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ORIGINAL ARTICLE

Altered gene profile of placenta from women with intrahepatic cholestasis of pregnancy

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Abstract
Objectives To investigate the alterations in gene profile of placenta from pregnant women with intrahepatic cholestasis of pregnancy (ICP) and to enhance the insight of etiology and pathogenesis of ICP.
Methods Ten pregnant women diagnosed ICP were recruited and 10 healthy pregnant women served as control. Four samples were taken from each placenta and RNA was isolated. Gene expression was analyzed with microarray and real time PCR was used to validate the differentially expressed genes.
Results 392 genes were found differentially expressed. Among these differentially expressed genes, 280 were up-regulated and 112 were down-regulated. These differentially expressed genes involved 20 categories including genes involved in transportation, cell growth, apoptosis and immune response that were putatively participated the pathogenesis of ICP.
Conclusions 293 differentially expressed genes of 20 categories were found in ICP placenta, suggesting the diversity of gene expression alteration and the complexity of etiology and pathogenesis of ICP.

Keywords Intrahepatic cholestasis of pregnancy · Placenta · Gene profile · Microarray

Introduction

Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific complication in the second and third trimester and is characterized by pruritus, jaundice and the disturbances of liver tests, especially the elevation of bile acid [1, 2]. The maternal prognosis of ICP is usually favorable. Pruritus usually disappears in the first few days following delivery, which was accompanied by normalization of serum bile acid concentrations and other liver tests and the women with ICP generally have no hepatic sequelae [3]. However, ICP poses significant effects on fetus. It increases the risk of preterm labor [4–6], fetal distress [4, 7, 8] and even stillbirth [4, 9, 10]. The etiology and pathogenesis of ICP remain elusive and incompletely understood. It appears to be a multifactorial disease. Hormone factors, genetic predisposition and environmental factor have been thought to be involved in pathogenesis of the disease [1]. Although much work has been done to explore the etiology and pathogenesis, no specific factors, genetic or environmental, were identified.

The role of placenta in the development of ICP has been noticed because the disappearance of pruritits and the normalization of liver tests would occur after the delivery of placenta. In addition, alteration of placental gene expression was reported. Elevated expression of bax and Fas, reduced expression of bcl-2 and FasL and alteration of Bax/Bal-2 [11] and Fas/FasL [12] balance in placenta from ICP women were reported. It was also observed that the expression of tumor necrosis factor (TNF) α [13] were was significantly increased in placenta of ICP women as compared

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with that in normally pregnant women. Previously, we observed the presence of epidermal growth factor (EGF) receptor [14] in placental trophoblast in ICP and healthy pregnancy and found that the expression of EGFR was significantly decreased in ICP compared with healthy pregnancy. However, there was no high-throughout investigation exploring the gene expression file of ICP placenta.

Microarray technology provides a powerful tool to survey differential expression of gene products [15]. The simultaneous expression screening of thousands of transcripts facilitates the discovery of gene expression patterns that are associated with a specific condition such as that under a disease condition, and may thereby identify transcripts that may be involved in the disease development or may be altered during the disease development [16, 17].

To enhance the insight into the pathogenesis of ICP and the effect of ICP on placental gene expression, we performed a microarray analysis to identify the differentially expressed transcripts that may participate the disease development.

Materials and methods

Human subjects

This investigation was conducted after the approval of the Hospital Ethical Committee and informed consents were obtained from all the participants. All subjects were nulliparous Chinese women with singleton pregnancy. Ten pregnant women who was diagnosed ICP were recruited and ten age-matched healthy pregnant women served as control. Demographic and pregnancy characteristics the recruited subjects were presented in Table 1.

The diagnostic criteria for ICP as described previously [18–20] were (a) the presence of marked pruritus with no rash and quick disappearance after delivery, (b) biochemical evidences of intrahepatic cholestasis, including marked elevation of cholylglycine or total bile acid and mild to moderate elevation of aminotransferases in fasting serum in the second and third trimester and quick recovery after delivery, (c) absence of obstructive gallstone disease as diagnosed clinically and by ultrasonography, (d) absence of viral hepatitis as judged by negative serology to hepatitis A, B, and C, (e) absence of fever or general malaise, and (f) absence of medication that could produce the elevation of bile acid and liver enzymes.

To avoid the confusion of the diseases and multiple pregnancies on the result, the exclusion criteria were multiple pregnancies, chronic hypertension, diabetes mellitus, diseases of heart, lung, and kidney, and other underlying organ diseases. No other obstetric complications existed.

Table 1 Demographic and pregnancy characteristics

No.	History	Maternal age (year)	Gestational age (week)	Birth weight (g)	Cholylglycine level (μg/dl)
Normal pregnancy					
1	G1P0	27	40	3,400	494.77
2	G1P0	23	39	3,250	156.31
3	G1P0	30	39	3,900	125.95
4	G1P0	26	39	3,650	286.37
5	G1P0	30	39	3,700	639.50
6	G1P0	32	39	3,200	420.68
7	G1P0	25	39	2,800	51.08
8	G1P0	24	38	3,400	294.41
9	G1P0	28	39	3,950	244.22
10	G1P0	29	39	3,600	360.18
ICP					
1	G1P0	26	37	2,600	5554.30
2	G1P0	25	36	2,850	6081.4
3	G1P0	29	37	3,150	8398.50
4	G1P0	27	34	2,150	3188.89
5	G1P0	36	37	3,650	5499.6
6	G1P0	22	32	2,100	5900.80
7	G1P0	27	36	2,800	3001.57
8	G1P0	30	36	2,600	2622.93
9	G1P0	27	39	3,800	3184.95
10	G1P0	20	35	2,050	3266.76

Placental tissue collection

All the placental samples both from ICP and normal pregnancy were obtained immediately after Caesarean sections. To avoid the further effect of labor on the expression profile, only placental samples that were obtained from the women who had not undergone labor were included. Four samples were taken at 0, 3, 6 and 9 o'clock of middle zone of each placenta after the deciduas and amnionic membranes were removed. We then dissected $1 \times 1 \times 1$ cm sections of placental vili between basal and chorionic plates. After vigorous washing of the maternal blood with saline, tissues were immediately frozen in liquid nitrogen and stored at $-80°C$ until assay.

RNA extraction and probe preparation

Total RNA was extracted from placenta tissues with Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacture's instructions. The quality of the RNA samples was determined by electrophoresis through denaturing agarose gels and staining with ethidium bromide. The RNA was quantified and evaluated for purity by UV spectrophotometry.

RNA was purified using RNeasy (Qiagen, Valencia, CA, USA). Despite the power of high through-put technologies to reveal general patterns of gene expression, variations in gene expression of among individuals must be considered. One potential approach is to make a pool of RNA for each group to reduce the in individual variation [21]. Thus RNA samples of 16 tissue samples from four women were pooled for control group or ICP group. Double stranded cDNA was synthesized using Superscript Choice system (Invitrogen Life Technologies, Carlsbad, CA) and a T7T21 oligonucleotide primer (GenSet, La Jolla, CA). Biotin-labeled RNA was synthesized by in vitro transcription using Enzo Bioarray RNA labeling kit (Enzo Diagnostics, Farmingdale, NY). Labeled cRNA was purified using the Qiagen Rneasy Mini Kit spin Columns.

Hybridization

The Affymetrix HG-U133 plus 2.0 oligonucleotide microarray (Affymetrix, Santa Clara, CA, USA) that consists of 47,000 known transcripts and expressed sequence tags (ESTs), representing approximately 38,500 human genes, was used in the current investigation. Arrays were hybridized, washed, stained, and scanned following the standard Affymetrix protocol. Hybridization was performed at 45°C for 16 h in a hybridization oven with constant rotation (60 rpm). The microarrays were then automatically washed and stained with streptavidinphycoerythrin conjugate (SAPE; Molecular Probes, Eugene, OR) in an Affymetrix Genechip Fluidics Station and fluorescence intensities were scanned with a GeneArray Scanner (Affymetrix).

Data analysis

Data analysis was performed using GCOS software (Affymetrix, Santa Clara, CA, USA). After intensity dependent normalization, the expression levels relative to the control were calculated as a ratio and the expression profile was compared between ICP and control groups. A gene with ratio more than +2.0 or less than −2.0 was considered up- or down-regulated. Each differently regulated gene was classified according to its gene ontology (GO), in which genes were organized into hiearchial categories based on the biological process, molecular function and cellular process. Genesifter software (VizX labs,Seattle,USA) was used to perform the GO analysis.

Real-time PCR

To validate the microarray data, real-time PCR was used to detect the expression of randomly selected genes and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) was used as the internal control to normalize mRNA level. Total RNA was isolated as described above in 40 placental samples obtained from 10 women including those enrolled in microarray. Reverse transcription was performed in a reaction system containing buffer, oligo(dT), dNTP mixture and reverse transcriptase. PCR was conducted in duplicate using the SYBR Green I assay on ABI PRISM 7900 (Perkin-Elmer, Foster city, CA, USA). The cycling conditions were as following: 2 min at 95°C and then 30 s at 95°C, 30 s at 56°C repeats 40. The sequences of primers were listed in Table 2. The expression of detected genes was calculated as previously described. Statistical significance was determined by Student's *t* test and *P* values of <0.05 were regarded as significant.

Results

Clinical characteristics of subjects

Demographic and pregnancy characteristics of each group were presented in Table 1. Maternal ages were similar in

Table 2 Primers and annealing temperatures for real time-PCR

Gene symbol	Primers (F: forward, R: reverse)	Annealing temperature
IL1RL1	F: 5′-GCTGCTTAAATTGTTCGT-3′	55
	R: 5′-TCAGTTTACGGTTGTTGG-3′	
PLSCR1	F: 5′-CTCTGGACCTTGTCTCGC-3′	58
	R: 5′-GAATGCTGTCGGTGGATA-3′	
PAEP	F: 5′-GGATTCATCAGGGCTTTC-3′	58
	R: 5′-GGGTGGGAGTCTGGTCTT-3′	
IGFBP1	F: 5′-AGAGCACGGAGATAACTGA-3′	56
	R: 5′-CCATTCCAAGGGTAGACG-3′	
IGF2	F: 5′-CTTGGACTTTGAGTCAAATTGG-3′	58
	R: 5′-CCTCCTTTGGTCTTACTGGG-3′	
GAPDH	F: 5′-TGCACCACCAACTGCTTAGC-3′	58
	R: 5′-GGCATGGACTGTGGTCATGAG-3′	

Table 3 Categories of differentially expressed genes

Category	GO No.	Down		Up	
		n	%	*n*	%
Cell process	0009987	43	10.97	113	28.83
Metabolic process	0008152	27	6.89	73	18.62
Biological regulation	0065007	15	3.83	42	10.71
Regulation of biological process	0050789	14	3.57	41	10.46
Multicellular organismal process	0032501	10	2.55	36	9.18
Developmental process	0032502	9	2.30	36	9.18
Establishment of localization	0051234	8	2.04	23	5.87
Localization	0051179	8	2.04	29	7.40
Response to stimulus	0050896	8	2.04	37	9.44
Negative regulation of biological process	0048519	4	1.02	12	3.06
Biological adhesion	0022610	3	0.76	6	1.53
Immune system process	0002376	2	0.51	11	2.81
Positive regulation of biological process	0048518	2	0.51	10	2.55
Growth	0040007	1	0.26	4	1.02
Multi-organism process	0051704	1	0.26	6	1.53
Rhythmic process	0048511	1	0.26	2	0.51
Reproductive process	0022414	0		7	1.79
Reproduction	0000003	0		12	3.06
Pigmentation	0043473	0		1	0.26
Viral reproduction	0016032			1	0.26

ICP patients and controls (26.9 ± 4.38 vs. 27.4 ± 2.91). As expected, ICP women delivered at earlier gestational age than control (35.9 ± 1.91 vs. 39 ± 0.47) and neonatal birth weight were lower in ICP group than in control (3,485 ± 348 vs. 2,775 ± 614, $P = 0.005$). ICP women had significantly increased level of CG compared with control (307.34 ± 178.60 vs. 4,669.97 ± 1,892, $P < 0.001$).

Differential expression of genes in placenta of ICP

By comparison with the control, the expression of 392 genes was found significantly altered: 280 genes were up- and 112 down-regulated in ICP group. The names and gene IDs of differentially expressed genes were listed in the supporting material.

By performing GO analysis, the differentially expressed genes were categorized into 20 categories according to their biological process. As listed in Table 3, cell process, metabolic process, biological regulation, regulation of biological process, multicellular organismal process, developmental process, response to stimulus, biological adhesion, immune system process, growth, reproductive process and reproduction were among the these categories.

Because placental transportation abnormality, growth dysfunction and apoptosis, as well as immune maladaptation and disordered lipid metabolism were proposed to be important pathophysiology of ICP and were possibly involved in the pathogenesis of disease, differentially expressed genes of these categories were outlined in Table 4.

To verify the results of microarray, the mRNA levels of IL-1RL1, PLSCR-1 and PAEP were analyzed in 40 samples of 10 ICP patients and compared with appropriate controls. We found that the expression of IL-1RL1 and PLSCR-1 were significantly elevated while the expression of PAEP decreased in ICP group compared with that of control $P < 0.05$ for all, Fig. 1). The change trend was consistent with that found in microassay analysis, which validated the results of microarray to certain degree.

Discussion

By conducting the current investigation, we revealed for the first time the profile of differentially expressed genes in placenta of ICP women. It was found that 280 genes were up-regulated and 122 down-regulated in ICP placenta and that these gene were categorized into 20 categories, indicating that alterations in placenta of ICP patients were multifaceted. Our study provided the evidences that confirmed the complexity of the pathogenesis of ICP and role of placenta in the disease development. To the best of our knowledge, this is the first report of a genome-wide analysis of gene expression in ICP placenta. However, previous studies have

Table 4 Putatively involved genes of differentially expression in ICP

Gene symbol	Gene name	GenBank accession no.	Biological process
Cell Growth			
ENOX2	Ecto-NOX disulfide-thiol exchanger 2	NM_182314	Regulation of cell; growth; ultradian rhythm
GAP43	Growth associated protein 43	NM_002045	Activation of protein kinase C; multicellular organismal development; nervous system development; regulation of cell growth; cell differentiation
IGFBP1	Insulin-like growth factor binding protein 1	NM_000596	Regulation of cell growth; signal transduction
IGF2	Insulin-like growth factor 2	NM_000612	Egulation of progression through cell cycle; skeletal development; imprinting; development; physiological process; cell proliferation; insulin receptor signaling pathway; chromatin assembly or disassembly; metabolism
NOV	Nephroblastoma overexpressed gene	NM_002514	Regulation of cell growth
Immunity			
ADA	Adenosine deaminase	NM_000022	Immune response; purine nucleotide; metabolic process; purine ribonucleoside monophosphate biosynthetic process
CXCL1	Chemokine (C-X-C motif) ligand 1	NM_001511	Actin cytoskeleton organization and biogenesis; chemotaxis; G-protein coupled receptor protein signaling pathway; Immune response; inflammatory response; intracellular signaling cascade; negative regulation of cell proliferation; nervous system development
CIITA	Class II, major histocompatibility complex, transactivator	NM_000246	Defense response; immune response; regulation of transcription; regulation of transcription, DNA-dependent
CR1	Complement component (3b/4b) receptor 1	NM_000651	Complement activation, classical pathway; innate immune response
GAL	Galanin	NM_015973	Growth hormone secretion; inflammatory response; insulin secretion; negative regulation of cell proliferation; negative regulation of lymphocyte proliferation; nervous system development; neuropeptide signaling pathway; positive regulation of apoptosis; regulation of glucocorticoid metabolic process; response to drug; response to estrogen stimulus; response to stress; smooth muscle contraction
IL1RAPL2	Interleukin 1 receptor accessory protein-like 2	NM_017416	Central nervous system development; innate immune response; signal transduction
IL1R2	Interleukin 1 receptor, type II	NM_004633	Immune response
PF4	Platelet factor 4 (chemokine (C-X-C motif) ligand 4)	NM_002619	Cytokine and chemokine mediated signaling pathway; immune response; leukocyte chemotaxis; negative regulation of megakaryocyte differentiation; platelet activation
PPBP	Pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)	NM_002704	Cell proliferation; chemotaxis; defense response to bacterium; glucose transport; immune response
PRL	Prolactin	NM_000948	Cell proliferation; cell surface receptor linked signal transduction; female pregnancy; hemocyte development; lactation

Table 4 continued

Gene symbol	Gene name	GenBank accession no.	Biological process
PRG2	Proteoglycan 2, bone marrow (natural killer cell activator, eosinophil granule major basic protein)	NM_002728	Defense response to bacterium; immune response; inflammatory response
SP2	Sp2 transcription factor	NM_003110	Transcription; regulation of transcription from RNA polymerase II promoter; immune response; regulation of transcription, DNA-dependent
IL1RL1	Interleukin 1 receptor-like 1	NM_016232	Innate immune response; signal transduction
Apoptosis			
PHLDA1	Pleckstrin homology-like domain, family A, member 1	NM_007350	Apoptosis
DNASE1L3	Deoxyribonuclease I-like 3	NM_004944	DNA catabolic process, apoptosis
KIAA0367	KIAA0367	NM_015225	Apoptosis, induction of apoptosis, G1 phase
TP63	Tumor protein p73-like	NM_003722	Apoptosis, induction of apoptosis, multicellular organismal development, negative of cell growth, negative of transcription, DNA-dependent; positive regulation of Notch signaling pathway; response to tumor cell, protein homotetramerization
RNF36	Ring finger protein 36	NM_080745	Apoptosis
TNFRSF18	Tumor necrosis factor receptor superfamily, member 18		
CGB	Chorionic gonadotropin, beta polypeptide	NM_000737	Apoptosis; signal transduction; cell-cell signaling; female gamete generation
Lipid metabolism			
DHCR7	7-dehydrocholesterol reductase	NM_001360	Blood vessel development; cell differentiation; cholesterol biosynthetic process; lung development; regulation of cell proliferation
ELOVL4	Elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 4	NM_022726	Fatty acid biosynthetic process; response to stimulus; visual perception
LPL	Lipoprotein lipase	NM_000237	Blood circulation; fatty acid metabolic process; lipid catabolic process; phospholipid metabolic process; triacylglycerol metabolic process
PLA2G4C	Phospholipase A2, group IVC	NM_003706	Arachidonic and metabolic process; glycerophospholipid catabolic process; inflammatory response; intracellular signaling cascade; lipid catabolic process; metabolic process; parturition
LIPF	Lipase, gastric	NM_004190	Lipid catabolic process; lipid metabolic process; triacylglycerol metabolic process
APOD	Adenosine deaminase	NM_001647	Lipid metabolic process; transport
Transportation			
TRPV5	Transient receptor potential cation channel, subfamily V, member 5	NM-019841	Calcium ion transport; ion transport; protein transport

Table 4 continued

Gene symbol	Gene name	GenBank accession no.	Biological process
SLC35D1	Solute carrier family 35 (UDP-glucuronic acid/UDP-*N*-acetylgalactosamine dual transporter), member D1	NM_015139	Carbohydrate transport; UDP-N-acetylgalactosamine transport
SLC16A3	Solute carrier family 16 (monocarboxylic acid transporters), member 3	NM_004207	Monocarboxylic acid transport; organic anion transport
SLC12A8	Solute carrier family 12 (potassium/chloride transporters), member 8	NM_024628	Ion transport; potassium ion transport
KCNJ15	Potassium inwardly rectifying channel, subfamily J, member 15	NM_170737	Ion transport; potassium ion transport
CADPS2	Ca2+-dependent activator protein for secretion 2	NM_017954	Exocytosis; protein transport
ATP11B	ATPase, Class VI, type 11B	NM_014616	Phospholipid transport
ATP9A	ATPase, Class II, type 9A	NM_006045	Phospholipid transport; metabolic process
ATP2C2	ATPase, Ca++ transporting, type 2C, member	NM_014861	Calcium ion transport; cationcationcation transport; proton transport

documented that abnormal immunity and cell growth, lipid metabolism, placental apoptosis and probably altered material transportation are involved in the disease pathogenesis.

Abnormal immunity has been described in ICP and considered an important pathophysiology of ICP. Previously, we reported that serum neopterin, a marker of monocyte/macrophage activity, and soluble interleukin-2 receptor (sIL-2R), a product of activated T lymphocyte and a quantitative marker of T lymphocyte activity, were significantly increased in ICP women relative to normal pregnancy, which suggested the abnormal activation of both monocyte/macrophage and T lymphocyte and the abnormality of innate and adaptive immunity [22]. Peng and Liu [23] found that the production of Th1-type cytokine by peripheral lymphocyte was increased and that of Th2-type cytokine decreased and that the balance of Th1/Th2 shifted toward Th1 in ICP women. Ling et al. [24] observed increased NK cells and NKT cells, decreased T cells and over-secretion of IFN-γ in the decidual parietalis of ICP women, suggesting the involvement of cell-mediated immunity imbalance in the pathophysiology of ICP. We also found the suppressed mixed lymphocyte reaction between the mother and fetus in ICP setting, implying abnormality of maternal-fetal immunity recognition [20]. These findings point to the importance of altered immunity in the pathophysiology of ICP.

In the current investigation, at least six genes were found differentially expressed in ICP placenta relative to normal placenta. Adenosine deaminase (ADA), "class II, major histocompatibility complex, transactivator" (CIIA), chemokine (C-X-C motif) ligand1 (CXCL1), chemokine (C-X-C motif) ligand 4 (CXCL4) and chemokine (C-X-C motif) ligand 7 (CXCL7) were up-regulated and Interleukin 1 receptor-like 1 (IL1RL1) were down-regulated. Among these genes, the change in the expression of and IL1RL1 was confirmed by real-time PCR.

Adenosine deaminase (ADA) is an enzyme of purine metabolism that catalyzes the deamination of adenosine to inosine and deoxyadenosine to deoxyinosine. ADA is a marker of T-cell activation and considered important for lymphocyte differentiation and growth [25, 26]. IL1RL1, also known as ST2, is a member of the IL-1R family and is expressed preferentially on Th2 effector cells but not on Th1 cells [27–29]. The interaction of IL1RL1 (ST2) and soluble fusion protein ST2L resulted in the suppression of Th2 cell differentiation, suggesting a functional role for ST2L and ST2 in the development of Th2 cells [30]. The up-regulation of ADA and down-regulation of IL1RL1 pointed to abnormality of cell-mediated immunity and the imbalance of Th1/Th2 at the maternal–fetal interface in ICP. Our finding of a group of differentially expressed genes of immunity indicates complex alteration of immunity at maternal–fetal interface.

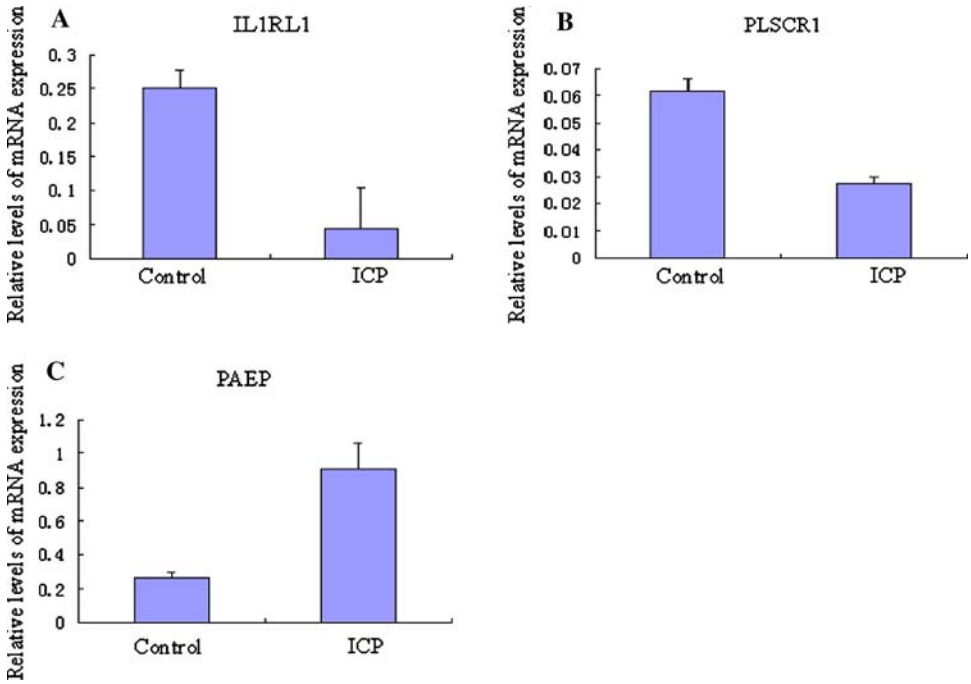

Fig. 1 Validation of differentially expressed genes by real time PCR. The expression of IL1RL1, PLSCR1 and PAEP were significantly different between control and ICP ($P < 0.05$ for all)

Abnormal lipid metabolism and altered lipid profile are another important pathophysiology of ICP. It was documented that serum levels of low-density lipoprotein cholesterol (LDL-Ch), apolipoprotein B-100 and total cholesterol were significantly increased while that of high-density lipoprotein cholesterol (HDL-Ch) decreased in ICP compared with normal pregnancy [31]. However, the underlying mechanisms have not been investigated. In the current study, six genes involved in lipid metabolism process were found differentially expressed in the placenta of ICP: LPL (lipoprotein lipase), PLA2G4C (phospholipase A2, group IVC), ELOVL4 (elongation of very long chain fatty acids - like 4), APOD (apolipoprotein D), LIPF (lipase, gastric) and DHCR7 (7-dehydrocholesterol reductase).

DHCR7 is rate-limiting enzyme of cholesterol synthesis. The up-regulation of DHCR7 indicates the increase of cholesterol synthesis. APOD was identified an HDL component and was in association with LCAT, apoA-I and CETP (cholesteryl ester transfer protein), where it could be part of a complex responsible for the transport of cholesterol from peripheral tissues to the liver for its further catabolism [32]. The down-regulation of APOD reduces the transportation and metabolism of cholesterol in peripheral tissues and may lead to the increase of cholesterol in circulation. Our findings of differentially expressed genes involved in lipid metabolism provided evidence that placenta may participate the disordered lipid metabolism in ICP and indicated that the alteration in lipid metabolism is multifaceted.

Previous studies revealed that the expression of vascular endothelial growth factor (VEGF) [33] and epidermal growth factor receptor (EGFR) [14] in placenta was significantly lower in ICP than in normal pregnancy, indicating the altered expression of VEGF and EGFR may be involved in ICP. Microarray analysis did not detect the alteration of VEGF and EGFR expression but detected the up-regulation of insulin-like growth factor binding protein 1 (IGFBP1) and insulin-like growth factor 2 (IGF2) and the down-regulation of nephroblastoma overexpressed gene (NOV).

The insulin-like growth factor (IGF)—IGF-binding protein (IGFBP) system is crucial for placental development and fetal growth. Fetal growth was retarded in Igf 1 -/- or Igf 2 -/- mouse [34, 35]. By binding IGF, which prevents from being degraded or prevents from interacting with it receptor, IGFBP affects IGF activity. NOV, also known as CCN3, is a member of CCN family of angiogenic regulators, that consists of six members (CCN1-6) and that are extracellular matrix proteins involved in the regulation of cellular processes like adhesion, migration, proliferation and differentiation[36, 37]. In early onset pre-eclamptic placenta, the expression of NOV at both mRNA and protein level was significantly decreased compared with normal pregnancy [38].

Chen et al. [39] reported that the apoptotic cell increased and that pro-apoptotic gene bax was up-regulated while anti-apoptotic gene bcl-2 was down-regulated in ICP placenta, resulting in the increase in bax/bcl-2 ratio. Perez [40] found that maternal obstructive cholestasis during pregnancy (OCP) causes apoptosis in rat placenta by demonstrating the increase in the ratio of Bax/Bcl-2 mRNA. In the current study, we found that deoxyribonuclease I-like 3 (DNASE1L3), tumor protein p73-like (TP73L), ring finger protein 36 (RNF36) and KIAA0367 were differentially expressed. These genes are all pro-apoptotic and were

found up-regulated in ICP placenta. All these findings indicate that apoptosis of placental trophoblast is an important pathology of ICP, although the cause-effect relationship is not known yet.

The alteration in transportation carrier of bile acid was certainly the focus of investigation in this field. Huang et al. [41] observed the expression of farnesoid X receptor (FXR) and bile salt excretory pump (BSEP) in ICP placenta with immunohistochemistry and found that the expression of FXR was increased and that of BSEP was decreased relative to normal pregnancy. In this study, we did not find any differentially expressed gene of transportation carrier of bile acid, however, we did find some differentially expressed genes involved in material transportation. These differentially expressed genes included transient receptor potential cation channel, subfamily V, member 5 (TRPV5), Solute carrier family 6, member 17 (SLC16A17), ATPase, Class II, type 9A (ATP9A) and others. Although the involvement of these genes in ICP remains unknown, it is reasonable to speculate that the abnormalities of placental material transportation carrier might be important pathophysiology of ICP.

Mutations or polymorphisms of genes expressing hepatobiliary transport proteins, at least including ABCB4 (MDR3), FIC1 (ATP8B1) and ABCB11 (BSEP), may contribute to the development and/or severity of ICP [42, 43]. These genes were expressed in human placentas probably at a low level [44, 45]. The low expression of these genes, limited sensitivity of microarray to identify differentiation of lowly expressed genes and relatively small sample size might be among the causes for lack of differential expression of these genes.

Except for above-mentioned genes, genes of categories at least including signal transduction, response to stimulus, reproduction, multi-organism process, biological adhesion, developmental process were differentially expressed, indicating the diversity of gene expression in placenta of ICP and the complexity of etiology and pathogenesis of ICP. Although the current investigation was primary, we believe that our experiment provided some clue for the further investigation of ICP etiology and pathogenesis.

In summary, 293 differentially expressed genes of 20 categories were found in ICP placenta, which suggests the diversity of gene expression alteration and the complexity of etiology and pathogenesis of ICP.

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