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BASIC RESEARCH

A comparison of gene expression in mouse liver and kidney in obstructive cholestasis utilizing high-density oligonucleotide microarray technology

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Abstract

AIM: To assess the effects of obstructive cholestasis on a wider range of gene expression using microarray technology.

METHODS: Male C57BL/6J mice underwent common bile duct ligation (BDL) and were matched with pair-fed sham-operated controls. After 7 d, the animals were sacrificed and total RNA was isolated from livers and kidneys. Equal amounts of RNA from each tissue were pooled for each group and hybridized to Affymetrix GeneChip®MG-U74Av2 containing a total of 12488 probe sets. Data analysis was performed using GeneSpring® 6.0 software. Northern analysis and immunofluorescence were used for validation.

RESULTS: In sham-operated and BDL mice, 44 and 50% of 12488 genes were expressed in livers, whereas 49 and 51% were expressed in kidneys, respectively. Seven days after BDL, 265 liver and 112 kidney genes with GeneOntology annotation were up-regulated and 113 liver and 36 kidney genes were down-regulated in comparison with sham-operated controls. Many genes were commonly regulated in both tissues and metabolism-related genes represented the largest functional group.

CONCLUSION: Following BDL, microarray analysis reveals a broad range of gene alterations in both liver and kidney.

Key words: Bile duct ligation; Cholestasis; Kidney; Liver; Microarray

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INTRODUCTION

Cholestasis, defined as impairment of bile secretion, is a feature of many hepatic disorders and systemic diseases. The recent cloning and functional characterization of different transport proteins for bile acids, organic anions and cations in hepatocytes and cholangiocytes have provided new insights into the molecular biology and physiology of bile formation and have increased understanding of the pathophysiology of cholestatic disorders[1]. Thus it is now established that a number of transport proteins in the basolateral and canalicular hepatocyte membrane undergo adaptive regulation in response to cholestatic liver injury to minimize the hepatic accumulation of toxic substances, such as hydrophobic bile acids[2-4]. Previous studies have indicated that in addition to the liver, adaptive regulation of these transporters in cholestasis also occurs in extrahepatic tissues, including the kidney[5] and the intestine[6]. Other alterations in cholestasis affect hepatic signal transduction[7,8], vesicular transport[7], apoptosis[9,10], metabolism[11], and the structure of the extracellular matrix[12,13].

Given the wide range of signaling, regulatory, and metabolic pathways, structural elements, and transport proteins which may be affected in cholestasis, much

further research will be necessary to more fully understand the extent of these adaptations. High-density DNA microarrays containing thousands of DNA fragments and oligonucleotides are a potentially promising approach to identify additional genes of interest that play a role in this pathophysiologic process. Based on their ability to monitor large numbers of genes at a time, high-density DNA microarrays are a sensitive, time-saving, and efficient tool in determining gene expression and finding regulatory pathways[14].

In the present study, we, therefore, have utilized high-density oligonucleotide microarray technology to screen for gene alterations in the liver and kidney following bile duct ligation (BDL) in mice, an established model of obstructive cholestasis. This study has allowed a comprehensive gene expression profile to be obtained in cholestatic mouse liver and kidney as well as it has highlighted a number of genes whose expression is particularly altered by this process.

MATERIALS AND METHODS

Animals and animal treatment

Male C57BL/6J mice (8-12-wk-old) purchased from Jackson Lab (Bar Harbor, ME) underwent BDL or sham-surgery as previously described[15]. The common bile duct was identified, ligated twice close to the liver hilum immediately below the cystic duct, and then divided between the ligatures. Control mice underwent sham-surgery in which the common bile duct was exposed but not ligated. Since sham-operated mice tend to consume more food than BDL mice and the expression of some genes may be affected by caloric intake, food intake of BDL mice was monitored daily and sham-operated mice were pair-fed so as to receive the same amount of food as BDL mice. Animals were sacrificed 7 d after surgery and livers and kidneys were harvested. The protocol was approved by the Yale Animal Care and Use Committee, and the animals received humane care as outlined in the "Guide for the Care and Use of Laboratory Animals" (NIH publication 86-23, revised 1985).

Isolation of total RNA

Blood-free livers and kidneys were homogenized in GTC solution containing 4 mol/L guanidinium thiocyanate, 25 mmol/L Na-citrate, and 5 g/L N-lauroylsarcosine and subjected to CsCl gradient centrifugation. The recovered total RNA was further purified by phenol/chloroform extraction and ethanol precipitation. The RNA concentration was determined spectrophotometrically and the RNA quality was confirmed by formaldehyde-agarose gel electrophoresis. Equal amounts of liver and kidney, respectively, total RNA from each of four BDL and four sham-operated mice were pooled to minimize inter-animal variations and used for biotin-labeling.

DNA microarray hybridization and analysis

The biotin-labeled RNA from the different groups was hybridized with two replicates for each condition to individual high-density oligonucleotide microarray chips (GeneChip®MG-U74Av2) from Affymetrix (Santa Clara, CA) containing a total of 12488 probe sets. Microarray expression data were generated with Affymetrix Microarray Suite 5.0 software and further analysis was carried out with GeneSpring®6.0 (Silicon Genetics, Redwood City, CA). Raw intensity values from each chip were normalized to the 50th percentile of the measurements taken from that chip to reduce chip-wide variations in intensity. Each gene was normalized to the average measurement of that gene in the respective paired controls to enable comparison of relative changes in gene expression levels between different conditions. Cross-gene error model was active based on the replicates. Comparisons of gene expression data were made between BDL and sham-operated mice. Signal and detection flag from Microarray Suite 5.0 were used as quality controls. Only genes with a minimum signal intensity of 600, a detection flag present in both replicates in at least one of the comparison conditions, and a two-fold and above change in gene expression were used for further analysis. For identification of differentially expressed genes in the different groups, a one-sample *t*-test with a *P* value cutoff of 0.05 was performed to determine if the average log of the ratio of the replicates was significantly different from 1.0, which was the value of the control samples after normalization. Finally, genes were categorized into GeneOntology (GO) and annotated using NetAffx®, an analysis web interface from Affymetrix.

Northern analysis

To validate alterations in gene expression on the microarray, changes in the expression of selected genes were confirmed in aliquots of the same RNA samples used for the microarray by Northern analysis as previously described[16]. The following primers were used for the generation of specific probes: cytochrome P450 7b1 (GenBank accession number U36993): 5'-GAATCTC AGCTTAGAGAGTAAGAG-3' (sense), 5'-TTTGTA CCTAAAGGAGACGGCAG-3' (antisense); organic cation transporter 1 (Oct1) (GenBank accession number U38652): 5'-GCAGCCTGCCTCCTCATGATC-3' (sense), 5'-GGTAAATCGTGTTTTCTTTGGCC-3' (antisense); similar to putative integral membrane transport protein (GenBank accession number AI647632): 5'-TGATTACA AGAAATGTCAAGCAGG-3' (sense), 5'-CCTCTTCCTG ACTCCATCCATG-3' (antisense).

Immunofluorescence

Indirect immunofluorescence with a polyclonal antibody against Oct1[17,18] (dilution 1:100; kindly provided by Prof. Dr. H. Koepsell, Würzburg, Germany) was conducted on liver specimens from sham-operated and BDL mice as previously described[19].

RESULTS

Gene expression profile in mouse liver in obstructive cholestasis

Of the total of 12488 genes on the microarray chip, 44 and 50% were expressed in the livers of sham-operated and BDL mice, respectively. After 7 d of obstructive cholestasis 265 genes with GO annotation were up-regulated and 113 were down-regulated in livers of BDL

mice by a factor of two or more in comparison with sham-operated pair-fed controls. Metabolism-related genes represented the largest functional group among the altered genes after BDL in liver (Table 1). It should be noted that the grouping of the altered genes was primarily done to achieve a clearer arrangement for the reader. Since a considerable number of the encoded proteins have multiple, little characterized or even unknown functions, we want to point out that the classification provided is subject to the personal opinions and emphasis of the authors (Table 1). Upon request, a complete list of the altered genes including genes without GO annotation that are not mentioned here can be obtained from the authors. Alternatively, the complete list of altered genes can be accessed via http://livercenter.yale.edu/datalist.html.

Gene expression profile in mouse kidney in obstructive cholestasis

In the kidneys of sham-operated and BDL mice, 49 and 51% of the 12 488 genes on the microarray chip were expressed, respectively. Seven days after surgery, 112 genes with GO annotation were up-regulated and 36 were down-regulated in the kidneys of BDL mice at least two-fold when compared with the sham-operated pair-fed controls. Thus the number of altered genes in kidney seven days after BDL was considerably smaller than that in liver (148 *vs* 378). Of the 112 GO genes up-regulated in kidney after BDL, 53 were also up-regulated in cholestatic liver. In contrast, of the 36 genes down-regulated in kidney, 7 were also down-regulated in liver (Table 1). What was particularly striking is that many of the most highly up-regulated genes in liver were also the same genes that were most highly up-regulated in kidney, irrespective of their functional class (Table 1). This suggests that both the liver and the kidney may be responding to similar transcriptional signaling molecules in this cholestatic model. For example, the acute phase gene, *serum amyloid A3*, was up-regulated 10.0-fold in liver and 36.1-fold in kidney, the gene encoding *chemokine (C-X-C motif) ligand 1* was increased 9.6-fold in liver and 4.2-fold in kidney, and the gene encoding the transport molecule *lipocalin 2* was up-regulated 13.1-fold in liver and 66.5-fold in kidney. In addition, a number of cell adhesion and extracellular matrix genes were similarly up-regulated in both liver and kidney. However, only one membrane transporter gene was up-regulated in both tissues, the gene encoding the *β1 subunit of the voltage-gated sodium channel* (Table 1). Interestingly, several genes for nucleic acid binding proteins were also highly up-regulated in both liver and kidney including the genes encoding the transcription factors *FBJ osteosarcoma oncogene* (alias *c-Fos*), *CCAAT/enhancer binding protein (C/EBP), delta*, and *activating transcription factor 3*.

In contrast, only seven genes were commonly down-regulated in both liver and kidney. These included the *RIKEN cDNA 1700013L23 gene* and the genes encoding *similar to putative integral membrane transport protein, major urinary protein 2*, *transthyretin*, *cytochrome P450 7b1* (GenBank accession numbers AV141027 and U36993), and *thioether S-methyltransferase*.

Northern analysis of selected genes

Gene expression results from the microarray were confirmed by Northern analysis for selected genes that included *cytochrome P450 7b1*, *organic cation transporter 1 (Oct1; solute carrier family 22, member 1)* and *similar to putative integral membrane transport protein* from aliquots of the RNA samples utilized for the microarray (Figure 1).

Tissue immunofluorescence of Oct1 in liver

Indirect immunofluorescence was performed to illustrate the decreased expression of the organic cation transporter Oct1 in BDL mouse liver. Figures 2A and B demonstrate that the findings are consistent with the microarray and the Northern blot results and corroborate that obstructive cholestasis leads to a down-regulation of Oct1 in mouse liver similarly as demonstrated previously in rat liver following BDL[19,20].

DISCUSSION

Ligation of the common bile duct in rodents is a well-established model of obstructive cholestasis. While most previous studies have been limited to investigations of small numbers of genes and their encoded proteins, we have been able to simultaneously monitor the responses of large numbers of genes in this cholestatic model by using high-density oligonucleotide microarray technology. In contrast to a recent study which investigated gene expression in obstructive cholestasis only in the livers of BDL mice[21], we additionally monitored alterations of gene expression in the kidneys because the kidney is functionally closely linked to the liver and provides an alternative excretory route for cholephilic substances in cholestasis[5]. One of the interesting conclusions from this analysis is the finding that many of the most highly up-regulated genes were shared in both liver and kidney, possibly due to a common response to similar transcriptional signaling molecules in both tissues. The interpretation and discussion of our data is based on the assumption that changes in gene expression lead to changes in protein expression although it is known that changes at the mRNA level do not always result in changes in protein expression in certain time periods[22]. As others have done, we first evaluated the observed changes in gene expression in terms of what is already known about the effects of cholestasis. We then attempted to identify novel regulatory processes that have not yet been investigated[22].

For example, our microarray data largely confirm previous results obtained by conventional determination of transcription in obstructive cholestasis, such as the up-regulation of the canalicular cation transporter *multidrug resistance P-glycoprotein 1a* (*Mdr1a*, Abcb1a)[23] or the down-regulation of the basolateral *sodium-taurocholate cotransporting polypeptide* (*Ntcp*, Slc10a1)[24]. In addition, our gene expression profile obtained from cholestatic liver also closely matched the gene expression profile recently generated by Campbell *et al*[21], although there are a substantial number of additional gene alterations in our data set. This difference can be explained since Campbell

Table 1 Fold increase/decrease in liver and kidney, GenBank accession number, and classification of altered genes in mice 7 d after bile duct ligation in comparison with pair-fed sham-operated controls

Liver	Kidney	Accession number	Description
Cell death			
3.6		AF011428	CD5 antigen-like
3.2		AW046181	Serum/glucocorticoid regulated kinase
2.6		AV373612	Bcl2-associated athanogene 3
-2.6		X65128	Growth arrest specific 1
-2.7		AA770736	Induced in fatty liver dystrophy 2
	2.8	M61737	Fat-specific gene 27
	2.7	AV003873	Clusterin
	2.3	D14077	Clusterin
	-7.0	AJ000062	Deoxyribonuclease I
Stress response			
10.0	36.1	X03505	Serum amyloid A 3
5.0		M13521	Serum amyloid A 2
2.8		M12566	Orosomucoid 2
2.5		J04633	Heat shock protein 1, alpha
2.5		X60676	Serine (or cysteine) proteinase inhibitor, clade H, member 1
	7.4	M96827	Haptoglobin
	-2.2	Z36774	Serine (or cysteine) proteinase inhibitor, clade F, member 2
Immune and inflammatory response			
31.9	5.0	M94584	Chitinase 3-like 3
17.3		M19681	Chemokine (C-C motif) ligand 2
10.7		X53798	Chemokine (C-X-C motif) ligand 2
9.6	4.2	J04596	Chemokine (C-X-C motif) ligand 1
9.4		AW120786	Chemokine (C-X-C motif) ligand 14
9.2		U18424	Macrophage receptor with collagenous structure
8.4		AV370035	Chemokine (C-C motif) receptor 5
7.1		U56819	Chemokine (C-C) receptor 2
6.9	5.5	AF002719	Secretory leukocyte protease inhibitor
6.1		M18237	Immunoglobulin kappa chain variable 8 (V8)
5.9		U34277	Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)
5.4		M83218	S100 calcium binding protein A8 (calgranulin A)
3.6	3.7	X04673	Adipsin
3.6		AI844520	Interferon gamma inducible protein 30
3.6		AF081789	Complement component 1, q subcomponent, receptor 1
3.5		X12905	Properdin factor, complement
3.3		L32838	Interleukin 1 receptor antagonist
3.2		U96752	Histocompatibility 2, Q region locus 1
3.2	3.4	M22531	Complement component 1, q subcomponent, beta polypeptide
3.2		X15591	Cytotoxic T lymphocyte-associated protein 2 alpha
3.1		X63782	Lymphocyte antigen 6 complex, locus D
2.9		M58004	Chemokine (C-C motif) ligand 6
2.9		M21932	Histocompatibility 2, class II antigen A, beta 1
2.9		U16985	Lymphotoxin B
2.9		M14639	Interleukin 1 alpha
2.9	4.2	X58861	Complement component 1, q subcomponent, alpha polypeptide
2.8		U77461	Complement component 3a receptor 1
2.8		M31314	Fc receptor, IgG, high affinity I
2.8		X52643	Histocompatibility 2, class II antigen A, alpha
2.7		AB007599	Lymphocyte antigen 86
2.6		X15592	Cytotoxic T lymphocyte-associated protein 2 beta
2.6		AF013715	Periplakin
2.6	2.1	X66295	Complement component 1, q subcomponent, gamma polypeptide
2.5	3.1	L38444	T-cell specific GTPase
2.5		AA596710	Leukotriene B4 12-hydroxydehydrogenase
2.5		AB019505	Interleukin 18 binding protein
2.4		M34815	Chemokine (C-X-C motif) ligand 9
2.4	2.0	X00496	Ia-associated invariant chain
2.4	2.8	AJ007970	Guanylate nucleotide binding protein 2
2.3		D86382	Allograft inflammatory factor 1
2.3		L22181	Formyl peptide receptor 1
2.2		AF038149	Paired-Ig-like receptor B
2.1		AW060457	Immunoglobulin superfamily, member 7
2.1		U03003	Defensin related cryptdin 6
2.0		M29855	Colony stimulating factor 2 receptor, beta 2, low-affinity (granulocyte-macrophage)
2.0		AF003525	Defensin beta 1
-2.8		M29007	Complement component factor h

Liver	Kidney	Accession number	Description
-4.6		L22977	X-linked lymphocyte-regulated 3b
	13.6	U47810	Complement component factor i
	6.5	K02782	Complement component 3
	6.0	X06454	Complement component 4 (within H-2S)
	4.2	AI563854	Tumor-associated calcium signal transducer 2
	3.8	AA986114	T-cell immunoglobulin and mucin domain containing 2
	3.5	U49513	Chemokine (C-C motif) ligand 9
	3.0	Y08830	Tumor-associated calcium signal transducer 2
	2.4	AA270365	Cytokine receptor-like factor 1
	2.2	AI152789	Sema domain, immunoglobulin domain (Ig), and GPI membrane anchor, (semaphorin) 7A
Signal transduction			
12.9		U88328	Suppressor of cytokine signaling 3
5.4		Z48043	Coagulation factor II (thrombin) receptor-like 1
5.0	2.7	M14044	Annexin A2
4.0	2.2	AJ001633	Annexin A3
3.6		AI641895	Shroom
3.6		U90715	Coxsackievirus and adenovirus receptor
3.6		AI317205	Mitogen activated protein kinase kinase kinase 1
3.4		J03023	Hemopoietic cell kinase
3.1		AW209098	IQ motif containing GTPase activating protein 1
3.0		AW049806	RIKEN cDNA 1700093E07 gene
3.0		X84797	Hematopoietic cell specific Lyn substrate 1
2.9	3.0	AB015978	Oncostatin M receptor
2.6		X93328	EGF-like module containing, mucin-like, hormone receptor-like sequence 1
2.3		D63423	Annexin A5
2.3	2.1	M69260	Annexin A1
2.2		M68902	Hemopoietic cell phosphatase
2.2		AF020313	Amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein
2.1	3.6	AV374868	Suppressor of cytokine signaling 3
2.1		AA608387	Interleukin 13 receptor, alpha 1
-2.0		AC002397	Gene rich cluster, C9 gene
-2.0		AW125649	Guanine nucleotide binding protein, alpha 12
-2.4		AI839138	Thioredoxin interacting protein
-2.6		AV321519	Sorting nexin 17
-2.7		AA691492	RIKEN cDNA D530020C15 gene
-5.6		D17444	Leukemia inhibitory factor receptor
-11.7		AV349152	Regulator of G-protein signaling 16
-15.3		U94828	Regulator of G-protein signaling 16
	2.3	AF084466	Ras-related associated with diabetes
	2.1	AF009246	RAS, dexamethasone-induced 1
	-2.1	AF054623	Frizzled homolog 1 (Drosophila)
	-2.2	D85605	Cholecystokinin A receptor
	-2.2	AI834895	Membrane progestin receptor alpha
	-2.3	AW046638	PDZ domain containing 1
Cell growth and maintenance			
8.7		M33960	Serine (or cysteine) proteinase inhibitor, clade E, member 1
6.9		X98471	Epithelial membrane protein 1
5.8	4.9	X66449	S100 calcium binding protein A6 (calcyclin)
5.4		AF055638	Growth arrest and DNA-damage-inducible 45 gamma
5.1		M17298	Nerve growth factor, beta
3.6		AI849928	Cyclin D1
3.5		X59846	Growth arrest specific 6
3.2		M64292	B-cell translocation gene 2, anti-proliferative
3.2		AW048937	Cyclin-dependent kinase inhibitor 1A (P21)
3.1		AF009366	Neural precursor cell expressed, developmentally down-regulated gene 9
2.7		M21019	Harvey rat sarcoma oncogene, subgroup R
2.7		X06368	Colony-stimulating factor 1 receptor
2.2		X81579	Insulin-like growth factor binding protein 1
2.1		AI851454	Cysteine rich protein 2
2.0		AA529583	Mortality factor 4 like 2
-2.1		X95280	G0/G1 switch gene 2
-2.2		M31680	Growth hormone receptor
-2.5		U15012	Growth hormone receptor
	3.4	AI852641	Nuclear protein 1
	2.8	M34094	Midkine
	2.8	AF058798	Stratifin
	2.1	X81580	Insulin-like growth factor binding protein 2
Protein biosynthesis			
2.3		Y11460	Integrin beta 4 binding protein

Liver	Kidney	Accession number	Description
2.1		NM_011690	Valyl-tRNA synthetase 2
-2.0		AV055186	Ribosomal protein, large, P1
Proteolysis and protein degradation			
7.6	2.4	X61232	Carboxypeptidase E
6.1		AW060527	Ubiquitin-conjugating enzyme E2 variant 2
4.0		AJ000990	Legumain
4.0	5.0	AJ223208	Cathepsin S
3.7		AL078630	Ubiquitin D
2.0		U35833	Ubiquitin-like 1 (sentrin) activating enzyme E1B
-2.2		AI844932	F-box only protein 8
-2.4		L21221	Proprotein convertase subtilisin/kexin type 4
-2.6		AV359471	Ubiquitin specific protease 15
	-2.2	J04946	Angiotensin converting enzyme
	-2.5	L15193	Meprin 1 beta
Protein amino acid phosphorylation and dephosphorylation			
3.2		D89728	Serine/threonine kinase 10
3.2		M97590	Protein tyrosine phosphatase, non-receptor type 1
2.6		D37801	Protein tyrosine phosphatase, non-receptor type 21
2.0		X61940	Dual specificity phosphatase 1
-2.1		L31783	Uridine monophosphate kinase
Cell adhesion and extracellular matrix			
24.7		L36244	Matrix metalloproteinase 7
20.6		U43525	Proteinase 3
10.7		M82831	Matrix metalloproteinase 12
10.4	2.3	D00613	Matrix gamma-carboxyglutamate (gla) protein
9.0	3.1	X16834	Lectin, galactose binding, soluble 3
8.9		L02918	Procollagen, type V, alpha 2
8.1		M31039	Integrin beta 2
7.2		M62470	Thrombospondin 1
6.2		X13986	Secreted phosphoprotein 1
5.9	2.4	U03419	Procollagen, type I, alpha 1
5.8		D14010	Regenerating islet-derived 1
4.9	2.1	X52046	Procollagen, type III, alpha 1
4.7	2.5	M90551	Intercellular adhesion molecule
4.2		X58251	Procollagen, type I, alpha 2
4.0		L57509	Discoidin domain receptor family, member 1
3.4	4.3	U12884	Vascular cell adhesion molecule 1
3.2	3.3	M84487	Vascular cell adhesion molecule 1
3.2		L29454	Fibrillin 1
3.0		Z22532	Syndecan 1
2.9		M23552	Serum amyloid P-component
2.8		X04017	Secreted acidic cysteine rich glycoprotein
2.7		M38337	Milk fat globule-EGF factor 8 protein
2.7		AA763466	Procollagen, type I, alpha 1
2.5		AA919594	Elastin
2.5		M70642	Connective tissue growth factor
2.5		D88577	C-type (calcium dependent, carbohydrate recognition domain) lectin, superfamily member 13
2.3		M15832	Procollagen, type IV, alpha 1
2.2		X59990	Catenin alpha 1
2.2		U82624	Amyloid beta (A4) precursor protein
2.1		X53928	Biglycan
2.1		U89915	F11 receptor
2.0		X04647	Procollagen, type IV, alpha 2
2.0	2.2	V00755	Tissue inhibitor of metalloproteinase 1
2.0		X91144	Selectin, platelet (p-selectin) ligand
-2.1		AF101164	CEA-related cell adhesion molecule 2
-2.2		AI840501	Camello-like 1
	2.1	L19932	Transforming growth factor, beta induced
Cytoskeleton and structural elements			
5.0	7.3	M36120	Keratin complex 1, acidic, gene 19
4.8		V00830	Keratin complex 1, acidic, gene 10
4.5		U38967	Thymosin, beta 4, X chromosome
3.6	2.3	AI852553	Thymosin, beta 10
3.6		U42471	Wiskott-Aldrich syndrome homolog (human)
3.4		U29539	Lysosomal-associated protein transmembrane 5
3.4		M22479	Tropomyosin 1, alpha
3.2	2.2	M28739	Tubulin, beta 2
3.2		AW215736	RIKEN cDNA 2310057H16 gene

Liver	Kidney	Accession number	Description
3.2	4.5	M22832	Keratin complex 1, acidic, gene 18
3.1		AI505453	Myosin heavy chain IX
3.0		X15662	Keratin complex 2, basic, gene 8
2.8		X60671	Villin 2
2.7		D49733	Lamin A
2.7		AW125446	Golgi phosphoprotein 2
2.6		AW050256	Tubulin, beta 3
2.6		AI839417	Moesin
2.4		AW125698	Myosin heavy chain IX
2.4		AW212775	Actin-related protein 2/3 complex, subunit 1B
2.4		AV356071	Lysosomal-associated protein transmembrane 5
2.2		M28727	Tubulin, alpha 2
2.2		AI835858	Tropomyosin 4
2.2		M12347	Actin, alpha 1, skeletal muscle
2.1		D88793	Cysteine and glycine-rich protein 1
2.1		AF020185	Dynein, cytoplasmic, light chain 1
2.1		AI837625	Cysteine and glycine-rich protein 1
2.1	3.2	X54511	Capping protein (actin filament), gelsolin-like
2.0	2.1	X04663	Tubulin, beta 5
2.0		AI841606	Actin-binding LIM protein 1
2.0		M21495	Actin, gamma, cytoplasmic
2.0		AI849152	Clathrin, light polypeptide (Lcb)
2.0		M60474	Myristoylated alanine rich protein kinase C substrate
-2.2		AW123904	Gamma-aminobutyric acid (GABA(A)) receptor-associated protein-like 1
	3.3	AB000713	Caudin 4
	2.6	AA755126	Keratin complex 2, basic, gene 7
	2.6	AF087825	Claudin 7
	2.3	AI195392	Actinin, alpha 1
Transport			
13.1	66.5	X81627	Lipocalin 2
12.1	2.0	L48687	Sodium channel, voltage-gated, type I, beta polypeptide
7.2		U04827	Fatty acid binding protein 7, brain
3.7		M24417	ATP-binding cassette, sub-family B (MDR/TAP), member 1A
3.4		AI842825	Glycolipid transfer protein
3.2		NM_033444	Chloride intracellular channel 1
3.2		X99347	Lipopolysaccharide binding protein
2.9		L13732	Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1
2.8		U72680	FXYD domain-containing ion transport regulator 5
2.8		X60367	Retinol binding protein 1, cellular
2.5		U27315	Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4
2.4		AI842065	Expressed sequence AW538430
2.3		AI849583	RIKEN cDNA 6330416G13 gene
2.3		AI852578	Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2
2.1		D87661	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide
2.1		U28960	Phospholipid transfer protein
-2.0	-2.2	AA670737	RIKEN cDNA 1700013L23 gene
-2.1		M16360	Major urinary protein 5
-2.1		AF072757	Solute carrier family 27 (fatty acid transporter), member 2
-2.1		M16358	Major urinary protein 4
-2.1		L28836	ATP-binding cassette, sub-family D (ALD), member 3
-2.3		U38652	Solute carrier family 22 (organic cation transporter), member 1
-2.3		M16357	Major urinary protein 3
-2.3		U95131	Solute carrier family 10 (sodium/bile acid cotransporter family), member 1
-2.3	-2.3	AV355798	Major urinary protein 2
-2.3		AV104178	Serine (or cysteine) proteinase inhibitor, clade A, member 6
-2.4		U95132	Solute carrier family 10 (sodium/bile acid cotransporter family), member 1
-2.4		M16359	Major urinary protein 1
-2.4		AB028737	ATP-binding cassette, sub-family C (CFTR/MRP), member 6
-2.6	-2.0	D00073	Transthyretin
-2.9		AJ011080	Afamin
-3.8	-3.8	AI647632	Similar to putative integral membrane transport protein
-4.4		AI255271	Major urinary protein 2
-6.4		Y14660	Fatty acid binding protein 1, liver
-6.6		X70533	Serine (or cysteine) proteinase inhibitor, clade A, member 6
	4.0	M55413	Group-specific component
	2.7	AF047838	Chloride channel calcium activated 1
	2.7	AI849587	Protein distantly related to the gamma subunit family
	2.6	D00466	Apolipoprotein E
	2.4	AI661431	Aquaporin 2
	2.3	AI197481	Amiloride binding protein 1 (amine oxidase, copper-containing)
	-2.1	AI606956	Solute carrier family 2 (facilitated glucose transporter), member 5

Liver	Kidney	Accession number	Description
	-2.1	AW122706	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 8
	-2.2	AI120514	Solute carrier family 26 (sulfate transporter), member 1
	-2.3	AI837530	Solute carrier family 9 (sodium/hydrogen exchanger), member 8
Cell surface markers and membrane proteins			
12.9		X13333	CD14 antigen
6.7		X97227	CD53 antigen
6.4		M65027	Glycoprotein 49 A
5.9		D16432	CD63 antigen
5.3	2.3	AW209486	Prostate stem cell antigen
5.0	3.4	AF024637	TYRO protein tyrosine kinase binding protein
3.6		M58661	CD24a antigen
3.3		U37438	Deleted in malignant brain tumors 1
3.3		M55561	CD52 antigen
3.3		AI854863	RIKEN cDNA 1200015A22 gene
3.0		AF039663	Prominin 1
2.7		AI849180	Integral membrane protein 2C
2.6		AI787183	RIKEN cDNA 0610011I04 gene
2.5	2.5	X68273	CD68 antigen
2.2		AB031386	RIKEN cDNA 1810009M01 gene
2.0		L11332	CD38 antigen
-2.5		AI843959	RIKEN cDNA 5730403B10 gene
	3.3	AW261569	RIKEN cDNA D630035O19 gene
	2.0	AI847784	CD34 antigen
	-2.5	L23108	CD36 antigen
Transcription factors and nucleic acid binding proteins			
8.1	7.2	V00727	FBJ osteosarcoma oncogene
6.2	3.6	AW124113	Brain abundant, membrane attached signal protein 1
4.9	2.2	AW049031	Core promoter element binding protein
4.0		M90397	B-cell leukemia/lymphoma 3
3.8		M31885	Inhibitor of DNA binding 1
3.8	3.0	AA614971	Molecule possessing ankyrin-repeats induced by lipopolysaccharide
3.2	3.6	X61800	CCAAT/enhancer binding protein (C/EBP), delta
3.2		AF017258	Ribonuclease, RNase A family, 2
2.7		AB016424	RNA binding motif protein 3
2.6	2.4	U19118	Activating transcription factor 3
2.5		AF016294	E74-like factor 3
2.4		L03215	SFFV proviral integration 1
2.3		AI642098	RIKEN cDNA 4921515A04 gene
2.3		U20735	Jun-B oncogene
2.2		M60523	Inhibitor of DNA binding 3
2.2		D26089	Minichromosome maintenance deficient 4 homolog (S. cerevisiae)
2.1	2.2	U20344	Kruppel-like factor 4 (gut)
-2.0		U36799	Retinoblastoma-like 2
-2.0		AF038995	DEAD (Asp-Glu-Ala-Asp) box polypeptide 6
-2.1		L20450	Zinc finger protein 97
-2.1		X77602	Upstream transcription factor 2
-2.2		AF064088	TGFB inducible early growth response 1
-2.2		U95945	One cut domain, family member 1
-2.4		U62674	Histone 2, H2aa1
-2.4		AA002843	Nuclear factor I/X
-2.7		AI834950	Nuclear receptor subfamily 1, group D, member 1
-2.8		AW047343	D site albumin promoter binding protein
-3.4		X57638	Peroxisome proliferator activated receptor alpha
	4.7	AI840339	Ribonuclease, RNase A family 4
	2.7	M28845	Early growth response 1
	2.3	X16995	Nuclear receptor subfamily 4, group A, member 1
Metabolism			
8.5		M13018	Cysteine-rich protein 1 (intestinal)
6.1		AV327760	Stearoyl-Coenzyme A desaturase 2
6.0	37.5	X51547	P lysozyme structural
5.9		AW046124	Cytochrome b-245, alpha polypeptide
5.1	4.6	M21050	Lysozyme
4.9		X97047	Pyruvate kinase, muscle
4.2		AV368209	Pyruvate kinase, muscle
4.1		U43384	Cytochrome b-245, beta polypeptide
4.1		AA726364	Lipoprotein lipase
4.0		AI846517	Cytochrome b-561
4.0		AI854821	RIKEN cDNA 0610041P13 gene
3.8		U13705	Glutathione peroxidase 3
3.8		U12961	NAD(P)H dehydrogenase, quinone 1

Liver	Kidney	Accession number	Description
3.6	2.1	M26270	Stearoyl-Coenzyme A desaturase 2
3.6		M34141	Prostaglandin-endoperoxide synthase 1
3.6		X07888	3-hydroxy-3-methylglutaryl-Coenzyme A reductase
3.5		M31775	Cytochrome b-245, alpha polypeptide
3.5		AI847162	RIKEN cDNA 1300017C10 gene
3.4		U87147	Flavin containing monooxygenase 3
3.4		AA690863	ATPase, class VI, type 11A
3.4		J04696	Glutathione S-transferase, mu 2
3.3		X56824	Heme oxygenase (decycling) 1
3.0		AJ238894	Acyl-Coenzyme A thioesterase 3, mitochondrial
3.0		D42048	Squalene epoxidase
2.9		AW060927	Lanosterol synthase
2.8		J03953	Glutathione S-transferase, mu 3
2.4		U49350	Cytidine 5'-triphosphate synthase
2.4		AI594518	Chitinase, acidic
2.2		J02980	Alkaline phosphatase 2, liver
2.2		U27455	Serine palmitoyltransferase, long chain base subunit 2
2.2		AI327450	Phospholipase A2, group IB, pancreas
2.2		AF077527	Syndecan binding protein
2.2		AA710635	Colipase, pancreatic
2.1		M62766	3-hydroxy-3-methylglutaryl-Coenzyme A reductase
2.1		AW049778	Mevalonate (diphospho) decarboxylase
2.1		AF057368	7-dehydrocholesterol reductase
2.0		U49385	Cytidine 5'-triphosphate synthase 2
-2.0		AW123316	Methylcrotonoyl-Coenzyme A carboxylase 1 (alpha)
-2.0		AA824102	Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7
-2.0		AF098009	Fatty acid amide hydrolase
-2.1		AV216468	Expressed in non-metastatic cells 1, protein
-2.1		L42996	Dihydrolipoamide branched chain transacylase E2
-2.1		AI846934	Lipin 1
-2.1		AV071102	Cytochrome c oxidase, subunit VIc
-2.1		AI839995	Sarcosine dehydrogenase
-2.1		X61397	Carbonic anhydrase 8
-2.1		AF022894	Sulfotransferase family 1B, member 1
-2.1		U24493	Tryptophan 2,3-dioxygenase
-2.2		AA675075	Proline dehydrogenase (oxidase) 2
-2.2		AV276715	Aldehyde dehydrogenase family 3, subfamily A2
-2.3		L11333	Esterase 31
-2.3		L11163	Acyl-Coenzyme A dehydrogenase, short chain
-2.3		AI840013	Peroxisomal delta3, delta2-enoyl-Coenzyme A isomerase
-2.4		M27347	Elastase 1, pancreatic
-2.4		U32684	Paraoxonase 1
-2.4		M77015	Hydroxysteroid dehydrogenase-3, delta<5>-3-beta
-2.4		AF030343	Enoyl coenzyme A hydratase 1, peroxisomal
-2.4		AF047542	Cytochrome P450, family 2, subfamily c, polypeptide 37
-2.4		AF047727	Cytochrome P450, family 2, subfamily c, polypeptide 40
-2.4		Z14050	Dodecenoyl-Coenzyme A delta isomerase (3,2 trans-enoyl-Coenyme A isomerase)
-2.5		D17674	Cytochrome P450, family 2, subfamily c, polypeptide 29
-2.5		AI844846	2,4-dienoyl CoA reductase 1, mitochondrial
-2.6		X83202	Hydroxysteroid 11-beta dehydrogenase 1
-2.6		U14390	Aldehyde dehydrogenase family 3, subfamily A2
-2.6		AW012588	3-ketoacyl-CoA thiolase B
-2.7		AI530403	Acetyl-Coenzyme A acyltransferase 1
-2.7		X51971	Carbonic anhydrase 5a, mitochondrial
-2.7		AF031170	Hydroxysteroid dehydrogenase-6, delta<5>-3-beta
-2.8		AI266885	RIKEN cDNA 1700124F02 gene
-2.9		AF030513	Retinol dehydrogenase 6
-3.0		U15977	Fatty acid Coenzyme A ligase, long chain 2
-3.0		X04283	Cytochrome P450, family 1, subfamily a, polypeptide 2
-3.4		X63349	Dopachrome tautomerase
-3.6		M15268	Aminolevulinic acid synthase 2, erythroid
-4.0		D63764	Pyruvate kinase liver and red blood cell
-4.1		AF026074	Sulfotransferase related gene X1
-4.1		Y14004	Cytosolic acyl-CoA thioesterase 1
-4.3	-3.8	AV141027	Cytochrome P450, family 7, subfamily b, polypeptide 1
-4.3		AJ132098	Vanin 1
-4.6		AW226939	Carboxylesterase 3
-5.1		U49861	Deiodinase, iodothyronine, type I
-6.1	-3.4	U36993	Cytochrome P450, family 7, subfamily b, polypeptide 1
-6.4		U12791	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2
-6.6	-2.8	M88694	Thioether S-methyltransferase
-6.9		AF090317	Cytochrome P450, family 8, subfamily b, polypeptide 1

Liver	Kidney	Accession number	Description
-14.0		AB018421	Cytochrome P450, family 4, subfamily a, polypeptide 10
-17.9		Y11638	Cytochrome P450, family 4, subfamily a, polypeptide 14
-28.0	2.5	AJ006474	Carbonic anhydrase 3
-37.8		M21855	Cytochrome P450, family 2, subfamily b, polypeptide 9
-93.8		L41519	Hydroxysteroid dehydrogenase-5, delta<5>-3-beta
	6.4	AB006034	Cytochrome P450, family 27, subfamily b, polypeptide 1
	4.8	U49430	Ceruloplasmin
	3.0	AF032466	Arginase type II
	2.9	J05277	Hexokinase 1
	2.6	Z19521	Low density lipoprotein receptor
	2.6	U04204	Aldo-keto reductase family 1, member B8
	2.6	AI848668	Sterol-C4-methyl oxidase-like
	2.6	U31966	Carbonyl reductase 1
	2.5	U49915	Adipocyte complement related protein
	2.4	AW124337	Microsomal glutathione S-transferase 1
	2.3	U18975	UDP-N-acetyl-alpha-D-galactosamine:(N-acetylneuraminyl)-galactosylglucosylceramide-beta-1, 4-N-acetylgalactosaminyltransferase
	2.2	L06047	Glutathione S-transferase, alpha 4
	2.1	AA718169	Resistin
	2.1	D88994	AMP deaminase 3
	2.0	AA710564	N-acetylneuraminate pyruvate lyase
	-2.0	U19265	Glucosaminyl (N-acetyl) transferase 1, core 2
	-2.0	AB005450	Carbonic anhydrase 14
	-2.1	M75886	Hydroxysteroid dehydrogenase-2, delta<5>-3-beta
	-2.1	AB020239	Adenylate kinase 4
	-2.2	U48896	UDP-glucuronosyltransferase 8
	-2.2	U89352	Lysophospholipase 1
	-2.2	M12330	Ornithine decarboxylase, structural
	-2.3	U90535	Flavin containing monooxygenase 5
	-2.3	AF009605	Phosphoenolpyruvate carboxykinase 1, cytosolic
	-2.3	AB015426	Fucosyltransferase 9
	-2.4	U89906	Alpha-methylacyl-CoA racemase
	-2.5	AA840463	Lysophospholipase 1
	-3.5	X06358	UDP-glucuronosyltransferase 2 family, member 5
Other			
18.6	8.2	U69488	G7e protein
10.9	2.4	X67644	Immediate early response 3
7.6		U78770	Trefoil factor 2 (spasmolytic protein 1)
2.9		AI117936	Mus musculus 11 days embryo head cDNA, RIKEN full-length enriched library, clone: 6230409N14 product:unknown EST, full insert sequence
2.7		AI852545	Transgelin 2
2.6	2.0	AW121336	RIKEN cDNA 1600023A02 gene
2.6		X58196	H19 fetal liver mRNA
2.5	2.2	U25844	Serine (or cysteine) proteinase inhibitor, clade B, member 6a
2.4		AA980164	SPARC related modular calcium binding 2
2.4		D38410	Trefoil factor 3, intestinal
2.2		U44426	Tumor protein D52
2.1		U22262	Apolipoprotein B editing complex 1
2.1	4.6	AW230891	Leucine-rich alpha-2-glycoprotein
-2.1		U32170	Regucalcin
-2.3		AI854813	Mus musculus 3 days neonate thymus cDNA, RIKEN full-length enriched library, clone: A630086H07 product:RAS GTPASE-ACTIVATING-LIKE PROTEIN IQGAP2 homolog [Homo sapiens], full insert sequence
-2.3		AW049373	RIKEN cDNA 2310016A09 gene
-2.8		AI326963	Angiopoietin-like 4
-3.0		AA797604	Angiopoietin-like 4
-3.4	10.7	AB011030	Protein related to DAN and cerberus
	9.8	AA986050	Fibrinogen, gamma polypeptide
	6.8	M64086	Serine (or cysteine) proteinase inhibitor, clade A, member 3N
	5.0	AA880891	Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 10
	2.6	AI876446	Fibrinogen, alpha polypeptide
	2.1	X61597	Serine (or cysteine) proteinase inhibitor, clade A, member 3C
	2.0	X59520	Cholecystokinin
	2.0	D13003	Reticulocalbin
	-2.1	AI314227	RIKEN cDNA 0610006H10 gene
	-2.2	AW122036	Mus musculus transcribed sequence with strong similarity to protein ref:NP_005351.2 (H.sapiens) v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian); v-maf musculoaponeurotic fibrosarcoma (avian) oncogene homolog; Avian musculoaponeurotic fibrosarcoma (MAF) protooncogene [Homo sapiens]
	-3.4	M93264	Pregnancy zone protein

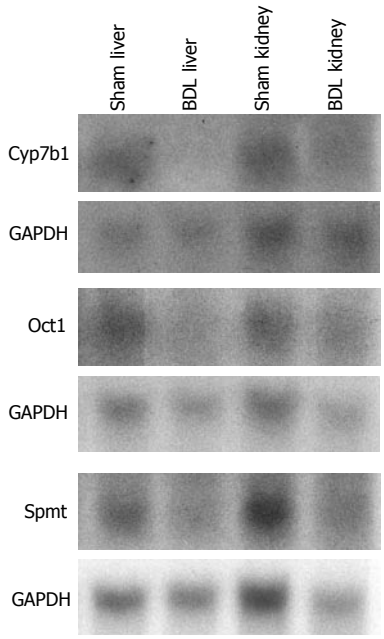

Figure 1 Confirmation of microarray data by Northern analysis of selected genes. Northern blots were performed with aliquots of pooled RNA from livers and kidneys from each 4 pair-fed sham-operated and 4 bile duct ligated mice, 7 d after surgery. Cyp7b1: Cytochrome P450 7b1; Oct1: Organic cation transporter 1; Spmt: Similar to putative integral membrane transport protein; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; Sham: Sham-surgery; BDL: Bile duct ligation.

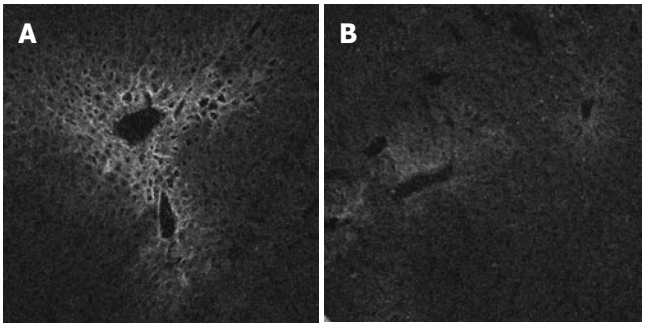

Figure 2 Indirect immunofluorescence of organic cation transporter 1 in murine liver sections. **A:** A low magnification view (x 20) shows antibody labeling at the basolateral membranes of hepatocytes of the pericentral zone of the liver lobule in the liver section of a sham-operated mouse, 7 d after surgery; **B:** In contrast, there is only a weak signal for organic cation transporter 1 after bile duct ligation.

et al[21] excluded genes with expression levels of less than 1 000, whereas we included genes with a mininum signal intensity of 600 and above. This approach led to the identification of a number of novel gene alterations of functional significance for the cholestatic phenotype. For instance, the decrease in expression of the gene encoding *Oct1* in BDL liver in the present microarray, an alteration not reported by Campbell *et al*[21] but previously reported by Ogawa *et al*[20] in the rat, led us to study this important basolateral cationic drug transporter in more detail. We were subsequently able to demonstrate that Oct1 is indeed down-regulated in rat liver, but not in kidney, in obstructive cholestasis at the mRNA as well as the protein levels and that this decrease results in reduced hepatic uptake of the Oct1 substrate tetraethylammonium[19]. Northern analysis and immunofluorescence microscopy of hepatic Oct1 performed in the present study indicated a similar pattern in mouse and confirmed the results of our microarray.

A number of other observations emerge from this analysis that deserve further study. For example, among the cell growth-related genes, the number of genes up-regulated in liver after BDL surpassed by far the number of down-regulated genes, a pattern which might reflect the extensive fibroproliferative process and tissue remodeling that takes place in this model of obstructive cholestasis. Similarly, a large number of genes related to cell adhesion, the extracellular matrix, and the cytoskeleton were found to be altered that have not been identified yet. We presume that many of these genes may play an important but as yet to be identified role in the fibrogenic response of the liver to bile duct obstruction. Alterations in the composition of the extracellular matrix are typical features of hepatic fibrosis[13], including substantial increases of collagens and non-collagenous components[25,26]. Accordingly, we observed a uniform up-regulation of genes encoding the *procollagen types* $I\alpha_1$, $I\alpha_2$, $III\alpha_1$, $IV\alpha_1$, $IV\alpha_2$, and $V\alpha_2$ in this mouse model of obstructive cholestasis. In addition, two members of the matrix metalloproteinase family, the *matrix metalloproteinases 7* and *12*, were up-regulated more than ten-fold following BDL when compared with the sham-operated controls. Matrix metalloproteinases represent a group of calcium-dependent enzymes involved in physiological and pathological degradation of extracellular matrix and tissue-remodeling[27]. *Matrix metalloproteinase 7 (matrilysin)*, an enzyme which is associated with poor prognosis in hepatocellular[28] and cholangiocellular carcinomas[29], has been closely related to the fibro-proliverative process in chronic hepatitis C[30] but not in cholestatic liver diseases. In contrast, *matrix metalloproteinase 12*, to our knowledge, has not been associated with liver fibrosis before and deserves future attention. Interestingly, the genes encoding *tissue inhibitor of metalloproteinase 1*, *vascular cell adhesion molecule 1* and *intercellular adhesion molecule* were up-regulated both in liver and kidney of BDL mice. Genes encoding the *procollagen types* $I\alpha_1$ *and* $III\alpha_1$ were also increased in the kidney of BDL mice although at lower levels than in the liver. The up-regulation of fibrosis-associated factors in kidney following BDL might be due to a paracrine action of fibrogenic mediators such as *connective tissue growth factor* whose hepatic expression is increased in cholestasis as previously described[31,32] and confirmed in our microarray. However, the functional relevance of the increased expression of these fibrotic genes in the kidney remains to be determined. Alternatively, the simultaneous up-regulation of important regulators of transcription following BDL such as *FBJ osteosarcoma oncogene*, *core promoter element binding protein*, and *activating transcription factor 3* in both liver and kidney supports the idea of coordinated gene regulation in different tissues as response to a specific stimulus. Another non-collagenous component of the extracellular matrix which was up-regulated in BDL liver is the gene for the matricellular protein *secreted acidic cysteine rich glycoprotein*. Matricellular proteins are a group of matrix-associated factors that mediate cell-matrix interactions but do not serve primarily as structural elements[13]. In particular, the expression of *secreted acidic cysteine rich glycoprotein* has been associated with cell proliferation, migration, and extracellular matrix remodeling in tissues, and *secreted acidic cysteine rich glycoprotein* has been found to be increased in different models of hepatic fibrosis[33].

The expression of a number of genes encoding

membrane proteins and transporters that were not previously known to be affected by cholestasis was also of interest. For example, the gene encoding the *β1 subunit of the voltage-gated sodium channel* which is important for the maturation and function of this channel[34] was up-regulated in liver as well as in kidney of BDL mice. In contrast, the expression of the gene encoding the ATP-binding cassette transporter *multidrug resistance-associated protein 6* (*Mrp6,* Abcc6) was reduced in cholestatic mouse liver as previously described for the rat[20]. Since mutations of human MRP6 are associated with pseudoxanthoma elasticum, a disorder characterized by calcification of the elastic fibres and abnormalities of the collagen fibrils[35], it is tempting to speculate that reduced hepatic Mrp6 expression in cholestasis might have functional implications for the development of liver fibrosis. Other genes up-regulated in cholestatic liver were the genes encoding the macrophage receptor markers *CD14 antigen* and *CD68 antigen*. Hepatic expression of both markers is increased in patients with biliary atresia[36], and expression of *CD68 antigen* may be an indicator of prognosis[37]. The functional significance of the concomitant *CD68 antigen* elevation in BDL kidney is unclear at the moment but illustrates again the close linkage between liver and kidney in this model of cholestasis and supports again a concept of coordinated gene regulation in different tissues.

In accordance with previous studies[38], obstructive cholestasis decreased the expression of a number of genes encoding *cytochrome P450* isoenzymes in liver. Since BDL results in an increase in liver concentrations of bile acids[15], the down-regulation of the *cytochrome P450 7b1 (oxysterol 7α-hydroxylase)* and *cytochrome P450 8B1 (sterol 12α-hydroxylase)* genes, that encode key enzymes in the conversion of cholesterol to bile acids[39], may represent adaptive responses to minimize the liver levels of cytotoxic bile salts. The increase of the gene encoding *cytochrome P450 27b1 (25-hydroxyvitamin D_3 1α-hydroxylase)* in BDL kidney is another interesting observation. *Cytochrome P450 27b1* catalyzes the conversion of 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3, the last step in vitamin D activation, which takes place in kidney[40]. Thus the increase in renal *cytochrome P450 27b1* expression may reflect an adaptive response to compensate for 25-hydroxyvitamin D deficiency in cholestasis. This may be a pathophysiologically important mechanism since patients with primary biliary cirrhosis often present with deficiencies of 25-hydroxyvitamin D but normal or even elevated levels of 1, 25-dihydroxyvitamin D[41].

In summary, the present study provides a comprehensive gene expression profile from mouse liver and kidney in obstructive cholestasis. Changes in gene expression were validated by Northern analysis, immunofluorescence, or comparison with the literature. The findings in this study provide new insights for generating novel hypotheses concerning the adaptive responses of gene expression in this mouse model of cholestasis.

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REFERENCES

1 **Trauner M**, Boyer JL. Bile salt transporters: molecular characterization, function, and regulation. *Physiol Rev* 2003; **83**: 633-671
2 **Gartung C**, Ananthanarayanan M, Rahman MA, Schuele S, Nundy S, Soroka CJ, Stolz A, Suchy FJ, Boyer JL. Down-regulation of expression and function of the rat liver Na^+/bile acid cotransporter in extrahepatic cholestasis. *Gastroenterology* 1996; **110**: 199-209
3 **Dumont M**, Jacquemin E, D'Hont C, Descout C, Cresteil D, Haouzi D, Desrochers M, Stieger B, Hadchouel M, Erlinger S. Expression of the liver Na^+-independent organic anion transporting polypeptide (oatp-1) in rats with bile duct ligation. *J Hepatol* 1997; **27**: 1051-1056
4 **Vos TA**, Hooiveld GJ, Koning H, Childs S, Meijer DK, Moshage H, Jansen PL, Müller M. Up-regulation of the multidrug resistance genes, Mrp1 and Mdr1b, and down-regulation of the organic anion transporter, Mrp2, and the bile salt transporter, Spgp, in endotoxemic rat liver. *Hepatology* 1998; **28**: 1637-1644
5 **Lee J**, Azzaroli F, Wang L, Soroka CJ, Gigliozzi A, Setchell KD, Kramer W, Boyer JL. Adaptive regulation of bile salt transporters in kidney and liver in obstructive cholestasis in the rat. *Gastroenterology* 2001; **121**: 1473-1484
6 **Soroka CJ**, Cai SY, Boyer JL. Effects of cholestasis on the regulation of membrane transporter expression in intestine and kidney. *Hepatology* 2002; **36**: 462A
7 **Beuers U**, Denk GU, Soroka CJ, Wimmer R, Rust C, Paumgartner G, Boyer JL. Taurolithocholic acid exerts cholestatic effects *via* phosphatidylinositol 3-kinase-dependent mechanisms in perfused rat livers and rat hepatocyte couplets. *J Biol Chem* 2003; **278**: 17810-17818
8 **Beuers U**, Probst I, Soroka C, Boyer JL, Kullak-Ublick GA, Paumgartner G. Modulation of protein kinase C by taurolithocholic acid in isolated rat hepatocytes. *Hepatology* 1999; **29**: 477-482
9 **Rust C**, Gores GJ. Apoptosis and liver disease. *Am J Med* 2000; **108**: 567-574
10 **Patel T**, Gores GJ. Apoptosis and hepatobiliary disease. *Hepatology* 1995; **21**: 1725-1741
11 **Oude Elferink RP**, Groen AK. Mechanisms of biliary lipid secretion and their role in lipid homeostasis. *Semin Liver Dis* 2000; **20**: 293-305
12 **Bedossa P**, Paradis V. Liver extracellular matrix in health and disease. *J Pathol* 2003; **200**: 504-515
13 **Schuppan D**, Ruehl M, Somasundaram R, Hahn EG. Matrix as a modulator of hepatic fibrogenesis. *Semin Liver Dis* 2001; **21**: 351-372
14 **Holloway AJ**, van Laar RK, Tothill RW, Bowtell DD. Options available--from start to finish--for obtaining data from DNA microarrays II. *Nat Genet* 2002; **32 Suppl**: 481-489
15 **Bohan A**, Chen WS, Denson LA, Held MA, Boyer JL. Tumor necrosis factor alpha-dependent up-regulation of Lrh-1 and Mrp3(Abcc3) reduces liver injury in obstructive cholestasis. *J Biol Chem* 2003; **278**: 36688-36698
16 **Lee JM**, Trauner M, Soroka CJ, Stieger B, Meier PJ, Boyer JL. Expression of the bile salt export pump is maintained after chronic cholestasis in the rat. *Gastroenterology* 2000; **118**: 163-172
17 **Meyer-Wentrup F**, Karbach U, Gorboulev V, Arndt P, Koepsell H. Membrane localization of the electrogenic cation transporter rOCT1 in rat liver. *Biochem Biophys Res Commun* 1998; **248**: 673-678
18 **Karbach U**, Kricke J, Meyer-Wentrup F, Gorboulev V, Volk C, Loffing-Cueni D, Kaissling B, Bachmann S, Koepsell H. Local-

ization of organic cation transporters OCT1 and OCT2 in rat kidney. *Am J Physiol Renal Physiol* 2000; **279:** F679- F687

19 **Denk GU**, Soroka CJ, Mennone A, Koepsell H, Beuers U, Boyer JL. Down-regulation of the organic cation transporter 1 of rat liver in obstructive cholestasis. *Hepatology* 2004; **39**: 1382-1389

20 **Ogawa K**, Suzuki H, Hirohashi T, Ishikawa T, Meier PJ, Hirose K, Akizawa T, Yoshioka M, Sugiyama Y. Characterization of inducible nature of MRP3 in rat liver. *Am J Physiol Gastrointest Liver Physiol* 2000; **278**: G438- G446

21 **Campbell KM**, Sabla GE, Bezerra JA. Transcriptional reprogramming in murine liver defines the physiologic consequences of biliary obstruction. *J Hepatol* 2004; **40**: 14-23

22 **Deaciuc IV**, Doherty DE, Burikhanov R, Lee EY, Stromberg AJ, Peng X, de Villiers WJ. Large-scale gene profiling of the liver in a mouse model of chronic, intragastric ethanol infusion. *J Hepatol* 2004; **40**: 219-227

23 **Schrenk D**, Gant TW, Preisegger KH, Silverman JA, Marino PA, Thorgeirsson SS. Induction of multidrug resistance gene expression during cholestasis in rats and nonhuman primates. *Hepatology* 1993; **17**: 854-860

24 **Zollner G**, Fickert P, Silbert D, Fuchsbichler A, Stumptner C, Zatloukal K, Denk H, Trauner M. Induction of short heterodimer partner 1 precedes downregulation of Ntcp in bile ductligated mice. *Am J Physiol Gastrointest Liver Physiol* 2002; **282**: G184- G191

25 **Rojkind M**, Giambrone MA, Biempica L. Collagen types in normal and cirrhotic liver. *Gastroenterology* 1979; **76**: 710-719

26 **Schuppan D**. Structure of the extracellular matrix in normal and fibrotic liver: collagens and glycoproteins. *Semin Liver Dis* 1990; **10**: 1-10

27 **Benyon RC**, Arthur MJ. Extracellular matrix degradation and the role of hepatic stellate cells. *Semin Liver Dis* 2001; **21**: 373-384

28 **Yamamoto H**, Itoh F, Adachi Y, Sakamoto H, Adachi M, Hinoda Y, Imai K. Relation of enhanced secretion of active matrix metalloproteinases with tumor spread in human hepatocellular carcinoma. *Gastroenterology* 1997; **112**: 1290-1296

29 **Miwa S**, Miyagawa S, Soeda J, Kawasaki S. Matrix metalloproteinase-7 expression and biologic aggressiveness of cholangiocellular carcinoma. *Cancer* 2002; **94**: 428-434

30 **Lichtinghagen R**, Michels D, Haberkorn CI, Arndt B, Bahr M, Flemming P, Manns MP, Boeker KH. Matrix metalloproteinase (MMP)-2, MMP-7, and tissue inhibitor of metalloproteinase-1 are closely related to the fibroproliferative process in the liver during chronic hepatitis C. *J Hepatol* 2001; **34**: 239-247

31 **Paradis V**, Dargere D, Vidaud M, De Gouville AC, Huet S, Martinez V, Gauthier JM, Ba N, Sobesky R, Ratziu V, Bedossa P. Expression of connective tissue growth factor in experimental rat and human liver fibrosis. *Hepatology* 1999; **30**: 968-976

32 **Sedlaczek N**, Jia JD, Bauer M, Herbst H, Ruehl M, Hahn EG, Schuppan D. Proliferating bile duct epithelial cells are a major source of connective tissue growth factor in rat biliary fibrosis. *Am J Pathol* 2001; **158**: 1239-1244

33 **Frizell E**, Liu SL, Abraham A, Ozaki I, Eghbali M, Sage EH, Zern MA. Expression of SPARC in normal and fibrotic livers. *Hepatology* 1995; **21**: 847-854

34 **Kupershmidt S**, Yang T, Roden DM. Modulation of cardiac Na^+ current phenotype by beta1-subunit expression. *Circ Res* 1998; **83**: 441-447

35 **Bergen AA**, Plomp AS, Schuurman EJ, Terry S, Breuning M, Dauwerse H, Swart J, Kool M, van Soest S, Baas F, ten Brink JB, de Jong PT. Mutations in ABCC6 cause pseudoxanthoma elasticum. *Nat Genet* 2000; **25**: 228-231

36 **Tracy TF Jr**, Dillon P, Fox ES, Minnick K, Vogler C. The inflammatory response in pediatric biliary disease: macrophage phenotype and distribution. *J Pediatr Surg* 1996; **31:** 121-125; discussion 125-126

37 **Kobayashi H**, Puri P, O'Briain DS, Surana R, Miyano T. Hepatic overexpression of MHC class II antigens and macrophage-associated antigens (CD68) in patients with biliary atresia of poor prognosis. *J Pediatr Surg* 1997; **32**: 590-593

38 **Tateishi T**, Watanabe M, Nakura H, Tanaka M, Kumai T, Kobayashi S. Liver damage induced by bile duct ligation affects CYP isoenzymes differently in rats. *Pharmacol Toxicol* 1998; **82**: 89-92

39 **Chiang JY**. Bile acid regulation of gene expression: roles of nuclear hormone receptors. *Endocr Rev* 2002; **23**: 443-463

40 **Omdahl JL**, Bobrovnikova EV, Annalora A, Chen P, Serda R. Expression, structure-function, and molecular modeling of vitamin D P450s. *J Cell Biochem* 2003; **88**: 356-362

41 **Kaplan MM**, Elta GH, Furie B, Sadowski JA, Russell RM. Fat-soluble vitamin nutriture in primary biliary cirrhosis. *Gastroenterology* 1988; **95**: 787-792

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