Published in final edited form as:

Am J Obstet Gynecol. 2010 May ; 202(5): 474.e1–474.20. doi:10.1016/j.ajog.2010.02.034.

Human Effector / Initiator Gene Sets That Regulate Myometrial Contractility During Term and Preterm Labor

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Abstract

Objective—Distinct processes govern transition from quiescence to activation during term (TL) and preterm labor (PTL). We sought gene sets responsible for TL and PTL, along with the effector genes necessary for labor *independent* of gestation and underlying trigger.

Methods—Expression was analyzed in term and preterm +/- labor (n =6 subjects/group). Gene sets were generated using logic operations.

Results—34 genes were similarly expressed in PTL/TL but absent from nonlabor samples (*Effector Set*). 49 genes were specific to PTL (*Preterm Initiator Set*) and 174 to TL (*Term Initiator Set*). The gene ontogeny processes comprising *Term Initiator* and *Effector Sets* were diverse, though inflammation was represented in 4 of the top 10; inflammation dominated the *Preterm Initiator Set*.

Comments—TL and PTL differ dramatically in initiator profiles. Though inflammation is part of the *Term Initiator* and the *Effector Sets*, it is an overwhelming part of PTL associated with intraamniotic inflammation.

Keywords

effector; initiator; labor; myometrium; preterm birth

Background and Objectives

Successful parturition requires the synchronization of uterine events (myometrial activation, myometrial contraction, cervical ripening, cervical dilation and rupture of the fetal membranes)

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Presented in part at the 2006 annual meeting of the Society for Gynecologic Investigation

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¹. The myometrium specifically must undergo a series of molecular and biochemical changes that transition it from the phase of quiescence, which is characterized by a loss of responsiveness to contractile agents, to the phase of activation with subsequent onset of labor ^{2, 3}. Many prior investigators have assumed the triggers of parturition are similar regardless of the gestational age ^{2, 4–9} and focused on the control of term labor as a surrogate for preterm labor. This approach has not met with great success.

Genomics helps expand and characterize the number of molecular pathways that potentially define an underlying condition. Since our initial report in 2000¹⁰, oligonucleotide / cDNA microarrays have been used by many groups for the study of biological processes involved in normal term labor ^{11–18}. The results of such studies using myometrium obtained from term laboring women suggest that contractile stimulators of labor include but are not limited to hormone receptors, cell adhesion molecules, interleukins, prostaglandins, and gap junctions ^{10, 11, 13–15, 17}. Others have attempted to correlate human myometrial transcriptional levels at term with samples from idiopathic, spontaneous preterm labor, and concluded the mechanisms underlying parturition at all stages of pregnancy are related^{1, 13, 17}. This is surprising as there are several well accepted ‘causes’ of spontaneous preterm birth ^{19, 20}. While molecules within these groups may well influence parturition during normal labor, there is little study of myometrial gene expression in women with either preterm or dysfunctional term/preterm labor. Array type investigations have also been used to explore myometrial gene expression in the pregnant rodent ^{11, 18, 21}. However, the resulting conclusions should be interpreted cautiously as the gestational and hormonal patterns of these species are not homologous to the human. And while these studies provide insight, they tend as a group to be biased by the limited number of genes included on the array and the number of genes selected for confirmatory study.

Rather than compare term labor to one of several models for preterm birth, we investigated human pregnancy and hypothesized there should be a core set of genes whose expression was necessary for the process of labor independent of gestational age (GA) and the underlying trigger for the labor. We further hypothesized this *Effector Gene Set* should relate to myometrial contractility and the cellular activities necessary to sustain it. We also hypothesized there must be *Initiator Gene Sets* responsible for the transition of the myometrium from quiescence to activation. But unlike the *Effector Set*, which would be unaffected by the underlying labor stimulus, there should be separate *Initiator Gene Sets* for term and preterm labor that reflect the underlying mechanism of labor. The potential result of identifying these gene sets could be the development of alternative treatment options targeted at preterm and term or dysfunctional labor. The purpose of this investigation was to test our hypotheses in a series of women undergoing cesarean delivery at term or preterm, in labor or absent labor.

Material and Methods

Study Design

Myometrium was obtained from the upper pole of the transverse lower uterine segment incision of 4 groups of women (n=6 per group) at the time their primary cesarean section at Yale University: (i) preterm not in labor and no inflammation (PTNL; mean GA 28.8w, range 25.4–32.5w); (ii) preterm in labor with inflammation (PTL; mean GA 29.7w, range 25.1–32.6w); (iii) term not in labor (TNL; mean GA 39.3w, range 38.4–41.0w); and (iv) term labor (TL; mean GA 40.2w, range 39.0–41.2w). The Yale University IRB approved the protocol for sample collection, and all women provided informed written consent. Labor was defined by presence of regular uterine contractions accompanied by progressive cervical dilation. The diagnosis of intra-amniotic inflammation was based on an amniotic fluid Mass Restricted (MR) Score of 3 or 4 plus more than 100 WBCs/ μL ³ in the context of a positive amniotic fluid culture in a sample obtained by transabdominal amniocentesis ^{22–25}. These tests provide the most accurate tools currently available to maximize the likelihood of sample homogeneity. The MR

Score provides qualitative information regarding the presence or absence of intra-amniotic inflammation. Briefly, the Score ranges from 0 to 4, depending on the presence (assigned a value of 1) or absence (assigned a value of 0) of each of four protein biomarkers²⁵. A score of 3–4 indicates inflammation, whereas a score of 0–2 excludes it. This biomarker pattern is predictive of preterm birth, histological chorioamnionitis and adverse neonatal outcome. A detailed description of the MR method has previously been published^{22–25}. Indications for cesarean delivery in the PTNL group were all related to preeclampsia, and breech presentation in the TNL cesarean deliveries. The indication for cesarean in the TL group was an arrest of cervical dilation at 6 cm or greater. No normal laboring patient at term underwent cesarean section in this sample. Clinical data were retrieved from the medical records and statistical analysis of patient demographics performed using one-way ANOVA followed by Newman Keuls Post Hoc test. All laboratory studies were performed at either the University of Kansas School of Medicine or the University of Maryland Baltimore School of Pharmacy.

Isolation of RNA

Total RNA was isolated using TRIzol™ Reagent (Invitrogen™, Carlsbad, CA) according to the manufacturer's protocol. The purity and integrity of each RNA sample was assessed by spectroscopy and formaldehyde-agarose gel electrophoresis.

Microarray Preparation

Myometrial gene profiling was performed on each individual RNA sample using the Affymetrix GeneChip Human Genome U133 Plus 2.0™ microarray (Affymetrix, Santa Clara, CA) containing some 38,500 human genes. RNA quality was reassessed prior to spotting using the Agilent 2100 Bioanalyzer™ (Agilent Technologies, Palo Alto, CA). Once the quality was confirmed, biotin-labeled cRNA was generated and 20 µg hybridized to the microarrays using the manufacturer's standard conditions. Image processing utilized an Affymetrix GeneArray 3000™ scanner.

Microarray Data Processing and Statistical Analysis

Oligonucleotide microarrays were analyzed using the Affymetrix Expression Console™. Gene expression levels were normalized using the R Statistical package and software available through the Bioconductor Project (www.bioconductor.org). The process normalizes gene expression using a background adjustment procedure and a sequence-specific expression method as described by Wu et al²⁶. Normalized microarray data were then subject to further discriminative analysis. A detection P value was used to make a reliable call of gene expression (present, marginal, or absent). Genes present in less than 4 of the six patient samples were classified as absent overall and not considered further.

To identify the *Effector* and *Initiator Gene Sets*, we conducted a series of logic operations based on gene presence (detection P value) and illustrated by the Venn diagram in Figure 1. We first generated four groups of genes based on the following:

Group A = (PTL-PTNL) Group C = (TL-TNL)
Group B = (Group A-TNL) Group D = (Group C-PTNL)

The *Effector Gene Set* was defined as those genes common to Groups B and D. The *Effector Set* was further filtered to exclude genes whose expression during PTL changed 2-fold or more in either direction compared to TL, reasoning there was something unique to the disease state influencing gene expression. The *Preterm Initiator Gene Set* was defined as those genes in Group A exclusive to PTL (minus Group C). And lastly, the *Term Initiator Gene Set* was defined as those genes in Group C exclusive of term labor (minus Group A).

Network Analysis and Biological Classification

Identification and visual analysis of gene networks were performed using MetaCore™ analytical suite version 2.0 (GeneGo, St. Joseph, MI). There were few direct interactions identified among the individual proteins of the *Effector*, *Preterm Initiator*, and *Term Initiator* genes. Therefore, we analyzed the possible networks that could be built from such relatively large gene sets using an algorithm that generates sub-networks highly saturated with input genes from each dataset (i.e., Effector set, Preterm Initiator set, and Term Initiator set). In this case, the network algorithm takes a list of root nodes (i.e., genes from Effector, Preterm Initiator, and Term Initiator sets) and for each one creates shortest paths networks to other root nodes in the list and stops the network expansion at a size of 50 nodes (standard number predefined in MetaCore). The resulting networks are evaluated and ranked according to their statistical significance (p-values). This high trust p-value calculation was also used to evaluate the network's relevance to Gene Ontology (GO) biological processes classification. In general, GO is a method of standardizing the representation of gene and gene product attributes across species and databases. Three Gene Ontology functional ontologies (processes, molecular functions, and localizations) are used in Metacore™ for enrichment analysis. The advantage of using this network algorithm is that it may find a well-connected cluster of root nodes without any predefined restrictions and as a result offers more flexibility in identifying possible connections. These interactions were then assigned to specific biological processes, cellular components, and/or molecular functions to further characterize the underlying condition to yield insight into the underlying mechanisms. We selected some genes of interest for confirmation by q-rtPCR based on their participation in sub-networks with a low p-value. Other genes selected from the input list were selected for q-rtPCR analysis based on their statistically significant association with GO processes.

Quantitative Real Time PCR (Q-rtPCR)

Total RNA from the original samples used for the microarrays was used to quantify each gene. Primer sequences for amplifications were based on previously published cDNA sequences using the Beacon Designer program (BioRad, Hercules, CA). SYBR green (BioRad, Hercules, CA) was used for amplicon detection. All primer sets were tested to ensure the efficiency of amplification over a wide range of template concentrations. PCR reactions were carried out in the iQ5 real-time PCR detection system (BioRad, Hercules, CA). A melting curve was performed after amplification to ensure all samples exhibited a single amplicon. Gene expression of each individual myometrial sample was first normalized to the expression of 18S rRNA (internal control) using the ΔCT method²⁷ and then compared to the average of the corresponding control samples. *mRNA relative expression is equal to $2^{-\Delta \Delta CT}$ and determined by the following equations:*

$$\begin{aligned}\Delta\Delta CT &= \Delta CT 1 \text{ (each individual sample)} - \Delta CT 2 \text{ (control group mean)} \\ \Delta CT 1 &= \text{gene CT value} - 18S \text{ CT value of the same sample; and} \\ \Delta CT 2 &= \text{average of (gene CT value of control group} - 18S \text{ CT value of control group)}\end{aligned}$$

The point at which the fluorescence crosses the threshold is called the CT value, and is read by the RT-PCR instrument as a specific parameter.

Results

Clinical Characteristics of Myometrial Samples

The clinical characteristics of the pregnancies included are listed in Table 1. There were no statistically significant differences in gestational age or birth weight among women at term (no

labor vs. labor) or preterm (no labor vs. labor). There were no statistically significant differences in maternal age as determined by one-way ANOVA. Thus, the observed differences in genes in the initiator groups are most likely the result of labor (term or preterm) rather than any variation in gestational age or maternal age.

Identification of Effector Set and the Term Initiator and Preterm Initiator Sets

Sixty seven (67) genes were present in both PTL and TL but absent from all non-labor samples (n=6) (Groups B and D). This group was termed the *Effector Set* since the genes comprising it represent a distinct group that might act to sustain the labor itself rather than contributing to its onset. This *Effector Set* was then further filtered to exclude genes showing at least a 2-fold difference between PTL and TL based on the assumption there was something about the disease process which led to differential regulation. This left 34 genes in the *Effector Set* (Table 2).

While the *Effector Gene Set* was by definition independent of GA, we suspected there must exist gene sets that enabled the initiation of the labor either term or preterm. We detected 49 genes specific to preterm labor alone (*Preterm Initiator Set*, Table 3) and 174 specific to term labor alone (*Term Initiator Set*, Table 4). In light of the gestational ages of the preterm subjects, it is highly likely the selected gene sets represent or at least include molecular groups responsible for initiating the transition from myometrial quiescence to activation in their respective categories.

Characterization of Initiator and Effector Gene Sets

It is not adequate to categorize genes based solely on a given condition. Rather, there must be an understanding of how the encoded proteins interact. The result of these interactions is a universe representing the functional potential in terms of complex protein associations, such as pathways with successive reactions and transient structural complexes. We reasoned that characterization of networks based on the identified *Initiator* and *Effector* Gene Sets would add insight into the molecular events regulating preterm and term labor. Thus, we enriched the *Initiator* and *Effector* sets using the GO classifications. Unlike standard GO classification systems that produce a vague and general understanding of the molecular events underlying the given networks, GeneGo™ GO classifies gene groups within a specified realm of biological processes, molecular function, and cellular components. GeneGo processes are network modules of main cellular processes manually created on the basis of GO processes and pathway maps.

The specificity of each gene set allows for a gene to be present in only one of the three groups. The *Effector Set* (Figure 2a) and the *Term Initiator Set* (Figure 3a) were each dominated by inflammatory GO processes, though distinctly different from each other. Each had 4 significant processes (based on P-value and FDR calculation) within the top ten GeneGo processes identified involved with inflammation. These findings clearly support the concept that inflammation plays an important role in term labor.

Perhaps not surprisingly, considering the patient selection criterion, the *Preterm Initiator Set* was also heavily associated with inflammation- all but two of the top ten GeneGo processes (Figure 4a). Of interest was the appearance of 'Hypoxia and Oxidative Stress' (number five in Figure 4a) as we have recently demonstrated that chronic hypoxia alone stimulates a response that is clinically the same as the Fetal Inflammatory Response Syndrome²⁸.

Visualization of Term Initiator, Preterm Initiator, and Effector Networks

The traditional approach to gene array analyses includes a list with the genes clustered based on biological similarity (Tables 2–4). While this method provides insight into the transcriptional activity of major biological processes, it fails to express how the encoded

proteins might interact with each other and surrounding proteins. We previously demonstrated that the identification of gene networks provides a more robust tool by which to extract information from the microarrays than gene lists¹⁶. These networks incorporate genomic data and represent the encoded proteins in the form of predicted interactions and reactions compiled from different experiments and experimental conditions.

There were few direct interactions identified among the proteins of the 34 *Effector* genes, the 49 *Preterm Initiator* and 174 *Term Initiator* genes. This supports our supposition of gene set uniqueness. We then analyzed the possible networks that could be built from such gene sets using an algorithm that generates sub-networks highly saturated with the selected objects which are represented in Figures 2b, 3b, and 4b with a large red circle next to the network object. We selected sub networks based on their P-values. Figures 2b, 3b and 4b illustrate the top ranked identified sub networks in the *Effector* (n=9), *Preterm Initiator* (n=11) and *Term Initiator Sets* (n=30), respectively. The inward and outward bound lines extending from the selected genes (genes in experimental sets (red circles)) illustrate the interactive environment surrounding the selected genes and can be used to show how their encoded proteins are affected (positively (green), negatively (red), unknown (grey)) upstream or downstream within the network pathway. This information could have significant implications for the development and design of therapeutic entities targeted at inhibiting or stimulating the activity of the targeted gene or protein in the pathway.

Validation of Initiator and Effector Gene Sets

The high sensitivity of Q-rtpCR makes it likely small changes in gene expression exist that were undetected by the microarray analyses. And while we assume for microarray analyses that relevant genes were described by gene expression above a set expression threshold, we recognize the sensitivity of Q-rtpCR might reveal enhanced expression in genes unselected in the microarray analyses. Thus, we focused on sub network genes with high mRNA levels.

The idea that a unique group of encoded proteins is responsible for sustaining myometrial contractility during labor irrespective of gestational age (*Effector set*) has not been investigated previously. Based on our analyses, we selected HNT (neutrimin precursor), KCNAB2 (voltage-gated potassium channel, shaker-related subfamily) and RRAD (Ras-related associated with diabetes) (Figure 5). The expression of these genes has not previously been localized to the myometrium; nor have they been associated with labor. The microarray analyses and the logic process were confirmed to identify genes that were in similarly increased during labor independent of gestational age and mechanism.

By applying a similar analytic approach, we selected five genes for confirmation in the *Term Initiator Set* (Figure 6): PROK1 (prokineticin 1), IL9R (interleukin 9 receptor), EREG (epiregulin), BDKRB1 (bradykinin receptor B1), and IL13RA2 (interleukin 13 receptor, alpha 2). Of the 5, only PROK1 has previously been reported to be expressed in the reproductive tract (endometrial glands of nonpregnant women²⁹). In each instance except for IL9R (which declined significantly with labor), the microarray analyses and the logic process confirmed the identified genes were more highly expressed during term labor, though each was also effected by preterm labor but to a significantly smaller extent. There was a more than fourfold increase in expression of BDKRB1, EREG, and IL13RA2 during TL compared to PTL, and both EREG and BDKRB1 were increased in TL compared to TNL.

The genes selected for validation in the *Preterm Initiator genes* were CATSPER2 (cation channel, sperm associated 2 pseudogene 1) and PTPRZ1 (protein tyrosine phosphatase, receptor-type, Z polypeptide 1). The existence of the latter in rodent myometrium has previously been suggested^{30, 31}. We both confirm the presence of mRNA from both genes in

human myometrium, and that they are specifically increased in preterm labor associated with inflammation (Figure 7).

Comments

Models of labor previously proposed include endocrine and paracrine regulation, calcium signaling, inflammation secondary to infection, etc. Rather than compare term labor to one of many models of preterm birth, we hypothesized that labor is defined by a core set of genes whose expression is unaltered by gestational age and independent of the underlying labor trigger. We applied functional gene array profiling of myometrium obtained from preterm and term non-laboring and laboring women. While this is not the first time gene array technology has been used to map the molecular networks of labor (we first published such in 2006¹⁶), it is apparently the first attempt to differentiate genomically the molecular events that initiate and maintain myometrial activity in human labor at term and preterm. In our original report, we noted how genomic networks could be used to profile myometrial events during pregnancy and herein apply more advanced methodology to generate signature gene networks.

It is often assumed the triggers or initiators of human labor are similar regardless of gestation, hence an investigative focus on term labor as a model to understand preterm labor. Numerous researchers have shown term labor is characterized by an inflammatory response, and at least the majority of spontaneous preterm births are associated with inflammation^{8, 32–35}. The findings of the present study support the premise that inflammation is a central mechanism at term as such genes were heavily represented in the *Effector* and the *Term Initiator Gene Sets*. While many clinicians chose a tocolytic agent which targets inflammation (e.g., indomethacin), this approach has failed to reduce the prevalence of spontaneous preterm birth or dysfunctional labor. The results of the present investigation are more than a simple cataloging of genomic events. We identify activity along several pathways not previously known to be involved which are part of the *Effector* and *Initiator Gene Sets*. In fact, a large percentage of the involved genes identified in the current investigation have not previously been described in myometrium, much less shown to be altered by disorders of labor. We recognize that this is a first step. It remains now to explore the functional roles of the identified pathways to see how altering them affects the process of labor.

The *Effector Gene Set* (Figure 2a) encompasses genes whose products are involved in chemotaxis, cell adhesion, reproduction and neurohormone signaling, signal transduction, muscle contraction, vascularization and as noted earlier, inflammation. Microarray findings were confirmed for HNT, KCNAB2, and RRAD. HNT is grouped in GO processes related to biological and cell adhesion and cell recognition, all logical needs to sustain labor. The protein encoded is a member of the Ig domain containing glycosylphosphatidylinositol-anchoring adhesion molecules. As such, HNT may play a role in the coordination of myometrial cell contractions. Its expression has not previously been shown in human myometrium or to be affected by labor.

We previously demonstrated that pregnancy and sex hormones (estrogen and progesterone) alter G-protein mediated signaling³⁶ at least in part by modulating myometrial GTPase activity. RRAD encodes a GTPase molecule previously identified in the myocardiocyte and shown to interact with calmodulin, tropomyosin and CaM kinase II among others. Its expression in laboring myometrium is a new finding. Analyses placed it into the node with the most significant likelihood of not having occurred at random (gScore = 50.44). As a member of the *Effector Gene Set*, it could play a role coordinating and modulating the strength of the myometrial contraction.

KCNAB2 is another *Effector Gene Set* member of interest. This voltage-gated potassium channel represents the most complex class of voltage-gated ion channels from both functional and structural standpoints and fell into the second highest GO group (gScore 33.6). In other anatomic structures, they are involved in the regulation of the contraction rate in regularly beating muscles. A number of investigators have concluded voltage-gated potassium channels are fundamentally involved in the regulation of rodent myometrial contractility^{37–39}. Other voltage-gated potassium channels are regulated by sex hormones^{40, 41}. We previously demonstrated that the human chorion produces a factor or factors that inhibit myometrial contractility via the opening of a large conductance calcium-activated potassium channels⁴². KCNAB2 may help generate the increased frequency of myometrial contractions associated with labor regardless of gestational age.

The majority of pregnancy passes with the myometrium in a state of quiescence, a genomically distinct phase that renders it relatively unresponsive to contractile agonists. Labor follows myometrial activation, possibly due to complex alterations in the signaling mechanism(s). We hypothesized that in contrast to the *Effector Set*, there must exist specific *Initiator Gene Sets* responsible for that transition from quiescence to activation that reflect the specific underlying stimulus. The results of the current study support that working hypothesis. For example, the cytokine network is in sensitive balance throughout uncomplicated pregnancy, and we suspect a similar balance exists for encoded cytokines during pregnancy. However, despite the presence of several GO processes in the *Effector Gene Set* that reflect inflammation, interleukins are not seen in that grouping save for interleukin 8 receptor, beta. In myocardium, interleukin (IL)-8 is up regulated in areas of infarction and may induce neutrophil infiltration⁴³. In contrast to the *Effector Gene Set*, several cytokine genes are present in the *Term Initiator Gene Set*, including interleukin 1 receptor-associated kinase 3 (IRAK3), interleukin 24 (IL24), interleukin 1 beta (IL1B), IL13RA2, and IL9R (the latter was the only conflict between microarray and Q-rtPCR). Not found in any of the gene sets was interleukin 10 (IL-10), which has been implicated as an anti-inflammatory modulator of cytokine synthesis initiated by intrauterine infection^{44–48}. Its absence suggests it does not have a significant role in either inflammation preterm labor or term labor.

We have previously noted that myometrial quiescence and activation are associated with altered G protein expression and GTPase activity in both the guinea pig and human^{16, 36}. In addition to the expression of RRAD in the *Effector Gene Set*, there were several G-protein related proteins included in the *Term Initiator Gene Set* including G protein-coupled receptor 84 (GPR84), rab39 member of ras oncogene family (RAB39), ras-like family 11 member A (RASL11A), and ras association domain family 1 (RASSF1), most of which there is little known about and not previously documented to be expressed in the myometrium.

The presence of BDKRB1 and EREG expression in the *Term Initiator Set* are both provocative. There was more than a 4 fold increase in expression of BDKRB1 and EREG compared to PTL (Figure 6), suggesting preferential involvement in term labor. Contractile responses of nonpregnant human myometrium to bradykinin are reduced during the luteal phase⁴⁹. Wassdal et al noted that bradykinin contracted rodent myometrium via the same pathway as did oxytocin⁵⁰. In cultured decidua-derived cells, bradykinin stimulates the release of arachidonic acid, interleukin 6 (IL-6), and interleukin 8 (IL-8). These effects are prevented by the specific B2R antagonist Hoe 140⁵¹. Further, human decidua-derived cells express the B2R, and its expression is up regulated in response to IL- β , and bradykinin stimulates the secretion of further mediators by these cells. Finally, bradykinin is purported to trigger the release of PGE₂ from human myometrium⁵². Each of these characteristics of bradykinin could facilitate the onset of term labor. Epiregulin is expressed in myometrium from pregnant mice but not myometrium of pseudo pregnant mice⁵³. Induction of its synthesis has been linked to the RAF/MEK/ERK pathway⁵⁴.

In accordance with our current working hypothesis, we found that gene events associated with myometrial activation and term labor are not the same as those associated with preterm labor. Not surprisingly in light of the patient sample chosen for study, the *Preterm Initiator Gene Set* was dominated by inflammatory processes. The present study focused on inflammation mediated preterm labor as it is the most plentiful and the best described. Though not tested, our hypothesis requires the initiator set differ in women who experience preterm labor unassociated with inflammation (e.g., over distension with multiple gestation).

Despite the strong weighting of GeneGo processes toward inflammation in the *Preterm Initiator Gene Set*, there were a number of other genes worthy of follow-up in the future. For example, GSTT2, glutathione S-transferase theta 2 is a member of a superfamily of proteins that catalyze the conjugation of reduced glutathione to a variety of electrophilic and hydrophobic compounds. Human GSTs are subdivided into five classes: Alpha, Mu, Pi, Theta, and Zeta. The theta members GSTT1 and GSTT2 share 55% amino acid sequence identity and are both thought to have an important role in human carcinogenesis. An increase in its protein could reflect an increase in oxygen free radical production. We previously demonstrated that inflammation related preterm labor is associated with a pro-oxidant state in the newborn characterized by a decrease in reduced glutathione ⁵⁵.

The *Preterm Initiator Set* also included the transporter, ATP-binding cassette, sub-family A member 3 (ABCA3) whose protein is a member of the ABC1 subfamily. Members of the ABC1 subfamily comprise the only major ABC subfamily found exclusively in multicellular eukaryotes and may be involved in the development of resistance to xenobiotics and engulfment during programmed cell death. The function of ABCA3 in myometrium is unknown, but one possible role in the initiation of inflammation associated preterm labor could involve the exposure and presentation of potential antigens that trigger inflammation as a secondary response to the initiating event. The present study is the first report of its expression in myometrium and the impact of spontaneous preterm labor upon it.

It is becoming increasingly clear that the pattern of cytokine expression differs in preterm and term labor ⁵⁶. Therefore, cytokine expression may also differ among various tissues (i.e. myometrium) at different gestations. Increased protein and/or mRNA concentrations of IL-1 β , tumor necrosis factor alpha (TNF- α) and IL-6 in myometrium have each previously been associated with labor ⁴⁴. In the present study, none were included in the *Preterm Initiator Gene Set* suggesting the production is a downstream event or not specific to preterm labor. This conclusion is consistent with findings of previous investigators who found that the levels of cytokines such as IL-1 β , IL-6, and IL-8 were higher in TL compared to PTL myometrial samples ⁵⁷. The results of the current study do not support the use of TNF- α as a biomarker for inflammation specifically associated preterm labor. The TNF- α gene encodes a multifunctional proinflammatory cytokine that belongs to the TNF superfamily. It is involved in the regulation of a wide spectrum of biological processes, including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. Elevated transcription and accumulation of TNF- α and other cytokines in gestational tissues is proposed to activate prostaglandin synthesis which, in turn, leads to the myometrial contractions ^{58, 59}. Though not present in the *Preterm Initiator Gene Set*, it is a component of the *Term Initiator Gene Set* and thus may play a role with normal labor.

Regional differences in gene expression between the fundus, lower segment and cervix were reported in a prior study ¹². In the current study, we used biopsies from the upper edge of the lower uterine segment and emphasize our findings must be interpreted in this context. However, we previously determined that the collagen/muscle ratio is 20%/80% in lower segment biopsies collected from the upper lip of the incision⁶⁰. Thus, we believe that the muscle component to our biopsies is significant. Importantly, we found a significant increase in the contribution of

the muscular component in laboring tissues indicating that the lower segment participates morphologically in the process of labor. In the current study, our non-reliance on differences in levels of gene expression to derive biological significance, but rather by intersecting the presence of genes in biologically relevant instances should circumvent minor differences in regionalization of the myometrium versus the collagen component with either gestational age or labor.

Finally, it is interesting to look at the genes not included on any of the three gene sets described herein. It is striking that no list includes oxytocin or an oxytocin receptor. As it is recognized that oxytocin can induce its own receptor in rodent myometrium, it is surprising neither gene was present in the Effector Set. Nor does one of the three lists include representation of the eicosanoids or their related enzymes. And while there are numerous cytokines or cytokine pathway elements included in one of the three sets, IL-6, its receptor, TNF- alpha or its receptor, or IL-10 are not included. Their absence does not suggest these compounds are not involved in human parturition. Rather, it suggests their primacy in the process is other than anticipated and that their actions may be downstream events. Alternatively, there is no uniqueness in their expression during spontaneous term or inflammation associated preterm labor.

In conclusion, we have identified through gene profiling a specific set of labor activation/repression genes (the *Effector Gene Set*) whose expression is unaffected by the timing of the labor. Future searches for new tocolytic agents might benefit from a focus on these unique gene products. And while the *Effector Gene Set* is unchanged by the gestation of the labor, preterm labor and term labor differ dramatically in their *Initiator Gene Sets*. This finding is consistent with the suggestion that there are alternative pathways for preterm and term labor that trigger a common phenotype. Lastly, we confirm that term labor and preterm labor associated with inflammation are characterized by an inflammatory response, but they are not the same responses and they do not include several previously 'in vogue' genes. Thus, the classic evidence for inflammation as a cause of spontaneous preterm labor may well be a downstream event from myometrial gene activation.

Acknowledgments

Funding: This work was supported in part by grants from the PHS (U01 DP000187-02 [CPW] and R01HL049041-12 [CPW])

References

1. Breuiller-Fouche M, Germain G. Gene and protein expression in the myometrium in pregnancy and labor. *Reproduction* 2006;131:837–50. [PubMed: 16672349]
2. Carbillon L, Seince N, Uzan M. Myometrial maturation and labour. *Ann Med* 2001;33:571–8. [PubMed: 11817651]
3. Norwitz ER, Robinson JN, Challis JR. The control of labor. *N Engl J Med* 1999;341:660–6. [PubMed: 10460818]
4. Buxton IL. Regulation of uterine function: a biochemical conundrum in the regulation of smooth muscle relaxation. *Mol Pharmacol* 2004;65:1051–9. [PubMed: 15102932]
5. Hillhouse EW, Grammatopoulos DK. Role of stress peptides during human pregnancy and labour. *Reproduction* 2002;124:323–9. [PubMed: 12201805]
6. Lopez Bernal A. Mechanisms of labour--biochemical aspects. *BJOG* 2003;110 (Suppl 20):39–45. [PubMed: 12763110]
7. Mesiano S. Myometrial progesterone responsiveness and the control of human parturition. *J Soc Gynecol Investig* 2004;11:193–202.
8. Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel LA, Nien JK. Inflammation in preterm and term labour and delivery. *Semin Fetal Neonatal Med* 2006;11:317–26. [PubMed: 16839830]

9. Wray S, Jones K, Kupittayanant S, et al. Calcium signaling and uterine contractility. *J Soc Gynecol Investig* 2003;10:252–64.
10. Aguan K, Carvajal JA, Thompson LP, Weiner CP. Application of a functional genomics approach to identify differentially expressed genes in human myometrium during pregnancy and labour. *Mol Hum Reprod* 2000;6:1141–5. [PubMed: 11101697]
11. Bethin KE, Nagai Y, Sladek R, et al. Microarray analysis of uterine gene expression in mouse and human pregnancy. *Mol Endocrinol* 2003;17:1454–69. [PubMed: 12775764]
12. Bukowski R, Hankins GD, Saade GR, Anderson GD, Thornton S. Labor-associated gene expression in the human uterine fundus, lower segment, and cervix. *PLoS Med* 2006;3:e169. [PubMed: 16768543]
13. Charpigny G, Leroy MJ, Breuiller-Fouche M, et al. A functional genomic study to identify differential gene expression in the preterm and term human myometrium. *Biol Reprod* 2003;68:2289–96. [PubMed: 12606369]
14. Esplin MS, Fausett MB, Peltier MR, et al. The use of cDNA microarray to identify differentially expressed labor-associated genes within the human myometrium during labor. *Am J Obstet Gynecol* 2005;193:404–13. [PubMed: 16098862]
15. Havelock JC, Keller P, Muleba N, et al. Human myometrial gene expression before and during parturition. *Biol Reprod* 2005;72:707–19. [PubMed: 15509731]
16. Mason CW, Swaan PW, Weiner CP. Identification of interactive gene networks: a novel approach in gene array profiling of myometrial events during guinea pig pregnancy. *Am J Obstet Gynecol* 2006;194:1513–23. [PubMed: 16731067]
17. Rehman KS, Yin S, Mayhew BA, Word RA, Rainey WE. Human myometrial adaptation to pregnancy: cDNA microarray gene expression profiling of myometrium from non-pregnant and pregnant women. *Mol Hum Reprod* 2003;9:681–700. [PubMed: 14561811]
18. Salomonis N, Cotte N, Zambon AC, et al. Identifying genetic networks underlying myometrial transition to labor. *Genome Biol* 2005;6:R12. [PubMed: 15693941]
19. Gibb W, Challis JR. Mechanisms of term and preterm birth. *J Obstet Gynaecol Can* 2002;24:874–83. [PubMed: 12417902]
20. Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel L, Hassan S. The role of inflammation and infection in preterm birth. *Semin Reprod Med* 2007;25:21–39. [PubMed: 17205421]
21. Girotti M, Zingg HH. Gene expression profiling of rat uterus at different stages of parturition. *Endocrinology* 2003;144:2254–65. [PubMed: 12746283]
22. Buhimschi CS, Bhandari V, Hamar BD, et al. Proteomic profiling of the amniotic fluid to detect inflammation, infection, and neonatal sepsis. *PLoS Med* 2007;4:e18. [PubMed: 17227133]
23. Buhimschi CS, Buhimschi IA, Abdel-Razeq S, et al. Proteomic biomarkers of intra-amniotic inflammation: relationship with funisitis and early-onset sepsis in the premature neonate. *Pediatr Res* 2007;61:318–24. [PubMed: 17314690]
24. Buhimschi IA, Buhimschi CS, Weiner CP, et al. Proteomic but not enzyme-linked immunosorbent assay technology detects amniotic fluid monomeric calgranulins from their complexed calprotectin form. *Clin Diagn Lab Immunol* 2005;12:837–44. [PubMed: 16002632]
25. Buhimschi IA, Christner R, Buhimschi CS. Proteomic biomarker analysis of amniotic fluid for identification of intra-amniotic inflammation. *BJOG* 2005;112:173–81. [PubMed: 15663581]
26. Wu Z, Irizarry RA, Gentleman R, Martinez-Murillo F, Spencer F. A Model-Based Background Adjustment for Oligonucleotide Expression Arrays. *Journal of the American Statistical Association* 2004;99:909–917.
27. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) Method. *Methods* 2001;25:402–8. [PubMed: 11846609]
28. Dong Y, Hou W, Wei J, Weiner CP. Chronic hypoxemia absent bacterial infection is one cause of the fetal inflammatory response syndrome (FIRS). *Reproductive Sciences* 2009;16:650–656. [PubMed: 19351964]
29. Battersby S, Critchley HO, Morgan K, Millar RP, Jabbour HN. Expression and regulation of the prokineticins (endocrine gland-derived vascular endothelial growth factor and Bv8) and their receptors in the human endometrium across the menstrual cycle. *J Clin Endocrinol Metab* 2004;89:2463–9. [PubMed: 15126578]

30. Palmier B, Vacher M, Harbon S, Leiber D. A tyrosine kinase signaling pathway, regulated by calcium entry and dissociated from tyrosine phosphorylation of phospholipase Cgamma-1, is involved in inositol phosphate production by activated G protein-coupled receptors in myometrium. *J Pharmacol Exp Ther* 1999;289:1022–30. [PubMed: 10215683]
31. Phillippe M, Bradley DF, Engle D, Sweet L. SHP protein tyrosine phosphatase expression in rat uterine tissue. *J Soc Gynecol Investig* 2006;13:338–42.
32. Bowen JM, Chamley L, Keelan JA, Mitchell MD. Cytokines of the placenta and extra-placental membranes: roles and regulation during human pregnancy and parturition. *Placenta* 2002;23:257–73. [PubMed: 11969336]
33. Kelly RW. Inflammatory mediators and parturition. *Rev Reprod* 1996;1:89–96. [PubMed: 9414445]
34. Kelly RW. Inflammatory mediators and cervical ripening. *J Reprod Immunol* 2002;57:217–24. [PubMed: 12385844]
35. Yellon SM, Mackler AM, Kirby MA. The role of leukocyte traffic and activation in parturition. *J Soc Gynecol Investig* 2003;10:323–38.
36. Weiner CP, Mason C, Hall G, Ahmad U, Swaan P, Buhimschi IA. Pregnancy and estradiol modulate myometrial G-protein pathways in the guinea pig. *Am J Obstet Gynecol* 2006;195:275–87. [PubMed: 16681987]
37. Aaronson PI, Sarwar U, Gin S, et al. A role for voltage-gated, but not Ca²⁺-activated, K⁺ channels in regulating spontaneous contractile activity in myometrium from virgin and pregnant rats. *Br J Pharmacol* 2006;147:815–24. [PubMed: 16415906]
38. Smith RC, McClure MC, Smith MA, Abel PW, Bradley ME. The role of voltage-gated potassium channels in the regulation of mouse uterine contractility. *Reprod Biol Endocrinol* 2007;5:41. [PubMed: 17980032]
39. Suzuki T, Takimoto K. Differential expression of Kv4 pore-forming and KChIP auxiliary subunits in rat uterus during pregnancy. *Am J Physiol Endocrinol Metab* 2005;288:E335–41. [PubMed: 15454398]
40. Knock GA, Tribe RM, Hassoni AA, Aaronson PI. Modulation of potassium current characteristics in human myometrial smooth muscle by 17beta-estradiol and progesterone. *Biol Reprod* 2001;64:1526–34. [PubMed: 11319161]
41. Song M, Helguera G, Eghbali M, et al. Remodeling of Kv4.3 potassium channel gene expression under the control of sex hormones. *J Biol Chem* 2001;276:31883–90. [PubMed: 11427525]
42. Carvajal JA, Thompson LP, Weiner CP. Chorion-induced myometrial relaxation is mediated by large-conductance Ca²⁺-activated K⁺ channel opening in the guinea pig. *Am J Obstet Gynecol* 2003;188:84–91. [PubMed: 12548200]
43. Frangogiannis NG. Chemokines in the ischemic myocardium: from inflammation to fibrosis. *Inflamm Res* 2004;53:585–95. [PubMed: 15693606]
44. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med* 1991;174:1209–20. [PubMed: 1940799]
45. Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A. IL-10 inhibits cytokine production by activated macrophages. *J Immunol* 1991;147:3815–22. [PubMed: 1940369]
46. Greig PC, Herbert WN, Robinette BL, Teot LA. Amniotic fluid interleukin-10 concentrations increase through pregnancy and are elevated in patients with preterm labor associated with intrauterine infection. *Am J Obstet Gynecol* 1995;173:1223–7. [PubMed: 7485325]
47. Mitchell MD, Simpson KL, Keelan JA. Paradoxical proinflammatory actions of interleukin-10 in human amnion: potential roles in term and preterm labour. *J Clin Endocrinol Metab* 2004;89:4149–52. [PubMed: 15292362]
48. Simpson KL, Keelan JA, Mitchell MD. Labor-associated changes in interleukin-10 production and its regulation by immunomodulators in human choriodecidua. *J Clin Endocrinol Metab* 1998;83:4332–7. [PubMed: 9851773]
49. Janicek F, Franova S, Nosalova G, Visnovsky J. In vitro contractile response of human myometrium to oxytocin, PGF₂alpha, bradykinin and ET-1. *Bratisl Lek Listy* 2007;108:174–8. [PubMed: 17694800]

50. Wassdal I, Nicolaysen G, Iversen JG. Bradykinin causes contraction in rat uterus through the same signal pathway as oxytocin. *Acta Physiol Scand* 1998;164:47–52. [PubMed: 9777024]
51. Buchinger P, Rehbock J. The bradykinin B2-receptor in human decidua. *Semin Thromb Hemost* 1999;25:543–9. [PubMed: 10632476]
52. Abbas F, Clayton J, Marshall K, Senior J. A preliminary study of prostaglandin release by bradykinin (BK) on isolated human myometrium. *Immunopharmacology* 1996;33:130–2. [PubMed: 8856131]
53. Das SK, Das N, Wang J, et al. Expression of betacellulin and epiregulin genes in the mouse uterus temporally by the blastocyst solely at the site of its apposition is coincident with the “window” of implantation. *Dev Biol* 1997;190:178–90. [PubMed: 9344537]
54. Cho MC, Choi HS, Lee S, et al. Epiregulin expression by Ets-1 and ERK signaling pathway in K-ras-transformed cells. *Biochem Biophys Res Commun* 2008;377:832–7. [PubMed: 18948081]
55. Buhimschi IA, Buhimschi CS, Pupkin M, Weiner CP. Beneficial impact of term labor: nonenzymatic antioxidant reserve in the human fetus. *Am J Obstet Gynecol* 2003;189:181–8. [PubMed: 12861160]
56. Keelan JA, Blumenstein M, Helliwell RJ, Sato TA, Marvin KW, Mitchell MD. Cytokines, prostaglandins and parturition--a review. *Placenta* 2003;24 (Suppl A):S33–46. [PubMed: 12842412]
57. Tattersall M, Engineer N, Khanjani S, et al. Pro-labour myometrial gene expression: are preterm labour and term labour the same? *Reproduction* 2008;135:569–79. [PubMed: 18367515]
58. Challis JR, Sloboda DM, Alfaidy N, et al. Prostaglandins and mechanisms of preterm birth. *Reproduction* 2002;124:1–17. [PubMed: 12090913]
59. Mohan AR, Loudon JA, Bennett PR. Molecular and biochemical mechanisms of preterm labour. *Semin Fetal Neonatal Med* 2004;9:437–44. [PubMed: 15691781]
60. Buhimschi CS, Buhimschi IA, Yu C, et al. The effect of dystocia and previous cesarean uterine scar on the tensile properties of the lower uterine segment. *Am J Obstet Gynecol* 2006;194:873–83. [PubMed: 16522428]

FIGURE 1. Venn diagram illustrating the logic process for the derivation of the three unique gene sets. The number refers to the number of genes identified in the various subgroups.

FIGURE 2.

FIGURE 2a. The Gene Ontology table was generated from the genes of the *Effector* set in MetaCore. The top 10 Genego GO pathways are listed in the table based on the number of selected genes that saturate each biological area. These processes are prioritized by MetaCore™ based on their statistical significance ($-\log(p \text{ value})$) with respect to genes expressed in the dataset. A false discovery rate filter set at 10% was also used to illustrate significant processes. The bars representing experiment mappings that do not pass through this significance level are semi-transparent.

FIGURE 2b. Interactive network of the *Effector Gene Set* was determined using the *analyze network* algorithm. This algorithm designs sub-networks that are highly saturated with genes of the *Effector Set*. Selection of the network was based on significance (P-value). The p-value calculation was used to evaluate network's relevance to GO biological processes classification. The network illustrates gene and gene interactions within *Effector Set*. Canonical pathways are illustrated by a thick light blue line and represent linear stretches of carefully defined sets of consecutive signals confirmed as a whole by experimental data. The small colored hexagons

on vectors (lines) between nodes describe positive (green), negative (red), unspecified (black) interactions, or logical relationships (blue). A node corresponds to a network object (i.e. gene or protein) and is marked with a graphical symbol that reflects the type of the network object represented (i.e. enzyme, transcription factor, ligand, etc.). The large red circle next to the network object corresponds to those genes detected in the *Effector Set*. The network objects are placed in specific vectors of this network according to their sub-cellular localization. The localization sectors are Nucleus, Cytoplasm, Membrane, Extracellular, and Unspecified.

FIGURE 3. FIGURE 3a. Similar to figure 2a, this Gene Ontology table is representative of the Term Initiator Gene Set. The genes included are more varied than those of the Preterm Initiator Set. FIGURE 3b. Similar to figures 2b, this Term Initiator sub-network was generated using the *analyze network* algorithm (Metacore™). The large red circle next to the network object corresponds to those genes detected in the *Term Initiator Group*. Selection of the network was based on significance (P-value).

FIGURE 4.
FIGURE 4a. This figure illustrates the major Gene Ontology pathways underlying the *Preterm Initiator Gene Set*. There is a high correlation between inflammation and the *Preterm Initiator Gene Set*.
FIGURE 4b. The *Preterm Initiator Set* sub-network was generated using the *analyze network* algorithm (Metacore™). The large red circle next to the network object corresponds to those genes detected in the *Preterm Initiator Group*. Selection of the network was based on significance (P-value).

FIGURE 5.

The *Effector Set* represents genes that sustain myometrial contractility and are unaffected by stimulus. HNT, KCNAB2, RRAD were selected for study to verify the microarray results. mRNA levels were determined in the myometrium of term (n=6; 39.7w) and preterm with inflammation (n=6; 30.9w) pregnant women in active labor and not-in-labor (term n=6; 39.6w, preterm n=6; 28.9w) via Q-rtPCR using the Livak Method. Microarray findings were confirmed for all but one gene tested. In general, the findings confirm both the microarray findings and the presence of core genes associated with labor regardless of its timing.

FIGURE 6.

Term Initiator Set genes EREG, BDKRB1, IL13RA2 mRNA levels were significantly greater at term labor (open bar) than preterm labor (close bar) in myometrium. mRNA levels were determined in the myometrium of term (n=6; 39.7w) and preterm with inflammation (n=6; 30.9w) pregnant women in active labor and not-in-labor (term n=6; 39.6w, preterm n=6; 28.9w) via Q-rtPCR using the Livak Method. Microarray findings were confirmed for all but one gene tested. EREG and BDKRB1 mRNA levels were significantly higher at labor (open bar) than non labor (close bar) in term myometrium. Only IL9R Q-rtPCR results different from the microarray.

FIGURE 7.

Preterm Initiator Set- mRNA levels were determined as indicated previously. Changes in the mRNA levels for genes *Catsper2* and *Ptpz1* were consistent with the array data.

Table 1

Clinical Characteristics

Group	Gestational Age (weeks)	Maternal Age (years)	Birthweight (grams)	Indication for CS	Histological Chorioamnionitis
TNL	39 ± 1	30 ± 3	3490 ± 618	breech	NA
TL	40 ± 1	28 ± 6	3490 ± 333	failure to progress	NA
PNL	29 ± 3 a,b	26 ± 9	971 ± 380 a,b	preeclampsia	Stage 0
PTL	30 ± 3 a,b	34 ± 6	1299 ± 344 a,b	PPROM/ sPTL	Stage III

Data presented as mean ± S.D. CS, cesarean section; PPRM, premature rupture of membranes; sPTL, spontaneous preterm labor; PE, preeclampsia; NA, not applicable.

^a $p < 0.05$ based on Newman-Keuls test compared to TNL

^b $p < 0.05$ based on Newman-Keuls test compared to TL

Table 2

Core Myometrial Genes of Labor (Effector Set).(excluding hypothetical and other unidentified/unnamed proteins)

Gene Symbol	Gene Name	Entrez ID
ABRA	Actin-binding rho activating protein	137735
ALDH16A1	Aldehyde dehydrogenase 16 family, member a1	126133
ANKS3	Ankyrin repeat and sterile alpha motif domain containing 3	124401
ATRIP/TREX1	ATR-interacting protein/Three prime repair exonuclease 1	84126
BAIAP2L1	Bai1-associated protein 2-like 1	55971
CADM2	Cell adhesion molecule 2 precursor	253559
CCDC36	Coiled-coil domain containing 36	339834
CFB	Complement factor b	629
CORO2A	Coronin, actin binding protein, 2a	7464
D4S234E	Dna segment on chromosome 4 (unique) 234 expressed sequence	27065
EGFL7	Egf-like-domain, multiple 7	51162
FPGS	Folylpolyglutamate synthase	2356
GPATCH3	G patch domain containing 3	63906
H2AFY2	H2a histone family, member y2	55506
HNT	Neurotrimin	50863
HTRA4	Htra serine peptidase 4	203100
IL8RB	Interleukin 8 receptor, beta	3579
KCNAB2	Potassium voltage-gated channel, shaker-related subfamily, beta member 2	8514
KRTAP8-1	Keratin associated protein 8-1	337879
LY6G5C	Lymphocyte antigen 6 complex, locus g5c	80741
MPP3	Membrane protein, palmitoylated 3 (maguk p55 subfamily member 3)	4356
P2RY8	Purinergic receptor p2y, g-protein coupled, 8	286530
PLAC8L1	Plac8-like 1	153770
PLCXD1	Phosphatidylinositol-specific phospholipase c, x domain containing 1	55344
POLR3D	Polymerase (rna) iii (dna directed) polypeptide d, 44kda	661
RRAD	Ras-related associated with diabetes	6236
SIRPB2	Signal-regulatory protein beta 2	284759
SKIV2L	Superkiller viralicidic activity 2-like (s. Cerevisiae)	6499
SLPI	Secretory leukocyte peptidase inhibitor	6590
ST3GAL3	St3 beta-galactoside alpha-2,3-sialyltransferase 3	6487
ZNF552	Zinc finger protein 552	79818
ZNF718	Zinc finger protein 718	255403
ZNF74	Zinc finger protein 74 (cos52)	7625
ZNF788	Zinc finger protein 788	388507

Table 3

Myometrial Genes Initiating Preterm Labor (Preterm Initiator Set) (excluding hypothetical and other unidentified/unnamed proteins)

Genes Symbol	Gene Name	Entrez ID
ABCA3	Atp-binding cassette, sub-family a (abc1), member 3	21
ABP1	Amiloride binding protein 1 (amine oxidase (copper-containing))	26
ARSA	Arylsulfatase a	410
CD3E	Cd3e antigen, epsilon polypeptide (tit3 complex)	916
DGKQ	Diacylglycerol kinase, theta 110kda	1609
GJA3	Gap junction protein, alpha 3, 46kda (connexin 46)	2700
GSTT2	Glutathione s-transferase theta 2	2953
HLA-DOA	Major histocompatibility complex, class ii, do alpha	3111
HYAL1	Hyaluronoglucosaminidase 1	3373
IFNG	Interferon, gamma	3458
IRF5	Interferon regulatory factor 5	3663
LLGL1	Lethal giant larvae homolog 1 (drosophila)	3996
LYL1	Lymphoblastic leukemia derived sequence 1	4066
MYCL1	V-myc myelocytomatosis viral oncogene homolog 1, lung carcinoma derived (avian)	4610
PDE1C	Phosphodiesterase 1c, calmodulin-dependent 70kda	5137
MAPK11	Mitogen-activated protein kinase 11	5600
PTPRZ1	Protein tyrosine phosphatase, receptor-type, z polypeptide 1	5803
ZNF208	Zinc finger protein 208	7757
ABCA7	Atp-binding cassette, sub-family a (abc1), member 7	10347
CXCL13	Chemokine (c-x-c motif) ligand 13 (b-cell chemoattractant)	10563
OGFR	Opioid growth factor receptor	11054
ELOVL2	Elongation of very long chain fatty acids (fen1/elo2, sur4/elo3, yeast)-like 2	54898
CPXM	Carboxypeptidase x (m14 family)	56265
AARSL	Alanyl-trna synthetase like	57505
TINAGL1	Tubulointerstitial nephritis antigen-like 1	64129
DIO3OS	Deiodinase, iodothyronine, type iii opposite strand	64150
NDRG4	Ndr family member 4	65009
VPS33A	Vacuolar protein sorting 33a (yeast)	65082
LRFN4	Leucine rich repeat and fibronectin type iii domain containing 4	78999
TLE6	Transducin-like enhancer of split 6 (e(sp1) homolog, drosophila)	79816
TIGD6	Tigger transposable element derived 6	81789
KRTAP4-8	Keratin associated protein 4-8	83898
CHCHD6	Coiled-coil-helix-coiled-coil-helix domain containing 6	84303
ZNF341	Zinc finger protein 341	84905
CCDC97	Coiled-coil domain-containing protein 97	90324
STK11IP	Serine/threonine kinase 11 interacting protein	114790
SORCS1	Sortilin-related vps10 domain containing receptor 1	114815
CATSPER2	Cation channel, sperm associated 2	117155
LRGUK	Leucine-rich repeats and guanylate kinase domain containing	136332

Genes Symbol	Gene Name	Entrez ID
FAM69B	Family with sequence similarity 69, member b	138311
BEAN	Brain expressed, associated with nedd4	146227
GRASP	Grp1 (general receptor for phosphoinositides 1)-associated scaffold protein	160622
ZNF579	Zinc finger protein 579	163033
DENND1B	Denn/madd domain containing 1b	163486
SAMD14	Sterile alpha motif domain containing 14	201191
ALS2CL	Als2 c-terminal like	259173
STK32C	Serine/threonine kinase 32c	282974
CATSPER2P1	Cation channel, sperm associated 2 pseudogene	440278
GSTT2B	Similar to glutathione s-transferase theta 2 (gst class-theta 2)	653689

Table 4

Genes Initiating Term Labor (Term Initiator Set) in Myometrium (excluding hypothetical and other unidentified/unnamed proteins)

Genes Symbol	Gene Name	Entrez ID
A1BG	Alpha-1-b glycoprotein	1
ADCYAP1R1	Adenylate cyclase activating polypeptide 1 (pituitary) receptor type i	117
ALDOC	Aldolase c, fructose-bisphosphate	230
AQP9	Aquaporin 9	366
AREG	Amphiregulin (schwannoma-derived growth factor)	374
ARHGAP4	Rho gtpase activating protein 4	393
BCL2A1	Bcl2-related protein a1	597
BDKRB1	Bradykinin receptor b1	623
BUB1	Bub1 budding uninhibited by benzimidazoles 1 homolog (yeast)	699
BUB1B	Bub1 budding uninhibited by benzimidazoles 1 homolog beta (yeast)	701
CALCA	Calcitonin/calcitonin-related polypeptide, alpha	796
RUNX3	Runt-related transcription factor 3	864
CCNE1	Cyclin e1	898
CD48	Cd48 antigen (b-cell membrane protein)	962
CDC20	Cdc20 cell division cycle 20 homolog (s. Cerevisiae)	991
CHI3L1	Chitinase 3-like 1 (cartilage glycoprotein-39)	1116
CSF3	Colony stimulating factor 3 (granulocyte)	1440
CSN2	Casein beta	1447
DDIT3	Dna-damage-inducible transcript 3	1649
EFNB1	Ephrin-b1	1947
EREG	Epiregulin	2069
EYA4	Eyes absent homolog 4 (drosophila)	2070
ESRRA	Estrogen-related receptor alpha	2101
FPRL1	Formyl peptide receptor-like 1	2358
GALE	Udp-galactose-4-epimerase	2582
GCHFR	Gtp cyclohydrolase i feedback regulator	2644
GK	Glycerol kinase	2710
GRIN2D	Glutamate receptor, ionotropic, n-methyl d-aspartate 2d	2906
NRG1	Neuregulin 1	3084
HK2	Hexokinase 2	3099
NR4A1	Nuclear receptor subfamily 4, group a, member 1	3164
IL1B	Interleukin 1, beta	3553
IL9R	Interleukin 9 receptor	3581
IL13RA2	Interleukin 13 receptor, alpha 2	3598
INHBB	Inhibin, beta b (activin ab beta polypeptide)	3625
KRT7	Keratin 7	3855
LIF	Leukemia inhibitory factor (cholinergic differentiation factor)	3976
MMP3	Matrix metalloproteinase 3 (stromelysin 1, progelatinase)	4314
MYH3	Myosin, heavy polypeptide 3, skeletal muscle, embryonic	4621

Genes Symbol	Gene Name	Entrez ID
NEFH	Neurofilament, heavy polypeptide 200kda	4744
NEK2	Nima (never in mitosis gene a)-related kinase 2	4751
NPPB	Natriuretic peptide precursor b	4879
SERPINB2	Serpin peptidase inhibitor, clade b (ovalbumin), member 2	5055
PCSK1	Proprotein convertase subtilisin/kexin type 1	5122
PFKFB4	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4	5210
PGK2	Phosphoglycerate kinase 2	5232
SERPINF2	Serpin peptidase inhibitor, clade f (alpha-2 antiplasmin, pigment epithelium derived factor), member 2	5345
POLE	Polymerase (dna directed), epsilon	5426
PRKCD	Protein kinase c, delta	5580
S100P	S100 calcium binding protein p	6286
CCL11	Chemokine (c-c motif) ligand 11	6356
CCL19	Chemokine (c-c motif) ligand 19	6363
CXCL6	Chemokine (c-x-c motif) ligand 6 (granulocyte chemotactic protein 2)	6372
SELE	Selectin e (endothelial adhesion molecule 1)	6401
ST3GAL1	St3 beta-galactoside alpha-2,3-sialyltransferase 1	6482
SLAMF1	Signaling lymphocytic activation molecule family member 1	6504
SLC9A2	Solute carrier family 9 (sodium/hydrogen exchanger), member 2	6549
SPAG4	Sperm associated antigen 4	6676
STXBP2	Syntaxin binding protein 2	6813
TAC1	Tachykinin, precursor 1 (substance k, substance p, neurokinin 1, neurokinin 2, neuromedin 1, neurokinin alpha, neuropeptide k, neuropeptide gamma)	6863
VAV1	Vav 1 oncogene	7409
VIL1	Villin 1	7429
ZNF31	Zinc finger protein 31 (kox 29)	7579
GCS1	Glucosidase i	7841
PLA2G7	Phospholipase a2, group vii (platelet-activating factor acetylhydrolase, plasma)	7941
NR4A3	Nuclear receptor subfamily 4, group a, member 3	8013
FOSL1	Fos-like antigen 1	8061
GPR68	G protein-coupled receptor 68	8111
SLC7A5	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 5	8140
ENC1	Ectodermal-neural cortex (with btb-like domain)	8507
CPZ	Carboxypeptidase z	8532
TNFRSF6B	Tumor necrosis factor receptor superfamily, member 6b, decoy	8771
PROM1	Prominin 1	8842
RRP9	RRP9, small subunit (SSU) processome component, homolog (yeast)	9136
MSC	Musculin (activated b-cell factor-1)	9242
CYP7B1	Cytochrome p450, family 7, subfamily b, polypeptide 1	9420
IL27RA	Interleukin 27 receptor, alpha	9466
IKBKE	Inhibitor of kappa light polypeptide gene enhancer in b-cells, kinase epsilon	9641
DHX34	Deah (asp-glu-ala-his) box polypeptide 34	9704
SEC14L5	Sec14-like 5 (s. Cerevisiae)	9717
CENTB1	Centaurin, beta 1	9744

Genes Symbol	Gene Name	Entrez ID
CLSTN3	Calsyntenin 3	9746
DHRS2	Dehydrogenase/reductase (sdr family) member 2	10202
TACC3	Transforming, acidic coiled-coil containing protein 3	10460
DPYSL4	Dihydropyrimidinase-like 4	10570
IL24	Interleukin 24	11009
LILRA1	Leukocyte immunoglobulin-like receptor, subfamily a (with tm domain), member 1	11024
CIT	Citron (rho-interacting, serine/threonine kinase 21)	11113
KPTN	Kaptin (actin binding protein)	11133
RASSF1	Ras association (ralgds/af-6) domain family 1	11186
IRAK3	Interleukin-1 receptor-associated kinase 3	11213
ATXN2L	Ataxin 2-like	11273
RRP12	Ribosomal RNA processing 12 homolog (S. Cerevisiae)	23223
SMG6	Smg-6 homolog, nonsense mediated mrna decay factor (c. Elegans)	23293
NUP188	Nucleoporin 188kda	23511
CLEC5A	C-type lectin domain family 5, member a	23601
CLEC4E	C-type lectin domain family 4, member e	26253
SIGLEC7	Sialic acid binding ig-like lectin 7	27036
SULT1B1	Sulfotransferase family, cytosolic, 1b, member 1	27284
CECR2	Cat eye syndrome chromosome region, candidate 2	27443
DKFZP434P211	Pom121-like protein	29774
EMR2	Egf-like module containing, mucin-like, hormone receptor-like 2	30817
ANGPTL4	Angiopoietin-like 4	51129
ABI3	Abi gene family, member 3	51225
RTEL1	Regulator of telomere elongation helicase 1	51750
P2RY13	Purinergic receptor p2y, g-protein coupled, 13	53829
GPR84	G protein-coupled receptor 84	53831
SOX18	Sry (sex determining region y)-box 18	54345
SDK2	Sidekick homolog 2 (chicken)	54549
RAB39	Rab39, member ras oncogene family	54734
GRAMD1C	Gram domain containing 1c	54762
MCM10	Mcm10 minichromosome maintenance deficient 10 (s. Cerevisiae)	55388
AJAP1	Adherens junction associated protein 1	55966
CCL28	Chemokine (c-c motif) ligand 28	56477
ASAH2	N-acylsphingosine amidohydrolase (non-lysosomal ceramidase) 2	56624
PDXP	Pyridoxal (pyridoxine, vitamin b6) phosphatase	57026
ATP10A	Atpase, class v, type 10c	57194
LOC57228	Small trans-membrane and glycosylated protein	57228
LRRN1	Leucine rich repeat neuronal 1	57633
NLRC4	NLR family, CARD domain containing 4	58484
PROK2	Prokineticin 2	60675
CARD9	Caspase recruitment domain family, member 9	64170
CDCP1	Cub domain containing protein 1	64866

Genes Symbol	Gene Name	Entrez ID
ZNF643	Zinc finger protein 643	65243
ZNF557	Zinc finger protein 557	79230
IQCA	Iq motif containing with aaa domain	79781
VASH2	Vasohibin 2	79805
DENND2D	Denn/madd domain containing 2d	79961
LRRC8E	Leucine rich repeat containing 8 family, member e	80131
ZC3H12A	Zinc finger ccch-type containing 12a	80149
DEPDC2	Dep domain containing 2	80243
CLPB	Clpb caseinolytic peptidase b homolog (e. Coli)	81570
PPP1R14C	Protein phosphatase 1, regulatory (inhibitor) subunit 14c	81706
KIF18A	Kinesin family member 18a	81930
TMUB1	Transmembrane and ubiquitin-like domain containing 1	83590
URP2	Unc-112 related protein 2	83706
MND1	Meiotic nuclear divisions 1 homolog (s. Cerevisiae)	84057
PROK1	Prokineticin 1	84432
DOT1L	Dot1-like, histone h3 methyltransferase (s. Cerevisiae)	84444
SLC12A8	Solute carrier family 12 (potassium/chloride transporters), member 8	84561
IGSF21	Immunoglobulin superfamily, member 21	84966
PNMA6A	Paraneoplastic antigen like 6a	84968
TSLP	Thymic stromal lymphopoietin	85480
PPP1R3F	Protein phosphatase 1, regulatory (inhibitor) subunit 3f	89801
MTG1	Mitochondrial gtpase 1 homolog (s. Cerevisiae)	92170
SLC38A5	Solute carrier family 38, member 5	92745
DDIT4L	Dna-damage-inducible transcript 4-like	115265
ZMYND19	Zinc finger, mynd-type containing 19	116225
SLC16A10	Solute carrier family 16, member 10 (aromatic amino acid transporter)	117247
OSCAR	Osteoclast-associated receptor	126014
WTIP	Wilms tumor 1 interacting protein	126374
PUSL1	Pseudouridylate synthase-like 1	126789
B3GNTL1	Udp-glcnac:betagal beta-1,3-n-acetylglucosaminyltransferase-like 1	146712
FAM132B	Family with sequence similarity 132, member B	151176
SGOL1	Shugoshin-like 1 (s. Pombe)	151648
PRELID2	PRELI domain containing 2	153768
CRYGN	Crystallin, gamma n	155051
TMEM30B	Transmembrane protein 30b	161291
CCDC138	Coiled-coil domain containing 138	165055
SLC30A8	Solute carrier family 30 (zinc transporter), member 8	169026
PPAPDC1A	Phosphatidic acid phosphatase type 2 domain containing 1a	196051
ABHD7	Abhydrolase domain containing 7	253152
OFCC1	Orofacial cleft 1 candidate 1	266553
VMO1	Vitelline membrane outer layer 1 homolog (chicken)	284013
GLDN	Gliomedin	342035

Genes Symbol	Gene Name	Entrez ID
FMN1	Formin 1	342184
AADACL2	Arylacetamide deacetylase-like 2	344752
CA13	Carbonic anhydrase xiii	377677
RASL11A	Ras-like, family 11, member a	387496
APOBEC4	Apolipoprotein b mrna editing enzyme, catalytic polypeptide-like 4 (putative)	403314
SPINK6	Serine peptidase inhibitor, kazal type 6	404203
SNORD68	Small nucleolar RNA, C/D box 68	606500
FAM110C	Family with sequence similarity 110, member C	642273
PHLDB3	Similar to pleckstrin homology-like domain, family b, member 1	653583