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Optimizing microarray in experimental hypertension

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Optimizing microarray in experimental hypertension.

Background. Genetic noise between outbred animals can potentially be a major confounder in the use of microarray technology for gene expression profiling. The study of paired organs from the same animal offers an alternative approach (e.g., for studies of the kidney in experimental hypertension). The present study was undertaken to determine the level of genetic noise between outbred adult Sprague-Dawley (SD) rats, and to determine the effects of unilateral nephrectomy on changes in gene expression as a basis for the design of microarray studies in experimental hypertension.

Methods. Male SD rats (approximately 130 g) were acclimatized before measurement of tail-cuff systolic blood pressure (SBP) for 6 control days and 4 days of saline treatment. Left kidney nephrectomy was performed, and the tissue snap-frozen in liquid nitrogen for subsequent RNA extraction. Two weeks later, SBP was measured over 4 control and 8 saline treatment days, and the remaining right kidney removed and frozen. Total RNA purification, preparation of cRNA, hybridization, and scanning of the Rat U34A Affymetrix arrays were performed, and data analyzed using MAS5 software Affymetrix Suite (v5), Bioconductor, as well as statistical methods motivated by relevant simulations.

Results. Gene expression profiles in the left control kidney were extremely consistent across animals. The expression profiles of pairs of kidneys from the same animal were, however, more similar than those of kidneys from different animals. Nephrectomy had little effect on the gene expression profiles in the time frame examined.

Conclusion. Despite the outbred nature of the rats used in this study, they are useful for gene expression profiling comparisons. The use of paired organs from an individual animal ensures even further genetic identity, allowing determination of genes modified by the treatment of interest.

'For gene hunting in hypertension the kidney is an ideal choice as several elegant transplantation experiments showed that hypertension always travels with the kidney' [1].

Microarray technology allows assessment of gene expression in a variety of physiologic, pathologic, and experimental conditions. However, a potential drawback of the use of this technique is the lack of genetic identity between the outbred animals used in many of these studies. Thus, in analyzing microarray data in physiologic experiments, it may be difficult to distinguish specific changes in gene expression from noise due to genetic variability. Thus, there is a need to understand the level of variability in gene expression profiles for outbred rats used in hypertension studies. One way of dealing with this problem is to use the animal as its own control, and the kidney, as a paired organ, is ideal in this regard. However, any experimental protocol using paired kidneys must take into account the possible changes produced by nephrectomy.

This study was undertaken to determine the effects of both genetic variability and nephrectomy in the adult outbred Sprague-Dawley rat on changes in gene expression in the kidney as a basis for the design of microarray studies in experimental hypertension.

METHODS

Experimental animals and design

Eight male Sprague-Dawley rats (~130 g body weight; Animal Resource Centre, WA, Australia) were housed in a room with constant temperature (21–23°C), with a 12-hour alternating light dark cycle (700-1900 hour), and given free access to a commercial rodent diet and tap water. Two rats were studied in each of 4 identical experiments. After an acclimatization period of 1-week holding and 1-week adaptation, systolic blood pressure (SBP) measurements (tail-cuff) and weights were recorded on alternate days for 6 control days and 4 treatment days [saline, daily 1 mL/kg SC or heparinized saline solution (10 IU/mL) at 5 μL/hr via an osmotic mini-pump (Alza Corp., Palo Alto, CA, USA)]. Left (L) nephrectomy was performed on treatment day 4 on the 8 animals, and

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Table 1. Experimental design for control left kidneys and nephrectomy right kidneys

Experiment	Sample name	Treatment
1	L1	Control rat 1
	L2	Control rat 2
	R2	Nephrectomy rat 2
2	L3	Control rat 3
	L4	Control rat 4
	R4	Nephrectomy rat 4
3	L5	Control rat 5
	L6	Control rat 6
	R6	Nephrectomy rat 6
4	L7	Control rat 7
	L8	Control rat 8
	R8	Nephrectomy rat 8

the kidney snap-frozen in liquid nitrogen for subsequent RNA extraction. These kidney samples are referred to as L1-L8. The rats were then allowed to recover for 2 weeks. After this recovery period, SBP and weight were measured on alternate days for 4 days control, and then over 8 days of saline treatment as above. At sacrifice, the right (R) kidney was removed from rats 2, 4, 6, and 8, weighed, and snap-frozen and referred to as R2, R4, R6, and R8 (Table 1). Kidneys were stored at -70°C. Rats 1–6 were housed at The Australian National University, and rats 7 and 8 were housed at Monash University. The Animal Experimental Ethics Committees from the Australian National University (JHB.01.99 & JHB.08.01) and Monash University (PHYS 2002/09) approved these studies.

RNA isolation, preparation of cRNA, and gene chip hybridization

Total RNA was extracted from each of the kidneys using Tri-Reagent (T-9424; Sigma, St. Louis, MO, USA), and further purified using the RNeasy spin column kit (74104; Qiagen, Hilden, Germany). The integrity of the RNA was assessed by analysis on 1.2% formaldehyde-agarose gels. Preparation of cRNA, hybridization, and scanning of the Test3 arrays and Rat U34A arrays were performed according to the manufacturer's protocol (Affymetrix, Santa Clara, CA, USA). Briefly, double-stranded cDNA was synthesized from 5 to 8 μg of each RNA sample via oligo T_7-$(dT)_{24}$ primer-mediated reverse transcription. Biotin-labeled cRNA was generated using the Enzo BioArray kit (Affymetrix), purified using RNeasy spin columns (Qiagen), and then quantified by spectrophotometer. Fifteen to 20 μg of each biotin-labeled fragmented cRNA sample was used to prepare 300 μL of hybridization mixture. Aliquots of each sample (100 μL) were hybridized onto Test3 arrays to check the quality of the samples prior to hybridization (200 μL) onto the Rat U34A arrays (∼7000 full-length sequences and ∼1000 EST clusters). The arrays were washed with optimized wash protocols, stained with strepdavidin/phycoerythrin followed by antibody amplification, and scanned with the Agilent GeneArray Scanner (Affymetrix). During the course of these experiments, the scanner setting was altered on advice from Affymetrix to prevent saturation. Experiment 1 was, therefore, scanned on the old scanner setting, while all other experiments were scanned on the new settings.

Data analysis

Quality control (QC) was carried out using both MAS5 criteria (background, scale factor, control gene hybridization) and appropriate statistical plots of the $\log_2$ CEL file intensities, including box plots for each array and density plots for all arrays plotted separately and together (because these different displays allow comparisons of different features of the distributions between arrays), histograms of PM (perfect match) transformed intensities (to detect saturation) and histograms, scatter plots and Q–Q plots (to compare each pair of arrays). Box plots of the Affymetrix CEL files were generated using the box plot function in the base package in R (Bioconductor version 1.8.0) with all input parameters set to default values. Affymetrix CEL files were imported into R using the ReadAffy function in the affy package available from Bioconductor (www.bioconductor.org) [2]. Different analysis methods use different normalization procedures and scale factors, and these can have large effects on subsequent analyses. Thus, we have used a number of different methods to analyze these data. Data were first analyzed using the MAS5 software (Affymetrix Suite v5.0), where pairs of samples (either control pairs from different animals or pairs of kidneys from the same animal) were compared using both absent/present calls and signal values. Hierachic clustering of arrays, with appropriate gene filtering, was carried out on the $\log_2$ Affymetrix signals from each array using the hclust function from the mva package in R (Bioconductor version 1.8.0) [2]. The Euclidean distance metric and average agglomeration methods were used. A third analysis method was used which was based on synthetic data simulation in accord with current biological and technical understanding of microarray data [3]. Gene expression values were converted to $\log_2$ values, both the samples and genes centered (mean 0), the samples standardized (variance 1) [3]. Then, gene filtering was carried out for further analysis. A useful ordination method, the GE-biplot, is obtained from the Singular Value Decompositor of the resultant matrix. On this plot, the genes and chips are both represented [3].

RESULTS

There was no significant change in blood pressure in response to nephrectomy (112 ± 2 before and 119 ± 2 mm

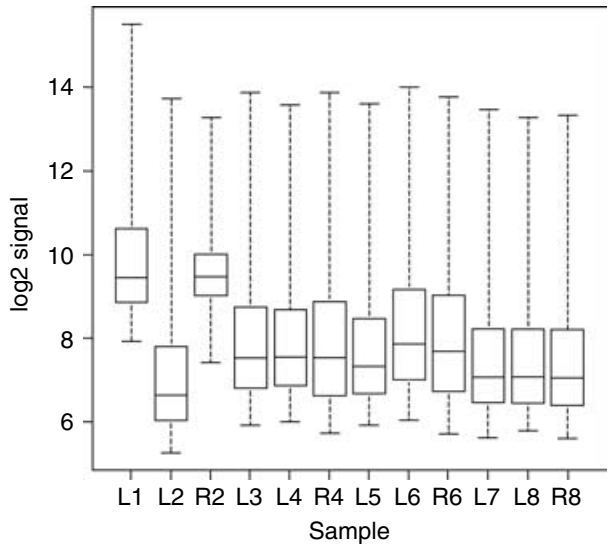

Fig. 1. Box plots of the $\log_2$ Affymetrix signal values for all the samples L1-L8 and R2, 4, 6, and 8 as output by Bioconductor.

Hg, after unilateral nephrectomy, $P = 0.2$). Renal hypertrophy was evident in the right kidney following nephrectomy, both in absolute terms (L 1.32 ± 0.08, R 2.09 ± 0.10 g, $P = 0.001$) and relative to body weight (4.24 ± 0.19 to 5.41 ± 0.31 mg/g, respectively; $P = 0.01$).

MAS5 analysis of the 8 control and 4 nephrectomy chips showed that one of the control samples, L2, had a higher background than the other samples in the same experiment. Further QC analysis of the entire data set also demonstrated this, and clearly identified those chips (L1, L2 and R2) that were scanned on the old scanner settings (Fig. 1). Some laser saturation effects were also evident (plots not shown). Because of these problems, experiment 1 was not included in any further data analysis.

Hierarchic clustering in R using hclust and $\log_2$ of the Affymetrix signal values [filtered for genes present on at least 3 of the chips (4242genes)] with the remaining 6 left kidneys showed that pairs of samples prepared and analyzed in the same "batch" were more similar to each other (Fig. 2A) than to any other left kidney. This experimental clustering was also evident in a *GE*-biplot representation of the L3-L8 samples (Fig. 2B). The advantage of the *GE*-biplot is that samples and genes can be represented simultaneously on the one plot, and interpreted with respect to each other. The most salient features of this plot are: (*1*) samples that have approximately the same relative indices for all the genes will cluster together; (*2*) the Euclidean distance from a gene point to the origin is an approximation to the standard deviation of the gene's expression index; and (*3*) genes that lie in the direction of, for these data, any pair of samples (e.g., L3/L4) are relatively up-regulated in this pair compared with the other 2 pairs, while those lying in the opposite direction are relatively down-regulated. One can observe differential

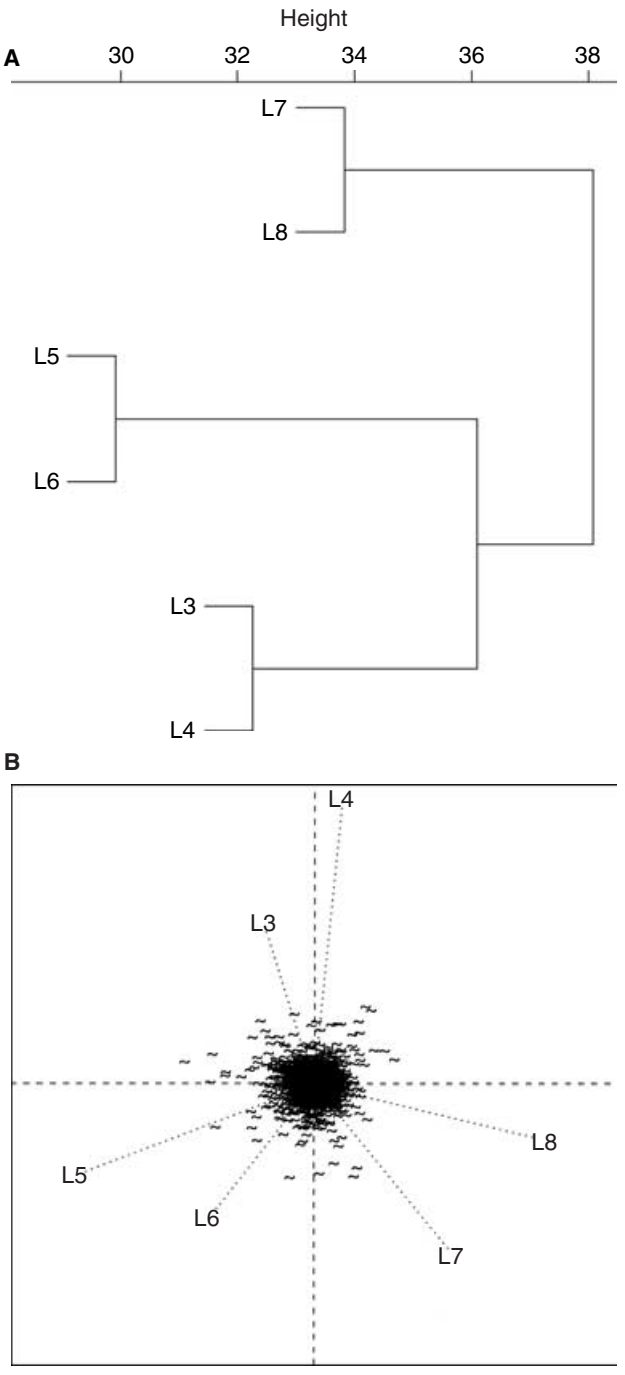

Fig. 2. Clustering of control samples L3–L8. Hierarchic clustering was carried out using hclust in R on the $\log_2$ Affymetrix signal values using the unweighted pair group method with arithmetic mean and Euclidean distance as the similarity measure. (*A*). Genes were selected based on their presence on at least 3 of the chips using the Affymetrix present/absent calls. GE-biplot of control samples L3–L8 using 4242 genes selected on the basis of their presence on at least 3 of the chips (*B*). The position of each chip is shown as a label joined by a dotted line to the origin, and each gene is shown as ∼.

expression between the 3 pairs of samples for many of the genes, and it was necessary to evaluate whether these differences are statistically significant. This evaluation was done using analysis of variance (ANOVA) with the null

Table 2. List of genes with significant F test, $P \leq 0.001$ from an ANOVA analysis of the data for control samples L3-L8

Probeset	Gene title	Gene symbol	L3[a]	L3[b] signal	L4[a]	L4[b] signal	L5[a]	L5[b] signal	L6[a]	L6[b] signal	L7[a]	L7[b] signal	L8[a]	L8[b] signal
AB016536_s_at	Heterogeneous nuclear ribonucleoprotein A/B	Hnrpab	1.63	967.6	1.63	941.4	1.56	809.7	1.57	816	1.67	955.1	1.68	998.2
AF033109_g_at	Syntaxin 8	Stx8	0.62	116.7	0.61	116.4	0.65	135.7	0.65	137.1	0.75	153.5	0.76	153.8
AJ007016_at	Protein tyrosine phosphatase type IVA, member 2	Ptp4a2	1.27	448.7	1.25	435.1	1.09	322.6	1.07	309.7	1.28	433.5	1.27	437.7
D89340_at	Dipeptidylpeptidase III	Dpp3	1.11	325.8	1.13	335.2	0.88	212.3	0.91	229.1	1.19	364.5	1.21	383.9
M19359mRNA#2_at	Rat gamma-crystallin gene cluster		−0.11	25.2	−0.1	***26.8***[c]	0.14	49.6	0.19	***55.8***	−0.19	***23.6***	−0.17	23.9
rc_AA799525_at	EST 189022	–	1.27	449.5	1.29	472.6	1.15	364.9	1.19	395	1.52	704.6	1.51	703.1
rc_AA800743_at	EST 190240 drebrin-like	Dbnl	0.84	184.2	0.82	180.1	0.6	122.3	0.57	117.3	1	248.4	0.97	237.4
rc_AA891949_at	EST 195752	–	1.9	1692.5	1.9	1675	1.78	1242.4	1.79	1260.9	1.89	1466.1	1.88	1496.1
rc_AA892086_at	EST 195889	–	1.89	1646.8	1.9	1659.1	1.77	1227.7	1.76	1199.8	1.87	1414.8	1.88	1477.4
rc_AA892296_at	EST 196099	Hoxb8	0.35	65.9	0.36	69.4	−0.93	***6.1***	−0.83	***7.8***	−0.15	25.4	−0.09	28
rc_AI104388_at	Hspb1	Hspb1	1.11	326.1	1.12	330.7	0.92	229.5	0.94	240.1	1.21	378	1.24	408.1
rc_AI172247_at	EST218247 xanthine dehydrogenase	Xdh	1.07	296.9	1.07	301.2	0.97	253.8	0.98	262.5	1.23	391.7	1.2	378.4
rc_AI228548_g_at	EST225243	–	1.18	374.7	1.15	353.5	1.14	354	1.12	344.5	0.96	230.6	0.96	234
S48190_at	S48190 type II activin receptor	–	−0.39	14	−0.41	14.2	−0.31	***20.7***	−0.31	21.3	0.04	37.1	0.01	34.5
U20643mRNA#2_f_at	RNU20643 Rattus norvegicus aldolase A	Aldoa	1.47	681.4	1.45	654.9	1.86	1454.2	1.85	1413.1	1.79	1209.3	1.82	1304.8
U93692_at	Rattus norvegicus nucleoporin Nup84	Prei2	0.32	62.2	0.31	62.4	0.23	59.6	0.23	60.7	0.36	70.8	0.38	72.1

[a] Log_2 chip scaled Affymetrix signal values for the gene listed in the row and the sample listed in the column.
[b] Affymetrix signal value for the gene listed in the row and the sample listed in the column.
[c] Those values in bold and italics were called as absent or marginal by the Affymetrix MAS5 software. All other values were called present.

Fig. 3. Clustering of 3 pairs samples of nephrectomy samples (L4:R4; L6:R6; L8:R8). Hierarchic clustering using hclust in R was carried out on the $\log_2$ Affymetrix signal values using the unweighted pair group method with arithmetic mean and Euclidean distance as the similarity measure (*A*). Genes were selected based on their presence on at least one of the chips using the Affymetrix present/absent calls. GE-biplot of 3 pairs of nephrectomy samples (L4:R4; L6:R6; L8:R8) using 4885 genes selected on the basis of their presence on at least 1 of the chips (*B*). The position of each chip is shown as a label joined by a dotted line to the origin, and each gene is shown as ∼.

hypothesis of no difference in mean gene expression between the 3 pairs for the selected (4242) genes. We used *P* values of 0.01 and 0.001, and found that 122 (data not shown) and 15 genes (Table 2), respectively, showed

Table 3. List of genes with significant paired *t* test values ($P \leq 0.01$) when pairs of right and left kidneys were compared following uninephrectomy

Probeset	Gene title	Gene symbol	L4[a]	L4[b] signal	R4[a]	R4[b] signal	L6[a]	L6[b] signal	R6[a]	R6[b] signal	L8[a]	L8[b] signal	R8[a]	R8[b] signal
AB000098_at	MIPP65 protein	Mipp65	0.43	79.2	0.52	98.6	0.33	73.8	0.4	80.6	0.51	93.9	0.61	111.7
AB010743_at	Uncoupling protein 2	Ucp2	1.44	644.1	1.35	551.3	1.38	565.3	1.28	465	1.43	598.9	1.33	483.7
AF016252_at	Nuerabin 2	Neb2	0.77	160.2	0.67	133.3	0.78	176.9	0.68	139.7	0.72	142.9	0.61	113.7
AF061971_at	Palmitoyl-protein thioesterase 2	Ppt2	0.93	222.4	0.89	211	1.08	318	1.05	292.6	1.03	266.8	0.99	245.2
D12770_s_at	Solute carrier family 25 (mitochondrial adenine nucleotide translocator) member 4	Slc25a4	1.91	1688.8	1.83	1469.2	1.93	1644.4	1.83	1397.2	1.9	1556.6	1.83	1322.8
J02773_at	Fatty acid binding protein 3	Fabp3	1.45	649.1	1.52	786.5	1.55	788.2	1.64	970.1	1.55	768.6	1.65	917.9
J04943_at	Nucleophosmin 1	Npm1	1.07	298.2	0.95	240.9	1.01	274.7	0.89	214.1	1.22	393.9	1.09	299.9
L37333_s_at	Glucose-6-phosphatase	G6pc	1.3	482.6	1.41	622.3	1.65	953.8	1.77	1258.9	1.73	1088.8	1.86	1411.4
M67465_at	Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid deltaisomerase	Hsd3b	−0.27	***18.8***[c]	−0.67	***8.3***	−0.2	***26.2***	−0.67	***9.4***	−0.22	21.7	−0.71	***7.8***
rc_AA799396_g_at	EST188893	–	0.45	83.9	0.33	65.8	0.4	84.9	0.23	56.5	0.36	69.6	0.22	51.7
rc_AA799680_at	Rattus norvegicus similar to cDNA sequence BC013529 (LOC361448)	–	0.64	122.6	0.72	149	0.8	182.4	0.9	216.6	0.71	141.4	0.82	173.9
rc_AA800593_at	EST190090	–	−0.21	21.4	−0.05	30	−0.02	37.4	0.16	49.1	−0.32	17.7	−0.17	23.2
rc_AA801286_at	Inositol (myo)-1(or 4)-monophosphatase 1	Impa1	1.18	371.3	1.13	345	1.16	367.9	1.11	332.9	1.27	429.6	1.22	388.8
rc_AA875023_at	Rattus norvegicus similar to RIKEN cDNA 2410005K17 (LOC362578)	–	0.51	93.7	0.58	111.2	0.43	89.5	0.53	103.3	0.5	91.6	0.59	107.5
rc_AA875261_at	Ul-R-E0-ce-a-06-0-Ul.s1	–	0.43	79.7	0.33	66.3	0.35	***76.5***	0.22	***55.9***	0.28	58.9	0.17	***46***
rc_AA875269_at	Stearoyl-Coenzyme A desaturase 2	Scd2	0.6	114.2	1.03	285	1.29	479.5	1.61	902.7	1.01	255.7	1.42	582.4
rc_AA891220_at	EST195023	–	0.43	78.9	0.56	107.5	0.53	108.3	0.64	130.7	0.35	67.8	0.48	86.3
rc_AA891727_at	Rattus norvegicus similar to RIKEN cDNA 2900010M23 (LOC361805)	–	0.38	72.6	0.5	94.7	0.33	73.9	0.45	88.9	0.45	82.7	0.55	100
rc_AA892014_s_at	HLA-B-associated transcript 1A	Bat1a	0.85	191.6	0.93	228.2	0.81	186.7	0.87	207.2	0.8	168.6	0.86	185.4
rc_AA894297_at	EST198100	–	0.65	126	0.71	146.9	0.53	109.7	0.6	119.9	0.69	135.8	0.75	150.3
rc_AI007824_at	Rattus norvegicus similar to Guanine nucleotide exchange factor MSS4 (RAB interacting factor) (LOC295109)	–	1.96	1876.1	1.98	2010.5	2.33	3612.1	2.35	4009.2	2.18	2703.4	2.19	2773.8
rc_AI030175_s_at	Sorbitol dehydrogenase	Sord	1.73	1169.8	1.8	1403.5	1.74	1144.8	1.81	1357.3	1.77	1195.5	1.87	1437.4
rc_AI058941_s_at	Rattus norvegicus similar to dimethylarginine dimethylaminohydrolase 1 (LOC362055)	Ddah1	1.06	291.5	1.15	364.9	1.2	398.8	1.28	467.4	1.24	408.7	1.34	489.2
rc_AI231164_s_at	Splicing factor, arginine/serine-rich (transformer 2 Drosophila homolog) 10	Sfrs10	0.78	162.5	0.66	132.3	0.77	172.7	0.63	126.4	0.81	173.3	0.69	132.7
rc_AI234604_s_at	Heat shock protein 8	Hspa8	2.26	3511.1	2.17	3019	2.12	2390.1	2.03	2094.8	2.34	3725	2.26	3174.3
rc_AI638971_g_at	Occludin	Ocln	0.07	37.9	0.19	49.8	0.22	59.4	0.32	67.6	−0.02	32.5	0.09	39.7
rc_AI639203_at	Rattus norvegicus similar to RIKEN cDNA 2410006F12 (LOC298688)	–	0.21	50.9	0.08	39.8	0.24	62.3	0.09	43.4	0.26	57.2	0.12	42.1
rc_AI639425_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx003133	–	0.99	251.5	0.9	217.9	0.91	226.3	0.83	191.4	0.99	244.6	0.91	205.5

Table 3. (Continued.)

Probeset	Gene title	Gene symbol	L4[a]	L4[b] signal	R4[a]	R4[b] signal	L6[a]	L6[b] signal	R6[a]	R6[b] signal	L8[a]	L8[b] signal	R8[a]	R8[b] signal
U03416_at	Olfactomedin related ER localized protein	Olfml	−0.02	31.5	−0.14	25.3	0.08	45.3	−0.05	32.4	0.16	46.6	0.02	34.4
U38180_at	Solute carrier family 19, member 1	Slc19a1	1.22	405.3	1.23	427.4	1.24	432.8	1.25	442.6	1.29	447.3	1.3	450.6
U75932_at	Protein kinase, cAMP dependent regulatory, type I, alpha	Prkar1a	1.16	361.1	1.12	341.5	1.17	380.5	1.13	345.4	1.28	441	1.23	396.2
U78167_at	cAMP-regulated guanine nucleotide exchange factor 1 (cAMP-GEF1)	Epac	0.43	***80.1***	0.5	93.9	0.37	***79.2***	0.44	86.7	0.37	70.8	0.44	80.6
X51706cds_g_at	RRRPL9 Rat mRNA for ribosomal protein L9	–	1.96	1861	1.88	1642.1	1.95	1720.8	1.89	1582.8	1.99	1847.8	1.92	1584.1
X51707cds_s_at	RRRPS19 Rat mRNA for ribosomal protein S19	–	1.75	1208	1.68	1074.1	1.68	1012.6	1.6	889.6	1.84	1370.9	1.75	1121.8
X52815cds_f_at	RRGAMACT Rat mRNA for cytoplasmic-gamma isoform of actin	–	2.1	2525.7	2.04	2304.4	2.08	2212.2	2.03	2095.9	2.22	2964.3	2.18	2697.3
X58200mRNA_g_at	Ribosomal protein L23	Rpl23	2.09	2453.1	1.99	2080.2	2.04	2040	1.95	1777.8	2.16	2593.2	2.06	2110.5
X74834cds_s_at	Cholinergic receptor, nicotinic, gamma polypeptide	Chrnng	0.55	102	0.49	91.8	0.56	***116.3***	0.5	***97***	0.51	94.8	0.46	83

[a] Log_2 chip scaled Affymetrix signal values for the gene listed in the row and the sample listed in the column.
[b] Affymetrix signal value for the gene listed in the row and the sample listed in the column.
[c] Those values in bold and italics were called as absent or marginal by the Affymetrix MAS5 software. All other values were called present.

evidence of significant differences at these levels. These data show that there are relatively few differences between the gene expression profiles in the control kidneys of these outbred rats.

To determine what effect unilateral nephrectomy had on the gene expression pattern in the remaining kidney, expression profiles were generated from pairs of kidneys before and after nephrectomy (i.e., L4:R4, L6:R6, L8:R8), and again, hierarchic clustering and the *GE*-biplot were used to evaluate similarities and differences. Using both approaches, the expression profiles of kidney pairs from the same animal were more similar than those from different animals (Fig. 3A and B). Because the samples cluster according to animals, and not before and after nephrectomy, it appears that nephrectomy does not lead to major global changes in gene expression, at least when measured 3 to 4 weeks following the operation. To evaluate whether there are statistically significant differences between left (control) and right (nephrectomy) kidneys from the paired animals, paired *t* tests were performed. Only 37 genes showed evidence of significant differences ($P < 0.01$) between kidney pairs (Table 3). For these evaluations, the data were normalized to mean 0, variance 1 within the samples, and the values for those genes showing differential expression levels between left and right kidneys were found to have relatively small differences.

DISCUSSION

A possible problem in interpretation of microarray data is genetic variability between outbred animals in physiologic experiments. To our knowledge, microarray studies have not been carried out on outbred rats, but it has been predicted from computation studies of microarray analysis on human tissues that variation due to lack of genetic identity should be a major issue, at least in human populations [4]. The use of outbred SD rats is common in studies of hypertension. Thus, it is important to determine if the genetic variability between these animals leads to substantial changes in gene expression profiles. Our detailed comparison here of 6 of these animals leads us to conclude that there is only a low level of variation in gene expression between the kidneys of these animals. This is despite the fact that 2 of the animals were housed separately, and the experiments were performed in pairs over a period of 24 months. The only clustering effect observed was a "batch" effect (i.e., samples prepared at the same time were more similar to each other than samples prepared at different times). Thus, it should be possible to combine gene expression data from a number of replicate rats to generate meaningful results when examining the effect of specific treatments.

Unilateral nephrectomy is known to lead to significant changes in both structure and function in the remaining

kidney [5, 6]. In the present study, we observed the expected increase in weight in the remaining kidney after the removal of the contralateral kidney. Nephrectomy is not uncommon clinically in adults; in living donor transplantation, following trauma, and in various disease states (e.g., malignancy). In general, the clinical sequelae, if any, are mild. Nephrectomy is also used experimentally in a range of circumstances (e.g., DOCA-salt hypertension [7] or studies of direct renal infusion of drugs or hormones [8]). Changes appear to depend on age at nephrectomy and are greater in younger animals [9] and humans [10]. Sex differences have also been reported [11]. In the rat models of particular interest to us, ACTH hypertension and angiotensin II mediated vascular changes, nephrectomy does not modify the timing or extent of the blood pressure change [8, 12]. In this study, we used young male SD rats (body weight at nephrectomy approximately 310 g) and found that there are a group of genes whose expression does change upon nephrectomy, but the degree of change is generally very low. These genes included a group involved in fatty acid metabolism, transport, mitochondrial function, and signal transduction. These genes are possible candidates involved in the genesis of compensatory renal hypertrophy. A literature search suggests genes which might be involved include those for VEGF [13], IGF-I and II [13, 14], GH [12, 14], TGF β [15], amylin [16], protooncogenes c-myc and c-fos [17], cell-cycle dependent hypertrophy (G1 cell cycle kinases) [18], angiotensin II, AVP, and endothelin I [19]. These genes were all represented in the data set, but none appeared to be altered in our studies. Accordingly, these experiments show that it is possible to use outbred SD rats for studies of gene expression in the kidney, and that the use of paired organs from individual animals as control and test ensures even closer genetic identity and provides ideal conditions for the use of microarrays to determine which genes are modified by the treatment of interest.

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