (*VEGFB*). Genes unique to rat renal expression include: glutathione *S*-transferases A3, A4 and A5 (*Gsta3, Gsta4, Gsta5*) and three members of the solute carrier gene family (*Slc12a3, Slc17a8* and *Slc5a11*). Genes unique to baboon renal expression include: collagen alpha 1 (*COL1A1*), renin binding protein (*RENBP*) and three members of the solute carrier family (*SLC17A3, SLC4A1* and *SLC4A2*). Principal component analyses of genes common to baboon and rat indicate that baboon cortex and medulla gene expression are more similar to each other than to either rat cortex or medulla. In addition, rat cortex and rat medulla have less similar expression profiles than baboon cortex and medulla. These results demonstrate genetic differences in renal gene expression between rodents and primates (Figure 1).

Table 4 QRT-PCR validation of gene array results for baboon renal cortex

Gene ID	GAPDH	ACE	GAL	IGF1
Mean no. cycles	32.05	29.86	25.78	30.82
ΔCT		10.09	5.64	11.36

In addition to comparing rodent and primate renal gene expression, we used this array platform to begin to identify differentially expressed genes in the rat renal cortex and medulla during the condition of hypertension. The DOCA-salt model of hypertension provided an opportunity to examine genes that were altered due to the hypertensive state without prior genetic manipulations. Surprisingly, of

Table 5 Differentially expressed genes in the renal cortex – oligonucleotide array

Gene name	Gene ID	Sham ± SEM	DOCA ± SEM	Ratio	Direction	*P* value
Annexin A1	ANXA1	1.316 ± 0.321	2.312 ± 0.283	20.0	Up	0.049
Glutamate-cysteine ligase	GCLC	3.738 ± 0.174	2.645 ± 0.364	2.1	Down	0.018
Glutathione *S*-transferase 5	Gsta5	5.572 ± 0.246	4.215 ± 0.411	2.6	Down	0.016

SEM, standard error of the mean; DOCA, deoxycorticosterone acetate

the hypertension-related genes investigated, there were few significantly different between the rat kidney cortex and medulla, with five genes up-regulated and 20 down-regulated. Only two genes, the glutamate-cysteine ligase, catalytic subunit (*Gclc*) and glutathione *S*-transferase A5 (*Gsta5*), were differentially expressed in both the kidney cortex and medulla; both of which were significantly down-regulated in DOCA-salt hypertension. An additional advantage of this study was the number of individual samples used from each group ($N = 6$), allowing for statistical analyses of all genes represented on the array and providing a more complete evaluation of the genetic changes. Thus, the stringent statistical analyses may be one reason for the limited number of genes found to be differentially expressed.

Rat gene expression profiling

Anxa1 is a Ca^{2+}-regulated phospholipid-binding and membrane-binding protein[8] that is up-regulated in the renal cortex and medulla from DOCA-salt treated rats. Annexins have both intracellular as well as extracellular roles in cell function that include membrane scaffolding, membrane/protein transport, anticoagulant activity (annexin A5), endothelial cell-surface receptor for plasminogen and tissue-type plasminogen activator (annexin A2), and anti-inflammatory actions (*Anxa1*).[8] Walther *et al.*[9] demonstrated that *Anxa1* acts through the formyl peptide receptor on human neutrophils to cause inhibition of the transendothelial migration of neutrophils and a desensitization of neutrophils toward a chemoattractant challenge. Touyz *et al.*[10] found blunted expression of transient receptor potential melastatin 7 (*Trpm7*) in a genetic model of hypertension, the spontaneously hypertensive rat (SHR), which was associated with attenuated annexin-1 translocation and decreased vascular smooth muscle cell $[Mg^{2+}]_i$ in SHR.[10] Anxa1 can also mimic the effect of steroids, mediate anti-inflammatory activity, and act as a signaling molecule involved in various processes.[11] Thus, the 20-fold increase in Anxa1 expression in DOCA-salt-treated rats suggests that this may be a molecular response to inflammation and protein trafficking changes within renal cortex cells.

Glutathione *S*-transferase A5 (*Gsta5*), which was down-regulated in the renal cortex in response to DOCA-salt treatment, belongs to the Gst superfamily (specifically alpha) and catalyzes RX + glutathione to HX + *R*-*S*-glutathione and can also function as a peroxidase and isomerase.[12,13] Members of the Gst isoenzyme family alpha, mu and pi are known to increase in response to chemical and oxidative stress and protect against cytotoxicity.[14,15] While little has been published specifically on *Gsta5* function, it is known that these families of enzymes are important for protection against oxidative stress and contribute to maintenance of cellular redox state. McBride *et al.*[16] found another member of this family, glutathione *S*-transferase μ type 1 (*Gstm1*), has reduced expression in kidneys from stroke-prone SHR. Recent studies suggest that reactive oxygen

Table 6 Differentially expressed genes in renal medulla – oligonucleotide array

Gene name	Gene ID	Sham ± SEM	DOCA ± SEM	Ratio	Direction	*P* value
Annexin A1	Anxa1	2.021 ± 0.161	2.979 ± 0.385	1.94	Up	0.037
Apolipoprotein E	Apoe	1.525 ± 0.062	2.346 ± 0.255	1.77	Up	0.008
Aquaporin 1	Aqp1	3.112 ± 0.161	2.335 ± 0.316	1.71	Down	0.046
Arginine vasopressin receptor 2	Avpr2	2.086 ± 0.219	1.425 ± 0.115	1.58	Down	0.033
Catalase	Cat	4.109 ± 0.175	3.141 ± 0.383	1.96	Down	0.037
FXYD domain containing ion transport regulator 2	Fxyd2	5.809 ± 0.186	5.065 ± 0.275	1.67	Down	0.047
Glucagon	Gcg	1.380 ± 0.157	0.325 ± 0.411	2.08	Down	0.030
Glucagon-like peptide 1 receptor	Glp1r	2.204 ± 0.147	1.315 ± 0.118	1.85	Down	0.001
Glutamate-cysteine ligase, catalytic subunit	Gclc	4.603 ± 0.171	3.520 ± 0.255	2.12	Down	0.005
Glutathione *S*-transferase theta 1	Gstt1	1.825 ± 0.171	0.983 ± 0.224	1.79	Down	0.014
Glutathione *S*-transferase, theta 2	Gstt2	1.600 ± 0.245	0.673 ± 0.319	1.90	Down	0.043
Glutathione transferase YA subunit	Gsta5	4.818 ± 0.250	3.431 ± 0.538	2.62	Down	0.035
Insulin-like growth factor 1	Igf1	2.238 ± 0.242	1.062 ± 0.278	2.26	Down	0.011
Integral membrane protein 2B	Itm2b	5.205 ± 0.128	4.682 ± 0.199	1.44	Down	0.048
Lipoprotein lipase	Lpl	2.076 ± 0.196	1.436 ± 0.194	1.56	Down	0.047
Malate dehydrogenase 1, NAD	Mdh1	3.440 ± 0.190	2.516 ± 0.253	1.90	Down	0.016
Potassium inwardly-rectifying channel J1	Kcnj1	2.905 ± 0.152	1.724 ± 0.235	2.27	Down	0.002
Solute carrier family 9 isoform 3 regulator 2	Slc9a3r2	2.415 ± 0.212	1.547 ± 0.207	1.82	Down	0.018
Stromal cell derived factor receptor 1	Sdfr1	4.212 ± 0.105	3.468 ± 0.306	1.67	Down	0.035
Tropomyosin 1 (alpha)	Tpm1	1.825 ± 0.096	2.554 ± 0.204	1.66	Up	0.008
Tumor protein p53	Tp53	1.540 ± 0.095	1.933 ± 0.101	1.31	Up	0.020
Uromodulin	Umod	4.888 ± 0.165	3.991 ± 0.313	1.86	Down	0.026

SEM, standard error of the mean; DOCA, deoxycorticosterone acetate

Table 7 QRT-PCR validation of gene array results for the rat renal cortex

Gene ID	Gapdh	Fxyd2	Lpl	Nos3
Mean no. cycles	16.56	15.66	21.70	23.62
ΔCT		−0.79	5.14	7.17

species are centrally involved in the pathophysiology of hypertension in laboratory animals and in human beings.[17–19] In summary, these data support the hypothesis that this gene may be involved in altered reactive oxygen species found in hypertension.

Glutamate-cysteine ligase, catalytic subunit (*Gclc*), also down-regulated in the renal cortex of DOCA-salt-treated rats, is the first rate-limiting enzyme in glutathione biosynthesis. Glutathione is an important intravascular scavenger that protects endothelial cells from atherosclerosis.[20] Recent studies found that the Gclc T allele, together with hypertension and the male sex, has been associated with cardiovascular events within a human Italian subpopulation.[20] Allelic variants of this gene are associated with hemolytic anemia and myocardial infarction. Although little has been published on the specific function of this gene and its product, its function in glutathione biosynthesis suggests it may play a role in response to oxidative stress in the renal cortex of DOCA-salt treated rats consistent with *Gsta5* gene expression response.

Five of the genes differentially expressed in the medulla are known to play a role in sodium management or blood pressure regulation: *Anxa1, Aqp1, Fxyd2*, potassium inwardly rectifying channel J1 (*Kcnj1*) and solute carrier family 9 (sodium/hydrogen exchanger) isoform 3 regulator 2 (*Slc9Aa3r2*). Two differentially expressed genes are known to play roles in renal function: arginine vasopressin receptor 2 (*Avpr2*) and uromodulin (*Umod*). Six genes have functions related to oxidative damage: *Apoe*, catalase (*Cat*), glutathione *S*-transferase theta 1 (*Gatt1*), glutathione *S*-transferase, theta 2 (*Gstt2*), *Gsta5* and malate dehydrogenase 1 NAD (*Mdh1*). Two of these genes, *Gstt1* and *Gstt2*, are

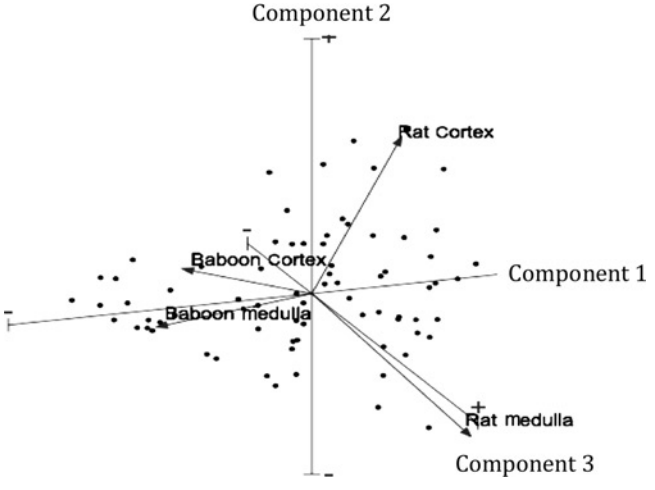

Figure 1 Principal components analysis of expressed genes common to baboon and rat renal cortex and medulla. Component 1 accounts for the greatest amount of variation in the data-set and Component 3 the least amount of variance

candidate genes for regulation of SLC activity in CEPH families.[7] Five genes are related to growth and metabolism: glucagon (*Gcg*), glucagon-like peptide 1 receptor (*Glp1r*), *Gclc, Tp53*, and insulin-like growth factor 1 (*Igf1*). One gene, *Tpm1*, is involved with vascular function. Three genes, which were included on the array for a group of genes expressed in SLC baboons,[21] have no known functions that link them to sodium management or blood pressure regulation: integral membrane protein 2B (*Itm2n*), *Lpl* and stromal cell-derived factor receptor 1 (*Sdfr1*).

Of the 22 differentially expressed genes in the rat renal medulla, four genes were up-regulated in response to DOCA-salt treatment: *Anxa1*, *Apoe*, *Tp53* and *Tpm1*. *Apoe* is involved in lipid metabolism. When the DOCA-salt model of hypertension is applied to an apoE$^{-/-}$ mouse model, there is a dramatic increase in the atherosclerotic disease extent.[22] With regard to *Tp53*, angiotensin induces an up-regulation of *p53* as well as other pro-apoptotic proteins, suggesting that the angiotensin system, which is suggested to be deregulated in hypertension may influence apoptotic processes within the kidney. These apoptotic mechanisms may be yet another mechanism in the kidney involved in hypertension.[23] Sodium−lithium countertransport has been associated with patients with a history of hypertension.[24] This abnormality has been associated with the actin-binding protein tropomyosin.[25]

Eighteen genes were down-regulated in the renal medulla from DOCA-salt hypertensive rats compared with sham animals (*Aqp1, Avp2, Cat, Fxyd2, Gcg, Glp1r, Gclc, Gstt1, Gstt2, Igf1, Itm2b, Lpl, Mdh1, Kcnj1, Sdfr1* and *Umod*). Interestingly, the protein levels for *AQP1* are associated with enhanced activity of the arginine vasopressin (AVP)/cyclic adenosine monophosphate (cAMP) pathway.[26] Avp translates its signal primarily through the arginine vasopressin receptor 1 (*Avpr1*). *Avp1* is known to play a critical role in blood pressure maintenance of DOCA-salt hypertension.[27] Elevated *AVP* is associated with downregulation of *Avp2* receptors within the kidney in DOCA-salt hypertension and our data further support this.[28] Expression of aquaporin-1 (*Aqp1*) and aquaporin-2 (*Aqp2*) within the kidney are critical in the maintenance of water homeostasis. *Aqp1* also has non-water transport functions such as transporting nitric oxide across the cell membrane.[29] Catalase breaks down hydrogen peroxide to water, and thus a down-regulation of this gene may indicate that during hypertension, there may be elevated levels of hydrogen peroxide in the kidney. Taylor and Cowley[30] have found increased medullary H_2O_2 production, and this may contribute to hypertension in salt-sensitive rats. Catalase infusion significantly reduced H_2O_2 and attenuated the hypertension.[30] The *Fxyd* gene family of small ion transport regulators associates in a tissue-specific manner with Na,K-ATPase.[31] Glucagon is a hormone that is involved in carbohydrate metabolism and is released when the glucose level is low in the blood. Recombinant glucagon-like peptide 1 receptor binds glucagon-like peptide (*Glp1*). *Glp1* is produced by L-type cells in the intestine and is released after a meal.[32] Increased recombinant *Glp1* has been found to have cardiac and renal protective effects in Dahl S rats fed a high-salt diet, due to its diuretic and natriuretic effects.[33] Insulin-like

growth factor infusion into WKY rats increased glomerular filtration rate and renal plasma flow; however, this does not occur in SHR animals, suggesting that modulation of renal hemodynamics by *IGF-1* is absent in SHR, and is suggested to be related to the pathophysiology of the development of hypertension.[34] While there is no direct connection to hypertension as of yet, it has been proposed that *Itm2b* is involved in a p53-independent apoptotic pathway involved in cell death.[35] Association of *Itm2b* with mitochondria correlates with loss of mitochondrial membrane potential, release of cytochrome c and ultimately in apoptosis.[35] Further studies will determine whether *Itm2b* is indeed involved in the maintenance and/or development of hypertension. Most NADH oxidation occurs via a novel malate/oxaloacetate and malate/aspartate shuttle (to a much lesser extent) which is increased with the development of hypertension in SHR.[36] *Lpl* is involved in plasma triglyceride hydrolysis, and is down-regulated in the hearts of SHR.[37] Variants in the *Lpl* gene that are associated with lower LPL activity are associated with altered plasma triglyceride levels and increased risk of coronary heart disease.[38] *Sdfr1* is a chemokine receptor that has been shown to activate the p44/p42 mitogen-activated protein kinase (MAPK) pathway.[39] While at this time *Sdfr1* is not directly related to hypertension *per se*, the MAPK signaling cascade is known to be involved in hypertension. Moreover, *Sdfr1* can stimulate adjacent fibroblasts to produce matrix metalloproteinases, also thought to be involved in hypertension. Inhibition of metalloproteinases ameliorated hypertension and preceded vascular dysfunction in a two-kidney, one-clip model of hypertension.[40]

There were marked differences in the number of genes altered in the renal cortex compared with the renal medulla. In addition, there was differential expression of genes whose function is clearly relevant to hypertension and sodium management (*Anxa1, Aqp1, Fxdy2, Kcnj1, Slc9a3r2);* we observed differential expression of genes whose function may be relevant to hypertension and sodium management (*Avpr2, Umod, Apoe, Cat, Gstt1, Gstt2, Gsta5, Mdh1, Tpm1)*; and there was differential expression of genes with no known specific relevance to hypertension and sodium management (*Gcg, Glp1r, Gclc, Tp53 and Igf1*). Several differentially expressed genes in this study were reported as differentially expressed in other gene array studies of salt-sensitive hypertension including *Apoe, Lpl, Umod*[41] and *Anxa1.*[42] Interestingly, many of the systems that are commonly thought to play major roles in high blood pressure, nitric oxide synthase, superoxide and the renin − angiotensin − aldosterone system, showed very little, if any, changes between the sham and DOCA-salt treatment. No genes detected in this array were similar to those found by Kinoshita *et al.*[43] when they performed a whole rat DNA array survey for candidate genes in kidneys from three SHR substrains at two stages of age, including an analysis of kidneys from animals treated with the antihypertensive agent hydralizine hydrochloride. While this is a genetic-based model of hypertension, it punctuates the need for further studies.

It is interesting to note that several of the genes that were differentially expressed in the sham versus DOCA rat kidneys were not detected in baboon kidney. These results may mean that baboon gene expression for these genes will only be detected when the baboon is salt-challenged or may indicate that renal salt responsive genetic networks differ between rodents and primates. If the latter hypothesis is correct, then this will provide another example regarding the importance of studying genetics underlying salt-sensitive hypertension in a non-human primate model. Future studies will define the role of genes identified here that are differentially expressed in rat and determine if these genes are activated in response to salt challenge in primates.

Author contributions: All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript. CAN, JPG, LAC conducted the experiments; RES, CH-L, GDF, JRH and LAC assisted in design of the array; CMK, JPG, CAN and LAC analyzed the data; and CAN and LAC wrote the manuscript.

ACKNOWLEDGEMENTS

This work was supported by National Institutes of Health grants P01 HL028972, P51 RR013986, 5R01HL68180–4 and K99/R00 HL087927. This investigation was conducted in part in facilities constructed with support from Research Facilities Improvement Program Grant Number C06 RR013556 and C06 RR015456 from the National Center for Research Resources, National Institutes of Health.

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(Received May 31, 2011, Accepted August 15, 2011)