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# ITGB3 and associated molecules as critical biomarkers in Cesarean Scar Pregnancy

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## Abstract

**Background** Cesarean scar pregnancy (CSP) is a life-threatening condition with a rising incidence in China. The pathogenesis of CSP remains poorly understood, partly due to the limited availability of comprehensive datasets constrained by spatiotemporal factors.

**Objective** This study aimed to explore key regulatory molecules and mechanisms involved in CSP through a multi-omics approach.

**Methods** Proteomic analysis was performed on decidual and villous tissues from clinical patients ($n=6$, including 3 CSP cases and 3 controls). Gene expression datasets ($n=9$) were obtained from the GEO and SRA databases. Bioinformatics analyses were conducted using DAVID, Metascape, and STRING, with transcription factor prediction performed via the JASPAR database. Data analysis was conducted using SPSS 27, with a significance threshold set at $P<0.05$.

**Results** CSP shared differentially expressed genes (DEGs) with cesarean delivery (CD) and embryo implantation (EI). Enrichment analysis revealed that biological processes and KEGG pathways related to adhesion, with Integrin Subunit Beta 3 (ITGB3), Integrin Subunit Alpha 2b (ITGA2B), and Vitronectin (VTN) playing significant roles. ITGB3 expression was significantly downregulated following CD compared to spontaneous delivery, followed by upregulation in subsequent pregnancies. The transcription factor GATA4 was identified as a key regulator of ITGB3, potentially contributing to CSP pathogenesis.

**Conclusion** Our findings suggest that CSP development is closely associated with CD and EI, with ITGB3 and its regulatory network playing a crucial role. GATA4-mediated regulation of ITGB3 may represent an important molecular mechanism contributing to CSP formation.

**Keywords** Cesarean Scar Pregnancy, Integrin beta3, Bioinformatics analysis, Proteomics

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## Introduction

Cesarean scar pregnancy (CSP) is a severe complication of cesarean section, where the fertilized egg implants at the previous cesarean scar site. With increasing cesarean section rates, CSP incidence is also rising globally, particularly in China [1]. Due to limited clinical research, the exact pathogenesis of CSP remains unclear. Previous studies suggest that incomplete scar healing after cesarean delivery can create microscopic channels, allowing embryo implantation at the scar site [2, 3]. Additionally, Gnainsky et al. found that inflammation and damage repair responses mechanisms enhance embryo implantation success [4]. However, the nonspecific symptoms of CSP often lead to misdiagnosis, resulting in serious complications such as hemorrhage, uterine rupture, and maternal death [5]. A review showed that 107 of 751 (13.6%) cases of CSP were misdiagnosed as cervical pregnancy, spontaneous abortion, or low intrauterine pregnancy, and a similar number of cases may have gone underdiagnosed [6]. The high rate of misdiagnosis significantly increases maternal morbidity and mortality, emphasizing the need for improved understanding and diagnostic approaches for CSP [7].

Previously, studies on CSP pathogenesis were limited due to small-scale tissue-specific analyses and molecular investigations constrained by spatial and temporal factors. As a result, no standardized guidelines exist for CSP diagnosis and treatment.

Gene expression and protein-level changes are the most visible manifestation of changes in life activity. Given the lack of comprehensive CSP pathogenesis studies, bioinformatics and proteomics offer powerful tools for uncovering key regulatory pathways. With global information sharing and technological advances, integrating bioinformatics with proteomic analysis has become an emerging trend in disease research [8, 9].

In this study, we used bioinformatics analysis and label-free proteomics quantitative proteomics to analyze clinical samples and differential expression datasets. Moreover, we investigated hub genes that may play a key role in CSP and mined potential biomarkers of CSP. This study has proposed the hypothesis that the transcription factor GATA4 regulates the rebound effect of ITGB3, thus shedding light on the underlying molecular mechanisms of CSP.

## Materials and methods

### Ethics approval and consent to participate

This study complies with the Declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital of Kunming Medical University. Informed consent was obtained from all study participants prior to inclusion.

### Patient sample collection and proteomics

Pregnant women were recruited between August 1, 2019, and June 30, 2020, at the First Affiliated Hospital of Kunming Medical University. A case-control study design was used to compare CSP patients with matched controls. Curettage was performed to collect decidual and villous tissue samples. Key regulatory proteins were identified using label-free quantitative proteomics. Detailed proteomic sequencing methods are available in the supplementary materials. Differentially expressed proteins (DEPs) were identified based on fold change (FC) and statistical significance. FC was calculated as the ratio of mean relative quantification values between groups. Log2 transformation was applied to the data before performing Student's t-test to ensure normal distribution. Proteins with p-value $\leq 0.05$ and FC $\geq 1.5$ (or $\leq 1/1.5$) were considered significantly differentially expressed.

### Bioinformatics analysis

Gene expression datasets were retrieved from the GEO (https://www.ncbi.nlm.nih.gov/GEO/) and SRA databases (https://trace.ncbi.nlm.nih.gov/traces/SRA/) using keywords such as "Cesarean Section," "Embryo implantation," "Pregnancy," and "Postpartum Period". A total of 9 datasets were obtained, including GSE149436 (whole blood, 101 samples in spontaneous labor group, 160 samples in cesarean delivery group), GSE18850 (placenta, 5 samples in spontaneous labor group, 5 samples in cesarean delivery group), GSE195795 (uterus, 6 samples at the embryo implantation stage, 6 samples at the labor stage), GSE2957 (myometrium, 14 non-pregnant human samples, 4 pregnant human samples), PRJNA516344 (cord blood, 8 samples in spontaneous labor group, 8 samples in cesarean delivery group), PRJNA687512 (myometrium, 5 samples in spontaneous labor group, 5 samples in cesarean delivery group), PRJNA417762 (endometrium, 39 samples with implantation failure, 31 health samples), PRJNA667091 (endometrium, 49 samples with implantation failure, 24 health samples), and PRJNA725184 (placenta, 20 samples with repeated implantation failure, 16 health samples).

After normalization, batch effects were carefully corrected by DESeq2 and limma packages in R, thereby minimizing technical variability. Differential expression analysis was subsequently conducted using the limma and DESeq2 to obtain differentially expressed genes (DEGs). Both methods screened for DEGs by the following selection criteria: absolute value of logFC (log2 fold change) $> 0.5$ and adjusted p-value $< 0.05$, where genes with logFC $> 0.5$ were defined as up-regulated DEGs and genes with logFC $< -0.5$ were defined as down-regulated DEGs.

The intersection of DEGs from different datasets was determined using the online bioinformatics tool (http:/

/www.bioinformatics.com.cn). Functional and pathway enrichment analyses of the intersecting genes were conducted using DAVID v6.8 (https://DAVID.ncifcrf.gov/tools.jsp), the QuickGO online database (https://www.ebi.ac.uk/QuickGO/), and the gene enrichment analysis site Metascape (http://www.Metascape.org/gp/index.html). Additionally, the interaction network of DEGs was constructed using the STRING database (https://cn.STRING-db.org/) and Cytoscape v. 3.8.2 software, with core genes extracted using MCODE and Cytohubba.

### Statistical analysis

All statistical analyses were performed using SPSS 27. The chi-square test was used for categorical data comparisons, with significance set at $P<0.05$. Graphs were generated using GraphPad Prism 8.

## Results

### Study design and clinical sample details

A total of 300 pregnant women completed the screening questionnaire during the study period (Fig. 1), with CSP cases identified based on ultrasound findings (Fig. 2). The typical ultrasound findings include [2]: (1) no gestational sac observed within the uterine cavity and cervical canal; (2) the gestational sac is implanted at the site of the previous cesarean section scar; (3) significant thinning or even absence of the uterine myometrium between the gestational sac and the bladder. Participants were selected according to the inclusion/exclusion criteria shown in Fig. 2. For matching, control subjects were selected within one month after CSP patients were admitted as outpatients, based on the criteria of age ± 1 year and gestational age ± 4 days to avoid temporal bias. Ultimately, three pregnant women without CSP and three women with CSP were selected as study subjects (Supplementary Table 1, Fig. 2).

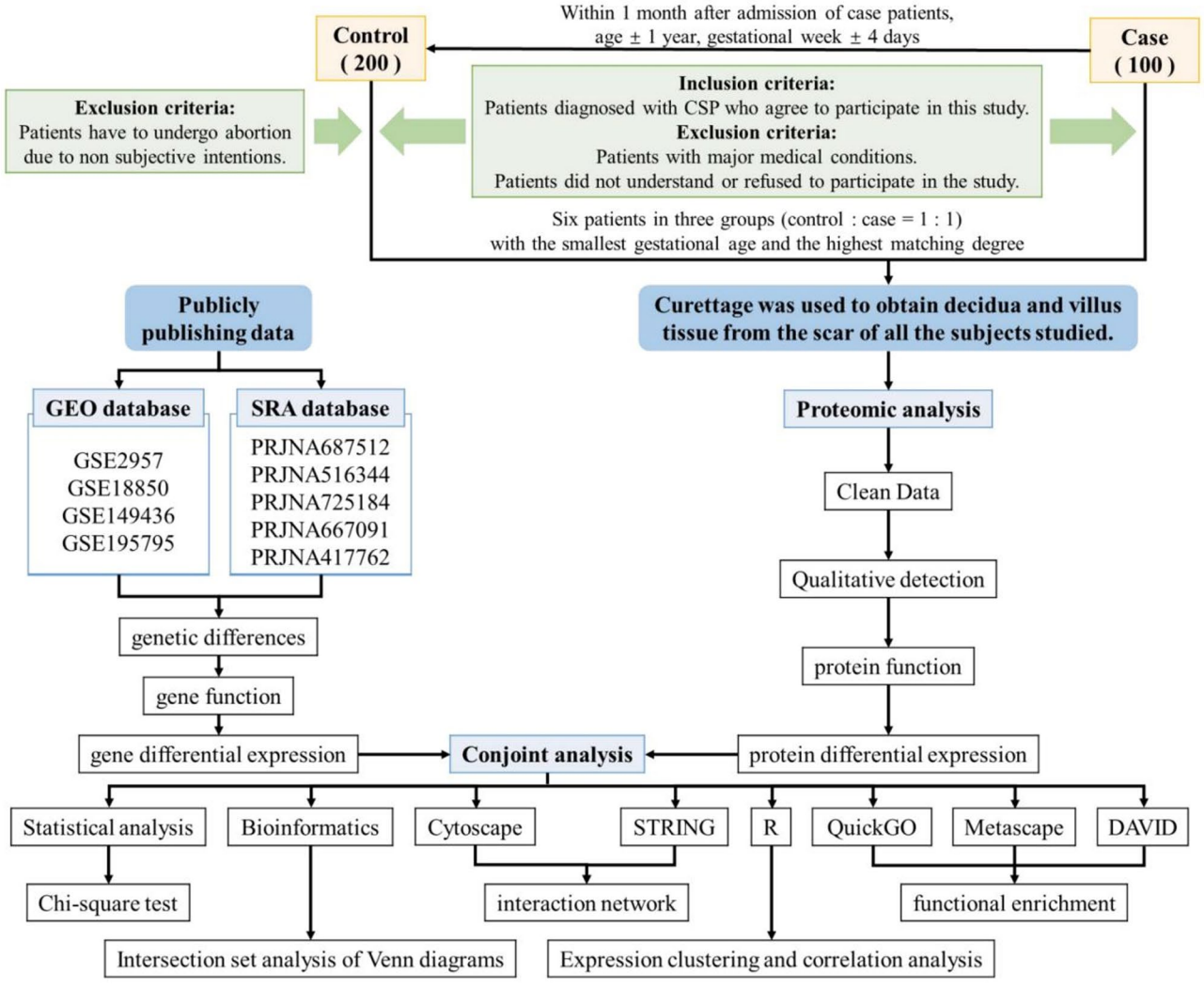



**Fig. 1** Flow chart of the study design

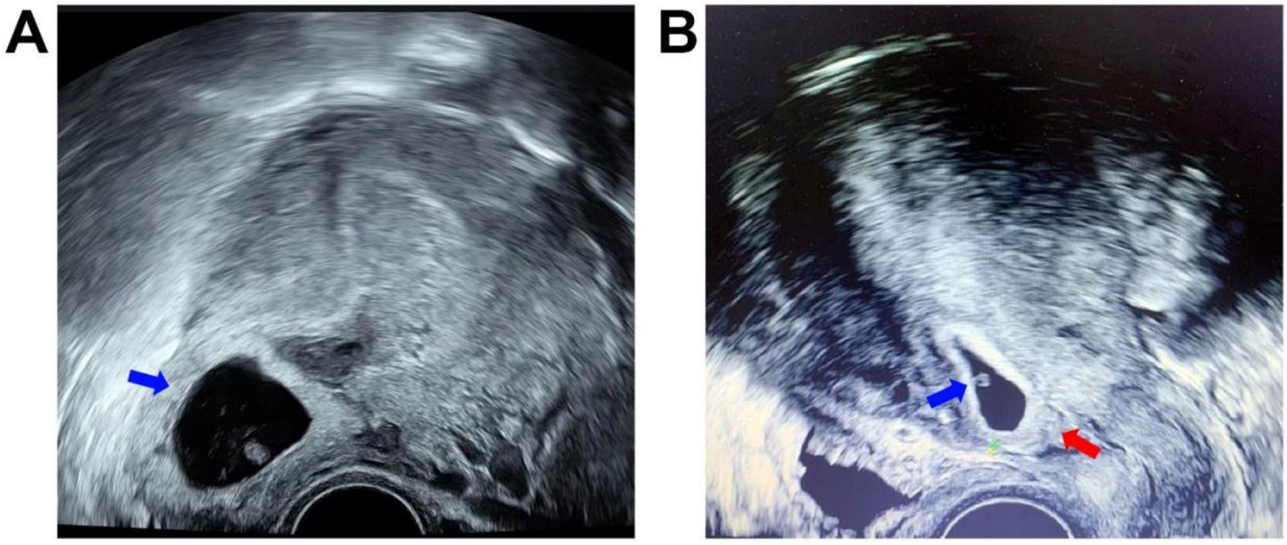



**Fig. 2** Ultrasound images of pregnancies in clinical subjects. **A**. Uterus of a woman with a normal pregnancy, with the gestational sac indicated by a blue arrow. **B**. Uterus of a woman with CSP, with the gestational sac indicated by a blue arrow and the scar from the previous cesarean section indicated by a red arrow

Decidual and villous tissues were extracted from the scars of all subjects using curettage. The villi, which are small white hair-like structures floating in water after curettage, and the decidua, which is the membranous tissue covering the uterine wall and gestational sac, were collected. The scar pregnancy samples in this study were endogenous (Type 1 CSP). According to the Society for Maternal-Fetal Medicine (SMFM) guidelines, CSP can be divided into endogenous (Type 1, 'on the scar') and exogenous (Type 2, 'in the niche') types. Type 1 CSP progresses toward the endometrial cavity, while Type 2 CSP grows deeply into the myometrium and toward the serosal surface, often requiring laparoscopic surgical management due to the risk of uterine rupture [10]. The tissues collected by curettage from each group were then categorized: CSP decidua (SD), CSP villi (SV), and the respective D and V tissues from normal pregnancies, with three samples per group. After washing with 0.9% saline, the tissue samples were stored at -80℃ until proteomic analysis was conducted.

#### Transcriptome data collection, quality control, and analysis

Two GEO datasets (GSE149436, GSE18850) and two SRA datasets (PRJNA516344, PRJNA687512) were analyzed to compare CD vs. spontaneous labor (SL). Furthermore, three additional SRA datasets (PRJNA417762, PRJNA667091, PRJNA725184) were used to examine DEGs in EI vs. recurrent implantation failure (RIF). Quality assessment of the raw next-generation sequencing data was performed using FastQC, discarding bases with a quality score below 30. Differential analysis was conducted using DESeq2. After removing duplicates and empty gene names, and merging the four CD vs. SL datasets, we identified 3,007 DEGs (CD_DEGs). In the EI vs. RIF comparison, 16,040 DEGs (EI_DEGs) were identified. Additionally, we found 7,103 DEGs in GSE2957, which compared normal pregnancy to non-pregnancy (P vs. NP), with 398 genes upregulated and 6,705 genes downregulated. Analysis of GSE195795 revealed 7,533 DEGs related to postpartum and normal pregnancy (PP vs. P), with 3,327 genes upregulated and 4,206 genes downregulated.

#### Integrated analysis of proteomics and transcriptomics

##### *Proteomics analysis results*

Following database searches, a series of quality control measures were performed to ensure that the results met the required standards, as shown in Supplementary Fig. 1. The results indicated that the peptides predominantly ranged from 7 to 20 amino acids in length, meeting quality control requirements. Most proteins were associated with at least two peptides and exhibited less than 20% coverage. Protein distributions above 10 kDa were relatively uniform, ensuring no significant molecular weight bias.

Proteomic analysis identified 7,276 proteins (RAW) in all samples. There were 1,063 DEPs between SD and D groups (SD vs. D), including 781 down-regulated and 282 up-regulated proteins. Similarly, 1,023 DEPs were detected between SV and V groups (SV vs. V), with 757 down-regulated and 266 up-regulated proteins. KEGG pathway enrichment indicated that DEPs were primarily involved in focal adhesion, formation of various diseases, and synthesis of nucleic acids, suggesting a role in CSP pathogenesis (Fig. 3A). Previous studies have reported that focal adhesion helps maintain the recognition and

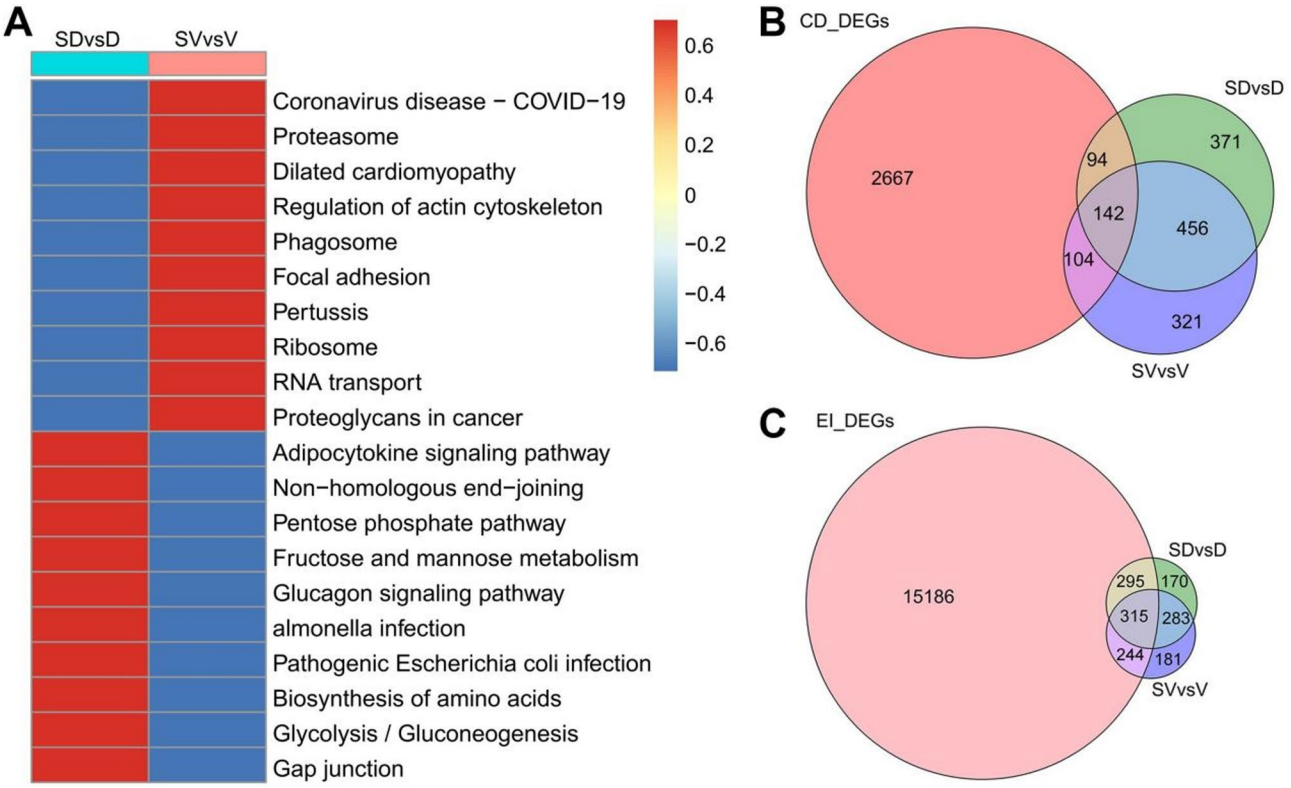



**Fig. 3** **A**. KEGG enrichment heatmap of DEPs, **B**. Venn diagram of DEGs1 obtained by the intersection of SD vs. D, SV vs. V and CD_DEGs, **C**. Venn diagram of DEGs2 obtained by the intersection of SD vs. D, SV vs. V and EI_DEGs

**Table 1** Chi-square test showed that CSP-altered proteins are enriched for genes changed in CD or EI

| | Group | Overlapping DEGs | Non overlapping DEGs | $X^2$ | *P* (Sig.) |
|---|---|---|---|---|---|
| 1 | DEGs1 (CD_DEGs∩SD vs. D & SV vs. V) | 142 (9.54%) | 1346 (90.46%) | 21.14 | < 0.001 |
| | CD_DEGs∩RAW | 1427 (13.88%) | 8856 (86.12%) | | |
| 2 | DEGs2 (EI_DEGs∩SD vs. D & SV vs. V) | 315 (21.17%) | 1173 (78.83%) | 8.23 | 0.004 |
| | EI_DEGs∩RAW | 4243 (18.20%) | 19,073 (81.8%) | | |

tight connection between the uterine and placental surfaces [11]. Considering the impact on the site of embryo implantation, focal adhesion may be involved in the formation of CSP.

Through intersection analysis, we found that SD vs. D and SV vs. V shared 142 common differentially expressed genes (DEGs1, Fig. 3B) with CD_DEGs, and 315 common differentially expressed genes (DEGs2, Fig. 3C) with EI_DEGs. This indicates that CD and EI are closely related to the occurrence of CSP, with a significant number of commonly altered genes. To determine whether these overlaps were statistically significant and not due to random coincidence, we performed a chi-square test. Specifically, we tested the hypothesis that CSP-altered proteins are enriched for genes changed in CD or EI. We compared the intersection of CD_DEGs and EI_DEGs with DEPs (SD vs. D & SV vs. V), against the background of all proteins (RAW) detected in the CSP samples ("the rest" referring to non-overlapping genes). As shown in Table 1, the overlaps between CD/EI DEGs and DEPs (142 and 315 genes, respectively) were significantly greater than expected by chance ($P = 0.004$ & $P < 0.001$). This indicates that these overlapping genes are unlikely to be due to chance ($P < 0.005$) and thus warrant further investigation.

### *Cell adhesion pathways and hub genes in CSP*

The GO function enrichment of DEGs1 and DEGs2 were implemented using DAVID v6.8. Combining the two sets of GO_BP, we found that cell-cell adhesion is involved in both (Fig. 4A), and cell adhesion is the ancestral BP of cell-cell adhesion (Supplementary Fig. 2). Considering the characteristics of CSP disease, it is reasonable to believe that cell adhesion is the most relevant biological process for CSP. However, to ensure all possibilities are considered, we conducted an interaction analysis of all enriched DEGs. After K-means clustering, the 12 points were divided into 3 groups (Fig. 4B). The MCODE (Molecular Complex Detection) algorithm was used to

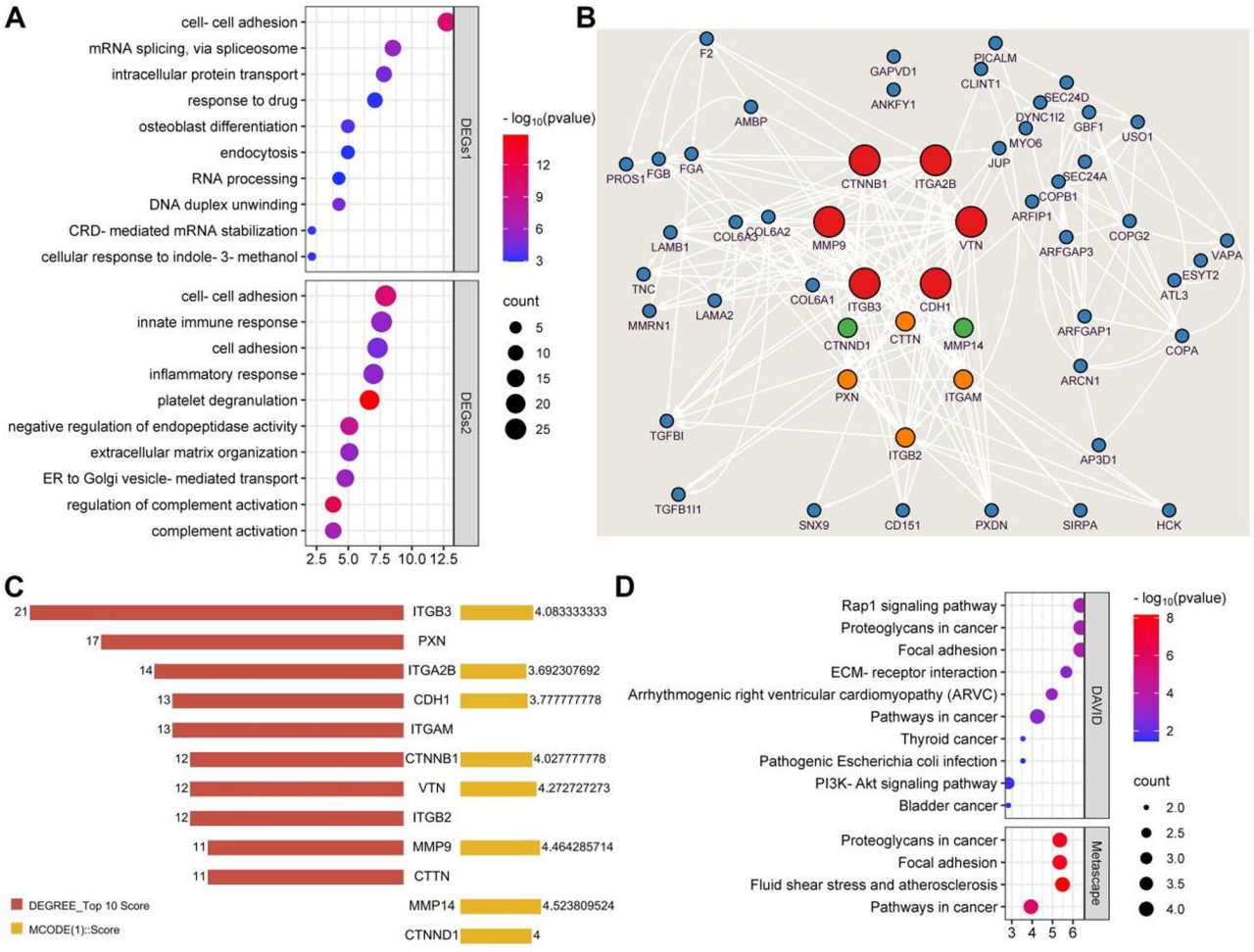



**Fig. 4** Hub genes in cell adhesion pathways and the occurrence of CSP. **A**. Enrichment bubble chart of biological processes (BP) for DEGs1 and DEGs2. **B**. PPI network after K-means clustering into 3 groups. **C**. The butterfly plot illustrates a comparison of hub gene rankings obtained using the MCODE and Degree algorithms. **D**. KEGG enrichment bubble chart for the 6 hub genes in DAVID and Metascape databases. In the bubble charts, larger dots represent higher counts, and redder dots indicate smaller p-values

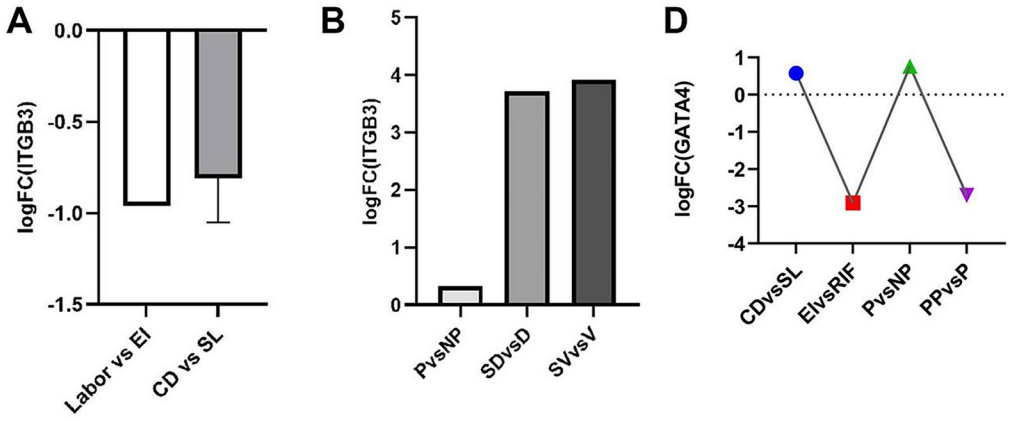
identify densely connected sub-networks within the PPI network [12]. To further prioritize hub genes, the Degree method implemented in the cytoHubba plugin was applied, ranking nodes based on the number of direct connections [13]. Next, by combining the two algorithm, we identified 6 hub genes, including MMP9, VTN, ITGB3, CTNNB1, CDH1, and ITGA2B (Fig. 4C). This further screening showed that these six hub genes are key genes more likely to be involved in the regulation of CSP.

To further explore the signaling pathways through which the formation of CSP is activated, we conducted KEGG pathway enrichment analysis for the 6 hub genes. Analyses from both the DAVID and Metascape websites revealed that focal adhesion, which was co-enriched, is the most likely signaling pathway for the formation of CSP (Fig. 4D). The genes enriched in this pathway are VTN, ITGB3, and ITGA2B.

### *The transcription factor GATA4 potentially regulates ITGB3*

Next, we reviewed the expression of these three genes across various datasets and found that ITGB3 was downregulated postpartum. Moreover, the downregulation of ITGB3 postpartum was more pronounced following a cesarean section compared to natural delivery (Fig. 5A). Compared to non-pregnant women, ITGB3 expression was upregulated after conception, with a more significant increase in villous and decidual tissues following CSP (Fig. 5B). This suggests that, compared to natural delivery, the compensatory upregulation of ITGB3 postpartum after cesarean section leads to a sharp increase in ITGB3 at the scar site during subsequent pregnancies, attracting the gestational sac to implant at the scar. The p-values of the above differentially expressed genes were less than 0.05. Therefore, ITGB3 is a key molecule in the occurrence of CSP.

To investigate how ITGB3 regulates CSP, we predicted the transcription factors (TF) of ITGB3 using the JASPAR




C

| Name | motif | Score | Relative score | Gene | Start | End | Strand | Predicted sequence |
|---|---|---|---|---|---|---|---|---|
| MA0482.3 | | 14.39 | 1.00 | ITGB3 | 1266 | 1273 | - | CCTTATCT |
| MA0482.2 | | 13.96 | 0.96 | | 1264 | 1275 | - | CCCCTTATCTCC |
| MA0482.3 | | 11.15 | 0.93 | | 875 | 882 | - | ACTTATCT |
| MA0482.3 | | 11.00 | 0.92 | | 1318 | 1325 | - | CTTTATCT |

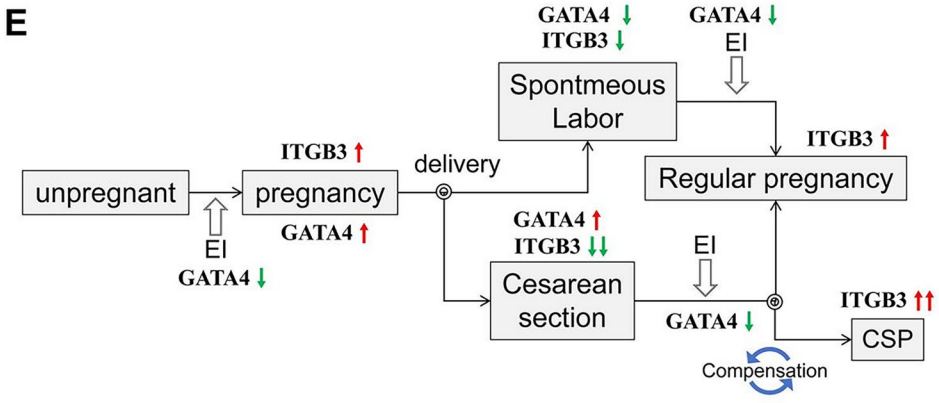



**Fig. 5** The transcription factor GATA4 regulates ITGB3. **A**-**B**. Levels of ITGB3 during the conception process. **C**. Binding sites of GATA4 with ITGB3, where a higher score indicates a greater likelihood of this transcription factor binding to the input sequence. **D**. Levels of GATA4 during the conception process. **E**. Changes in the expression of GATA4 and ITGB3 in women during the conception process

database and found four binding sites for GATA4 on ITGB3 (Fig. 5C). This indicates that GATA4 has the ability to bind and regulate ITGB3, typically by specifically inhibiting the expression of target genes [14]. Similarly, we analyzed the changes in GATA4 expression during the course of pregnancy (Fig. 5D). To clarify the roles of GATA4 and ITGB3 in CSP, we displayed their expressions at different stages of pregnancy in women (Fig. 5E). It was found that GATA4 is downregulated during blastocyst implantation, leading to a weakened inhibitory effect on ITGB3, resulting in increased ITGB3 expression post-conception. To balance ITGB3 levels, GATA4 also increases. Due to differences in delivery methods, the molecular expressions in women vary: (1) During natural delivery, both ITGB3 and GATA4 decrease, restoring ITGB3 balance. In subsequent pregnancies, further GATA4 reduction elevates ITGB3 levels. (2) During a cesarean section, ITGB3 decreases significantly, and increased GATA4 drives ITGB3 even lower. During blastocyst implantation, GATA4 expression is reduced to

compensate, allowing ITGB3 to increase; adequate compensation leads to normal pregnancy, but excessive compensation significantly upregulates ITGB3, making the cesarean scar more conducive to implantation, resulting in CSP.

## Discussion

With the rise in cesarean deliveries, reports of CSP are increasing worldwide [15]. However, due to insufficient research, delayed diagnoses can lead to uterine rupture and hemorrhage, which can be fatal [16]. Therefore, exploring CSP mechanisms is essential for better diagnosis, treatment, and prognosis evaluation. In this study, we conducted proteomic analysis on tissue samples from six women and analyzed nine gene expression datasets from GEO and SRA. Venn diagrams identified common genes between CD and CSP. Bioinformatics analysis revealed that focal adhesion is a key pathway involving hub genes VTN, ITGB3, and ITGA2B. Review of gene level changes showed that ITGB3 levels rebound in CSP women, and GATA4 is identified as TF for ITGB3.

### The interconnection between CSP, Cesarean delivery, and embryo implantation

Our Venn diagram analysis shows a significant gene overlap between CD, EI, and CSP. Chi-square tests confirm that these shared regulatory genes are closely related ($P < 0.05$). Reports indicate that the prevalence of CSP in women with a history of CD is 0.15%, accounting for 6.1% of ectopic pregnancies [5, 17]. Studies also show that the risk of placental implantation errors increases with the number of CD surgeries [18]. As a type of pregnancy, CSP is inherently linked to embryo implantation, confirming the close relationship between EI and CSP. Additionally, Gull's research team found that women with previous CD have a threefold increased risk of CSP [19]. The occurrence of CSP is independent of the time after CD, with many cases occurring after a single CD [5, 20]. Therefore, the association between cesarean delivery and CSP is likely not coincidental.

### Key genes for CSP formation: ITGA2B, VTN, and ITGB3 in focal adhesion signaling

KEGG enrichment analysis using DAVID and Metascape identified focal adhesion as the critical pathway, involving upregulated genes ITGB3, VTN, and ITGA2B. Literature review indicates higher ITGB3 levels in placental tissues than in the secretory endometrium during pregnancy, highlighting its role in EI [21]. Adhesion molecules and extracellular matrix proteins, crucial for embryo implantation, were noted to mediate cell-extracellular matrix interactions [22, 23]. Focal adhesion mediates cell-extracellular matrix interactions [24]. The role of VTN in endometrial epithelial cell adhesion further supports this connection [25]. It is worth noting that the results of both GO_BP and KEGG are related to the adhesion process. These results are consistent with observations from earlier studies that cell adhesion molecules have an important role in maintaining the integrity of cells and tissues during the development of tissues and organs and throughout the life course [26, 27]. Based on these data, we can infer that Focal adhesion plays a crucial role in the development of CSP.

Analysis of the PRJNA417762 dataset revealed downregulated ITGB3 and VTN in the pre-secretory endometrium compared to the mid-secretory phase, where ITGA2B was upregulated. This indicates that ITGB3 and VTN enhance endometrial receptivity, while ITGA2B aids embryo implantation. Liu's research supports ITGB3 as a marker of good endometrial receptivity [28]. VTN acts as a key controller of tissue repair [29], while ITGA2B has a documented role in embryo implantation in canines [30]. Proteomic analysis confirmed the upregulation of ITGB3, VTN, and ITGA2B in CSP samples, suggesting their increased expression improves endometrial receptivity in a scarred uterus, promoting CSP formation. Thus, ITGB3, VTN, and ITGA2B, enriched in the focal adhesion pathway, are crucial in CSP development.

### GATA4 as a potential regulator of CSP

Our study found that ITGB3 expression decreases postpartum, with a more pronounced downregulation following cesarean delivery compared to spontaneous delivery. Previous reports have documented the cycle-dependent expression of ITGB3 in the human endometrium [31], suggesting that the postpartum endometrium is initially less receptive to embryo implantation, particularly after cesarean delivery. GATA4, a member of the GATA transcription factor family, not only plays a crucial role in the development of the heart and major blood vessels [32] but also influences the uterine environment and the implantation of the embryo [33]. Therefore, GATA4 may be involved in the regulation of the ITGB3 gene. We predicted and identified four binding sites of the transcription factor GATA4 within the ITGB3 gene, and this relationship was further corroborated by expression changes observed in pregnancy-related datasets. Overall, the above evidence strongly supports the notion that ITGB3 is a target gene of GATA4.

Additionally, data from GSE2957 indicate higher ITGB3 levels in pregnant women compared to non-pregnant women, while our proteomics analysis shows that ITGB3 expression is significantly upregulated in both decidua and villi in CSP compared to normal pregnancies. These findings are consistent with the study by Qian et al. [34], which suggests that changes in endometrial receptivity and embryo implantation in CSP may be related to increased expression of ITGB3. This

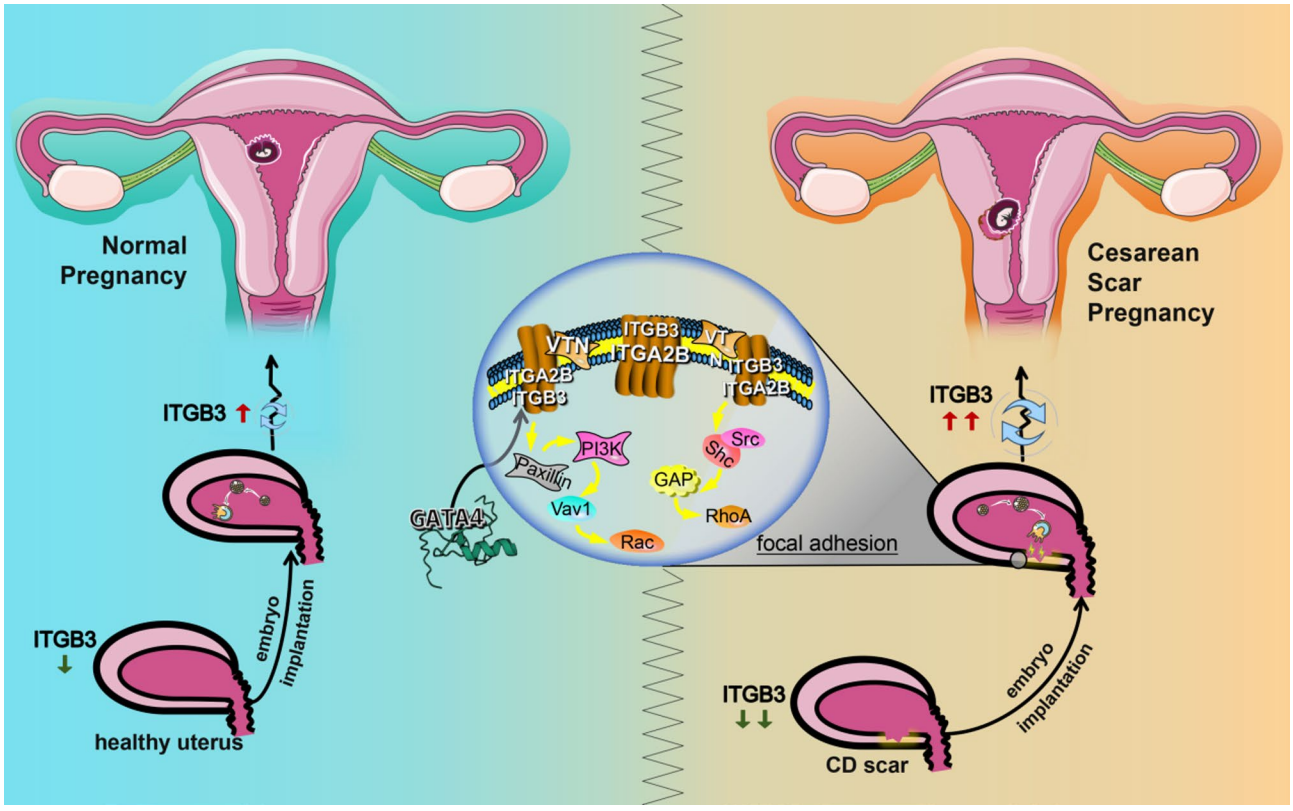



**Fig. 6** Hypothesis of ITGB3-Induced Rebound Effect in CSP. The greater the downregulation of ITGB3 after cesarean section, the more excessive the compensatory regulation of ITGB3 by GATA4 during the scar healing process, leading to the cesarean section scar becoming a more attractive site for embryo implantation through activation of focal adhesion signaling pathways, thus forming CSP

implies that elevated ITGB3 in cesarean scars may attract embryos, contributing to the occurrence of CSP.

Based on this, we propose a plausible hypothesis of the ITGB3 rebound effect in CSP. Specifically, after cesarean delivery, there is a significant downregulation of ITGB3, followed by a marked upregulation during scar repair, potentially regulated by GATA4. This compensatory upregulation may activate the focal adhesion signaling pathway, making the cesarean scar a more favorable implantation site and contributing to CSP (Fig. 6).

## Limitations and prospects

Using a combined proteomics and bioinformatics approach, this study offers deeper insights into the pathogenesis of CSP and provides a theoretical basis for new prevention and treatment strategies. It may also shed light on other obstetrical and gynecological diseases. However, several questions remain unanswered. Currently, CSP lacks a reliable animal model, which limits in vivo validation of key gene changes. For cesarean scar defect (CSD), a rat full-thickness uterine scar model has been widely used. However, CSD is a static defect, while CSP is caused by embryo implantation into the scar. Therefore, the CSD model cannot fully represent CSP. Further efforts will be needed to develop more specific and representative models for CSP in future research. Current proteomic analyses compared women with CSP to those with normal pregnancies, but including samples from normal pregnancies after cesarean sections would enhance comparisons. The unpredictable occurrence of CSP complicates the collection of specimens for further analysis. Given the incidence and severe consequences of CSP, further research is crucial to fully understand this condition. In future work, we plan to conduct further experimental validation, including CUT&Tag, Western blot, and functional assays, once sufficient clinical materials are collected. These efforts will help to strengthen the conclusions and provide more direct evidence for the molecular mechanisms underlying CSP.

### Abbreviations

| | |
|---|---|
| CSP | Cesarean Scar Pregnancy |
| CD | Cesarean Delivery |
| EI | Embryo Implantation |
| ITGB3 | Integrin Subunit Beta 3 |
| ITGA2B | Integrin Subunit Alpha 2b |
| VTN | Vitronectin |
| DEGs | Differentially Expressed Genes |
| DEPs | Differentially Expressed Proteins |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12884-025-07752-4.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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The Figure 6. was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

**Author contributions**

Zaiqing Qu, Bei Zhu, and Xiaoning Lu made significant contributions to the design of the work and collected clinical samples, Zhanqing Luo and Yutao Wang analyzed the data and wrote the manuscript, Xue Cao critically revised the work and finally approved the version to be published, Chenyu Xing and Dingyun You agree to be responsible for all aspects of the work to ensure that issues related to the accuracy or completeness of any part of the work are properly investigated and resolve.

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**Data availability**

The data underlying this article are available in the article and in its online supplementary material. The open datasets used and/or analyzed during this study are available from GEO: GSE18850, GSE149436, GSE195795, GSE2957 ( https://www.ncbi.nlm.nih.gov/geo/) and SRA: RJNA687512, PRJNA516344, PRJNA725184, PRJNA667091, PRJNA417762 (https://www.trace.ncbi.nlm.nih.gov/Traces/sra/) were obtained.

## Declarations

**Ethics approval and consent to participate**

This study complies with the Declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital of Kunming Medical University. Informed consent was obtained from all study participants prior to inclusion.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare no competing interests.

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