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• *BASIC RESEARCH* •

Expression profiling suggests a regulatory role of gallbladder in lipid homeostasis

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Abstract

AIM: To examine expression profile of gallbladder using microarray and to investigate the role of gallbladder in lipid homeostasis.

METHODS: ^{33}P-labelled cDNA derived from total RNA of gallbladder tissue was hybridized to a cDNA array representing 17 000 cDNA clusters. Genes with intensities $\geqslant$2 and variation <0.33 between two samples were considered as positive signals with subtraction of background chosen from an area where no cDNA was spotted. The average gray level of two gallbladders was adopted to analyze its bioinformatics. Identified target genes were confirmed by touch-down polymerase chain reaction and sequencing.

RESULTS: A total of 11 047 genes expressed in normal gallbladder, which was more than that predicted by another author, and the first 10 genes highly expressed (high gray level in hybridization image), e.g., ARPC5 (2 225.88±90.46), LOC55972 (2 220.32±446.51) and SLC20A2 (1 865.21±98.02), were related to the function of smooth muscle contraction and material transport. Meanwhile, 149 lipid-related genes were expressed in the gallbladder, 89 of which were first identified (with gray level in hybridization image), e.g., FASN (11.42±2.62), APOD (92.61±8.90) and CYP21A2 (246.11±42.36), and they were involved in each step of lipid metabolism pathway. In addition, 19 of those 149 genes were gallstone candidate susceptibility genes (with gray level in hybridization image), e.g., HMGCR (10.98±0.31), NPC1 (34.88±12.12) and NR1H4 (16.8±0.65), which were previously thought to be expressed in the liver and/or intestine tissue only.

CONCLUSION: Gallbladder expresses 11 047 genes and takes part in lipid homeostasis.

Key words: Gallbladder; Microarray; Gene expression; Lipid homeostasis

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INTRODUCTION

Cholesterol cholelithiasis is an extremely common, economically significant digestive disease that affects some 10-15% of the global population[1]. Gallstone is also the main cause of gallbladder carcinoma, biliary pancreatitis and iatrogenic lesions of the biliary tract. It has been reported that the USA spends 8-10 billion dollars on gallstone disease annually[2]. It was suggested from the data in the late 1980s that about 5.6% of the population in China was affected with gallstones[3], and the incidence may be increased in recent years. The patients with gallstone-related diseases hospitalized in the surgical department of our hospital accounted for 47% in 2001. Clearly, it is an important disease that deserves more attention.

Cholesterol saturated bile secreted by the liver is the prerequisite of gallstone formation, so liver is the place of lipid metabolism and becomes the focus of study. However, gallbladder is the place of stone formation and its relationship with lipid metabolism has been seldom investigated. Furthermore, the molecular mechanisms of gallstone formation in gallbladder-related with lipid metabolism are far from clear. Only about 40 genes (including gallstone susceptible gene loci) have been identified presently, and most of the previous studies were based on the changes in a single gene. Obviously, a total list of genes expressed in the gallbladder should be identified. The relatively new advent of cDNA array technology has provided a powerful method for large-scale expression profiling[4], and has led to the elucidation of a number of regulatory pathways involved in complex biological processes especially in tumor-related areas[5]. In this study, we used a powerful tool to examine expression profile of gallbladder and to investigate the role of gallbladder in lipid homeostasis. This would build a basis for understanding the physiological function of gallbladder, especially the mechanism of gallstone formation.

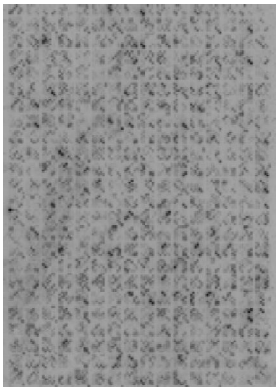

Figure 1 Half of an array hybridization image from a normal gallbladder sample.

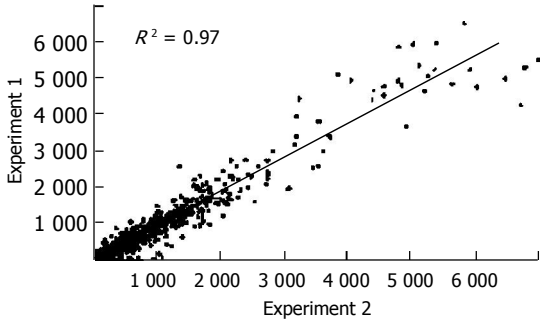

Figure 2 Scatterplot of two independent cDNA array analyses of the same sample. Each point stands for a gene or cDNA cluster, with the *X* coordinate representing the gene expression level in one test and the *Y* representing the value of the other test. An R^2 of 0.97 indicates high reproducibility of the cDNA array assay.

MATERIALS AND METHODS

cDNA array construction

cDNA clones were derived from liver and hepatocarcinoma cell lines, isolated from hypothalamus-pituitary-adrenal libraries[6] or purchased from Research Genetics (Huntsville, AL, USA). The assembled cDNA array contained 17 000 cDNA clones (representing the same number of independent cDNA clusters), of which 7 565 clusters were homologous to those found in the UniGene Database. All cDNA fragments were amplified and verified by electrophoresis. The average length of the cDNA fragments was -1 kb. PCR products were precipitated in isopropanol, redissolved in 10 μL of denaturing buffer (1.5 mol/L NaCl, 0.5 mol/L NaOH), and spotted on two 8×12-cm Hybond-N nylon membranes (Amersham Pharmacia, Buckinghamshire, UK) with an arrayer (BioRobotics, Cambridge, UK). Each spot carried -100 nL in volume and was 0.4 mm in diameter each cDNA fragment was placed in two different spots (double-offset). Lambda phage and pUC18 vector DNAs were spotted as negative controls.

Hybridization intramembrane control

Eight housekeeping genes were used as hybridization intramembrane controls (HIC): ribosomal protein S9 (RPS9), β-actin (ACTB), glyceraldehyde-3-phosphate dehydrogenase, hypoxanthine phosphoribosyltransferase 1, M_r 23 000 highly basic protein (RPL13A), ubiquitin C, phospholipase A2, and ubiquitin thiolesterase (UCHL1). These were evenly distributed in 12 places each per 8×12-cm array. Hybridization data was considered invalid if among the 12 spots representing the same gene, the intensity of the darkest spot exceeded 1.5-fold that of the weakest spot.

Clinical samples

Normal gallbladders were removed within 4 h postmortem from two adult males (aged 25 and 20 years, respectively) who died in traffic accidents. The Institute of Biomedical Science, Shanghai Second Medical University, approved the study, and all samples were obtained with informed consent. Tissues were frozen in liquid nitrogen immediately after separation, and kept frozen until used.

RNA extraction and probe preparation

Total RNA was extracted using a standard TRIzol RNA isolation protocol (Life Technologies, Inc., Grand Island, NY, USA). Poly (A)+ mRNA was then isolated from total RNA using a poly (dT) resin (Qiagen, Hilden, Germany). Approximately 1-2 μg of mRNA was labeled in a reverse transcription reaction in the presence of 200 μCi [α-^{33}P] deoxyadenosine 5'-triphosphate (DuPont NEN, Boston, MA, USA) using Moloney murine leukemia virus reverse transcriptase as per the manufacturer's instructions (Promega Corp., Madison, WI, USA).

Hybridization and image processing

Prehybridization was carried out in 20 mL of prehybridization solution (6× SSC, 0.5% SDS, 5× Denhardt's, and 100 μg/mL denatured salmon sperm DNA) at 68 ℃ for 3 h. Overnight hybridization with the ^{33}P-labeled cDNA in 6 mL of hybridization solution (6× SSC, 0.5% SDS, and 100 μg/mL salmon sperm DNA) was followed by stringent washing (0.1× SSC and 0.5% SDS at 65 ℃ for 1 h). Membranes were exposed to phosphor screens overnight and scanned using an FLA-3000A Plate/Fluorescent Image Analyzer (Fuji Photo Film, Tokyo, Japan). The radioactive intensity of each spot was linearly digitalized to 65 536 gray-grade in a pixel size of 50 μm in an Image Reader, and recorded with Array Gauge software (Fuji).

Data collection and analysis

After subtraction of background values (3±3) measured in an area where no cDNA was spotted, genes with intensities ⩾2 were considered positive signals; this ensured that positives were distinguished from the background with a statistical confidence of >99.9%. Normalization among arrays was based on the sum of background-subtracted signals from all genes on the membrane[7]. The average hybridization intensities of two gallbladders were adopted to analyze the bioinformatics.

Touchdown reverse transcription polymerase chain reaction

For most of the genes, mRNA levels in the gallbladder samples were too low to be detected by standard dot blot and hybridization *in situ*, or even by conventional PCR, we used touch-down PCR to confirm the array hybridization results. All PCR products were verified by sequencing to avoid false positives.

Table 1 The top 10 highly expressed genes in normal gallbladder tissue

Name of gene	Symbol	Gray level (mean±SD)
Breakpoint cluster region protein, uterine leiomyoma, 2	BCRP2	2 738.74±23.23
Actin-related protein 2/3 complex, subunit 5 (16 ku)	ARPC5	2 225.88±90.46
Eukaryotic translation initiation factor 4A, isoform 1	EIF4A1	2 223.86±274.74
Mitochondrial carrier family protein	LOC55972	2 220.32±446.51
Solute carrier family 20 (phosphate transporter), member 2	SLC20A2	1 865.21±98.02
Cytochrome b5 reductase 1 (B5R.1)	LOC51706	1 851.01±298.45
ADP-ribosylation factor 1	ARF1	1 844.63±353.31
FERM, RhoGEF (ARHGEF) and pleckstrin domain protein 1 (chondrocyte-derived)	FARP1	1 654.95±126.85
Proteasome (prosome, macropain) activator subunit 2 (PA28 beta)	PSME2	1 635.33±148.15
cAMP responsive element binding protein-like 1	CREBL1	1 579.36±71.68

RESULTS

Establishment of cDNA array system

Human cDNA clones randomly picked from cDNA libraries were terminally sequenced and compared with the Unigene database prior to their use in creating a cDNA array representing 17 000 genes or cDNA clusters (Figure 1). The reproducibility of the cDNA array analysis was evaluated in multiple replicated tests in which cDNA probes independently generated from the same mRNA sample were hybridized to different replicates of the cDNA arrays. The results from these experiments were almost perfectly concordant with a scatterplot R^2 (the square of the Pearson correlation coefficient, which measures similarity in gene expression patterns) of 0.97-0.98 (Figure 2). Of the 17 000 genes, only 0.2% showed >two-fold differences in their expression levels across different measurements. This showed that the cDNA array system was highly reproducible.

Gene list expressed in the gallbladder

In our work, a catalog of genes expressed in the human gallbladder was identified by cDNA array hybridization. The radio-intensities of corresponding spots on two parallel arrays were averaged. If the value was >2 and the variation <0.33 between the two samples, the signal was considered efficient. Of the 17 000 genes tested, a total of 11 047 genes were expressed in human normal gallbladder tissue, which is more than the number of 3 754 predicted by Lewis[8].

Top 10 genes expressed in the gallbladder

The 10 most highly expressed genes are listed in Table 1, and the top 3 breakpoint cluster region protein, uterine leiomyoma, 2; actin-related protein 2/3 complex, subunit 5 (16 ku) and eukaryotic translation initiation factor 4A, isoform 1, respectively.

Lipid metabolism-related genes and gallstone candidate genes in gallbladder

Totally 149 lipid metabolism-related genes were expressed in the gallbladder (Table 2). Eighty-three of them were identified for the first time in gallbladder (results after searching Unigene and Pubmed). Lammert *et al* have listed 45 possible gallstone candidate genes based on previous knowledge, 24 of which were assembled in our array, and 19 of these 24 genes were lipid-related genes and expressed in the gallbladder. We selected four lipid-related genes randomly, and by touchdown reverse transcription polymerase chain reaction (RT-PCR) and sequencing, confirmed the results in cDNA array (Figure 3).

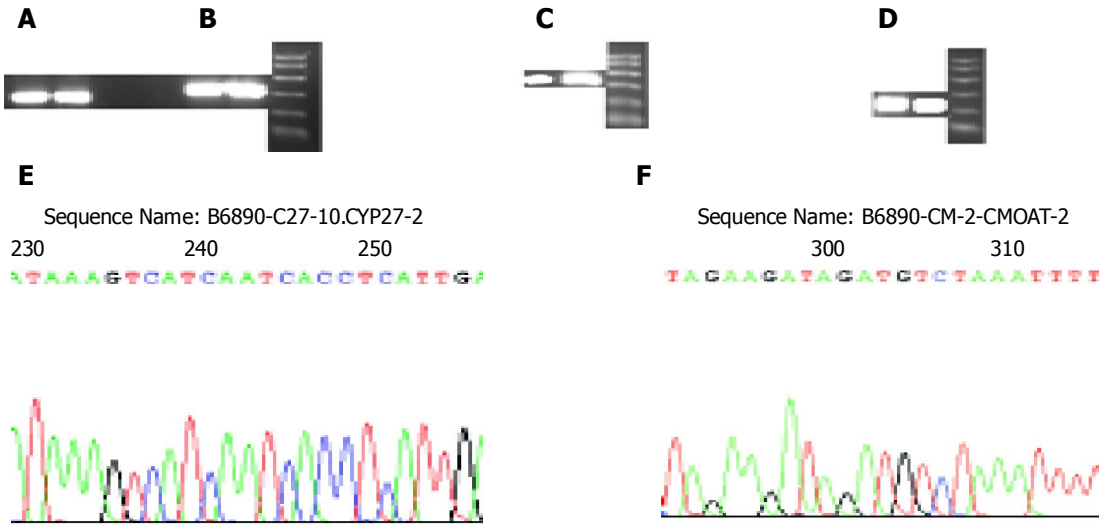

Figure 3 RT-PCR and sequencing confirmation of genes that were expressed in the gallbladder. RT-PCR electrophoresis result: A: CYP27A1 (320 bp); B: NR1H4(352 bp); C: CMOAT(353 bp); D: AKR1C3(240 bp). Sequencing result of PCR product: E: CYP27A1; F: CMOAT.

Table 2 Lipid-related genes expressed in normal gallbladder

GenBank ID	Gene name	Symbol	Reported or not	Gray level (mean±SD)
NM_000859	3-Hydroxy-3-methylglutaryl-Coenzyme A reductase[2]	HMGCR	P[3]	10.98±0.31
U66669	3-hydroxyisobutyryl-Coenzyme A hydrolase	HIBCH		11.17±0.09
U29344	Fatty acid synthase	FASN		11.42±2.62
AA460901	ATPase, aminophospholipid transporter-like, Class I, type 8A, member 2	ATP8A2		11.53±1.08
BE730527	Lipase protein	LOC57406	P[3]	11.58±0.85
AL043165	Homolog of mouse transient receptor potential-phospholipase C-interacting kinase CHaK; hypothetical protein FLJ20117	LTRPC7		11.59±0.69
U22662	Nuclear receptor subfamily 1, group H, member 3[2]	NR1H3		11.77±0.85
M14564	Cytochrome P450, subfamily XVII (steroid 17-alpha-hydroxylase), adrenal hyperplasia	CYP17		12.06±1.35
M93107	3-Hydroxybutyrate dehydrogenase (heart, mitochondria)	BDH	P[3]	12.10±2.22
NM_019844	Solute carrier family 21 (organic anion transporter), member 8[2]	SLC21A8	P[3]	12.14±0.77
AK000184	Acid sphingomyelinase-like phosphodiesterase	ASM3A		12.22±0.65
±X04506	±Apolipoprotein B (including Ag(x) antigen)	APOB	U[1]	12.23±1.17
X87176	Hydroxysteroid (17-beta) dehydrogenase 4	HSD17B4	U[1]	12.25±0.57
AF095703	L-3-hydroxyacyl-Coenzyme A dehydrogenase, short chain	HADHSC		12.37±1.66
AW022180	ESTs, weakly similar to S14747 sphingomyelin phosphodiesterase [*H. sapiens*]			12.39±2.40
U32576	Apolipoprotein C-IV	APOC4	U[1]	12.58±0.28
AI133376	Human DNA sequence from clone RP11-16L21 on chromosome 9. Contains the gene for NADP-dependent leukotriene B4 12-hydroxydehydrogenase, the gene for a novel DnaJ domain protein similar to Drosophila, *C. elegans* and Arabidopsis predicted proteins, the GNG10			12.75±1.64
AV658073	Homolog of mouse transient receptor potential-phospholipase C-interacting kinase CHaK; hypothetical protein FLJ20117	LTRPC7		12.83±1.45
X03635	Estrogen receptor 1	ESR1		12.83±0.34
AF165514	Hydroxysteroid (17-beta) dehydrogenase 7	HSD17B7	P[3]	12.99±0.56
NM_018557	Low density lipoprotein-related protein 1B (deleted in tumors)	LRP1B		13.06±0.29
U22526	Lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)	LSS		13.14±0.70
X83618	3-Hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)	HMGCS2	P[3]	13.34±0.03
D14662	Anti-oxidant protein 2 (non-selenium glutathione peroxidase, acidic calcium-independent phospholipase A2)	KIAA0106	U[1]	13.40±0.78
M62839	Apolipoprotein H (beta-2-glycoprotein I)	APOH		13.49±0.20
Z99716	Sterol regulatory element binding transcription factor 2[2]	SREBF2	P[3]	13.53±2.31
AF126799	Fatty acid desaturase 2	FADS2		13.62±2.16
AV662152	EST, moderately similar to LPHUC1 apolipoprotein C-I precursor [*H. sapiens*]			13.63±1.57
M37238	Phospholipase C, gamma 2 (phosphatidylinositol-specific)	PLCG2	P[3]	13.79±2.16
AF129756	Apolipoprotein M G3A			13.90±0.29
AW873435	Lipase A, lysosomal acid, cholesterol esterase (Wolman's disease)	LIPA		13.98±1.71
U09117	Phospholipase C, delta 1	PLCD1	P[3]	14.06±0.58
AC007954	Glutathione transferase zeta 1 (maleylacetoacetate isomerase)	GSTZ1		14.13±0.76
AA706930	Fatty acid binding protein 1, liver	FABP1	U[1]	14.19±0.25
AB011153	Phosphoinositide-specific phospholipase C-beta 1	PLCB1		14.65±2.22
AF038440	Phospholipase D2	PLD2		14.66±5.01
N42553	Homolog of mouse transient receptor potential-phospholipase C-interacting kinase CHaK; hypothetical protein FLJ20117	LTRPC7		14.68±2.50
AL110209	LCAT-like lysophospholipase	LLPL		14.88±2.24
X13916	Low density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor) [2]	LRP1		14.91±1.56
AI955289	ESTs, weakly similar to DXHUBH 11beta-hydroxysteroid dehydrogenase [*H.sapiens*]			14.96±1.66
AF077046	Ganglioside expression factor 2	GEF-2		15.11±1.98
Z34975	Low density lipoprotein receptor defect C complementing	LDLC		15.18±2.11
BE271295	Group XII secreted phospholipase A2	PLA2G12		15.64±1.27
U49248	ATP-binding cassette, sub-family C (CFTR/MRP), member 2[2]	ABCC2	U[1]	15.78±0.45
N78156	Homolog of yeast long chain polyunsaturated fatty acid elongation enzyme 2	HELO1		15.95±5.40
AB016247	Sterol-C5-desaturase (fungal ERG3, delta-5-desaturase)-like	SC5DL	P[3]	16.09±3.33
NM_000954	Prostaglandin D2 synthase (21 ku, brain)	PTGDS		16.19±3.18
U60205	Sterol-C4-methyl oxidase-like	SC4MOL		16.22±1.29
AA557324	ESTs, weakly similar to fatty acid omega-hydroxylase [*H.sapiens*]			16.31±2.98
X66435	3-Hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	HMGCS1	P[3]	16.46±2.32
AL117352	Human DNA sequence from clone RP5-876B10 on chromosome 1q42.12-43. Contains the 3' end of the GNPAT gene for glyceronephosphate O-acyltransferase (DHAPAT, DAPAT, dihydroxyacetone phosphate acyltransferase, EC 2.3.1.42), the gene for a novel protein (ortho)			16.74±0.16
BE737965	Caveolin 1, caveolae protein, 22 ku[2]	CAV1		16.74±2.50

U68233	Nuclear receptor subfamily 1, group H, member 4**	NR1H4		16.80±0.65
AF035284	Fatty acid desaturase 1	FADS1	U[1]	16.82±0.92
X04898	Apolipoprotein A-II	APOA2	U[1]	16.96±2.01
AL031295	Lysophospholipase II	LYPLA2	P[3]	17.01±0.19
AL031295	3-Hydroxymethyl-3-methylglutaryl-Coenzyme A lyase (hydroxymethylglutaricaciduria)	HMGCL		17.01±0.19
M55150	Fumarylacetoacetate hydrolase (fumarylacetoacetase)	FAH		17.41±2.04
M31210	Endothelial differentiation, sphingolipid G-protein-coupled receptor, 1	EDG1		17.56±0.62
BE395256	Lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)	LSS		17.63±1.71
NM_000483	Apolipoprotein C-II	APOC2	U[1]	17.71±1.70
L76465	Hydroxyprostaglandin dehydrogenase 15-(NAD)	HPGD		17.86±0.79
S68287	Aldo-keto reductase family 1, member C4 (chlordecone reductase; 3-alpha hydroxysteroid dehydrogenase, type I; dihydrodiol dehydrogenase 4)	AKR1C4	P[3]	18.11±2.89
M54927	Proteolipid protein 1 (Pelizaeus-Merzbacher disease, spastic paraplegia 2, uncomplicated)	PLP1	P[3]	18.21±1.67
BE714757	Lipase A, lysosomal acid, cholesterol esterase (Wolman's disease)	LIPA		18.26±2.02
AJ238243	Phospholipase A2-activating protein	PLAA		18.73±3.07
AF077820	Low density lipoprotein receptor-related protein 5	LRP5		18.98±2.79
AL031778	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 2	APOBEC2	P[3]	19.21±3.45
AC004770	Fatty acid desaturase 1	FADS1	U[1]	19.22±9.65
AC004770	Fatty acid desaturase 3	FADS3		19.22±9.65
AA280051	Fatty acid binding protein 1, liver	FABP1	U[1]	19.81±3.80
X67698	Niemann–Pick disease, type C2 gene	NPC2		20.53±1.84
AL049748	Apolipoprotein L, 5	APOL5		20.61±0.01
X51416	Estrogen-related receptor alpha	ESRRA		20.63±1.82
AF065215	Phospholipase A2, group IVB (cytosolic)	PLA2G4B	P[3]	20.71±3.25
AB006746	Phospholipid scramblase 1	PLSCR1	U[1]	21.33±0.62
M59979	Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	PTGS1	P[3]	21.55±0.74
X02162	Apolipoprotein A-I	APOA1	U[1]	21.70±0.75
AF079167	Oxidised low density lipoprotein (lectin-like) receptor 1	OLR1	P[3]	22.12±7.75
U55764	Sulfotransferase, estrogen-preferring	STE		22.15±2.72
J03459	Leukotriene A4 hydrolase	LTA4H		22.32±4.28
U03090	Phospholipase A2, group V	PLA2G5	P[3]	22.88±2.74
M76665	Hydroxysteroid (11-beta) dehydrogenase 1	HSD11B1	P[3]	22.98±3.71
NM_016108	Androgen induced protein	AIG-1	P[3]	23.02±3.33
AV651650	ESTs, highly similar to AF237982 1 oxysterol 7 alpha-hydroxylase [*H.sapiens*]			23.62±0.57
U11313	Sterol carrier protein 2[2]	SCP2	U[1]	23.92±0.18
NM_001645	Apolipoprotein C-I	APOC1	U[1]	25.02±12.59
X54741	Cytochrome P450, subfamily XIB (steroid 11-beta-hydroxylase), polypeptide 2	CYP11B2		25.13±3.81
X01388	Apolipoprotein C-III	APOC3		25.17±4.82
R98624	Bile acid Coenzyme A: amino acid *N*-acyltransferase (glycine *N*-choloyltransferase)	BAAT	P[3]	25.26±16.74
U93305	Proteolipid protein 2 (colonic epithelium-enriched)	PLP2	P[3]	25.41±2.44
U67963	Lysophospholipase-like	HU-K5		26.75±5.28
D86096	Prostaglandin E receptor 3 (subtype EP3)	PTGER3	P[3]	26.79±0.19
AL022398	Hydroxysteroid (11-beta) dehydrogenase 1	HSD11B1		26.82±8.70
X76488	Lipase A, lysosomal acid, cholesterol esterase (Wolman's disease)	LIPA		27.10±11.84
AF019225	Apolipoprotein L	APOL1	U[1]	28.12±1.76
AL034374	Homolog of yeast long chain polyunsaturated fatty acid elongation enzyme 2	HELO1		28.76±4.09
AI675602	EST, moderately similar to I65981 fatty acid omega-hydroxylase [*H.sapiens*]			28.85±13.77
U89281	Oxidative 3 alpha hydroxysteroid dehydrogenase; retinol dehydrogenase; 3-hydroxysteroid epimerase	RODH		29.09±7.19
AL022318	Phorbolin (similar to apolipoprotein B mRNA editing protein)	DJ742C19.2		29.26±5.26
X07228	Lipase, hepatic	LIPC	P[3]	30.57±4.34
AL031230	Glycosylphosphatidylinositol specific phospholipase D1	GPLD1		30.88±1.21
AA128778	Tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	TFPI		32.76±0.09
Z99390	L-3-hydroxyacyl-Coenzyme A dehydrogenase, short chain	HADHSC		33.87±0.98
AF002020	Niemann–Pick disease, type C1[2]	NPC1		34.88±12.12
X55764	Cytochrome P450, subfamily XIB (steroid 11-beta-hydroxylase), polypeptide 1	CYP11B1		34.95±1.61
L34081	Bile acid Coenzyme A: amino acid *N*-acyltransferase (glycine N-choloyltransferase)	BAAT	P[3]	37.8±3.91
AF002668	Degenerative spermatocyte (homolog Drosophila; lipid desaturase)	DEGS		37.93±4.50
AF034544	7-Dehydrocholesterol reductase	DHCR7		38.05±2.80
D82073	Prostaglandin D2 synthase, hematopoietic	PGDS		38.83±13.58
Z37986	Emopamil-binding protein (sterol isomerase)	EBP		41.25±12.24
L21934	Sterol O-acyltransferase (acyl-Coenzyme A: cholesterol acyltransferase) 1	SOAT1	P[3]	44.08±1.76
L21934	Sterol O-acyltransferase (acyl-Coenzyme A: cholesterol acyltransferase) 1[2]	SOAT1	P[3]	44.08±1.76

M12792	Cytochrome P450, subfamily XXIA (steroid 21-hydroxylase, congenital adrenal hyperplasia), polypeptide 2	CYP21A2		44.19±7.57
L07077	Enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase	EHHADH		45.78±6.21
BE566894	Human DNA sequence from clone RP11-16L21 on chromosome 9. Contains the gene for NADP-dependent leukotriene B4 12-hydroxydehydrogenase, the gene for a novel DnaJ domain protein similar to Drosophila, *C. elegans* and Arabidopsis predicted proteins, the GNG10		U[1]	47.45±1.75
NM_016371	Hydroxysteroid (17-beta) dehydrogenase 7	HSD17B7		48.38±6.73
AF151638	Phosphatidylcholine transfer protein[2]	PCTP	P[3]	53.38±6.96
M12529	Apolipoprotein E[2]	APOE	P[3]	53.51±9.77
AL034369	Human DNA sequence from clone 149D17 on chromosome Xq22.2-23. Contains part of a PLRP2 (PNLIPRP2, pancreatic lipase-related protein 2 Precursor, EC 3.1.1.3) LIKE gene and 5' exons of the COL4A5 and alternatively spliced COL4A6 genes for Collagen, type IV,			54.46±5.61
M22430	Phospholipase A2, group IIA (platelets, synovial fluid)	PLA2G2A	P[3]	58.57±9.02
AK000339	Long-chain fatty acid Coenzyme A ligase 5	FACL5	U[1]	58.82±3.86
NM_013389	NPC1 (Niemann–Pick disease, type C1, gene)-like 1	NPC1L1	U[1]	59.9±13.58
M63959	Low density lipoprotein-related protein-associated protein 1 (alpha-2-macroglobulin receptor-associated protein 1)[2]	LRPAP1	U[1]	60.24±6.70
AF263613	Intracellular membrane-associated calcium-independent phospholipase A2 gamma	IPLA2(GAMMA)		60.96±13.23
Z29481	3-Hydroxyanthranilate 3,4-dioxygenase	HAAO		62.47±0.09
D38081	Thromboxane A2 receptor	TBXA2R		66.03±19.67
X71973	Glutathione peroxidase 4 (phospholipid hydroperoxidase)	GPX4		69.27±12.44
AW662196	Apolipoprotein L, 2	APOL2	P[3]	79.49±5.89
AI590076	3-Hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	HMGCS1	P[3]	83.08±4.33
AL022398	*Homo sapiens* DNA sequence from PAC 434O14 on chromosome 1q32.3.-41. Contains the HSD11B1 gene for hydroxysteroid (11-beta) dehydrogenase 1, the ADORA2BP adenosine A2b receptor LIKE pseudogene, the IRF6 gene for Interferon Regulatory Factor 6 and two novel			85.30±14.15
AL022394	Phospholipase C, gamma 1 (formerly subtype 148)	PLCG1	P[3]	85.54±21.21
Z82215	Apolipoprotein L	APOL1	U[1]	89.89±16.36
J02611	Apolipoprotein D	APOD		92.61±8.90
U19487	Prostaglandin E receptor 2 (subtype EP2), 53 ku	PTGER2	P[3]	95.40±18.27
X59812	Cytochrome P450, subfamily XXVIIA (steroid 27-hydroxylase, cerebrotendinous xanthomatosis), polypeptide 1[2]	CYP27A1	P[3]	100.70±10.81
AF070675	Apolipoprotein L, 3	APOL3	U[1]	105.58±12.73
U00968	Sterol regulatory element binding transcription factor 1[2]	SREBF1	P[3]	107.4±8.65
AB018580	Aldo-keto reductase family 1, member C3 (3-alpha hydroxysteroid dehydrogenase, type II) [2]	AKR1C3	P[3]	110.69±31.00
X98332	Solute carrier family 22 (organic cation transporter), member 1[2]	SLC22A1	P[3]	112.02±22.09
M10617	Fatty acid binding protein 1, liver[2]	FABP1	U[1]	117.5±11.59
L11702	Glycosylphosphatidylinositol specific phospholipase D1	GPLD1		149.16±33.78
X06290	Lipoprotein, Lp(a)	LPA	P[3]	156.94±26.59
X14723	Clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J)	CLU	U[1]	163.92±11.19
BE018577	3-Hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	HMGCS1	P[3]	169.72±16.05
U23942	Cytochrome P450, 51 (lanosterol 14-alpha-demethylase)	CYP51		219.74±47.13
AL049547	Cytochrome P450, subfamily XXIA (steroid 21-hydroxylase, congenital adrenal hyperplasia), polypeptide 2	CYP21A2		246.11±42.36
AF081281	Lysophospholipase I	LYPLA1	P[3]	348.56±34.15
L40802	Hydroxysteroid (17-beta) dehydrogenase 2	HSD17B2		396.94±5.65

[1]U: Genes expressed in the gallbladder through searching Unigene Database; [2]Gallstone candidate genes; [3]P: Genes expressed in the gallbladder suggested by previous report.

DISCUSSION

A cDNA array representing 17 000 human genes or cDNA clusters was established. Compared with the cDNA array without hybridization intra membrane controls, the HIC in the cDNA array system significantly contributed to the evenness of hybridization among different parts of the array membrane and therefore improved the reliability of the array analysis (data not shown). Replicated examinations of the same sample indicated that only 0.2-0.3% of the genes spotted or 0.5% informative genes, might be false-positive signals. Because we chose the genes that were lower than 0.33 in the variation of average on two samples, the possibilities of false-positives were remote.

Totally 11 047 genes were expressed in the gallbladder, which is almost thrice the number of 3 754 predicted by Lewis. The results remind us the importance of the role of gallbladder in the human body.

So far, two measures can be used to study gene expression profile, namely constructing a cDNA library following sequencing or thorough cDNA array. The former is a

labor-consuming and accurate work, but it needs complex procedures, and can be affected by many factors, thus, it is not so sensitive. On the contrary, cDNA array is a high-throughput and relatively less expensive technology.

The top 10 highly expressed genes with a clear function are displayed in Table 1, they are: BCRP2: breakpoint cluster region protein, uterine leiomyoma, 2; ARPC5: actin-related protein 2/3 complex, subunit 5 (16 ku); EIF4A1: eukaryotic translation initiation factor 4A, isoform 1; LOC55972: mitochondrial carrier family protein; SLC20A2: solute carrier family 20 (phosphate transporter), member 2; LOC51706: cytochrome b5 reductase 1 (B5R.1); ARF1: ADP-ribosylation factor 1; FARP1: FERM, RhoGEF (ARHGEF) and pleckstrin domain protein 1 (chondrocyte-derived); PSME2: proteasome (prosome, macropain) activator subunit 2 (PA28 beta); CREBL1: cAMP responsive element binding protein-like 1. Their functions are associated with smooth muscle contraction and material transport, thus, they may participate in the contraction and concentration of gallbladder bile[9-16].

The human body contains both exogenous and endogenous cholesterol. Exogenous cholesterol comes from diet while the endogenous is synthesized inside the body by liver cells. Excess total cholesterol in the plasma will eventually become deposited on the arterial walls, leading to atherosclerosis[17]. High-density lipoprotein is the only lipoprotein that can transport cholesterol to the liver for degradation; this is called reverse cholesterol transport[18]. Liver cells catalyze cholesterol into bile acid, which is secreted into the biliary tract. Supersaturated cholesterol remains in the gallbladder bile, and can result in the formation of cholesterol monohydrate crystals, and finally gallstones[19]. Normally, the gallbladder epithelium absorbs high amounts of biliary cholesterol and phosphatidylcholine in a proportion determined by their molar ratio in the bile entering the lumen. This physiological lipid absorption continuously reduces biliary cholesterol molar percentage in the gallbladder, thus avoiding crystal precipitation. In contrast, gallbladder epithelium in patients affected with cholesterol gallstone disease may be hyperplastic and/or hypertrophic, and consistently absorbs cholesterol and phosphaticylcholine less efficiently, leading to a higher likelihood of cholesterol crystal precipitation[20]. A number of lipid metabolism genes or proteins are reportedly expressed in gallbladder tissue, including scavenger receptor class B type I(SR-BI)[21], Apo B[22], acyl-coA: cholesterol acyltransferase (Soat1)[23], sodium-dependent bile acid transporter and organic anion transporting polypeptide(Oatp1)[24], and the multidrug resistance protein (MRP)[25]. However, most of the lipid metabolism-related genes we identified in this study (Table 2) have not previously been reported in the gallbladder. From our results, we hypothesize that supersaturated lipids in bile lead to impaired regulation of the buffering ability of the gallbladder, resulting in the formation of gallstones. In our previous work, we observed that high cholesterol diet decreased the expression of cholecystokinin-A receptor in guinea pig, which led to the formation of gallstone[26]. Usually, it is nucleation factors or impaired motility of gallbladder that results in the formation of gallstone[27]. However, we found 19 lipid metabolism-related genes expressed in the gallbladder in our previous research[28,29], and currently a total of 149 lipid metabolism-related genes were expressed in the gallbladder, and most of them were firstly identified (83 genes), thus, we think it is necessary to consider the regulation ability of gallbladder in lipid homeostasis while we study the mechanism of gallstone formation.

Lammert *et al*[2] collected 45 possible candidate genes in gastroenterology. Twenty-four of the 45 genes were included in our array, and 21 of them were expressed in the gallbladder. Among the 21 genes, 19 genes were lipid-related genes, and they can be divided into five groups: lipid regulatory enzymes, lipoprotein receptors and related proteins, intracellular lipid transporters, membrane lipid transporters and lipid regulatory transcription factors. Previously those genes were believed to be expressed in the liver and/or intestinal cells only, not in gallbladder, and they could not be searched in Unigene and Pubmed for their association with gallbladder except LRPAP1[30], FABP1[31], SCP2 and ABCC2[32]. It is well known that lipid metabolism has a close relationship with liver, but we found those genes were expressed in the gallbladder, too, suggesting gallbladder takes part in both digestion and lipid homeostasis. Those genes of patients with gallstones expressed in the gallbladder are involved in each step of gallstone formation, thus, if we study the differentially expressed genes between normal and gallstone affected gallbladder by cDNA hybridization, we may find some valuable gallstone-related genes.

In summary, we established a cDNA array and identified a catalog of genes expressed in normal gallbladder tissue, the number is greater than previous predictions. In addition, we identified the expression of 139 lipid metabolism-related genes, eighty-three of them were first discovered, suggesting that the gallbladder takes part in lipid homeostasis.

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