Oligonucleotide microarray for detection of the hereditary liver diseases-associated genes in rat liver regeneration☆

Zikuo Tang[1], Shoubing Zhang[1], Cunshuan Xu[2,*]

[1]*Key Laboratory for Cell Differentiation Regulation, Xinxiang, Henan 453007, China;* [2]*College of Life Science, Henan Normal University, Xinxiang, Henan 453007, China*

Received December 17, 2006

Abstract

Objective. This paper mainly explores the roles of hereditary liver disease-associated genes during liver regeneration (LR) at transcriptional level. *Methods.* The hereditary liver disease-associated genes were obtained by collecting and referring to the data of relevant databases, and the gene expression changes in the rat regenerating liver were checked by the GeneChip Rat Genome 230 2.0 Array. *Results.* 65 genes were found to be associated with LR. The numbers of initial and total expression of these genes occurring in initiation (0.5 – 4 hours after PH), G0/G1 transition (4 – 6 hours after PH), cell proliferation (6 – 66 hours after PH), redifferentiation and structure-function reconstruction phase (72 – 168 hours after PH) were 24, 7, 39, 2 and 46, 29, 236, 75, respectively, illustrating that the associated genes mainly were triggered at the initial stage of LR (0.5 – 4 hours after PH), and continue to develop at the different stages. Overall, 228 genes were found up-regulated and 129 were down-regulated, demonstrating that expression of the majority of genes was enhanced during LR, while that of the minority was weakened. *Conclusion.* Metabolisms of bilirubin, iron, copper, chlorine ion, lipids, carbohydrate and amino acids are active during LR. The resistance of regenerating liver to diseases and infection is increased. Among them, 65 genes associated with LR play an important role. [Life Science Journal. 2007; 4(2): 56 – 63] (ISSN: 1097 – 8135).

Keywords: partial hepatectomy; Rat Genome 230 2.0 Array; hereditary liver diseases; genes; liver regeneration

1 Introduction

Liver has strong regenerating capacity[1]. The remaining liver cells are activated and enter into cell cycle to compensate for the lost liver mass after partial hepatectomy (PH)[2], and this process is called liver regeneration (LR)[3]. In addition to the protection mechanism of hepatocytes being activated after PH, the activities of removing damaged cells and repairing injured cells, cell proliferation, apoptosis, extracellular matrix formation and reconstruction of structure-function are enhanced[4]. Meanwhile, bioactive ingredients synthesis and detoxification in hepatocyte are still carried on[5].

The activities mentioned above are carried out under the control of genes, and gene mutation or expression disorder will influence the normal function of liver and lead to hereditary liver diseases[6]. For example, mutation of *ireb2* can lead to hereditary hemochromatosis (HHC)[7]; mutation of *atp7b* to Wilson disease (WD)[8]; mutations of *pfkm* and *phkg2* to glycogen storage disease (GSD)[9]; expression disorders of *tcf1, muc1, hmox1* and *prkaa2* to cystic fibrosis (CF)[10]; mutations of *apoe, smpd1, pex14, abcc2, shbg, gale, psap, gstz1* and *map2k5* to familial hypercholesterolemia (FH)[11], Niemann-Pick disease[12], Zellweger syndrome[13], Dubin-Johnson's syndrome[14], intrahepatic cholestasis[15], galactosemia[16], Gaucher disease[17], tyrosinemia[18], Caroli's disease[19] respectively.

To study the actions of the above genes during LR, the gene expression changes of the regenerating liver after 2/3 hepatectomy are checked by the Affymetrix

☆Supported by the National Natural Science Foundation of China, No. 30270673.

*Corresponding author. Tel: 86-373-3326001; Fax: 86-373-3326524; Email: xucs@x263.net

GeneChip Rat Genome 230 2.0 harboring 127 hereditary liver disease-associated genes, and 65 genes are identified to be associated with LR. The known gene functions, gene deficiency phenotypes and their expression changes during LR are compared to clarify the actions of these genes during LR.

2 Materials and Methods

2.1 Regenerating liver preparation

Healthy Sprague-Dawley rats weighing 200 – 250 g were obtained from the Animal Center of Henan Normal University. The rats were separated into 44 groups randomly, 22 hepatectomized groups and 22 sham-operation (SO) groups (n = 6; male: female = 1:1). PH was performed according to Higgins and Anderson[20], by which the left and middle lobes of liver were removed. The rats were sacrificed by cervical dislocation at 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 54, 66, 72, 120, 144 and 168 hours after PH and the regenerating livers were observed at the corresponding time point. The livers were immediately removed and rinsed three times with 1 × PBS and the tissues were quickly dissected on ice. The liver tissues of each group were pooled from about 100 – 200 mg of the middle parts of right lobe of each liver and stored at –80ºC. The SO group was handled as the same as hepatectomized group except the liver lobes were not removed. The laws of animal protection of China were enforced strictly.

2.2 RNA isolation and purification

Total RNA was isolated from the frozen livers according to the manual of Trizol reagent (Invitrogen Corporation, Carlsbad, California, USA)[21] and then purified following the RNeasy mini protocol (Qiagen, Inc, Valencia, CA, USA)[22]. The quality of total RNA samples were assessed by optical density measurement and agarose electrophoresis (180 V, 0.5 hour) with a 260/280 nm ratio 1.8 – 2.0 and 28S rRNA:18S rRNA ratio of 2.0[23].

2.3 cDNA, cRNA synthesis and purification

The 1 – 8 μg of total RNA as template was used for cDNA synthesis. cDNA purification, biotinylated cRNA synthesis and purification were performed following the Affymetrix protocol[24]. The quality and concentration of cDNA, cRNA were examined using the same procedure described above.

2.4 cRNA fragmentation and microarray detection

According to Affymetrix protocol, the 15 μl of the cRNA were incubated with 5 × fragmentation buffer at 94ºC for 35 min to generate 35 – 200 bp fragments and hybridized to the Rat Genome 230 2.0 expression microarray at 45ºC for 16 hours on a rotary mixer at 60 rpm. The microarray was then washed and stained with an Affymetrix GeneChip fluidics station 450 and scanned on GeneChip Scan 3000 (Affymetrix Inc, Santa Clara, CA, USA), and the signal values of gene expression were processed to collect raw data with GeneChip Operating Software (GCOS) 1.2[25].

2.5 Microarray data analysis

The normalized signal values, signal detections (P, A, M) and experiment/control (Ri) were obtained by quantifying and normalizing the signal values using GCOS 1.2[25].

2.6 Normalization of the microarray data

To minimize the technical error from the microarray analysis, each sample was hybridized three times to the GeneChips. The average value of three measurements was normalized, and statistics and cluster analysis were conducted on these values with GeneMath, GeneSpring (Silicon Genetics, San Carlos, CA) and Microsoft Excel Software (Microsoft, Redmond, WA)[25–27].

2.7 Identification of genes associated with LR

Firstly, the curated annotations describing hereditary liver diseases were adopted from the GENEONTOLOGY database (www.geneontology.org/), and input into the databases at NCBI (www.ncbi.nlm.nih.gov/) and RGD (rgd.mcw.edu/) to identify the rat, mouse and human genes associated with the above pathological process. Then, we collated the influential genes according to maps of biological pathways embodied by GENMAPP (www.genmapp.org), KEGG (www.genome.jp/kegg/pathway.html) and BIOCARTA (www.biocarta.com/genes/index.asp). The associated-genes were cross-checked through literature searches of the pertinent articles. Besides the rat genes, the genes, which were now thought to only exist in mouse and/or human and which exhibited more than two-fold change in the rat regenerating livers, were referred to as rat homologous genes. The genes that displayed reproducible result with the three independent analysis with the Rat Genome 230 2.0 Array, that revealed more than two-fold change in expression at least at one time point, and that showed a significant difference ($P \leq 0.05$) or an extremely significant difference ($P \leq 0.01$) between PH and SO, were included as being associated with LR.

3 Results

3.1 The changes in expression of the hereditary liver disease-associated genes during LR

According to the data of databases at NCBI, GENMAPP, KEGG and BIOCARTA, 166 genes were associated with hereditary liver diseases. 127 genes were contained in Rat Genome 230 2.0 Array. Among them, 65 genes revealed meaningful expression changes at least at one time point after PH , showed significant difference or extremely significant difference in expression when comparing PH with SO and displayed reproducible results with three analysis with Rat Genome 230 2.0 Array detection. The result suggested that the genes were associated with LR. The analysis indicated that 25 genes were up-regulated, 21 genes down-regulated, and 19 genes up/down-regulated in regenerating liver. The range of up-regulation was from 2 to 9.2 times higher than control, and that of down-regulation was 2 – 13.9 folds (Table 1).

Table 1. Expression profiles of 65 hereditary liver disease-associated genes during LR

Name	Gene Abbr.	Involved in others	Recovery time (h) after partial hepatectomy (PH)																						
			0	0.5	1	2	4	6	8	12	16	18	24	30	36	42	48	54	60	66	72	96	120	144	168
1 Hypercholesterolemia																									
UDP glycosyltransferase 1 family polypeptide A1	Ugt1a1		1.0	0.9	1.4	1.3	1.2	1.1	1.0	0.7	2.1 [1]	1.0	0.9	1.4	1.0	1.2	1.0	1.0	1.1	1.5	1.3	0.7	1.4	1.1	1.1
ATP-binding cassette sub-family B member 4	Abcb4		1.0	0.4 [2]	0.7	0.8	0.7	1.1	0.8	0.9	1.1	0.9	1.7	1.2	0.9	0.7	0.7	1.1	1.0	1.9	1.1	1.1	0.9	0.7	0.8
ATPase class I type 8B member 1	Atp8b1		1.0	0.5	0.8	0.6	0.5 [2]	0.8	0.6	0.7	1.4	1.0	1.1	1.1	0.5 [2]	1.3	0.6	0.6	0.4 [2]	1.1	0.9	1.4	1.1	1.0	1.1
ATP-binding cassette sub-family D member 2	Abcd2	12	1.0	0.6	0.4 [2]	0.7	0.7	0.6	0.6	0.9	0.5 [2]	0.4 [2]	0.5	0.7	0.5 [2]	0.8	0.3 [2]	0.4 [2]	0.6	0.8	0.7	1.5	0.5 [2]	0.4 [2]	0.5 [2]
sex hormone binding globulin	Shbg		1.0	1.7	0.7	1.3	1.6	0.9	1.3	1.5	1.0	0.5 [2]	0.1 [2]	2.8 [1]	0.3 [2]	0.8	0.2 [2]	0.3 [2]	0.3 [2]	0.7	0.9	0.7	0.7	0.8	1.1
ATP-binding cassette sub-family B member 11	Abcb11		1.0	1.1	1.2	1.1	1.3	2.0 [1]	1.4	1.0	2.1 [1]	0.9	0.8	0.5 [2]	0.6	0.9	0.4 [2]	1.1	0.7	1.1	1.0	1.2	1.2	1.1	1.2
2 Hemochromatosis																									
transferrin receptor 2	Trfr2		1.0	1.2	1.1	1.0	1.1	1.1	1.2	1.1	1.2	1.3	1.3	2.2 [1]	0.7	1.3	0.9	1.2	1.0	1.6	1.3	3.2 [1]	1.9	1.7	1.6
iron responsive element binding protein 2	Ireb2		1.0	0.5	1.3	1.2	1.8	1.8	2.1 [1]	2.9 [1]	3.3 [1]	1.0	0.8	2.0 [1]	1.3	1.9	0.6	0.7	0.8	1.7	1.6	3.0 [1]	2.3 [1]	2.8 [1]	2.6 [1]
transferrin receptor	Tfrc		1.0	1.2	1.7	1.0	1.1	2.0 [1]	1.7	1.0	0.9	1.9	1.7	1.1	1.7	1.4	1.6	1.7	2.1 [1]	3.5 [1]	1.8	1.2	2.7 [1]	2.6 [1]	2.9 [1]
aconitase 1	Aco1		1.0	0.7	0.9	0.8	0.6	0.5	0.5	0.5 [2]	1.1	0.8	0.8	1.1	0.9	1.1	0.9	1.1	1.0	1.4	1.2	0.9	1.2	1.1	1.0
solute carrier family 40 member 1	Slc40a1		1.0	0.6	0.8	0.7	0.6	0.6	0.7	0.5	0.9	0.7	0.7	0.9	0.7	0.9	0.5 [2]	0.7	0.8	1.1	0.9	0.9	1.5	1.2	1.4
ubiquitin-conjugating enzyme E2D 1	Ube2d1		1.0	1.3	1.9	0.3 [2]	0.2 [2]	0.7	1.0	0.8	4.6 [1]	1.6	1.2	1.5	1.0	5.1 [1]	2.0 [1]	1.9	1.1	1.5	1.7	1.5	1.3	0.9	1.0
3 Cystic fibrosis																									
transcription factor 1	Tcf1		1.0	1.0	6.1 [1]	3.2 [1]	2.1 [1]	6.1 [1]	6.2 [1]	6.1 [1]	2.6 [1]	4.9 [1]	2.7 [1]	3.2 [1]	4.6 [1]	3.6 [1]	6.5 [1]	5.3 [1]	3.2 [1]	6.5 [1]	6.8 [1]	3.5 [1]	3.7 [1]	3.0 [1]	3.0 [1]
S100 calcium binding protein A8	S100a8		1.0	1.2	1.3	3.5 [1]	6.5 [1]	4.0 [1]	3.3 [1]	4.0 [1]	2.1 [1]	3.0 [1]	3.5 [1]	4.5 [1]	3.0 [1]	3.4 [1]	2.8 [1]	3.7 [1]	2.6 [1]	4.9 [1]	3.4 [1]	1.7	2.1 [1]	0.9	0.9
S100 calcium binding protein A9	S100a9		1.0	1.1	1.5	3.0 [1]	4.9 [1]	2.6 [1]	2.0 [1]	2.1 [1]	0.8	1.0	1.1	1.3	1.3	1.0	2.6 [1]	2.1 [1]	1.0	1.1	2.6 [1]	0.7	2.0 [1]	0.7	0.8
transforming growth factor beta 1	Tgfb1		1.0	0.9	0.5	1.1	1.1	1.1	1.0	1.1	2.1 [1]	3.7 [1]	4.0 [1]	1.8	1.2	2.7 [1]	2.1 [1]	0.9	1.1	1.6	0.7	1.4	0.9	0.8	0.9
STIP1 homology and U-box containing protein 1	Stub1		1.0	1.0	1.1	1.0	1.0	1.4	1.4	1.5	2.2 [1]	1.5	1.3	0.9	1.2	1.0	0.9	1.2	1.0	1.4	1.2	1.0	0.9	0.9	0.9
transporter 1 ATP-binding cassette sub-family B	Tap1		1.0	1.4	0.9	1.0	1.1	1.0	0.6	0.8	0.7	0.7	1.3	0.9	1.3	1.0	2.2 [1]	0.7	0.7	1.5	1.4	1.0	1.0	1.1	1.2
chloride channel 2	Clcn2		1.0	1.1	1.2	0.6	0.7	1.6	1.0	1.5	2.8 [1]	1.6	0.9	1.0	1.3	2.7 [1]	1.6	1.1	2.1 [1]	1.1	1.0	2.8 [1]	1.1	2.0 [1]	1.5
ATP-binding cassette sub-family B member 11	Abcb1		1.0	0.5	0.6	0.9	0.8	0.9	0.7	1.3	0.6	4.6 [1]	3.7 [1]	0.5	3.0 [1]	0.6	1.4	1.6	1.3	4.0 [1]	2.8 [1]	0.5	1.2	0.7	0.9
chloride channel calcium activated family member 2	Clca2		1.0	1.0	1.8	1.3	1.1	0.9	0.8	0.7	1.1	0.5 [2]	0.6	1.9	0.7	1.8	0.5 [2]	0.7	0.8	0.9	0.9	1.0	0.8	1.1	0.9
chloride channel calcium activated 4	Clca4		1.0	0.5 [2]	0.3 [2]	0.7	2.3 [1]	5.7 [1]	0.6	0.8	0.8	0.5 [2]	0.5 [2]	0.6	0.8	0.3 [2]	0.9	0.8	3.0 [1]	0.7	1.1	0.7	0.7	0.9	0.3 [2]
sulfotransferase family cytosolic 2B member 1	Sult2b1		1.0	1.0	1.0	1.0	0.9	0.9	0.9	0.8	1.0	1.8	0.5 [2]	1.0	1.3	1.0	3.1 [1]	0.7	1.2	0.4 [2]	0.8	1.1	0.4 [2]	0.7	0.4 [2]
ubiquitin-conjugating enzyme E2D 1	Ube2d1		1.0	1.3	1.9	0.3 [2]	0.2 [2]	0.7	1.0	0.8	4.6 [1]	1.6	1.2	1.5	1.0	5.1 [1]	2.0 [1]	1.9	1.1	1.5	1.7	1.5	1.3	0.9	1.0
4 Familial hypercholesterolemia																									
amyloid beta precursor protein	App	5	1.0	1.1	1.0	1.3	1.2	1.6	1.6	1.3	0.7	2.9 [1]	2.5 [1]	0.8	2.1 [1]	0.7	1.7	2.4 [1]	2.6 [1]	5.8 [1]	3.1 [1]	0.9	5.0 [1]	5.2 [1]	6.4 [1]
ATP-binding cassette sub-family B member 1A	Abcb1a	5	1.0	0.5	0.6	0.9	0.8	0.9	0.7	1.3	0.6	4.6 [1]	3.7 [1]	0.5	3.0 [1]	0.6	1.4	1.6	1.3	4.0 [1]	2.8 [1]	0.5	1.2	0.7	0.9
low density lipoprotein receptor	Ldlr		1.0	0.7	1.2	1.1	0.8	4.4 [1]	0.8	1.0	2.5 [1]	3.1 [1]	5.1 [1]	5.8 [1]	2.0 [1]	1.2	2.2 [1]	4.1 [1]	1.0	1.7	0.8	1.5	1.1	1.1	1.8
apolipoprotein E	Apoe	5	1.0	0.9	1.2	0.9	0.9	1.1	1.0	0.8	0.2 [2]	0.9	1.1	0.1 [2]	0.9	0.2 [2]	1.0	1.0	0.9	1.1	1.2	0.3 [2]	1.4	1.2	1.1
paraoxonase 1	Pon1		1.0	1.2	1.0	1.1	1.1	0.9	0.9	1.1	0.8	0.6	1.0	1.1	0.6	1.3	0.4 [2]	0.6	0.5 [2]	0.9	1.1	0.7	1.0	0.9	1.0
arachidonate 12-lipoxygenase	Alox12		1.0	1.2	0.3 [2]	0.8	1.9	1.5	1.3	1.4	4.0 [1]	1.1	0.7	4.8 [1]	1.3	3.9 [1]	0.7	0.9	1.1	1.0	0.5 [2]	2.6 [1]	0.5 [2]	0.9	1.0
5 Niemann-Pick disease																									
amyloid beta precursor protein	App	4	1.0	1.1	1.0	1.3	1.2	1.6	1.6	1.3	0.7	2.9 [1]	2.5 [1]	0.8	2.1 [1]	0.7	1.7	2.4 [1]	2.6 [1]	5.8 [1]	3.1 [1]	0.9	5.0 [1]	5.2 [1]	6.4 [1]
ATP-binding cassette sub-family B member 1A	Abcb1a	4	1.0	0.5	0.6	0.9	0.8	0.9	0.7	1.3	0.6	4.6 [1]	3.7 [1]	0.5	3.0 [1]	0.6	1.4	1.6	1.3	4.0 [1]	2.8 [1]	0.5	1.2	0.7	0.9
tachykinin 1	Tac1		1.0	0.8	0.3 [2]	1.0	0.8	0.8	0.5	0.8	1.0	0.3 [2]	0.2 [2]	0.4 [2]	0.3 [2]	0.5	0.7	0.6	1.0	0.2 [2]	0.2 [2]	0.8	0.2 [2]	0.3 [2]	0.2 [2]
chitinase 1	Chit1	11	1.0	0.8	0.7	1.4	1.9	0.9	1.0	0.9	1.3	0.2 [2]	0.1 [2]	1.3	0.5 [2]	1.1	0.3 [2]	0.1 [2]	0.1 [2]	0.1 [2]	0.5	1.0	0.4 [2]	1.0	0.7
apolipoprotein E	Apoe	4	1.0	0.9	1.2	0.9	0.9	1.1	1.0	0.8	0.2 [2]	0.9	1.1	0.1 [2]	0.9	0.2 [2]	1.0	1.0	0.9	1.1	1.2	0.3 [2]	1.4	1.2	1.1
solute carrier family 6 member 1	Slc6a1		1.0	0.9	0.3 [2]	1.1	6.1 [1]	2.8 [1]	2.0 [1]	2.5 [1]	2.3 [1]	3.2 [1]	0.6	1.5	0.8	1.6	1.1	1.2	1.9	6.5 [1]	0.4 [2]	1.7	0.4 [2]	2.5 [1]	1.4
6 Wilson disease																									
copper metabolism domain containing 1	Commd1		1.0	1.1	1.8	1.0	0.8	0.8	0.7	0.7	4.9 [1]	4.8 [1]	2.0 [1]	3.9 [1]	1.4	5.5 [1]	1.5	2.0 [1]	2.0 [1]	2.1 [1]	1.7	9.2 [1]	1.1	0.8	0.9
prepronociceptin	Pnoc		1.0	0.9	1.6	1.7	1.1	1.0	1.0	4.9 [1]	1.9	1.1	0.5	0.7	1.2	1.5	0.8	2.5 [1]	1.7	0.7	2.6 [1]	1.0	2.6 [1]	2.0 [1]	3.5 [1]
solute carrier family 31 member 1	Slc31a1		1.0	1.0	1.3	1.3	1.0	0.8	0.8	0.9	1.4	1.1	1.0	1.4	1.1	1.2	0.8	1.0	1.0	1.7	1.3	1.2	2.0	2.2 [1]	2.0 [1]
tumor necrosis factor receptor superfamily member 11b	Tnfrsf11b		1.0	3.7 [1]	8.7 [1]	3.2 [1]	3.0 [1]	6.5 [1]	3.8 [1]	3.7 [1]	5.5 [1]	6.1 [1]	7.5 [1]	0.4 [2]	3.7 [1]	1.0	5.7 [1]	6.1 [1]	5.3 [1]	8.0 [1]	7.3 [1]	0.3 [2]	6.1 [1]	4.9 [1]	3.7 [1]
7 Glycogen storage disease																									
glucose-6-phosphatase catalytic	G6pc		1.0	7.6 [1]	11.2 [1]	4.6 [1]	3.7 [1]	3.4 [1]	3.9 [1]	4.2 [1]	3.7 [1]	3.0 [1]	4.0 [1]	1.4	1.8	1.5	2.0 [1]	3.2 [1]	2.9 [1]	4.0 [1]	6.0 [1]	0.6	5.5 [1]	7.0 [1]	7.3 [1]
phosphofructokinase muscle	Pfkm		1.0	0.9	0.7	1.3	1.3	1.4	1.1	1.3	0.8	1.3	1.7	9.6 [1]	0.9	6.9 [1]	1.3	1.4	2.1 [1]	0.9	0.7	1.4	0.6	0.7	0.7
solute carrier family 37 member 4	Slc37a4		1.0	1.9	1.3	1.3	1.1	0.9	0.6	0.5 [2]	1.0	0.8	1.0	1.1	0.5 [2]	1.0	0.5 [2]	0.6	0.4 [2]	1.2	1.0	0.9	1.0	1.4	1.3
glucosidase alpha acid	Gaa		1.0	0.9	0.9	1.0	0.7	0.8	0.9	0.8	1.2	0.7	0.5 [2]	1.3	0.6	1.4	0.6	0.6	0.8	1.1	1.0	1.2	1.4	1.3	1.5
phosphorylase kinase alpha 2	Phka2		1.0	0.9	0.8	1.2	1.3	1.2	0.9	1.2	1.4	0.5	0.6	1.8	0.7	1.5	0.4 [2]	0.7	0.8	0.8	0.7	1.1	0.7	0.7	0.7
liver glycogen phosphorylase	Pygl		1.0	1.7	2.0 [1]	1.4	1.3	0.8	0.5	0.4 [2]	1.2	0.8	1.2	1.0	1.3	0.8	1.2	1.6	1.3	1.6	1.4	0.8	1.6	1.5	1.4

amylo-1,6-glucosidase	Agl		1.0	0.5 [2]	1.1	1.1	0.4 [2]	0.3 [2]	0.3 [2]	0.4 [2]	1.0	1.2	0.6	2.2 [1]	0.9	0.8	0.5	1.1	0.6	1.4	1.0	1.2	1.6	1.3	1.5
muscle glycogen phosphorylase	Pygm		1.0	1.2	2.0 [1]	1.4	2.0	1.7	1.3	1.3	0.8	0.4 [2]	0.5 [2]	0.8	0.5 [2]	1.1	0.7	0.5	0.6	0.8	0.7	1.1	1.2	0.8	0.9
glucan branching enzyme 1	Gbe1		1.0	2.0 [1]	2.5 [1]	1.5	1.5	0.9	0.8	0.5 [2]	1.0	1.0	1.2	2.6 [1]	1.0	4.2 [1]	0.8	1.2	0.9	1.1	1.3	2.8 [1]	1.2	1.6	1.6
8 Galactosemia																									
galactose-4-epimerase	Gale		1.0	0.7	0.9	0.9	0.8	0.9	0.7	1.4	2.1 [1]	1.6	1.3	1.2	1.4	1.2	0.9	1.1	1.2	2.3 [1]	1.5	0.9	1.2	0.9	0.9
UDP-glucuronosyltransferase 8	Ugt8		1.0	1.0	0.4 [2]	1.2	1.2	1.2	1.0	1.3	1.8	0.4 [2]	0.4 [2]	1.1	0.5	1.6	0.8	0.9	1.6	0.7	1.9	1.8	0.5 [2]	0.5 [2]	0.5 [2]
galactokinase 1	Galk1		1.0	0.6	0.9	0.5	0.5 [2]	0.5	0.5 [2]	0.5 [2]	0.9	0.9	1.3	0.6	1.1	0.8	0.9	1.1	1.1	1.5	1.1	0.6	0.8	0.8	0.8
galactose-1-phosphate uridyl transferase	Galt		1.0	1.0	1.1	0.7	0.6	0.4 [2]	0.4 [2]	0.4 [2]	1.3	0.8	0.9	1.0	0.8	0.8	0.7	1.1	0.8	1.1	1.0	1.4	0.7	0.7	0.8
9 Tyrosinemia																									
fumarylacetoacetate hydrolase	Tat		1.0	1.7	2.3 [1]	2.0 [1]	2.3 [1]	2.8 [1]	2.7 [1]	1.0	1.9	0.9	0.8	1.3	0.6	1.6	0.8	0.8	0.8	1.1	1.4	1.5	1.9	1.9	1.9
fumarylacetoacetate hydrolase	Fah		1.0	1.1	1.4	1.0	0.9	0.9	0.7	0.4 [2]	0.8	0.6	0.6	1.0	0.7	1.0	0.9	1.0	1.0	1.4	1.4	0.8	1.2	1.1	1.1
glutathione transferase zeta 1	Gstz1		1.0	1.1	1.9	1.1	1.1	0.9	1.1	0.6	0.8	0.7	0.5	0.7	0.4 [2]	1.0	0.6	1.1	0.9	1.4	1.5	0.7	1.4	1.5	1.6
10 Congenital cyst of liver																									
tumor necrosis factor receptor superfamily member 11b	Tnf	11	1.0	0.9	0.6	1.2	1.4	1.1	1.2	0.9	0.9	0.7	0.7	0.8	1.4	0.8	1.6	1.1	1.1	0.9	0.5	1.2	3.2 [1]	0.8	0.7
endothelin 1	Edn1		1.0	1.2	0.6	1.6	1.1	1.3	1.1	1.2	0.7	0.4 [2]	0.4 [2]	2.6 [1]	1.0	1.5	1.0	0.7	1.3	0.8	0.7	1.3	0.8	0.7	0.7
11 Gaucher disease																									
tumor necrosis factor receptor superfamily member 11b	Tnf	10	1.0	0.9	0.6	1.2	1.4	1.1	1.2	0.9	0.9	0.7	0.7	0.8	1.4	0.8	1.6	1.1	1.1	0.9	0.5	1.2	3.2 [1]	0.8	0.7
B-cell leukemia/lymphoma 2	*Bcl2		1.0	0.8	0.6	1.1	1.0	1.2	1.2	1.2	0.8	0.3 [2]	0.4 [2]	1.8	0.3 [2]	0.8	0.4 [2]	0.4 [2]	0.5 [2]	0.8	0.4 [2]	0.8	0.5	0.7	0.6
chitinase 1	Chit1	5	1.0	0.8	0.7	1.4	1.9	0.9	1.0	0.9	1.3	0.2 [2]	0.1 [2]	1.3	0.5 [2]	1.1	0.3 [2]	0.1 [2]	0.1 [2]	0.1 [2]	0.5	1.0	0.4 [2]	1.0	0.7
12 Zellweger syndrome																									
isopentenyl-diphosphate delta isomerase	Idi1		1.0	0.3 [2]	0.7	0.7	0.4 [2]	0.3 [2]	0.4 [2]	0.5 [2]	1.1	0.6	0.2 [2]	1.3	0.6	1.2	0.2 [2]	0.6	0.8	1.1	0.6	1.0	0.5	0.4 [2]	0.4 [2]
ATP-binding cassette sub-family D member 2	Abcd2	1	1.0	0.6	0.4 [2]	0.7	0.7	0.6	0.6	0.9	0.5 [2]	0.4 [2]	0.5	0.7	0.5 [2]	0.8	0.3 [2]	0.4 [2]	0.6	0.8	0.7	1.5	0.5 [2]	0.4 [2]	0.5 [2]
peroxisomal biogenesis factor 14	Pex14		1.0	1.1	0.7	0.7	1.0	0.8	0.8	0.6	1.3	0.4 [2]	0.5	2.5 [1]	0.5	2.3 [1]	0.5	0.7	0.4 [2]	0.7	0.7	1.1	0.6	1.2	1.0
peroxisome biogenesis factor 26	Pex26		1.0	0.7	0.4 [2]	0.7	0.7	0.6	1.8	0.8	0.8	4.3 [1]	1.4	1.0	0.9	1.1	1.5	4.9 [1]	1.7	0.5 [2]	0.8	1.1	2.1 [1]	0.7	2.3 [1]
acyl-coenzyme A oxidase 2	Acox2		1.0	4.3 [1]	4.0 [1]	2.6 [1]	2.5 [1]	1.6	1.1	0.9	1.3	4.6 [1]	2.7 [1]	0.8	2.8 [1]	0.2 [2]	1.7	1.4	2.6 [1]	2.1 [1]	3.0 [1]	0.2 [2]	4.6 [1]	7.5 [1]	6.5 [1]
13 Hereditary fructose intolerance																									
aldolase B	Aldob		1.0	1.0	1.2	0.9	0.8	0.8	0.6	0.7	1.2	0.9	1.1	2.4 [1]	0.9	1.9	0.9	0.9	0.8	1.1	1.2	1.5	1.1	1.1	0.9
14 Essential fructosuria																									
ketohexokinase	Khk		1.0	1.3	1.6	1.1	1.1	0.7	0.5	0.5 [2]	0.5	0.6	0.8	3.6 [1]	0.6	2.1 [1]	0.6	1.0	0.8	1.5	1.2	1.0	1.1	1.2	1.2
15 Acute intermittent porphyria																									
hydroxymethylbilane synthase	Hmbs		1.0	1.0	1.1	0.9	0.6	0.5 [2]	0.8	1.0	1.2	1.1	1.5	0.9	1.2	1.3	1.1	1.3	1.1	1.9	1.4	1.6	0.9	1.1	0.9
16 Congenital hyperammonemia																									
ornithine transcarbamylase	Otc		1.0	1.3	2.0 [1]	1.2	1.1	1.0	0.8	0.4 [2]	0.6	0.7	0.7	0.9	0.5 [2]	1.0	0.7	0.8	0.9	1.2	1.3	1.0	1.1	1.3	1.3
17 Caroli's disease																									
mitogen activated protein kinase kinase 5	Map2k5		1.0	1.1	0.8	0.9	0.9	0.9	1.0	1.3	1.9	0.5 [2]	0.9	1.6	0.9	2.2 [1]	0.7	0.4 [2]	0.8	1.4	0.7	1.6	0.9	0.9	0.8

Asterisk represents the reported genes associated with liver regeneration; [1]: indicate genes with expression larger than or equal to 2-fold; [2]: represent genes with expression less than or equal to 2-fold

3.2 Initial expression time of the hereditary liver disease-associated genes during LR

At each time point of LR, the numbers of initial up-, down-regulated and total up-, down-regulated genes were shown as Table 2. In the respect of the initial expressions of the above 65 genes, 32 and 33 genes were initially up-regulated and down-regulated during LR, respectively. A detailed introduction is as follows: at the initiation stage (0.5 – 4 hours after PH), the G0/G1 transition phase (4 – 6 hours after PH), cell proliferation period (6 – 66 hours after PH), redifferentiation and the structure-function reorganization stage of LR (72 – 168 hours after PH), the number of initially up and down-regulated genes were 11 and 13, 3 and 4, 19 and 20, and 2 and 0. The whole situation of the genes expression was that total frequencies of up and down-regulated expression were respectively 228 and 129. Specifically, at the above-mentioned four phases of LR, the number of times of up-regulation and down-regulation was separately 29 and 17, 20 and 9, 148 and 88, 51 and 24 (Figure 1).

4 Discussion

The roles of hereditary liver disease-associated genes are analyzed during rat LR. Among genes associated with hereditary bilirubinemia, ABC family is associated with transport of bile salts[28]. ATP8B1 and SHBG are involved in transport of bile acid, and their mutations could lead

Table 2. The numbers of initially and totally expressed genes at every recovery time point after PH

Expression Change		Recover time (h)																						
		0.5	1	2	4	6	8	12	16	18	24	30	36	42	48	54	60	66	72	96	120	144	168
Initial expression	up regulation	4	5	2	0	3	2	1	6	3	0	3	0	0	2	0	0	0	0	0	1	1	0
	down regulation	4	6	1	2	2	0	4	1	7	2	0	1	0	3	0	0	0	0	0	0	0	0
Total expression	up regulation	4	9	7	9	11	9	8	16	13	12	15	8	12	10	10	11	13	11	7	12	11	10
	down regulation	4	7	1	5	4	4	11	2	12	11	4	10	3	11	5	7	4	4	3	7	4	6

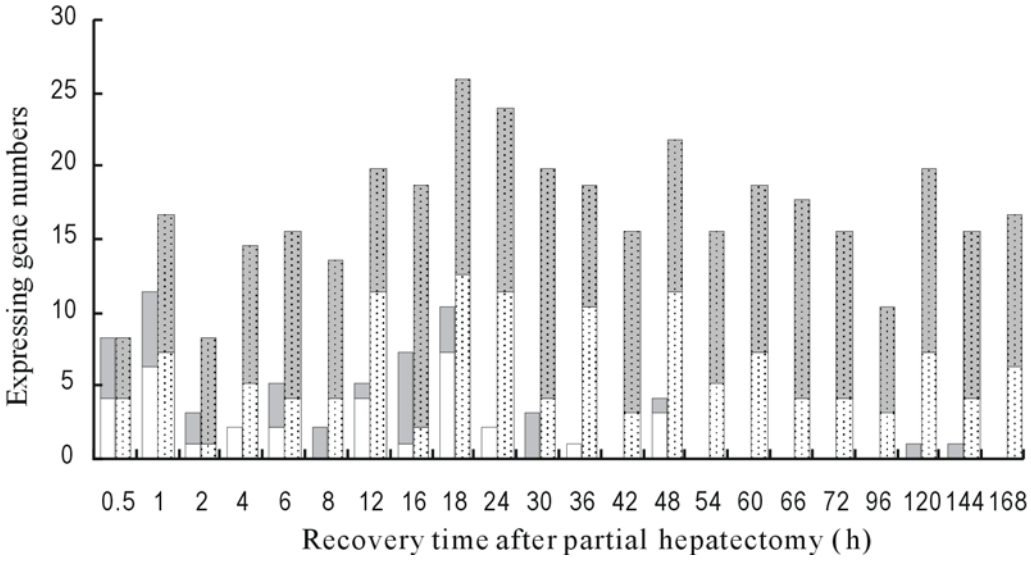

Figure 1. The initial and total expression profiles of 65 hereditary liver disease-associated genes at each time point of LR. Blank bars: Initially expressing gene number; Dotted bars: Total expressing gene number; Grey-background bars: Up-regulated genes; White-background bars: Down-regulated genes. Expression change of the genes spans the whole live regeneration. Initially up-regulated genes are predominate at 2 – 8, 16, 30 and 120 – 144 hours after PH; initially down-regulated genes are overwhelm at 1, 4, 12, 18 – 24, 36 and 48 hours; there are no genes initially expressed at 42, 54 – 96 and 168 hours.

to familial intrahepatic cholestasis[29,30]. UGT1A1 associated with bilirubin degradation *in vivo*, whose inactivity could lead to hereditary bilirubinemia[31]. The expression changes of the genes mentioned above were same or similar at some time points and different at other time points during LR, demonstrating they regulate bilirubin metabolism together.

Of HHC-associated genes, ACO1 regulates iron storage and transport[32]. SLC40A1 is responsible for iron absorption[33]. Both IREB2 and TFR are involved in iron transport[7,34]. The expression changes of the genes mentioned above showed the same or similar level at some time points, while different at other time points during LR, demonstrating that these genes adjust metabolism and utilization of iron during LR simultaneously.

Of CF-associated genes, *abcb1* promotes intracellular toxic substance excretion, and its mutation could lead to CF[35]. Up-regulation of the gene in the metaphase of LR indicates to detoxification of liver is enhanced in the corresponding period. *ube2d1* is involved in protein ubiquitination[36]. It is down regulated at 2 – 4 hours after PH and up at 16 and 42 – 48 hours, demonstrating that ubiquitination is rather simple during LR. *clcn2, clca2, clca4, tcf1* and *stub1* can promote chlorine ions transmembrane transport[37–40]. The expression changes of the genes were same or similar at some time points and different at others during LR, demonstrating they together regulate chloride balance in cell.

Of FH-associated genes, ABCB1A and APOE promote cholesterol transport[11,41]. APP is involved in cholesterol metabolism[42]. LDLR is responsible for cholesterol transport, and its mutation could lead to FH[11]. PON1 is associated with cholesterol release[43]. ALOX12 relating to FH is involved in low density lipoprotein absorption[44]. The expression changes of the genes mentioned above were same or similar at some time points and different at other time points during LR, demonstrating they modulate the metabolism of cholesterol together.

SLC6A1 and CHIT1, as Niemann-Pick disease-associated genes, the former is responsible for pain[45] and the latter is marker of Niemann-Pick disease[46]. *slc6a1* is up-regulated at 4 – 18 hours after PH, indicating an increase in pain response. *chit1* is down-regulated at 18 – 24, 36, 48 – 66 and 120 hours after PH indicating there is no expression of related protein.

Of WD-associated genes, COMMD1 regulates copper metabolism, and its mutation could cause copper poisoning[47]. SLC31A1 is response for transmembrane transport of copper[48]. Both of them separately are up-regulated at the metaphase and anaphase during LR, demonstrating copper metabolism and utilization are enhanced in the corresponding period. PNOC mediate immune response[49] and TNFRSF11B can prevent arterial calcification[50]. Of up-regulation of them during LR are possibly

associated with maintenance of normal structure of the blood vessel.

Of GSD-associated genes, G6PC may have to do with gluconeogenesis. GBE1 promotes glycogen synthesis. GAA, AGL, PHKA2, PFKM, PYGL and PYGM play the roles in glycogen degradation[9,51]. SLC37A4 hastens glucose transport[52], and mutation, deletion or abnormal expression of these genes can lead to GSD. The expression changes of the above genes were same or similar at some time points and different at other time points during LR, demonstrating that they together regulate glycogen metabolism and maintain glycogen balance in the corresponding period.

Of galactosemia-associated genes, GALE, GALK1 and GALT are all involved in galactose metabolism[16]. UGT8 facilitates biotransformation[53]. The expression changes of the genes mentioned above tend to be same or similar at some time points and different at other time points during LR, demonstrating that they regulate galactose metabolism in the corresponding period together.

TAT, GSTZ1 and FAH, associated with tyrosinemia, are all involved in tyrosine catabolic pathway[54–56]. Up-regulation of *tat* appears at 1 – 8 hours after PH, down of *fah* and *gstz1* take place respectively at 12 hours and 30 hours, demonstrating that they regulate tyrosine metabolism in the corresponding period together.

EDN1 and TNF relate to congenital cyst of liver and Gaucher disease, and the former promotes cell proliferation and prevents apoptosis[57], the latter suppresses tumorigenesis and triggers apoptosis[58]. They tend to be up-regulated at 30 hours and 120 hours after PH, suggesting they together hamper cell transformation and maintain quality and quantity of the regenerating liver.

Of Zellweger syndrome-associated genes, PHYH and ACOX2 are involved in fatty acid oxidation[59]. According to their up-regulation during LR, it can be conferred fatty acid metabolism maybe speed up in the corresponding period. PEX14 and PEX16 are involved in peroxisomal biogenesis, and could bring on Zellweger syndrome if mutation[60]. Both of them are up-regulated at some time points, and down at others, demonstrating they co-regulate peroxisome formation.

In addition, fructose metabolism-associated genes products ALDOB and KHK promote fructose degradation. HMBS, OTC and MAP2K5, which are separately products of acute intermittent porphyria-, congenital hyperammonemia- and Caroli disease-associated gene, involved in heme biosynthesis[61], urea formation[62] and regulation formation of bile[19] in sequence. The expression changes of the genes mentioned above were same or similar at some time points and different at other time points during LR, demonstrating they regulate cell metabolism of the corresponding period together.

In summary, 65 hereditary liver disease-associated genes are associated with LR and closely associated with many physiological and biochemical processes of the regenerating liver. For the future, we will use Northern blotting, protein array, and RNA interference etc. to confirm the above result at the cell level.

References

1. Taub R. Liver regeneration: from myth to mechanism. Nat Rev Mol Cell Biol 2004; 5 (10): 836 – 7.
2. Xu CS, Chang CF, Yuan JY, Li WQ, Han HP, Yang KJ, Zhao LF, Li YC, Zhang HY, Rahman S, Zhang JB. Expressed genes in regenerating rat liver after partial hepatectomy. World J Gastroenterol 2005; 11 (19): 2932 – 40.
3. Suzuki T, Tsukamoto I. Apoptosis induced by 5-(N, N-hexamethylene)-amiloride in regenerating liver after partial hepatectomy. European J Pharmacolog 2004; 503 (1-3): 1 – 7.
4. Xu CS, Zhao LF, Yang KJ, Zhang JB. The origin and function of liver stem cells. Acta Biologiae Experimentalis Sinica 2004; 37 (2): 72 – 7.
5. Su AI, Guidotti LG, Pezacki JP, Chisari FV, Schultz PG. Gene expression during the priming phase of liver regeneration after partial hepatectomy in mice. PNAS 2002; 17 (99): 11181 – 6.
6. Schilsky ML, Oikonomou I. Inherited metabolic liver disease. Curr Opin Gastroenterol 2005; 21 (3): 275 – 82.
7. Schilsky ML, Fink S. Inherited metabolic liver disease. Curr Opin Gastroenterol 2006; 22 (3): 215 – 22.
8. Cox DW. Review: molecular approaches to inherited liver disease. Focus on Wilson disease. J Gastroenterol Hepato 1997; 12 (9-10): 251 – 5.
9. Moses SW. Historical highlights and unsolved problems in glycogen storage disease type 1. Eur J Pediat 2002; 161: 2 – 9.
10. Schwarz KB, Rosensweig J, Sharma S, Jones L, Durant M, Potter C, Narkewicz MR. Plasma markers of platelet activation in cystic fibrosis liver and lung disease. J Pediatr Gastroenterol Nutr 2003; 37 (2): 187 – 91.
11. Choumerianou DM, Dedoussis GV. Familial hypercholesterolemia and response to statin therapy according to LDLR genetic background. Clin Chem Lab Med 2005; 43 (8): 793 – 801.
12. Erickson RP, Bhattacharyya A, Hunter RJ, Heidenreich RA, Cherrington NJ. Liver disease with altered bile acid transport in Niemann-Pick C mice on a high-fat, 1% cholesterol diet. Am J Physiol Gastrointest Liver Physiol 2005; 289 (2): 300 – 7.
13. Mochel F, Grebille AG, Benachi A, Martinovic J, Razavi F, Rabier D, Simon I, Boddaert N, Brunelle F, Sonigo P. Contribution of fetal MR imaging in the prenatal diagnosis of Zellweger syndrome. AJNR Am J Neuroradiol 2006; 27 (2): 333 – 6.
14. Fretzayas A, Kitsiou S, Papadopoulou A, Nicolaidou P. Clinical expression of co-inherited Dubin-Johnson and thalassaemic heterozygous states. Dig Liver Dis 2007; 39(4): 369 – 74.
15. Carlton VE, Pawlikowska L, Bull LN. Molecular basis of intrahepatic cholestasis. Ann Med 2004; 36 (8): 606 – 17.
16. Schwarz M, Wendel U. Inborn errors of metabolism (IEM) in adults. A

new challenge to internal medicine. Med Klin 2005; 100 (9): 547 – 52.
17. Germain DP, Mistry P. Phenotype variations in Gaucher disease. Rev Med Interne 2006; 27 (Suppl 1): S3 – 10.
18. Scott CR. The genetic tyrosinemias. Am J Med Genet C Semin Med Genet 2006; 142 (2): 121 – 6.
19. Gupta AK, Gupta A, Bhardwaj VK, Chansoria M. Caroli's disease. Indian J Pediatr 2006; 73 (3): 233 – 5.
20. Higgins GM, Anderson RM. Experimental pathology of the liver: restoration of the liver of the white rat following partial surgical removal. Arch Pathol 1931; 12: 186 – 202.
21. Knepp JH, Geahr MA, Forman MS, Valsamakis A. Comparison of automated and manual nucleic acid extraction methods for detection of enterovirus RNA. J Clin Microbiol 2003; 41 (8): 3532 – 6.
22. Nuyts S, Van Mellaert L, Lambin P, Anne J. Efficient isolation of total RNA from Clostridium without DNA contamination. J Microbiol Methods 2001; 44 (3): 235 – 8.
23. Arkin A, Ross J, McAdams HH. Stochastic kinetic analysis of developmental pathway bifurcation in phage lambda-infected *Escherichia coli* cells. Genetics 1998; 149 (4): 1633 – 48.
24. Li L, Roden J, Shapiro BE, Wold BJ, Bhatia S, Forman SJ, Bhatia R. Reproducibility, fidelity, and discriminant validity of mRNA amplification for microarray analysis from primary hematopoietic cells. J Mol Diagn 2005; 7 (1): 48 – 56.
25. Hood L. Leroy Hood expounds the principles, practice and future of systems biology. Drug Discov Today 2003; 8 (10): 436 – 8.
26. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. PNAS 1998; 95: (25) 14863 – 8.
27. Werner T. Cluster analysis and promoter modelling as bioinformatics tools for the identification of target genes from expression array data. Pharmacogenomics 2001; 2 (1): 25 – 36.
28. Van Mil SW, van der Woerd WL, van der Brugge G, Sturm E, Jansen PL, Bull LN, Berger R, Houwen RH, Klomp LW. Benign recurrent intrahepatic cholestasis type 2 is caused by mutations in ABCB11. Gastroenterology 2004; 127 (2): 379 – 84.
29. Klomp LW, Vargas JC, van Mil SW, Pawlikowska L, Strautnieks SS, van Eijk MJ, Juijn JA. Characterization of mutations in ATP8B1 associated with hereditary cholestasis. Hepatology 2004; 40 (1): 27 – 38.
30. Ropponen A, Aittomaki K, Vihma V, Tikkanen MJ, Ylikorkala. Effects of oral and transdermal estradiol administration on levels of sex hormone-binding globulin in postmenopausal women with and without a history of intrahepatic cholestasis of pregnancy. J Clin Endocrinol Metab 2005; 90 (6): 3431 – 4.
31. Jemnitz K, Lengyel G, Vereczkey L. *In vitro* induction of bilirubin conjugation in primary rat hepatocyte culture. Biochem Biophys Res Commun 2002; 291 (1): 29 – 33.
32. Neonaki M, Graham DC, White KN, Bomford A. Down-regulation of liver iron-regulatory protein 1 in haemochromatosis. Biochem Soc Trans 2002; 30 (4): 726 – 8.
33. Sham RL, Phatak PD, West C, Lee P, Andrews C, Beutler E. Autosomal dominant hereditary hemochromatosis associated with a novel ferroportin mutation and unique clinical features. Blood Cells Mol Dis 2005; 34 (2): 157 – 61.
34. Ned RM, Swat W, Andrews NC. Transferrin receptor 1 is differentially required in lymphocyte development. Blood 2003; 102 (10): 3711 – 8.
35. Loo TW, Bartlett MC, Clarke DM. Introduction of the most common cystic fibrosis mutation (Delta F508) into human P-glycoprotein disrupts packing of the transmembrane segments. J Biol Chem 2002; 277 (31): 27585 – 8.
36. Gehrke SG, Riedel HD, Herrmann T, Hadaschik B, Bents K, Veltkamp C, Stremmel W. UbcH5A, a member of human E2 ubiquitin-conjugating enzymes, is closely related to SFT, a stimulator of iron transport, and is up-regulated in hereditary hemochromatosis. Blood 2003; 101 (8): 3288 – 93.
37. Olsen ML, Schade S, Lyons SA, Amaral MD, Sontheimer H. Expression of voltage-gated chloride channels in human glioma cells. J Neurosci 2003; 23 (13): 5572 – 82.
38. Pauli BU, Abdel-Ghany M, Cheng HC, Gruber AD, Archibald HA, Elble RC. Molecular characteristics and functional diversity of CLCA family members. Clin Exp Pharmacol Physiol 2000; 27 (11): 901 – 5.
39. Mouchel N, Henstra SA, McCarthy VA, Williams SH, Phylactides M, Harris A. HNF1alpha is involved in tissue-specific regulation of CFTR gene expression. Biochem J 2004; 378 (Pt 3): 909 – 18.
40. Lewindon PJ, Pereira TN, Hoskins AC, Bridle KR, Williamson RM, Shepherd RW, Ramm GA. The role of hepatic stellate cells and transforming growth factor-beta in cystic fibrosis liver disease. Am J Pathol 2002; 160 (5): 1705 – 15.
41. Erickson RP, Kiela M, Devine PJ, Hoyer PB, Heidenreich RA. Mdr1a deficiency corrects sterility in Niemann-Pick C1 protein deficient female mice. Mol Reprod Dev 2002; 62 (2): 167 – 73.
42. Burns M, Gaynor K, Olm V, Mercken M, LaFrancois J, Wang L, Mathews PM, Noble W, Matsuoka Y, Duff K. Presenilin redistribution associated with aberrant cholesterol transport enhances beta-amyloid production *in vivo*. J Neurosci 2003; 23 (13): 5645 – 9.
43. Roest M, Jansen AC, Barendrecht A, Leus FR, Kastelein JJ, Voorbij HA. Variation at the paraoxonase gene locus contributes to carotid arterial wall thickness in subjects with familial hypercholesterolemia. Clin Biochem 2005; 38 (2): 123 – 7.
44. Zhao L, Cuff CA, Moss E, Wille U, Cyrus T, Klein EA, Pratico D. Selective interleukin-12 synthesis defect in 12/15-lipoxygenase- deficient macrophages associated with reduced atherosclerosis in a mouse model of familial hypercholesterolemia. J Biol Chem 2002; 277 (38): 35350 – 6.
45. Byun K, Kim J, Cho SY, Hutchinson B, Yang SR, Kang KS, Cho M, Hwang K, Michikawa M. Alteration of the glutamate and GABA transporters in the hippocampus of the Niemann-Pick disease, type C mouse using proteomic analysis. Proteomics 2006; 6 (4): 1230 – 6.
46. Brinkman J, Wijburg FA, Hollak CE, Groener JE, Verhoek M, Scheij S, Aten J, Boot RG, Aerts JM. Plasma chitotriosidase and CCL18: early biochemical surrogate markers in type B Niemann-Pick disease. J Inherit Metab Dis 2005; 28 (1): 13 – 20.
47. Forman OP, Boursnell ME, Dunmore BJ, Stendall N, van den Sluis B, Fretwell N, Jones C, Wijmenga C, Rothuizen J, van Oost BA. Characterization of the COMMD1 (MURR1) mutation causing copper toxico-

sis in Bedlington terriers. Anim Genet 2005; 36 (6): 497 – 501.
48. Klomp AE, Tops BB, Van Denberg IE, Berger R, Klomp LW. Biochemical characterization and subcellular localization of human copper transporter 1(hCTR1). Biochem J 2002; 364 (Pt 2): 497 – 505.
49. Mollereau C, Simons MJ, Soularue P, Liners F, Vassart G, Meunier JC, Parmentier M. Structure, tissue distribution, and chromosomal localization of the prepronociceptin gene. PNAS 1996; 93 (16): 8666 – 70.
50. Zannettino AC, Holding CA, Diamond P, Atkins GJ, Kostakis P, Farrugia A, Gamble J. Osteoprotegerin (OPG) is localized to the Weibel-Palade bodies of human vascular endothelial cells and is physically associated with von Willebrand factor. J Cell Physiol 2005; 204 (2): 714 – 23.
51. Viana R, Perez-Martinez G, Deutscher J, Monedero V. The glycolytic genes pfk and pyk from Lactobacillus casei are induced by sugars transported by the phosphoenolpyruvate:sugar phosphotransferase system and repressed by CcpA. Arch Microbiol 2005; 183 (6): 385 – 93.
52. Almqvist J, Huang Y, Hovmoller S, Wang DN. Homology modeling of the human microsomal glucose 6-phosphate transporter explains the mutations that cause the glycogen storage disease type Ib. Biochemistry 2004; 43 (29): 9289 – 97.
53. Lebea PJ, Pretorius PJ. The molecular relationship between deficient UDP-galactose uridyl transferase (GALT) and ceramide galactosyltransferase (CGT) enzyme function: a possible cause for poor long-term prognosis in classic galactosemia. Med Hypotheses 2005; 65 (6): 1051 – 7.
54. Sivaraman S, Kirsch JF. The narrow substrate specificity of human tyrosine aminotransferase–the enzyme deficient in tyrosinemia type II. FEBS J 2006; 273 (9): 1920 – 9.
55. Board PG, Anders MW. Human glutathione transferase zeta. Methods Enzymol 2005; 401: 61 – 77.
56. Dreumont N, Poudrier JA, Bergeron A, Levy HL, Baklouti F, Tanguay RM. A missense mutation (Q279R) in the fumary-lacetoacetate hydrolase gene, responsible for hereditary tyrosinemia, acts as a splicing mutation. BMC Genet 2001; 2: 9.
57. Dong F, Zhang X, Wold LE, Ren Q, Zhang Z, Ren J. Endothelin-1 enhances oxidative stress, cell proliferation and reduces apoptosis in human umbilical vein endothelial cells: role of ETB receptor, NADPH oxidase and caveolin-1. Br J Pharmacol 2005; 145 (3): 323 – 33.
58. Varfolomeev EE, Ashkenazi A. Tumor necrosis factor: an apoptosis JuNKie? Cell 2004; 116: 491 – 7.
59. Ding ST, Li YC, Nestor KE, Velleman SG, Mersmann HJ. Expression of turkey transcription factors and acyl-coenzyme oxidase in different tissues and genetic populations. Poult Sci 2003; 82 (1): 17 – 24.
60. Steinberg S, Chen L, Wei L, Moser A, Moser H, Cutting G, Braverman N. The PEX gene screen: molecular diagnosis of peroxisome biogenesis disorders in the Zellweger syndrome spectrum. Mol Genet Metab 2004; 83 (3): 252 – 63.
61. Gregor A, Schneider-Yin X, Szlendak U, Wettstein A, Lipniacka A, Rufenacht UB, Minder EI. Molecular study of the hydroxymethylbilane synthase gene (HMBS) among Polish patients with acute intermittent porphyria. Hum Mutat 2002; 19 (3): 310.
62. Qureshi K, Rao KV, Qureshi IA. Differential inhibition by hyperammonemia of the electron transport chain enzymes in synaptosomes and non-synaptic mitochondria in ornithine transcarbamylase-deficient spf-mice: restoration by acetyl-L-carnitine. Neurochem Res 1998; 23 (6): 855 – 61.