Cao et al. Cell Communication and Signaling (2025) 23:127
https://doi.org/10.1186/s12964-025-02120-3

Cell Communication and Signaling

RESEARCH Open Access

IL-1β stimulates ADAMTS9 expression and contributes to preterm prelabor rupture of membranes

Jiasong Cao[1,2,3†], Yixin Wang[4†], Qimei Lin[1,2,3†], Shuqi Wang[1,2], Yongmei Shen[1,2,3], Lei Zhang[1,2], Wen Li[1,2,3], Ling Chen[1,2], Chunliu Liu[5], Shihan Yao[2], Ling Shuai[1,6], Xu Chen[1,2], Zongjin Li[1,4,7*] and Ying Chang[1,2,8*]

Abstract

Background Preterm prelabor rupture of membranes (pPROM) is a leading cause of neonatal morbidity and mortality. While intra-amniotic infection is a well-established driver of pPROM, the role of sterile intra-amniotic inflammation remains unclear. Recent evidence suggests that interleukin-1 beta (IL-1β) promotes extracellular matrix (ECM) remodeling via downstream effectors, a disintegrin-like and metalloproteinase domain with thrombospondin type 1 motif 9 (ADAMTS9), while protein O-fucosyltransferase 2 (POFUT2) facilitates its O-fucosylation and secretion, amplifying ECM degradation. This study investigates how IL-1β-triggered nuclear factor kappa-B (NF-κB) activation promotes ADAMTS9 and POFUT2 expression, ultimately driving fetal membrane ECM remodeling and weakening in pPROM without signs of intra-amniotic infection.

Methods A nested case-control study included maternal serum and fetal membrane samples from 60 pregnant women (34 pPROM, 26 full-term births [FTB]). ELISA measured serum levels of IL-1β and ADAMTS9, and their correlations were analyzed. Mechanistic studies utilized primary human amniotic epithelial cells (hAECs) and fetal membrane-decidua explants with IL-1β treatment. The role of NF-κB was explored using chromatin immunoprecipitation (ChIP) and luciferase assays to assess NF-κB binding to the promoters of ADAMTS9 and POFUT2. A murine model of sterile intra-amniotic inflammation under ultrasound-guided IL-1β injection was used to validate in vitro findings and assess pregnancy outcomes.

Results Serum IL-1β and ADAMTS9 levels at 16 weeks of gestation were significantly higher in pPROM cases compared to FTB controls ($P < 0.001$). A combined model of these biomarkers demonstrated high predictive accuracy for pPROM (AUC = 0.83). Mechanistically, IL-1β activated NF-κB, leading to its binding to the promoters of ADAMTS9 and POFUT2. NF-κB activation promoted ADAMTS9 expression, while POFUT2 enhanced its secretion. Together, these processes drove versican degradation and ECM weakening. Intra-amniotic administration of IL-1β in mice induced

†Jiasong Cao, Yixin Wang and Qimei Lin contributed equally to this work.

*Correspondence:
Zongjin Li
zongjinli@nankai.edu.cn
Ying Chang
changying4470@sina.com

Full list of author information is available at the end of the article

fetal membrane weakening, preterm birth, and adverse neonatal outcomes, which were mitigated by the NF-κB inhibitor BAY 11-7082 treatment.

Conclusion Maternal serum ADAMTS9 levels at mid-gestation are promising non-invasive biomarkers for pPROM risk stratification. Mechanistically, IL-1β-induced NF-κB activation promotes ADAMTS9 expression and POFUT2-dependent secretion, contributing to fetal membrane weakening. These findings provide new insights into the role and potential therapeutic target for sterile intra-amniotic inflammation in pPROM.

Keywords Preterm prelabor rupture of fetal membranes, Interleukin-1 beta, ADAMTS9, NF-kappa B, Protein O-fucosyltransferase 2

Introduction

Preterm birth (PTB), defined as delivery before 37 weeks of gestation, significantly impacts 5–18% of pregnancies globally [1, 2]. It is a leading cause of neonatal morbidity and mortality, imposing long-term health risks on surviving infants [3]. Preterm prelabor rupture of membranes (pPROM) accounts for 30-40% of PTB cases. Due to the difficulty in predicting the onset and progression, pPROM has been a significant challenge in pregnancy management [4, 5]. These outcomes underscore the urgent need for detailed investigation into the mechanisms and biomarker identification for pPROM, thereby reducing the rate of preterm births.

The fetal membranes' structural integrity is essential for pregnancy maintenance. However, preterm fetal membrane weakening occurs more acutely than during full-term birth (FTB) [6, 7]. Notably, chorioamniotic separation (CAS) has been considered a precursor to the loss of membrane integrity in pPROM, occurring before the onset of clinical symptoms of membrane rupture [8]. Clinical studies have highlighted that extensive amniotic membrane detachment is associated with adverse perinatal fetal outcomes [9]. Although fetal membrane damage in preterm labor has been investigated over the years [10–12], the molecular mechanisms that initially trigger CAS in this process are still not fully understood.

Extracellular matrix (ECM) components are widely involved in maintaining fetal membranes' mechanical strength and functional integrity. Pathological studies suggest that CAS may originate from a proteoglycan-rich intermediate layer and be triggered by mechanical stress or biochemical degradation [13]. Previous studies have focused on the degradation of fibrous proteins such as collagen. However, the role of non-fibrous proteoglycan in pPROM is less understood. Versican (VCAN), a type of chondroitin sulfate proteoglycan (CSPG) and a member of the hyalectan family of ECM components, provides biomechanical strength, and its proteolytic cleavage may be closely linked to increased susceptibility to rupture [14]. Recent studies have found that its cleavage product versikine could significantly induce inflammatory factors in amniotic mesenchymal cells and be involved in the spontaneous rupture of the fetal membrane at parturition [15].

In addition to the external environment and maternal conditions, intra-amniotic infection caused by microbes is another known risk factor for pPROM, with its microbial pathogenic and cytotoxic mechanisms extensively studied. In clinical practice, only a minority of pPROM cases are diagnosed early based on detectable signs of intra-amniotic infection, which allows for timely intervention and is associated with improved clinical outcomes [16, 17], while they generally do not prevent preterm birth in cases without proven infection [18, 19]. Currently, many cases still lack microbial evidence, highlighting the potential role of sterile intra-amniotic inflammation in pPROM [20]. Previous studies have found that sterile intra-amniotic inflammation is mainly mediated by oxidative stress, senescent or apoptotic cells releasing damage-associated molecular patterns (DAMPs) [21, 22], which trigger signaling cascades through receptor-activated immune pathways such as p38 mitogen-activated protein kinase (p38MAPK) and nuclear factor kappa-B (NF-κB), ultimately leading to the upregulated pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and IL-1β [10, 23, 24]. These cytokines further exacerbate inflammation, weakening and even severely damaging the fetal membranes. Importantly, IL-1β has been identified not only as a mediator of inflammation but also as a regulator of ECM dynamics [25–27], possibly through its type I IL-1β receptor (IL-1R1)-mediated downstream pathways to affect a disintegrin and metalloproteinase with thrombospondin motifs 9 (ADAMTS9), an enzyme that modulates ECM components like versican [28–31]. Although IL-1β enhances ADAMTS9 expression in chondrosarcoma cells [32, 33], how IL-1β influences ADAMTS9 activity in the fetal membranes and its subsequent impact on versican degradation remains unclear, particularly without signs of intra-amniotic infection.

This study aims to investigate the potential of maternal serum IL-1β and ADAMTS9 levels at 16 weeks of gestation as novel non-invasive predictive biomarkers for pPROM without signs of intra-amniotic infection. Additionally, it seeks to explore their roles and underlying

mechanisms in the pathophysiological weakening of fetal membranes during the development of pPROM.

Materials and methods

Study design

This nested case-control study was conducted within a larger prospective cohort study initiated at Tianjin Central Hospital in Tianjin, China. The study initially enrolled 243 singleton pregnant women between 28^{+0} and 40^{+6} weeks of gestation. Maternal serum was collected at 16 weeks of gestation as a single-time-point sampling from all participants. After excluding cases with potential confounding factors, such as preterm rupture of membranes > 37 weeks of gestation, spontaneous preterm birth with intact membrane, preexisting or gestational diabetes mellitus, and in vitro fertilization, 155 women with full-term births (FTB, $n = 103$) and pPROM ($n = 52$) were included in the study. Management of pPROM has been previously described in detail [34, 35]. The protocol consists of an initial assessment and confirmation of membrane rupture, administration of corticosteroids for fetal lung maturation, and antibiotic prophylaxis to prevent infection, followed by careful monitoring for labor progression and signs of infection. The decision for timing of delivery is made based on gestational age, fetal and maternal well-being, and the presence of any complications.

Following the preliminary analysis of clinical data, a total of 27 women were excluded from the study due to the presence of various conditions: placental anomalies ($n = 9$), obstetric infections including clinical chorioamnionitis ($n = 11$) and bacterial vaginosis ($n = 6$), along with major fetal structural anomalies ($n = 1$). To minimize the influence of potential intra-amniotic infections on the study outcomes, an additional 7 women diagnosed with acute histological chorioamnionitis were excluded following pathological examinations. After stringent serum quality control ($n = 7$), 34 women with pPROM (gestational age > 31 weeks) and 26 age- and BMI-matched FTB women as controls were selected for the final measurement and comparison of serum IL-1β and ADAMTS9 levels to explore their potential as predictive biomarkers for pPROM without signs of intra-amniotic infection. pPROM was defined as membrane rupture before 37 weeks. This study evaluated signs of intra-amniotic infection according to the following criteria: (i) clinical chorioamnionitis; (ii) pathological acute histological chorioamnionitis [36, 37]. Clinical data were collected post-delivery (Table S1).

Fetal membrane tissues were collected within 15 min post-delivery, washed with 0.9% saline, and subsequently fixed in 4% formaldehyde. For histological examination, five cases from each group were randomly selected.

The study was approved by the Obstetrics and Gynecology Ethics Committee of Tianjin Central Hospital (Approval No. 2021KY113). The confidentiality of the patients was strictly protected, and no personal data were required for this study. All participants provided written informed consent.

Human fetal membrane-decidual explants

The human fetal membrane-decidual tissues used to establish an explant model of sterile intraamniotic inflammation were obtained from singleton pregnancies and women with complications undergoing elective cesarean section before the onset of labor. Briefly, fetal membrane-decidual tissues were cleared with HBSS containing antibiotic-antimycotic solution (Solarbio) and cultured in transwell chambers (0.4-μm pore size, BD Falcon). Before treating IL-1β (10 ng/mL, MedChemExpress), Amniotic epithelium was pretreated with shADAMTS9 or shNC lentivirus for 8 h. Following the successful establishment of the model, explants were collected for subsequent pathological staining. We also isolated hAECs, hAMCs, hCMCs, hCTCs, and hDECs from women with FTB for further analysis.

Human primary hAECs isolation and culture

The amnion was obtained from healthy pregnant women at full term undergoing cesarean section. Briefly, the amnion was cleared with HBSS containing antibiotic-antimycotic solution and digested into single-cell suspension with 0.25% trypsin. After centrifugation, the cell pellet was resuspended and cultured in DMEM/F12 medium containing 1% antibiotic-antimycotic, 10% FBS (Gibco), 1% sodium pyruvate (Gibco), 1% MEM nonessential amino acids (Gibco), 1% L-glutamine (Gibco), 0.1% β-mercaptoethanol (Thermo Fisher), and 0.01% EGF (Peprotech) (37 °C with 5% CO_2).

Animal study

All animal procedures, including euthanasia, were carried out following ARRIVE guidelines and approved by the Institutional Review Board of Nankai University. C57BL/6 mice (Vital River, China), aged 8–12 weeks, were housed under controlled conditions and mated overnight. The gestational day was counted as 0.5 dpc when a vaginal plug was present.

All mice were randomly assigned to three groups and numbered sequentially at 15.5 dpc: PBS ($n = 10$), IL-1β ($n = 16$), and IL-1β + BAY ($n = 14$). On 17.5 dpc, 1st half of the mice in each group were designated for pregnancy monitoring, including ultrasound scanning and fetal heart rate examination. After the monitoring, serum samples were collected. The mice were then euthanized by inhalation of isoflurane, and fetal and placental photos were taken during tissue dissection. The 2nd half in each

group was observed until 20.5 dpc to record delivery outcomes. Neonatal weight was obtained immediately after delivery. The fetal heart rate and neonatal weight are reported as an average per litter. The gestational length was calculated from the detection of the vaginal plug (defined as 0.5 days post-coitum, dpc) until the appearance of the first pup in the cage bedding. Preterm birth was defined as delivery before 18.5 dpc, and its rate was quantified by the percentage of preterm females relative to the total number of pregnant mice. The rate of neonatal mortality was quantified by the percentage of pups confirmed dead at birth out of the total number of pups delivered. The survival rate of neonates within four weeks was defined as the proportion of survival pups among the total number of infants at postnatal four weeks.

A mouse model of ultrasound-guided intra-amniotic inflammation was established as previously described [11, 28, 38]. Briefly, Mice were anesthetized on 15.5 dpc by inhalation of 1.75% isoflurane. Intra-amniotic administration of IL-1β (100 ng/20 μL, MedChemExpress) or PBS (1X/20 μL) was performed in each gestational sac under ultrasound guidance, using the Vevo 2100 Imaging System (VisualSonics) with a 29-gauge needle. Half an hour later, NF-κB inhibitor BAY 11-7082 (100 μg/100 μL, MedChemExpress) or PBS was administered via tail vein; injection was given every two days until delivery or 20.5 dpc. The dose of 100 ng/20 uL IL-1β for intra-amniotic injection was selected based on a comprehensive review of prior research [39–41], notably adjusting from the 10 μg used by Romero et al. in similar mouse experiments [29], which successfully simulated infection-induced severe or acute intra-amniotic inflammation and subsequent preterm labor. Considering the role of IL-1β in sterile intra-amniotic inflammation and its potential impact on fetal membrane stability, a lower dosage facilitates the simulation of a longer period of sterile inflammation by avoiding immediate preterm labor.

Others

To ensure a comprehensive understanding of all other methods employed in this study, additional laboratory techniques such as Western blotting, qRT-PCR, histological staining, immunohistochemistry, and immunofluorescence staining, as well as information on the corresponding antibodies and primers, are discussed in the supplementary file.

Statistical analysis

The Shapiro–Wilk test was used to assess the normality of continuous data. Continuous variables were compared using the Mann–Whitney U test and the Kruskal–Wallis H analysis, followed by Denn's test. The results were presented as a median with an interquartile range or minimum to maximum values. Categorical variables were compared using chi-squared or Fisher's exact test, and the results were presented as counts or percentages. Spearman's correlation analysis was used to detect correlations among non-normally distributed continuous variables. Receiver operating curves (ROCs) were constructed to evaluate the performance of serum IL-1β and ADAMTS9 levels at 16 weeks of gestation from the nested case-control study for predicting pPROM without signs of intra-amniotic infection, and the areas under the curve (AUC) were determined and compared by Delong test. A P-value calculated with two tails and <0.05 was considered statistically significant. All the Statistical analyses were performed using GraphPad Prism 10 and SPSS 26.0, except the Delong test conducted by MedCalc Software 22.032.

Results

Elevated serum Il-1β and ADAMTS9 levels predict pPROM in pregnant individuals

This was a nested case-control study conducted within a larger prospective cohort study. Serum samples were collected from all participants at 16 weeks of gestation. Initially, 243 women (FTB, $n=140$; PTB, $n=103$) were screened for study eligibility. The flow of participants is displayed in Fig. 1. After excluding cases due to serum samples failing quality control ($n=7$) and histological chorioamnionitis ($n=7$), 114 women were considered for final testing and analysis. 60 women were finally included for clinical and serological analysis, including 34 women with pPROM without signs of intra-amniotic infection and 26 age- and BMI-matched women with FTB as controls. No significant differences in demographics were observed except for gestational age at delivery, which was significantly lower in the pPROM group ($P<0.0001$; Table S1).

Serum levels of IL-1β and ADAMTS9 at 16 weeks of gestation were significantly elevated in women who went on to develop pPROM without signs of intra-amniotic infection compared to FTB controls ($P=0.0005$ and $P=0.0001$) (Fig. 2a and b). Specifically, the median measured concentrations of IL-1β and ADAMTS9 were 10.30 pg/mL (interquartile range [IQR], 7.14–21.28 pg/mL) and 6.17 ng/mL (IQR, 3.94–10.69 ng/mL) in pPROM, respectively, vs. 6.53 pg/mL (IQR, 4.90–9.70 pg/mL) and 3.13 ng/mL (IQR, 2.31–4.45 ng/mL), in FTB. A significant positive correlation between serum concentrations of IL-1β and ADAMTS9 was evident in pPROM ($r=0.51$, $P<0.01$) but not in FTB ($r=0.06$, $P=0.70$) (Fig. 2c and d).

The predictive accuracy of serum IL-1β and ADAMTS9 concentrations at 16 weeks of gestation for pPROM without signs of intra-amniotic infection was assessed using ROC analysis. The AUC values for pPROM were 0.77 (95% confidence interval [CI], 0.64–0.87), 0.78 (95% CI, 0.66–0.88), and 0.83 (95% CI, 0.71–0.91) for IL-1β,

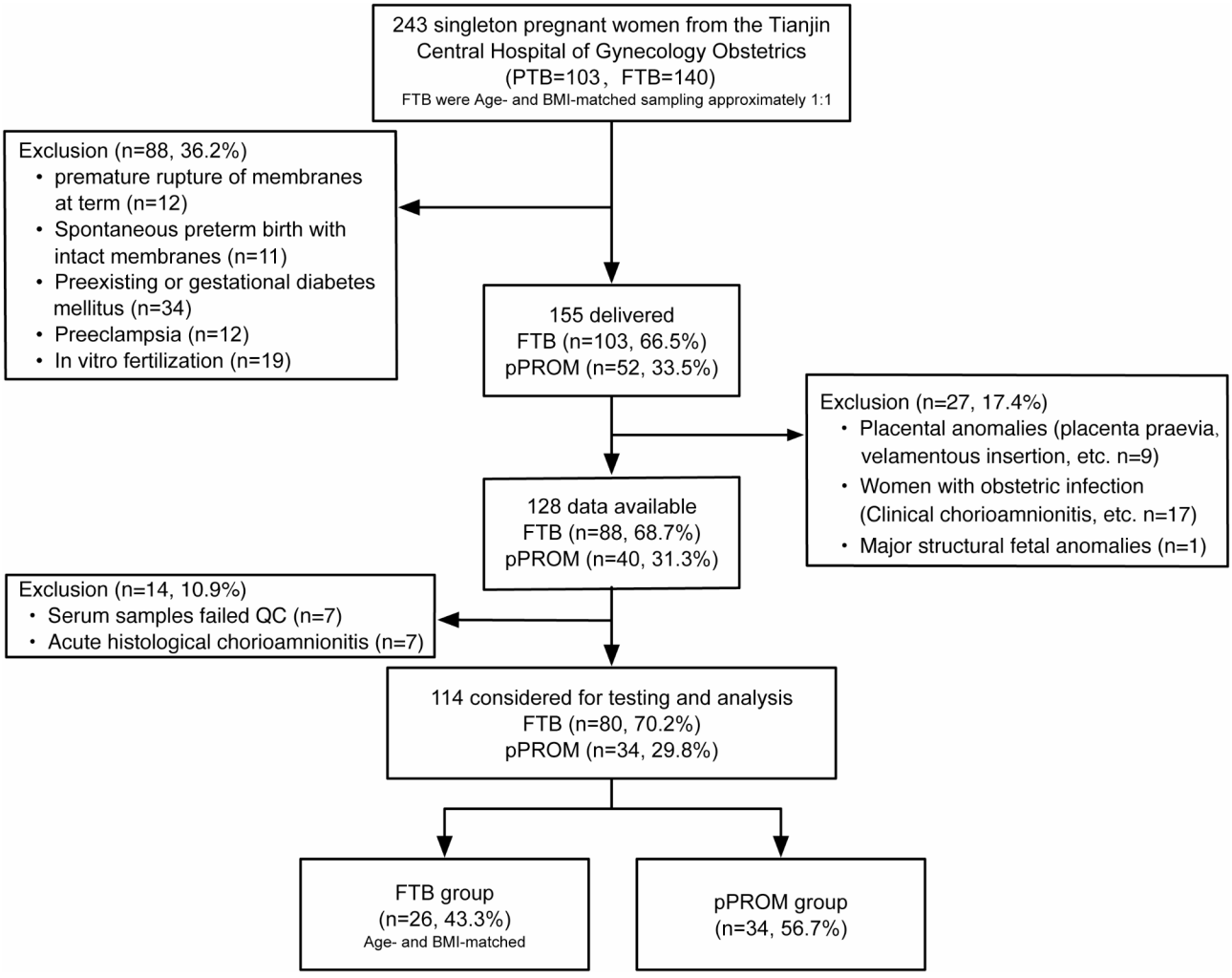

Fig. 1 The patient flow diagram of the study population

ADAMTS9, and the combined model of IL-1β and ADAMTS9, respectively, and all the two-tailed P-values are significant ($P<0.0001$) (Fig. 2e and Table S2). The AUC value for predicting pPROM determined using the combined model was the highest among the 3 variables, but it did not reach statistical significance when compared with IL-1β and ADAMTS9 individually (Table S3). In particular, the likelihood ratio for the combined model (8.17) far exceeds that of the individual markers (IL-1β: 1 0.86, ADAMTS9: 4.27), illustrating a significant increase in the odds of detecting pPROM when both biomarkers indicate positive results. ADAMTS9 also exhibited higher specificity (84.62%) than IL-1β (53.85%), indicating better accuracy in correctly identifying women who will not experience pPROM.

ADAMTS9 overexpression in hAECs correlates with reduced versican levels and weakened fetal membranes in pPROM

Given that ADAMTS9 demonstrated higher specificity than IL-1β in predicting pPROM, we investigated its involvement in breaking down versican [7, 42]. In fetal membrane tissues from FTB and pPROM cases, pPROM amnions exhibited looser microstructure, reduced proteoglycan deposition in the intermediate layer, and decreased cytokeratin 18 (CK18) compared to FTB (Fig. 3a and b). ADAMTS9 was primarily expressed in hAECs and significantly increased in pPROM samples, while versican levels were notably lower in pPROM patients (Fig. 3c). These findings were confirmed in primary hAECs from FTB and pPROM cases (Fig. 3d). hAECs from pPROM cases exhibited elevated ADAMTS9 and decreased CK18 and Vimentin levels (Fig. 3e and f, and Fig. S1). These results suggest that elevated ADAMTS9 expression in hAECs may contribute to pPROM by reducing versican levels and weakening fetal membranes.

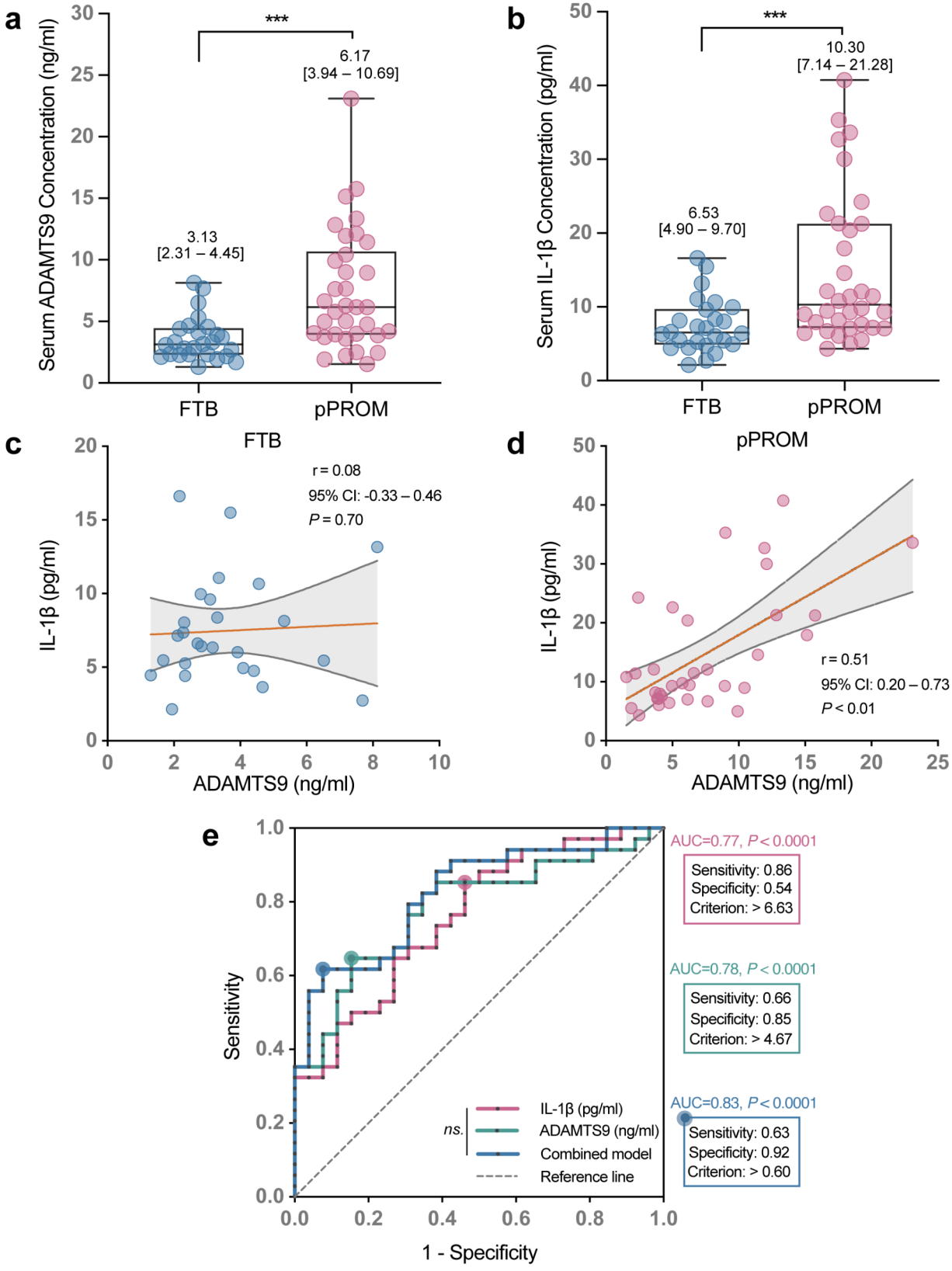

Fig. 2 (See legend on next page.)

(See figure on previous page.)

Fig. 2 The predictive efficacy of individual and composite biomarkers in predicting preterm prelabor rupture of membranes (pPROM). (**a**, **b**) Differences in prenatal serum IL-1β and ADAMTS9 concentrations between full-term birth (FTB; $n = 26$) and pPROM ($n = 34$) were determined using the Mann–Whitney U test. (**c**, **d**) The correlation between serum IL-1β and ADAMTS9 in women with FTB and pPROM was evaluated using Spearman's correlation coefficient (r). The orange line indicates the slope of the linear regression. The shadowed area indicates a 95% confidence interval, and the gray dashed line indicates the reference line. (**e**) Comparison of ROC curves of single and combined markers for predicting pPROM using the Delong method ($n = 60$). In the box-and-whisker plots, the midlines represent the medians, the boxes indicate the interquartile ranges, and the whiskers represent the minimum and maximum values. ***$P < 0.001$, ns. $P > 0.05$

IL-1β signaling enhances ADAMTS9 expression and versican depletion in hAECs

pPROM is associated with elevated levels of DAMPs [13]. When primary hAECs were exposed to DAMP-induced inflammatory cytokines, IL-1β notably upregulated ADAMTS9 transcription and secretion (Fig. 4a and b). Western blot analysis confirmed a significant increase in ADAMTS9 levels 24 h post-IL-1β treatment, coinciding with reduced versican protein expression after 24 h (Fig. 4c). To elucidate the role of ADAMTS9 in IL-1β-induced fetal membrane weakening, we used a fetal membrane-decidual explant model with hAECs pretreated with shADAMTS9 for 8 h (Fig. 4d). The downregulation of ADAMTS9 in hAECs confirmed the effectiveness of the model (Fig. 4f and Fig. S2). Additionally, IL-1β treatment reduced ECM deposition in amnion tissue and increased ADAMTS9 expression in hAECs; these effects were attenuated by ADAMTS9 knockdown (Fig. 4e). Immunohistochemical staining showed elevated IL-1R1 levels in hAECs from pPROM cases, consistent with increased ADAMTS9 expression (Fig. 4g). Furthermore, the IL-1R1 inhibitor AF12198 reduced both mRNA and protein levels of ADAMTS9 in primary hAECs cultured with IL-1β (Fig. 4h). These results collectively suggest that IL-1β signaling upregulates ADAMTS9 in hAECs, leading to versican depletion and fetal membrane weakening in pPROM.

IL-1β promotes ADAMTS9 transcription via NF-κB activation in pPROM

GSEA analysis of the GSE73685 dataset revealed upregulated IL-1R and NF-κB pathways in pPROM (Fig. S3). Immunohistochemical staining confirmed increased p-p65-positive hAECs in pPROM versus FTB (Fig. 5a). Immunofluorescence revealed elevated ADAMTS9 and p-p65 expression in hAECs from pPROM cases (Fig. 5b and c). In IL-1β-treated hAECs, p65 overexpression increased ADAMTS9, while BAY treatment reduced it (Fig. 5d-f). Activated p-p65 exerts transcriptional regulatory function by forming an NF-κB dimer with p50 [43]. Our hypothesis that activated NF-κB could bind to the ADAMTS9 promoter to enhance its transcription was confirmed by ChIP assays, which demonstrated p-p65 binding to the ADAMTS9 promoter in the presence of IL-1β (Fig. 5g and h). Luciferase reporter assays with mutant ADAMTS9 promoters showed that IL-1β-mediated ADAMTS9 promoter activity was abolished in mutants (Fig. 5i), suggesting that IL-1β promotes ADAMTS9 transcription by facilitating NF-κB recruitment to its promoter (Fig. 5j).

POFUT2 and NF-κB mediate IL-1β-induced ADAMTS9 expression and secretion in pPROM

Protein O-fucosyltransferase 2 (POFUT2) can regulate ADAMTS9 expression and secretion in various diseases [44, 45]. Immunohistochemical staining revealed increased POFUT2 protein expression in pPROM compared to FTB (Fig. 6a), with colocalized POFUT2 and ADAMTS9 in hAECs from pPROM (Fig. 6b). ChIP assay demonstrated that IL-1β treatment enhanced p-p65 binding to the POFUT2 promoter (Fig. 6c and d). Luciferase reporter assays with mutated POFUT2 promoter binding sites reduced IL-1β-induced promoter activity (Fig. 6e and f). Moreover, IL-1β upregulated POFUT2 mRNA levels in hAECs, which was abolished by BAY treatment or POFUT2 knockdown (Fig. 6g). Western blot analysis and ELISA revealed that POFUT2 knockdown or BAY treatment attenuated IL-1β-induced ADAMTS9 expression and secretion (Fig. 6h and i), suggesting that IL-1β enhances POFUT2 expression in hAECs through NF-κB recruitment, leading to increased ADAMTS9 secretion and potentially contributing to fetal membrane weakening in pPROM.

Inhibition of NF-κB signaling mitigates IL-1β-induced fetal complications and PTB

Using a murine model of intra-amniotic inflammation induced by IL-1β, we administered corresponding drugs through the ultrasound-guided intra-amniotic injection from gestational day 15.5 until the onset of labor or full term (Fig. 7a). IL-1β-exposed fetuses had lower fetal heart rates than controls, while BAY treatment improved fetal heart rates (Fig. 7b). IL-1β-injected mice displayed reduced fetal weights and sizes at birth, which were significantly improved by BAY treatment (Fig. 7c, d and Fig. S4).

We also assessed pregnancy duration, PTB rate, neonatal mortality, and short-term postnatal survival in preterm infants. Dams injected with IL-1β exhibited a higher PTB rate, neonatal mortality, and shorter gestation length than those receiving PBS. BAY treatment partially mitigated these effects, increasing pregnancy durations (Fig. 7e-g) and improving survival rates of neonates at week 4 (Fig. 7h). These results suggest that blocking the

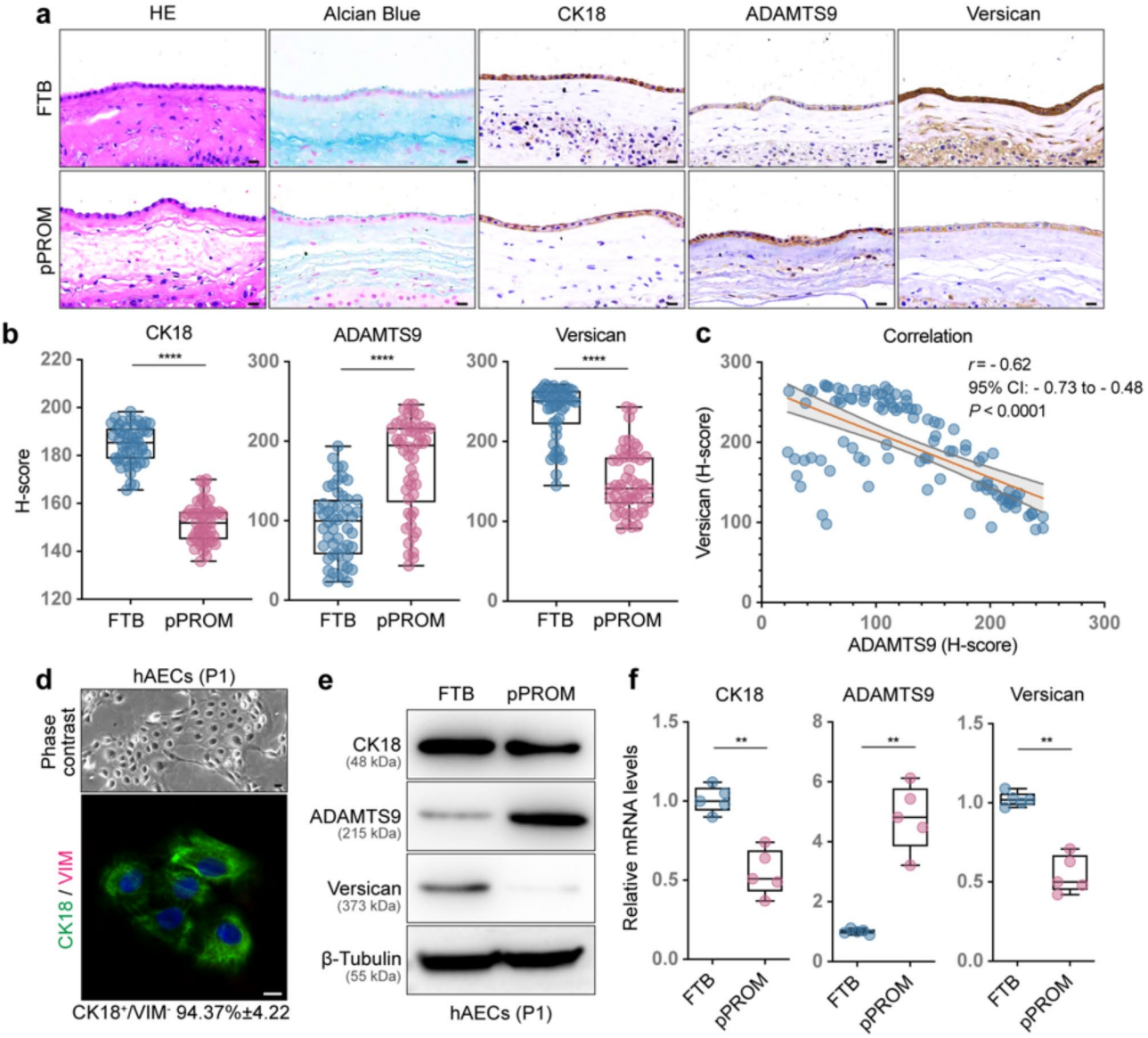

Fig. 3 The association between highly expressed ADAMTS9-induced versican reduction and fetal membrane weakening in pPROM. (a) Representative images of HE staining, Alcian staining, and immunohistochemical (IHC) staining of CK18, ADAMTS9, and versican in FTB and pPROM fetal membranes. Scale bars: 20 μm. (**b**) CK18, ADAMTS9, and versican expression were quantified using the H-score method in five random fields from FTB ($n = 50$) and pPROM ($n = 50$) fetal membranes (Mann–Whitney U test). (c) The correlation between amniotic ADAMTS9 and versican expression in FTB and pPROM was assessed using Spearman's correlation coefficient (r). The orange line indicates the slope of the linear regression. The shadowed area indicates a 95% confidence interval, and the gray dashed line indicates the reference line. (**d**) Brightfield image (top) and CK18/VIM expression (bottom) in primary hAECs. Scale bar: 10 μm. (**e**, **f**) CK18, ADAMTS9, and versican protein (**e**) and mRNA (**f**) expression in primary hAECs from FTB and pPROM (Mann–Whitney U test). In the box-and-whisker plots, the midlines indicate the medians, the boxes indicate the interquartile ranges, and the whiskers indicate the minimum and maximum values. $^{**}P < 0.01$, $^{****}P < 0.0001$

NF-κB signaling pathway decreases fetal growth restriction and stillbirth rates associated with pPROM.

Potential role of serum ADAMTS9 in predicting PTB and NF-κB/ADAMTS9 pathway in fetal membrane weakening

We investigated the potential of serum IL-1β and ADAMTS9 in predicting PTB. ELISA results demonstrated higher serum ADAMTS9 and IL-1β concentrations in IL-1β-injected mice compared to controls, with BAY treatment reducing ADAMTS9 levels while leaving IL-1β unchanged (Fig. 8a and b). A positive correlation was found between ADAMTS9 and IL-1β (Fig. 8c). ROC analysis showed that the combined model of serum IL-1β and ADAMTS9, along with individual ADAMTS9, exhibited superior predictive efficiency for PTB compared to IL-1β alone, though statistical

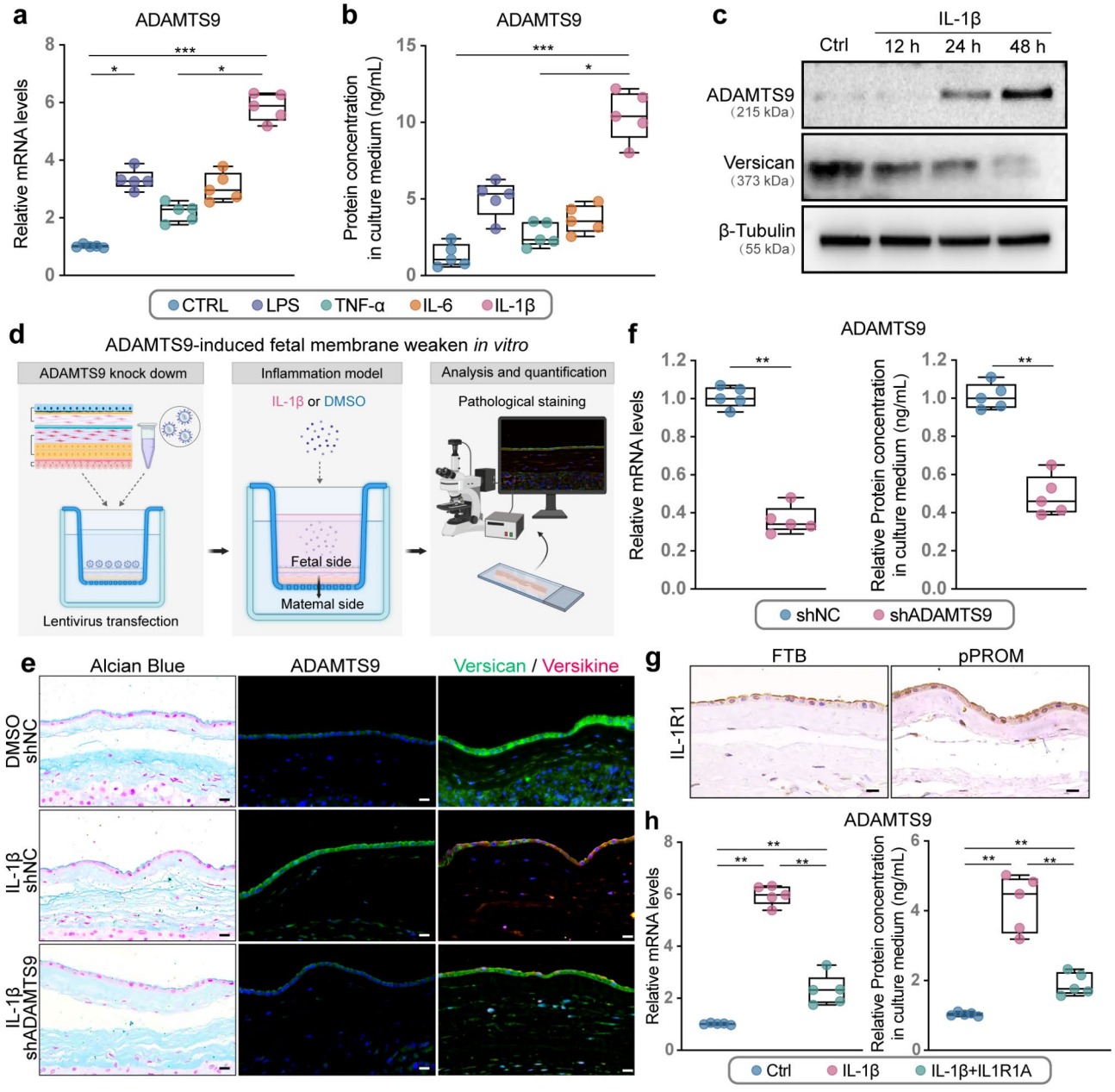

Fig. 4 IL-1β signaling promotes chorioamniotic membrane separation in fetal membrane-decidual explants by upregulating hAEC-derived ADAMTS9 expression. **(a. b)** ADAMTS9 mRNA (left) and secreted protein (right) expression in hAECs after the indicated treatment. Kruskal–Wallis and Dunn's post hoc tests were used for comparisons between groups. (**c**) IL-1β-induced cytosolic ADAMTS9 and membrane versican protein expression in hAECs. (**d**) A schematic illustration of the IL-1β-induced fetal membrane weakening explant model. (**e**) Representative images of Alcian staining and immunofluorescence (IF) staining of ADAMTS9 and versican/versiline in explants after treatment with IL-1β alone or combined with shADAMTS9. (**f**) Left: ADAMTS9 mRNA expression in hAECs isolated from the explant model (Mann–Whitney U test); Right: ADAMTS9 concentration in culture medium obtained from the explant model was tested by ELISA (Mann–Whitney U test). (**g**) Immunostaining of IL-1R1 in hAECs from the fetal membrane of FTB and pPROM. (**h**) ADAMTS9 mRNA expression in hAECs (left) and ADAMTS9 concentration in culture medium (right) after treatment with IL-1β alone or in combination with IL-1R1A (Kruskal–Wallis test with Dunn's post hoc multiple comparisons). Scale bar: 20 μm. In the box-and-whisker plots, the midlines indicate the medians, the boxes indicate the interquartile ranges, and the whiskers indicate the minimum and maximum values. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$

significance was not reached (Fig. 8d, Table S4, and Table S5). These findings support the consideration of serum ADAMTS9 as a potential predictor of PTB.

In addition, we assessed ECM deposition and NF-κB/ADAMTS9 activation in amnion tissues. Amnionic membranes from IL-1β-injected mice exhibited reduced ECM deposition and versican levels compared to PBS-injected mice, partially reversed by BAY treatment. Protein levels of p-p65, ADAMTS9, and POFUT2 were elevated in the amniotic membrane of IL-1β-injected

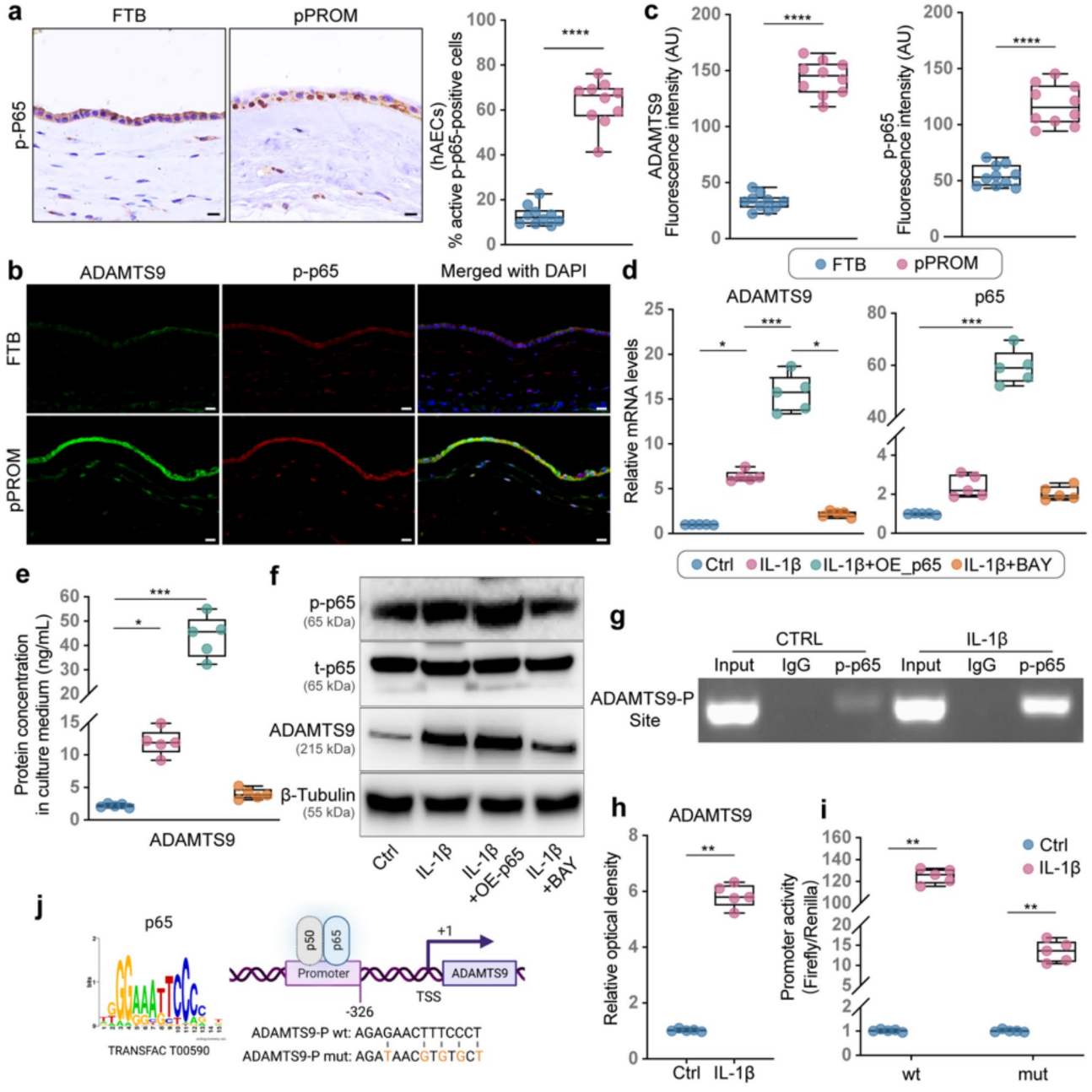

Fig. 5 hAECs-derived ADAMTS9 expression is driven by NF-κB-mediated promoter activation. **(a)** IHC analysis of p-p65 expression in hAECs from FTB ($n = 10$) and pPROM ($n = 10$) (Mann–Whitney U test). (**b**, **c**) Representative IF staining and semiquantitative analysis for ADAMTS9 (green) and p-p65 (red) in hAECs from FTB and pPROM (Mann–Whitney U test). (**d**) ADAMTS9 and p65 mRNA expression in hAECs. (**e**) Quantification of ADAMTS9 protein secreted into the cultured medium after the indicated treatments using ELISA (Kruskal–Wallis test with Dunn's post hoc multiple comparisons). (**f**) Protein expression of p-p65, total-p65 (t-p65), and ADAMTS9 in hAECs after the indicated treatments. **(g**, **h)** Chromatin immunoprecipitation (ChIP) in combination with qPCR was used to detect p-p65 directly binding within the ADAMTS9 promoter region upon IL-1β treatment (Mann–Whitney U test). (**i**) Dual luciferase reporter assay was conducted to measure the luciferase reporter activity of Luc-ADAMTS9-wild-type/mutant in hAECs treated with PBS or IL-1β (Mann–Whitney U test). (**j**) A schematic diagram of the binding site between p-p65 and the ADAMTS9 promoter. Scale bar: 20 μm. In the box-and-whisker plots, the midlines indicate the medians, the boxes indicate the interquartile ranges, and the whiskers indicate the minimum and maximum values. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$

mice, reduced by BAY treatment (Fig. 8e). The heatmap of H-scores indicated similar expression patterns among p-p65, ADAMTS9, and POFUT2, with negative correlations with versican (Fig. 8f). Immunofluorescence staining results were consistent with the findings above (Fig. S5a and S5b). Furthermore, immunofluorescence revealed that IL-1β treatment elevated the number of p-p65^{high}/ADAMTS9^{high} and p-p65^{high}/POFUT2^{high}

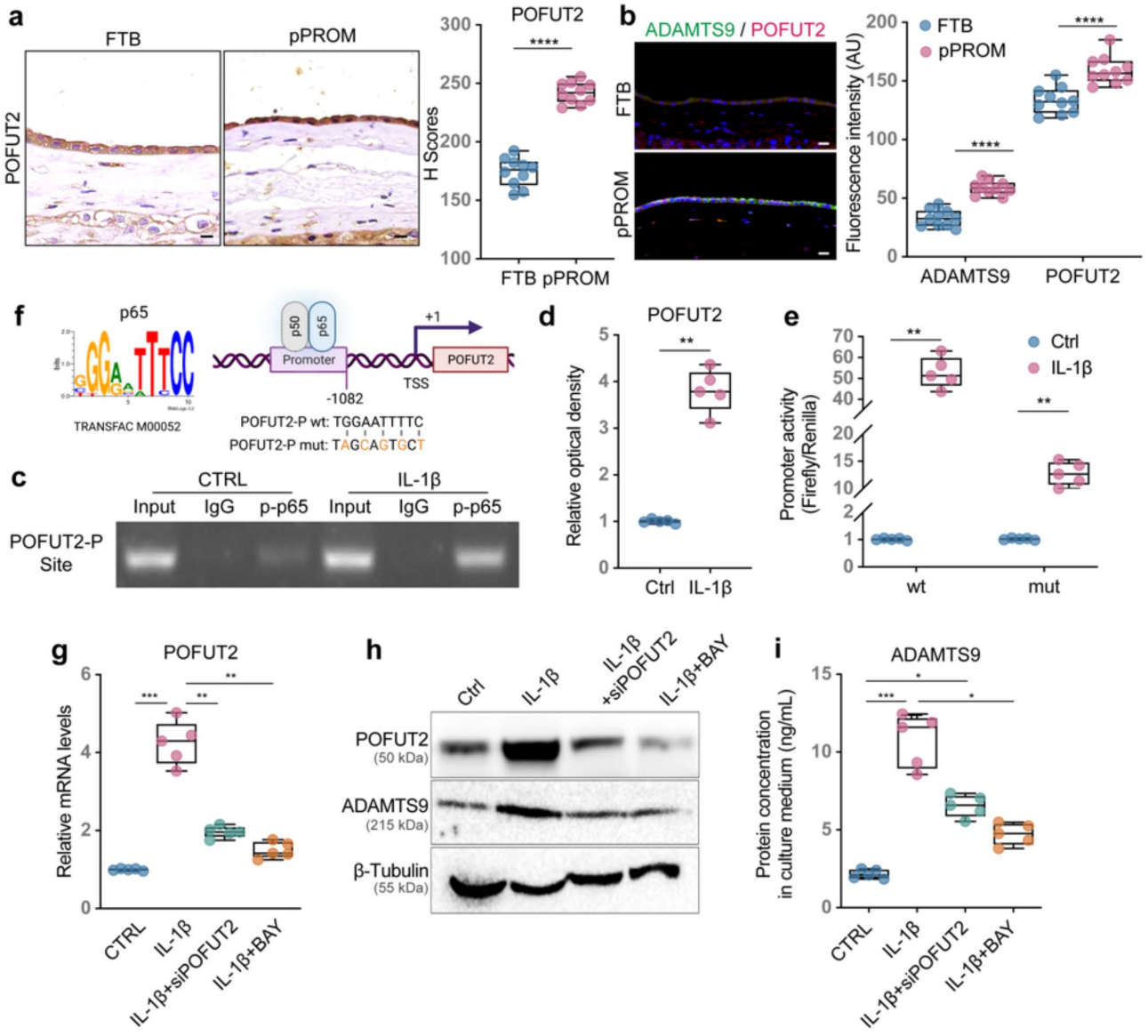

Fig. 6 ADAMTS9 secretion is enhanced by NF-κB-mediated POFUT2 promoter activation and transcriptional regulation. **(a)** IHC staining and quantification of POFUT2 in hAECs of the fetal membrane (Mann–Whitney U test). **(b)** IF staining of POFUT2 (red) and ADAMTS9 (green) in hAECs from the fetal membrane, with quantification based on fluorescence intensity (Mann–Whitney U test). (**c**, **d**) ChIP, in combination with qPCR, was used to detect p-p65 directly binding within the POFUT2 promoter region upon IL-1β treatment (Mann–Whitney U test). (**e**) A dual luciferase reporter assay was conducted to measure the luciferase reporter activity of Luc-POFUT2-wild-type/mutant in hAECs treated with PBS or IL-1β (Mann–Whitney U test). (**f**) A schematic diagram of the binding site between p-p65 and the POFUT2 promoter. (**g**) POFUT2 mRNA expression in hAECs after the indicated treatments (Kruskal–Wallis test with Dunn's post hoc multiple comparisons). (**h**) Protein expression of POFUT2 and ADAMTS9 in hAECs after the indicated treatments. (**i**) Quantification of ADAMTS9 protein secreted into the cultured medium after the indicated treatments using ELISA (Kruskal–Wallis test with Dunn's post hoc multiple comparisons). Scale bar: 20 μm. In the box-and-whisker plots, the midlines indicate the medians, the boxes indicate the interquartile ranges, and the whiskers indicate the minimum and maximum values. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

hAECs, attenuated by BAY (Fig. S5c-h). These findings suggest that blocking NF-κB/ADAMTS9 signaling may inhibit pPROM by preventing fetal membrane weakening (Fig 9).

Discussion

This study indicates that serum IL-1β and ADAMTS9 levels at 16 weeks of gestation could be non-invasive biomarkers for predicting pPROM with no signs of intra-amniotic infection. The AUC value for predicting pPROM calculated using a combination of the IL-1β and ADAMTS9 was higher than those alone, although it did not show statistical significance. The combined model

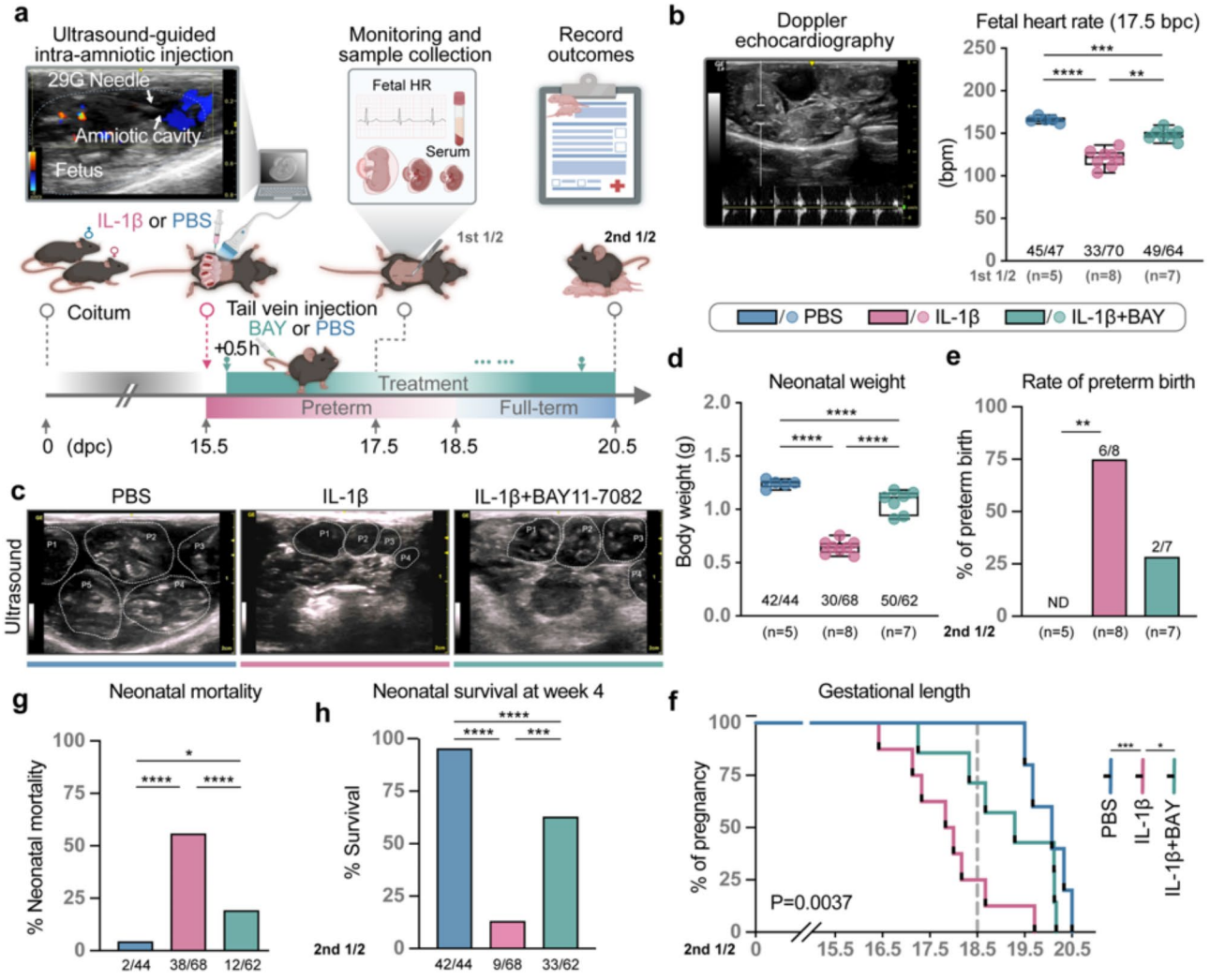

Fig. 7 Targeting the NF-κB/ADAMTS9 signaling axis ameliorates sterile intra-amniotic inflammation-induced preterm labor and adverse outcomes. **(a)** Schematic illustration of the experimental strategy. (**b**) Representative Doppler echocardiography of the fetal heart (left) and fetal heart rate (right) at 17.5 days post coitum (dpc; Kruskal–Wallis test with Dunn's post hoc multiple comparisons). (**c**) Representative ultrasound images of the fetal mouse at 17.5 dpc. **(d)** Neonatal body weight (Fisher's exact test). (**e**) Preterm birth rate (Kruskal–Wallis test with Dunn's post hoc multiple comparisons). (**f**) Gestational lengths were analyzed using the log-rank (Mantel-Cox) test. (**g**, **h**) Neonatal mortality and 4-week neonatal survival rates (Fisher's exact test). In the box-and-whisker plots, the midlines indicate the medians, the boxes indicate the interquartile ranges, and the whiskers indicate the minimum and maximum values. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

and ADAMTS9 showed higher specificity in accurately identifying the pPROM risk than IL-1β alone. Mechanistically, IL-1β significantly promoted ADAMTS9 expression and POFUT2-dependent secretion in hAECs by promoting the binding of activated NF-κB to their promoters. Ultimately, increased ADAMTS9 leads to the depletion of versican localized in the intermediate layer of the fetal membrane. This sequence of events contributes to the weakening and separation of the chorioamnion in pPROM with no signs of intra-amniotic infection. In the murine model of intra-amniotic inflammation induced by IL-1β, we have again demonstrated the above hypothesis and mechanism, highlighting the importance of blocking NF-κB/ADAMTS9 signaling, which may inhibit pPROM by preventing fetal membrane weakening.

The etiology of pPROM is multifactorial. While collagen degradation by MMPs in microbial-driven intra-amniotic infection has been intensively studied, more than 50% remain unexplained [46, 47]. Growing evidence suggests that pPROM may also be associated with sterile fetal membrane or intra-amniotic inflammation, though the initiating factors and pathophysiologic pathways remain unclear. Our study illuminates the significant elevation of serum IL-1β and ADAMTS9 at 16 weeks of gestation in women with pPROM compared to those with FTB, which is partially consistent with previous studies showing increased IL-1β levels of serum, amniotic fluids,

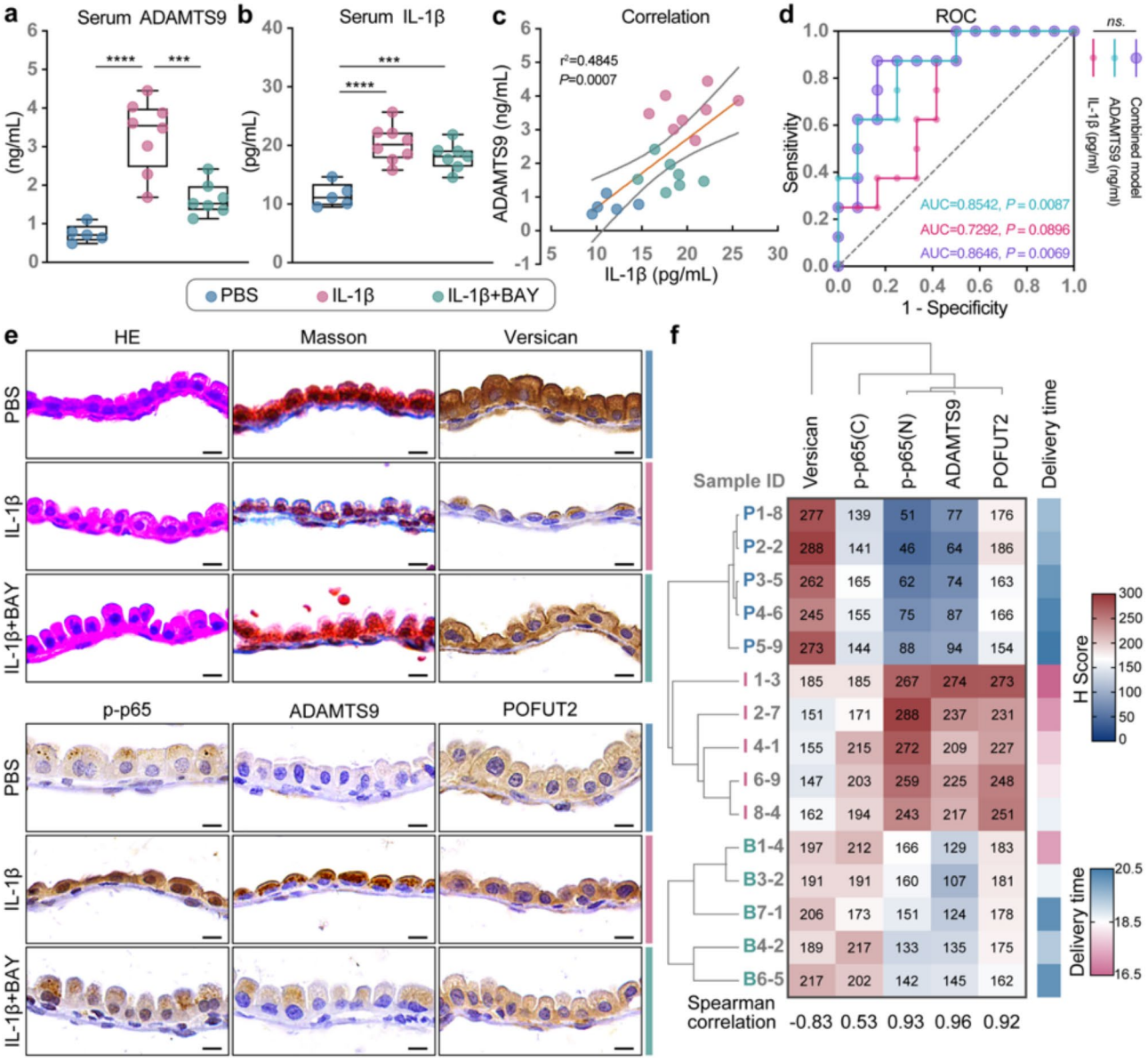

Fig. 8 The prediction performance of individual and composite biomarkers in a murine PTB model. (**a**, **b**) Concentrations of ADAMTS9 and IL-1β in serum (Kruskal–Wallis test with Dunn's post hoc multiple comparisons). (**c**) Pearson correlation analysis for serum ADAMTS9 and IL-1β concentrations. (**d**) ROC analysis of ADAMTS9 and IL-1β alone or in combination to predict PTB in pregnant mice, with a pairwise comparison of AUROC, used the Delong method. (**e**) Representative images of HE, Masson, and IHC staining of versican, p-p65, ADAMTS9, and POFUT2 in mouse amniotic membrane tissues. Scale bar: 20 μm. (**f**) A heatmap depicting the H-score for versican, p-p65, ADAMTS9, and POFUT2 in mouse amniotic membrane tissues. In the box-and-whisker plots, the midlines indicate the medians, the boxes indicate the interquartile ranges, and the whiskers indicate the minimum and maximum values. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$

and vaginal fluids in preterm delivery [48–50]. Compared to prior studies that primarily captured changes closer to the onset of labor or post-infection. Our findings extend the understanding of IL-1β and ADAMTS9 as mid-pregnancy biomarkers for pPROM, suggesting biochemical changes that can be detected before the onset of potential sterile inflammation-related clinical signs. Furthermore, ADAMTS9 displayed higher specificity than IL-1β, indicating higher accuracy in correctly distinguishing cases less likely to develop pPROM. Although serum IL-1β is promising in pPROM prediction, its optimal clinical utility may be achieved by combining it with other biomarkers and integrating it into comprehensive clinical assessments. In this study, the combined markers were identified as the prediction model with the best AUC value, and its likelihood ratio far exceeded that of the individual markers. This suggested that the chance of detecting pPROM increased significantly when both

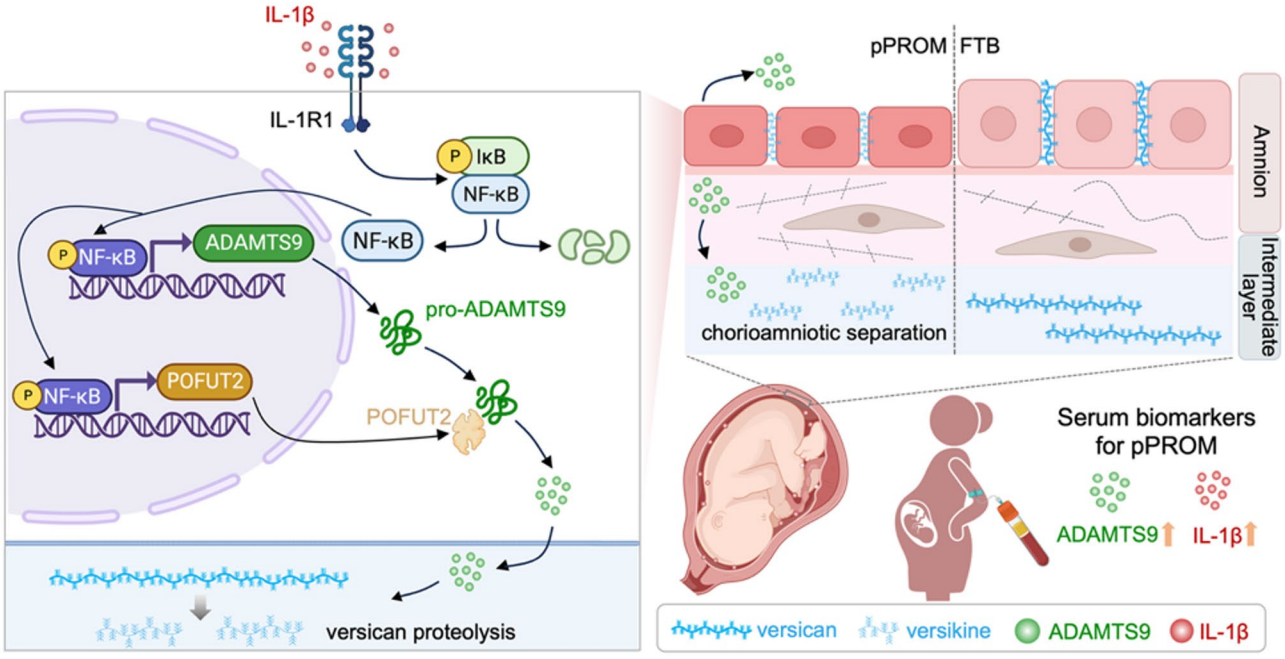

Fig. 9 A graphical summary of potential predictive markers for pPROM and how IL-1β drives the occurrence and progression of pPROM. ADAMTS9 and IL-1β, particularly ADAMTS9, have potential clinical value as early predictive markers of pPROM. hAECs-derived ADAMTS9 plays a role in the cleavage of versican, leading to the separation, weakening, and eventual rupture of fetal membranes in pPROM. Mechanistically, aseptic inflammation-induced IL-1β/NF-κB pathway activation significantly contributes to upregulating POFUT2/ADAMTS9 expression in hAECs. This figure was created with BioRender.com

biomarkers showed positive results. Overall, our findings propose a novel non-invasive approach for screening pPROM risk in pregnant women as early as 16 weeks of gestation.

Our findings underscore the critical role of ADAMTS9 in coordination with IL-1β and NF-κB signaling in fetal membrane weakening. Pregnancy maintenance depends on a delicate balance of inflammatory and anti-inflammatory effects. Disruption of this balance by excessive pro-inflammatory factors can lead to fetal membrane ECM degradation and pPROM, even without intra-amniotic infection [10]. The elasticity, integrity, and strength of fetal membranes rely heavily on ECM proteins in the mesenchymal layer, which are crucial in preventing pPROM [51]. In a healthy state, the proteoglycan-rich intermediate layer of the amnion allows for smooth movement over the chorion, absorbing physical stress. However, detachment of the amnion from the chorion compromises this stress-absorbing mechanism, increasing the pPROM risk [52]. An important proteoglycan component, Versican is implicated in various pathophysiological processes [53]. Furthermore, ADAMTS9, widely expressed in fetal tissues, contributes to tissue remodeling through versican proteolysis in the cell surface and the ECM [30, 54, 55]. During pregnancy, ADAMTS9 modulates the ECM of uterine smooth muscle cells, consequently affecting the timing of labor [56]. It can also regulate the ECM of the umbilical cord, thereby influencing the differentiation of umbilical vascular smooth muscle cells [57]. However, its involvement in pPROM remains unclear. Our results demonstrated that compared with FTB, hAECs-derived ADAMTS9 expression in pPROM was significantly elevated, whereas versican levels were reduced. These findings suggest that excess ADAMTS9 in hAECs may drive CAS by versican degradation of the intermediate layer and ultimately lead to pPROM. Our previous study has highlighted that lipopolysaccharide (LPS)-induced ADAMTS9 expression contributes to preterm birth [58], underscoring its role as a structural enzyme and a responsive element in inflammatory conditions. The dual roles of ADAMTS9 in ECM remodeling and inflammatory response highlight its potential as a therapeutic target for high-risk pregnancies associated with fetal membrane weakening.

NF-κB activation accelerates the expression of genes such as matrix metalloproteins (MMPs), chemokines, and prostaglandin E2, which are critical in labor initiation [59–62]. The ability of the fetal membrane to maintain an adjustable biological barrier and a semirigid physical barrier is mainly attributed to the amniotic ECM [42]. Extensive degradation of fetal membrane ECM induced by MMPs with IL-1β activation has been proposed as a mechanism for fetal membrane weakening [10, 63]. However, the mechanisms remain unclear. The present study found that IL-1β enhanced p65 phosphorylation and upregulated ADAMTS9 transcription by directly binding

to its promoter. The proteolytic activity of ADAMTS proteases in vivo is mainly regulated at the transcriptional and post-translational levels. Protein O-fucosyltransferase 2 (POFUT2) is localized in the endoplasmic reticulum and can promote the secretion of POFUT2/B3GLCT target proteins by stabilizing and accelerating the rate of TSR folding in the endoplasmic reticulum [64]. We demonstrated that POFUT2 expression is essential for ADAMTS9 secretion. These findings reveal that IL-1β/NF-κB signaling regulates ADAMTS9 through transcriptional and posttranslational modification.

To better simulate sterile intra-amniotic inflammation, we established a murine PTB model induced by ultrasound-guided intra-amniotic administration of IL-1β at 15.5 days of gestation. Consistent with previous findings in vitro, we also reported that maternal serum ADAMTS9 and IL-1β concentrations correlated well at 17.5 days. In addition, their single and combined models have good diagnostic performance in predicting preterm labor characterized by fetal membrane weakening. The NF-κB inhibitor significantly reduced fetal complications and IL-1β-induced expression of p-p65, ADAMTS9, and POFUT2 in hAECs and versican loss.

The strength of our study is that we combined clinical validation of the biomarkers with their mechanistic exploration in vitro and in vivo models, which provide a comprehensive view of IL-1β and ADAMTS9 contributing to pPROM without signs of intra-amniotic infection and the potential as predictive biomarkers. We employed a case-control design nested within a prospective cohort of healthy pregnant women, effectively reduced the recall bias, and quantified the predictive ability of our combined marker model of serum IL-1β and ADAMTS9 at 16 weeks of gestation for pPROM by ROC curve analysis, which may enable some high-risk pregnant women to benefit from early intervention. Additionally, the use of primary hAECs and a murine model of intra-amniotic inflammation improved the reproducibility and reliability of our findings on regulatory mechanisms of ADAMTS9. However, this study also has some limitations. Our study evaluated biomarker levels only at a single time point. However, the development of pPROM involves a complex process of dynamic imbalance in the inflammatory-immunomodulatory network and biomarker levels, whose levels may show nonlinear fluctuations with the pregnancy progression. It is difficult to capture the dynamic pattern of change in a single test, and its predictive value may be underestimated or overestimated. Second, inter-individual differences in baseline inflammatory status are indistinguishable from pregnancy-specific changes, potentially masking critical time-dependent risk evolution trajectories. In the future, longitudinal monitoring of biomarker levels throughout pregnancy may contribute to our understanding of the dynamic changes in IL-1β and ADAMTS9 and their relationship to pPROM progression. Despite including a reasonable number of participants, considering the complexity and potentially unique etiologies of pPROM, a larger sample size and the inclusion of comparisons with preterm labor and premature rupture of membranes are required to validate the reliability of our findings further. Although our study employed clinical assessments and postnatal histopathological examinations to exclude the possibility of microbial-induced intra-amniotic infection and chorioamnionitis rigorously, the absence of direct microbial evidence in amniotic fluid and fetal membranes somewhat limits the interpretability of our findings. At the same time, whether our predictors also apply to pregnant women with pPROM with histological chorioamniotic inflammation (regardless of bacteria/sterility in the amniotic cavity) remains to be further verified. In addition, a more thorough assessment of clinical applicability and intervention side effects is required.

Conclusion

In conclusion, this study emphasizes serum IL-1β and ADAMTS9 as potential non-invasive biomarkers for the clinical prediction of pPROM with no signs of intra-amniotic infection, highlighting their potential in early prediction and intervention for the high-risk groups of fetal membrane injury and subsequent pPROM. IL-1β promotes ADAMTS9 expression and secretion via NF-κB activation in hAECs and contributes to chorioamniotic membrane separation through versican depletion; this elucidates new mechanistic insights into the loss of structural integrity to fetal membranes. In vivo, studies based on mice models reaffirmed our above results and underscored the importance of targeting activated NF-κB as a potential therapeutic strategy for pPROM. Future research should focus on the long-term effects of these interventions on fetal health and development, emphasizing optimizing therapeutic approaches to enhance efficacy and safety across diverse populations.

Abbreviations

pPROM	preterm Premature Rupture of Membrane
FTB	Full Term Birth
sPTB	Spontaneous Preterm Birth
PTB	Preterm Birth
FGR	Fetal Growth Restriction
hAECs	Human Amniotic Epithelial Cells
hAMCs	Human Amniotic Mesenchymal Cells
hCMCs	Human Chorionic Mesenchymal Cells
hCTCs	Human Chorionic Trophoblasts Cells
hDECs	Human Decidual Cells
CK18	Cytokeratin 18
LPS	Lipopolysaccharide
IL-1R1	type I IL-1β Receptor
IL-1β	Interleukin-1β
TNF-α	Tumor Necrosis Factor-Alpha
IL-6	Interleukin-6
DAMP	Damage-Associated Molecular Pattern
NF-κB	Nuclear Factor kappa-B

p38MAPK	p38-Mitogen-Activated Protein Kinase
ECM	Extracellular Matrix
MMPs	Matrix Metalloproteins
CSPG	Chondroitin Sulfate Proteoglycan
VCAN	Versican
ADAMTS9	A Disintegrin and Metalloproteinases with Thrombospondin Motifs 9
POFUT2	Protein O-Fucosyltransferase 2
qRT-PCR	Real-Time quantitative Reverse Transcription-Polymerase Chain Reaction
DAB	Diaminobenzidine
IHC	Immunohistochemistry
IF	Immunofluorescence
HE	Hematoxylin–Eosin
ChIP	Chromatin Immunoprecipitation
ELISA	Enzyme Linked Immunosorbent Assay

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12964-025-02120-3.

Supplementary Material 1

Acknowledgements

We thank Professor Meifeng Zhu (Key Laboratory of Bioactive Materials, Ministry of Education, College of Life Sciences, State Key Laboratory of Medicinal Chemical Biology, Nankai University, Tianjin 300071, China) for their valuable discussion. We also thank all the nurses, especially Bingying Yu, Jing Yu, and Yunfei Bu, in the Department of Obstetrics of Tianjin Central Hospital of Obstetrics and Gynecology, for their assistance in sample collection. We also appreciate the administrative support provided by Xu Chen, Lan Cheng, and Hongyan Cui.

Author contributions

JS.C., YX.W., QM.L., SQ.W., YM.S., CL.L., and SH.Y. performed experiments and participated in data analysis and interpretation. JS.C., YX.W., and QM.L. wrote the manuscript. L.Z., L.C., and W.L. provided support in ultrasound and pathology image analysis. Y.C., ZJ.L., JS.C., X.C., and L.S. supervised the project design and execution. All authors reviewed the manuscript.

Funding

This work was supported by the Natural Science Foundation of Tianjin, China (21JCYBJC00100 to JSC and 23JCQNJC00950 to QML), and Tianjin Health Research Project (TJWJ2022MS044 and 2023007 to JSC). The funders had no role in study design, data collection and analysis, publication decisions, or manuscript preparation.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethical approval

Upon the declaration of Helsinki, the clinical sample was approved by the Obstetrics and Gynecology Ethics Committee of Tianjin Central Hospital (Approval No. 2021KY113). The confidentiality of the patients was strictly protected, and no personal data were required for this study. All participants provided written informed consent. All animal procedures, including euthanasia, were carried out following ARRIVE guidelines and approved by the Institutional Review Board of Nankai University.

Consent for publication

All authors have agreed to publish this manuscript.

Competing interests

The authors declare no competing interests.

Author details

[1]Tianjin Key Laboratory of Human Development and Reproductive Regulation, Tianjin Central Hospital of Gynecology Obstetrics, Tianjin 300100, China
[2]Nankai University Affiliated Hospital of Obstetrics and Gynecology, Tianjin 300100, China
[3]Tianjin Institute of Gynecology Obstetrics, Tianjin Central Hospital of Gynecology Obstetrics, Tianjin 300100, China
[4]School of Medicine, Nankai University, Tianjin 300071, China
[5]Tianjin Academy of Traditional Chinese Medicine Affiliated Hospital, Tianjin 300120, China
[6]State Key Laboratory of Medicinal Chemical Biology, College of Pharmacy, Nankai University, Tianjin 300350, China
[7]Key Laboratory of Bioactive Materials, Ministry of Education, Nankai University, Tianjin 300071, China
[8]Medical School, Tianjin University, Tianjin 300072, China

Received: 12 December 2024 / Accepted: 22 February 2025
Published online: 08 March 2025

References

1. Green ES, Arck PC. Pathogenesis of preterm birth: bidirectional inflammation in mother and fetus. Semin Immunopathol. 2020;42(4):413–29.
2. Liu Y, Gao L. Preterm labor, a syndrome attributed to the combination of external and internal factors. Maternal-Fetal Med. 2022;4(1):61–71.
3. Ballabh P, de Vries LS. White matter injury in infants with intraventricular haemorrhage: mechanisms and therapies. Nat Rev Neurol. 2021;17(4):199–214.
4. Suff N, Story L, Shennan A. The prediction of preterm delivery: what is new? Semin Fetal Neonatal Med. 2019;24(1):27–32.
5. van der Ham DP, Vijgen SM, Nijhuis JG, van Beek JJ, Opmeer BC, Mulder AL, et al. Induction of labor versus expectant management in women with preterm prelabor rupture of membranes between 34 and 37 weeks: a randomized controlled trial. PLoS Med. 2012;9(4):e1001208.
6. Strohl A, Kumar D, Novince R, Shaniuk P, Smith J, Bryant K, et al. Decreased adherence and spontaneous separation of fetal membrane layers–amnion and choriodecidua–a possible part of the normal weakening process. Placenta. 2010;31(1):18–24.
7. Arikat S, Novince RW, Mercer BM, Kumar D, Fox JM, Mansour JM, et al. Separation of Amnion from choriodecidua is an integral event to the rupture of normal term fetal membranes and constitutes a significant component of the work required. Am J Obstet Gynecol. 2006;194(1):211–7.
8. Amberg BJ, Hodges RJ, Rodgers KA, Crossley KJ, Hooper SB, DeKoninck PL. Why do the fetal membranes rupture early after fetoscopy? A review. Fetal Diagn Ther. 2021;48(7):493–503.
9. Govaerts J, Cryns P, Jacquemyn Y. Spontaneous chorioamniotic membrane separation discovered by preterm prelabor rupture of membranes. Clin Case Rep. 2019;7(4):762–5.
10. Menon R, Richardson LS. Preterm prelabor rupture of the membranes: A disease of the fetal membranes. Semin Perinatol. 2017;41(7):409–19.
11. Motomura K, Romero R, Garcia-Flores V, Leng Y, Xu Y, Galaz J, et al. The alarmin interleukin-1α causes preterm birth through the NLRP3 inflammasome. Mol Hum Reprod. 2020;26(9):712–26.
12. Severino ME, Richardson LS, Kacerovsky M, Menon R. Histologic evidence of Epithelial-Mesenchymal transition and autophagy in human fetal membranes. Am J Pathol. 2024;194(5):684–92.
13. Padron JG, Saito Reis CA, Kendal-Wright CE. The role of danger associated molecular patterns in human fetal membrane weakening. Front Physiol. 2020;11:602.
14. Wight TN, Kang I, Evanko SP, Harten IA, Chang MY, Pearce OMT, et al. Versican-A critical extracellular matrix regulator of immunity and inflammation. Front Immunol. 2020;11:512.
15. Li MD, Lu JW, Zhang F, Lei WJ, Pan F, Lin YK, et al. ADAMTS4 is a crucial proteolytic enzyme for versican cleavage in the Amnion at parturition. Commun Biol. 2024;7(1):301.
16. Lee J, Romero R, Kim SM, Chaemsaithong P, Park CW, Park JS, et al. A new antimicrobial combination prolongs the latency period, reduces acute histologic chorioamnionitis as well as funisitis, and improves neonatal outcomes in preterm PROM. J Matern Fetal Neonatal Med. 2016;29(5):707–20.

17. Kacerovsky M, Romero R, Stepan M, Stranik J, Maly J, Pliskova L, et al. Antibiotic administration reduces the rate of intraamniotic inflammation in preterm prelabor rupture of the membranes. Am J Obstet Gynecol. 2020;223(1):114.e1- e20.
18. Van den Broek NR, White SA, Goodall M, Ntonya C, Kayira E, Kafulafula G, et al. The APPLe study: a randomized, community-based, placebo-controlled trial of Azithromycin for the prevention of preterm birth, with meta-analysis. PLoS Med. 2009;6(12):e1000191.
19. Andrews WW, Goldenberg RL, Hauth JC, Cliver SP, Copper R, Conner M. Interconceptional antibiotics to prevent spontaneous preterm birth: a randomized clinical trial. Am J Obstet Gynecol. 2006;194(3):617–23.
20. Behnia F, Sheller S, Menon R. Mechanistic differences leading to infectious and sterile inflammation. Am J Reprod Immunol. 2016;75(5):505–18.
21. Cholтus H, Lavergne M, De Sousa Do Outeiro C, Coste K, Belville C, Blanchon L, et al. Pathophysiological implication of pattern recognition receptors in fetal membranes rupture: RAGE and NLRP inflammasome. Biomedicines. 2021;9(9):1123.
22. Menon R, Taylor BD. Exploring inflammatory mediators in fetal and maternal compartments during human parturition. Obstet Gynecol. 2019;134(4):765.
23. Bredeson S, Papaconstantinou J, Deford JH, Kechichian T, Syed TA, Saade GR, et al. HMGB1 promotes a p38MAPK associated non-infectious inflammatory response pathway in human fetal membranes. PLoS ONE. 2014;9(12):e113799.
24. Lappas M, Permezel M, Rice GE. N-acetyl-cysteine inhibits phospholipid metabolism, Proinflammatory cytokine release, protease activity, and nuclear Factor-κB deoxyribonucleic acid-binding activity in human fetal membranes in vitro. J Clin Endocrinol Metabolism. 2003;88(4):1723–9.
25. Robertson SA, Hutchinson MR, Rice KC, Chin PY, Moldenhauer LM, Stark MJ, et al. Targeting Toll-like receptor-4 to tackle preterm birth and fetal inflammatory injury. Clin Translational Immunol. 2020;9(4):e1121.
26. Shynlova O, Nedd-Roderique T, Li Y, Dorogin A, Nguyen T, Lye SJ. Infiltration of myeloid cells into decidua is a critical early event in the labour cascade and post-partum uterine remodelling. J Cell Mol Med. 2013;17(2):311–24.
27. Kumar D, Fung W, Moore RM, Pandey V, Fox J, Stetzer B, et al. Proinflammatory cytokines found in amniotic fluid induce collagen remodeling, apoptosis, and biophysical weakening of cultured human fetal membranes. Biol Reprod. 2006;74(1):29–34.
28. Sadowsky DW, Adams KM, Gravett MG, Witkin SS, Novy MJ. Preterm labor is induced by intraamniotic infusions of interleukin-1β and tumor necrosis factor-α but not by interleukin-6 or interleukin-8 in a nonhuman primate model. Am J Obstet Gynecol. 2006;195(6):1578–89.
29. Romero R, Tartakovsky B. The natural interleukin-1 receptor antagonist prevents interleukin-l-induced preterm delivery in mice. Am J Obstet Gynecol. 1992;167(4):1041–5.
30. Somerville RP, Longpre J-M, Jungers KA, Engle JM, Ross M, Evanko S, et al. Characterization of ADAMTS-9 and ADAMTS-20 as a distinct ADAMTS subfamily related to Caenorhabditis elegans GON-1. J Biol Chem. 2003;278(11):9503–13.
31. Wang LW, Nandadasa S, Annis DS, Dubail J, Mosher DF, Willard BB, et al. A disintegrin-like and metalloproteinase domain with thrombospondin type 1 motif 9 (ADAMTS9) regulates fibronectin fibrillogenesis and turnover. J Biol Chem. 2019;294(25):9924–36.
32. Uysal S, Ünal ZN, Erdoğan S, Akyol S, Ramazan Yiğitoğlu M, Hirohata S, et al. Augmentation of ADAMTS9 gene expression by IL-1β is reversed by NFκB and MAPK inhibitors, but not PI3 kinase inhibitors. Cell Biochem Funct. 2013;31(7):539–44.
33. Demircan K, Hirohata S, Nishida K, Hatipoglu OF, Oohashi T, Yonezawa T, et al. ADAMTS-9 is synergistically induced by interleukin-1β and tumor necrosis factor α in OUMS-27 chondrosarcoma cells and in human chondrocytes. Arthr Rhuem. 2005;52(5):1451–60.
34. Prelabor Rupture of Membranes. ACOG practice bulletin, number 217. Obstet Gynecol. 2020;135(3):e80–97.
35. Association CM. Guidelines for diagnosis and management of premature rupture of membranes (2015). Chin J Obstet Gynecol. 2015;18(3):161–7.
36. Jung E, Romero R, Suksai M, Gotsch F, Chaemsaithong P, Erez O, et al. Clinical chorioamnionitis at term: definition, pathogenesis, microbiology, diagnosis, and treatment. Am J Obstet Gynecol. 2024;230(3S):S807–40.
37. Romero R, Pacora P, Kusanovic JP, Jung E, Panaitescu B, Maymon E, et al. Clinical chorioamnionitis at term X: microbiology, clinical signs, placental pathology, and neonatal bacteremia - implications for clinical care. J Perinat Med. 2021;49(3):275–98.
38. Faro J, Romero R, Schwenkel G, Garcia-Flores V, Arenas-Hernandez M, Leng Y, et al. Intra-amniotic inflammation induces preterm birth by activating the NLRP3 inflammasome. Biol Reprod. 2019;100(5):1290–305.
39. Kallapur SG, Presicce P, Senthamaraikannan P, Alvarez M, Tarantal AF, Miller LM, et al. Intra-amniotic IL-1beta induces fetal inflammation in rhesus monkeys and alters the regulatory T cell/IL-17 balance. J Immunol. 2013;191(3):1102–9.
40. Nadeau-Vallee M, Quiniou C, Palacios J, Hou X, Erfani A, Madaan A, et al. Novel noncompetitive IL-1 Receptor-Biased ligand prevents Infection- and Inflammation-Induced preterm birth. J Immunol. 2015;195(7):3402–15.
41. Gomez-Lopez N, Romero R, Galaz J, Xu Y, Panaitescu B, Slutsky R, et al. Cellular immune responses in amniotic fluid of women with preterm labor and intra-amniotic infection or intra-amniotic inflammation. Am J Reprod Immunol. 2019;82(5):e13171.
42. Oyen ML, Calvin SE, Landers DV. Premature rupture of the fetal membranes: is the Amnion the major determinant? Am J Obstet Gynecol. 2006;195(2):510–5.
43. Li Q, Verma IM. NF-kappaB regulation in the immune system. Nat Rev Immunol. 2002;2(10):725–34.
44. Holdener BC, Percival CJ, Grady RC, Cameron DC, Berardinelli SJ, Zhang A, et al. ADAMTS9 and ADAMTS20 are differentially affected by loss of B3GLCT in mouse model of Peters plus syndrome. Hum Mol Genet. 2019;28(24):4053–66.
45. Holdener BC, Haltiwanger RS. Protein O-fucosylation: structure and function. Curr Opin Struct Biol. 2019;56:78–86.
46. DiGiulio DB, Romero R, Kusanovic JP, Gomez R, Kim CJ, Seok KS, et al. Prevalence and diversity of microbes in the amniotic fluid, the fetal inflammatory response, and pregnancy outcome in women with preterm pre-labor rupture of membranes. Am J Reprod Immunol. 2010;64(1):38–57.
47. Vadillo-Ortega F, Estrada-Gutierrez G. Role of matrix metalloproteinases in preterm labour. BJOG. 2005;112(Suppl 1):19–22.
48. Bhattacharjee R, Li T, Koshy S, Beard LL, Sharma K, Carter EP, et al. Stimulation of MAP kinase pathways after maternal IL-1beta exposure induces fetal lung fluid absorption in guinea pigs. Respir Res. 2007;8(1):27.
49. Shaffer Z, Romero R, Tarca AL, Galaz J, Arenas-Hernandez M, Gudicha DW et al. The vaginal immunoproteome for the prediction of spontaneous preterm birth: A retrospective longitudinal study. Elife. 2024;13.
50. Vitoratos N, Mastorakos G, Kountouris A, Papadias K, Creatsas G. Positive association of serum interleukin-1beta and CRH levels in women with pre-term labor. J Endocrinol Invest. 2007;30(1):35–40.
51. Strauss JF 3. Extracellular matrix dynamics and fetal membrane rupture. Reprod Sci. 2013;20(2):140–53.
52. Corroenne R, Yepez M, Barth J, Pan E, Whitehead WE, Espinoza J, et al. Chorioamniotic membrane separation following fetal myelomeningocele repair: incidence, risk factors and impact on perinatal outcome. Ultrasound Obstet Gynecol. 2020;56(5):684–93.
53. Nandadasa S, Foulcer S, Apte SS. The multiple, complex roles of versican and its proteolytic turnover by ADAMTS proteases during embryogenesis. Matrix Biol. 2014;35:34–41.
54. Clark ME, Kelner GS, Turbeville LA, Boyer A, Arden KC, Maki RA. ADAMTS9, a novel member of the ADAM-TS/ Metallospondin gene family. Genomics. 2000;67(3):343–50.
55. Nandadasa S, Kraft CM, Wang LW, O'Donnell A, Patel R, Gee HY, et al. Secreted metalloproteases ADAMTS9 and ADAMTS20 have a non-canonical role in ciliary vesicle growth during ciliogenesis. Nat Commun. 2019;10(1):953.
56. Mead TJ, Du Y, Nelson CM, Gueye N-A, Drazba J, Dancevic CM, et al. ADAMTS9-regulated pericellular matrix dynamics governs focal adhesion-dependent smooth muscle differentiation. Cell Rep. 2018;23(2):485–98.
57. Nandadasa S, Nelson CM, Apte SS. ADAMTS9-mediated extracellular matrix dynamics regulates umbilical cord vascular smooth muscle differentiation and rotation. Cell Rep. 2015;11(10):1519–28.
58. Li W, Zhao X, Li S, Chen X, Cui H, Chang Y, et al. Upregulation of TNF-alpha and IL-6 induces preterm premature rupture of membranes by activation of ADAMTS-9 in embryonic membrane cells. Life Sci. 2020;260:118237.
59. Hernandez-Guerrero C, Monzon-Bordonaba F, Jimenez-Zamudio L, Ahued-Ahued R, Arechavaleta-Velasco F, Strauss JF 3, et al. In-vitro secretion of Pro-inflammatory cytokines by human amniochorion carrying hyper-responsive gene polymorphisms of tumour necrosis factor-alpha and interleukin-1beta. Mol Hum Reprod. 2003;9(10):625–9.
60. Lappas M, Rice GE. The role and regulation of the nuclear factor kappa B signalling pathway in human labour. Placenta. 2007;28(5–6):543–56.

61. Ferrand PE, Parry S, Sammel M, Macones GA, Kuivaniemi H, Romero R, et al. A polymorphism in the matrix metalloproteinase-9 promoter is associated with increased risk of preterm premature rupture of membranes in African Americans. Mol Hum Reprod. 2002;8(5):494–501.
62. Fortunato SJ, Menon R. IL-1 beta is a better inducer of apoptosis in human fetal membranes than IL-6. Placenta. 2003;24(10):922–8.
63. Zhang Y, He F, Chen Z, Su Q, Yan M, Zhang Q, et al. Melatonin modulates IL-1β-induced extracellular matrix remodeling in human nucleus pulposus cells and attenuates rat intervertebral disc degeneration and inflammation. Aging. 2019;11(22):10499–512.
64. Takeuchi H, Yu H, Hao H, Takeuchi M, Ito A, Li H, et al. O-Glycosylation modulates the stability of epidermal growth factor-like repeats and thereby regulates Notch trafficking. J Biol Chem. 2017;292(38):15964–73.

Publisher's note