Table 4. Continued.

Probe sets	medulla/ papilla	cortex/ papilla	medulla/ cortex	GENE_SYMBOL	GENE_NAME	channel transporter	Metabolic enzymes	cytoskeleton /extracellular matrix
1398612_at	51.8	37.6	1.4	Akr1c12_predicted	aldo-keto reductase family 1, member C12 (predicted)			
1384639_at	51.6	49.0	1.1	Dpl1l_predicted	deleted in polyposis 1-like 1 (predicted)			
1368627_at	49.7	35.7	1.4	Rgn	regucalcin			
1368366_at	47.1	42.1	1.1	Cml2	Camello-like 2			
1387234_at	44.2	42.4	1.0	Azgp1	alpha-2-glycoprotein 1, zinc			
1368163_at	43.3	43.2	1.0	Dpp4	dipeptidylpeptidase 4			
1372841_at	41.7	32.3	1.3	Dpl1l_predicted	deleted in polyposis 1-like 1 (predicted)			
1398255_at	38.7	20.9	1.8	Slc15a2	solute carrier family 15 (H+/peptide transporter), member 2			
1367905_at	38.2	29.7	1.3	Enpp3	ectonucleotide pyrophosphatase/phosphodiesterase 3			
1370688_at	33.0	27.8	1.2	Gclc	glutamate-cystcine ligase, catalytic subunit			
1368374_a_at	31.9	29.2	1.1	Ggt1	gamma-glutamyltransferase 1			
1387218_at	30.2	23.1	1.3	Tff3	trefoil factor 3			
1370714_a_at	30.0	17.1	1.8	Siat1	sialyltransferase 1			
1373773_at	29.4	25.3	1.2	Gpm6a	glycoprotein m6a			
1387357_at	28.1	24.8	1.1	Tmlhe	trimethyllysine hydroxylase, epsilon			
1380962_at	27.6	23.3	1.2	Ace2	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 2			
1370144_at	26.9	18.1	1.5	Gtpbp4 /// LOC364763 /// LOC498786	G protein-binding protein CRFG /// similar to GTP-binding protein NGB /// similar to GTP-binding protein NGB			
1387209_at	24.6	22.3	1.1	Rgpr	regucalcin gene promotor region related protein			
1367838_at	24.5	17.9	1.4	Cth	CTL target antigen			
1390855_at	23.9	12.7	1.9	Prep	Prolyl endopeptidase			
1371913_at	23.4	11.8	2.0	Tgfbi	transforming growth factor, beta induced			
1368234_at	23.4	12.1	1.9	Prep	prolyl endopeptidase			
1388145_at	22.5	22.4	1.0	Tnxa	tenascin XA			
1370365_at	22.3	14.3	1.6	Gss	glutathione synthetase			
1381350_at	20.3	17.4	1.2	Idb4	inhibitor of DNA binding 4			
1394022_at	18.0	11.8	1.5	Idb4	inhibitor of DNA binding 4			
1368164_at	17.9	14.7	1.2	Blvra	biliverdin reductase A			
1379300_at	17.5	17.0	1.0	Chst2_predicted	carbohydrate sulfotransferase 2 (predicted)			
1387296_at	17.1	15.7	1.1	Cyp2j4	cytochrome P450, family 2, subfamily J, polypeptide 4			
1377408_at	16.9	16.2	1.0	Pla2g6	phospholipase A2, group VI			
1369407_at	16.8	10.4	1.6	Tnfrsf11b	tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)			
1382868_at	16.5	9.9	1.7	Sema6a_predicted	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A (predicted)			
1387819_at	16.3	16.3	1.0	Ela1	elastase 1, pancreatic			
1372523_at	16.3	14.9	1.1	Gclc	glutamate-cysteine ligase, catalytic subunit			

Table 4. Continued.

Probe sets	medulla/ papilla	cortex/ papilla	medulla/ cortex	GENE_SYMBOL	GENE_NAME	channel transporter	Metabolic enzymes	cytoskeleton /extracellular matrix
1387941_s_at	16.1	14.6	1.1	Pla2g6	phospholipase A2, group VI			
1372750_at	3.7	0.2	15.9	Fst	Follistatin			
1370072_at	15.6	12.1	1.3	Mme	membrane metallo endopeptidase			
1387966_at	15.5	11.4	1.4	Asrgl1	asparaginase-like sperm autoantigen			
1384831_at	15.1	11.7	1.3	Slc7a13_predicted	solute carrier family 7, (cationic amino acid transporter, y+ system) member 13 (predicted)			
1368189_at	14.8	11.3	1.3	Dhcr7	7-dehydrocholesterol reductase			
1367798_at	14.5	11.2	1.3	Ahcy	S-adenosylhomocysteine hydrolase			
1371059_at	14.5	14.4	1.0	Prkar2a	protein kinase, cAMP-dependent, regulatory, type 2, alpha			
1369158_at	14.4	14.3	1.0	Casr	calcium-sensing receptor			
1370030_at	14.1	11.5	1.2	Gclm	glutamate cysteine ligase, modifier subunit			
1398350_at	14.0	12.9	1.1	Basp1	brain abundant, membrane attached signal protein 1			
1369728_at	13.7	10.7	1.3	Hist1h4m_predicted	histone 1, H4m (predicted)			
1387223_at	13.6	12.2	1.1	Aadat	aminoadipate aminotransferase			
1370529_a_at	12.8	7.7	1.7	Pld1	phospholipase D1			
1384603_at	12.8	9.8	1.3	Abca4_predicted	ATP-binding cassette, sub-family A (ABC1), member 4 (predicted)			
1369494_a_at	12.0	6.6	1.8	Ghrhr	growth hormone releasing hormone receptor			
1367729_at	11.9	11.4	1.0	Oat	ornithine aminotransferase			
1374565_at	11.8	9.7	1.2	Nek6	NIMA (never in mitosis gene a)-related expressed kinase 6			
1368431_at	11.6	10.3	1.1	Hpn	hepsin			
1382274_at	11.5	5.5	2.1	Rarres1_predicted	retinoic acid receptor responder (tazarotene induced) 1 (predicted)			
1374871_at	11.2	7.3	1.5	Asrgl1	asparaginase-like sperm autoantigen			
1392965_a_at	11.0	2.3	4.8	Smoc2_predicted	SPARC related modular calcium binding 2 (predicted)			
1370163_at	11.0	6.5	1.7	Odc1	ornithine decarboxylase 1			
1390208_at	10.7	10.7	1.0	Htatip2_predicted	HIV-1 Tat interactive protein 2 (predicted)			
1370530_a_at	10.6	5.7	1.9	Pld1	phospholipase D1			
1376852_at	10.5	8.3	1.3	Mccc1_predicted	methylcrotonoyl-Coenzyme A carboxylase 1 (alpha) (predicted)			
1369184_at	10.5	6.8	1.5	Cldn16	claudin 16			
1385970_at	10.4	9.5	1.1	Sh2bp1_predicted	SH2 domain binding protein 1 (tetratricopeptide repeat containing) (predicted)			
1383742_at	10.1	9.4	1.1	Snx7_predicted	sorting nexin 7 (predicted)			

After selection by ANOVA ($p<0.01$) for the data of percellome normalization, genes maximally expressed in medulla were selected. The genes were aligned in the order of the ratio to the lower expression value, either in papilla or in cortex. As the genes listed here are expressed in medulla and cortex to a similar extent, exceptional cases (ratio >3) are shaded in the medulla/cortex column. The genes categorized to "channel/transporters", "metabolic enzymes", or "cytoskeleton/extracellular matrix" are also shaded. Proteases or enzymes involving signal transduction are not included in the category of "metabolic enzymes". For simplicity, genes with less than 10-fold specificity are omitted.

Table 5. A list of probe sets specifically expressed in cortex of kidney.

Probe sets	cortex/ papilla	medulla/ papilla	cortex/ medulla	GENE_SYMBOL	GENE_NAME	channel transporter	Metabolic enzymes	cytoskeleton /extracellular matrix
1387314_at	312.0	259.8	1.2	Sult1b1	sulfotransferase family 1B, member 1			
1387820_at	284.9	14.7	19.4	Klk7	kallikrein 7			
1388172_at	245.4	110.3	2.2	Ust1r	integral membrane transport UST1r			
1368064_a_at	230.1	19.6	11.8	Ddc	dopa decarboxylase			
1390591_at	224.2	166.1	1.3	Slc17a3	Na/Pi cotransporter 4			
1368467_at	217.1	117.6	1.8	Cyp4f2	cytochrome P450, family 4, subfamily F, polypeptide 2			
1368600_at	210.7	75.0	2.8	Slc26a1	solute carrier family 26 (sulfate transporter), member 1			
1396039_at	202.5	188.6	1.1	Slc22a12_predicted	solute carrier family 22 (organic anion/cation transporter), member 12 (predicted)			
1387230_at	193.9	13.2	14.7	Slc12a3	solute carrier family 12, member 3			
1368245_at	192.4	134.7	1.4	Upb1	ureidopropionase, beta			
1367917_at	192.3	124.6	1.5	Cyp2d26	cytochrome P450, family 2, subfamily d, polypeptide 26			
1367871_at	187.8	32.3	5.8	Cyp2e1	cytochrome P450, family 2, subfamily e, polypeptide 1			
1376267_at	185.1	13.9	13.4	Slc16a6	Solute carrier family 16 (monocarboxylic acid transporters), member 6			
1384877_at	183.4	73.9	2.5	Aqp11	aquaporin 11			
1398282_at	174.5	75.2	2.3	Kynu	kynureninase (L-kynurenine hydrolase)			
1370547_at	169.5	56.4	3.0	Pzp	pregnancy-zone protein			
1368563_at	149.7	96.7	1.5	Aspa	aspartoacylase			
1383111_at	149.3	60.1	2.5	Acmsd	2-amino-3-carboxymuconate-6-semialdehyde decarboxylase			
1370991_at	146.7	32.6	4.5	Cml3	camello-like 3			
1387188_at	144.5	86.8	1.7	RGD:620099	solute carrier family 17 (sodium phosphate), member 1			
1370936_at	143.4	91.3	1.6	Dmgdh	dimethylglycine dehydrogenase precursor			
1367804_at	142.8	21.8	6.5	Sap	serum amyloid P-component			
1368915_at	141.5	87.2	1.6	Kmo	kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)			
1398511_at	138.7	16.8	8.3	Susd2_predicted	sushi domain containing 2 (predicted)			
1387851_at	129.9	78.3	1.7	Pter	phosphotriesterase related			
1376051_at	127.0	63.2	2.0	Cryl1	crystallin, lamda 1			
1384112_at	125.1	80.6	1.6	Nt5	5 nucleotidase			
1393894_at	123.8	94.1	1.3	RGD:628846	cytochrome P450, 4a12			
1370725_a_at	116.9	15.4	7.6	G6pc	glucose-6-phosphatase, catalytic			
1386980_at	116.6	64.2	1.8	Apom	apolipoprotein M			
1377125_at	116.3	28.0	4.2	Dnajc6_predicted	DnaJ (Hsp40) homolog, subfamily C, member 6 (predicted)			
1368317_at	114.8	70.5	1.6	Aqp7	aquaporin 7			
1370615_at	114.4	28.0	4.1	RGD:708417	UDP-glucuronosyltransferase			

Table 5. Continued.

Probe sets	cortex/ papilla	medulla/ papilla	cortex/ medulla	GENE_SYMBOL	GENE_NAME	channel transporter	Metabolic enzymes	cytoskeleton /extracellular matrix
1368236_at	113.4	105.5	1.1	Mep1a	meprin 1 alpha			
1373386_at	113.2	110.8	1.0	Gjb2	gap junction membrane channel protein beta 2			
1369636_at	112.6	41.2	2.7	Sord	sorbitol dehydrogenase			
1368521_at	110.8	46.3	2.4	Napsa	napsin A aspartic peptidase			
1368150_at	110.4	78.2	1.4	Slc27a2 /// LOC497779	solute carrier family 27 (fatty acid transporter), member 2 /// hypothetical gene supported by NM_031736			
1369635_at	109.5	42.4	2.6	Sord	sorbitol dehydrogenase			
1368180_s_at	107.9	73.9	1.5	Gsta2	glutathione-S-transferase, alpha type2			
1368190_at	105.6	12.1	8.7	Ren1	renin 1			
1377051_at	104.7	17.9	5.8	Mpv17l_predicted	Mpv17 transgene, kidney disease mutant-like (predicted)			
1387336_at	102.7	89.7	1.1	Nat8	*N*-acetyltransferase 8 (camello like)			
1387631_at	102.4	59.8	1.7	Hpgd	15-hydroxyprostaglandin dehydrogenase			
1379885_at	100.7	91.4	1.1	Fmo4	flavin containing monooxygenase 4			
1368659_at	100.0	60.0	1.7	Agxt2	alanine-glyoxylate aminotransferase 2			
1370259_a_at	99.6	31.1	3.2	Pthr1	parathyroid hormone receptor 1			
1368188_at	94.6	25.6	3.7	Hpd	4-hydroxyphenylpyruvic acid dioxygenase			
1369200_at	93.4	56.3	1.7	Nt5	5 nucleotidase			
1387053_at	90.3	37.7	2.4	Fmo1	flavin containing monooxygenase 1			
1388569_at	88.3	50.7	1.7	Serpinf1	serine (or cysteine) proteinase inhibitor, clade F), member 1			
1390857_at	87.5	26.6	3.3	Xylb_predicted	xylulokinase homolog (H. influenzae) (predicted)			
1387375_at	86.9	64.4	1.4	Khk	ketohexokinase			
1387034_at	86.3	17.7	4.9	Pah	phenylalanine hydroxylase			
1397740_at	86.3	51.0	1.7	Sfxn1_predicted	sideroflexin 1 (predicted)			
1368736_at	84.2	18.9	4.4	Tsx	testis specific X-linked gene			
1398514_at	82.6	81.3	1.0	Hgd_predicted	homogentisate 1, 2-dioxygenase (predicted)			
1368515_at	81.1	7.2	11.3	Epb4.1l3	erythrocyte protein band 4.1-like 3			
1368794_at	81.0	78.4	1.0	Haao	3-hydroxyanthranilate 3,4-dioxygenase			
1370964_at	80.8	27.0	3.0	Ass	arginosuccinate synthetase			
1368077_at	79.6	43.0	1.9	Fbp1	fructose-1,6- biphosphatase 1			
1370397_at	77.6	68.8	1.1	Cyp4a14	cytochrome P450, family 4, subfamily a, polypeptide 14			
1368397_at	76.3	36.6	2.1	Ugt2b5 /// Ugt2b4	UDP-glucuronosyltransferase 2 family, member 5 /// UDP glycosyltransferase 2 family, polypeptide B4			
1368282_at	74.3	24.9	3.0	Dpep1	dipeptidase 1 (renal)			
1395026_at	73.7	59.0	1.2	Fmo4	flavin containing monooxygenase 4			
1380577_at	70.1	53.6	1.3	Abcg2	ATP-binding cassette, sub-family G (WHITE), member 2			

Table 5. Continued.

Probe sets	cortex/ papilla	medulla/ papilla	cortex/ medulla	GENE_SYMBOL	GENE_NAME	channel transporter	Metabolic enzymes	cytoskeleton /extracellular matrix
1387339_at	69.4	19.2	3.6	Sepp1	selenoprotein P, plasma, 1			
1382913_at	68.9	16.8	4.1	Cttnbp2	cortactin binding protein 2			
1376327_at	68.9	24.2	2.8	Tnfrsf14_predicted	tumor necrosis factor receptor superfamily, member 14 (herpesvirus entry mediator) (predicted)			
1368178_at	66.6	32.4	2.1	Pdzk1	PDZ domain containing 1			
1377672_at	66.3	37.3	1.8	Sult1c2	sulfotransferase family, cytosolic, 1C, member 2			
1387084_at	65.5	55.5	1.2	Dpp4	dipeptidylpeptidase 4			
1374512_at	63.7	35.5	1.8	Cdh7	Cadherin 7, type 2			
1371824_at	63.6	40.8	1.6	Ak3l1	adenylate kinase 3-like 1			
1369412_a_at	63.4	37.9	1.7	Slc19a1	solute carrier family 19, member 1			
1373803_a_at	63.1	39.2	1.6	Ghr	growth hormone receptor			
1387259_at	62.9	29.4	2.1	Cdh2 /// LOC497718	cadherin 2 /// hypothetical gene supported by NM_031333			
1389166_at	62.8	31.1	2.0	Cib2_predicted	calcium and integrin binding family member 2 (predicted)			
1371354_at	62.1	8.0	7.8	Tncc_predicted	troponin C, cardiac/slow skeletal (predicted)			
1372672_at	58.8	36.8	1.6	Qprt_predicted	quinolinate phosphoribosyltransferase (predicted)			
1369491_at	58.4	36.1	1.6	Dao1	D-amino acid oxidase			
1387111_at	57.3	33.4	1.7	Ddah1	dimethylarginine dimethylaminohydrolase 1			
1367988_at	57.1	22.1	2.6	Cyp2c23	cytochrome P450, family 2, subfamily c, polypeptide 23			
1368607_at	56.5	51.1	1.1	RGD:628846	cytochrome P450, 4a12			
1370881_at	55.8	22.9	2.4	Tst	thiosulfate sulfurtransferase			
1369259_at	55.6	28.4	2.0	Dio1	deiodinase, iodothyronine, type I			
1376709_at	55.2	42.5	1.3	Slc39a8_predicted	solute carrier family 39 (metal ion transporter), member 8 (predicted)			
1387013_at	55.2	27.3	2.0	Tmem27	kidney-specific membrane protein			
1387808_at	54.9	5.1	10.8	Slc7a7	solute carrier family 7 (cationic amino acid transporter, y+ system), member 7			
1368283_at	54.7	29.8	1.8	Ehhadh	enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase			
1373337_at	53.4	22.5	2.4	Grhpr_predicted	glyoxylate reductase/hydroxypyruvate reductase (predicted)			
1383654_a_at	53.2	14.7	3.6	Fnsk	similar to fructosamine-3-kinase			
1368924_at	51.8	42.4	1.2	Ghr	growth hormone receptor			
1368092_at	50.7	35.9	1.4	Fah	fumarylacetoacetate hydrolase			
1380171_at	49.4	39.5	1.3	Adra2b	Adrenergic receptor, alpha 2b			
1367952_at	45.8	26.5	1.7	Lrp2	low density lipoprotein receptor-related protein 2			

Table 5. Continued.

Probe sets	cortex/ papilla	medulla/ papilla	cortex/ medulla	GENE_SYMBOL	GENE_NAME	channel transporter	Metabolic enzymes	cytoskeleton /extracellular matrix
1369705_at	44.1	42.6	1.0	RGD:621651	X transporter protein 3			
1368680_a_at	43.6	25.3	1.7	Slc34a1	solute carrier family 34 (sodium phosphate), member 1			
1367627_at	43.5	14.8	2.9	Gatm	glycine amidinotransferase (L-arginine:glycine amidinotransferase)			
1379950_at	42.9	37.7	1.1	Cml2	Camello-like 2			
1367775_at	42.6	32.3	1.3	Amacr	alpha-methylacyl-CoA racemase			
1388176_at	42.4	24.0	1.8	Cml5	camello-like 5			
1368322_at	42.1	8.2	5.1	Sod3	superoxide dismutase 3, extracellular			
1372264_at	42.1	15.9	2.7	Pck1	phosphoenolpyruvate carboxykinase 1			
1397647_at	41.9	17.1	2.5	Slc25a15_predicted	solute carrier family 25 (mitochondrial carrier; ornithine transporter) member 15 (predicted)			
1369073_at	41.9	12.2	3.4	Nr1h4	nuclear receptor subfamily 1, group H, member 4			
1368877_at	41.6	13.7	3.0	Znf354a	zinc finger protein 354A			
1390119_at	41.4	4.9	8.4	Sfrp2	secreted frizzled-related protein 2			
1367774_at	41.1	31.8	1.3	Gsta5	glutathione S-transferase A5			
1376191_at	40.1	27.6	1.5	Hpgd	15-hydroxyprostaglandin dehydrogenase			
1397526_at	39.3	23.5	1.7	Gcdh_predicted	glutaryl-Coenzyme A dehydrogenase (predicted)			
1374384_at	38.8	16.3	2.4	Crygc	Crystallin, gamma C			
1387491_at	38.5	10.9	3.5	Gyk	glycerol kinase			
1386944_a_at	38.3	7.7	5.0	G6pc	glucose-6-phosphatase, catalytic			
1367999_at	37.7	22.6	1.7	Aldh2	aldehyde dehydrogenase 2			
1369182_at	37.6	9.6	3.9	F3	coagulation factor 3			
1382975_at	37.4	20.8	1.8	Ceacam1	CEA-related cell adhesion molecule 1			
1374200_at	36.1	16.7	2.2	Slc29a3	solute carrier family 29 (nucleoside transporters), member 3			
1369973_at	35.6	9.6	3.7	Xdh /// LOC497811	xanthine dehydrogenase /// hypothetical gene supported by NM_017154			
1372306_at	35.4	22.9	1.5	Ethe1_predicted	ethylmalonic encephalopathy 1 (predicted)			
1370818_at	34.6	12.5	2.8	Decr2	2-4-dienoyl-Coenzyme A reductase 2, peroxisomal			
1397797_at	33.3	29.3	1.1	Tigd3	Tigger transposable element derived 3 (predicted)			
1372323_at	32.9	23.4	1.4	Sardh	sarcosine dehydrogenase			
1368412_a_at	32.5	5.3	6.1	Ptpro	protein tyrosine phosphatase, receptor type, O			
1390036_at	32.5	6.0	5.4	Slc16a6	solute carrier family 16 (monocarboxylic acid transporters), member 6			
1397744_at	32.5	22.2	1.5	Sardh	Sarcosine dehydrogenase			
1368642_at	32.4	19.5	1.7	Cdh2 /// LOC497718	cadherin 2 /// hypothetical gene supported by NM_031333			
1373188_at	32.0	12.2	2.6	Scn4b	sodium channel, voltage-gated, type IV, beta			

Table 5. Continued.

Probe sets	cortex/ papilla	medulla/ papilla	cortex/ medulla	GENE_SYMBOL	GENE_NAME	channel transporter	Metabolic enzymes	cytoskeleton /extracellular matrix
1373667_at	31.8	14.0	2.3	Ccbl1_predicted	cysteine conjugate-beta lyase (predicted)			
1372031_at	31.7	7.5	4.2	Dab2	Disabled homolog 2 (Drosophila)			
1390585_at	31.7	10.9	2.9	Masp1	mannan-binding lectin serine peptidase 1			
1386981_at	31.6	3.7	8.4	Slc16a1	solute carrier family 16 (monocarboxylic acid transporters), member 1			
1368253_at	31.5	28.7	1.1	Gamt	guanidinoacetate methyltransferase			
1388537_at	31.4	15.2	2.1	Nipsnap1_predicted	4-nitrophenylphosphatase domain and non-neuronal SNAP25-like protein homolog 1 (C. elegans) (predicted)			
1387165_at	31.1	4.4	7.1	Maf	v-maf musculoaponeurotic fibrosarcoma (avian) oncogene homolog (c-maf)			
1384273_at	30.9	10.2	3.0	Carkl_predicted	carbohydrate kinase-like (predicted)			
1380393_at	30.9	30.7	1.0	Cryz_predicted	crystallin, zeta (predicted)			
1393947_at	30.7	12.7	2.4	Slc25a15_predicted	solute carrier family 25 (mitochondrial carrier; ornithine transporter) member 15 (predicted)			
1378197_at	30.4	9.5	3.2	KIFC2	kinesin family member C2			
1379582_a_at	30.1	14.5	2.1	Ccna2	cyclin A2			
1382434_at	30.0	23.5	1.3	Entpd5	ectonucleoside triphosphate diphosphohydrolase 5			

After selection by ANOVA ($p<0.01$) for the data of percellome normalization, genes maximally expressed in cortex were selected. The genes were aligned in the order of the ratio to the lower expression value, either in papilla or in medulla. Among the genes listed here, relatively specific ones for cortex (ratio>3) are shaded in the cortex/medulla column. The genes categorized to "channel/transporters", "metabolic enzymes", or "cytoskeleton/extracellular matrix" are also shaded. Proteases or enzymes involving signal transduction are not included in the category of "metabolic enzymes". For simplicity, genes with less than 30-fold specificity are omitted.

Table 6. Regionally specific genes in rat kidney.

	Channel/transporter	Metabolizing enzymes	Cytoskeleton/ extracellular matrix	Others of interest
Papilla	Slco4a1, Aqp3, Aqp4, Fxyd4, Slc4a11, Clcnk1, bsnd	Ptgs1, Ptgs2, Aldh1a3, Akr1b4, Ugt8, Lib4dh	Capg, Plekhb1, Col18a1, Spnb3, Hs3st1, Krt1-18	Hspa2, Hspa1a, Hspb1
Medulla	Scl21a1, Scl21a13, Slc23a3, Slc21a9, UST5r, Scl6a18, Scl38a3, Slc22a2, Slc7a12, Slc15a2	CYP2d9, CYP2c, Hnmt, Pklr, Fmo3, Dhrs7, Akr1c12, Gclc, Ggt1, Tmlhe		Prlr, Ace2
Cortex	Ustlr, Slc17a3, Slc26a1, Slc22a12, Slc12a3, Slc16a6, Aqp11, Aqp7, Gjb2, Slc27a2	Sult1b1, Klk7, Ddc, CYP2d26, CYP2e1, CYP4f2, Upb1, Aspa, Acmsd, Dmgdh, Kmo		Ren1

Genes categorized in channel/transporter, metabolizing enzyme and cytoskeleton/extracellular matrix from high rank in Tables 3, 4, 5 are summarized by using gene symbols which can be referred to in the preceding tables. Interesting genes discussed in the text are included as "others".

aminoglycoside causes necrosis mainly in the proximal tubules, while puromycin does the same on glomerulus (Schnellmann, 2001). In an organ with such a complex structure, analysis by GeneChip would give different results when it is done as a whole or separated into portions. When the potential nephrotoxicity of candidate chemicals is assessed using toxicogenomics technology, it would be ideal to perform GeneChip analysis for each portion. However, it is sometimes difficult in the usual toxicity tests to obtain enough sample material for toxicogenomics analysis after sampling for other histopathological and biochemical analyses. Furthermore, it is difficult to determine which portion is to be examined when the target region of the test drug is unknown. Analyzing all of the separated portions is impractical considering the cost.

In the present study, comparison of the gene expression profile was made among each portion, papilla, medulla, and cortex, as well as between the whole slice and each portion. Although various genes or proteins with region-specific expression have been reported, their localization was toward glomerulus, distal/proximal tubules, or collecting duct, i.e., tissues from specific cell types, not the anatomical location. This way is of course desirable for cell physiological study, but is inconvenient when a potential bias in the gene expression analysis based on the position of sampling is concerned, and reports focusing on this point are scarce. It can be generally said that papilla is enriched in collecting duct and relatively scarce in glomerulus, but their proportion varies with the sampling.

When comparison between each portion was made by correlation coefficient, the correlation was decreased between the portions compared to within the same portion. The correlation of gene expression profile between cortex and medulla was relatively high, whereas that between papilla and cortex or medulla was low. Based on the correlation of each portion to the whole slice, it was concluded that gene expression in the whole slice largely reflected that of cortex, followed by medulla. The main reason is that the volume of the portion comprising the whole slice is in this order: cortex > medulla > papilla (Fig. 1). If the production of mRNA per cell is constant throughout the portions, the region-specific gene can be extracted by global normalization. However, this was found to be inappropriate.

To extract region-specific genes, detection call (absent, present, and marginal) included in the GeneChip data was utilized, i.e., genes with present call in a particular region but absent call in all others were extracted, and they were checked as to whether they had present call in the whole slice. The important point of this result is that the majority (94%) of genes specifically expressed in papilla are not detectable in the whole slice. This is consistent with the result of the correlation coefficient. It is thus concluded that the expression changes of such genes occurring in papilla cannot be detected when they are decreased, and it is difficult to measure when they are increased, but their extent is not so large, as in the analysis of the whole slice. Attention should be paid when these genes are used for the marker genes in the toxicogenomics of kidney.

As obvious from Figs. 3 and 4, global normalization of the data leads to an incorrect result in the comparison among different regions. This might be due to the fact that the transcriptional activity in papilla is much less than that in the others, and subsequently the expression of each gene in papilla was over-estimated by the normalization, using a low value. This does not mean that the global normalization is useless when drug effects are tested on the samples separated into different portions. As global normalization gives relative values to the total mRNA amount, it efficiently reveals the drug effect unless the drug brings about a large change in the total mRNA. In the present case, it became problematic simply because the comparison was made among tissues with largely different mRNA contents. An alternative way is to normalize each gene by a house-keeping gene, e.g., β-actin or GAPDH. However, as already shown above, there is no guarantee that expression of these genes is constant throughout the different tissues.

Based on the data normalized by the percellome procedure, genes with region-specific expression were extracted and aligned in the order of their relative specificity. It was then found that the majority of genes with high region-specificity were related to channel/transporter and metabolic enzyme, suggesting a good correlation between gene expression and physiological function.

There are many known members of the solute carrier family (slc), whose distribution showed an interesting and marked difference among the three portions. In papilla, slc4a family members (anion transporter) were specifically expressed. In medulla, the expression of slc21a family (organic anion transporting polypeptides; OATPs), was outstanding. There were also other members such as Slc23a3 (xanthine/uracil permease), Scl6a18 (monocarboxylic acid trans-

porter), Scl38a3 (SNAT family; Gu *et al.*, 2005), Slc22a2 (organic cation transporter), Slc7a12 (cationic amino acid transporter), and Slc15a2 (H+/peptide transporter), which were specifically expressed. In cortex, Slc17a (Na-phosphate co-transporter), Slc22a12 and Slc12a3 (both anion transporters), Slc16a6 (monocarboxylic acid transporter), and Slc27a2 (fatty acid transporter) were found to be specific.

There have been some reports regarding region-specific transporters. As glucose transport is known to be operational in the proximal tubule, it is expected that glucose transporters are enriched in cortex and medulla compared with papilla. Among the genes judged as significant by ANOVA, slc2a4 and 2a13 were glucose transporters and their expression was about 3.5-fold higher in medulla and cortex compared with papilla (this number was not large enough to be shown in the tables). It is also known that slc14a2, involved in urea transport, is highly expressed in the collecting duct (Karakashian *et al.*, 1999). Although the ratio was not large enough to be in Table 3, its expression in papilla was twofold compared with cortex, consistent with the literature.

Aquaporin (Aqp) 3 and 4 were specifically expressed in papilla while Aqp 7 and 11 were in cortex (Table 6). Although the ratio was not large enough to be in Table 3, Aqp 2 was also preferentially expressed in papilla (1.9 and 2.3 fold compared with medulla and cortex, respectively). There have been supportive reports that Aqp2 (Jo *et al.*, 1997) and Aqp3 (Echevarria *et al.*, 1994) are enriched in collecting duct and Aqp7 is highly expressed in cortex (Nejsum *et al.*, 2000).

Kidney produces renin to control blood pressure. Renin is synthesized in juxtaglomerular cells and converts angiotensinogen to angiotensin I, which is further converted to angiotensin II by angiotensin converting enzyme (ACE). The renin gene (Ren1) was expressed highest in cortex while ACE (ace2) was highest in medulla (Table 6).

Probes for prolactin receptor are 4 sets on the chip and all of them showed quite low expression in papilla (expression ranged 60 - 90 fold in cortex and 70 - 130 fold in medulla, compared with papilla, for these 4 probe sets). Prolactin is known as a natriuretic hormone which interacts with the renal dopamine system, and its natriuretic response is associated with inhibition of proximal tubular Na,K-ATPase (Ibarra *et al.*, 2005). The location of its receptor in kidney (enriched in medulla and cortex) elucidated in the present study was consistent with the region where the hormone works.

NSAID-induced nephrotoxicity is well known as a typical toxicity toward kidney (Schnellmann, 2001). Its mechanism is believed to be inhibition of cyclooxygenase (COX). COX-1 is a constitutive, house-keeping enzyme and reported to be much present in the collecting duct. COX-2 is an inducible enzyme involved in the inflammatory process, while it is reported to be always present at a low level in kidney without inflammation (Harris *et al.*, 1994). The genes for these enzymes (ptgs1 and ptgs2) were both highest in papilla. Moreover, phospholipase A2 IVA was also highest in papilla. These observations are consistent with previous reports and might be related to the fact that nephrotoxicity by NSAID is frequently associated with necrosis in papilla (Schnellmann, 2001).

On the other hand, other prostaglandin-related genes were uniformly low in papilla. The expression of the gene of 15-hydroxyprostaglandin dehydrogenase (Hpgd), which is involved in prostaglandin metabolism, was 60 and 102 fold in medulla and cortex, respectively, compared with papilla. Other prostaglandin-related genes without large enough specificity for the table were prostaglandin D2 synthase (Ptgds), prostaglandin E receptor 1 (Ptger1), prostaglandin E receptor 3 (Ptger3), and prostaglandin E receptor 4 (Ptger4), which were 6- and 26-fold, 3- and 4-fold, 5- and 2-fold, 3- and 6-fold, in medulla and cortex, respectively, compared with papilla. The expression of prostaglandin E synthase (Ptges) was exceptionally the same in papilla and medulla, and 4.5-fold of these was found in cortex.

Kallikrein that produces bradykinin is biosynthesized in kidney cortex (Xiong *et al.*, 1989). Kallikrein 7 (Klk7) is found in Table 5 showing 15-fold and 285-fold expression in medulla and cortex, respectively, compared with papilla. This is again consistent with the anatomical feature.

One unique point in the tables is that genes related to cytoskeletal proteins and heat shock proteins (both HSP70 and HSP27) selectively expressed high in papilla (Table 3 and 6). If the analysis was done by global normalization, it could be that the relative expression of these genes was apparently overestimated because of the low expression of other gene populations, such as transporters or enzymes. However, the present analysis was based on percellome normalization, and the values are directly related to the copy numbers per cell (or DNA). Therefore, enrichment of these genes means that the copy numbers of these genes are actually high. The potential involvement of

HSP70 in nephrotoxicity has been investigated in relation to renal cell survival and apoptosis, and the relationship between hsp27 and cytoskeletal proteins has also been discussed in relation to renal injury after ischemia-reperfusion (van de Water, 2006). Its pathophysiological meaning is presently unclear, especially because data of modulation by nephrotoxicants is not available, so this point is quite interesting as a future study.

In summary, many of the genes related to kidney functions showed region-related differences in their expression and some of them were consistent with previous reports. There are also many genes with unique region-related differences in the table, which have not been described in the literature and it would be worthwhile to start new investigations based on these data. In the present study, analysis of gene expression was exclusively done in non-treated animals. It is of course important to investigate the regional difference in the responsiveness to drugs, and it should be the highest priority of future study. In conclusion, comprehensive comparison data of gene expression in the renal anatomical areas would greatly enhance studies of physiological function and mechanism of toxicity in kidney.

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REFERENCES

Echevarria, M., Windhager, E.E., Tate, S.S. and Frindt, G. (1994): Cloning and expression of AQP3, a water channel from the medullary collecting duct of rat kidney. Proc. Natl. Acad. Sci. USA, **91**, 10997-11001.

Gu, S., Villegas, C.J. and Jiang, J.X. (2005): Differential Regulation of Amino Acid Transporter SNAT3 by Insulin in Hepatocytes. J. Biol. Chem., **280**, 26055-26062.

Harris, R.C., McKanna, J.A., Akai, Y., Jacobson, H.R., Dubois, R.N. and Breyer, M.D. (1994): Cyclooxygenase-2 is associated with the macula densa of rat kidney and increases with salt restriction. J. Clin. Invest., **94**, 2504-2510.

Ibarra, F., Crambert, S., Eklof, A.C., Lundquist, A., Hansell, P. and Holtback, U. (2005): Prolactin, a natriuretic hormone, interacting with the renal dopamine system. Kidney Int., **68**, 1700-1707.

Jo, I., Nielsen, S. and Harris, H.W. (1997): The 17 kDa band identified by multiple anti-aquaporin 2 antisera in rat kidney medulla is a histone. Biochim. Biophys. Acta., **1324**, 91-101.

Kanno, J., Aisaki, K., Igarashi, K., Nakatsu, N., Ono, A., Kodama, Y. and Nagao, T. (2006): "Per cell" normalization method for mRNA measurement by quantitative PCR and microarrays. BMC Genomics, **7**, 64.

Karakashian, A., Timmer, R.T., Klein, J.D., Gunn, R.B., Sands, J.M. and Bagnasco, S.M. (1999): Cloning and characterization of two new isoforms of the rat kidney urea transporter: UT-A3 and UT-A4. J. Am. Soc. Nephrol., **10**, 230-237.

Nejsum, L.N., Elkjaer, M., Hager, H., Frokiaer, J., Kwon, T.H. and Nielsen, S. (2000): Localization of aquaporin-7 in rat and mouse kidney using RT-PCR, immunoblotting, and immunocytochemistry. Biochem. Biophys. Res. Commun., **277**, 164-170.

Schnellmann, R.G. (2001): Toxic responses of the kidney. In Casarett & Doull's Toxicology, 6th ed.(Klaassen, C.D., ed.), pp.491-514. McGraw-Hill.

Snedecor, G.W. and Cochran, W.G. (1989): Statistical Methods, 8th ed., Iowa State University Press.

Thukral, S.K., Nordone, P.J., Hu, R., Sullivan, L., Galambos, E., Fitzpatrick, V.D., Healy, L., Bass, M.B., Cosenza, M.E. and Afshari, C.A. (2005): Prediction of nephrotoxicant action and identification of candidate toxicity-related biomarkers. Toxicol. Pathol., **33**, 343-355.

Urushidani, T. and Nagao, T. (2005): Toxicogenomics: The Japanese initiative. In Handbook of Toxicogenomics - Strategies and Applications (Borlak, J., ed.), pp. 623-631. Wiley – VCH.

van de Water, B., de Graauw, M., Le Devedec, S. and Alderliesten, M. (2006): Cellular stress responses and molecular mechanisms of nephrotoxicity. Toxicol. Lett., **162**, 83-93.

Xiong, W., Chao, L. and Chao, J. (1989): Renal kallikrein mRNA localization by in situ hybridization. Kidney Int., **35**, 1324-1329.

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PROFILING OF GENE EXPRESSION IN RAT LIVER AND RAT PRIMARY CULTURED HEPATOCYTES TREATED WITH PEROXISOME PROLIFERATORS

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ABSTRACT — The Toxicogenomics project has been constructing a large-scale database of about 150 compounds exposed to rat (single dose, 3, 6, 9, 24 hrs and repeated dose for 3, 7, 14 28 days with 3 dose levels) and rat hepatocytes (2, 8, 24 hr with 3 concentrations) and data of transcriptome in liver using GeneChip, and the related toxicological measures are being accumulated. In the present study, the data of three ligands of peroxisome proliferator activated receptor α (PPARα), i.e., clofibrate, WY-14643 and gemfibrozil in our database were analyzed. Many of the β-oxidation-related genes were commonly induced *in vivo* and *in vitro*, whereas expression changes in genes related to cell proliferation, apoptosis, were detected *in vivo* (single and repeated dose) but not *in vitro*. Changes in those related to the immune response, coagulation and the stress response were also detectable exclusively *in vivo*. Using the genes mobilized in two or three PPARα agonists, hierarchical clustering was performed on 32 compounds stored in our database. In the profiling of an *in vivo* single dose, benzbromarone and aspirin were located in the same cluster of the three PPARα agonists. The clustering of *in vitro* data revealed that benzbromarone, three NSAIDs (aspirin, indomethacin and diclofenac sodium) and valproic acid belonged to the same cluster of PPARα agonists, supporting the reports that benzbromarone,valproic acid and some NSAIDs were reported to be PPARα agonists. Using the genes commonly up-regulated both *in vivo* and *in vitro*, principal component analysis was performed in 32 compounds, and principal component 1 was found to be the convenient parameter to extract PPARα agonist-like compounds from the database.

KEY WORDS: Toxicogenomics, Hepatotoxicity, Peroxisome proliferator

INTRODUCTION

The Toxicogenomics Project is a 5-year collaborative project conducted by the National Institute of Health Sciences (NIHS) and 17 pharmaceutical companies in Japan which started in 2002 (Urushidani and Nagao, 2005). In April 2005, some rearrangements were made and now the project is conducted by NIHS, the National Institute of Biomedical Innovation, and 15 pharmaceutical companies. Its aim is to construct a large-scale toxicology database of transcriptome for prediction of toxicity of new chemical entities in the early stage of drug development. About 150 chemicals, mainly medicinal compounds, have been selected, and the following are examined for each. The *in vivo* test using rats consists of a single administration test (3, 6, 9 and 24 hr with 4 dose levels including vehicle control) as well as a repeated administration test (3, 7, 14

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and 28 days with 4 dose levels including vehicle control) and the data of body weight, general symptoms, histopathological examination of liver and kidney, and blood biochemistry are obtained from each animal. The gene expression in liver (kidney in some cases) is comprehensively analyzed using Affymetrix GeneChip. An *in vitro* test using rat and human hepatocytes is also carried out to accomplish the bridging between the species. By the time the present study was performed, more than 100 chemicals covering wide medication categories had been finished or were ongoing, and the whole data set of 32 compounds had been stored in the database ready for analysis. We have started the analysis with three fibric acids in the database, i.e., clofibrate, WY-14643 and gemfibrozil (ligands of peroxisome proliferator-activated receptor α, PPARα). They have been extensively studied regarding their mechanism of toxicity, as we consider them excellent model cases for evaluating the quality of our database.

MATERIALS AND METHODS

Compounds

All compounds were of the highest grade obtainable from the suppliers listed in Table 1.

In vivo studies

Male Sprague-Dawley rats were purchased from Charles River Japan Inc., (Kanagawa, Japan) at 5-weeks of age. After a 7-day quarantine and acclimatization period, the animals were divided into groups of 5 animals using a computerized stratified random grouping method based on body weight for each age. The animals were individually housed in stainless-steel cages on a 12 hr light/dark cycle. Each animal was allowed free access to water and pellet food (CRF-1, sterilized by radiation, Oriental Yeast Co., Japan). The test compounds were suspended in 0.5% methylcellulose solution or corn oil. Animals were orally administered daily at three dose levels for 1, 3, 7, 14 and 28 days. The highest dose level for each was determined in a 1-week dose-finding study (data not shown), and 1/3 and 1/10 of that were set as middle and low doses, respectively. The dose levels are given in Table 1.

Blood samples were taken at 3, 6, 9, and 24 hr after single dosing and 24 hr after repeated dosing with a needle and a heparinized syringe from the abdominal artery of animals under ether anesthesia. Plasma biochemical assessments were conducted by using COBAS MIRA plus autoanalyzer (Roche Diagnostics, Basel, SZ). After collecting the blood, the animals were euthanized by exsanguination from the abdominal veins and arteries under ether anesthesia. Livers were collected from each animal and weighed, then a portion (about 30 mg) of each left lateral lobe was put into RNAlater® (Ambion, Austin, TX, USA) for expression profiling. The remaining liver samples were fixed in 10% buffered formalin solution for routine histological processing. Paraffin sections were stained with hematoxylin and eosin for histopathological examination. The experimental protocols were reviewed and approved by the Ethics Review Committee for Animal Experimentation of the National Institute of Health Sciences.

In vitro studies

Hepatocytes were isolated from 6-week-old male Sprague-Dawley rats under sodium pentobarbital (120 mg/kg, ip) by a modified two-step collagenase perfusion method. The liver was perfused via the portal vein for 10 min with divalent cation-free EGTA (0.5 mM)-supplemented HEPES buffered Hank's balanced salt solution followed by a 10-min perfusion with HEPES-buffered normal Hank's balanced salt solution containing soybean trypsin inhibitor (Sigma, T-2011, 0.05 g/L) and collagenase (WAKO 034-10533, 0.5 g/L) at a flow rate of 10 - 30 ml/min. Isolated cells were washed three times by 50 g for 1 min to obtain a parenchymal cell-enriched pellet. Hepatocytes were not used when their viability assessed by trypan blue exclusion was lower than 70%. The cells were seeded into collagen-coated six-well plates (BD BioCoat® Collagen I Cellware, BD Bioscience) at a density of 1×10^6 cells/well in 2 ml HMC Bulletkit medium (CAMBREX) supplemented with 10% fetal bovine serum. Following an attachment period of 3 hr, the medium was replaced and kept overnight before drug exposure at 37°C in an atmosphere of 5% CO_2. The test compounds were added to the medium directly or as a 1,000× stock solution in dimethylsulfoxide. The highest concentration of each compound was determined in a pilot test based on cytotoxicity (ca. 20% release of lactate dehydrogenase) and 1/5 and 1/25 of that were set as middle and lowest concentrations (data not shown). After 2, 8 and 24 hr-exposure, the cells were dissolved with RLT buffer (Qiagen) and collected for expression profiling. GeneChip® analysis was performed in a duplicated manner for each time and concentration point.

Expression profiling

The livers were homogenized using Mill Mixer

(Qiagen) and zirconium beads. Total RNA was isolated from the liver homogenate or the hepatocyte lysate using RNeasy kit. Purity of the RNA was checked by gel electrophoresis, and the OD260/280 nm ratio was between 2.0-2.2. Microarray analysis was conducted on 3 out of 5 samples for each group by using GeneChip®RAE230A probe arrays (Affymetrix, Santa Clara, CA, USA), containing 15923 probe sets. The

Table 1.

Compound	Abbreviation	*in vivo*		*in vitro*		Supplier
		Dose (mg/kg)	Vehicle	Concentration (μM)	Vehicle	
acetaminophen	APAP	300, 600, 1000	MC	300, 1000, 3000	Medium	Sigma
allopurinol	APL	15, 50, 150	MC	5.6, 28, 140	DMSO	Sigma
allyl alcohol	AA	3, 10, 30	OIL	4, 20	Medium	Tokyo kasei
alpha-naphthyl-isothiocyanate	ANIT	1.5, 5, 15	OIL	8, 40, 200	DMSO	Kanto chemical
aspirin	ASA	45, 150, 450	MC	120, 600, 3000	DMSO	Wako
benzbromarone	BBr	20, 60, 200	MC	0.6, 3, 15	DMSO	Sigma
bromobenzene	BBZ	30, 100, 300	OIL	8, 40, 200	DMSO	Tokyo kasei
carbamazepine	CBZ	30, 100, 300	MC	12, 60, 300	DMSO	Sigma
carbon tetrachloride	CCL4	10, 30, 100	OIL	300, 1000, 3000	DMSO	Wako
chlorpromazine	CPZ	4.5, 15, 45	MC	0.8, 4, 20	DMSO	Wako
clofibrate	CFB	30, 100, 300	OIL	12, 60, 300	DMSO	Wako
coumarin	CMA	15, 50, 150	OIL	12, 60, 300	DMSO	Tokyo kasei
cyclophosphamide	CPA	1.5, 5, 15	MC	8, 40, 200	Medium	Ardrich
diazepam	DZP	25, 75, 250	MC	5, 25, 125	DMSO	Wako
diclofenac sodium	DFNa	1, 3, 10	MC	16, 80, 400	DMSO	Tokyo kasei
ethionine	ET	25, 80, 250	MC	400, 2000, 10000	Medium	Tokyo kasei
gemfibrozil	GFZ	30, 100, 300	OIL	4, 20, 100	DMSO	Sigma
hexachlorobenzene	HCB	30, 100, 300	OIL	0.6, 3, 15	DMSO	Tokyo kasei
indomethacin	IM	0.5, 1.6, 5	MC	12, 60, 300	DMSO	Sigma
isoniazid	INAH	10, 50, 100	MC	400, 2000, 10000	Medium	Sigma
methapyrilene hydrochloride	MP	10, 30, 100	MC	0.6, 3, 15	Medium	Sigma
methotrexate	MTX	10, 30, 100 (Single) 0.1, 0.3, 1 (Repeated)	MC	20, 100, 500	Medium	Wako
nitrofurantoin	NFT	10, 30, 100	MC	5, 25, 125	DMSO	ICN
omeprazole	OPZ	100, 300, 1000	MC	4.8, 24, 120	DMSO	Wako
phenobarbital	PB	10, 30, 100	MC	300, 1000, 3000	Medium	Sigma
phenylbutazone	PhB	20, 60, 200	MC	16, 80, 400	DMSO	Sigma
phenytoin	PHE	60, 200, 600	MC	2.4, 12, 60	DMSO	Tokyo kasei
propylthiouracil	PTU	10, 30, 100	MC	160, 800, 4000	Medium	Tokyo kasei
rifampicin	RIF	20, 60, 200	MC	2.8, 14, 70	DMSO	Wako
thioacetamide	TAA	4.5, 15, 45	MC	400, 2000, 10000	Medium	Ardrich
valproate sodium	VPA	45, 150, 450	MC	400, 2000, 10000	Medium	Sigma
Wy-14,643	WY	10, 30, 100	OIL	8, 40, 200	DMSO	Tokyo kasei

Supplier information: Wako; Wako pure chemical industries, Osaka, Japan, Tokyo kasei; Tokyo kasei kogyo, Tokyo, Japan. ICN; ICN Biomedical Inc., Aurora, OH, USA, Sigma (St. Louis, MO, USA), Aldrich (St. Louis, MO, USA), Kanto chemical (Tokyo, Japan).

procedure was conducted basically according to the manufacturer's instructions using Superscript Choice System (Invitrogen, Carlsbad, CA, USA) and T7-(dT)24-oligonucleotide primer (Affymetrix) for cDNA synthesis, cDNA Cleanup Module (Affymetrix) for purification, and BioArray High yield RNA Transcript Labeling Kit (Enzo Diagnostics, Farmingdale, NY, USA) for synthesis of biotin-labeled cRNA. Twenty μg of the fragmented cRNA was hybridized to a RAE230A probe array for 18 hr at 45°C at 60 rpm, after which the array was washed and stained by streptavidin-phycoerythrin using Fluidics Station 400 (Affymetrix) and then scanned by Gene Array Scanner (Affymetrix). The data were analyzed by using GeneSpring® version 6.1 (Silicon Genetics, Santa Clara, CA, USA). Expression data were normalized using the mean value (global normalization). Filtering of the data was performed by flags (present or marginal call) in at least half of the samples, as well as the fold change, over the concurrent control value.

Principal components analysis

To test whether profiling on *in vivo* effects of compounds can be estimated from *in vitro* transcriptome data, principal component analysis (PCA) on the data of three time points (24 hr after *in vivo* single dose, 29 day after *in vivo* repeated dose, and 24 hr after *in vitro* exposure) was independently conducted using the expression of 41 genes which changed to the same direction both *in vitro* and *in vivo* (single or repeated dose). The method of gene selection is described in the results section.

For calculation, we took the mean signals from three (*in vivo*) or two (*in vitro*) samples from each dose group and calculated the ratio for each respective control, and subsequently the values were transformed to log ratio to create a matrix. Each row corresponds to a different gene, and each column corresponds to a different condition including three different dose groups of 32 compounds. To compute the principal components, the eigenvalues and their corresponding eigenvectors were calculated from the correlation matrix of conditions. The calculations were done using the R version 2.2.0 (www.r-project.org) statistical environment.

RESULTS

Pathology

Toxicological changes observed in repeated administration of three peroxisome proliferators are summarized in Fig. 1. All three chemicals showed a significant increase in the relative liver to body weight after 4 days or later of administration (Fig. 1A). They also showed a significant decrease in triglyceride after the 4th day of administration, known to be directly related to their activity of PPARα agonist (Fig. 1B). Signs of hepatotoxicity and an increase in ALT activity were observed in the 4th day of WY-14643- and the 29th day of gemfibrozil-treated groups (Fig. 1-C). In pathological examinations, granular degeneration of hepatocytes was noted in all compounds. Furthermore, an increase in hepatocyte proliferation was observed in WY-14643 and gemfibrozil. Treatment with WY-14643 also induced necrosis of hepatocyte and hypertrophy of the bile duct (data not shown).

Analysis of mRNA expression profile

Differentially expressed genes in liver treated with clofibrate, WY-14643 and gemfibrozil were extracted as follows. For *in vivo* study, genes with present call in at least 3 out of 6 samples for each experimental set (3 each from control and treated group) were selected and used for further analysis. In the case of the *in vitro* study, genes with present call in at least 2 out of 4 samples in each experimental set (2 each from control and treated) were selected. Genes showing at least a 1.5 fold increase (or 1/1.5 fold decrease) both in middle and high dose vs. control at $p<0.05$ by Student's *t*-test were extracted for each time point. In the case of *in vitro* study, the same procedure without use of *t*-test was employed. In the next step, the common genes selected in at least 2 out of three peroxisome proliferators for single, repeated, and *in vitro* experiments were extracted. The numbers of genes extracted as above were as follows: the up-regulated genes, 115/195/89 for *in vivo*-single/*in vivo*-repeated/*in vitro*, respectively; and the down-regulated genes, 181/221/38, for *in vivo*-single/*in vivo*-repeated/ *in vitro*, respectively. The overlapping of these genes is depicted in a Venn diagram in Fig. 2.

Comparing the extracted genes between the protocols, 71, 29, and 37 up-regulated genes were in common between the single and repeated administration, between single administration and *in vitro*, and between repeated administration and *in vitro*, respectively. For down-regulated genes, 44 genes were in common between single and repeated administration, whereas no common genes were present between *in vivo* and *in vitro*. These genes are listed in Table 2 (up-regulated) and Table 3 (down-regulated).

Most of the genes categorized in β-oxidation and

fatty acid and cholesterol synthesis, and the peroxisomal protein, were commonly changed both *in vivo* and *in vitro*. In general, genes related to lipid metabolism were commonly up-regulated *in vivo* and *in vitro* except that apolipoprotein A-IV, apolipoprotein M, and lipoprotein-binding protein were down-regulated only *in vivo* (mainly repeated dose). As for genes related to lipid metabolism other than that listed above, most of the up-regulated ones were common between *in vivo* and *in vitro*, whereas the down-regulated ones were only observed *in vivo* (single and repeated dose).

Of the genes related to carbohydrate metabolism, elevation of pyruvate dehydrogenase kinase 4 was observed both *in vivo* (single and repeated dose) and *in vitro*, whereas reduction of "pyruvate kinase liver and RBC" was observed only *in vivo* (mainly repeated dose) and others were increased *in vivo* (single and repeated dose).

The genes classified as cell proliferation were up- or down-regulated only *in vivo* (single and repeated dose). Some genes, including cyclin D1, showed more marked changes in single dose than in repeated dose. The changes of genes categorized in apoptosis were only detectable *in vivo* and their changes were also more prominent in single dose than in repeated dose.

Obvious changes were noted in the genes related to "drug and xenobiotic metabolism" and most of them occurred exclusively *in vivo* (single and repeated dose). The only observable changes in growth factor-related genes were a reduction of the *in vivo* repeated dose. Changes in the expression of genes related to "cellular morphogenesis" as well as the "stress response" were mainly noted *in vivo* (single and repeated dose). Many other genes categorized to "transcription activation and repression", "transporter", "cell adhesion", "immune response", "blood coagulation", "regulation of blood

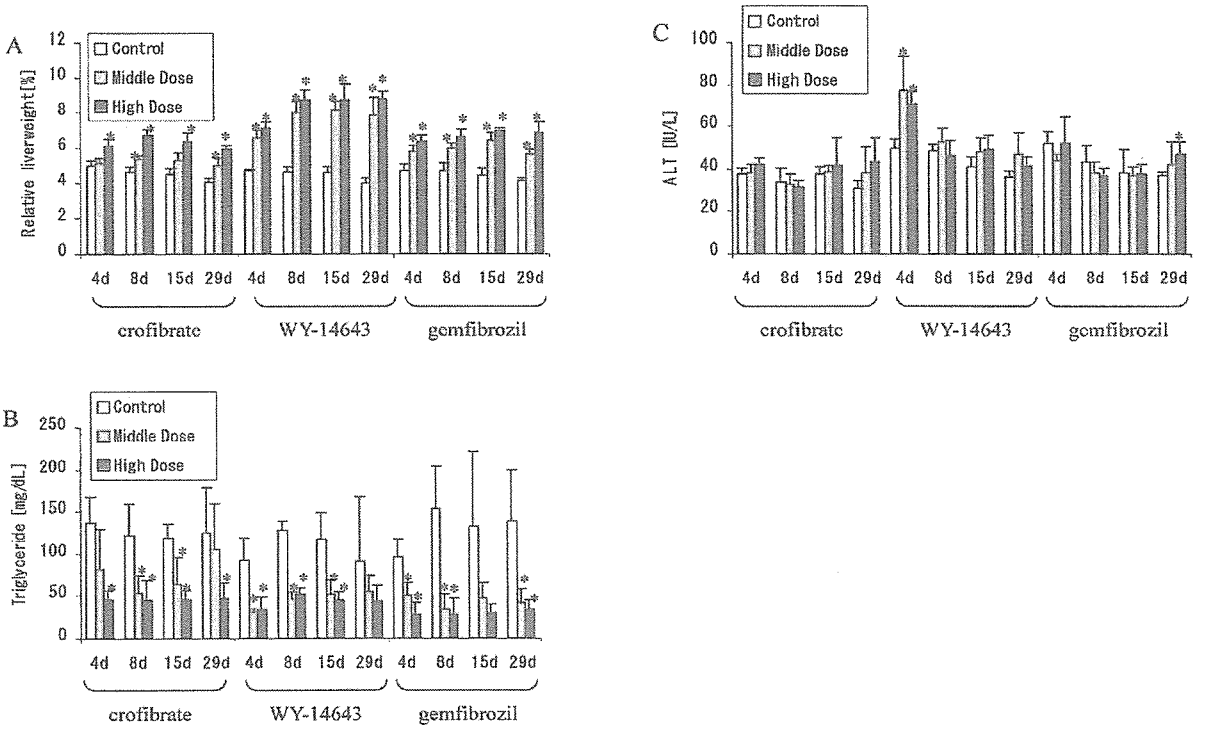

Fig. 1. Effects of clofibrate, WY-14643 and gemfibrozil on relative liver weight (A), plasma triglyceride (B) and plasma alanine amino transferase (ALT; C). Values were obtained 24 hr after repeated administration of each drug for 3, 7, 14, and 28 days. Middle and high dose was 100 and 300 mg/kg for clofibrate, 30 and 100 mg/kg for WY-14643, and 100 and 300 mg/kg for gemfibrozil, respectively. For simplicity, data of the low dose was omitted. *Significantly different from control ($p<0.05$, Student's *t*-test, N=5).

pressure" were found to be down-regulated *in vivo* without any changes *in vitro*.

Hierarchical cluster analysis

Hierarchical clustering (complete linkage method, Euclidean distance) was performed in the 32 compounds stored in our database (the number at that stage of our analysis) using the data of gene expression *in vivo* or *in vitro* (24 hr after middle and high dose). The probe sets used for analysis of *in vivo* data were 36 (up-regulated) and 35 (down-regulated) which showed a more than 1.5 fold change with $p<0.05$ by Student's *t*-test both in middle and high dose of more than 2 compounds out of clofibrate, WY-14643 and gemfibrozil. The probe sets used for analysis of *in vitro* data were 49 (up-regulated) and 6 (down-regulated) which showed a more than 1.5 fold change with $p<0.05$ by Student's *t*-test both in the middle and the high doses of more than 2 out of the three compounds. In clusters of single-dose experiments, benzbromarone and aspirin were classified into the same cluster of the three peroxisome proliferators (Fig. 3). In clusters of the *in vitro* experiments, benzbromarone, three non-steroidal anti-inflammatory drugs (aspirin, indomethacin, and diclofenac sodium), valproic acid, and ANIT were classified into the same cluster of the three peroxisome proliferators (Fig. 4).

PCA-based estimation of PPARα activity

As previously shown in Fig. 2, no common down-regulated genes existed between *in vivo* and *in vitro*, whereas 41 up-regulated genes were found to be common genes (between *in vivo* and *in vitro*) by PPARα agonist treatment. We expected that these genes could be useful for prediction of *in vivo* effects from *in vitro* effects, so PCA analysis was conducted on the 32 compounds using these 41 genes.

A projection on the first two principal components for each condition, together with the contribution rate of the first three components, is shown in Fig. 5. It was obvious from the figure that the first principal component score (PC1) with high contribution (about 60%) for all three experimental sets, was negatively correlated to the dose levels of these PPARα ligands. Therefore, we reasoned that this negative PC1 score could be used to estimate the PPARα activity in general.

Fig. 6 shows the plotting of negative PC1 values from the 32 compounds examined *in vivo* and *in vitro*. In this figure, a clearer comparison of putative PPARα activity between the compounds can be performed. All the PPARα agonists showed high scores both *in vivo* and *in vitro*. Benzbromarone, aspirin and valproic acid again showed high scores both *in vivo* and *in vitro*. It was also easy to pick up the compounds that showed high scores *in vitro* rather than *in vivo*, such as diclofenac and indomethacin.

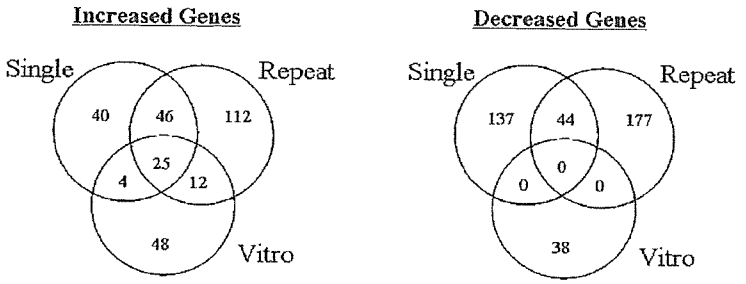

Fig. 2. Venn diagram of extracted genes. Differentially expressed genes in liver treated with clofibrate, WY-14643 and gemfibrozil were extracted as follows. For *in vivo* study (single and repeated) genes with present call in at least 3 out of 6 samples in each experimental set that show at least a 1.5-fold increase (or 1/1.5-fold decrease) for both middle and high dose vs. control (at $p<0.05$ by Student's *t*-test) were extracted for each time point. For the *in vitro* study, genes with present call at least 2 out of 4 samples in each experimental set showing the same criteria as *in vivo* (without using *t*-test) were extracted. The numbers in the diagram show the numbers of extracted genes in each category.

Table 2. Up-regulated genes that are discussed in the text.

	ProbeID	GeneName	Gene Symbol	Vivo		Vitro[c]
				Single[a]	Repeated[b]	
	β-oxidation					
*	1387783_a_at	acetyl-Coenzyme A acyltransferase 1	Acaa1	3.9	5.8	8.4
	1367735_at	acetyl-Coenzyme A dehydrogenase, long-chain	Acadl	1.6	2.0	1.8
*	1367897_at	acyl-Coenzyme A dehydrogenase, very long chain	Acadvl	2.2	2.6	2.8
	1367680_at	acyl-Coenzyme A oxidase 1, palmitoyl	Acox1	2.7	3.1	4.3
*	1367836_at	Carnitine palmitoyltransferase 1 liver	Cpt1a	5.3	7.4	6.6
*	1386946_at	carnitine palmitoyltransferase 1, liver	Cpt1a	4.3	4.6	8.5
*	1386927_at	carnitine palmitoyltransferase 2	Cpt2	5.5	6.2	3.0
*	1367659_s_at	dodecenoyl-coenzyme A delta isomerase	Dci	6.4	7.0	3.6
*	1367777_at	2,4-dienoyl CoA reductase 1, mitochondrial	Decr1	2.4	3.3	3.0
*	1370818_at	2-4-dienoyl-Coenzyme A reductase 2 peroxisomal	Decr2	3.5	3.5	2.5
*	1386885_at	enoyl coenzyme A hydratase 1, peroxisomal	Ech1	8.9	11.6	4.6
*	1368283_at	enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase	Ehhadh	7.1	8.4	83.0
	1370164_at	Hydroxyacyl-Coenzyme A dehydrogenase alpha subunit	Hadha	3.1	4.2	1.9
	1367694_at	hydroxyacyl-Coenzyme A dehydrogenase beta subunit	Hadhb	3.1	3.2	1.5
*	1388210_at	Mitochondrial acyl-CoA thioesterase 1	Mte1	24.1	32.1	3.9
*	1388211_s_at	Mitochondrial acyl-CoA thioesterase 1	Mte1	162.8	175.5	7.6
	Lipid mobilization					
	1370024_at	fatty acid binding protein 7, brain	Fabp7	2.4	3.0	1.5
*	1368150_at	solute carrier family 27 (fatty acid transporter), member 2	Slc27a2	1.7	1.9	3.3
	1398249_at	solute carrier family 25 member 20	Slc25a20	3.7	3.7	2.4
*	1367950_at	solute carrier family 22 member 5	Slc22a5	4.1	5.5	3.1
*	1367689_a_at	Cd36 antigen	Cd36	5.8	16.4	3.7
*	1386901_at	cd36 antigen	Cd36	5.2	9.6	3.8
*	1375247_at	Monoglyceride lipase	Mgll	3.2	7.4	3.3
*	1370831_at	Monoglyceride lipase	Mgll	4.2	7.4	3.5
*	1388644_at	Monoglyceride lipase	Mgll	7.2	9.4	3.9
	1368317_at	aquaporin 7	Aqp7	3.4	22.0	1.3
	Fatty acid and cholesterol synthesis					
	1367857_at	fatty acid desaturase 1	Fads1	1.5	1.8	1.2
	1368453_at	fatty acid desaturase 2	Fads2	2.4	3.8	1.3
*	1370355_at	Stearoyl-Coenzyme A desaturase 1	Scd1	1.6	2.6	2.2
	1372318_at	ELOVL family member 6 elongation of long chain fatty acids yeast	rELO2	4.6	7.2	1.5
*	1388108_at	ELOVL family member 6 elongation of long chain fatty acids yeast	rELO2	5.4	9.6	2.1
	1367767_at	3-hydroxy-3-methylglutaryl CoA lyase	Hmgcl	1.9	3.4	1.5
*	1370310_at	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	Hmgcs2	1.9	1.9	11.5
	Ohter lipid methabolism related					
*	1386880_at	acetyl-Coenzyme A acyltransferase 2	Acaa2	2.0	2.5	4.3
	1373778_at	Acetyl-Coenzyme A carboxylase beta	Acacb	2.0	3.6	1.4

Table 2. Continued.

	ProbeID	GeneName	Gene Symbol	Vivo		Vitro[c]
				Single[a]	Repeated[b]	
	1367763_at	Acetyl-coenzyme A acetyltransferase 1	Acat1	2.2	3.4	2.1
	1368177_at	acyl-CoA synthetase long-chain family member 3	Acsl3	2.4	5.8	3.6
*	1371886_at	Carnitine acetyltransferase	Crat	9.7	17.4	2.5
	1368426_at	carnitine *O*-octanoyltransferase	Crot	2.8	3.1	2.0
	1387183_at	Carnitine *O*-octanoyltransferase	Crot	6.4	6.4	3.6
*	1398250_at	cytosolic acyl-CoA thioesterase 1	Cte1	537.8	971.5	1906.3
	1368206_at	peroxisomal acyl-CoA thioesterase 1	Pte1	3.6	5.8	1.5
	1369485_at	cytoplasmic acetyl-CoA hydrolase	rACH	2.3	2.9	2.7
*	1388924_at	Angiopoietin-like protein 4	Angptl4	5.3	5.6	5.5
	Peroxisomal Protein					
*	1379361_at	Peroxisomal biogenesis factor 11A	Pex11a	16.5	12.1	4.9
*	1387740_at	peroxisomal biogenesis factor 11A	Pex11a	14.8	13.3	7.4
	Carbohydrate methabolism					
*	1369150_at	pyruvate dehydrogenase kinase, isoenzyme 4	Pdk4	9.7	17.9	15.9
	1370509_at	Pyruvate dehydrogenase phosphatase isoenzyme 2	Pdp2	2.3	1.9	1.3
	1369560_at	glycerol-3-phosphate dehydrogenase 1 (soluble)	Gpd1	3.3	3.6	2.8
	1371363_at	Glycerol-3-phosphate dehydrogenase 1 soluble	Gpd1	2.4	2.3	2.2
	1387670_at	Glycerol-3-phosphate dehydrogenase 2	Gpd2	6.2	7.5	1.5
	1370870_at	Malic enzyme 1	Me1	5.0	15.0	1.2
	1370067_at	malic enzyme 1	Me1	5.1	12.0	1.1
	Proliferation					
	1371150_at	Cyclin D1	Ccnd1	2.2	1.7	1.1
	1383075_at	Cyclin D1	Ccnd1	3.7	1.6	1.1
	1368947_at	growth arrest and DNA-damage-inducible 45 alpha	Gadd45a	3.6	4.0	1.3
	1368308_at	myelocytomatosis viral oncogene homolog (avian)	Myc	2.2	5.7	1.2
	1373473_a_at	Nucleosome assembly protein 1-like 1	Nap1l1	3.3	3.9	1.2
	1370826_at	Nucleosome assembly protein 1-like 1	Nap1l1	2.0	2.2	1.1
	1387977_at	Nibrin	Nbn	3.3	2.3	1.3
	1387062_a_at	checkpoint kinase 1 homolog (S. pombe)	Chek1	2.8	2.7	1.3
	1371352_at	High mobility group protein 17	Hmgn2	1.8	1.9	1.5
	1370334_at	Evectin-1	Plekhb1	3.4	2.2	1.4
	1370413_at	Pregnancy specific beta-1-glycoprotein 4	Psg4	1.8	1.7	1.2
	1389403_at	Bone morphogenetic protein 7	Bmp7	1.7	1.9	1.2
	1368143_at	Annexin A7	Anxa7	2.3	3.5	1.0
	Growth factor					
	1369608_at	fibroblast growth factor 16	Fgf16	1.8	1.8	1.2
	Apoptosis					
	1368888_a_at	Reticulon 4	Rtn4	2.5	8.9	1.1
	1388027_a_at	Reticulon 4	Rtn4	3.5	13.3	1.1
	1387521_at	programmed cell death 4	Pdcd4	1.3	2.3	1.2
	Cellular morphogenesis					
*	1368475_at	collagen-like tail subunit of asymmetric acetylcholinesterase	Colq	7.8	7.7	2.4

Table 2. Continued.

	ProbeID	GeneName	Gene Symbol	Vivo		Vitro[c]
				Single[a]	Repeated[b]	
	1368355_at	myosin 5B	Myo5b	4.2	7.0	1.1
	1398281_at	occludin	Ocln	2.7	3.0	1.1
	1367655_at	thymosin, beta 10	Tmsb10	2.4	5.0	1.9
	Drug and xenobiotic methabolism					
	1387296_at	cytochrome P450, family 2, subfamily J, polypeptide 4	Cyp2j4	2.8	4.6	1.6
	1370706_a_at	Cytochrome P450 family 2 subfamily j polypeptide 9	Cyp2j9	1.1	2.0	1.1
*	1370397_at	Cytochrome P450 family 4 subfamily a polypeptide 14	Cyp4a14	3.4	3.3	21.9
*	1368934_at	cytochrome P450, family 4, subfamily b, polypeptide 1	Cyp4b1	6.6	6.1	9.9
	1368738_at	Cytochrome P450 subfamily 11B polypeptide 1	Cyp11b1	2.2	2.4	3.5
	1387123_at	cytochrome P450, family 17, subfamily a, polypeptide 1	Cyp17a1	5.8	8.7	4.2
	1369111_at	alcohol dehydrogenase 4 (class II), pi polypeptide	Adh4	1.4	1.2	10.7
	1370313_at	alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide	Adh7	3.3	4.6	2.8
	1370613_s_at	UDP glycosyltransferase 1 family polypeptide A6	Ugt1a6	1.4	2.0	1.2
	1387759_s_at	UDP glycosyltransferase 1 family polypeptide A6	Ugt1a6	2.1	2.5	1.2
	Stress response					
	1367577_at	heat shock 27kDa protein 1	Hspb1	4.7	8.7	1.2
	1374105_at	Hypoxia induced gene 1	Hig1	2.4	1.9	1.7
	1387636_a_at	P11 protein	Cdtw1	3.3	3.6	1.3
	1368552_at	GrpE-like 1, mitochondrial	Grpel1	1.9	2.3	1.2
	1387023_at	glutathione *S*-transferase, mu type 3 (Yb3)	Gstm3	1.3	2.1	2.0
	1375357_at	Dystonia 1 torsion autosomal dominant torsin A	Dyt1	1.6	1.6	1.1
	Other probes which were used in PCA analysis					
*	1374265_at	NA	NA	4.7	5.6	2.6
*	1374556_at	Similar to spermine oxidase	NA	2.1	3.0	2.8
*	1376076_at	Transcribed locus strongly similar to NP_076005.1 RIKEN cDNA 2310016C08 gene Mus musculus	NA	5.8	5.9	4.4
*	1377037_at	Similar to peroxisomal acyl-CoA thioesterase 2B likely ortholog of mouse peroxisomal acyl-CoA thioesterase 2B	NA	17.5	10.6	7.9
*	1377867_at	Similar to Glutaminyl-peptide cyclotransferase precursor QC Glutaminyl-tRNA cyclotransferase Glutaminyl cyclase	NA	10.5	43.2	6.1
*	1383205_at	Similar to dapper2	NA	3.5	4.2	1.9
*	1388756_at	Similar to RIKEN cDNA 6330579B17 gene	NA	2.5	2.5	2.7
*	1389253_at	Vanin 1	Vnn1	14.7	29.9	5.6
*	1390383_at	Adipose differentiation-related protein	ADRP	4.7	2.4	2.0

[a]: The largest fold change among the single dose studies of CFB, WY and GFZ is shown. [b]: The largest fold change among the repeated dose studies of CFB, WY and GFZ is shown. [c]: The largest fold change among *in vitro* studies of CFB, WY and GFZ is shown. The columns are shaded when the corresponding probe sets appear in Fig. 2. *: Probes which were used in the PCA analysis.

Table 3. Down-regulated genes that are discussed in the text.

Probe ID	Gene Name	Gene Symbol	Vivo		Vitro[c]
			Single[a]	Repeated[b]	
Lipid mobilization					
1368520_at	apolipoprotein A-IV	Apoa4	−4.0	−255.0	−1.2
1386980_at	apolipoprotein M	Apom	−1.4	−5.3	−1.1
1398859_at	Lipoprotein-binding protein	Hdlbp	−1.8	−1.9	−1.1
Other lipid Methabolism related					
1387959_at	Lysophospholipase	LOC246266	−2.2	−3.4	−2.3
1370530_a_at	Phospholipase D1	Pld1	−2.0	−1.8	−1.4
1369526_at	acyl-Coenzyme A dehydrogenase, short/branched chain	Acadsb	−2.0	−2.9	−1.8
Carbohydrate methabolism					
1368651_at	Pyruvate kinase liver and RBC	Pklr	−6.1	−5.8	−1.4
1387263_at	pyruvate kinase, liver and RBC	Pklr	−11.1	−15.7	−3.1
Proliferation					
1387129_at	X-ray repair complementing defective repair in Chinese hamster cells 1	Xrcc1	−1.9	−1.4	−1.2
1372863_at	MYC binding protein 2	Mycbp2	−2.3	−3.0	−1.2
1373291_at	Deleted in liver cancer 1	Dlc1	−2.0	−2.7	−1.3
1373332_at	Casein kinase 1 delta	Csnk1d	−1.9	−1.7	−1.1
1398273_at	ephrin A1	Efna1	−2.1	−1.6	−1.3
Transcription activation and repression					
1371202_a_at	Nuclear factor I B	Nfib	−2.6	−3.2	−1.3
1388167_at	Nuclear factor I B	Nfib	−2.5	−2.8	−1.4
1370946_at	Nuclear factor I X	Nfix	−2.5	−4.1	−1.3
1368221_at	nuclear receptor subfamily 3, group C, member 1	Nr3c1	−2.3	−2.5	−1.3
1369244_at	aryl hydrocarbon receptor nuclear translocator	Arnt	−1.8	−2.2	−1.3
1372601_at	Activating transcription factor 5	Atf5	−1.9	−2.8	−1.1
1367601_at	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	Cited2	−3.1	−2.2	−1.3
1367602_at	Cbp p300-interacting transactivator with Glu Asp-rich carboxy-terminal domain 2	Cited2	−3.0	−2.3	−1.1
1387165_at	v-maf musculoaponeurotic fibrosarcoma (avian) oncogene homolog (c-maf)	Maf	−2.0	−3.7	−1.9
1371781_at	Signal transducer and activator of transcription 3	Stat3	−2.2	−2.6	−1.1
Growth factor					
1370830_at	Epidermal growth factor receptor	Egfr	−5.9	−3.8	−1.3
1373829_at	Fibroblast growth factor receptor 2	Fgfr2	−1.6	−2.5	−1.3
1370941_at	Platelet derived growth factor receptor alpha polypeptide	Pdgfra	−1.5	−2.7	−1.3
1367652_at	insulin-like growth factor binding protein 3	Igfbp3	−1.6	−2.6	−1.3
Apoptosis					
1369941_at	death-associated protein	Dap	−2.0	−2.8	−1.1
1369902_at	Bcl2 modifying factor	Bmf	−12.3	−22.2	−3.4
1370512_at	Androgen receptor-related apoptosis-associated protein CBL27	Cbl27	−2.9	−3.1	−1.3
1371491_at	Notch gene homolog 1 Drosophila	Notch1	−1.9	−1.9	−1.4