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Identification of Differentially Expressed Genes and Signaling Pathways in Placenta Tissue of Early-Onset and Late-Onset Pre-Eclamptic Pregnancies by Integrated Bioinformatics Analysis

Authors' Contribution:
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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Pre-eclampsia (PE) can be divided into 2 sub-groups: early-onset and late-onset PE. Although these sub-groups show overlapping molecular and cellular mechanisms and similar clinical manifestations, they are regarded as 2 different phenotypes with heterogeneous manifestations. The pathophysiological mechanisms underlying early-onset and late-onset PE still remain unclear. Therefore, the present study aimed to identify the key genes and pathways related to early-onset and late-onset PE, and to investigate the molecular mechanisms that are involved in gene regulation.

Material/Methods: Our analysis involved the Gene Expression Series (GSE) 74341 and GSE22526 from the National Center of Biotechnology Information (NCBI) Gene Expression Omnibus Database. These 2 microarray datasets included 15 patients with early-onset PE and 15 patients with late-onset PE.

Results: Our analyses identified 15 differentially expressed genes (DEGs), including *CGA*, *EGR1*, *HBB*, *HBA2*, *LEP*, and *LHB*. Gene Ontology (GO) functional annotation showed that the biological functions of these DEGs were mainly associated with steroid biosynthetic, oxidative stress, angiogenesis, and sex differentiation. Signaling pathway analyses showed that these DEGs were mainly involved in the prolactin signaling pathway, hormone metabolism, the AMPK signaling pathway, and the FoxO signaling pathway. Protein-protein interaction (PPI) network analysis identified 4 genes with the highest degree of interaction. The hub genes for this selection of DEGS were *EGR1*, *LEP*, and *HBB*.

Conclusions: Integrated bioinformatic analyses provide us with a new approach to further understand the pathophysiology and molecular mechanisms underlying early-onset and late-onset PE. The DEGs identified in this study represent potential biomarkers for the early diagnosis of PE and may provide significant options the treatment of these 2 subtypes of PE.

MeSH Keywords: Microarray Analysis • Pre-Eclampsia • Pregnant Women

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Background

Pre-eclampsia (PE) is a pregnancy-related disorder. The characteristics of PE are elevated blood pressure, and proteinuria, both of which lead to a variety of pathophysiological processes, including endothelial dysfunction, systemic inflammation, and impaired implantation [1]. PE occurs in approximately 3% of all pregnant women, and it is the main cause of more than 60 000 global maternal deaths every year [2]. The prevalence of PE has been increasing relentlessly [3]. However, as yet, there are no effective methods for screening or managing PE. Following delivery, the clinical symptoms of this disorder disappear. Even without any fetal-derived tissue, patients with a hydatidiform mole can also develop PE. The general consensus of opinion is that the placenta is linked to the etiology of this complication.

Genome-wide profiling approaches, such as microarray analysis, provide researchers with an efficient way of investigating the etiology of placental disorders. PE has 2 subtypes: early-onset (<34 weeks) and late-onset (>34 weeks) [4]. The clinical features and biochemical markers of these 2 subtypes differ [5]. Early-onset PE is life-threatening, and the maternal mortality associated with this subgroup is 20 times higher than normal delivery [6]. Furthermore, previous studies that have analyzed the expression of serum cytokines, and leukocyte function, revealed that early-onset PE is a special subtype of PE compared to late-onset [7]. Women with a history of early-onset PE tend to have an increased risk of chronic hypertension, or ischemic heart disease. The latter could lead to earlier cardiovascular death [8]. Therefore, comparing the expression profiles of the 2 subtypes of PE is of critical clinical significance. By investigating the etiologies underlying these 2 subgroups of PE could help us to develop new therapies for the treatment of this pregnancy disorder.

In this study, we identified numerous differentially expressed genes (DEGs) by comparing the gene expression profiles of placental tissue between patients with the 2 subtypes of PE. Upon screening, we conducted Gene Ontology (GO) enrichment analysis, signaling pathways enrichment analysis, and protein-protein interaction (PPI) network analysis, in an attempt to identify the molecular interactions that are implicated in the two sub-classifications of PE.

Material and Methods

Microarray data

We acquired GSE74341 and GSE22526's series matrix file(s) from the Gene Expression Omnibus (GEO) database (*http://www.ncbi.nlm.nih.gov/geo*); these databases store microarray data that are accessible to all researchers. GSE74341 was generated on the GPL16699 platform (Agilent039494 SurePrintG3 HumanGEv2 Microarray039381). GSE22526 was generated on the GPL10579 platform (GynObsLU Human 800 PE-associated cDNA).

Identification of DEGs

In order to identify DEGs, we used the R software and annotation package to transfer the downloaded platform and series of matrix file(s). The probe number was then changed into a universal gene name (gene symbol) within a CSV document. We then identified DEGs in early-onset and late-onset PE samples in the GSE74341 and GSE22526 using R software, and the Limma package [9]. The threshold for differential expression genes was defined as a *P*-value <0.05 and |log2(fold change)| >1.

Enrichment analyses

To assess the function of DEGs in early-onset and late-onset PE, we performed GO term enrichment analyses using the ClusterProfiler package in R software [10], including biological process, cellular component, and molecular function; a *P*-value <0.05 was set as the threshold. We performed signaling pathway enrichment analyses, including KOBAS-Kyoto Encyclopedia of Genes and Genomes, KEGG), for all DEGs using a specific website (*http://kobas.cbi.pku.edu.cn*). The threshold was set to a *P*-value <0.05.

PPI network analyses

We used the STRING online tool (*http://string-db.org*) to explore the relationships between the DEGs. Then, we attempted to reveal the biomolecular interactions that occur in the 2 different forms of PE. Hub genes were identified using Cytohubba, a plug-in of the Cytoscape software package [11].

Human participants

Ethical approval was granted by the Ethics Committee of The First Affiliated Hospital of China Medical University (Shenyang, China) and methods were carried out in accordance with the committee guidelines (Ethical Application Reference Number: AF-SOP-07-1.1-01). All participating patients signed informed consent. The diagnosis criteria for PE were published previously by Steegers et al. [12]. Patients enrolled in the PE group had no history of pre-existing or chronic hypertension, although they exhibited ≥140 mmHg systolic or ≥90 mmHg diastolic blood pressure on 2 occasions at least 4 hours apart after 20 weeks of gestation, and ≥300 mg per 24-hour urine collection after 20 weeks of gestation. Early-onset PE was defined as onset of the disease before 34 weeks of gestation (between 20 and 34 completed gestational weeks); late-onset was

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Table 1. Clinical characteristics of pregnant women enrolled in the study.

	Early-onset PE (n=5)		Late-onset PE (n=5)		P
Maternal age (years)	28	(23–34)	30	(27–35)	0.399
BMI (kg/m^2)	20.4	(17.9–22.3)	21.5	(19.5–23.1)	0.347
Systolic pressure (mmHg)	153	(141–173)	157	(142–177)	0.465
Diastolic pressure (mmHg)	100	(78–115)	97	(82–110)	0.602
24h proteinuria (mg/L)	4484	(319–8428)	3420	(412–7361)	0.754
Gestational age at delivery (days)	225	(215–235)	262	(253–273)	0.009
Birthweight (grams)	1256	(950–1790)	2788	(2100–3590)	0.009
Placental weight (grams)	338	(180–510)	592	(490–750)	0.016

BMI – body mass index; PE – pre-eclampsia.* Comparing early-onset PE group with late-onset PE group using independent-samples Mann-Whitney U test.

Table 2. Sequence of primers used for validation of expression levels of core DEGs.

Genes	Genes ID	Sequence
CGA left primer	NM_000735.3	tctccattccgctcctgat
CGA right primer		gggagaagaatgggttttcc
EGR1 left primer	NM_001964.2	tctgaacaacgagaaggtgct
EGR1 right primer		gggcagtcgagtggtttg
HBA2 left primer	NM_000517.6	GTCCCCACAGACTCAGAGA
HBA2 right primer		GGGAAGGACAGGAACATCC
HBB left primer	NM_000518.4	gcacgtggatcctgagaact
HBB right primer		cactggtggggtgaattctt
LEP left primer	NM_000230.2	tgacatttcacacacgcagtc
LEP right primer		atgaagtccaaaccggtgac
LHB left primer	NM_000894.2	atcctggctgtcgagaagg
LHB right primer		gtaggtgcacaccacctgag

DEG – differentially expressed genes.

defined as onset of the disease after 34 weeks of gestation (between 34 and 41 completed gestational weeks). The clinical characteristics of the pregnant women enrolled in this study are given in Table 1.

Validation of the mRNA expression of crucial genes

We selected 10 placental tissue samples for verification by quantitative reverse transcription polymerase chain reaction (PCR): 5 samples of early-onset PE (<34 weeks gestation), and 5 samples of late-onset PE (>36 weeks gestation). Chorionic tissues were obtained from 4 different parts of the placenta, from which the amniotic membrane and maternal decidual tissues were removed. Tissues were frozen and stored at –80°C until use. RNA isolation was performed with TRIzol (Invitrogen) in accordance with the manufacturer's instructions. First-strand cDNA was reverse transcribed using the GoScript Reverse Transcription System (Promega). Quantitative PCR was then performed using GoTaq qPCR Master Mix (Promega), and amplification was quantified by the CFX-96 system (Bio-Rad). Expression levels were then normalized to *Gapdh*. At least 3 biological replicates were performed for each experiment. Primer sequences are given in Table 2.

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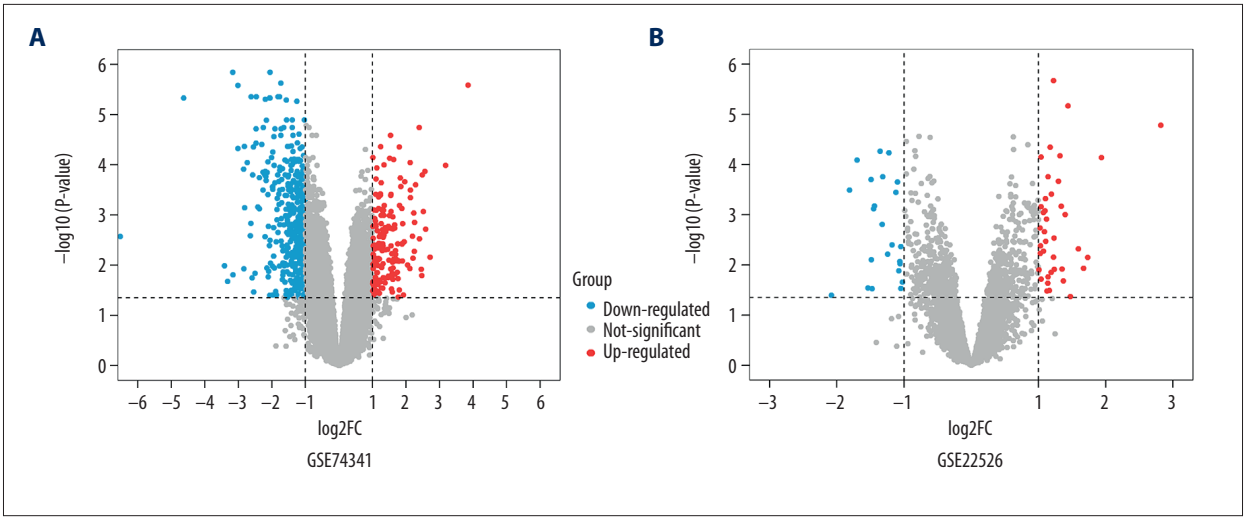

Figure 1. Volcano plot of gene expression profile data in early-onset pre-eclampsia and late-onset ones. (**A**) Volcano plot of GSE74341. (**B**) Volcano plot of GSE22526.

Results

Identification of DEGs

GSE74341 and GSE22526 data were downloaded from GEO, including 30 placenta samples (15 early-onset and 15 late-onset). We then used the Limma package to identify DEGs by comparing early-onset and late-onset PE. The Limma package identified 172 upregulated genes and 455 downregulated genes in the GSE74341 dataset (a total of 627 genes; Figure 1A). In total, 62 DEGs were acquired from the GSE22526 datasets, including 23 upregulated genes and 39 downregulated genes (Figure 1B).

The expression levels of the top 100 DEGs in GSE74341, and all the DEGs in GSE22526, are presented in a heatmap (Figure 2). After merging all DEGs in GSE74341 and GSE22526, 15 DEGs were revealed, including 4 upregulated genes and 11 downregulated genes: *HBB*, *HBA2*, *AOC3*, *EGR1*, *CLEC3B*, *AOC1*, *EGFL7*, *HIGD1B*, *KLF2*, *CCND1*, *COL18A1*, *CGB3*, *LHB*, *CGA*, and *LEP* (Figure 3). Of these, *HBB*, *HBA2*, *AOC3*, *EGR1*, *CLEC3B*, *AOC1*, *HIGD1B*, *KLF2*, *CCND1*, and *COL18A1*, were novel and provided us with a new direction with which to investigate the etiopathogenetic mechanisms underlying PE.

Enrichment analyses

In terms of biological processes, our analyses showed that DEGs were significantly enriched in oxidative stress, the biosynthesis of steroids, the regulation of nitric oxide biosynthesis, angiogenesis, and sex differentiation. With regards to cellular components, DEGs were significantly enriched in the endocytic vesicles, blood microparticles, and extracellular matrix. With regards to molecular function, DEGs were significantly enriched in peroxidase activity, oxidoreductase activity, and antioxidant activity (Figure 4). Enrichment analyses suggested that the DEGs were mainly implicated in oxidative stress and related function.

Signaling pathway analyses

Several enriched pathways of DEGs were identified between early-onset and late-onset PE (Table 3). The top 10 *P*-values included the prolactin signaling pathway, peptide hormone metabolism, the take up of oxygen by erythrocytes and the subsequent release of carbon dioxide, glycoprotein hormones, hormone ligand-binding receptors, peptide hormone biosynthesis, the metabolism of steroid hormones, the AMP-activated protein kinase (AMPK) signaling pathway, and the FoxO signaling pathway. Hormone metabolism was identified as the most predominant signaling pathway when compared between early-onset and late-onset PE.

PPI network analysis and hub genes

All 15 DEGs were entered into the STRING online database. PPI networks were then constructed (Figure 5). The PPI enrichment *P*-value was 4.13×10^{-6}. The top 5 combined scores were 0.995 (between *HBB* and *HBA2*), 0.981 (between *LHB* and *CGA*), 0.775 (between *LEP* and *CCND1*), 0.737 (between *CGB3* and *CGA*), and 0.715 (between *EGR1* and *CCND1*). Three genes (*EGR1*, *LEP* and *HBB*) were identified as hub genes (Figure 5B).

The validation of hub gene expression in clinical samples

To further determine the expression of hub genes in placental tissue from early-onset PE and late-onset PE, we used real-time quantitative PCR to detect the expression of the 3 hub genes

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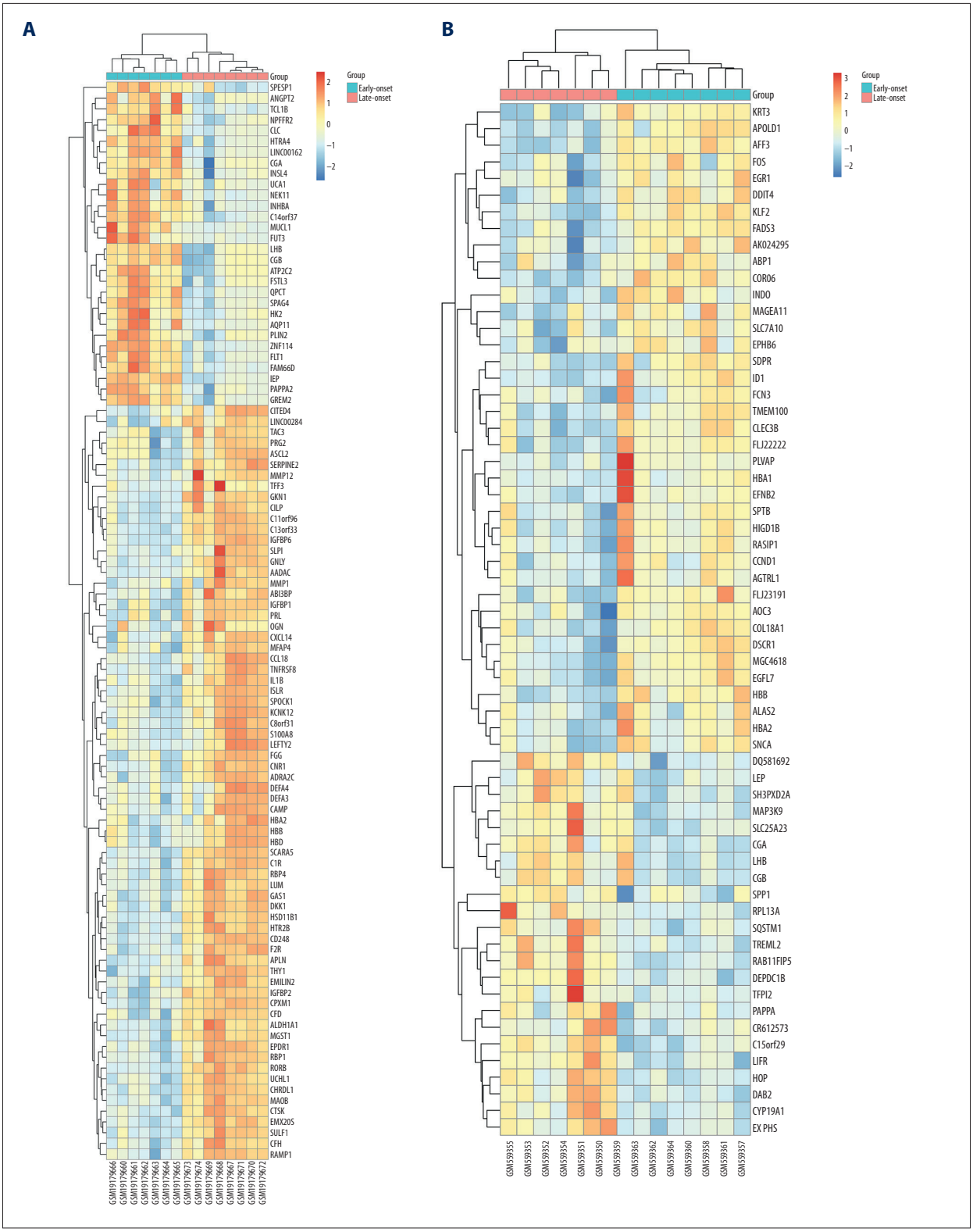

Figure 2. Heat map of DEGs. (**A**) Top 100 of GSE74341. (**B**) GSE22526, blue represents a lower expression level, red represents higher expression levels,and white represents that there is no different expression amongst the genes. Each column represents one dataset and each row represents one gene. The gradual color ranged from blue to red represents the changing process from downregulation to upregulation. DEG – differentially expressed gene.

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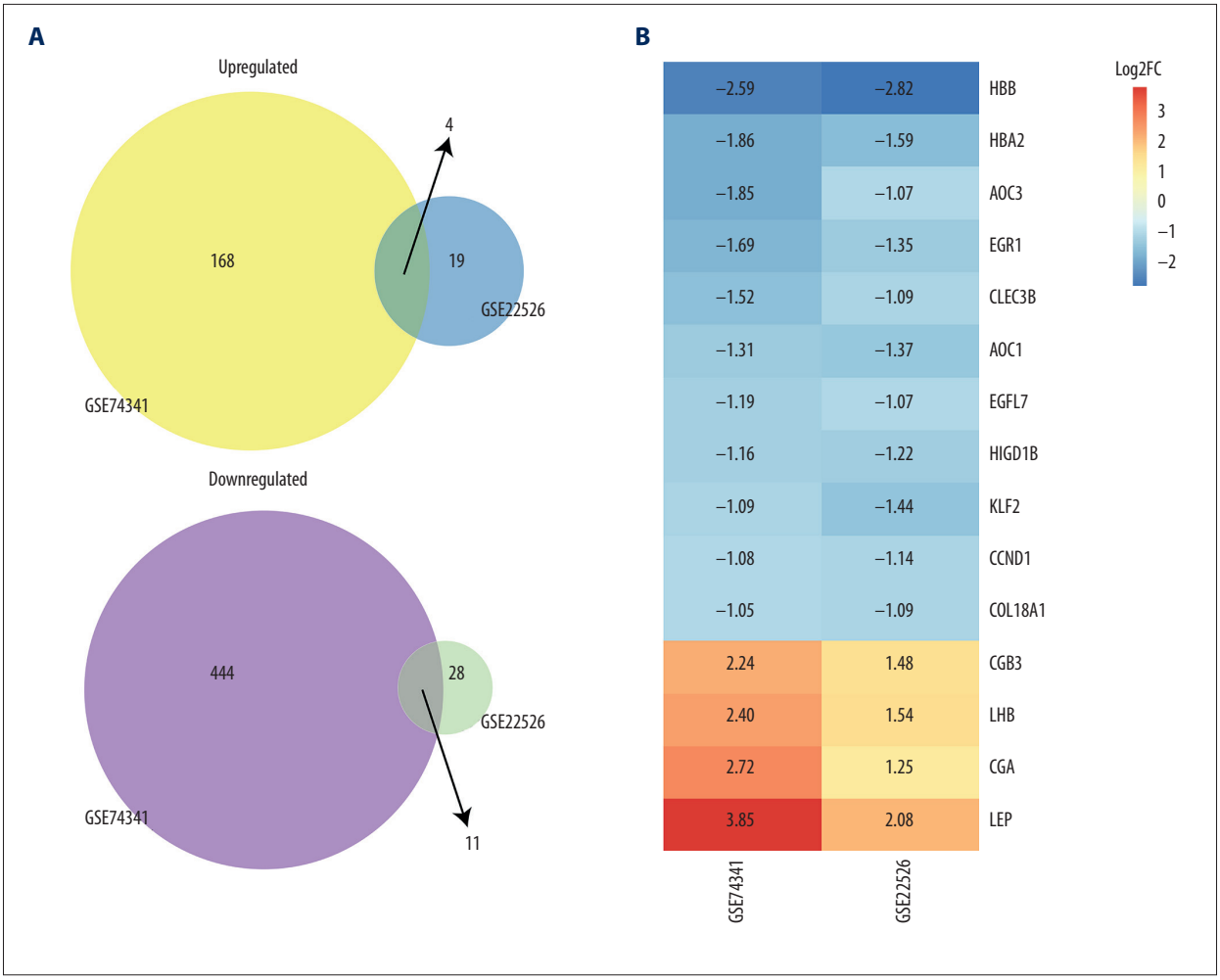

Figure 3. DEGs after merged. (**A**) Venn diagram shows that 4 upregulated and 11 downregulated genes. (**B**) Heat map of DEGs after merged GSE74341 and GSE22526. The number in each rectangle represents the normalized gene expression level. DEG – differentially expressed gene.

in placental tissue, and 2 pairs of proteins showing the highest interaction. Placentas were collected from patients with early-onset PE and late-onset PE. We confirmed that *EGR1*, *HBB,* and *HBA2*, were significantly downregulated in early-onset PE when compared with late-onset PE. Furthermore, *LHB*, *CGA*, and *LEP* were significantly upregulated in early-onset PE compared with late-onset PE (Figure 6). These results were consistent with our bioinformatic results.

Discussion

Early-onset and late-onset PE should be viewed as distinct nosological entities rather than different clinical forms of the same illness. The differences between these conditions are not limited only to the time that clinical symptoms emerge; and it should be noted that this issue is still debated [13]. Previous studies performed gene expression analyses of early-onset and late-onset PE to identify DEGs and investigate their potential for exploring the role of genetic heterogeneity in the pathogenesis of the 2 sub-classifications of PE [14–16]. These genome-wide expression profiling techniques were valuable given the significant availability of complementary data. Genome-wide approaches have also been used to explore differential expression in independent placental tissues of early-onset and late-onset PE. However, the underlying molecular mechanisms associated with early-onset and late-onset PE have not been investigated; many of the previous studies have focused on a single gene, or results were derived from a single-cohort study. In the current study, we acquired placental gene expression data from early-onset and late-onset patients, and used bioinformatic analysis to explore the importance of key genes. Using 2 genome-wide transcriptomic datasets, we identified several DEGs between early-onset and late-onset PE, including 4 upregulated genes and 11 downregulated genes. These genes may represent crucial biomarkers, with mechanistic

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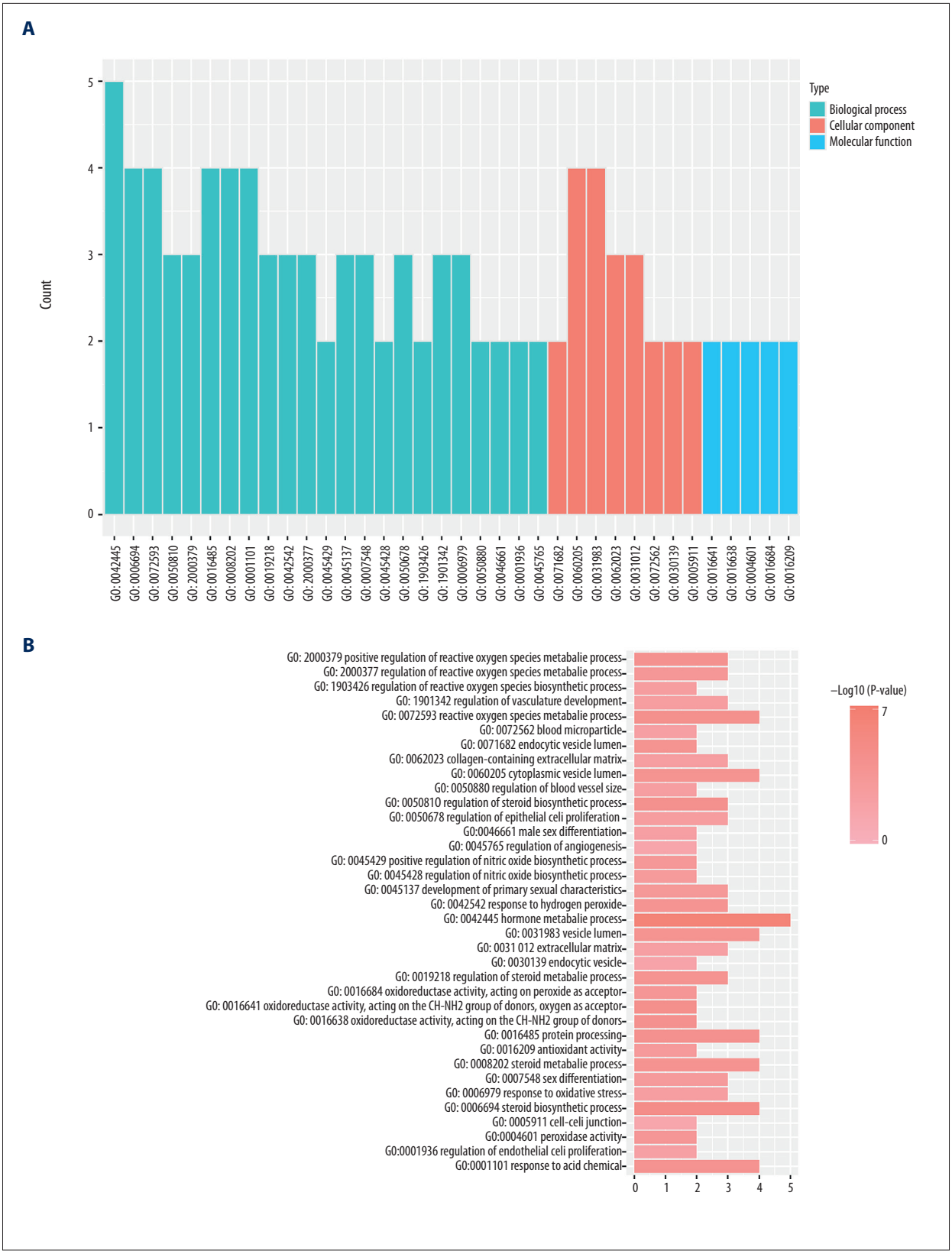

Figure 4. (**A, B**) GO functional annotation of DEGs between early-onset and late-onset pre-eclampsia. GO – Gene Ontology; DEG – differentially expressed gene.

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Table 3. Signaling pathway enrichment.

Term	Database	ID	Gene number	*P*-value	Corrected *P*-value	Genes
Prolactin signaling pathway	KEGG pathway	hsa04917	3	1.82E-06	0.000278974	LHB, CCND1, CGA
Peptide hormone metabolism	Reactome	R-HSA-2980736	3	2.56E-06	0.000278974	LEP, LHB, CGA
Erythrocytes take up oxygen and release carbon dioxide	Reactome	R-HSA-1247673	2	5.42E-06	0.000361262	HBA2, HBB
Glycoprotein hormones	Reactome	R-HSA-209822	2	5.42E-06	0.000361262	LHB, CGA
Hormone ligand-binding receptors	Reactome	R-HSA-375281	2	6.50E-06	0.000383313	LHB, CGA
Peptide hormone biosynthesis	Reactome	R-HSA-209952	2	7.68E-06	0.000395435	LHB, CGA
Metabolism of steroid hormones	Reactome	R-HSA-196071	2	5.18E-05	0.000964628	LHB, CGA
Metabolism	Reactome	R-HSA-1430728	5	0.000279689	0.003168314	HBA2, HBB, LHB, CGA, AOC3
AMPK signaling pathway	KEGG pathway	hsa04152	2	0.000771072	0.006385017	LEP, CCND1
FoxO signaling pathway	KEGG pathway	hsa04068	2	0.000883228	0.007065826	KLF2, CCND1
Signal Transduction	Reactome	R-HSA-162582	4	0.006567989	0.021934604	LEP, LHB, CCND1, CGA
Metabolism of proteins	Reactome	R-HSA-392499	3	0.009201322	0.025954137	LEP, LHB, CGA

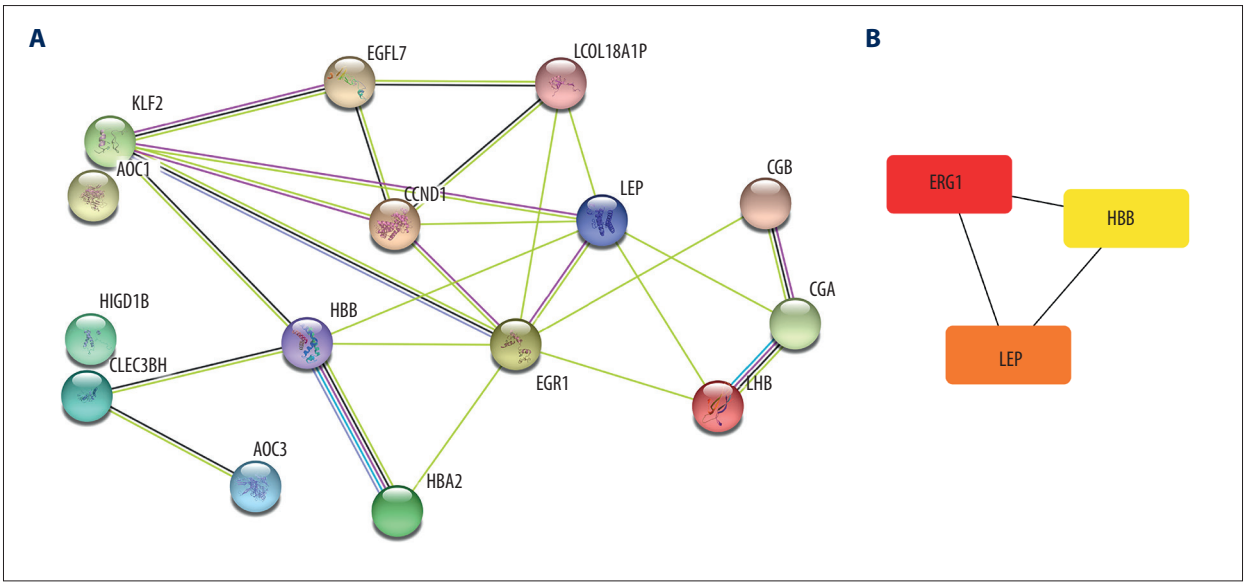

Figure 5. The PPI network and the hub genes of DEGs. (**A**) The PPI network was analyzed by String online website. (**B**) The 3 hub genes of DEGs. DEG – differentially expressed gene; PPI – protein–protein interaction.

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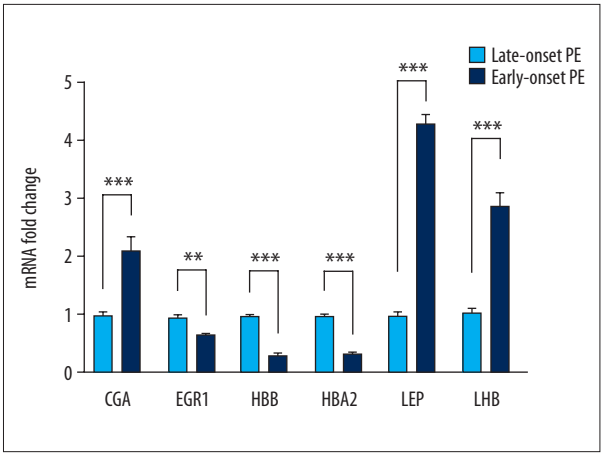

Figure 6. The validation of hub genes expression in clinical samples. Real-time quantitative PCR revealed that *EGR1*, *HBB*, and *HBA2* were downregulated in early-onset pre-eclampsia group compared with late-onset pre-eclampsia. While *LHB*, *CGA*, and *LEP* were significantly upregulated.

associations, for early-onset pathogenesis and prediction. Five of these 15 DEGs, *EGFL7*, *CGB3*, *LHB*, *CGA*, and *LEP*, were differentially expressed when compared between early-onset PE and healthy controls [17].

GO functional annotation showed that several biological processes were associated with the enriched genes, including steroid biosynthesis, oxidative stress, angiogenesis, and sex differentiation. Steroids, such as estradiol (E2), can modulate the synthesis of stress factors and both angiogenic and vascular endothelium functions. Furthermore, E2 increases the synthesis of angiogenic factors and nitric oxide (NO) [18], thus mediating vascular tone and increasing uterine blood flow in normal pregnancies [19]. Previous work demonstrated that *CGA* is an estrogen receptor-responsive gene [20]. Further research is necessary to determine if the association between *CGA* and estrogen is involved in the pathogenetic mechanisms underlying early-onset PE. Oxidative stress is one of the most important humoral factors to consider when dealing with a poorly perfused placenta. Previous studies have indicated that oxidative stress is more significant in early-onset PE compared to late-onset PE [21]. Furthermore, oxidative stress in the syncytiotrophoblast is a particular hallmark of PE, particularly early-onset PE [22]. A complex blend of factors released from a stressed syncytiotrophoblast can lead to a systemic inflammatory response, the predominant clinical feature of PE. Recent studies have suggested that fetal gender might act as a critical risk factor for PE. It was suggested that nearly half of the cases of PE are X-linked and arise due to problems associated with X-inactivation [23,24]. Moreover, women carrying male fetuses show higher levels of vascular resistance in terms of uterine artery pulsatility index [25]. Therefore, the male fetus may be less adaptable to an unfavorable environment due to the fact that they are more sensitive to suboptimal placentation [26]. The existence of a more "pro-inflammatory environment" in the male fetus could also explain the clear bias of early-onset PE towards female fetuses [27]. Above all, our GO analysis of DEGs indicated that early-onset PE is primarily due to a serious defective placentation and poor uterine blood flow, and shares common pathophysiology with other disorders of placentation; these findings were similar to those reported previously [28].

Furthermore, the enriched DEGs were associated with the prolactin signaling pathway, hormone metabolism, the AMPK signaling pathway, and the FoxO signaling pathway. Prolactin is released from multiple extra-pituitary sites during pregnancy, including adipose tissue, lymphocytes, myometrium, prostate, breast, and the decidua [29]. Prolactin generated from decidua can regulate placental angiogenesis and immunological functions at the feto-maternal interface [29]. The full-length, 23 kiloDalton (kDa) prolactin protein can promote placenta angiogenesis, while the proteolytic 16 kDa fragment can suppress placenta angiogenesis [30]. Disfunction associated with the 23 kDa and 16 kDa fragments could explain both impaired early placentation, and subsequent endothelial dysfunction; this represents the core pathophysiology of PE. The elevation of levels of the 16 kDa prolactin fragment in the circulation, amniotic fluid, and urine of PE patients also fits this model [31]. AMPK is a one of the central regulators of cellular and organismal metabolism in eukaryotes. During pregnancy, AMPK plays an important role in placental differentiation, maternal and fetal energy homeostasis, nutrient transportation, and fetal membrane protection [32]. In a previous paper, Nadiye et al. showed that the levels of AMPK were significantly elevated in patients with severe PE compared to controls and those with mild PE. Importantly, there was a positive relationship between the levels of AMPK and blood pressures, and there was a negative relationship between the levels of AMPK and the gestational week at birth and fetal weight [33]. These findings indicate that AMPK might act as a novel marker to predict disease severity. *FoxO1* is a transcription factor expressed in syncytiotrophoblasts; its phosphorylated form (p-*FoxO1*), and acetylated form (ac-*FoxO1*) are expressed in cytotrophoblasts and syncytiotrophoblasts [34]. In addition to regulating apoptosis and oxidative stress, *FoxO1* regulates various other cellular processes that are important for the placenta, including cellular proliferation and differentiation, cell cycle regulation, DNA repair, and metabolism. A previous study found that the number of *FoxO1*-negative syncytiotrophoblast nuclei were increased, and *FoxO1*-positive syncytiotrophoblast nuclei were significantly decreased, in mild PE compared to controls [35]. The abnormal expression of *FoxO1* may be involved in the impaired placental cellular morphogenesis observed in mild PE. Activation of *FoxO1* could repress the expression of

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CCND1 [36,37], a novel DEG identified in the present study. The downregulation of *CCND1* suggests that the activation of *FoxO1* might be more serious in early-onset PE.

The PPI network of DEGs was constructed by STRING. In this study, the pairing of *CGA* with *LHB*, and the pairing of *HBB* with *HBA2,* showed the highest levels of interaction in the PPI network. Human chorionic gonadotropin (hCG) is associated with placental disorders [38]. This glycoprotein consists of 2 dissimilar peptide subunits, designated alpha (α) and beta (β). CGA encodes the alpha subunit. Previous studies have found that α-hCG is correlated with PE [38,39]. These studies indicated that hCG is a reliable trophoblastic marker, and that α-hCG could reflect chronic placental hypoxia [38]. One of the DEGs identified in the present study was *CGA*; this indicates that α-hCG could represent a potential serum biomarker with which to make a differential diagnosis between early-onset and late-onset PE. *LHB* is a member of the glycoprotein hormone beta chain family and encodes the beta subunit of luteinizing hormone (LH). Many studies have found that *LHB* was unregulated in early-onset PE or PE, largely due to the abnormal physiology of trophoblasts [17,40]. LH is primarily expressed in syncytiotrophoblasts, thus indicating that altered pathways are related to placental oxidative stress or uteroplacental vascular insufficiency [41]. As LH is a characteristic for PE in the maternal circulation, it could also be used as a novel screening biomarker for early-onset PE via serum testing [42]. Hemoglobin subunit alpha 2 (*HBA2*) is a protein-coding gene and was one of the novel DEGs identified in the present study. Although previous research has not identified a role for *HBA2* in PE, the diseases most associated with *HBA2* include hemoglobin H disease, and alpha-thalassemia; these are also significantly related to certain pregnancy complications, including fetal growth restriction, automatic miscarriages, and perinatal mortality [43,44]. Oxidative stress as a result of iron overload, and placental hypoxia caused by maternal anemia, appear to provoke such complications. It is possible that the oxidative stress associated with *HBA2* is implicated in the pathogenetic mechanisms underlying PE. This concept is worth exploring in future research.

Three hub genes were identified in our analyses. Earlier studies showed that *EGR1* protein plays a key role in implantation [45,46]; while *LEP* plays a major role in the regulation of energy homeostasis and can regulate the cytokine pathway by connecting the inflammatory and immune systems [47]. Recently, *LEP* was also shown to have vital roles in various physiological processes, including angiogenesis and the regulation of arterial blood pressure [48]. The level of serum *LEP* may therefore act as a biomarker to differentiate between early-onset and late-onset PE [49]. Moreover, *LEP* could activate AMPK [50], which is also elevated in PE and associated with blood pressure. Blocking the activation of AMPK stimulated by *LEP* could also be proposed as a promising drug target for PE. *HBB*'s basic function is oxygen transportation. Placental hypoxia has been suggested to play a key role in the mechanisms underlying PE. Alternations in gene expression in the placenta have further confirmed that upregulated pathways are related to hypoxia in PE [51]. The expression of *HBB* was shown to be altered because of its responses to oxidative or nitrosative stress, and/or hypoxia, in the rhesus macaques fetal/neonatal brain [52]. Owing to its ability to reversibly bind O_2 and other gaseous ligands in erythrocytes, *HBB* could also be used as a therapeutic target to improve the clinical manifestations caused by hypoxia.

Conclusions

We conducted comprehensive bioinformatics analysis of gene expression profiles in placental tissue from early-onset and late-onset PE, and identified 15 DEGs, including 3 hub genes. Abnormal placentation and hypoxia are more serious in early-onset PE, where placental and fetal tissues are subjected to adverse exposure from early pregnancy onwards. Defective placentation appears to play a much more critical role in the pathophysiology of early-onset PE than late-onset PE, with more adverse consequences for the fetus, such as fetal growth restriction, thus supporting the current results. Integrated bioinformatics analyses therefore yielded several promising serum biomarkers, such as *LEP, LH*, and α-hCG encoded by *CGA*, for the early diagnosis of the 2 different subtypes of PE. Furthermore, blocking the activation of AMPK stimulated by *LEP*, and using the ability of *HBB* to transport oxygen, could represent new therapeutic targets for early-onset PE.

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