Journal of Animal and Veterinary Advances 13 (5): 297-301, 2014
ISSN: 1680-5593


# Hepatic Gene Expression Profiles in Pre and Postnatal Stages in Pigs

Jian Xiao, Jideng Ma, Long Jin, Xuewei Li, Mingzhou Li and An-An Jiang
College of Animal Science and Technology, Institute of Animal Genetics and Breeding,
Sichuan Agricultural University, Ya'an, Sichuan, P.R. China

**Abstract:** The liver is a vital organ that plays a major role in metabolism and other functions in the body. In this study, researchers used microarray analyses to investigate the gene expression profile during the development of the liver in pigs (*Suss crofa*). Researchers identified various differentially expressed genes in the liver between the prenatal and postnatal stages. The upregulated genes in the prenatal liver were related to the cell cycle and developmental process. The genes that were upregulated in the postnatal liver were active in the immunological process. The study presented gene expression profiles of the porcine liver during the pre and post-natal stages which contribute to the understanding porcine liver development.

**Key words:** Microarray, pig, liver, development, metabolism

## INTRODUCTION

Liver, the largest internal organ is involved in essential biochemical functions such as metabolic, exocrine and endocrine pathways (Chapple *et al*., 2013; Lee *et al*., 2012). Specific gene expression in hepatocytes and other livercell types determines the phenotype and function of the liver (Jochheim *et al*., 2003). Porcine fetal liver development can be divided into three periods: differentiation (18-40 days post-conception), metabolic activity (40-80 days post-conception) and glycogen accumulation (80-113 post-conception) (Ponsuksili *et al*., 2007). After birth, the liver functions as a major filter organ and is important in innate immunity, contributing to antiviral, antibacterial and antitumor defenses (Gao *et al*., 2008; Le Rouzic *et al*., 2011). During ontogeny, the metabolic functions of liver undergo adaptive changes (Li *et al*., 2012). Many genes have been identified to play a vital role in liver organogenesis and liver differentiation (Jochheim *et al*., 2003). Thus, the regulation of the prenatal liver transcriptome is thought to be different from those in the postnatal liver. Here, using microarray technology researchers provided an insight into the gene expression profiles of porcine pre and post-natal livers. This study contributes to the understanding of the time-restricted gene expression patternsduring liver development.

## MATERIALS AND METHODS

**Animals and tissue collection:** Three female fetuses from a sow and three female piglets from another sow (a Chinese Jinhua breed) were used in the study. Fetuses and piglets were slaughtered at 80 days of pregnancy and 30 days old, respectively. The animals were humanely sacrificed in compliance with guidelines for the use of experimental animals established by the Ministry of Agriculture of China. The livers were separated rapidly from each carcass, frozen in liquid nitrogen and stored at -80°C until RNA extraction.

**RNA isolation:** TRIzol (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from the six samples and further purification was performed using an RN easy column (Qiagen, Hilden, Germany). A NanoDrop ND-1000 spectrophotometer (Nano Drop, Wilmington, DE, USA) determined the quality of the RNA at 260/280 nm (ratio >2.0). A Bioanalyzer 2100 (Model 2100; Agilent Technologies, Palo Alto, CA, USA) determined the integrity of the RNA with a RIN No. >6.0.

**Microarray analyses:** Three total RNA from same stage were pooled for subsequent microarray analyses. Agilent Oligo microarrays were used to profile the Global gene expression of two liver libraries representing the two stages. Hybridization, washing and scanning were done according to standard agilent protocols.

**Function enrichment analysis of genes:** The DAVID Software (Huang *et al*., 2008) was used to perform Gene Ontology (GO) to investigate the biological mechanisms associated with the genes that were correlated with liver development.

**Q-PCR validation of gene expression:** Quantitative PCR (Q-PCR) was used to confirm the expression pattern

**Corresponding Author:** An-An Jiang, College of Animal Science and Technology, Institute of Animal Genetics and Breeding, Sichuan Agricultural University, Ya'an, Sichuan, P.R. China

Table 1: Analysis of differential expressed genes

| Categories | Terms | Count | p-values |
|---|---|---|---|
| **Gene Ontology (GO) categories enriched for up-regulated genes in porcine prenatal liver** | | | |
| GOTERM_BP | M phase of mitotic cell cycle | 23 | $4.93\times10^{-6}$ |
| GOTERM_BPT | Organelle fission | 23 | $5.55\times10^{-6}$ |
| GOTERM_BP | M phase | 28 | $7.13\times10^{-6}$ |
| GOTERM_BP | Mitosis | 23 | $1.06\times10^{-5}$ |
| GOTERM_BP | Nuclear division | 23 | $1.06\times10^{-5}$ |
| GOTERM_BP | Cell cycle phase | 30 | $2.62\times10^{-5}$ |
| GOTERM_BP | Chromosome segregation | 13 | $8.54\times10^{-5}$ |
| GOTERM_BP | Mitotic cell cycle | 25 | $1.05\times10^{-3}$ |
| GOTERM_BP | Cell cycle | 39 | $1.36\times10^{-3}$ |
| GOTERM_BP | Cell division | 21 | $2.55\times10^{-3}$ |
| GOTERM_BP | Cell cycle process | 31 | $2.80\times10^{-3}$ |
| GOTERM_BP | Microtubule-based process | 19 | $3.33\times10^{-3}$ |
| GOTERM_BP | Microtubule cytoskeleton organization | 14 | $4.51\times10^{-3}$ |
| GOTERM_BP | DNA replication | 16 | $4.63\times10^{-3}$ |
| GOTERM_BP | Mitotic sister chromatid segregation | 7 | 0.020 |
| GOTERM_BP | Sister chromatid segregation | 7 | 0.02 |
| GOTERM_BP | Regulation of cell proliferation | 35 | 0.03 |
| GOTERM_BP | DNA packaging | 11 | 0.03 |
| **Gene Ontology (GO) categories enriched for up-regulated genes in porcine prenatal liver** | | | |
| GOTERM_BP | Immune response | 47 | $4.71\times10^{-7}$ |
| GOTERM_BP | Positive regulation of immune response | 19 | $8.06\times10^{-6}$ |
| GOTERM_BP | Response to wounding | 37 | $8.14\times10^{-6}$ |
| GOTERM_BP | Defense response | 41 | $8.22\times10^{-6}$ |
| GOTERM_BP | Activation of immune response | 15 | $2.11\times10^{-5}$ |
| KEGG_PATHWAY | Complement and coagulation cascades | 14 | $2.46\times10^{-5}$ |
| GOTERM_BP | Inflammatory response | 27 | $2.64\times10^{-5}$ |
| GOTERM_BP | Innate immune response | 17 | $6.05\times10^{-5}$ |
| GOTERM_BP | Complement activation, classical pathway | 9 | $6.95\times10^{-5}$ |
| GOTERM_BP | Positive regulation of immune system process | 22 | $7.51\times10^{-5}$ |
| GOTERM_BP | Humoral immune response mediated by circulating immunoglobulin | 9 | $1.10\times10^{-4}$ |
| GOTERM_BP | Fatty acid metabolic process | 19 | $2.58\times10^{-4}$ |
| GOTERM_BP | Adaptive immune response | 12 | $3.84\times10^{-4}$ |
| GOTERM_BP | Adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains | 12 | $3.84\times10^{-4}$ |
| GOTERM_MF | Cofactor binding | 20 | $5.49\times10^{-3}$ |
| GOTERM_MF | Electron carrier activity | 18 | $7.35\times10^{-3}$ |
| GOTERM_MF | MHC protein binding | 6 | 0.03 |
| GOTERM_MF | MHC class I protein binding | 5 | 0.04 |

BP: Biological Process; MF: Molecular Function

observed in the microarray results. Q-PCR was performed on a CF96 real-time PCR detection system (Bio-Rad, Hercules, CA, USA) using the SYBR® Green real-time PCR master mix (TaKaRa, Dalian, China). The PCR primer sequences are shown in Table 1. Porcine ACTB, TBP and TOP2B were used simultaneously as endogenous control genes. The $2^{-\Delta\Delta C}$T Method was used to determine the relativem RNA abundance for the surveyed samples.

## RESULTS AND DISCUSSION

To obtain high-confidence gene expression data, researchers mapped 43,603 probes (60-mers) to the pig reference genome allowing up to 1 mismatch. Of these 27,955 probes were mapped uniquely and among them 4,983 probes were mapped uniquely toexons of genes in Ensembl (>60% sequence overlap). Researchers filtered out the multiple probes that mapped to the same or different exons of the same specific gene. Ultimately, 3,074 probes representing 3,074 genes were used in subsequent analyses.

The differential expressed genesin porcine liver were compared between the pre and postnatal stages. Among the 3,074 high-confidence and well annotated probe-targeted genes, researchers identified the significantly differentially expressed genes (>2 fold changes) between the 2 stages and found 397 and 435 genes that were specifically upregulated in prenatal and postnatal liver, respectively (Fig. 1).

Researchers found that the genes that were upregulated in the fetal liver were significantly enriched in the Gene Ontology (GO) categories of cell cycle (i.e., mitosis, nuclear division, M phase and regulation of cell proliferation) (Table 1). For example, Cyclin-dependent kinase 2 (Cdk2) and minichromosome maintenance complex component 8 (MCM8) were upregulated in the fetal liver. Both genes are involved in

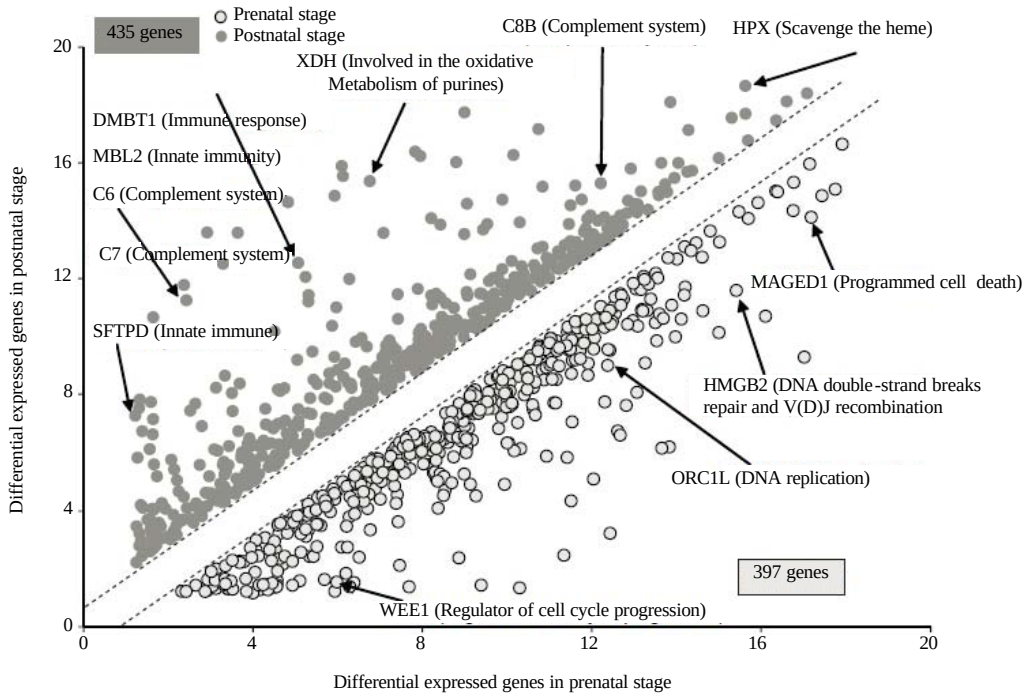


Fig. 1: The differential expressed genes in porcine pre and postnatal liver

the G1-S transition duringthe cell cycle. Cdk 2 initiates both DNA replication and centrosome duplication during the G1-S transition during the cell cycle (Moroy and Geisen, 2004). Down regulation of MCM8 could interfere with G1-S transition during the cell cycle (Volkening and Hoffmann, 2005). The upregulation of the two genes showed that they may influence mitosis during porcine fetal liver development. Another gene, Wee1 homolog (*S. pombe*) (Wee1) whose product is involved in mitosis was upregulated in the fetal liver. Wee1 negatively regulates mitotic entry in the G2 phase by suppressing cyclin B-Cdc2 activity (Masuda *et al*., 2011). Fibroblast Growth Factor 9 (FGF9) and Bone Morphogenetic Protein 7 (BMP7) are important regulators of growth, differentiation and repair in various tissues (Antoine *et al*., 2007; Sugimoto *et al*., 2007). FGF9 plays a key role in embryonic development of the lung, heart, cecum and testes (Geske *et al*., 2008). BMP7 mediates sprouting of the liver bud from the central foregut endoderm which eventually gives rise to hepatocytes (Sugimoto *et al*., 2007). Both FGF9 and BMP7 were upregulated in the porcine fetal liver suggesting that the two genes are involved in liver organogenesis and development (Lemaigre and Zaret, 2004; Sugimoto *et al*., 2007). The cell cycle is a vital process by which certain internal organs mature. The results showing that genes involved in the cell cycle were highly expressed in the fetal liver conform to the characteristics of liver development are consistent with a previous study (Li *et al*., 2009).

The genes upregulated in postnatal liver of pig were significantly enriched for the immune response (i.e., immune response, complement activation, response to wounding and oxidation reduction) (Table 1). Many defense-related genes were identified in the postnatal liver of pig. For example, terminal complement components (such as C6, C7, C8B and C9) were upregulated in postnatal liver. Terminal complement components play important roles in complement system which is a mechanism of the innateimmune systemkills microorganisms directly and modulates phagocytosis inflammation, humoral and cellular immune responses (Wimmers *et al*., 2011). In addition, genes that recognize viruses were upregulated in the postnatal liver. For example, Toll-Like Receptor 3 (TLR3) and Toll-Like Receptor 7 (TLR7) which recognize double-stranded RNA and single-stranded RNA found in the genomes of RNA viruses, respectively (Sang *et al*., 2008). Furthermore, Cytochrome P450 2E1 (CYP2E1) is a xenobiotic-metabolizing enzyme and is involved in the oxidative metabolism of a small range of substrates (Nebert and Russell, 2002). In the study, CYP2E1 was upregulatedin the postnatal liver which is consistent with a previous study where the expression level of

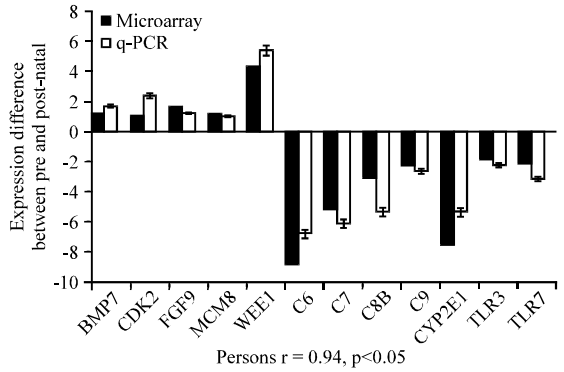


Fig. 2: Quantitative PCR validation of the microarray deduced expressions of 12 selected genes

CYP2E1 quickly increased after birth in mice (Hart *et al*., 2009). Researchers also found that genes upregulated in the postnatal liver were over-represented in the complement and coagulation cascades pathway which plays an important role in the context of life-threatening tissue injury and inflammation (Amara *et al*., 2010).

The expressions of the 12 genes mentioned earlier were validated by quantitative real-time PCR (q-PCR). As shown in Fig. 2, the expression levels of these genes showed good correlation (Person's r = 0.94, $p<0.05$) between the q-PCR and microarray results which highlighted the high confidence inthe results obtained using the microarray approach.

## CONCLUSION

The present study describes a genome-wide analysis ofthe gene expression profiles between pre and postnatal livers of pig. The results showed the genes upregulated in the prenatal liver were related to cell cycle and development processes while in those upregulated in the postnatal liver were prone to be active in immunological process. This study profiled the time-restricted gene expression and the data represent a basis resource for further research on porcine liver development.

## ACKNOWLEDGEMENTS

This research was supported by grants from the National Special Foundation for Transgenic Species of China (2014ZX0800950B and 2011ZX08006-003) the Fund for Distinguished Young Scientists of Sichuan Province (2013JQ0013), Program for Changjiang Scholars and Innovative Research Team in University (IRT13083) and the Specialized Research Fund of Ministry of Agriculture of China (NYCYTX-009).

## REFERENCES

Amara, U., M.A. Flierl, D. Rittirsch, A. Klos and H. Chen *et al*., 2010. Molecular intercommunication between the complement and coagulation systems. J. Immunol., 185: 5628-5636.

Antoine, M., W. Wirz, C.G. Tag, A.M. Gressner and M. Marvituna *et al*., 2007. Expression and function of Fibroblast Growth Factor (FGF) 9 in hepatic stellate cells and its role in toxic liver injury. Biochem. Biophys. Res. Commun., 361: 335-341.

Chapple, R.H., P.C. Tizioto, K.D. Wells, S.A. Givan and J. Kim *et al*., 2013. Characterization of the rat developmental liver transcriptome. Physiol. Genomics, 45: 301-311.

Gao, B., W.I. Jeong and Z. Tian, 2008. Liver: An organ with predominant innate immunity. Hepatology, 47: 729-736.

Geske, M.J., X. Zhang, K.K. Patel, D.M. Ornitz and T.S. Stappenbeck, 2008. Fgf9 signaling regulates small intestinal elongation and mesenchymal development. Development, 135: 2959-2968.

Hart, S.N., Y. Cui, C.D. Klaassen and X.B. Zhong, 2009. Three patterns of cytochrome P450 gene expression during liver maturation in mice. Drug Metab. Disposition, 37: 116-121.

Huang, D.W., B.T. Sherman and R.A. Lempicki, 2008. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protocols, 4: 44-57.

Jochheim, A., A. Cieslak, T. Hillemann, T. Cantz, J. Scharf, M.P. Manns and M. Ott, 2003. Multi-stage analysis of differential gene expression in BALB/C mouse liver development by high-density microarrays. Differentiation, 71: 62-72.

Le Rouzic, V., J. Corona and H. Zhou, 2011. Postnatal development of hepatic innate immune response. Inflammation, 34: 576-584.

Lee, J.S., W.O. Ward, G. Knapp, H. Ren and B. Vallanat *et al*., 2012. Transcriptional ontogeny of the developing liver. BMC Genomics, Vol. 13. 10.1186/1471-2164-13-33.

Lemaigre, F. and K.S. Zaret, 2004. Liver development update: New embryo models, cell lineage control and morphogenesis. Curr. Opin. Genet. Dev., 14: 582-590.

Li, C., S. Yu, X. Zhong, J. Wu and X. Li, 2012. Transcriptome comparison between fetal and adult mouse livers: implications for circadian clock mechanisms. PloS One, Vol. 7. 10.1371/journal. pone.0031292.

Li, T., J. Huang, Y. Jiang, Y. Zeng and F. He *et al*., 2009. Multi-stage analysis of gene expression and transcription regulation in C57/B6 mouse liver development. Genomics, 93: 235-242.

Masuda, H., C.S. Fong, C. Ohtsuki, T. Haraguchia nd Y. Hiraoka, 2011. Spatiotemporal regulations of Wee1 at the G2/M transition. Mol. Biol. Cell, 22: 555-569.

Moroy, T. and C. Geisen, 2004. Cyclin E. Int. J. Biochem. Cell Biol., 36: 1424-1439.

Nebert, D.W. and D.W. Russell, 2002. Clinical importance of the cytochromes P450. Lancet, 360: 1155-1162.

Ponsuksili, S., E. Murani, C. Walz, M. Schwerin and K. Wimmers, 2007. Pre-and postnatal hepatic gene expression profiles of two pig breeds differing in body composition: Insight into pathways of metabolic regulation. Physiol. Genomics, 29: 267-279.

Sang, Y., J. Yang, C.R. Ross, R.R. Rowland and F. Blecha, 2008. Molecular identification and functional expression of porcine Toll-Like Receptor (TLR) 3 and TLR7. Vet. Immunol. Immunopathol., 125: 162-167.

Sugimoto, H., C. Yang, V.S. LeBleu, M.A. Soubasakos, M. Giraldo, M. Zeisberg and R. Kalluri, 2007. BMP-7 functions as a novel hormone to facilitate liver regeneration. FASEB J., 21: 256-264.

Volkening, M. and I. Hoffmann, 2005. Involvement of human MCM8 in prereplication complex assembly by recruiting hcdc6 to chromatin. Mol. Cell. Biol., 25: 1560-1568.

Wimmers, K., S. Schutze, E. Murani and S. Ponsuksili, 2011. The three-way relationship of polymorphisms of porcine genes encoding terminal complement components, their differential expression and health-related phenotypes. BMC Proc., Vol. 5. 10.1186/1753-6561-5-S4-S19.