Identification of Key Candidate Genes and Pathways in Preterm Birth by Integrated Bioinformatical Analysis

Wen-Fei Zheng, Pingping Peng, Xiaojing Yuan, Jufang Qin, Chenghong Xie, Aihua Chen*
Department of Gynecology and Obstetrics, The People’s Hospital of China Three Gorges University/ The First People's Hospital of Yichang, Hubei 443000, China

*Corresponding author: Aihua Chen, Department of Gynecology and Obstetrics, The People’s Hospital of China Three Gorges University/ The First People’s Hospital of Yichang, Hubei 443000, China

Received date: March 11, 2021; Accepted date: April 04, 2021; published date: April 09, 2021

Citation: Wen-Fei Zheng, Pingping Peng, Xiaojing Yuan, Jufang Qin, Chenghong Xie, Aihua Chen.(2021) Identification of Key Candidate Genes and Pathways in Preterm Birth by Integrated Bioinformatical Analysis J.Women Health Care and Issues 4 (2); DOI: 10.31579/2642-9756/046

Abstract

Background: Preterm birth (PTB) is a primary cause of neonatal morbidity and mortality, the pathogenic mechanisms of PTB still remain largely unexplored. In the present study, we aimed to identify potential key genes and pathway associated with PTB by bioinformatics analysis.

Methods: The GSE46510 dataset was obtained from GEO database. Differentially expressed genes (DEGs) were identified using the limma package in R software, the functional enrichment analysis was performed, and the protein-protein interaction (PPI) network was constructed by Cytoscape software. The network topology was analyzed using MCODE.

Results: A total of 335 DEGs were identified from the dataset. The majority of up-regulated DEGs were significantly enriched in inflammatory response, while down-regulated DEGs were mainly enriched in mitotic nuclear division. The top 5 hub up regulated genes were ITGAM, IL1B, ITGAX, NFKB1, and SOCS3. Pathway analysis indicated enrichment in Cytokine-cytokine receptor interaction, signaling by Interleukins. The top 5 hub down regulated genes were CXCR4, ANAPC10, ANAPC4, UBE2V2, UBA3. Pathway analysis indicated enrichment in Ubiquitin mediated proteolysis, Phosphorylation of the APC/C.

Conclusion: Our study indicated genes and pathways in PTB by bioinformatics analysis, which may provide novel insights for unraveling pathogenesis of PTB.

Key words: preterm birth; differentially expressed genes; bioinformatics analysis

Running title: Genes and Pathways in Preterm Birth

Introduction

As one of the most common and serious complications of pregnancy, preterm birth (PTB) is defined as delivery before 37 weeks of gestation [1]. Every year, about 15 million babies are born before 37 weeks’ gestation worldwide, and such number is still increasing, with rates varying from 5% to 18% [2]. PTB is a primary cause of neonatal morbidity and mortality, causing some serious complications. In addition, PTB may lead to increased risk of adult-onset chronic diseases, placing a heavy burden on families and society [2, 3].

In the past few decades, important advances and efforts have been made in research on pregnancy and PTB. For example, the initiation of PTB is closely related to the change of inflammatory medium and its signaling pathway, such as IL-6, IL-8 and TNF-α [4, 6]. Cell-free fetal DNA (cffDNA) can engage TLR-9 and induce an inflammatory response, and individuals with high concentrations of cffDNA are associated with increased risk for spontaneous PTB (sPTB) [7, 8]. The pathogenic mechanisms of PTB still remain largely unexplored. Therefore, it is urgently necessary to identify potential target genes associated with PTB in order to prevent and predict PTB.

In the present study, we aimed to identify potential genes and miRNAs associated with PTB, and explore the underlying mechanisms in the PTB development based on the GSE46510 dataset from the Gene Expression Omnibus (GEO) database [9]. Moreover, we assessed the gene expression profiles to identify differentially expressed genes (DEGs) of individuals with an sPTB within 48 h of admission. Furthermore, functional analysis was performed, and a protein-protein interaction (PPI) network was constructed. In addition, the target miRNAs for DEGs were identified accordingly.
Material and Methods

Microarray Data

The gene expression dataset of GSE46510 was obtained from GEO (http://www.ncbi.nlm.nih.gov/geo/) database, which was analyzed using Affymetrix Human Genome U133 plus 2.0 Array. There were 154 samples, which were divided into two groups as follows: women who did (n = 48) and did not have a sPTB (n = 106) within 48 h of admission. Peripheral blood was collected at hospital admission from 154 women with threatened preterm labor (TPTL) before any medical treatment.

Data Processing and Screening of Degs

The original data in CEL format were processed into expression values by the robust multi-array average (RMA) method through the Affy [10] in R software(version 1.52.0; http://bioconductor.org/packages/release/bioc/html/affy.html) Secondly, the probe level data were transformed by R/Bioconductor platform notes package.

Identification of Degs

DEGs were identified by Bayes methods using the limma package [11] version 3.30.3 (www.bioconductor.org/packages/release/bioc/html/limma.html) in R software. The cut-off criteria were adjusted as P<0.05 and |log2 fold change (FC)| >1

Functional and Pathway Enrichment Analyses

The Database for Annotation, Visualization and Integrated Discovery (DAVID, version 6.8, https://david.ncifcrf.gov) provides a comprehensive set of functional annotation tools for investigators to understand biological meaning behind a large list of genes [12], including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of up-regulated and down-regulated DEGs. GO enrichment analysis, consisting of biological processes (BP), cell component (CC) and molecular function (MF), was performed by DAVID. Pathway enrichment analysis for screening of DEGs with DAVID, Reactome(Available online: http://www.reactome.org). The cut-off criteria of GO terms and KEGG pathways enriched with DEGs were P<0.05.

Ppi Network Construction

The PPI network was constructed with all DEGs using an online database: STRING (version 10.5, https://string-db.org) [13]. PPI links with a combined score >0.4 were identified for constructing the PPI network. PPI network was constructed with Cytoscape software (version 3.6.0, http://cytoscape.org/) [14], and hub genes were ranked by MCODE.

Ethics

All analyses were based on the data from public database, so ethics approval and patient consent were not required.

Results

Identification of Degs

After integrated bioinformatical analysis, out of a collection of 19009 Genes from patients indicated dysregulation of 2% (157 up-regulated and 178 down-regulated transcripts). 37DEGs were excluded due to duplication and low expression level (Figure 1).

Figure 1: Brief microarray results of genes.

Expression levels of 19009 genes were assessed in 154 samples. Compared with women not delivered within 48h of hospital admission, 372 genes (2%) had significant changes in expression levels (fold change>1, p<0.05). A total of 37 genes were precluded due to duplication and low expression level. A total of 335 genes were then identified from the screen, with 157 upregulated and 178 down regulated

Hierarchical clustering revealed the DEGs expression in blood women delivered within 48h of hospital admission and not delivered samples (Figure 2).
**Figure 2:** Heat Maps: Differentially expressed genes for women not delivered within 48h of hospital admission and delivered within 48h of hospital admission were hierarchically clustered. “Red” indicates high relative expression, and “green” indicates low relative expression.

**Degs Gene Ontology and Signaling Pathway Enrichment Analysis in Preterm Birth**

To investigate underlying biological associations, DEGs gene ontology analysis (GO) were performed with DAVID. As shown in **Figure 3** and **Table 1**, in the biological process group, up-regulated genes mainly enriched in inflammatory response, myeloid dendritic cell differentiation, apoptotic process, cell adhesion, regulation of cell proliferation; Down-regulated genes mainly enriched in mitotic nuclear division, cellular response to DNA damage stimulus, cell division, regulation of cell cycle.
Figure 3: Gene Ontology analysis and significant enriched GO terms of DEGs in preterm birth. GO analysis classified the DEGs into 3 groups (molecular function, biological process and cellular component)

| Category          | Term                                      | Count | %     | P value    |
|-------------------|-------------------------------------------|-------|-------|------------|
| Up-regulated      | GO:0006954~inflammatory response          | 13    | 0.047163 | 4.90E-04  |
|                   | GO:0043011~myeloid dendritic cell differentiation | 4     | 0.014512 | 8.77E-04  |
|                   | GO:0006915~apoptotic process               | 15    | 0.054419 | 0.001909  |
|                   | GO:0007155~cell adhesion                   | 13    | 0.047163 | 0.002514  |
|                   | GO:0037877~cellular response to DNA damage stimulus | 8     | 0.029023 | 0.002837  |
|                   | GO:0070062~extracellular exosome           | 47    | 0.170512 | 1.39E-04  |
|                   | GO:0005811~lipid particle                  | 5     | 0.01814  | 0.003727  |
|                   | GO:002020~protease binding                 | 6     | 0.021768 | 0.003828  |
In the cellular component group, up-regulated genes mainly enriched in extracellular exosome, membrane, endosome, early endosome, lipid particle; Down-regulated genes mainly enriched in nucleus, nucleoplasm, nucleolus, nuclear heterochromatin, NatA complex. In the molecular function group, up-regulated genes mainly enriched in protease binding, protein binding, ATP binding, transcription factor activity, RNA polymerase II core promoter proximal region sequence-specific binding, transferase activity, transferring glycosyl groups; Down-regulated genes mainly enriched in protein binding, RNA binding, voltage-gated cation channel activity, Rab geranylgeranyl transferase activity, nucleic acid binding. These results showed that most of the DEGs were significantly enriched in cell cycle, nucleus, protein binding. Signaling Pathway Analysis showed both up-regulated and down-regulated DEGs were mainly enriched in Immune System, Interleukins signaling pathway and Chemokine signaling pathway (Figure 4 and Table 2).

**Table 1: GO analysis of differentially expressed genes in preterm birth (P<0.05)**

| GO term                                               | Count | *P*-value   | Log10(p-value) |
|-------------------------------------------------------|-------|-------------|----------------|
| GOTERM MF DIRECT GO:0005515~protein binding           | 108   | 0.39185     | 0.005681       |
| GOTERM MF DIRECT GO:0005524~ATP binding               | 25    | 0.090698    | 0.018335       |
| GOTERM MF DIRECT GO:0009982~transcription factor activity, RNA polymerase II core promoter proximal region sequence-specific binding | 3     | 0.010884    | 0.022942       |
| GOTERM MF DIRECT GO:0016757~transferase activity, transferring glycosyl groups | 4     | 0.014512    | 0.024674       |

**Down-regulated**

| GO term                                               | Count | *P*-value   | Log10(p-value) |
|-------------------------------------------------------|-------|-------------|----------------|
| GOTERM BP DIRECT GO:0007067~mitotic nuclear division   | 10    | 0.032764    | 4.55E-04       |
| GOTERM BP DIRECT GO:0006974~cellular response to DNA damage stimulus | 9     | 0.029488    | 6.46E-04       |
| GOTERM BP DIRECT GO:0051301~cell division             | 11    | 0.036041    | 0.001403       |
| GOTERM BP DIRECT GO:0051726~regulation of cell cycle   | 6     | 0.019659    | 0.005557       |
| GOTERM BP DIRECT GO:0008380~RNA splicing               | 6     | 0.019659    | 0.018031       |
| GOTERM CC DIRECT GO:0005634~nucleus                    | 73    | 0.23918     | 2.88E-05       |
| GOTERM CC DIRECT GO:0005654~nucleoplasm                | 44    | 0.144163    | 1.06E-04       |
| GOTERM CC DIRECT GO:0005730~nucleus                    | 16    | 0.052423    | 0.008538       |
| GOTERM CC DIRECT GO:0005720~nuclear heterochromatin    | 3     | 0.009829    | 0.01577        |
| GOTERM CC DIRECT GO:0031415~NatA complex               | 2     | 0.006553    | 0.043139       |
| GOTERM MF DIRECT GO:0005515~protein binding            | 109   | 0.357131    | 3.23E-05       |
| GOTERM MF DIRECT GO:0003723~RNA binding                | 13    | 0.042594    | 0.005676       |
| GOTERM MF DIRECT GO:0022843~voltage-gated cation channel activity | 2     | 0.006553    | 0.01875        |
| GOTERM MF DIRECT GO:0004663~Rab geranylgeranyl transferase activity | 2     | 0.006553    | 0.03715        |
| GOTERM MF DIRECT GO:0003676~nucleic acid binding       | 16    | 0.052423    | 0.045125       |

**KEGG pathway**

- hsa04115:p53 signaling pathway
- hsa05134:Legionellosis
- hsa04062:Chemokine signaling pathway
- hsa04064:NF-kappa B signaling pathway
- hsa0520:Protein binding
- hsa04380:Osteoclast differentiation

**Reactome Pathway**

- Interleukin-4 and Interleukin-13...
- Signaling by Interleukins
- CLEC7A/inflammasome pathway
- Interleukin-10 signaling
- Immune System
- Innate Immune System
- Signaling by Interleukins
- Interleukin 4 and Interleukin 13...
- Neutrophil degranulation
- Immune System

Figure 4: Significantly enriched pathway terms of DEGs in preterm birth. DEGs functional And signaling pathway enrichment were conducted using online websites of KEGG PATHWAY, Reactome (“Red” indicates high relative expression, and “green” indicates low relative expression).
### Table 2: Signaling pathway enrichment analysis of differentially expressed genes function in preterm birth (P<0.05)

#### Ppi Network Analysis and Pathway of Hub Genes

Using String database and cytoscape software, a total of 251 genes were filtered into PPI network, contained 251 nodes and 510 protein pairs with a PPI score of >0.4, as shown in (Figure 5).
Figure 5: DEGs protein–protein interaction (PIP) network complex analysis. Using the STRING online database, total of 251 DEGs (Red standing for upregulation and Blue standing for downregulation) were filtered into the DEGs PPI network complex.

In the PPI network, nodes stand for DEGs, while edges represent interactions between two proteins. Using MCODE plug, 36 central node genes were identified with the filtering of degree cutoff ≥2 (Figure 6).

Figure 6: DEGs protein–protein interaction (PIP) network complex analysis modular analysis (36 central node genes were identified with the filtering of degree cutoff ≥2).

The most significant 10 (top 5 up-regulated and 5 down-regulated) node degree genes were CXCR4, ANAPC10, ANAPC4, UBE2V2, UBA3, ITGAM, IL1B, ITGAX, NFKB1, SOCS3 (Table 3).
Further analysis showed that up-regulated hub genes were mainly associated with Immune System, Signaling by Interleukins, Chemokine signaling pathway, down-regulated hub genes were mainly associated with Ubiquitin mediated proteolysis, Class I MHC mediated antigen processing & presentation and Phosphorylation of the APC/C (Fig7 A, B and table 4).

**Table 3:** Top 5 up-regulated and down-regulated nodes of the protein-protein interaction network by MCODE

| Gene     | Degree | Betweenness | Closeness | Eigenvector | LAC     | Network |
|----------|--------|-------------|-----------|-------------|---------|---------|
| **Down-regulated** |        |             |           |             |         |         |
| CXCR4    | 18     | 4070.189    | 0.036555  | 0.228093    | 5.44444 | 4.5     |
| ANAPC10  | 12     | 5288.686    | 0.036523  | 0.040881    | 3.16666 | 7.46464 |
| ANAPC4   | 11     | 8244.644    | 0.036646  | 0.04073     | 3.45454 | 9.28123 |
| UBE2V2   | 11     | 3420.311    | 0.036364  | 0.028726    | 3.45454 | 5.15273 |
| UBA3     | 9      | 2803.995    | 0.036528  | 0.038659    | 3.33333 | 15.45487 |
| **Up-regulated** |      |             |           |             |         |         |
| ITGAM    | 35     | 57885.53175 | 0.036841  | 0.385783    | 7.14285 | 5.68744 |
| IL1B     | 26     | 5821.9783   | 0.036824  | 0.309252    | 6.23076 | 6.55757 |
| ITGAX    | 23     | 1651.996713 | 0.036337  | 0.296355    | 6.86965 | 6      |
| NFKB1    | 18     | 8234.385291 | 0.036884  | 0.163043    | 3.55555 | 5      |
| SOCS3    | 17     | 6520.199086 | 0.03683   | 0.154799    | 4.58823 | 5.47402 |

**KEGG pathway** (up)

- hsa04064:NF-kappa B signaling pathway
- hsa04062:Chemokine signaling pathway
- hsa04621:NOD-like receptor signaling pathway
- hsa04060:Cytokine-cytokine receptor interaction
- hsa04668:TNF signaling pathway

**Reactomen pathway** (up)

- Cytokine Signaling in Immune system
- Innate Immune System
- Interleukin-4 and Interleukin-13 signaling
- Signaling by Interleukins
- Immune System

**Figure 7A:** Significantly enriched pathway terms of up-regulated DEGs in preterm borth(Orange standing for KEGG Pathway and Blue standing for Reactomen Pathway)
### KEGG and Reactome Pathway (down)

| Category | Term | Count | Genes | P value |
|-----------|------|-------|-------|---------|
| Up-regulated | | | | |
| hsa04668 | TNF signaling pathway | 4 | SOCS3, RIPK3, IL1B, NFKB1 | 0.005167075 |
| hsa04060 | Cytokine-cytokine receptor interaction | 6 | OSM, CCR1, IL4R, CXCR1, IL1B | 0.007920517 |
| hsa04621 | NOD-like receptor signaling pathway | 3 | IL1B, NFKB1, NLRP3 | 0.014759858 |
| hsa04062 | Chemokine signaling pathway | 5 | CCR1, HCK, CXCR1, NFKB1 | 0.023139865 |
| hsa04064 | NF-kappa B signaling pathway | 3 | CCR1, HCK, CXCR1, NFKB1 | 0.033682481 |
| R-HSA-168256 | Immune System | 44 | CD63;ITGAM;SLC2A3;GPR84;RELB;SOCS3;HK3;CXCR1;ITGAX;NLRP3;TIMP1;CCR1;FCER1G;SERPINB2;IL4R;DGAT1;RIPK3;OSM;MCEMP1;LILRB3;NFKB1;HCK;UBE2R2;IL1B;CYSTM1;TLR5;CD44 | 1.11E-16 |
| R-HSA-449147 | Signaling by Interleukins | 22 | CCR1;SOCS3;HCK;ITGAM;SERPINB2;IL4R;IL1B;ITGAX;OSM;TIMP1;NFKB1 | 4.44E-16 |
| R-HSA-6785807 | Interleukin-4 and Interleukin-13 signaling | 15 | SOCS3;ITGAM;IL4R;IL1B;ITGAX;OSM;TIMP1 | 1.78E-15 |
| R-HSA-168249 | Innate Immune System | 26 | CD63;FCER1G;ITGAM;SERPINB2;DGAT1;RIPK3;MCEMP1;SLC2A3;GPR84;LILRB3;NFKB1;REL;HK3;HCK;CXCR1;IL1B;ITGAX;NLRP3;CYSTM1;TLR5;CD44 | 2.18E-13 |
| R-HSA-1280215 | Cytokine Signaling in Immune system | 25 | CCR1;ITGAM;SERPINB2;IL4R;OSM;NFKB1;REL;SOCS3;HCK;IL1B;ITGAX;TIMP1;CD44 | 6.06E-13 |
| Down-regulated | | | | |
| hsa04914 | Progesterone-mediated oocyte maturation | 2 | ANAPC4, ANAPC10 | 0.037469108 |
| hsa04114 | Oocyte meiosis | 2 | ANAPC4, ANAPC10 | 0.047638096 |

**Figure 7B**: Significantly enriched pathway terms of down-regulated DEGs in preterm birth (Orange standing for KEGG Pathway and Blue standing for Reactome Pathway)
Table 4: Signaling pathway enrichment analysis of hub expressed genes function in preterm birth (P<0.05)

| DEG ID | Function Description                                      | P-value |
|--------|----------------------------------------------------------|---------|
| R-HSA-983168 | Antigen processing: Ubiquitination & Proteasome degradation | 3.23E-07 |
| R-HSA-983169 | Class I MHC mediated antigen processing & presentation | 1.87E-06 |
| R-HSA-176412 | Phosphorylation of the APC/C | 6.03E-05 |
| R-HSA-176407 | Conversion from APC/C/Cdc20 to APC/C/Cdh1 in late anaphase | 6.03E-05 |
| R-HSA-141430 | Inactivation of APC/C via direct inhibition of the APC/C complex | 6.67E-05 |

Discussion

These several decades, a lots of work has been done about preterm birth and recent prematurity rates seem to be on the decline is considered [15]. The prevention of preterm birth is a public health priority because of the potential to reduce infant and childhood morbidity and mortality related to this condition [16]. We need to recognize that PTB is caused by multiple factors, such as microbial-induced inflammation, decidual hemorrhage and vascular disease, disruption of maternal-fetal tolerance, decline in progesterone action, cell-free fetal DNA and so on [17]. It is critically important to understand the molecular mechanism of these factors.

In the current study, the dataset (GSE46510) were downloaded from GEO database to identify DEGs between sPTB and not sPTB samples using bioinformatics analysis. A total of 335 DEGs, including 157 up- and 178 down-regulated DEGs, were identified. These differentially expressed genes were classified into three groups by GO terms using online website (DAVID). Functional and signaling pathway enrichment were conducted using DAVID and Reactome, both of up and down regulated genes were mostly enriched in Immune System and Interleukin- signaling. After that, protein–protein interaction (PPI) network complex was developed using String and Cytoscape, 180 nodes/DEGs were identified with 518 edges, the most significant module was filtered using MCODE plug, 36 central node genes were identified and most of the corresponding genes were associated with Immune System, Signaling by Interleukins, Ubiquitin mediated proteolysis.

Through integrated bioinformatical analysis, we have identified 36 hub genes, ITGAM, IL1β, ITGAX, NFKB1, SOCS3, CXCR4, ANAPC10, ANAPC4, UBE2V2, UBA3, were listed at the top of the most changed genes, and their biological functions are involved in cell adhesion, inflammatory response and proteasome-mediated ubiquitin-dependent protein catabolic process. ITGAM and ITGAX encode the integrin alpha M and X chain, respectively. ITAGM and ITGAX also known as CD11B and CD11c, they play an important role in the adherence of neutrophils and monocytes to stimulated endothelium cells, and in the phagocytosis of complement coated particles. Gervasi et al [18] showed that preterm labour was associated with a significant increase in the expression of CD11b, CD15 and CD66b on neutrophils and CD11b and CD15 on monocytes, CD11a and b mediate binding to ICAM-1, which was up-regulated in endothelium of human cervix and myometrium during labour [19]. Once leukocytes emigrate to the myometrium and cervix, chemotaxis of more neutrophils and monocytes is mediated by their own increased expression of IL-8 and MCP-1, respectively [20]. Pro-inflammatory cytokines (such as IL-1, IL-6, IL-8 and TNF-α) can directly trigger the transition from a uterine quiescent state to a subsequent unscheduled activation of the uterus [21,23].

During labor, the IL1β level is increased due to the influx of leukocytes into intrauterine tissues, which can enhance the contractile potential of myometrial smooth muscle[24,25].In addition, it has been demonstrated that IL-1β can increase prostaglandin production and MMP9 expression via NF-kappa B signaling pathway [26,27], which are known to induce cervical ripening and myometrial contractions[28,29]. There is higher expression of the subunit of NF-κB in membranes, cervix and myometrium [30,31]. NF-κB can be activated by pro-inflammatory cytokines such as TNF and IL1β, and microbial or viral components that activate toll-like receptors (TLRs) [32].

Suppressor of cytokine signaling 3 (SOCS3) is a member of SOCS family, induced by various cytokines, including IL6, IL10, and interferon (IFN)-γamma [33]. Inflammatory mediators might enter the circulation and activate placental macrophages, leading to IL-1β release and subsequent SOCS activation as a feedback/response mechanism, play a role in the interaction of endothelial cells of the villous placenta with neighboring cells, participate in Placental inflammation [34].

Activation of NF-κB involves the phosphorylation of the NFKBIA protein. NFKBIA will be ubiquitinated and subsequently degraded by proteasomes [32]. Ubiquitin like modifier activating enzyme 3 (UBA3) encodes a member of the E1 ubiquitin-activating enzyme family, regulates cell division, signaling and embryogenesis. Ubiquitin-conjugating enzyme E2 variant proteins (UBE2V2), is a distinct subfamily within the E2 protein family, the protein encoded by this gene shares homology with ubiquitin-conjugating enzyme E2 variant 1. Both genes are down-regulated in premature births, and they may play a role in influencing NFKB pathway by reducing ubiquitination.

The anaphase-promoting complex (APC/C) is a multimeric RING E3 ubiquitin ligase, which is composed with many different subunits (APC1-1, APC9-11, and CDC26) and plays a crucial role in coordinating mitosis progression through targeting numerous regulators for destruction by the 26S proteasome[35,36]. It helps during ubiquitination of free polyubiquitin chain that leads to MAP3K7 activation that in turn, leads to the activation of NFκB via its respective activation pathways [37]. Anaphase promoting complex subunit 4 (ANAPC4/APC4) and 10 (ANAPC10/APC10), are subunit of the anaphase-promoting complex (APC), APC10 is the core subunit, plays a critical role in facilitating the activity of the APC to function as an E3 protein ubiquitin ligase [38].In the present study, ANAPC10, ANAPC4, UBE2V2, UBA3 down-regulated expression, play a role in preterm by influencing ubiquitination and proteasome degradation.

Cytokines are the major inducible products of immune system cells. CXCR4 encodes a CXC chemokine receptor specific for stromal cell-derived factor-1. CXCR4 is activated by NFKB, regulates decidual leukocyte recruitment during labor [39,40]. Study confirmed that CXCR4 was up regulated in labor and is related to inflammatory response [41], it is contrary to the results of this study. In this study, CXCR4 was down regulated. This is an interesting thing, more research is needed in the future.

There are several limitations in our analysis. First, all the predicted results need to be confirmed by experimental data. Second, there are fewer
samples of PTB. Third, we only chose genes, while transcription regulator (TF) and miRNA were not predicted. In future studies, large-scale samples are required to validate the expressions of above-mentioned DEGs. Moreover, future investigations should focus on the interactions of DEGs, regulatory associations between TFs, miRNAs and DEGs, and possible pathways underlying these gene alterations.

Conclusions

Using bioinformatical analysis, we have identified commonly changed 335 DEGs (including 157 up- and 178 down-regulated DEGs), and finally found 10 mostly changed hub genes, which significant enriched in several pathways, mainly associated with inflammatory response, ubiquitination and proteasome degradation. These findings significantly improve the understanding of the cause and underlying molecular events in preterm birth, and the candidate genes and pathways could be used as therapeutic targets.

Conflicts Of Interest

The author(s) declare(s) that they have no conflicts of interest related to the subject matter or materials discussed in this article

Funding

No

Abbreviations:

PTB: preterm birth
PPI: protein-protein interaction
DEG: Differentially expressed genes
IL-6: interleukin 6
IL-8: interleukin 8
TNF-α: tumour necrosis factor alpha-like
IL1B: interleukin 1 beta
TLR-9: toll-like receptors 9
GEO: Gene Expression Omnibus
GO: Gene Ontology
KEGG: Kyoto Encyclopedia of Genes and Genomes
CXCR4: C-X-C motif chemokine receptor 4
ANAPC10: Anaphase promoting complex subunit 10
ANAPC4: Anaphase promoting complex subunit 4
UBE2V2: Ubiquitin-conjugating enzyme E2 variant proteins
UBA3: Ubiquitin like modifier activating enzyme 3
ITGAM: integrin subunit alpha M
ITGAX: integrin subunit alpha X
NFKB1: nuclear factor kappa B subunit 1
SOCS3: Suppressor of cytokine signaling 3
APC: anaphase-promoting complex
TF: transcription regulator

References

1. Di Renzo GC, Cabero Roura L, Facchinetti F, et al. Preterm Labor and Birth Management: Recommendations from the European Association of Perinatal Medicine. The Journal of Maternal-Fetal & Neonatal Medicine 2017;30(17):2011-30
2. Theo A, Knijnenburg, Joseph G. Vockley, Nyasha Chambwe, David L. Gibbs, Crystal Humphries, Kathi C. Huddleston, Elisabeth Klein, Prachi Kothiyan, Ryan Tasseff, Varsha Dhankani, Dale L. Bodian, Wendy S. W. Wong, Gustavo Glusman, Denise E. Mauldin, Michael Miller, Joseph Slagel, Summer Elasady, Jared C. Roach, Roger Kramer, Kalle Leinonen, Jasper Linthorst, Rajiv Baveja, Robin Baker, Benjamin D. Solomon, Greg Eley, Ramsawamy K. Iyer, George L. Maxwell, Brady Bernard, Ilya Shmulevich, Leroy Hood, John E. Niederhuber. Genomic and molecular characterization of preterm birth. Proceedings of the National Academy of Sciences. 2019, 116 (12) 5819-5827.
3. Ren H, Du M. Role of Maternal Periodontitis in Preterm Birth. Front Immunol. 2017 Feb 13;8:139.
4. Chigusa Y, Kishore A, Mogami H, et al. Nrf2 Activation Inhibits Effects of Thrombin in Human Amnion Cells and Thrombin-Induced Preterm Birth in Mice. J Clin Endocrinol Metab 2016;101(6):2612-2621
5. Begnel ER, Drake AL, Kinuthia J, Matemo D, Huang ML, Asbjörnsdóttir Kh, Chohan V, Berma-Sofie K, John-Stewart G, Lehman D, Slyker J. Cervical cytomegalovirus reactivation, cytokines and spontaneous preterm birth in Kenyan women. Clin Exp Immunol. 2021 Mar;203(3):472-479.
6. SC C, SB C, L K, et al. TLR9 provokes inflammation in response to fetal DNA: mechanism for fetal loss in preterm birth and preeclampsia. Wilsonia-Sevryant A. Journal of immunology (Baltimore, Md : 1950) 2012;188(11):5706-5712
7. van Boeckel SR, Davidson DJ, Norman JE, Stock SJ. Cell-free fetal DNA and spontaneous preterm birth. Reproduction. 2018 Feb;155(3):R137-R145.
8. Heng YJ, Pennell CE, Chua HN, et al. Whole blood gene expression profile associated with spontaneous preterm birth in women with threatened preterm labor. PloS one 2014;9(5):e96901
9. Gautier L, Cope L, Bolstad BM, et al. affy--analysis of Affymetrix GeneChip data at the probe level. Bioinformatics (Oxford, England) 2004;20(3):307-315
10. Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Statistical applications in genetics and molecular biology 2004;3:Article3
11. Huang DW, Sherman BT, Tan Q, et al. DAVID Bioinformatics Resources: expanded annotation database and novel algorithms to better extract biology from large gene lists. Nucleic acids research 2007;35(Web Server issue):W169-75
12. Zdzislawczuk D, Morris HOR, Cook H, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. 2017:45(D1):D362-D68
13. Killcoyne S, Carter GW, Smith J, et al. Cytoscape: a community-based framework for network modeling. Methods in molecular biology (Clifton, NJ) 2009;563:219-239
14. Martin JA, Hamilton BE, Osterman MJ, et al. Births: final data for 2013. National vital statistics reports : from the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System 2015;64(1):1-65
15. Frey HA, Klebanoff MA. The epidemiology, etiology, and costs of preterm birth. Seminars in Fetal & Neonatal Medicine 2016;21(2):68-73
16. Romero R, Dey S, Fisher S. Preterm labor: one syndrome, many causes. Science 2014;345(6198):760-765
17. Gervasi MT, Chaiworapongsa T, Naccasha N, et al. Phenotypic and metabolic characteristics of maternal monocytes and granulocytes.
in preterm labor with intact membranes. American journal of obstetrics and gynecology 2001;185(5):1124-1129
19. Ledingham MA, Thomson AJ, Jordan F, et al. Cell adhesion molecule expression in the cervix and myometrium during pregnancy and parturition. Obstetrics and gynecology 2001;97(2):235-242
20. Yuan M, Jordan F, McInnes IB, et al. Leukocytes are primed in peripheral blood for activation during term and preterm labour. Molecular human reproduction 2009;15(11):713-724
21. Nadeau-Vallee M, Obari D, Quiniu C, et al. A critical role of interleukin-1 in preterm labor. Cytokine & growth factor reviews 2016;28:37-51
22. Muñoz-Pérez VM, Ortiz MI, Cariño-Cortés R, Fernández-Martínez E, Rocha-Zaavaleta L, Bautista-Ávila M. Preterm Birth, Inflammation and Infection: New Alternative Strategies for their Prevention. Curr Pharm Biotechnol. 2019;20(5):354-365.
23. Belousova VS, Svitich OA, Timokhina EV, Strizhakov AN, Bogomazova IM. Polymorphism of the IL-1β, TNF, IL-IRA and IL-4 Cytokine Genes Significantly Increases the Risk of Preterm Birth. Biochemistry (Moscow). 2019;84(9):1040-1046.
24. Nadeau-Vallée M, Boudreault A, Leimert K, Hou X, Obari D, Madaan A, Rouget R, Zhu T, Belar M, Barker G, Lappas M. SIRT6 is decreased with preterm labor in human myometrium and exacerbates uterine contractility†. Biol Reprod. 2019 Jun 1;100(6):1597-1604.
25. Peng J, Jiang J, Wang H, Feng X, Dong X. miR-199a 3p suppresses cervical epithelial cell inflammation by inhibiting the HMGB1/TLR4/NF-kB pathway in preterm birth. Mol Med Rep. 2020 Aug;22(2):926-938.
26. Peng Q, Liu Y, Dong M, Xu F, Huang J, Chen J, Li X, Zhang J, Zhang W. Interaction between NF-kB and AP-1 and their intracellular localization at labor in human late pregnant myometrial cells in vivo and in vitro. Medicine (Baltimore). 2018 Sep;97(38):e12494.
27. Lappas M, A20, an essential component of the ubiquitin-editing protein complex, is a negative regulator of inflammation in human myometrium and foetal membranes. Molecular human reproduction 2017;23(9):628-645
28. Barkai U, Prigent-Tessier A, Tessier C, et al. Involvement of SOCS-1, the suppressor of cytokine signaling, in the prevention of prolactin-responsive gene expression in decidual cells. Molecular endocrinology (Baltimore, Md) 2000;14(4):554-563
29. Blumenstein M, Keelan JA, Bowen-Shauver JM, et al. Suppression of cytokine signaling proteins in human preterm placental tissues. Journal of molecular endocrinology 2005;35(1):165-175
30. Chang L, Zhang Z, Yang J, et al. Atomic structure of the APC/C and its mechanism of proteinubiquitination. Nature 2015;522(7557):450-454
31. Peters JM. The anaphase promoting complex/cyclosome: a machine designed to destroy. Nature reviews Molecular cell biology 2006;7(9):644-656
32. Ghosh S, Dass JFP. Study of pathway cross-talk interactions with NF-kappaB leading to its activation via ubiquitination or phosphorylation: A brief review. Gene 2016;584(1):97-109
33. Au SW, Leng X, Harper JW, et al. Implications for the ubiquitination reaction of the anaphase-promoting complex from the crystal structure of the Doc1/Apc10 subunit. Journal of molecular biology 2002;316(4):955-968
34. CL T, RL J. Identification of chemokines associated with the recruitment of decidual leukocytes in human labour: potential novel targets for preterm labour. PloS one 2013;8(2):e56946
35. McIntosh RL, Maestas MM, Dobson JR, Quinn KE, Runyan CL, Ashley RL. CXCR4 signaling at the fetal-maternal interface may drive inflammation and syncytia formation during ovine pregnancy†. Biol Reprod. 2021 Feb 11;104(2):468-478.
36. CL T, SA H, CL T, et al. Transcriptomic profiling of human choriodedida during term labor: inflammation as a key driver of labor. American journal of reproductive immunology (New York, NY : 1989) 2015;73(1):36-55

Ready to submit your research? Choose Auctores and benefit from:
- fast, convenient online submission
- rigorous peer review by experienced research in your field
- rapid publication on acceptance
- authors retain copyrights
- unique DOI for all articles
- immediate, unrestricted online access

At Auctores, research is always in progress.

Learn more www.auctoresonline.org/journals/women-health-care-and-issues