OBSTETRICS

Preterm labor is a distinct process from term labor following computational analysis of human myometrium

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BACKGROUND: The onset of the term human parturition involves myometrial gene expression changes to transform the uterus from a quiescent to a contractile phenotype. It is uncertain whether the same changes occur in the uterus during preterm labor.

OBJECTIVE: This study aimed to compare the myometrial gene expression between term and preterm labor and to determine whether the presence of acute clinical chorioamnionitis or twin gestation affects these signatures.

STUDY DESIGN: Myometrial specimens were collected during cesarean delivery from the following 7 different groups of patients: term not in labor (n=31), term labor (n=13), preterm not in labor (n=21), preterm labor with acute clinical chorioamnionitis (n=6), preterm labor with no acute clinical chorioamnionitis (n=9), twin preterm not in labor (n=8), and twin preterm labor with no acute clinical chorioamnionitis (n=5). RNA was extracted, reverse transcribed and quantitative polymerase chain reactions were performed on 44 candidate genes (with evidence for differential expression in human term labor) using the Fluidigm platform. Computational analysis was performed using 2-class unpaired Wilcoxon tests and principal component analysis.

RESULTS: Computational analysis revealed that gene expression in the preterm myometrium, irrespective of whether in labor or not in labor, clustered tightly and is clearly different from the term labor and term not-in-labor groups. This was true for both singleton and twin pregnancies. Principal component analysis showed that 57% of the variation was explained by 3 principal components. These 44 genes interact in themes of prostaglandin activity and inflammatory signaling known to be important during term labor, but are not a full representation of the myometrium transcriptional activity.

CONCLUSION: The myometrial contractions associated with preterm labor are associated with a pattern of gene expression that is distinct from term labor. Therefore, preterm labor may be initiated by a different myometrial process or processes outside the myometrium.

Key words: acute histologic chorioamnionitis, chorioamnionitis, CXCL8, inflammation, myometrium, NFκB, pregnancy, preterm birth, preterm labor, prostaglandin, twin gestation

Introduction

Human parturition at term (≥37+0 weeks' gestation) marks the end of pregnancy and occurs spontaneously in 91.7% of women between 37+0 and 41+6 weeks' gestation based on recent Australian data.[1] Throughout gestation, the effects of progesterone,[2–5] human chorionic gonadotropin (hCG),[6–8] corticotrophin releasing hormone (CRH), and the secondary messenger cyclic adenosine monophosphate (cAMP)[8–13] maintain uterine quiescence. Term labor is associated with a removal of the brake on uterine quiescence via epigenetic and functional changes in progesterone and estrogen receptor subtypes,[4,14] which leads to increased expression of contraction-associated proteins (CAPs), such as gap-junctions,[15,16] ion channels,[17,18] and prostaglandin synthesizing enzymes.[19–21] CAPs alter the structure and connectivity of the myometrial smooth muscle, increasing calcium signaling and phosphorylation of myosin light chains, which enables actin-myosin crossbridge cycling to generate contractions.[22] Supporting this paradigm, analogs of prostaglandins and oxytocin are used to promote uterine contraction to induce labor or arrest postpartum hemorrhage caused by uterine atony.[23]

It therefore seems logical that therapeutics that act on the pathways associated with term labor would be effective in preventing contractions observed in preterm birth (PTB), defined as birth <37+0 weeks' gestation. Unfortunately, calcium channel blockers, oxytocin antagonists, and beta-sympathomimetics have not been shown to block preterm contractions indefinitely.[24,25] Hence, PTB remains a major obstetrical burden, affecting 15 million babies annually, and continues to be the largest contributor to childhood death under the age of 5 years.[26,27] PTB increases the risk for long-term neurodevelopment delays and has been extensively linked to adult noncommunicable diseases.[28]

The biological basis of preterm tocolysis therapy relies heavily on the assumption that the myometrium undergoes a similar transition during preterm labor as during term labor. It has been hypothesized that PTB is a syndrome with multiple causes, which prematurely activate uterine contractions.[29] Reported causes include hormonal dysregulation, in particular maternal and fetal stress response,[30,31] elevation of placentally-derived CRH production,[32–34] activation of toll-like receptors (TLR) through infection and damage-associated molecules, placental and decidual hemorrhage, and uterine

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AJOG at a Glance

Why was this study conducted?

Transcriptomic changes occur in the term myometrium that transforms the uterus from a quiescent to a contractile phenotype during the process of human labor at term. The mechanism of preterm labor is poorly understood, and it is not currently known whether similar transcriptomic changes occur during preterm labor.

Key findings

Principal component analysis of 44 candidate genes from 93 women across 7 different clinical groups demonstrate clear separation of the term laboring, term nonlaboring, and preterm myometrium. This analysis revealed that the laboring preterm myometrium gene expression is distinct from the laboring term myometrium regardless of the presence of a twin gestation or acute clinical chorioamnionitis. These 44 genes are related to important functions, such as prostaglandin and inflammatory signaling, but are not representative of the entire transcriptome of the myometrium.

What does this add to what is known?

The transcriptional activity (related to our 44 candidate genes) in the preterm laboring myometrium is different from the myometrium during normal term labor, which may suggest that preterm birth is regulated by different myometrial signatures or processes outside the myometrium.

stretch.[29,35–37] Although there is biological plausibility that these etiologies may all lead to increased contractility of the uterus, this has not been demonstrated unequivocally.[38,39] The expression of genes that encode known CAPs is substantially different between the term and preterm laboring myometrium, including oxytocin receptor (*OXTR*), prostaglandin-endoperoxide synthase 2 (*PTGS2*), and C-X-C motif chemokine ligand 8 (*CXCL8*), previously known as interleukin 8 (IL-8).[39]

Our group has reported a large number of differentially expressed genes in the term myometrium as it transitions into a laboring phenotype using a suppression subtractive hybridization (SSH) technique.[40] These differentially expressed genes were specifically involved in inflammatory signaling pathways and prostaglandin production and the production of gap junction ion channels, progesterone receptors, and estrogen receptors.[40] Similar results have been reported by other investigators.[41–48] We now report a comparison of the genes that change in the term myometrium at the onset of labor and the genes that change in the preterm myometrium with singleton pregnancies in labor in the presence or absence of acute chorioamnionitis, in the presence of labor with twin gestations, and in samples in the absence of labor with both twin and singleton gestations. We sought to determine whether our previously identified genes associated with term labor change in a similar way in various preterm clinical groups.

Materials and Methods

We performed quantitative polymerase chain reactions (qPCRs) for 44 genes on RNA extracted from term and preterm myometria with various phenotypes of labor collected over a 5-year period.

Myometrial sample collection

Myometrial samples were collected from the lower uterine segment incision of women who underwent cesarean deliveries (CDs). Samples were promptly washed in cold phosphate-buffered saline and snap frozen on dry ice and stored at −80°C.

Clinical groups

Myometrial samples from the following 7 groups of patients with different laboring phenotypes were collected: term not in labor (TNIL), term in labor (TL), preterm not in labor (PTNIL), preterm in labor with evidence of clinical chorioamnionitis (PTL-C), and preterm in labor with no evidence of clinical chorioamnionitis (PTL-NC). Patients with twin pregnancies were categorized into preterm not in labor (TWIN-PTNIL) and preterm labor with no evidence of clinical chorioamnionitis (TWIN-PTL-NC). There were no cases of clinical chorioamnionitis in any labor involving twin deliveries in our study. The diagnosis of labor was made by the presence of regular, painful uterine contractions with evidence of cervical effacement and dilation over 2 vaginal examinations or if the cervix was >4 cm dilated during 1 examination. The diagnosis of clinical chorioamnionitis was made by clinical assessment that included the following: fever ≥38°C (≥100.4°F) on ≥2 occasions, maternal tachycardia ≥100 beats per minute (bpm), fetal tachycardia ≥160 bpm, uterine tenderness, purulent-appearing vaginal discharge, or an elevated white cell count (>15,000 cells/mm^2). Patients with clinical chorioamnionitis had confirmatory placental histopathology demonstrating acute histologic chorioamnionitis. Both mono- and dichorionic twins were included in this study.

RNA extraction

Tissues were pulverized using a Precellys 24 homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France), and RNA was extracted using Trizol reagent (Thermo Fisher Scientific, Waltham, MA), phase-separated with chloroform, and precipitation with isopropanol.[49] RNA was treated with DNase and cleaned using the Zymo RNA Clean & Concentrator-5 Kit (Zymo Research, Irvine, CA). RNA quantity and purity were checked by ultraviolet absorption using a Nanodrop spectrometer (Thermo Fisher Scientific) and RNA integrity was confirmed using a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA).

Real-time quantitative reverse transcription-polymerase chain reaction

RNA was reverse transcribed using an Invitrogen SuperScript III Reverse Transcriptase kit (Thermo Fisher Scientific). A total of 44 TaqMan (Thermo Fisher Scientific) primers were assessed (Supplemental Table 1) using high-throughput quantitative PCR on a BioMark HD instrument (Fluidigm, South San Francisco, CA). First, cDNAs were preamplified in a single 14-cycle PCR reaction using the TaqMan PreAmp Mastermix (Fluidigm) on the Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Preamplified samples and TaqMan reagents were loaded on the integrated fluidic circuit (Fluidigm) following the manufacturer's protocol. Cycle threshold (Ct) values were calculated using the BioMark PCR system analysis software (Fluidigm). Messenger RNA abundance was calculated using the $2^{-\Delta\Delta CT}$ method using 3 housekeeper genes (*ARF1*, *EIF2B1, MRPL19*) known to have stable expression in myometrial tissue.[50]

Differentially expressed genes

Our 44 differentially expressed genes were derived from a previous SSH study[40] (Supplemental Table 1). There were 19 up-regulated genes associated with the onset of term labor that were confirmed by qPCR in term myometrial tissues (data not published here), and there were 10 genes deduced from bioinformatics analysis of the SSH up-regulated genes and 15 genes found by SSH to be down-regulated, but these were not previously confirmed in term myometrial samples.

Statistical analysis

Statistical testing was performed using R (a free software environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria) and the Significant Analysis of Microarrays (SAM) package. Two-class unpaired Wilcoxon tests were performed with a false discovery rate (FDR) of 0.1 and correction for multiple testing was achieved using shrinkage-based test and permutation correction methods.[51,52] The fold changes in the expression between the 2 groups and q values (which measure the proportion of false positives when the specific hypothesis is deemed significant) are presented.

Pathway analysis

Our gene list was subjected to protein-protein interaction analysis using STRING 8[53] with reference to the Gene Ontology (GO)[54,55] and Reactome databases.[56] This analysis provides a statistical means of evaluating interactions among a set of genes to determine whether they are, at least partially, biologically connected as a group, and if so, what functions they share.

Computational analysis

Principal component analysis (PCA) was used as a technique to reduce the dimensionality and complexity of data. PCA creates new variables called principal components (PCs) from the data set, which aim to maximize the variance among the clinical groups in this study. Plotting these PCs in 3 dimensions allow increased interpretability without losing any data and is particularly useful for large data sets. Our data were first imputed using the K-Nearest Neighbor (KNN) method before PCA of the 44 genes was performed. All analyses, including video visualizations, were carried out using R and the relevant libraries.[51]

Histology

Small pieces of tissue were fixed in 10% formalin for 24 to 48 hours and stored in 70% ethanol before paraffin embedding. Embedded tissues were sectioned at 4 μm and stained with hematoxylin and eosin. Reporting of the histopathology was performed by an expert clinical pathologist. A diagnosis of acute histologic chorioamnionitis was made based on placental histopathology defined by infiltration of neutrophils, which has previously been described by Redline et al.[57]

Ethics

Informed consent was obtained from patients before CD at the John Hunter Hospital (Newcastle, New South Wales, Australia) and Singapore KK Women's and Children's Hospital (Singapore, Singapore). This study was approved by the relevant ethics committees (approval number 2019/ETH12330).

Results

A total of 93 samples were collected across the 7 patient groups and confirmation of myometrial tissue type was achieved by histology. Baseline characteristics and indications for CDs for all patient groups are outlined in Table 1. Most patients had an elective CD in the TNIL group owing to a previous CD, whereas intrauterine growth restriction and hypertensive disorders of pregnancy were the most common indications for CD in the PTNIL and TWIN-PTNIL groups. Labor dystocia and fetal distress were the top indications for CD in the TL group, whereas breech presentation and fetal distress were the most common indications in the preterm laboring groups. Of note, 5 of 6 patients in the clinical chorioamnionitis (PTL-C) group had available placental histopathology, all of which demonstrated acute histologic chorioamnionitis. All 6 patients in this group had clinical evidence of acute chorioamnionitis. Patients in the PTL-NC group did not have symptoms suggestive of clinical chorioamnionitis. Among these 9 women, 8 had available placental histology of which 4 demonstrated no evidence of histologic chorioamnionitis (50%) and 4 demonstrated mild histologic chorioamnionitis (50%). Of note, patients with histologic chorioamnionitis within this group were all in advanced labor with cervical dilation ranging from 6 cm to fully dilated. The remaining 4 patients with no histologic chorioamnionitis had cervical dilation <4 cm.

The pairwise comparisons among the 7 groups are displayed in Figure 1 (FDR of 0.1 and significant fold change threshold ≥ 1.5). Comparing the TNIL myometrium with the TL myometrium revealed that 12 out of the 44 genes were differentially expressed between the 2 groups to a significant extent. When comparing the myometrium across nonlaboring groups, there were 17 differentially expressed genes between

TABLE 1
Patient characteristics separated by laboring phenotype

Characteristic	Term not in labor (TNIL)	Term labor (TL)	Preterm not in labor (PTNIL)	Preterm labor no chorioamnionitis (PTL-NC)	Preterm labor with chorioamnionitis (PTL-C)	Twin preterm not in labor (TWIN-PTNIL)	Twin preterm labor no chorioamnionitis (TWIN-PTL-NC)
Number	31	13	21	9	6	8	5
GA (wk)	38.4±0.9	38.7±1.1	32.3±3.3	30.9±3.6	29.3±2	32.5±4	32.9±2.4
Maternal age (y)	31.2±5.9	31.2±3.7	29.1±5.6	27.8±5.2	32±7.2	29.0±5	27.8±4.1
BMI (kg/m^2)	30.5±8.6	23.2±8.1	28.6±9.3	33.5±6.7	24.7±5.2	25.6±4.2	33.7±7.1
Parity, n (%)							
0	9 (33.3)	10 (76.9)	9 (42.9)	6 (66.7)	1 (16.7)	5 (62.5)	2 (40.0)
1	9 (33.3)	1 (7.7)	4 (19.0)	1 (11.1)	1 (16.7)	1 (12.5)	1 (20.0)
2	5 (18.5)	2 (15.4)	6 (28.6)	1 (11.1)	1 (16.7)	0 (0.0)	0 (0.0)
>3	5 (18.5)	0 (0.0)	2 (9.5)	1 (11.1)	3 (50.0)	2 (25)	2 (40.0)
Indication for delivery, n (%)							
Antepartum hemorrhage	0 (0.0)	0 (0.0)	2 (9.5)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)
Breech	6 (22.2)	1 (7.7)	0 (0.0)	7 (77.8)	2 (66.3)	0 (0.0)	2 (40.0)
Intrauterine growth restriction	0 (0.0)	0 (0.0)	11 (52.3)	0 (0.0)	0 (0.0)	4 (50.0)	1 (20.0)
Hypertensive disorders of pregnancy	0 (0.0)	0 (0.0)	8 (38.1)	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)
Labor dystocia[a]	0 (0.0)	6 (41.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Previous cesarean delivery	15 (55.6)	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fetal distress	2 (4.8)	3 (23.1)	0 (0.0)	1 (11.1)	4 (66.7)	1 (12.5)	1 (20.0)
Failed induction of labor	0 (0.0)	2 (15.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Maternal request	3 (9.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (25.0)	0 (0.0)
Previous perineal trauma	1 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Vasa previa	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (20.0)

Data are presented as number, number (percentage), or mean±standard deviation.

[a] Labor dystocia was defined as a failure in cervical dilation despite IV oxytocin infusion.

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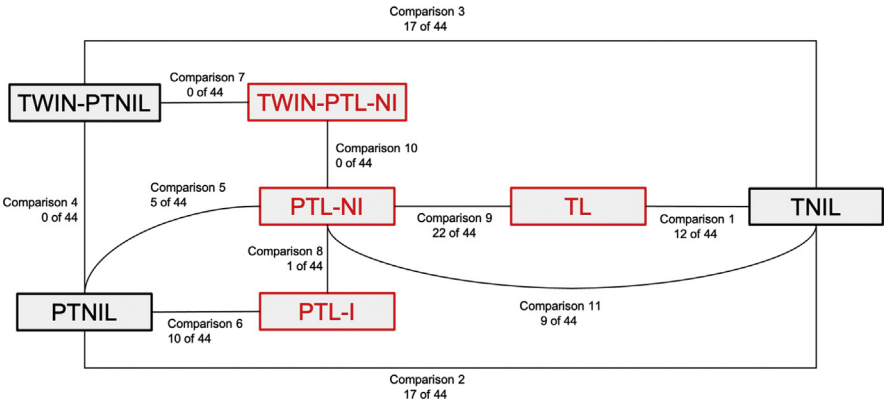
the TNIL and PTNIL groups and 17 differentially expressed genes between the TNIL and TWIN-PTNIL groups. No differences were identified between the PTNIL and TWIN-PTNIL groups. The largest number of differentially expressed genes (22 of 44 genes) were observed in the TL group than the PTL-NC group. Myometrial samples collected during preterm labor in the absence of acute clinical chorioamnionitis demonstrated no difference in gene expression between singleton and twin pregnancies. In the singleton preterm laboring myometrium, the presence of acute clinical chorioamnionitis was associated with differential expression in only 1 out of 44 genes. In the absence of acute clinical chorioamnionitis, there was differential expression in 6 of 44 genes in the PTNIL group than the PTL-NC group, and no genes were differentially expressed between the TWIN-PTNIL and TWIN-PTL-NC myometria. The magnitude of change and corresponding *q* values for each of the 2-class comparisons are provided in Supplemental Table 2 and the Supplemental Figure.

To better delineate intergroup differences, PCA was performed with the first 10 PCs reported in Table 2. Imputation of data was required because of missing data, however, >80% of the genes had <5% missing data. PCA demonstrated that the first 3 PCs explained 57% of the total variance across the 44 genes. Data the from the PCA including the first 3 PCs were demonstrated in a 2D plot (Figure 2) and a 3D video (Supplemental Video). The TNIL and TL groups seem to be separate entities when plotted based on the first 3 PCs. The remaining groups (PTNIL, TWIN-PTNIL, PTL-NC, and TWIN-PTL-NC) seem to occupy a similar 3-dimensional space. Although the PTL-C group clustered in a similar fashion to the other preterm samples, greater variation was observed.

To determine the functional significance of our data, a protein-protein interaction analysis of our 44 candidate genes was performed and it revealed that there were markedly more interactions among these genes than what would be expected from a random set of a similar size. There were 147 interactions represented by the edges (lines) between each node (candidate gene), which suggest that these candidates are at least partially biologically connected (Figure 3). The functions with the highest levels of enrichment included roles such as prostaglandin activity, NFkB signaling and cytokine signaling in the immune system (Supplemental 3). These closely resemble the reported pathways in term labor based on large data sets from RNA sequence studies[42] that showed overrepresentation in pathways associated with cytokine signaling, complement activity, and inflammation. This would suggest that our candidate genes may share important functions in term labor.

FIGURE 1
The two-class unpaired comparisons made between all myometrial phenotypes



The comparisons were based on 44 candidate genes that were compared using Wilcoxon tests with a false discovery rate of 0.1 and a significant fold change threshold of ≥1.5. The magnitude of expression differences in the genes with a *q* scores of <10% are reported. Further details for each comparison are reported in Supplemental Table 2.

PTL-I, preterm in labor with evidence of clinical chorioamnionitis; *PTL-NI*, preterm in labor with no evidence of clinical chorioamnionitis; *PTNIL*, preterm not in labor; *TL*, term in labor; *TNIL*, term not in labor; *TWIN-PTL-NI*, twin pregnancies—preterm labor with no evidence of clinical chorioamnionitis; *TWIN-PTNIL*, twin pregnancies—preterm not in labor.

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Comment

Principal findings

Our computational analysis demonstrated that the transcriptional pattern of the preterm myometrium is different from that of the term labor and TNIL myometrial samples when assessed in terms of the 44 candidate genes identified a priori. Our complex data set, including 93 women across 7 clinical groups for which 44 candidate genes

TABLE 2
The first 10 principal components of the principal component analysis reporting the standard deviation, proportion of variance, and cumulative proportion of variance

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Standard deviation	3.95	2.38	2.08	1.58	1.53	1.39	1.34	1.11	1.07	0.97
Proportion of variance	0.35	0.13	0.10	0.06	0.05	0.04	0.04	0.03	0.03	0.02
Cumulative proportion	0.35	0.47	0.57	0.62	0.68	0.72	0.76	0.79	0.81	0.83

Data are presented as number.

PC, principal component.

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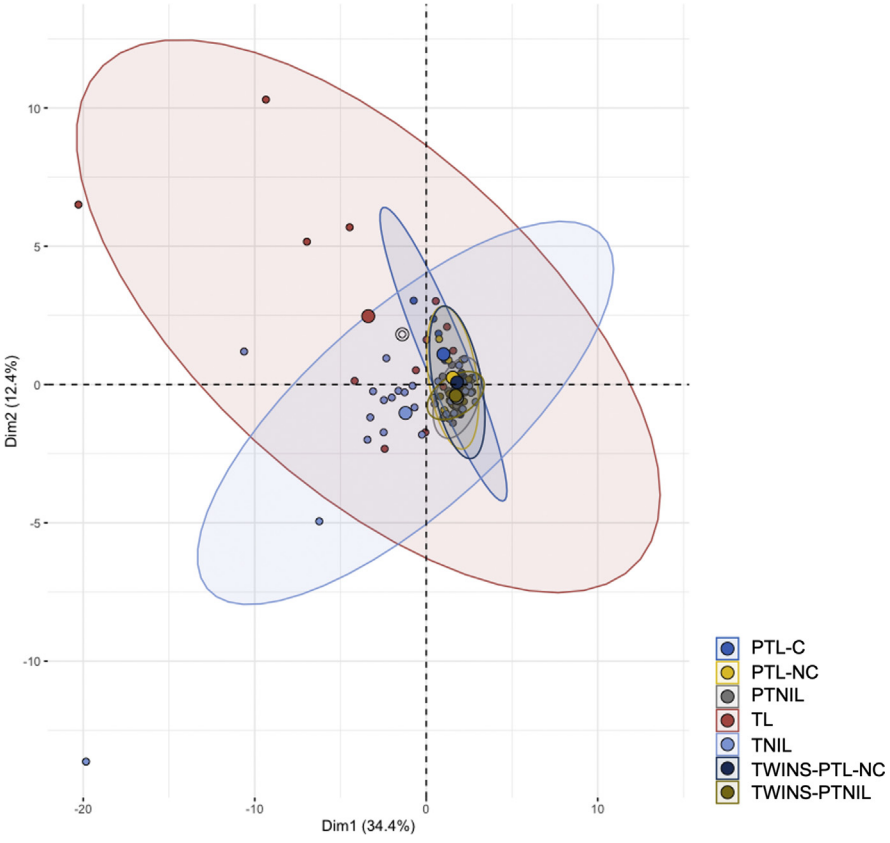
FIGURE 2
Principal component analysis



PCA of all 44 candidate genes for each myometrial phenotype plotted based on 3 principal components in 2 dimensions. A video demonstrating PCA of all 44 candidate genes for each myometrial phenotype plotted based on 3 principal components in 3 dimensions can be found in Supplementary Figure.

PCA, principal component analysis; *PTL-C*, preterm in labor with evidence of clinical chorioamnionitis; *PTL-NC*, preterm in labor with no evidence of clinical chorioamnionitis; *PTNIL*, preterm not in labor; *TL*, term in labor; *TNIL*, term not in labor; *TWINS-PTL-NC*, twin pregnancies—preterm labor with no evidence of clinical chorioamnionitis; *TWINS-PTNIL*, twin pregnancies—preterm not in labor.

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were assessed, was made interpretable through a PCA in which the first 3 PCs explained 57% of the data variance. Term myometrial samples from the TL and TNIL clinical groups clustered separately away from the preterm groups, which could suggest that preterm labor is a distinctly different process from term labor. Preterm myometrial samples from singleton and twin deliveries, regardless of laboring status or presence of acute clinical chorioamnionitis, failed to separate based on PCA. This suggests that the transcriptional behaviors of these tissues are similar despite the clinical differences among these preterm groups.

Results in the context of what is known

Although differences between term and preterm myometrial samples have been reported previously in literature, these have only been reported for a small number of genes, including *PTGS2*, *IL-8*, and *OXTR*[39] or the studies simply compared the changes in expression on the basis of gestational age alone,[58] neither of which provide global insights. Here we present the differences in expression among patient (myometrial) groups for a relatively large set of genes that represent known pathways of term labor, including inflammation, prostaglandin signaling, and hormone receptor signaling through estrogen and progesterone. Although SSH, which was used to derive this gene list, has been generally supplanted by RNA sequencing,[42] it is an alternative method that drastically reduces background data noise known to be problematic for RNA sequencing.

Based on previous studies,[59,60] we anticipated differences in the transcriptional activity between term and preterm laboring myometrial samples (particularly in the setting of clinical chorioamnionitis). Our data confirmed that the preterm myometrium seemed to be different from the term myometrium. However, we did not anticipate that the preterm laboring and preterm nonlaboring myometrium would be very closely clustered in the PCA. This was the case not only in singleton and twin pregnancies, but also in cases where there was evidence for clinical chorioamnionitis; however, the presence of chorioamnionitis did widen the data variance. This is in contrast with previous reports that demonstrated different *PTGS2* myometrial expression levels in the setting of chorioamnionitis.[41] Our data demonstrated that the transcriptional activity based on the 44 candidate genes that are important during term labor does not seem to have the same pattern of expression in the preterm myometrium. This would suggest that there may be different myometrial pathways responsible for preterm labor, that the magnitude of the change required may be different at preterm gestation, or that changes outside the myometrium (such as the cervix or fetal membranes) may be important for initiating and sustaining the process of preterm labor. There is a great body of evidence supporting that changes in the cervix[47,61,62] and fetal membranes[63] are important for term labor, however, the factors contributing to preterm gestation have yet to be fully elucidated. More recently, transcriptomic studies have revealed signatures in the cervix associated with preterm premature rupture of membranes and preterm labor,[64] highlighting the cervix as an important mediator of PTB.

We acknowledge that although the gestational age is a major confounder in

this study, the similarities among all preterm groups and their differences from term laboring myometrial samples support the concept that preterm and term labor are different. Other possible explanations include systematic errors in tissue sampling; however, our samples were confirmed to be myometrial in nature on histologic examinations (there were 46 histologic confirmations, all of which confirmed that the samples were from the myometrium), our biopsy techniques are identical to published reports that have demonstrated a 98% likelihood of sampling smooth muscle myometrium,[65] and we routinely utilized the biopsied myometrium samples to conduct contraction assays,[10,66–69] thus confirming the rhythmic contractile nature of the sampled tissue.

Clinical implications

All effective preventative treatments for managing PTB currently target cervical function, including vaginal progesterone or cervical cerclage.[70,71] Furthermore, cervical shortening is 1 of the most reliable clinical measures for predicting PTB,[72] whereas cervical fetal fibronectin is used for preterm labor prediction.[73] We again note that current therapeutics for tocolysis, which target uterine contractility, do not seem to be effective in stopping preterm labor and our data provide a possible explanation. Therapies to halt preterm labor should not be based on the physiology of myometrial activation at term.

Research implications

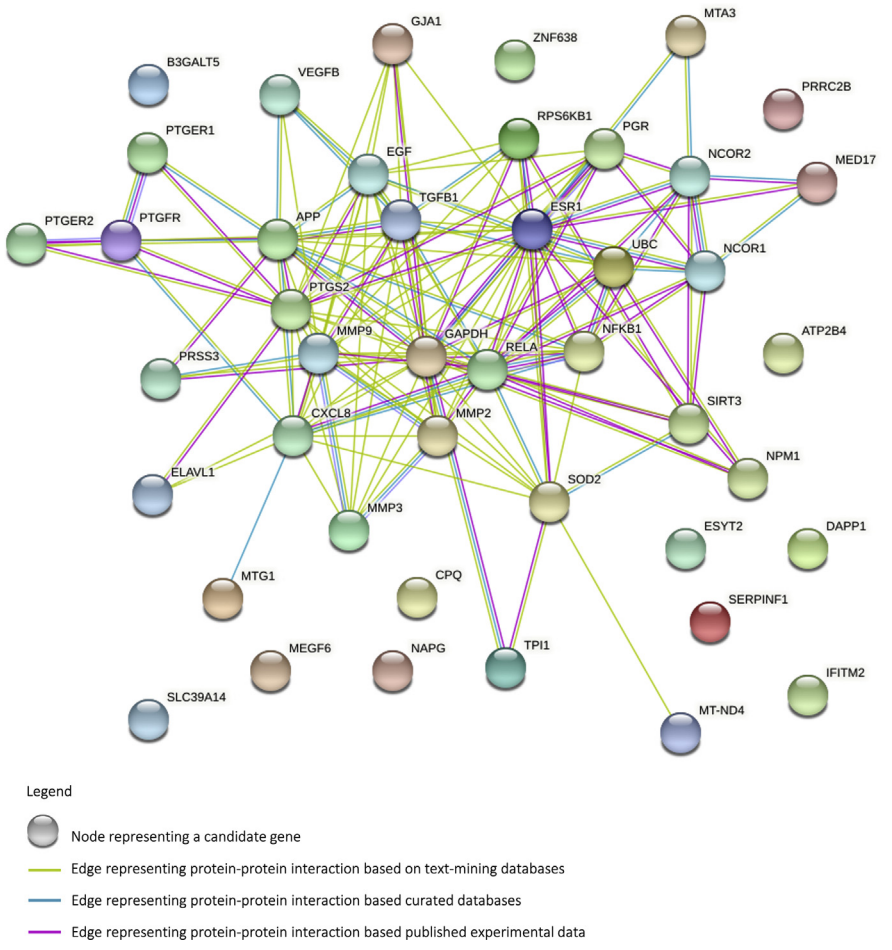
We believe that these data provide important contributions to what little is known about PTB and its underlying mechanisms. Furthermore, future research into the mechanisms of preterm labor should concurrently consider changes outside of the myometrium, which may include changes in the cervix or membranes.

Strengths and limitations

The strengths of our data include the well-characterized study populations, especially the preterm groups. This study characterized the expression of a large set of genes, considered to be important in term labor, by qPCR and demonstrated that the gene expression patterns in preterm labor are distinct from those of term labor in the human myometrium.

The limitations of our data include intergroup sample size differences, particularly the sample size within the laboring groups, and the heterogeneity introduced by the indications for CDs. This reflects the challenges of obtaining myometrial samples from laboring women. A further weakness is that the SSH data likely provided only a subset of the changes in gene expression among women during labor. It is possible that using a nontargeted approach to interrogate the myometrium may identify changes in gene expression in the preterm clinical groups not seen in the candidate genes presented here. Furthermore, our myometrial biopsies are taken from the lower uterine segment (which may not be representative of the myometrium in the fundus)[47] and are obtained following the delivery of the fetus (which may not be truly reflective of the laboring status). However, all myometrium samples were obtained within 10 minutes of delivery of the fetus and therefore although changes in expression of the candidate genes may have occurred, marked expression

FIGURE 3
The protein-protein interaction analysis of candidate genes



The protein-protein interaction analysis of the 44 candidate genes demonstrates significant interactions among these candidates with 143 edges with a protein-protein enrichment *P* value of $<1\times10^{-16}$; the expected number of edges of a randomly selected group of candidates of this size is 62.

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differences seem unlikely. This study did not include concurrent transcriptomic assessment of the fetal membranes and cervix, which are likely to be important during labor.

Finally, our diagnosis of acute clinical chorioamnionitis is limited by the lack of culture-proven evidence of infection and amniotic fluid assessment. It is well recognized that acute histologic chorioamnionitis is not synonymous with microbial-associated intraamniotic infection. In fact, histologic chorioamnionitis caused by sterile inflammation is markedly more common and is often associated with labor or prematurity.[74] Our data support this and show that 50% of the available placental histology in the PTL-NC group had histologic evidence for chorioamnionitis, however, subclinical, microbial-associated chorioamnionitis cannot be excluded in this group. In the absence of amniocentesis, our study has distinguished acute clinical chorioamnionitis (microbial-associated) from acute histologic chorioamnionitis by clinical signs, symptoms, and investigations previously defined by Gibbs et al.[75,76] The diagnostic accuracy of this criterion has recently been assessed in a preterm cohort and has been correlated with amniotic fluid assessments, which showed that 78% of patients with a diagnosis of acute clinical chorioamnionitis had either a positive microbial culture or evidence of histologic inflammation.[77] Future studies investigating acute chorioamnionitis should include amniotic fluid assessment comprising microbial culture, gram staining, and other measures of inflammation.

Conclusions

Our targeted gene study of 44 candidate genes showed that preterm labor is associated with a pattern of myometrial gene expression that is distinct from term labor. Consideration should be given to different myometrial processes or other tissues that may be responsible for preterm labor. Future therapeutic approaches to prevent preterm labor should therefore not be based solely on the physiology of term labor. ■

Glossary of Terms

Abbreviation	Terms
cAMP	Cyclic adenosine monophosphate
CAP	Contraction-associated protein
CRH	Corticotropin releasing hormone
CD	Cesarean delivery
FDR	False discovery rate
GO	Gene ontology
hCG	Human chorionic gonadotropin
KNN	K-nearest neighbor
PBS	Phosphate-buffered solution
PC	Principal component
PCA	Principal component analysis
PTB	Preterm birth
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
RNAseq	Ribonucleic acid sequencing
SSH	Suppression subtractive hybridization
TLR	Toll-like receptor

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Data available on request.

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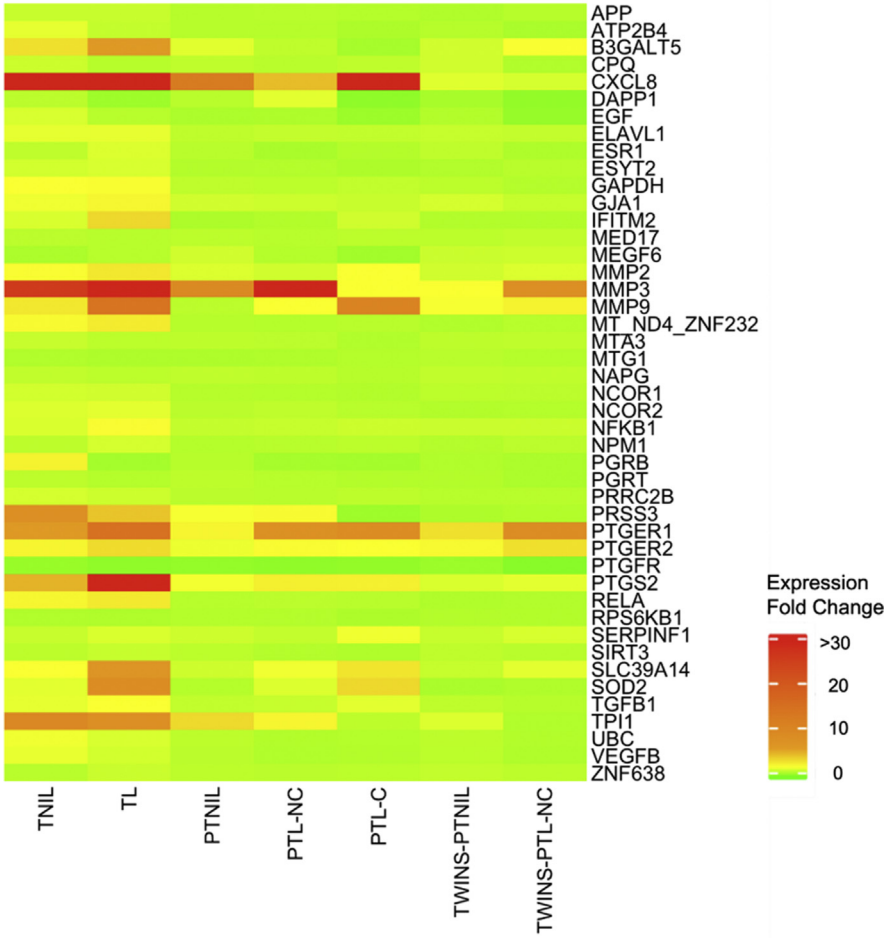
SUPPLEMENTAL FIGURE

Heatmap of average gene expression based on patient groups



PTL-C, preterm in labor with evidence of clinical chorioamnionitis; *PTL-NC*, preterm in labor with no evidence of clinical chorioamnionitis; *PTNIL*, preterm not in labor; *TL*, term in labor; *TNIL*, term not in labor; *TWINS-PTNIL*, twin pregnancies-preterm not in labor; *TWINS-PTL-NC*, twin pregnancies-preterm labor with no evidence of clinical chorioamnionitis.

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SUPPLEMENTAL TABLE 1
TaqMan assays for candidate genes

Gene symbol	Name	TaqMan assay ID
Suppression subtractive hybridization study: up-regulated genes		
CPQ	Carboxypeptidase Q	Hs01550609_m1
CXCL8	C-X-C motif chemokine ligand 8	Hs00174103_m1
IFITM2	Interferon induced transmembrane protein 2	Hs00829485_sH
MED17	Mediator complex subunit 17	Hs00188669_m1
MMP2	Matrix metallopeptidase 2	Hs01548727_m1
MMP3	Matrix metallopeptidase 3	Hs00968305_m1
MMP9	Matrix metallopeptidase 9	Hs00957562_m1
NAPG	NSF attachment protein gamma	Hs00909795_m1
NFκB1	Nuclear factor kappa B subunit 1	Hs00765730_m1
NPM1	Nucleophosmin 1	Hs02339479_g1
PTGER1	Prostaglandin E receptor 1	Hs00909194_g1
PTGER2	Prostaglandin E receptor 2	Hs00168754_m1
PTGFR	Prostaglandin F receptor	Hs00168763_m1
PTGS2	Prostaglandin-endoperoxide synthase 2	Hs00153133_m1
RELA	RELA proto-oncogene, NF-κB subunit	Hs01042014_m1
SERPINF1	Serpin family F member 1	Hs01106937_m1
SLC39A14	Solute carrier family 39 member 14	Hs00299262_m1
SOD2	Superoxide dismutase 2	Hs00167309_m1
TPI1	Triosephosphate isomerase 1	Hs03679721_g1
Suppression subtractive hybridization study: down-regulated genes		
ATP2B4	ATPase plasma membrane Ca^{2+} transporting 4	Hs00608066_m1
B3GALT5	Beta-1,3-galactosyltransferase 5	Hs00195943_m1
DAPP1	Dual adaptor of phosphotyrosine and 3-phosphoinositides 1	Hs01125914_m1
EGF	Epidermal growth factor	Hs01099990_m1
ESYT2	Extended synaptotagmin 2	Hs00294020_m1
MEGF6	Multiple EGF like domains 6	Hs00390990_m1
MT-ND4	Mitochondrially encoded NADH dehydrogenase 4	Hs02596876_g1
MTA3	Metastasis associated 1 family member 3	Hs00383033_m1
MTG1	Mitochondrial ribosome associated GTPase 1	Hs00536594_m1
PRRC2B	Proline rich coiled-coil 2B	Hs00261876_m1
PRSS3	Serine protease 3	Hs00605637_m1
RPS6KB1	Ribosomal protein S6 kinase B1	Hs00356367_m1
SIRT3	sirtuin 3	Hs00953477_m1
VEGFB	Vascular endothelial growth factor B	Hs00173634_m1
ZNF638	Zinc finger protein 638	Hs00963941_m1

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SUPPLEMENTAL TABLE 1
TaqMan assays for candidate genes *(continued)*

Gene symbol	Name	TaqMan assay ID
Up-regulated genes from bioinformatics and ingenuity pathway analysis of suppression subtractive hybridization study data		
APP	Amyloid beta precursor protein	Hs00169098_m1
ELAVL1	ELAV like RNA binding protein 1	Hs00171309_m1
ESR1	Estrogen receptor 1	Hs01046816_m1
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Hs02758991_g1
GJA1	Gap junction protein, alpha 1	Hs00748445_s1
NCOR1	Nuclear receptor corepressor 1	Hs01094541_m1
NCOR2	Nuclear receptor corepressor 2	Hs00196955_m1
PGR	Progesterone receptor	Hs04419616_s1
TGFB1	Transforming growth factor beta 1	Hs00998133_m1
UBC	Ubiquitin C	Hs00824723_m1

ATP, adenosine triphosphate; *EGF*, epidermal growth factor; *GTP*, guanosine triphosphate; *NADH*, nicotinamide adenine dinucleotide hydrogen; *NF-κB*, nuclear factor kappa B; *NSF*, N-ethylmaleimide-sensitive factor.

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SUPPLEMENTAL TABLE 2

Two-class unpaired comparisons using Wilcoxon tests with a false discovery rate of 0.1 and a significant fold change threshold ≥1.5 (threshold for down-regulated genes are expressed as <0.67)

Comparison 1: TL with TNIL Gene ID	Fold change	Nominal *P* value	*q* value
CXCL8	27.31	.02	<.01
EGF	9	.99	.04
PTGS2	7.44	.02	<.01
PTGER1	5.85	.08	<.01
SOD2	5.04	.02	<.01
MMP9	3.78	.03	.08
PRSS3	3.48	.71	<.01
SLC39A14	2.99	.03	<.01
MMP3	2.24	.03	<.01
IFITM2	2.07	.03	<.01
VEGFB	0.66	.18	.04
ATP2B4	0.56	.21	.04
Comparison 2: PTNIL with TNIL **Gene ID**	**Fold change**	**Nominal *P* value**	***q* value**
NCOR2	0.66	.03	<.01
ESYT2	0.63	.02	<.01
ELAVL1	0.63	.02	<.01
SLC39A14	0.62	.06	<.01
VEGFB	0.58	.02	<.01
SOD2	0.55	.03	<.01
UBC	0.53	.02	<.01
GAPDH	0.51	.02	<.01
IFITM2	0.51	.04	<.01
EGF	0.49	.03	<.01
PTGER1	0.42	.04	<.01
MT-ND4	0.39	.02	<.01
B3GALT5	0.39	.05	<.01
RELA	0.38	.02	<.01
PTGS2	0.35	.02	<.01
MMP9	0.31	.05	<.01
CXCL8	0.29	.02	<.01
Comparison 3: TWIN-PTNIL with TNIL **Gene ID**	**Fold change**	**Nominal *P* value**	***q* value**
NCOR2	0.64	.06	<.01
ESYT2	0.6	.03	<.01
VEGFB	0.57	.05	<.01
UBC	0.56	.06	<.01
SLC39A14	0.54	.06	<.01
SOD2	0.5	.03	<.01

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(continued)

SUPPLEMENTAL TABLE 2

Two-class unpaired comparisons using Wilcoxon tests with a false discovery rate of 0.1 and a significant fold change threshold ≥1.5 (threshold for down-regulated genes are expressed as <0.67) *(continued)*

Comparison 3: TWIN-PTNIL with TNIL Gene ID	Fold change	Nominal *P* value	*q* value
ATP2B4	0.5	.05	<.01
IFITM2	0.5	.15	.06
GAPDH	0.46	.03	<.01
PTGFR	0.45	.11	.05
EGF	0.41	.04	<.01
RELA	0.36	.02	<.01
MT-ND4	0.36	.02	<.01
PTGS2	0.32	.05	<.01
MMP3	0.26	.03	<.01
TPI1	0.18	.04	<.01
CXCL8	0.06	.15	.06
Comparison 4: TWIN-PTNIL with PTNIL			
No differences			
Comparison 5: PTL-NC with PTNIL **Gene ID**	**Fold change**	**Nominal *P* value**	***q* value**
MMP3	6.99	.05	<.01
CXCL8	4.56	.03	<.01
PTGER1	3.47	.04	<.01
MMP9	2.34	.03	<.01
SOD2	1.68	.03	<.01
Comparison 6: PTL-C with PTNIL **Gene ID**	**Fold change**	**Nominal *P* value**	***q* value**
CXCL8	12.7	.05	<.01
MMP9	9.98	.02	<.01
SOD2	4.55	.04	<.01
PTGER1	3.54	.05	<.01
SLC39A14	3.14	.04	<.01
IFITM2	1.85	.03	<.01
PRSS3	1.52	.17	.09
MEGF6	0.52	.12	.06
EGF	0.49	.03	<.01
TPI1	0.32	.1	.06
Comparison 7: TWIN-PTL-NC with TWIN-PTNIL			
No differences			
Comparison 8: PTL-C with PTL-NC **Gene ID**	**Fold change**	**Nominal *P* value**	***q* value**
SERPINF1	1.59	.03	<.01

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SUPPLEMENTAL TABLE 2

Two-class unpaired comparisons using Wilcoxon tests with a false discovery rate of 0.1 and a significant fold change threshold ≥1.5 (threshold for down-regulated genes are expressed as <0.67) *(continued)*

Comparison 9: PTL-NC with TL Gene ID	Fold change	Nominal *P* value	*q* value
DAPP1	2.56	.03	.01
PRSS3	1.77	.08	.02
ELAVL1	0.65	.04	<.01
TGFB1	0.62	.12	.01
VEGFB	0.61	.05	<.01
EGF	0.61	.11	.01
UBC	0.59	.03	<.01
ESYT2	0.59	.05	<.01
GJA1	0.53	.02	<.01
NFKB1	0.51	.06	<.01
B3GALT5	0.5	.06	<.01
GAPDH	0.45	.02	<.01
MMP2	0.45	.02	<.01
TPI1	0.44	.08	<.01
RELA	0.35	.02	<.01
PTGFR	0.34	.11	.01
MT-ND4	0.33	.02	<.01
IFITM2	0.27	.02	<.01
SLC39A14	0.27	.03	<.01
SOD2	0.17	.03	<.01
PTGS2	0.06	.03	<.01
CXCL8	0.04	.03	<.01
Comparison 10: TWIN-PTL-NC with PTL-NC			
No differences			
Comparison 11: PTL-NC with TNIL **Gene ID**	**Fold change**	**Nominal *P* value**	***q* value**
ELAVL1	0.65	.04	<.01
ESYT2	0.63	.02	<.01
GAPDH	0.51	.05	<.01
VEGFB	0.49	.02	<.01
UBC	0.47	.02	<.01
EGF	0.43	.03	<.01
RELA	0.42	.04	<.01
MT-ND4	0.41	.03	<.01
PTGFR	0.32	.03	<.01

PTL-C, preterm in labor with evidence of clinical chorioamnionitis; *PTL-NC*, preterm in labor with no evidence of clinical chorioamnionitis; *PTNIL*, preterm not in labor; *TL*, term in labor; *TNIL*; term not in labor; *TWIN-PTL-NC*, twin pregnancies-preterm labor with no evidence of clinical chorioamnionitis; *TWIN-PTNIL*, twin pregnancies-preterm not in labor.

Phung et al. Preterm laboring myometrium is distinct from term laboring myometrium. Am J Obstet Gynecol 2022.