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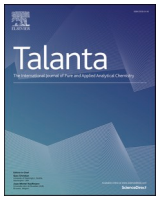

Proteomic and metabolomic analysis of plasma for pain at different labor stages

Yating Wang [a,1], Yi Qin [b,1], Shanshan Zeng [c], Ziyue Zhang [c], Wanshan Liu [c], Jingjing Wan [a], Kun Qian [c], Shunxiang Li [c,*], Jie Xiao [b,**]

[a] School of Chemistry and Molecular Engineering, East China Normal University, Shanghai, 200241, China
[b] Department of Anesthesiology, Renji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, 200127, China
[c] State Key Laboratory of Systems Medicine for Cancer, School of Biomedical Engineering, Institute of Medical Robotics and Med-X Research Institute, Shanghai Jiao Tong University, Shanghai, 200030, China

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ABSTRACT

Labor pain has an important impact on maternal labor experience, mood, and postpartum depression. It is of great emotional significance to pay attention to the pain stress response of pregnant women and take necessary intervention measures in the labor process to weaken the sense of delivery experience and reduce the risk of complications. To better understand the molecular alteration of pain and stress changes during the delivery, we analyzed the metabolomic and proteomic of the plasma collected during the labor process at different stages, revealing the significant changes in metabolites and proteins and the key regulatory pathways. The KEGG enrichment analysis showed the differentially expressed metabolites and differentially expressed proteins were mainly enriched in glutamate metabolism, glutathione metabolism, oxidative phosphorylation, glycolysis/gluconeogenesis, and citrate cycle (TCA cycle). In particular, the glutathione metabolism played a major role in the metabolic pathway of the whole labor process. The result demonstrated the potential significance of the glutathione metabolic pathway in pain regulation.

1. Introduction

Labor pain is one of the most severe physiological pains and occurs throughout the entire delivery process [1,2]. The discomfort of labor can cause changes in maternal heart rate and blood pressure, resulting in stress response of the cardiovascular system and increasing the burden of the heart [3–5]. Additionally, labor pain can induce alterations in maternal metabolism and the immune system, affecting essential physiological processes, such as energy metabolism and immune functionality [6,7]. Due to labor pain and the effects of anesthesia, the prevalence of chronic pain ranges from 6% to 18% after cesarean, and the prevalence is between 4% and 10% after eutocia [8]. Meanwhile, some studies have suggested that there is an association between labor pain and postpartum depression [9]. For instance, the intense pain of delivery, prolonged labor, dystocia, and other conditions could increase the physical and psychological stress of the participants, potentially leading to psychological imbalance [10]. Moreover, it is easy to cause postpartum depression due to the significant changes in hormone levels before and after delivery. Therefore, monitoring pain changes and further understanding biological changes in the maternal body during delivery could provide a more scientific basis for clinical practice and the health of pregnant and postpartum women.

Metabolomic focuses on studying changes in small molecule metabolites and identifying specific metabolites associated with diseases, which can serve as biomarkers for early diagnosis or monitoring disease progression [11,12]. Proteomic, positioned upstream of metabolomic, involves the comprehensive study of protein expression, function, interactions, and metabolic pathways to achieve a deeper understanding of an organism's physiological and pathological states [13]. By integrating proteomic and metabolomic data, it is possible to benefit the comprehensive understanding of the biological processes that occur in living organisms [14,15]. Simultaneously, the rapid development of the liquid chromatography-tandem mass spectrometry (LC-MS/MS) technology, along with its outstanding high-throughput and high-sensitivity

* Corresponding author.
** Corresponding author.
E-mail addresses: lishunxiang@sjtu.edu.cn (S. Li), applexiaomz@163.com (J. Xiao).
[1] These authors contributed equally to this work.

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0039-9140/© 2024 Elsevier B.V. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

analysis capabilities, has significantly advanced the application of metabolomic and proteomic in cutting-edge biomedical fields and the discovery of potential biomarkers [16,17]. To better understand the pain and stress changes during delivery, we analyzed both metabolomic and proteomic of the plasma collected during the labor process of pregnant women, revealing the significant changes in metabolites and proteins and the key regulatory pathways during delivery.

2. Materials and methods

2.1. Sample collection

The cohort samples were recruited from Shanghai Jiaotong University affiliate Renji Hospital, and a total of 185 plasma samples were collected from 32 participants at different stages in the labor process according to the Declaration of Helsinki guidelines. The freshly collected blood samples were treated with anticoagulants, and the upper plasma was extracted into sterile tubes after high-speed centrifugation and stored at −80 °C until further investigation.

2.2. Plasma sample preparation and LC-MS/MS analysis for metabolomic

The plasma samples were mixed with organic solvents (methanol/ACN, v/v = 1:1), vortexed for 30 s, and sonicated for 10 min, followed by incubation at −20 °C for 2 h. After precipitating proteins, the solution was centrifugated at 12,000 rpm for 15 min at 4 °C, and the supernatant was collected and evaporated to dryness in a vacuum concentrator. The extracted samples were resuspended in 100 μL of ACN: H_2O (v: v = 1:1) and sonicated for 10 min. The mixture was centrifuged at 12,000 rpm for 15 min at 4 °C, and the supernatant was collected and stored at −80 °C for further LC-MS analysis. The analysis of metabolomic used a Vanquish UHPLC system (Thermo Scientific, USA) coupled with a Q Exactive plus Mass spectrometer (Thermo Scientific, USA), and the Raw LC-MS data were analyzed using TraceFinder software (Thermo Scientific, USA) with an m/z mass tolerance of five parts per million to annotate the metabolites.

2.3. Plasma sample preparation and LC-MS/MS analysis for proteomic

Plasma sample preparation of proteomic was pretreated with LABP magnetic beads (OSFP0002, Omicsolution, Shanghai, China) for low abundance protein enrichment. The workflow contained the process of low abundance protein enrichment, protein denaturation, reduction, and alkylation, as well as the digestion and peptide cleanup. Sample clean-up and desalting were carried out by the C18 peptide cleaning column. Peptides were eluted twice with 30 μL of elution buffer (90% ACN, 0.2% TFA) and then dried in a speed vacuum concentrator. The peptides were re-dissolved in 0.1% formic acid (FA) in water and analyzed by Orbitrap Eclipse coupled to a Vanquish Neo UHPLC system (Thermo Fisher Scientific, MA, USA). The peptide was separated by AUR3-15075C18 column (15 cm length, 75 μm i.d, 1.7 μm particle size, 120 Å pore size, IonOpticks) with 90 min-gradient starting at 2.2% buffer B (80% ACN with 0.1% FA), followed by a stepwise increase to 44% in 78 min, 90% in 6 min and stayed there for 6 min. The column flow rate was maintained at 400 nL/min with the column temperature of 55 °C. The mass spectrometer was run under data independent acquisition mode with a hybrid data strategy.

Raw Data acquired by LC-MS/MS was processed and analyzed by Spectronaut 18 (Biognosys AG, Switzerland) with default settings. The database was Homo sapiens (Human) (version 2022, 20610 entries), which was downloaded from UniProt (https://www.uniprot.org/). Trypsin was the digestion enzyme and specific was the digest type. Carbamidomethyl on cysteine was specified as the fixed modification. Oxidation on methionine and acetyl on Protein N-term were defined as the variable modifications. The retention time prediction type was set to dynamic iRT. Data extraction was determined by Spectronaut based on the extensive mass calibration. Spectronaut will dynamically determine the ideal extraction window depending on iRT calibration and gradient stability. The Q-value (FDR) cutoff on the precursor level was 1%, and the protein level was 1%. Peptides that passed the 1% Q-value cutoff were used to calculate the major group quantities with the MaxLFQ method.

2.4. Metabolomic and proteomic data analysis

Prior to statistical analysis, a series of data processing steps were performed on the raw data, including deletion, filling, and normalization of missing values. The preprocess data matrix was statistically analyzed to screen differential metabolites and proteins.

Partial least-squares discriminant analysis (PLS-DA) was performed using the mixOmics package to assess the difference distribution among the different groups and evaluate the stability of the analysis process. The volcano map showed the distribution of proteins and metabolites in each group, with the log2 value of Fold Change (FC) as the horizontal axis, the -log10 change value of P-value (p) as the vertical axis, and the threshold of significance change as the dividing line. The differentially expressed proteins (DEPs) were selected by FC > 1.5 or FC < 0.67 and p-value < 0.05 thresholds, and differentially expressed metabolites (DEMs) were chosen by FC > 1.5 or FC < 0.67, p-value < 0.05, and variable important in projection (VIP) > 1 compared with the baseline groups. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway-based analysis of DEMs and DEPs was obtained from the Human Metabolome Database (https://hmdb.ca/) and UniProt, and Fisher's precision probability test was used to perform KEGG enrichment analysis.

3. Results and discussion

The study included 185 plasma samples from 32 eutocia women for label-free proteomic and untargeted metabolomic analysis. The plasma collection points were strategically planned: before entering the delivery room (serving as the baseline, the first time point), at the time of labor pain (the second time point), and 15 min (the third time point), 30 min (the fourth time point), 60 min (the fifth time point), and 120 min (the sixth time point) after the anesthetic intervention during delivery (Fig. 1A). We utilized a high-resolution liquid chromatography/tandem mass spectrometry to acquire data for metabolic and proteomic spectrum respectively. The metabolites and proteins were identified by data preprocessing and database retrieval, and then the enrichment pathway of differentially expressed molecules was achieved by statistical and bioinformatics analysis (Fig. 1B). We investigated the changes of small molecular metabolites and large molecular proteins in pregnant women during labor process, aiming to find the pain-related regulatory pathways, which is expected to further explore the monitoring of molecules related to pain and stress in pregnant women, pay attention to the status of pregnant women during labor process, and alleviate their fear of delivery.

3.1. Metabolomic analysis of maternal plasma

We investigated the plasma samples collected at six time points for metabolomic analysis and 1399 metabolites were detected covering a wide range of metabolite species. The intensity change of these metabolites, including lipids and lipid-like molecules, organic acids and derivatives, organic oxygen compounds, and other classes of metabolites, spanned five orders of magnitude (Fig. S1A).

We ranked a mapping of the intensity variations of all metabolites identified throughout the labor process and performed a time-series analysis on the 15 highest and 15 lowest abundant metabolites, which included amino acids, fatty acids, carbohydrates, purines, etc. Additionally, we analyzed the dynamic fluctuations of the 30 metabolites throughout different delivery stages. The top abundant 15 metabolites

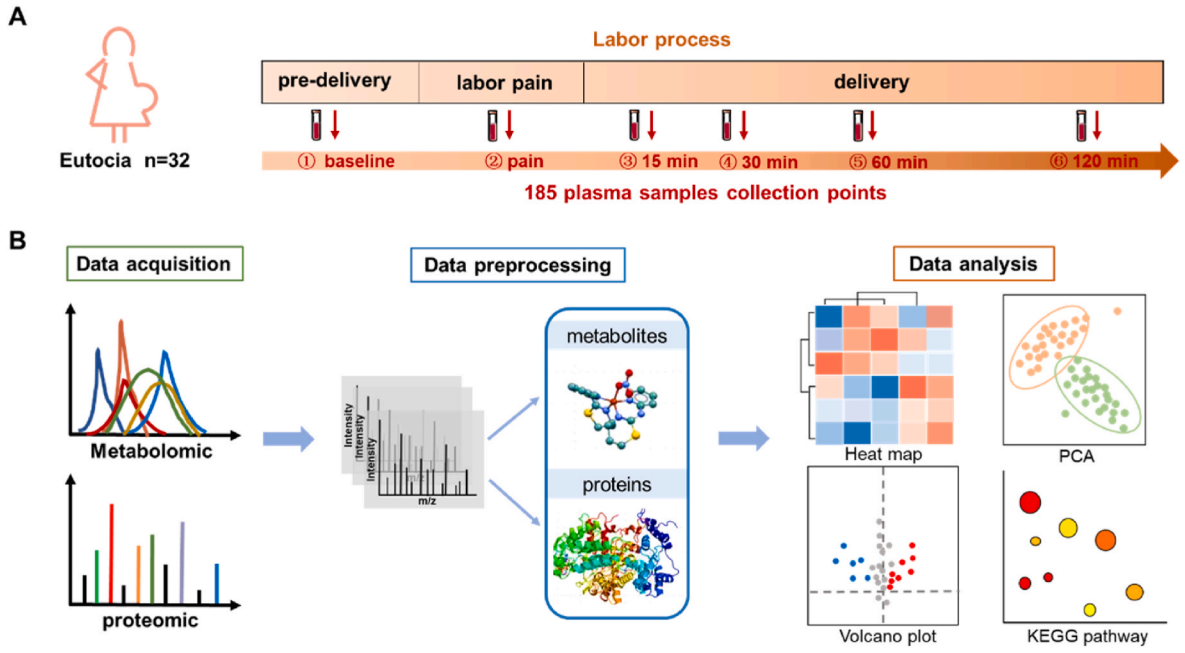

Fig. 1. The workflow of plasma metabolomic and proteomic profiling in pregnant women. (A) The plasma samples collection method in the labor process. (B) The data acquisition, preprocessing and analysis of metabolomic and proteomic.

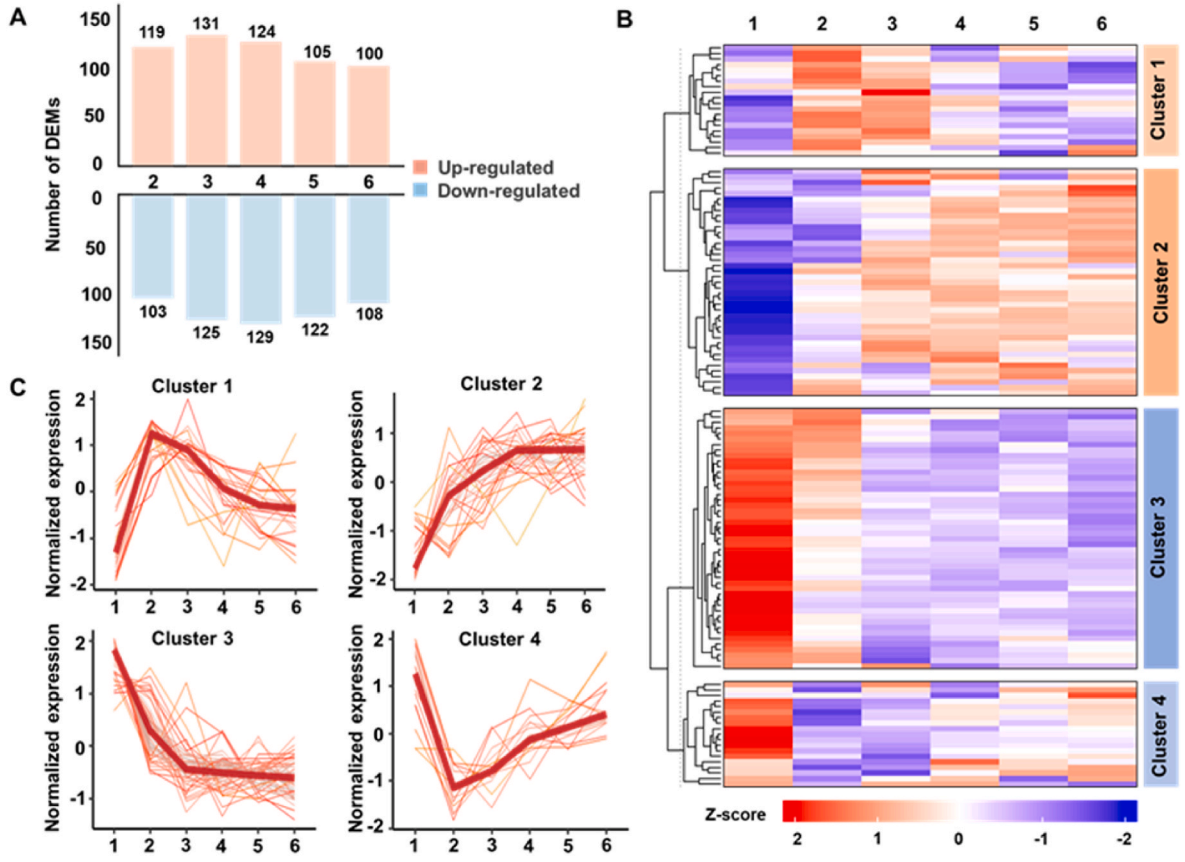

Fig. 2. Significantly differential metabolite analysis. (A) The bar chart showed the number of differentially expressed metabolites (DEMs) identified at different time points in the labor process, with the red column representing up-regulated metabolites and the blue column representing down-regulated metabolites. The screening criterion was the fold change beyond 1.5 or below 0.67 with p-value lower than 0.05 and Variable important in projection beyond 1. (B) Heatmap showing the four dynamic expression patterns of DEMs, and (C) the four hierarchical clusters of the DEMs using mFuzz.

showed an overall upward expression trend during the labor stages (Fig. S1B). Among these metabolites, there were two metabolites showed significant fluctuations, which were identified as 4-hydroxybutyric acid (GHB) and polidocanol, while the others showed a slightly increased trend. The GHB is a precursor and metabolite of gamma-aminobutyric acid (GABA) which acts as a neuromodulator of the central nervous system, exerting its effects through specific GABA and GHB receptors or modulating dopamine transmission [18,19]. Meanwhile, the low abundant caffeine and paraxanthine were found to be decreased aligned with the labor progress (Fig. S1C). Several in vitro and in vivo studies have demonstrated that caffeine modulates innate and adaptive immune responses [20,21]. For instance, studies indicated that caffeine and its major metabolite paraxanthine suppress neutrophil and monocyte chemotaxis and suppress the pro-inflammatory cytokine tumor necrosis factor (TNF) alpha production from human blood [22,23]. However, the mental state of women before entering the delivery room and at the time of labor was highly anxious and tense. This anxiety may be a reflection of the noticeable metabolism of caffeine. With the progress of labor and the implementation of analgesic intervention, maternal anxiety gradually eased, resulting in a corresponding decrease in caffeine metabolite levels.

To further investigate metabolic changes during delivery, we discovered that over 200 differentially expressed metabolites (DEMs) (compared with baseline) were associated with different stages of delivery using the combination of multivariate statistical analysis and unidimensional statistical analysis to screen the difference variables (FC $>$ 1.5 or FC $<$ 0.67, p-value $<$ 0.05, and VIP $>$ 1). The volcano plots showed the distribution of DEMs (Fig. S2), and the number of these DEMs increased significantly at the 15 min and 30 min of the delivery and then decreased with the progress of labor (Fig. 2A) which demonstrated that the physiological changes were obvious in the first half hour of labor, and it was of great concern to pay attention to the status of pregnant women. Furthermore, metabolites found to be significantly different between the baseline and the other five time points were applied to the metabolomic pathway topology analysis. Pathways were significantly enriched at the significance level of 0.05, including glutathione metabolism, cysteine and methionine metabolism, arginine and proline metabolism, and tryptophan metabolism (Fig. S3). The results indicated that the metabolism of various amino acids played an important role in the labor process [24].

To gain a more detailed understanding of metabolite changes at each stage of delivery, we clustered the differential metabolites in the heat map which showed significant differences between different time points during the labor process (Fig. 2B). For delineating the metabolite trajectories in the different stages of delivery, we performed the Mfuzz clustering using the differential metabolites among six stages and identified four distinct clusters (Fig. 2C). Metabolites in cluster 1 showed a marked increase in the labor pain stage and then decreased (e.g., hexanoyl glutamine, L-cysteine, estrone sulfate, dodecanedioic acid) (Figs. S4A, B, C) whereas metabolites in cluster 4 first decreased to the lowest point and then increased in the labor pain stage (e.g., 1-pyrroline-5-carboxylic acid, sorbitan palmitate) (Figs. S4A and B), suggesting that the metabolic changes were very significant in the labor pain stage, possibly marking a shift in the modes of energy consumption and production. Notably, several metabolites in cluster 2 and cluster 3 showed a stepwise increase or decrease from the pre-delivery stage to the completion of delivery (Fig. 2C). The level of hydroxybutyric acid was slightly increased whereas the level of 17-hydroxyprogesterone in the androgen and estrogen metabolism was moderately decreased (Figs. S4A and C). We also observed that some metabolites involved in the lipid peroxidation process displayed a constant upward trend significantly, while some metabolites exhibited a remarkable downward trend along with the labor process (Fig. S4B) indicating that the lipid peroxidation process played a critical role in the labor process.

We have further discussed the major metabolic pathways involved in the four clusters in the labor process, including glutamate metabolism, lipid peroxidation process, and androgen and estrogen metabolism. Glutamate is an excitatory neurotransmitter in the central nervous system and plays a pivotal role in regulating neuronal excitability and synaptic transmission [25]. Research has shown that fetal delivery can activate specific metabolic pathways, including glutamate metabolic pathways, by stimulating primary afferent neurons in the cervix [26]. This stimulation facilitates glutamate release and activates particular neural receptors, such as mGluR5, influencing the development of the fetal nervous system and its adaptive responses during delivery [27]. In addition, changes in glutamate metabolism may also affect the mother's experience during labor, including pain perception and emotional state. Therefore, glutamate and its metabolic processes are significant not only for the development of the fetal nervous system and ensuring adaptability to delivery but also in affecting the maternal experience of delivery. Lipid peroxidation is a process in which polyunsaturated fatty acids react with free radicals, leading to the destruction of lipid molecular structure and loss of function [28]. During delivery, oxidative stress in the body increases, which may lead to elevated lipid peroxidation levels. Lipid peroxidation products may cause damage to cell membranes, affect cell function, and potentially induce inflammatory responses [29]. However, the human body has antioxidant defense mechanisms such as glutathione, vitamin E, etc., which could help neutralize free radicals and protect cells from damage [30]. During delivery, estrone sulfate and 17-hydroxyprogesterone levels varied significantly (Fig. S4C). These hormonal changes successfully promoted cervical maturation, increased the uterus's sensitivity to oxytocin, and triggered contractions [31]. These metabolic changes may serve as biomarkers to assess the progress of labor, and it is possible to better understand the progress of the labor stage and make adjustments or interventions accordingly by monitoring these changes.

3.2. Proteomic analysis of maternal plasma

We also performed plasma proteomic profiling of samples collected before entering the delivery room (baseline, the first time point), at the time of labor pain (the second time point), 30 min (the fourth time point), and 120 min (the sixth time point) after the anesthetic intervention during delivery. Using data-independent acquisition (DIA) technology [32], we obtained high-quality quantitative data for 3906 protein groups. PLS-DA was used to evaluate the differential protein profiles of different time points compared to the baseline (Fig. 3A, B, C). The PLS-DA score plot clearly separated three time points and baseline, as well as the two adjacent time points, which indicated a significant and continuous proteome change as time progressed during the labor process (Fig. S5). We further analyzed the differentially expressed proteins (DEPs) with the conditions of FC $>$ 1.5 or FC $<$ 0.67 and p $<$ 0.05 for distinguishing the samples of three time points from baseline as shown in the volcano plot (Fig. 3D, E, F). Compared with the baseline, there were 119, 97, 162 up-regulated and 66, 89, 64 down-regulated proteins of these DEPs in the second, fourth, and sixth time points. The DEPs were clustered using mFuzz into significant discrete clusters, respectively, to illustrate the relative expression changes of the proteomic during the labor process (Fig. 3G). There were four clusters showing significant changes in protein expression, while the rest remained slight fluctuations. The proteins in cluster 1 showed a marked decrease, especially from the time of labor pain to 30 min after the anesthetic intervention during the delivery (the fourth time point). The expression changes of proteins in cluster 2 increased to the highest point at the labor pain stage and then began to decline, and the proteins in cluster 6 also first increased to the highest point at the labor pain stage and then decreased to the lowest point at 30 min after the anesthetic intervention during the delivery (the fourth time point), suggesting that the significant decrease in the expression changes of related proteins between the two stages might be due to the effect of anesthesia reducing the labor pain. The proteins in cluster 3 had a stable upward trend which may suggest that it was related to the inflammatory response and immune adjustment

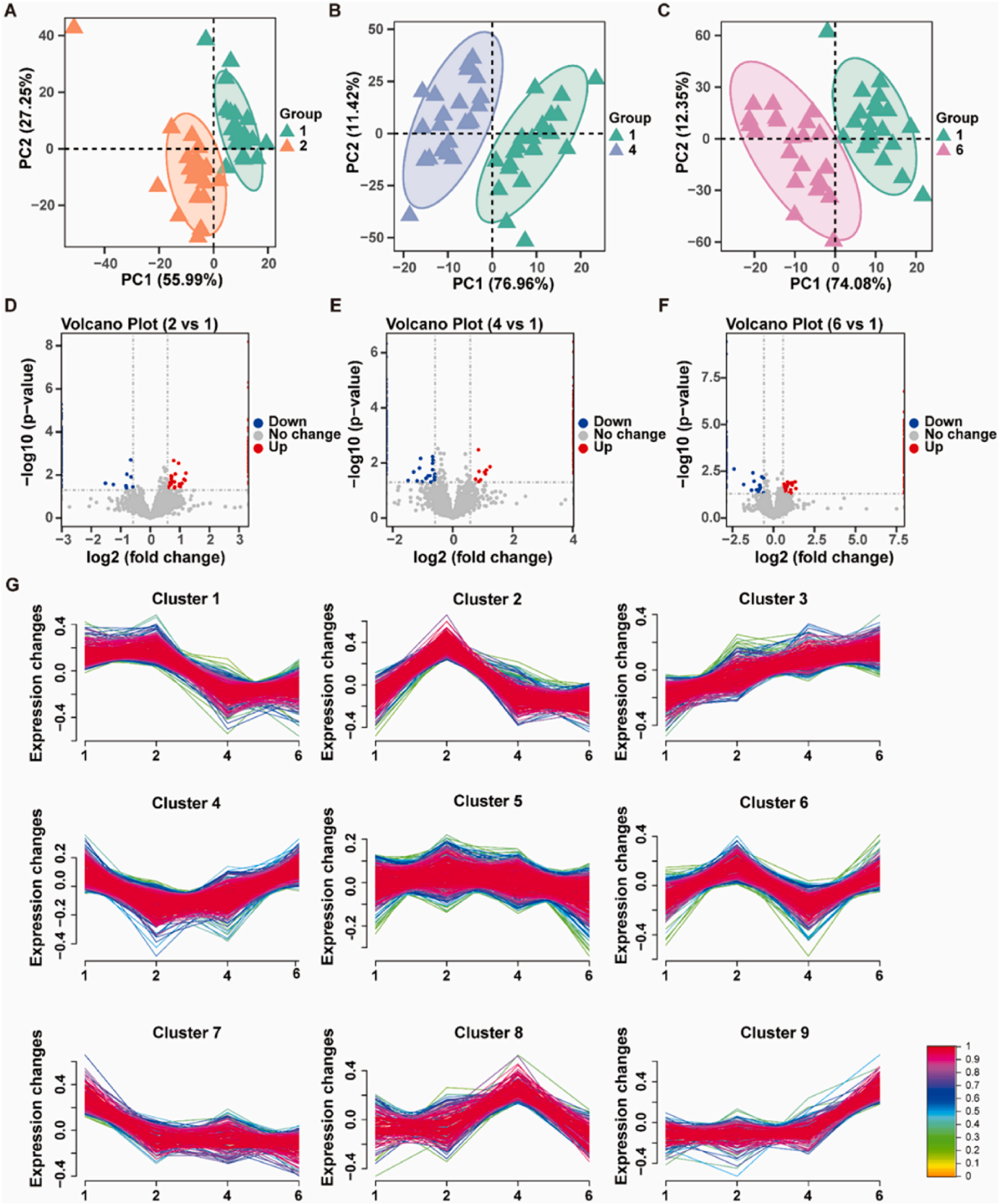

Fig. 3. Significantly differential protein analysis. Partial least squares discriminant analysis (PLS-DA) of (A) the time of labor pains (the second time point), (B) 30 min (the fourth time point), (C) 120 min (the sixth time point) after the anesthetic intervention during the delivery compared with the baseline (the first time point). Volcano plots showed differentially expressed proteins (DEPs) in different labor stages at (D) the time of labor pains (the second time point), (E) 30 min (the fourth time point), and (F) 120 min (the sixth time point) after the anesthetic intervention during the delivery compared with the baseline. Proteins with fold change beyond 1.5 or below 0.67 with p-value lower than 0.05 were considered as DEPs. Number of significantly up- (red) and down- (blue) regulated proteins were shown on the top. (G) Identification of specific clusters of proteins in 185 plasma samples using mFuzz.

during the labor process in pregnant women.

We performed KEGG enrichment pathway analysis to further investigate the significant biological information of DEPs in these four clusters to understand their roles in various biological processes during delivery. KEGG pathway analysis of cluster 1 showed that DEPs were enriched in pathways in glycolysis/gluconeogenesis and glutathione metabolism (Fig. 4A), which were related to carbohydrate metabolism and amino acid metabolism. Meanwhile, we also found the differential expression of amino acid metabolism, such as glutamate metabolites, in metabolomic analysis which was in accordance with the pathway alteration in proteome study. In cluster 2, the variation of DEPs peaked at the second time point, when in the process of labor pain, and then began to decline, and its KEGG pathway was significantly enriched in the ribosome and riboflavin metabolism (Fig. 4B), which belonged to the pathway of translation and metabolism of cofactors and vitamins. The DEPs in cluster 3 showed a continuous upward trend. Through KEGG pathway analysis, it was found that oxidative phosphorylation in energy metabolism was enriched in cluster 3 (Fig. 4C), indicating an increase in energy supply during delivery in pregnant women. Additionally, due to the pain during delivery, the process of oxidative phosphorylation not only provides energy for delivery but also helps to cope with and alleviate oxidative stress [33]. The DEPs in cluster 6 displayed significant changes before and after the anesthetic intervention, with pathway enrichment showing the presence of the citrate cycle (TCA cycle) and the synaptic vesicle cycle (Fig. 4D). The TCA cycle is a key metabolic pathway in cells [34]. During delivery, there is a significant increase in uterine muscle activity, and the TCA cycle provides the necessary energy to support sustained muscle activity and pain coping mechanisms for pregnant women [35]. The synaptic vesicle cycle is the process of neurotransmitter release and reuptake, which is crucial for perceiving and regulating pain [36]. At the same time, we found that the immune system was enriched in pathways across all four clusters, demonstrating the importance of immune regulation during the delivery process.

3.3. Pathway analysis of integrative metabolomic and proteomic

We further analyzed the KEGG enrichment of the DEMs and DEPs at the different time points of the labor process to gain insight into the pain stress pathways of pregnant women and the metabolic and protein enrichment pathways associated with significant changes. A combination of metabolomic and proteomic profiling, the DEMs and DEPs at the second time point were enriched in aspartate and glutamate metabolism, suggesting the nervous system modulation during this stage due to intense labor pains (Figs. S6A and D). In the fourth time point, the DEMs were enriched in glutathione metabolism and alanine, aspartate and glutamate metabolism (Fig. S6B), while the DEPs were enriched in oxidative phosphorylation and glycolysis/gluconeogenesis (Fig. S6E). It demonstrated that the body was still adapting to pain at the metabolic level, and the protein upstream had made corresponding changes in response to produce energy to cope with the high-intensity biological process and pain response. In the sixth time point, the DEMs and DEPs were enriched in the citrate cycle (TCA cycle), indicating that energy requirements increase significantly during the last stage of the labor process (Figs. S6C and F). It was worth noting that DEMs were

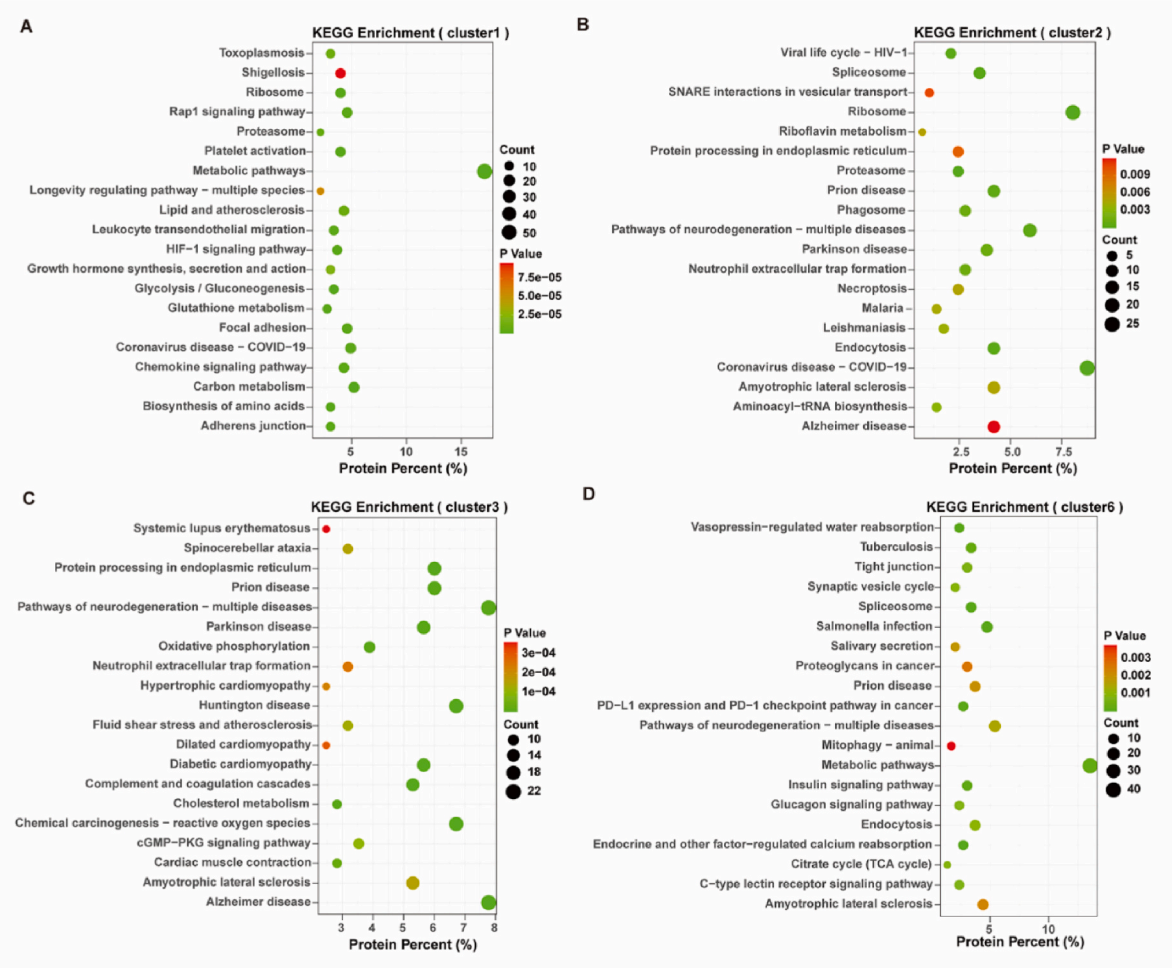

Fig. 4. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of (A) cluster 1, (B) cluster 2, (C) cluster 3, and (D) cluster 6. The 20 most enriched protein pathways were shown.

consistently enriched in glutathione metabolism throughout the whole labor process. This result emphasized the potential significance of the glutathione metabolic pathway in pain regulation [37].

Maternal labor pain is a significant concern that could be studied through the integration of proteomic and metabolomic technologies. By studying the protein expression profiles and metabolic characteristics of pregnant women, customized treatment and relief programs could be developed to minimize the side effects and limitations of traditional approaches. Proteomic and metabolomic enable the identification and semi-quantification of pain-related proteins and metabolites, facilitating the discovery of specific biomarkers. These markers enhance our understanding of pain mechanisms and support personalized pain management strategies. Besides, the pain process involves multiple signaling pathways, and these omics technologies would elucidate the interactions among these pathways, providing a comprehensive view of molecular dynamics. By integrating multiple omics datasets, researchers can precisely map the molecular networks involved in pain onset and propagation, offer detailed insights into various pain models, and highlight potential therapeutic targets. Nonetheless, the challenge with multi-omics technology lies in managing vast and complex datasets and performing integrated analyses. It is necessary to develop more efficient bioinformatics tools and machine learning algorithms that enhance the processing capabilities of omics data. Additionally, constructing multi-level data fusion models, incorporating both proteomic and metabolomic, will enable a more thorough understanding of molecular basis to increase the accuracy and interpretability of research findings. The quantification of biomarkers remains challenging due to the low abundance, instability, and complex purification processes associated with some varied biomolecules. Additionally, rigorous validation is required for biomarkers identified through non-targeted techniques in order to ensure their reliability for targeted analyses. The detection of interesting molecules from untargeted scanning to targeted LC-MS/MS monitoring in clinical settings revolves around bridging the gap between large-scale discovery strategy and precise, quantifiable diagnostic demand in therapeutic applications. While the process involves significant challenges, particularly in biomarker validation in an extensive sample cohort and infrastructural adjustments, the potential for improving patient outcomes through more precise and personalized medicine stands as a compelling incentive.

4. Conclusion

In summary, the metabolomic and proteomic analysis of collected plasma of the labor process using untargeted LC-MS/MS clarified metabolic and protein changes under pain and stress. The statistical analysis results indicated that over 200 metabolites and proteins underwent significant changes at different time points of the labor process. The KEGG enrichment analysis showed this differential expression mainly enriched in glutamate metabolism, glutathione metabolism, oxidative phosphorylation, glycolysis/gluconeogenesis, and citrate cycle (TCA cycle), which helped us better understand the pathological and physiological processes of the labor process through changed metabolic and proteomic pathways. In particular, the glutathione metabolism played a major role in the metabolic pathway of the whole labor process, which provided us with important information for the regulation of maternal labor pain. The monitoring and regulating pain during delivery is of great clinical significance in improving the quality of labor and reducing the risk of complications. This requires further strengthening of pain research in delivery to provide scientific and effective pain interventions.

CRediT authorship contribution statement

Yating Wang: Writing – original draft. **Yi Qin:** Validation. **Shanshan Zeng:** Validation. **Ziyue Zhang:** Data curation. **Wanshan Liu:** Methodology. **Jingjing Wan:** Supervision. **Kun Qian:** Conceptualization. **Shunxiang Li:** Writing – review & editing. **Jie Xiao:** Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2024.126905.

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