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# Predicting the Risk of Preterm Birth Throughout Pregnancy Based on a Novel Transcriptomic Signature

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## Abstract

**Objective:** This study focused on the prediction of preterm birth (PTB). It aimed to identify the transcriptomic signature essential for the occurrence of PTB and evaluate its predictive value in early, mid, and late pregnancy and in women with threatened preterm labor (TPTL).

**Methods:** Blood transcriptome data of pregnant women were obtained from the Gene Expression Omnibus database. The activity of biological signatures was assessed using gene set enrichment analysis and single-sample gene set enrichment analysis. The correlation among molecules in the interleukin 6 (IL6) signature and between IL6 signaling activity and the gestational week of delivery and latent period were evaluated by Pearson correlation analysis. The effects of molecules associated with the IL6 signature were fitted using logistic regression analysis; the predictive value of both the IL6 signature and IL6 alone were evaluated using receiver operating characteristic curves and pregnancy maintenance probability was assessed using Kaplan-Meier analysis. Differential analysis was performed using the DEseq2 and limma algorithms.

**Results:** Circulatory IL6 signaling activity increased significantly in cases with preterm labor than in those with term pregnancies (normalized enrichment score (NES) = 1.857, $P = 0.001$). The IL6 signature (on which IL6 signaling is based) was subsequently considered as the candidate biomarker for PTB. The area under the curve (AUC) values for PTB prediction (using the IL6 signature) in early, mid, and late pregnancy were 0.810, 0.695, and 0.779, respectively; these values were considerably higher than those for IL6 alone. In addition, the pregnancy curves of women with abnormal IL6 signature differed significantly from those with normal signature. In pregnant women who eventually had preterm deliveries, circulatory IL6 signaling activity was lower in early pregnancy (NES = –1.420, $P = 0.031$) and higher than normal in mid (NES = 1.671, $P = 0.002$) and late pregnancy (NES = 2.350, $P < 0.001$). In women with TPTL, the AUC values for PTB prediction (or PTB within 7 days and 48 hours) using the IL6 signature were 0.761, 0.829, and 0.836, respectively; the up-regulation of IL6 signaling activity and its correlation with the gestational week of delivery ($r = –0.260$, $P = 0.001$) and latency period ($r = –0.203$, $P = 0.012$) were more significant than in other women.

**Conclusion:** Our findings suggest that the IL6 signature may predict PTB, even in early pregnancy (although the predictive power is relatively weak in mid pregnancy) and is particularly effective in symptomatic women. These findings may contribute to the development of an effective predictive and monitoring system for PTB, thereby reducing maternal and fetal risk.

**Keywords:** Premature birth; IL6 signature; Transcriptome; Clinical prediction

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## Introduction

The World Health Organization defines preterm birth (PTB) as delivery before 37 completed gestational weeks. It is a serious global health problem and a major perinatal challenge.[1] As the leading cause of neonatal death, it is responsible for approximately one-third of all cases. PTB is also a critical independent risk factor for under-five mortality and is strongly associated with increased hospitalization and mortality rates even in adulthood.[2,3] It occurs in approximately 10% of pregnancies worldwide (ie, 15 million per year). Despite improvements in perinatal management over the past 2 decades, there has been no decline in the incidence of PTB; it therefore continues to represent a serious threat to maternal and fetal health.[4]

Early prediction of PTB risk and consequent individualized medical intervention are essential for reducing associated complications. However, there are no predictive tools are currently available for accurately identifying this serious condition in the clinic. Prediction mainly relies on a history of PTB, cervical length, and assessment of fetal fibronectin (fFN) and placental alpha microglobulin-1 (PAMG-1) levels. As these factors have limitations in the predictive

accuracy and the time of application, patients often obtain suboptimal medical benefits.[5,6] As a systemic multifactorial syndrome, PTB involves disorders in multiple maternal and fetal tissues. Therefore, various novel methods for PTB prediction have been studied in recent years; these include the assessment of genetic loci, circulatory protein biomarkers, the vaginal microbiome, and placental elasticity, among others. Although some methods show promise, they have not been implemented on a large scale.[7]

Recent advances in high-throughput technologies have led to the use of the transcriptome as an effective tool for predicting health outcomes.[8] Transcriptomic signatures change in response to altered pathological conditions in vivo; the identification and assessment of these signatures may therefore help determine the biological status of various tissues. Peripheral blood is usually considered as the excellent sample for transcriptomic molecular prediction of pregnancy outcomes, as it serves as the mediator of signal communication across various maternal tissues, allows maternal-fetal exchange, and is easily accessible compared to other samples such as amniotic fluid.[9] Given that the underlying pathophysiological process of disease is often mediated by a cluster of closely linked molecules and dynamic and complex molecular changes throughout pregnancy, prediction based on peripheral blood transcriptomic signatures may offer higher accuracy and entail less time constraints than traditional strategies based on a single (or a few) markers.[10,11]

Therefore, this study aimed to screen for a transcriptomic signature that is essential for PTB occurrence and assess its predictive value for PTB in early, mid, and late pregnancy and in women with threatened preterm labor (TPTL). It also aimed to provide a new clinical strategy for assessing PTB risk at earlier weeks of gestation. This may lead to timely stratified and targeted medical management of pregnant, thereby minimizing the deleterious effects of PTB on the mother and fetus.

## Materials and Methods

### Data collection and pre-processing

The transcriptomic data (ribonucleic acid sequencing (RNA-seq) and microarray) and the associated clinical data pertaining to peripheral blood samples of pregnant women were obtained from the Gene Expression Omnibus database. These included: (1) 84 samples obtained during the first trimester of pregnancy, (2) 281 samples obtained during the second trimester of pregnancy, (3) 223 samples obtained during the third trimester of pregnancy, (4) 15 samples from preterm labor pregnancies and 23 samples from gestational week-matched term pregnancies, and (5) 154 samples from cases of TPTL (please see the details in the supplemental digital content document, http://links.lww.com/MFM/A36). At each period mentioned above, these samples were classified into preterm and term groups depending on whether delivery eventually occurred before 37 weeks (ie, PTB). All the microarray data were log2 processed, and their probe IDs were converted to official gene symbols. The molecule list of the interleukin 6 (IL6) signature was downloaded from the Molecular Signatures Database (MSigDB).

All the datasets analyzed in this study were obtained from the Gene Expression Omnibus databases (publicly available and open access), which were approved by the ethics committee of their submitting institutions. Therefore, the Medical Research Ethics Committee of Women and Children's Hospital of Chongqing Medical University (Chongqing Health Center for Women and Children) ruled that no formal ethics approval was required in this particular case.

### Differential analysis

For RNA-seq data, the DEseq2 package of R was used to obtain the details of differential genes between groups.[12] This process eliminated genes that were not detected in more than half of the samples. For microarray data, differential analysis was performed using the limma R package[13] The default parameters of DEseq2 and limma were retained.

### Gene set enrichment analysis (GSEA) and single-sample gene set enrichment analysis (ssGSEA)

GSEA was performed using official GSEA software (version 3.0), which was downloaded from https://www.gsea-msigdb.org/gsea/index.jsp. The hallmark gene sets were obtained from the MSigDB database (http://software.broadinstitute.org/gsea/msigdb/). The gene list ranked by fold change was considered as the input file, and the result terms with a $P$ values of less than 0.05 were considered statistically significant.[14]

The GSVA package of R was used to perform ssGSEA, using the gene expression matrix as the input file: the method was set as "ssgsea." The IL6_JAK_STAT3_SIGNALING.gmt gene set was obtained from MSigDB, and the activity score of IL6 signaling in each sample was calculated. The results were finally presented in the form of a score matrix.

### Statistical analysis

Logistic regression analysis was performed using the SPSS software package (version 25.0; SPSS, Chicago, IL) to fit the effects of multiple molecules of the IL6 signature. Receiver operating characteristic (ROC) analysis was performed and the results were visualized using the GraphPad Prism (version 9.1) software.

The pregnancy curve (Kaplan-Meier survival curve) was analyzed and visualized using R (version 3.6.2) software. The maxstat package of R was used to calculate the optimal cutoff value for the RiskScore, based on which pregnant women were divided into 2 groups (with normal and abnormal IL6 signatures). The difference in pregnancy maintenance between the 2 groups was analyzed using the survfit function in the survival package of R. Differences between the pregnancy curves were tested using the log-rank test, and $P$ values of less than 0.05 were considered significant.

Normally distributed continuous data were presented as means ± standard deviation. The independent samples $t$-test was used to assess the significance of differences between the 2 groups. The $t$-test, linear regression, and Pearson correlation analysis were performed using R software (version 3.6.2) with its built-in functions. The threshold of statistical significance was set at a $P$ value of less than 0.05.

All the results were visualized using R (version 3.6.2), GraphPad Prism (version 9.1) and Cytoscape (version 3.6.1) software.

## Results

### *The IL6 signature is dysregulated in cases with preterm onset of labor*

As biological activities are often performed jointly by a group of closely related molecules, we screened candidate molecular clusters for predicting PTB via identifying the biological activities directly associated with PTB (based on peripheral blood samples from cases of preterm labor and gestational week-matched term pregnancies). GSEA showed the activity of 29 terms to be significantly altered in PTB (preterm group ($n$=15) *vs.* term group ($n$=23)), including IL6_JAK_STAT3_SIGNALING (normalized enrichment score (NES) = 1.857, $P$ = 0.001) (Fig. 1A and B). As IL6 is one of the classical inflammatory factors of PTB, and IL6 signaling plays an important role in its occurrence, we speculated that the molecular cluster (i.e., the IL6 signature) on which IL6_JAK_STAT3_SIGNALING is based may be the regulator and candidate biomarker for PTB. As shown in Figure 1C, the expression levels of 23 molecules in the IL6 signature were generally higher in PTB. In particular, a strong correlation was observed between these molecules in this condition (Fig. 1D). Thus, we subsequently validated its predictive value for PTB at different periods of pregnancy.

### *Predictive value of the IL6 signature for PTB in the first trimester of pregnancy*

During early pregnancy (9 to $13^{+6}$ weeks), the tumor necrosis factor (in the IL6 signature from peripheral blood) differed most significantly between the preterm and term groups (preterm group ($n$ = 47) *vs.* term group ($n$ = 37), $P$ = 0.034) (Fig. 2A).

During early pregnancy, IL6 alone demonstrated negligible predictive value for PTB (area under the curve (AUC) = 0.517); however, the combined IL6 signature showed satisfactory predictive value (AUC = 0.810; preterm group: $n$ = 47, term group: $n$ = 37) (Fig. 2B). Pregnant women with abnormal IL6 signature had a shortened duration of gestation (abnormal group, $n$ = 57; normal group, $n$ = 27; median survival times: abnormal group = 35.6 weeks, normal group = 38.6 weeks; $P$=0.004) and a shortened latency period (abnormal group, $n$ = 57; normal group, $n$ = 27; median survival times: abnormal group = 22.8 weeks, normal group = 26.7 weeks; $P$ = 0.020) (Fig. 2C).

From the biological perspective, IL6 signaling activity (in peripheral blood) during early pregnancy was markedly reduced in women who delivered prematurely (preterm group ($n$ = 47) *vs.* term group ($n$ = 37), NES = –1.420, $P$ = 0.031)

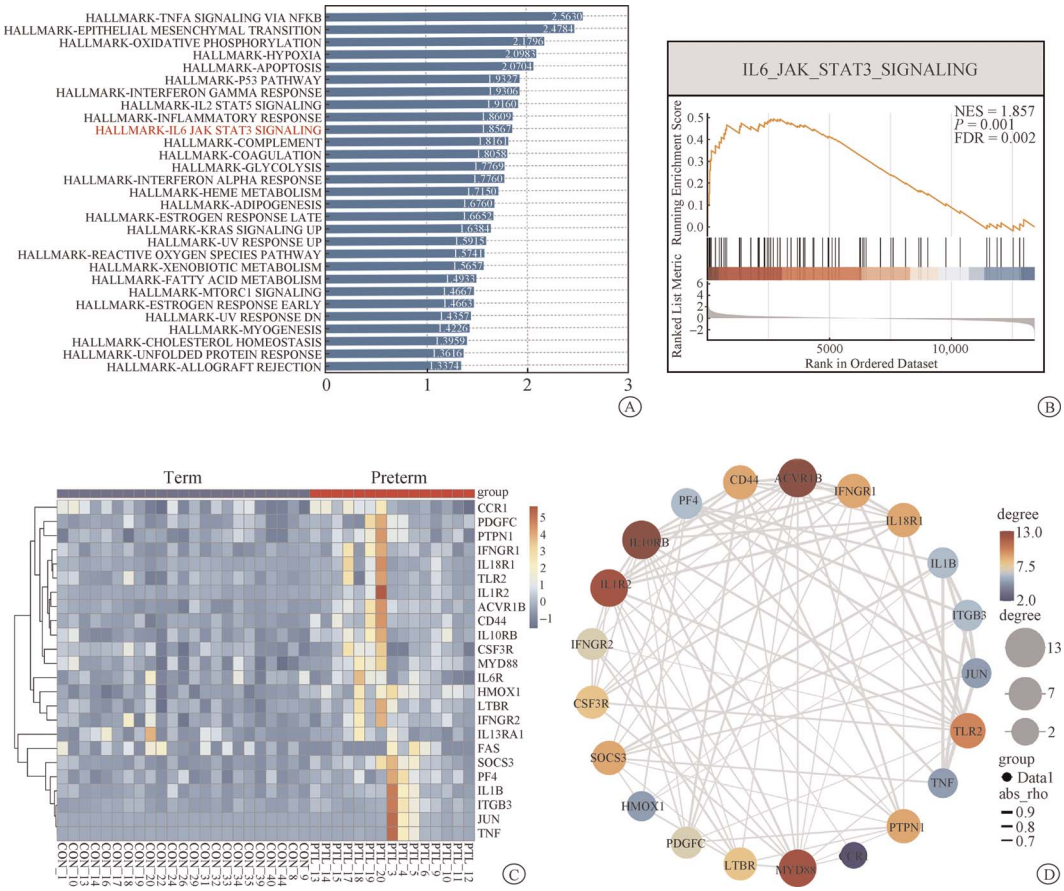


**Figure 1.** IL6 signature characteristics in PTB. A Significantly different biological processes between preterm and term groups (preterm group, $n$ = 15; term group, $n$ = 23). B Activity score of IL6_JAK_STAT3_signaling in PTB. C IL6 signature molecule expression. D Correlation network of IL6 signature molecules ($P$ < 0.050 and $r$ > 0.600). Circle size: number of associated genes; line thickness: strength of correlation. IL6: Interleukin 6; NES: Normalized enrichment score; PTB: Preterm birth.

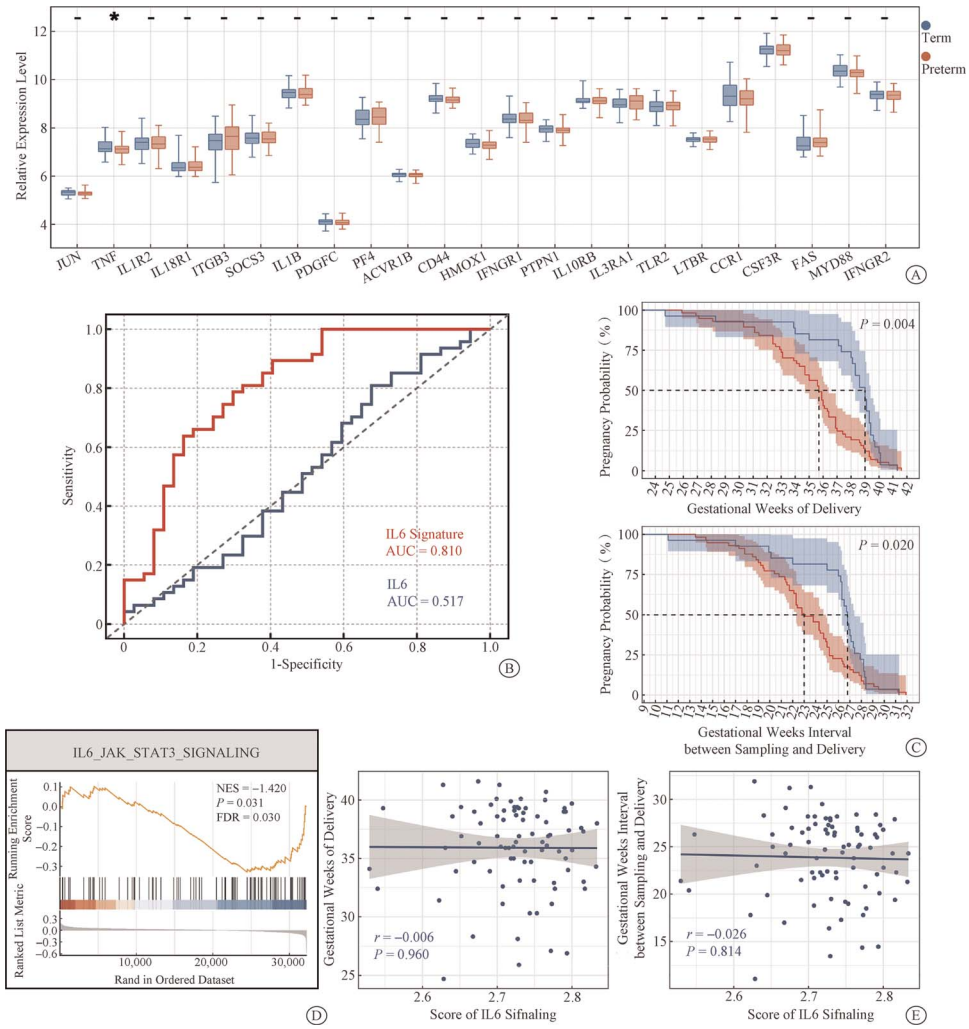


Figure 2. IL6 signature in the first trimester of pregnancy. A IL6 signature molecule expression in preterm and term groups (preterm group, $n$ = 47; term group, $n$ = 37; *$P$ < 0.050). B ROC analysis for PTB prediction of IL6 and combined IL6 signature. C Kaplan–Meier analysis of pregnancy maintenance probability in the first trimester with different levels of IL6 signature. Red line: abnormal IL6 signature; blue line: normal IL6 signature. D Activity score of IL6_JAK_STAT3_signaling in PTB. E Linear regression analysis of IL6 signaling activity scores and gestational week of delivery (left) and gestational week interval between sampling and delivery (right) ($n$ = 84). IL6: Interleukin 6; NES: Normalized enrichment score; PTB: Preterm birth; ROC: Receiver operating characteristic.

(Fig. 2D). However, a linear correlation was not observed between IL6 activity and the length of gestation ($n$ = 84, $r$ = –0.006, $P$ = 0.960) and latency period ($n$ = 84, $r$ = –0.026, $P$ = 0.814) (Fig. 2E).

## *Predictive value of the IL6 signature for PTB in the second trimester of pregnancy*

During mid-pregnancy (14 to $27^{+6}$ weeks), the IL6 signature (in peripheral blood) molecule that showed the most significant difference between the preterm and term groups changed to IL10RB (preterm group ($n$ = 180) *vs*. term group ($n$ = 101), $P$ = 0.031) (Fig. 3A).

The combined predictive value of the IL6 signature (AUC = 0.695) was considerably higher than that of IL6 alone (AUC = 0.533) (preterm group, $n$ = 180; term group, $n$ = 101) (Fig. 3B); this finding was similar to that observed in early pregnancy. Both the length of gestation (abnormal group, $n$ = 202; normal group, $n$ = 79; median survival time: abnormal group = 35.9 weeks, normal group = 38.3 weeks; $P$ < 0.001) and latency period (abnormal group, $n$ = 201; normal group, $n$ = 80; median survival time: abnormal group = 13.4 weeks, normal group = 26.7 weeks; $P$ = 0.034) differed significantly between pregnant women with normal and abnormal IL6 signatures (Fig. 3C).

Notably, pregnant women who eventually delivered prematurely had significantly higher IL6 signaling activity in the peripheral blood (preterm group ($n$ = 180) *vs*. term group ($n$ = 101), NES = 1.671, $P$ = 0.002) during this period (Fig. 3D). In addition, the activity demonstrated negative linear correlation with the length of gestation ($n$ = 281, $r$ = –0.114, $P$ = 0.056) and latency period ($n$ = 281, $r$ = –0.116, $P$ = 0.052); however, the correlation was not statistically significant (Fig. 3E).

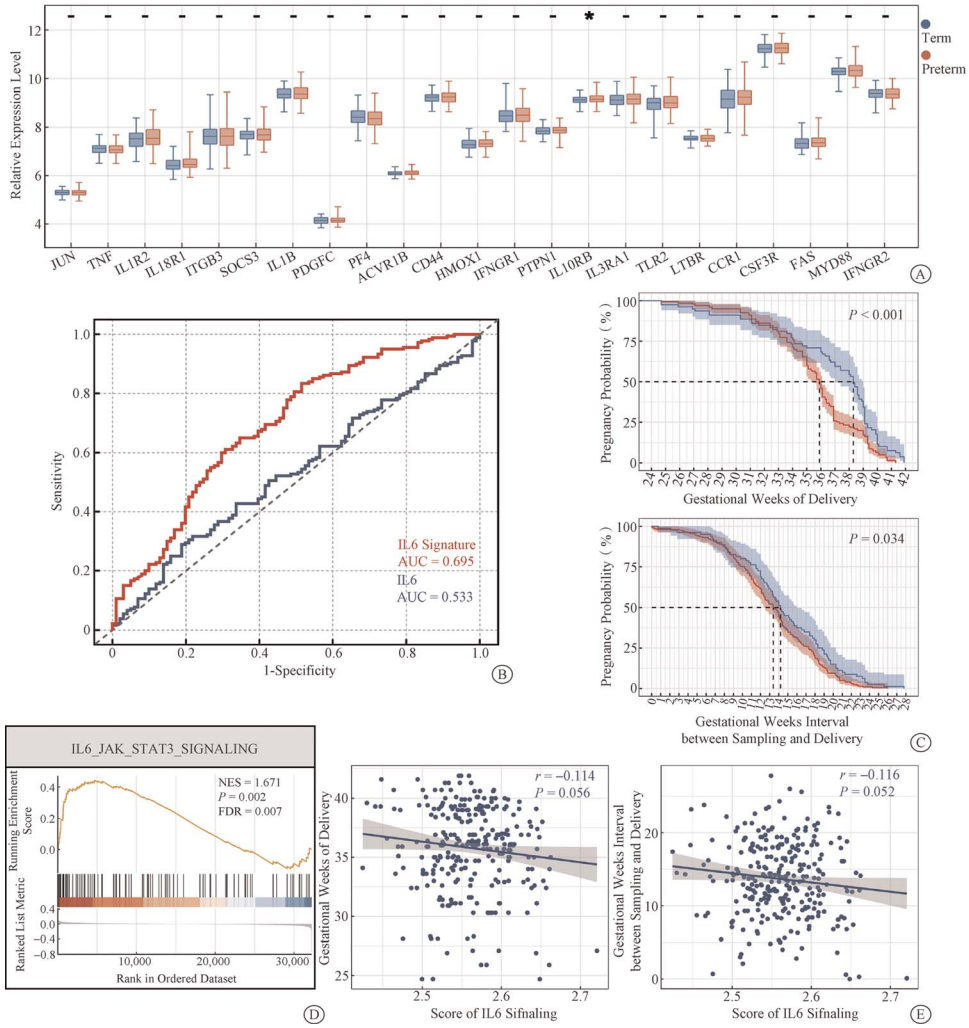


**Figure 3.** IL6 signature in the second trimester of pregnancy. A IL6 signature molecule expression in preterm and term groups (preterm group, $n$ = 180; term group, $n$ = 101) (*$P$ < 0.05). B ROC analysis for PTB prediction of IL6 and combined IL6 signature. C Kaplan–Meier analysis of pregnancy maintenance probability in the second-trimester with different levels of IL6 signature. Red line: abnormal IL6 signature; blue line: normal IL6 signature. D Activity score of IL6_JAK_STAT3_signaling in PTB. E Linear regression analysis for IL6 signaling activity score and gestational week of delivery (left) and the gestational week interval between sampling and delivery (right) ($n$ = 281). IL6: Interleukin 6; NES: Normalized enrichment score; PTB: Preterm birth; ROC: Receiver operating characteristic.

## *Predictive value of the IL6 signature for PTB in the third trimester of pregnancy*

During late gestation (28 to $36^{+6}$ weeks), the changes among the molecules of the IL6 signature (in peripheral blood) were found to be more pronounced; several molecules showed markedly differential expression between the preterm and term groups (preterm group ($n$ = 128) *vs*. term group ($n$ = 95)) (Fig. 4A).

At this stage, the combined predictive value of the IL6 signature (AUC = 0.779) was higher than that at the mid-pregnancy stage; in addition, it continued to be significantly higher than that of IL6 alone (AUC = 0.556) (preterm group, $n$ = 128; term group, $n$ = 95) (Fig. 4B). Similar to the findings in mid- and early gestation, the group with abnormal level of IL6 signature at this stage experienced delivery at earlier gestational weeks (abnormal group, $n$ = 68; normal group, $n$ = 155; median survival time: abnormal group = 35.4 weeks, normal group = 37.6 weeks; $P$ < 0.001) and had a shorter latency period (abnormal group, $n$ = 70; normal group, $n$ = 153; median survival time: abnormal group = 2.9 weeks, normal group = 5.1 weeks; $P$ < 0.001) (Fig. 4C).

IL6 signaling activity remained significantly upregulated in the preterm group: $n$ = 128 *vs*. term group: $n$ = 95, NES = 2.350, $P$ < 0.001). An increase in IL6 signaling activity gradually shortened the latency period ($n$ = 223, $r$ = −0.141, $P$ = 0.035) (Fig. 4D and E).

## *Predictive value of the IL6 signature for PTB in women with TPTL*

In women with TPTL, the levels of molecules in IL6 signature (in peripheral blood) were found to be considerably dysregulated in cases where PTB eventually occurred or occurred within 48 hours or 7 days (preterm group: $n$ = 75 *vs*.

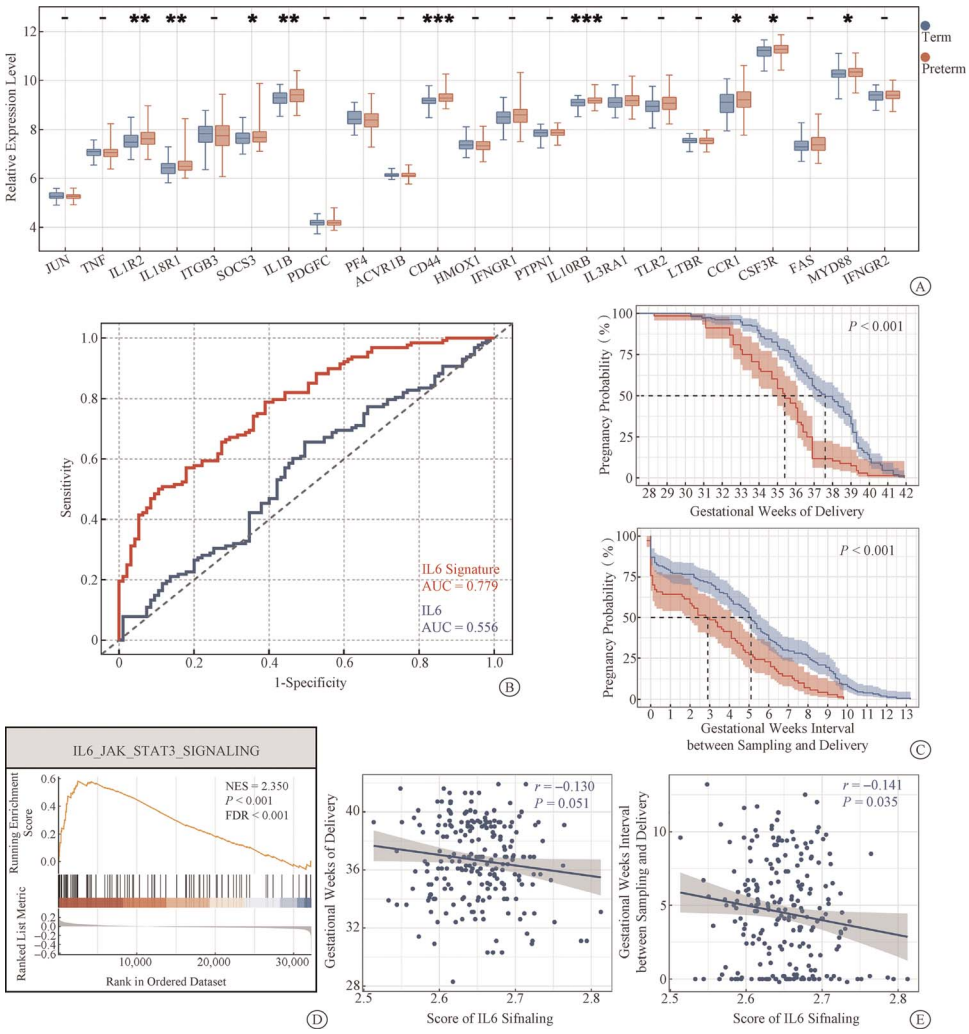


**Figure 4.** IL6 signature in the third trimester of pregnancy. A IL6 signature molecule expression in preterm and term groups (preterm group, $n$ = 128; term group, $n$ = 95; *$P$ < 0.05; **$P$ < 0.01; ***$P$ < 0.001). B ROC analysis for PTB prediction of IL6 and combined IL6 signature. C Kaplan–Meier analysis of pregnancy maintenance probability in the third-trimester with different IL6 signatures. Red line: abnormal IL6 signature; blue line: normal IL6 signature. D Activity score of IL6_JAK_STAT3_signaling in PTB. E Linear regression analysis for the IL6 signaling activity score and gestational week of delivery (left) and the gestational week interval between sampling and delivery (right) ($n$ = 223). IL6: Interleukin 6; NES: Normalized enrichment score; PTB: Preterm birth; ROC: Receiver operating characteristic.

term group: $n$ = 79; PTB <7 days: $n$ = 60 *vs*. delivery > 7 days ($n$ = 94); PTB <48 hours: $n$ = 48 *vs*. delivery > 48 hours ($n$ = 106)) (see supplementary data, Fig. S1, http://links.lww.com/MFM/A37).

The IL6 signature showed good predictive efficacy (AUC = 0.761–0.836) for future PTB occurrence at different time points during the current pregnancy; the interval between the time of testing and that of PTB was inversely proportional to the predicted AUC (preterm group, $n$ = 75; term group, $n$ = 79; PTB <7 days, $n$ = 60; delivery >7 days, $n$ = 94; PTB <48 hours, $n$ = 48; delivery >48 hours, $n$ = 106) (Fig. 5A). The women with TPTL who had abnormal IL6 signature experienced a significantly shorter length of gestation (abnormal group, $n$ = 46; normal group, $n$ = 108; median survival time: abnormal group = 32.9 weeks, normal group = 38 weeks; $P$ < 0.001) and latency period (abnormal group, $n$ = 54; normal group, $n$ = 100; median survival time: abnormal group = 0.2 weeks, normal group = 5.5 weeks; $P$ < 0.001) (Fig. 5B).

IL6 signaling activity was considerably upregulated in women who experienced PTB (preterm group: $n$ = 75 *vs*. term group: $n$ = 79; NES = 1.917, $P$ < 0.001); PTB within 7 days (PTB < 7 days: $n$ = 60 *vs*. delivery > 7 days: $n$ = 94; NES = 2.096, $P$ <0.001); and PTB within 48 hours (PTB < 48 hours: $n$ = 48 *vs*. delivery > 48 hours: $n$ = 106; NES =1.908, $P$ = 0.001) (Fig. 5C). In addition, IL6 activity correlated negatively with the gestational week of delivery ($n$ = 154, $r$ = –0.260, $P$ = 0.001) and length of the latency period ($n$ = 154, $r$ = –0.203, $P$ = 0.012) (Fig. 5D).

## Discussion

Timely and accurate prediction of PTB is necessary for developing an effective clinical management strategy that may reduce maternal and fetal morbidity and death. As the issue of PTB is a continuing challenge in perinatal medicine, we aimed to identify a reliable predictor for its occurrence. In this study, we screened for the IL6 signature as it

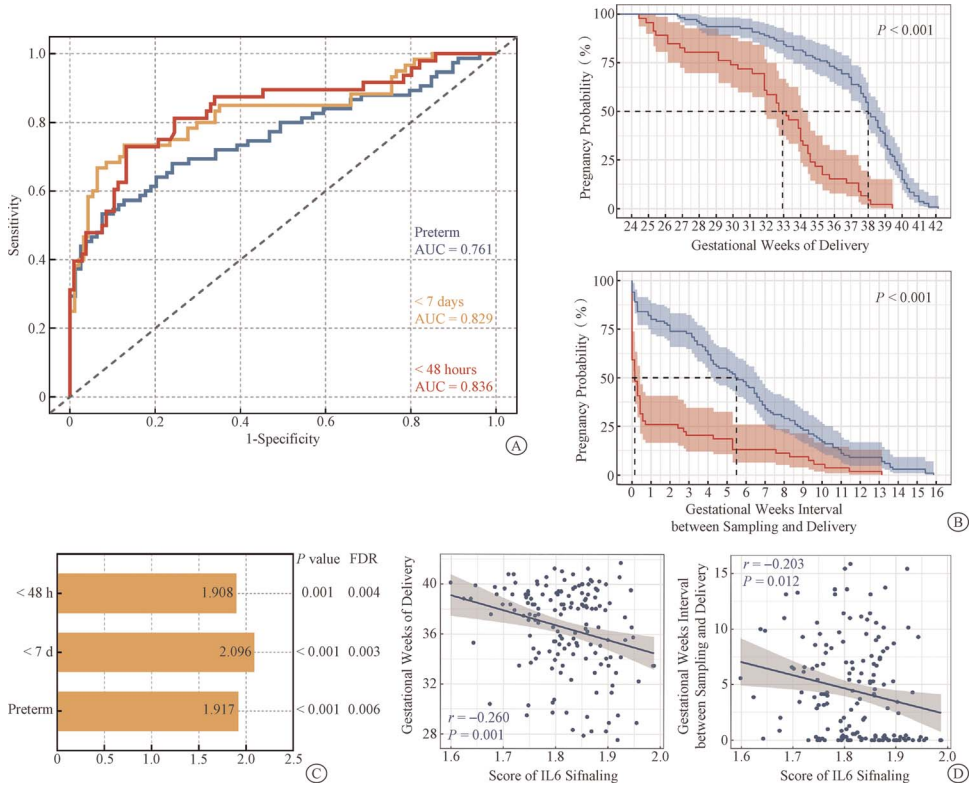


**Figure 5.** IL6 signature in TPTL pregnancies. A ROC analysis of IL6 signature predicting PTB, PTB within 7 days, and PTB within 48 hours (preterm group, *n* = 75; term group, *n* = 79; PTB < 7 days, *n* = 60; delivery > 7 days, *n* = 94; PTB < 48 hours, *n* = 48; delivery > 48 hours, *n* = 106). B Kaplan–Meier analysis of the probability of pregnancy maintenance in TPTL with different levels of IL6 signature. Red line: abnormal IL6 signature; blue line: normal IL6 signature. C Activity score of IL6_JAK_STAT3_signaling in PTB, PTB within 7 days, and PTB within 48 hours. D Linear regression analysis for the IL6 signaling activity score and gestational week of delivery (left) and the gestational week interval between sampling and delivery (right) (*n* = 154). IL6: Interleukin 6; PTB: Preterm birth; ROC: Receiver operating characteristic; TPTL: Threatened preterm labor.

represents an essential molecular basis for PTB at the transcriptome level; the findings suggested that it plays a significant role in prospectively predicting PTB in the early, mid, and late stages of pregnancy(although the predictive power is relatively weak in mid pregnancy). The biological activity of the IL6 signature was also found to be linearly related to the gestational week of delivery and latency period in women with TPTL.

Notably, none of the biomarkers in current clinical use can predict PTB accurately.[15] In addition to fFN and PAMG-1, several new biomarkers have been identified in recent years; most of these are based on cumulative potential biological alterations that occur before labor and delivery.[16] Reports suggest that C reactive protein and the complement system (representing increased levels of immune inflammation),[17,18] matrix metalloproteinases (representing the destruction of maternal-fetal interface structures such as the chorion, amnion, and decidua, among others),[19] circulatory metabolic molecules (representing increased metabolic activity),[20] and extracellular vesicles and fetal free nucleic acid (representing increased signal transmission from the fetus to mother),[9,21] are some of the markers that may potentially predict PTB. These physiological and pathological activities intensify/become more significant as the time to labor approaches; the predictive value of these biomarkers also increase.[22] As these biomarkers are usually evaluated in late pregnancy or only in pregnant women with clinical symptoms, the time available for clinical intervention is usually insufficient. However, tools for early assessment of PTB risk are lacking. As genetic susceptibility plays a substantial role in PTB, it is likely that underlying pathological factors that affect pregnancy and contribute to PTB may be found throughout pregnancy; these may be used to predict PTB earlier.[15,23]

It is generally accepted that an integrated prediction system consisting of multiple molecules has higher value than a single biomarker in assessing the risk of complex diseases. Recent studies have shown that the biomolecular signature holds promise in assessing the risk of complex diseases; it has been demonstrated to have considerable monitoring or predictive power for the occurrence, progression, status, and prognosis of diseases such as cancers.[24,25] As IL6 signaling may play a major role in the pathological process of PTB, we screened for this signature (on which IL6 signaling is based) and proposed that it has the potential to predict PTB.

IL6 is a widely expressed and functionally pleiotropic cytokine and one of the classical regulators of the immune inflammatory response in PTB. Studies from different ethnic populations have revealed that polymorphisms of the *IL6* gene are associated with PTB. The SNP IL6-174 (also known as rs1800755) GG/GC genotype is a risk factor for PTB, as it contributes to inflammation (while the CC

genotype is a protective one).[26,27] Notably, IL6 levels in maternal serum, amniotic fluid, vaginal secretions, and other samples increase significantly before the onset of PTB. Not only does IL6 signaling directly stimulate tissue cells to produce inflammatory factors, it also activates the expression of matrix metalloproteinases; this disrupts the biological microstructure of the maternal-fetal interface.[28,29] In addition, it induces the expression of oxytocin receptors in the myometrium and the secretion of oxytocin and prostaglandin E2 by maternal-fetal interface tissues, thereby promoting uterine contraction.[30] Notably, IL6 signaling strongly contributes to the activation and proliferation of immune cells, including neutrophils, macrophages, and lymphocytes (especially Th17 cells), and their migration to the maternal-fetal interface; this leads to formation of the immuno-inflammatory cascade necessary for PTB.[26,31] Our group previously demonstrated a significant increase in IL6 levels in the circulation and at the maternal-fetal interface in a lipopolysaccharide-induced inflammatory PTB mouse model.[32] In their study on fetal mice, Wakabayashi *et al.*[33] found that targeted inhibition of IL6 signaling in this model could reduce the PTB rate without adverse effects. Despite the strong association between IL6 and PTB, IL6 has demonstrated suboptimal predictive efficiency as an independent biomarker for PTB[34,35]; the findings from our study are consistent with these findings This may be due to the fact that various functions and activities of IL6 signaling (under different conditions) are mediated by dynamic and complex interactions between molecules in IL6 signature, and are not solely determined by IL6 expression levels. The IL6 signature is therefore a better predictor of PTB than IL6 alone.

Labor and delivery occur because of the accumulation of biological signals represented by immune inflammation.[36,37] In this study, the predictive power of the IL6 signature for PTB was essentially based on the activity of IL6-regulated biological processes. Thus, the AUC value for PTB prediction (by the IL6 signature) increased from 0.695 to 0.779 during the mid- to late-trimester. The predictive accuracy was more significant in women with TPTL who had clinical symptoms and a higher level of intrinsic biological response. This is consistent with the aforementioned importance of IL6 signaling in the development of PTB.[26] Interestingly, our results suggested that the AUC for predicting PTB (by the IL6 signature) is even higher in the first trimester than in the second and third trimesters. This may be related to the biological status of the maternal-fetal system during different gestational periods and the functional diversity of IL6. In early pregnancy, a moderate inflammatory response is necessary for promoting embryo implantation and placentation, and normal levels of IL6 signaling is important for this process. IL6 signaling not only regulates endometrial tolerance (to provide a favorable immune microenvironment for the embryo), but also regulates invasion and adhesion of trophoblasts and the release of hormones (such as human chorionic gonadotrophin, among others) to promote placenta formation and development.[38,39] An abnormal increase in IL6 signaling disrupts maternal immune tolerance, leading to an excessive inflammatory response and abortion.[40,41] In contrast, suboptimal IL6 signaling may affect early development of the embryo and placenta; this represents a hidden danger for pregnancy outcomes even if no obvious clinical manifestations are observed in early pregnancy.[31] Correspondingly, we found that IL6 signaling activity had decreased significantly in the first trimester and had increased in the second and third trimesters in women who eventually experienced PTB. These results suggest that the IL6 signature may contribute to the development of PTB-related pathology in early and late pregnancy, while the biological processes regulated by the IL6 signature may differ across different stages. Thus, the activity levels in both these stages may be used as a reference for evaluating PTB risk. However, no significant linear correlation was observed between IL6 signaling activity and the gestational week of delivery and latent period in early pregnancy; the correlation became progressively clearer from late-pregnancy, especially in women with TPTL. It is possible that a longer period until labor onset increases the number of influencing factors (both beneficial and harmful) that a pregnant woman is exposed to; this disturbs the correlation.

Although the findings from this study are promising, the following limitations need to be considered and addressed in our next study. First, individualized risk factors (PTB history, and age, among others) and clinical examination results (routine blood among others) may improve the accuracy PTB prediction using the IL6 signature. Second, the risk of morbidity and mortality is higher in PTB before 34 gestational weeks (than after 34 weeks). The ability of the IL6 signature in predicting and discriminating between these two phenotypes needs to be evaluated, as it may offer benefits in terms of fine risk stratification and clinical management. Third, the underlying molecular mechanisms (of the IL6 signature) involved in the pathological processes of PTB throughout the different stages of pregnancy remain unclear; these need further evaluation via systematic experiments.

## Conclusion

In conclusion, we found that the IL6 signature performed well in predicting the risk of PTB across all gestation periods (although the predictive power is relatively weak in mid pregnancy), even as early as the first trimester of pregnancy. Pregnancies with different levels and activity of the IL6 signature have distinct pregnancy curves and latent periods. Our findings will help assess the risk of PTB earlier and more accurately; they will also aid in the individualized medical management of pregnant women, thereby protecting maternal and fetal health.

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## Author Contributions

Yamin Liu and Hongbo Qi designed the research. Yuxin Ran and Dongni Huang performed the bioinformatic and statistical analysis and wrote the manuscript. Yanqing Wen helped to solve the clinical problems. Nanlin Yin and Yan Jiang contributed to data collection. All authors read and approved the final manuscript.

## Conflicts of Interest

None.

## Editor Note

Hongbo Qi is an editorial board member of *Maternal-Fetal Medicine*. The article was subject to the journal's standard procedures, with peer review handled independently of this editor and the associated research groups.

## Data Availability

All data generated for this study are included in the article, and further inquiries can be directed to the corresponding author.

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