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Characterization of the myometrial transcriptome in women with an arrest of dilatation during labor

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Abstract

Objective—The molecular basis of failure to progress in labor is poorly understood. This study was undertaken to characterize the myometrial transcriptome of patients with an arrest of dilatation (AODIL).

Study design—Human myometrium was prospectively collected from women in the following groups: 1) spontaneous term labor (TL; n=29); and 2) arrest of dilatation (AODIL; n=14). Gene expression was characterized using Illumina® HumanHT-12 microarrays. A moderated student *t*-test and false discovery rate adjustment were used for analysis. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) of selected genes was performed in an independent sample set. Pathway analysis was performed on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database using Pathway Analysis with Down-weighting of Overlapping Genes (PADOG). The Metacore knowledge base was also mined for pathway analysis.

Results—1) 42 genes differentially expressed were identified in women with an AODIL; 2) gene ontology analysis indicated enrichment of biological processes, which included: regulation of angiogenesis, response to hypoxia, inflammatory response, and chemokine-mediated signaling pathway. Enriched molecular functions included: transcription repressor activity, Heat shock protein (Hsp) 90 binding, and nitric oxide synthase (*NOS*) activity; 3) Metacore analysis identified immune response chemokine (C-C motif) ligand 2 (CCL2) signaling, muscle contraction regulation of eNOS activity in endothelial cells, and Triiodothyronine and Thyroxine signaling as significantly over-represented (FDR<0.05); 4) qRT-PCR confirmed overexpression of Nitric oxide synthase 3 *NOS3*; hypoxic ischemic factor (*HIF1A*), Chemokine (C-C motif) ligand 2 (*CCL2)*;

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angiopoietin-like 4 (*ANGPTL4*), ADAM metallopeptidase with thrombospondin type 1, motif 9 (*ADAMTS9*), G protein-coupled receptor 4 (GPR4), metallothionein 1A (*MT1A*), *MT2A*, selectin E (*SELE*) in an AODIL.

Conclusion—The myometrium of women with arrest of dilatation have a stereotypic transcriptome profile. This disorder was associated with a pattern of gene expression involved in muscle contraction, an inflammatory response, and hypoxia. This is the first comprehensive and unbiased examination of the molecular basis of an AODIL.

Keywords

angiopoietin-like 4 (ANGPTL4); arrest disorders; chemokine (C-C motif) ligand 2 (CCL2); metallothionein (MT); myometrium; nitric oxide synthase (NOS); pregnancy; parturition; systems biology; TGF signaling pathway; transcriptomics

Introduction

Parturition, a key event for the survival of viviparous species, is a complex process involving myometrial activation, cervical ripening and membrane/decidual activation [6,25,30–32,36,74,94,107–109,111,129,134,137,139,148,150,157,177,184,189,190,194]. The myometrium is responsible for the contractile force required to propel the fetus through the birth canal. During pregnancy and active labor, extensive changes in the anatomy, physiology, and composition of the myometrium have been identified [28,29,40,43,46,48,54,55,62,67–72,76–78,92,112–114,117,118,120,136,142,164,166,168,169,176,183,185,186].

Dystocia, broadly defined as slow or abnormal progression of labor [1], is responsible for 18 percent of primary cesarean deliveries, 60 percent of all cesarean deliveries, and is considered one of the most common indications for intrapartum cesarean delivery [14,21,75,174]. Moreover, this condition is associated with 8% of maternal deaths worldwide [75,90,187]. Dystocia includes protraction disorders (slow dilatation) or arrest disorders (complete cessation of progress). The diagnosis of arrest of dilatation (AODIL) occurs when a patient in the active phase of labor does not have cervical dilatation for at least 2 hours [1,66]. The most common cause of an arrest of dilatation is thought to be cephalopelvic disproportion. However, the observation that patients with an arrest of dilatation in the first pregnancy often deliver a larger neonate vaginally in a subsequent pregnancy suggests that the problem is not one of disproportion [24]. Instead, a functional disorder in myometrial contractility has been postulated to be responsible for such disorder.

High-dimensional biology techniques (such as genomics, transcriptomics, and proteomics) have been used to gain insight into the molecular basis of parturition in the myometrium [4,27,34,60,88,124,135], the uterine cervix [85–87], and the chorioamniotic membranes [131]. Recently, the transcriptome of the myometrium in patients with an “arrest of descent” [123] and “labor dystocia” have been reported [23]. However, the transcriptome of human myometrium in AODIL has not yet been investigated. We undertook this study in order to characterize the transcriptome of the myometrium in patients with this condition.

Materials and Methods

A prospective study was performed in which human myometrium was obtained from women undergoing primary cesarean delivery at term (≥ 37 week of gestation) in the following groups: 1) spontaneous term labor (TL) (n=29); and 2) AODIL (n=14). Labor was diagnosed in the presence of spontaneous regular uterine contractions occurring at a minimum frequency of 2 every 10 minutes with cervical changes that led to progressive cervical dilatation. Women in the term labor group underwent cesarean delivery because of a non-reassuring fetal heart rate tracing (as determined by the physician) or fetal malpresentation. All patients presented in spontaneous labor and delivered an infant with a birth weight between the 10^{th} and 90^{th} percentiles [5]. Patients with clinical or histological chorioamnionitis, underlying medical or obstetrical complications, and those undergoing labor induction were excluded.

The diagnosis of AODIL was diagnosed in women in the active phase of labor who did not progress despite adequate contractions after 2 hours [1]. The placentas of all participating women were examined to exclude histological chorioamnionitis by experienced pathologists who were blinded to the clinical diagnosis.

All women provided written informed consent prior to the collection of myometrial samples. The collection and utilization of the samples for research purposes was approved by the Institutional Review Board of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD/NIH/DHHS, Bethesda, Maryland), and the Human Investigation Committees of Wayne State University (Detroit, Michigan) and the Sotero del Rio Hospital (Santiago, Chile).

Sample collection

Myometrial tissue samples were obtained from the lower uterine segment at the time of cesarean delivery, after placenta detachment. Biopsies were obtained from the midpoint of the superior aspect of the uterine incision using Metzenbaum scissors. Specimens measured approximately 1.0 × 1.0 × 1.0 cm. Tissue was ground under liquid nitrogen, placed in TRI Reagent® (Applied Biosystems, Foster City, CA) and kept at −80° Celsius until analysis.

Total RNA extraction

Total RNA was isolated from snap-frozen myometrium using TRI Reagent® combined with the Qiagen RNeasy Lipid Tissue kit protocol (Qiagen, Valencia, CA) following the manufacturers' recommendations. RNA concentrations and the A260nm/A280nm ratio were assessed using a NanoDrop 1000 (Thermo Scientific, Wilmington, DE). RNA integrity numbers (RINs) were determined using the Bioanalyzer 2100 (Agilent Technologies, Wilmington, DE). An A260nm/A280nm ratio of 1.66, a 28S/18S ratio of 0.2, and a RIN of 3.8 were the minimum requirements for inclusion in the expression analysis.

Microarray experiments

The Illumina® HumanHT-12 version 3 expression microarray (Illumina, San Diego, CA) platform was used to determine the expression levels in each unpooled specimen per

manufacturer's instructions. In brief, after purification of RNA using an RNeasy Mini Kit (Qiagen), 500 ng of total RNA was amplified and biotin-labeled with the Illumina® TotalPrep RNA Amplification Kit (Ambion, Austin, TX). Labeled complementary RNAs were hybridized to the Illumina HumanHT-12 version 3 expression BeadChip and imaged using a BeadArray Reader. Raw data was obtained using the BeadStudio Software (Illumina).

Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR)

A larger set of specimens for each group (spontaneous TL: n= 31; AODIL: n=18) were obtained for qRT-PCR assays of a selected group of genes found to be differentially expressed by microarray analysis. Total RNA (3 μg) was reverse transcribed using the SuperScript® III First-Strand Synthesis System and oligo(dT)$_{20}$ primers (Invitrogen, Carlsbad, CA). qRT-PCR analyses were performed with TaqMan® Gene Expression Assays (*HIF1A*: Hs00936368_m1, *GPR4*: Hs00947870_m1, *EXOG*: Hs00270782_m1, ID1: Hs00357821_g1, *ID3*: Hs00171409_m1, *NOS3*: Hs01574659_m1, *MT1A*: Hs00831826_s1; *MT2A*: Hs02379661_g1; and *SBNO2*: Hs00209130_m1; Applied Biosystems, Foster City, CA, USA). Human *18S*, *GAPDH*, and ACTIN were used as reference genes. The gene specific TaqMan® assays and the *RPLPO* housekeeping gene were run in triplicate (50 ng) for each case to allow for the assessment of technical variability.

Statistical analysis

Clinical data—Student's t tests and Fisher exact tests were used for comparisons of continuous and discrete demographic variables, respectively. The tests were conducted using the R statistical language and environment (R Development Core Team 2012). A p-value < 0.05 was considered significant.

Microarray analysis—The Illumina BeadStudio software suite (Illumina, Inc., San Diego, CA, USA) was used to extract gene expression values from the array images. The data quality was assessed based on Illumina's positive and negative control probes on each array as well as by inspection of the distributions of probe intensities. Data was normalized using the quantile normalization method [18]. Probes that were considered present (detection p-value <0.1) in at least 5 samples were retained for further analysis. A moderated t-test implemented in the *limma* library of Bioconductor (Fred Hutchinson Cancer Research Center, Seattle, WA, USA) was applied to test differential expression, and a false discovery rate (FDR) adjustment of the p-values was performed to correct for multiple testing[173]. Probes were considered significant different if their adjusted p-value were < 0.25 and the fold change difference was at least 1.5.

Gene ontology analysis was performed using an over-representation approach implemented in the GO stats software package[61]. Pathway analysis was performed on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database using Pathway Analysis with Down-weighting of Overlapping Genes (PADOG) [181], which computes and enrichment score for each pathway from moderated t-scores of all genes in the pathway while giving more weight to genes that are pathway-specific. Metacore pathway analysis

(Thomson, Reuters, NY, USA) was also performed using their proprietary pathways database and over-representation method.

qRT-PCR assays—Quantitative RT-PCR was performed for 9 genes selected among the top candidates from the microarray results and biological function. Data analysis was performed using an equal variance two-sample one-tailed t-test based on the hypothesis provided by the microarray data. Confirmation of microarray data with qRT-PCR was considered significant using a 0.05 threshold.

Results

Demographic, clinical and obstetrical characteristics are displayed in Table 1 according to study group: 1) pregnant women with spontaneous TL microarray (n=29); 2) patients with AODIL microarray (n=14); 3) spontaneous TL qRT-PCR (n=31); and 4) patients with AODIL qRT-PCR (n=18). Among samples used in microarray analysis, there was a significantly higher mean body mass index (BMI) in the spontaneous TL group than in the AODIL group (p = 0.0028).

Microarray analysis

Table 2 lists 42 differentially expressed genes (43 probes) between (spontaneous TL and AODIL) ranked by *P* values. Differential expression results are depicted in Figure 1. The volcano plot (Figure 1) shows the magnitude (fold change) of the changes in the x-axis an the significance of gene expression changes in the y-axis.

Gene ontology (GO) meta-analysis was used to gain insight into the biology related to the stereotypic differences between the myometrial transcriptome of AODIL and spontaneous TL. Significant enrichment of 106 distinct biological processes includes regulation of angiogenesis, response to hypoxia, inflammatory response and regulation of nitric-oxide synthase activity (Table 3). Pathway Analysis with PADOG ranked the TGF-β signaling pathway and Protein processing in endoplasmic reticulum pathways with a nominal p-value <0.01 (non-significant after adjusting for the 227 pathways tested). The MetaCore database was also mined (Table 4). The three pathways ranked at the top by Metacore were Regulation of metabolism were: 1) Immune response chemokine (C-C motif) ligand 2 (CCL2) signaling (p=0.001); 2) Muscle Contraction - Regulation of eNOS Activity in Endothelial Cells (p=0.01); and 3) Triiodothyronine and Thyroxine signaling (p=0.009). Figure 2A depicts immune response CCL2 signaling with CCL2 regulating the transcription of *HIF1A*. Figure 2B demonstrates the Muscle Contraction - Regulation of eNOS Activity in Endothelial Cells pathway with the eNOS regulating the transcription of *HIF1A*. *CCL2, eNOS, and HIF1A* were upregulated in the myometrium of patients with AODIL.

Quantitative RT-PCR (qRT-PCR)

Confirmation of microarray results was performed using qRT-PCR. An extended set of myometrium samples was used to perform qRT-PCR assays on 9 selected genes based upon the microarray data and biological significance.

qRT-PCR confirmed differential expression of all 9 genes identify with microarray analysis. Significantly overexpressed genes in AODIL included nitric oxide synthase 3 (NOS3), Angiopoietin-like 4 (*ANGPTL4*), ADAM metallopeptidase with thrombospondin type 1 motif, 9 *(ADAMTS9)*, chemokine (C-C motif) ligand 2 (*CCL2*), G protein-coupled receptor 4 (*GPR4*), metallothionein *(MT1A), MT2A*, selectin E *(SELE)*, hypoxia inducible factor 1 alpha subunit *(HIF1A)* (Figure 3). A comparison of the PCR results with the microarray data of the selected genes is shown in Table 5.

Discussion

Principal findings of the study

1) the myometrial transcriptome of patients with an AODIL has been characterized; differential expression of 42 genes was identified; 2) Gene Ontology analysis revealed enrichment of multiple biological processes and molecular functions impacting regulation of angiogenesis, response to hypoxia, inflammatory response, chemokine-mediated signaling pathway, apoptosis, stress response, and muscle contraction in an AODIL; 3) using Metacore pathway analysis, we identified enrichment of the immune response CCL2 signaling pathway, muscle contraction - regulation of eNOS Activity in the Endothelial Cells pathway, and Triiodothyronine and Thyroxine signaling; and 4) qRT-PCR performed in an independent sample group confirmed overexpression of *NOS3, ANGPTL4, ADAMTS9, CCL2, GPR4, MT1A, MT2A, SELE, HIF1A* in an AODIL. This is the first study to describe the transcriptome of myometrium in patients with AODIL.

Differentially expressed genes related to muscle contraction

Although action potentials in myometrium are initiated by a cellular influx of Ca^{2+} ions, repolarization depends on K^+ ion efflux combined with inhibition of Ca^{2+} ion channels. A growing body of evidence supports the essential role of ion channels in uterine contractility [9,13,16,19,20,35,37,53,68,79,101,103,105,126,132,140,144,160,166,172,193,200,202]. Microarray analysis showed significant overexpression of *NOS3* and *HIF1A* in patients with an AODIL ($P<0.001$); confirmatory qRT-PCR assays were also significant ($P<0.05$). A role for these 2 genes in the regulation of myometrial contractility has been proposed. During pregnancy, the placenta [42,50,147,161,182,201], myometrium [57,127,147,182], and chorioamniotic membranes [49,50] produce nitric oxide (NO) to maintain uterine quiescence [38,106,130,133,170,171,195–198] by stimulation of guanylate cyclase, leading to the production of cGMP and a reduction in intracellular Ca^{2+} [26,51,52,104,110,125,133,170,197]. Previous studies have demonstrated that uterine NO production has been up-regulated during pregnancy and down-regulated during term and preterm labor [130,171,196–198]. Overexpression of NO synthase in AODIL may change the availability of NO in parturition.

Hypoxia is associated with the reduction of blood flow to the uterus during contractions [22,80,96]. Moreover, hypoxia/ischemia of the myometrium has been linked to decreased uterine contractility, a potential cause for dysfunctional labor [84,141,145,178,179,191,192]. HIF1A is an oxygen-sensitive transcription factor that allows adaptation to hypoxic environments and response to metabolic, hypoxic or inflammatory stress [165]. In human

pregnancy, HIF1A plays a role in the survival of hematopoietic precursors, embryonic vasculature [146] and trophoblast differentiation [3,44,119] during development [2]. Mittal *et al* [123] demonstrated a higher expression of *HIF1A* in the myometrium of patients with an arrest of descent (AOD) than in those with spontaneous term labor. Our findings suggest that during the course of failure to progress in labor (AODIL or AOD), a change in the expression of factors responsible for sensing tissue hypoxia occurs.

Differentially expressed genes related to inflammatory and immune response signaling in arrest of dilatation

CCL-2, also known as monocyte chemoattractant protein 1 (MCP1), is a member of a large chemokine family of soluble chemoattractant cytokines, which locally mediate leukocyte migration into various tissues [82,115]. CCL-2 is produced by endothelial cells, fibroblasts, monocytes, lymphocytes, and smooth muscle cells [47,89]. Recent evidence suggests that CCL-2 is up-regulated in human myometrium and amniotic fluid [58,91] as well as rat myometrium during labor [167]. Esplin et al. demonstrated that the amniotic fluid concentration of CCL-2 increased in women with preterm labor [59]. Moreover, mechanical strain caused by the growing fetus and experimental mechanical stretch of rat myometrial smooth muscle cells induces the release of CCL-2, and promotes chemotaxis of rat monocytes [167,168]. Hua et al [91] also reported that stretch and inflammatory cytokines (IL-1β and TNF α) induce marked increases in the expression of CCL-2 and CXCL-8 (IL8) in human term myometrium. We have previously reported that IL-8 [154] and IL-1 [155,156] were increased in the amniotic fluid of women in spontaneous labor at term. Our findings indicate that patients with an AODIL have overexpression of *CCL-2* in myometrium. qRT-PCR confirmed a significant increase in *CCL-2* expression in AODIL.

Biological functions of a select group of differentially expressed genes

Angiopoietin-like protein 4 (ANPTL4) is a multifunctional signal protein and expressed in the liver [102], adipose tissue [56], intestine [12] and placenta [100,188,199,203]. ANGPTL4 has been recognized as a central molecule in energy homeostasis. One of its actions is to inhibit lipoprotein lipase (LPL). This molecule also activates specific integrins to facilitate wound healing, modulate vascular permeability, and regulates reactive oxygen species (ROS) to promote tumorigenesis [81,204]. ANPTL4 is considered a positive acute phase protein since its expression in liver, heart, muscle and adipose tissue, and plasma are up regulated after LPS, TNF alpha, IL-1β, and interferon gamma treatment in mice [116]. We found a significantly higher expression of *ANPTL4* in the myometrium of pregnant women with an AODIL than in those with spontaneous TL, and this finding was confirmed by qRT-PCR data.

We also found a significantly higher expression of metallothionein *(MT1A)* and *MT2A* in the myometrial of patients with an AODIL than in those with spontaneous TL. Metallothioneins have been recognized to protect against oxidative stress [93,97,162,163]. Prior studies have demonstrated increase oxidative stress in the human placenta during labor [39]. Moreover, there is an increase in pro-inflammatory cytokines/chemokines [11,17,33,45,83,99,128,138,158,175,180] and eicosanoids [73,98,121,122] and lipooxygenase arachidonate products [143,151–153] during labor. These changes

accompany the increase in PMN migration and increased production of ROS in the myometrium [41,95,149]. Overexpression of *MT1A* and *MT2A* in the myometrium of patients with AODIL is consistent with previous studies demonstrating that Metallothionein genes are transcriptionally activated in cells and tissues in response to oxidative stress [7,8,159]. This overexpression in AODIL may be attributed to an increase in ROS production.

Pathway analysis

Most gene set analysis methods treat genes equally, regardless of how specific they are to a given gene set. PADOG is a new gene set analysis method that computes a gene set score as the mean of absolute values of weighted moderated gene T-scores. The gene weights are designed to emphasize the genes specific to a given pathway [181]. PADOG significantly improves gene set ranking and boosts sensitivity of analysis compared to methods that treat genes equally [181]. Using PADOG we identified enrichment of the TGF- Beta signaling Pathway and Protein processing in endoplasmic reticulum having unadjusted p-values <0.01. However, the adjusted p value was not significant (q-value=0.81 for both pathways). The Metacore database demonstrated a significantly enriched pathway; “immune response CCL2 signaling” and “muscle contraction-regulation of eNOS activity in endothelial cells” in the myometrium from patients with AODIL.

Muscle contraction-Regulation of eNOS activity in endothelial cells

Endothelial nitric oxide synthase (eNOS), one of the three distinct isoforms of nitric oxide synthases, is expressed in endothelial cells, cardiac myocytes, platelets, neurons of the brain, syncytiotrophoblasts of human placenta and kidney tubular epithelial cells [63,65]. eNOS plays a role in blood vessel vasodilatation, blood pressure regulation, platelet and leukocyte adhesion to endothelium and myometrial contractility [10,15,63]. Batlett et al reported eNOS expression (immunoblot, mRNA and protein) in the vascular endothelium of human pregnant myometrium, but not in cultured myometrial smooth muscle cells [15]. The diffusion of NO generated within the endothelium of the myometrial vasculature has implicated in uterine relaxation [15]. eNOS activity is regulated by mechanical forces such as shear stress [63] and humoral factors including estrogen, vascular endothelial growth factor, insulin and bradykinin [63]. eNOS is calcium-dependent; therefore, a higher concentration of intracellular calcium leads to eNOS activation [64].

Comparison with previous reports of functional genomics in labor dystocia

Our findings indicate that AODIL is associated with changes in the genes involved in inflammatory processes. These findings are consistent with prior reports from our group that focus on arrest of descent. [123]. Mittal et al. [123] first characterized the myometrial transcriptome of patients with an arrest of descent; 400 genes were differentially expressed. Impacted pathways included inflammation and muscle function. qRT-PCR confirmed the overexpression of *HIF1A*, *IL-6* and prostaglandin-endoperoxide synthase 2 or cycloozygenase-2(*COX2*) in patients with an arrest of descent. However, our results are different from those of Brennan et al [23] who reported that 70 genes were differentially expressed in nulliparous women with dystocia (n=4) and women in spontaneous TL (n=4).

Enrichment was observed in genes involved in immune response, transcription, and DNA replication [23]. Technical validation with qRT-PCR confirmed overexpression for endoplasmic reticulum aminopeptidase 2 (*ERAP2*), major histocompatibility complex, class II, DQ beta 1; (*HLADQB1*), cluster of differentiation *(CD) 28*, and leukocyte immunoglobulin-like receptor, subfamily A, member 3 (*LILRA3*) [23]. Potential explanations for the apparent differences include: 1) the criteria to diagnose labor disorders were different between our study and that of Brennan et al. We focused on AODIL, while Brennan et al studied “labor dystocia”. Yet, the two conditions are different; and 2) the sample sizes between the two studies were also different [23].

Strengths and Limitations

Strengths of this study include its prospective design, the use of a large sample set, stringent inclusion and exclusion criteria, and qRT-PCR confirmation in an independent set of specimens (biological replication).

A potential limitation of the study is that the frequency of nulliparous women was higher in the group with spontaneous labor at term than in patients with an AODIL. Yet, there was no effect modification of parity on gene expression by clinical groups (AODIL vs. TL), with one exception: KIF5C: kinesin family member 5C. Another limitation was that women in the AODIL group had lower BMI and received intrapartum oxytocin more frequently than women in spontaneous term labor [71.4% (10/14) vs. 27.6% (8/29), p=0.006]. However, we did not detect a significant interaction between AODIL and BMI or oxytocin treatment in myometrium gene expression. Lastly, the myometrial samples in the current study were taken from the lower uterine segment. It has been reported that gene expression of the lower uterine segment may be different from that of upper uterine segment [27].

Conclusion

The myometrial transcriptome of patients with an arrest of dilatation has been characterized. This disorder has been associated with a pattern of gene expression involved in muscle contraction, an inflammatory response, and hypoxia. The findings reported herein provide insight into the molecular basis, biological processes, and pathway associated with an arrest of dilatation.

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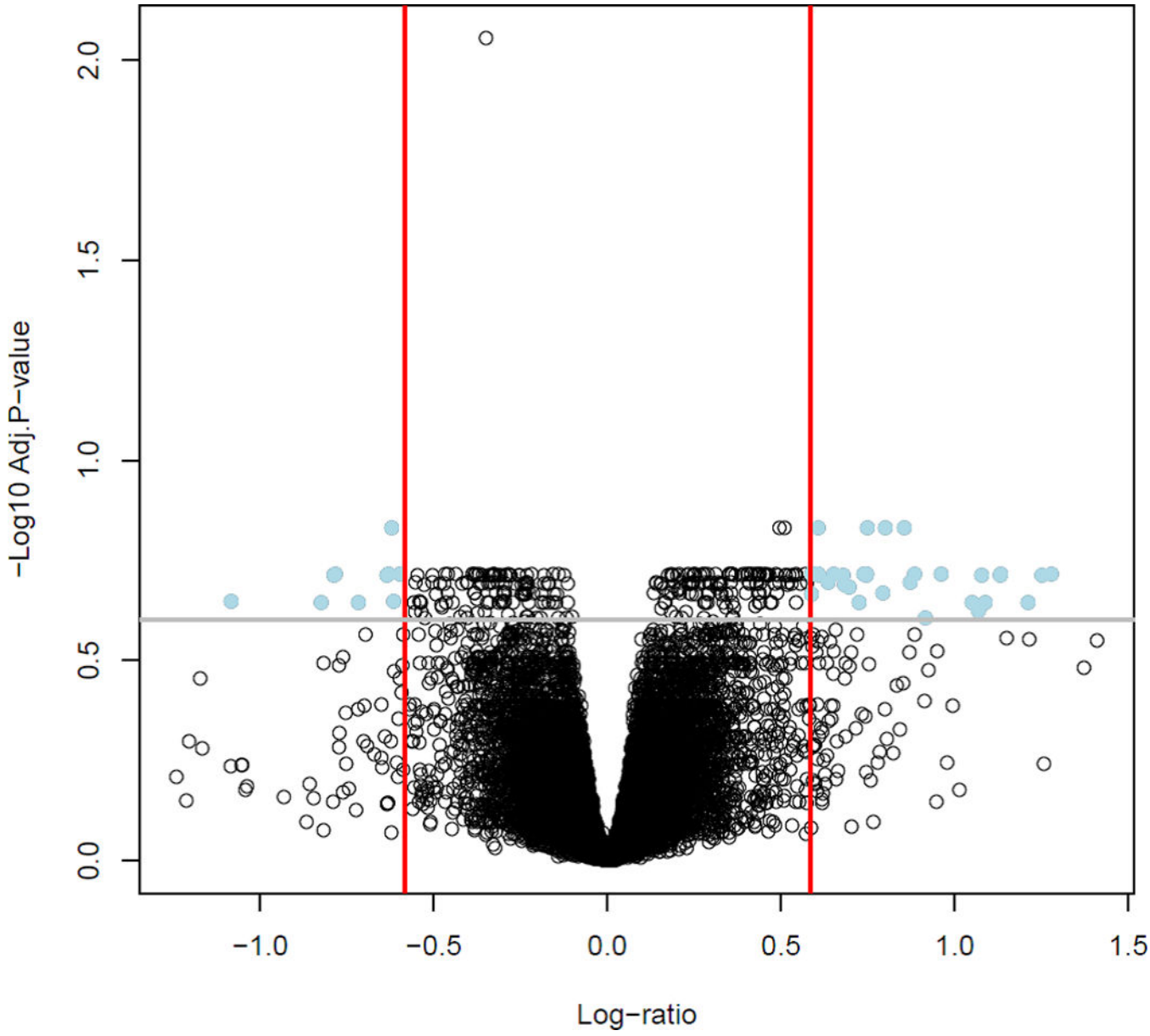
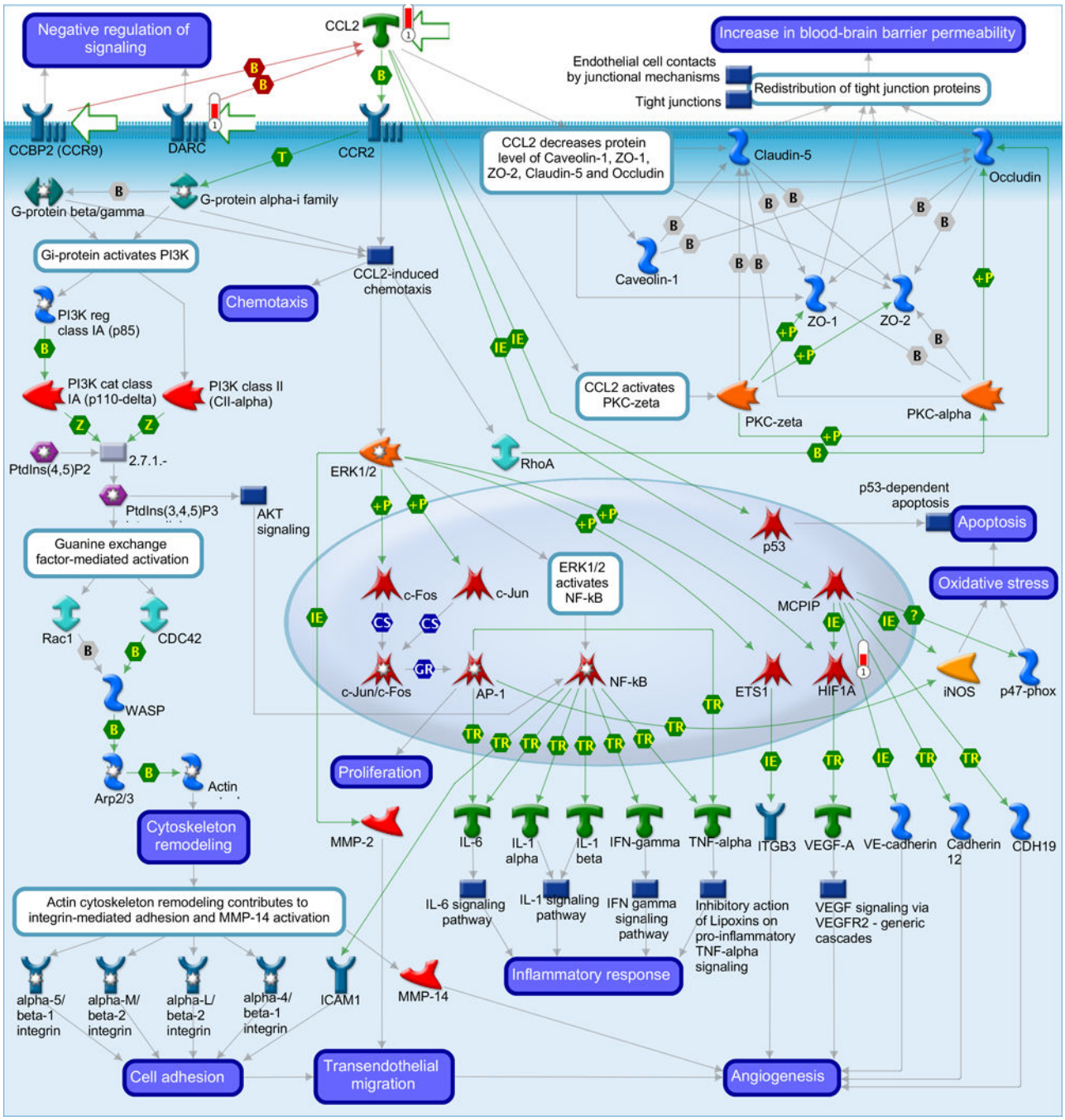


Figure 1. Microarray analysis of the gene expression profiles of myometrium in spontaneous term labor (TL) and arrest of dilatation (AODIL)

A Volcano plot shows differentially gene expression between AODIL and TL. Dots in the upper right and left quadrants represent genes with a fold change greater than 1.5 and a false discovery rate corrected p-value < 0.25. With these criteria, 42 genes were differentially expressed between the myometrial transcriptome of the two groups.



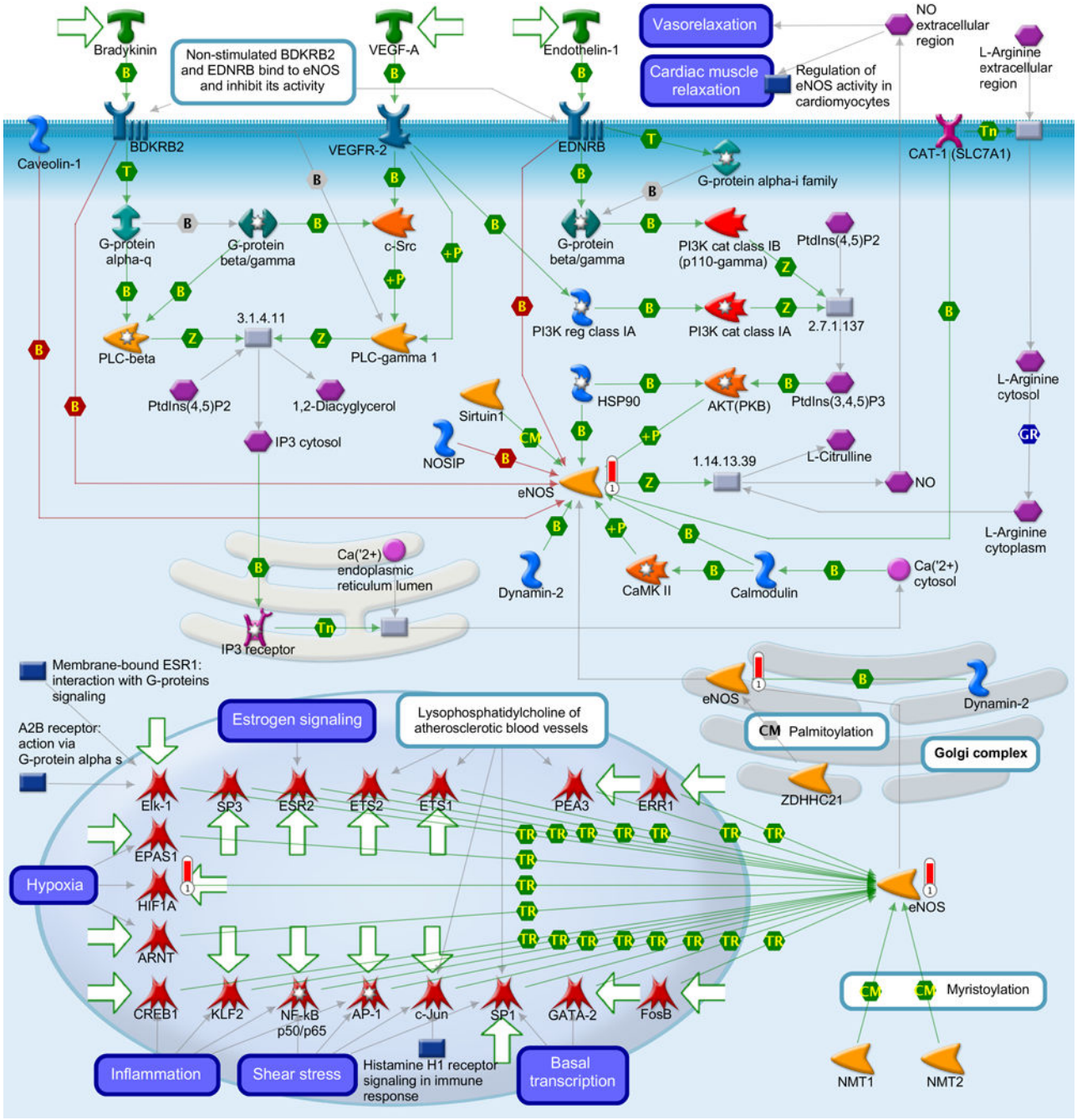


Figure 2.

A: Display of differentially expressed genes in the arrest of dilatation group on the most significantly over-represented MetaCore pathway: chemokine (C-C motif) ligand 2 (CCL2) signaling

B: Display of differentially expressed genes in the arrest of dilatation group on the most significantly over-represented MetaCore pathway: Muscle Contraction Regulation of eNOS activity in endothelial cells

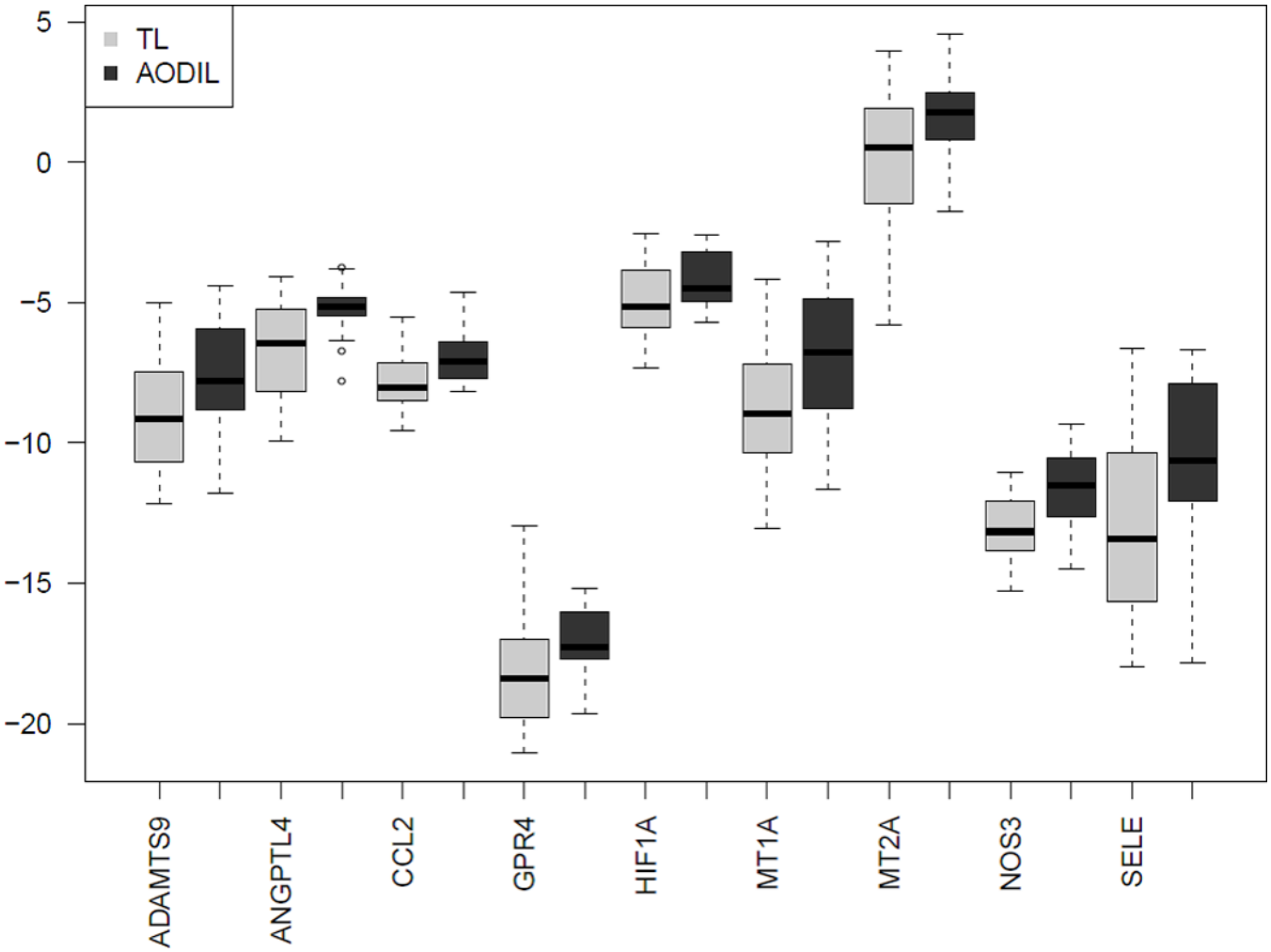


Figure 3.

Boxplot of qRT-PCR data for all 9 tested genes in an expanded sample set including those used in the microarray experiment. The data is presented as the −ΔCt values (surrogate of $\log_2$ expression). The boxes encompass 50% of the data from the 1st quartile to the 3rd quartile. The middle line represents the median value (50%) quartile. The whiskers extend to the most extreme data point, but do not exceed values >1.5 times the interquartile range from the box. The circles represent outliers. TL=spontaneous term labor; AODIL= arrest of dilatation.

Table 1

Demographic and clinical characteristics of the study groups

	Microarray			qRT-PCR		
	Term labor (n=29)	**Arrest of dilatation (n=14)**	**p-value**	**Term labor (n=31)**	**Arrest of dilatation (n=18)**	**p-value***
Maternal age (years)	26.0 ± 6.5	24.4 ± 6.5	0.433	26.4 ± 6.6	24.1 ± 6.5	0.24
BMI (kg/m^2)	32.6 ± 9.3	24.1 ± 2.8	0.0028	29.1 ± 7.3	32.2 ± 7.7	0.20
Nulliparity, %	55 (16/29)	50 (7/14)	1	38.7 (12/31)	66.7 (12/18)	0.06
African-American, %	55 (16/29)	71 (10/14)	0.82	64.5 (20/31)	83.3 (15/18)	0.25
Smoking, %	8 (2/26)	14 (2/14)	0.602	6.5 (2/31)	22.22 (4/18)	0.12
Gestational age at delivery (weeks)	39.3 ± 1.7	39.3 ± 1.3	0.956	39.4 ± 1.4	39.9 ± 1.2	0.21
Birth weight (grams)	3223 ± 440	3489 ± 371	0.06	3266 ± 441.3	3385.4 ± 456.7	0.37

Values are expressed as percentage (number) or mean ± standard deviation
BMI: body mass index.
P value: compare between term labor microarray and arrest of dilatation microarray

P value*: compare between term labor qRT-PCR and arrest of dilatation qRT-PCR

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Table 2

List of all probes with differential expression between the arrest of dilatation and spontaneous term labor groups

Illumina probe	SYMBOL	Name	p-value	q-value*	Fold Change	Direction
ILMN_1708041	*PLEKHF1*	pleckstrin homology domain containing, family F (with FYVE domain) member 1	0.0000	0.1472	1.54	Down
ILMN_1775224	*NOS3*	nitric oxide synthase 3 (endothelial cell)	0.0000	0.1472	1.74	Up
ILMN_1664861	*ID1*	inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	0.0000	0.1472	1.81	Up
ILMN_2074477	*GPR4*	G protein-coupled receptor 4	0.0000	0.1480	1.68	Up
ILMN_1794501	*HAS3*	hyaluronan synthase 3	0.0000	0.1480	1.52	Up
ILMN_1737314	*BCL6*	B-cell CLL/lymphoma 6	0.0001	0.1924	1.57	Up
ILMN_1710268	*ZNF385D*	zinc finger protein 385D	0.0001	0.1924	1.52	Up
ILMN_1805543	*ADAMTS9*	ADAM metallopeptidase with thrombospondin type 1 motif, 9	0.0001	0.1924	2.19	Up
ILMN_1660436	*HSPA1B*	heat shock 70kDa protein 1B	0.0001	0.1924	1.95	Up
ILMN_1666503	*DENND2A*	DENN/MADD domain containing 2A	0.0001	0.1924	1.55	Down
ILMN_1732296	*ID3*	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	0.0002	0.1924	1.68	Up
ILMN_1712888	*HSPH1*	heat shock 105kDa/110kDa protein 1	0.0002	0.1924	1.52	Up
ILMN_1808811	*SBNO2*	strawberry notch homolog 2 (Drosophila)	0.0002	0.1924	1.50	Up
ILMN_1742461	*UAP1*	UDP-N-acteylglucosamine pyrophosphorylase 1	0.0003	0.1924	1.67	Up
ILMN_1723684	*DARC*	Duffy blood group, chemokine receptor	0.0003	0.1924	1.85	Up
ILMN_2226917	*KIAA0247*	KIAA0247	0.0003	0.1924	1.52	Up
ILMN_1748538	*ALDH1A2*	aldehyde dehydrogenase 1 family, member A2	0.0004	0.1924	1.72	Down
ILMN_1709747	*EXOG*	endo/exonuclease (5'-3'), endonuclease G-like	0.0005	0.1924	2.43	Up
ILMN_2316386	*GPBAR1*	G protein-coupled bile acid receptor 1	0.0005	0.1924	1.51	Down
ILMN_1710514	*BCL3*	B-cell CLL/lymphoma 3	0.0005	0.1935	1.68	Up
ILMN_1656920	*CRIP1*	cysteine-rich protein 1 (intestinal)	0.0005	0.1935	1.73	Down
ILMN_2136089	*MT1IP*	metallothionein 1I, pseudogene	0.0005	0.1935	2.38	Up
ILMN_1691156	*MT1A*	metallothionein 1A	0.0005	0.1935	2.19	Up
ILMN_2379788	*HIF1A*	hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	0.0006	0.1935	1.60	Up
ILMN_1686664	*MT2A*	metallothionein 2A	0.0006	0.1935	2.11	Up
ILMN_2116299	*FAM110D*	family with sequence similarity 110, member D	0.0007	0.1935	1.53	Up

Illumina probe	SYMBOL	Name	p-value	q-value*	Fold Change	Direction
ILMN_1719170	*WBSCR27*	Williams Beuren syndrome chromosome region 27	0.0008	0.1935	1.55	Down
ILMN_2329914	*SPRY1*	sprouty homolog 1, antagonist of FGF signaling (Drosophila)	0.0009	0.2026	1.55	Up
ILMN_1674243	*TFRC*	transferrin receptor (p90, CD71)	0.0010	0.2026	1.83	Up
ILMN_1704730	*CD93*	CD93 molecule	0.0013	0.2047	1.61	Up
ILMN_1776998	*DNAJA4*	DnaJ (Hsp40) homolog, subfamily A, member 4	0.0014	0.2080	1.62	Up
ILMN_1789074	*HSPA1A*	heat shock 70kDa protein 1A	0.0017	0.2156	1.73	Up
ILMN_2086105	*SPRY4*	sprouty homolog 4 (Drosophila)	0.0018	0.2170	1.50	Up
ILMN_1659631	*LOC440905*	uncharacterized LOC440905	0.0019	0.2254	2.12	Down
ILMN_1763852	*ACACB*	acetyl-CoA carboxylase beta	0.0019	0.2254	1.53	Down
ILMN_2053103	*SLC40A1*	solute carrier family 40 (iron-regulated transporter), member 1	0.0020	0.2265	1.77	Down
ILMN_1720048	*CCL2*	chemokine (C-C motif) ligand 2	0.0020	0.2265	2.32	Up
ILMN_1797744	*TPPP3*	tubulin polymerization-promoting protein family member 3	0.0021	0.2265	1.64	Down
ILMN_1801504	*RUNX1*	runt-related transcription factor 1	0.0021	0.2265	1.65	Up
ILMN_1707727	*ANGPTL4*	angiopoietin-like 4	0.0022	0.2267	2.07	Up
ILMN_1739393	*SELE*	selectin E	0.0022	0.2267	2.13	Up
ILMN_1761281	*MT2A*	metallothionein 2A	0.0025	0.2382	2.10	Up
ILMN_1685540	*SHROOM3*	shroom family member 3	0.0027	0.2482	1.89	Up

*P-values adjusted for multiple comparisons using the False Discovery Rate method

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Table 3

Gene Ontology analysis: top 40 biological processes associated with differentially expressed genes between arrest of dilatation and term labor

GO ID	GO Name	DE genes	GO Size	Odds Ratio	p-value	q-value
GO:0045765	regulation of angiogenesis	6	129	22.3	0.0000	0.001
GO:0006915	apoptotic process	13	1380	5.2	0.0000	0.007
GO:0012501	programmed cell death	13	1391	5.2	0.0000	0.007
GO:0022603	regulation of anatomical structure morphogenesis	8	480	8.1	0.0000	0.007
GO:0045766	positive regulation of angiogenesis	4	69	26.4	0.0000	0.007
GO:0072358	cardiovascular system development	9	653	6.9	0.0000	0.007
GO:0072359	circulatory system development	9	653	6.9	0.0000	0.007
GO:0048646	anatomical structure formation involved in morphogenesis	9	666	6.7	0.0000	0.007
GO:0002376	immune system process	13	1492	4.8	0.0001	0.008
GO:0008219	cell death	13	1529	4.7	0.0001	0.008
GO:0016265	death	13	1531	4.7	0.0001	0.008
GO:0045926	negative regulation of growth	5	169	13.4	0.0001	0.008
GO:0001568	blood vessel development	7	426	7.7	0.0001	0.010
GO:0050793	regulation of developmental process	11	1143	5.0	0.0001	0.010
GO:0010043	response to zinc ion	3	37	36.7	0.0001	0.011
GO:0046677	response to antibiotic	3	37	36.7	0.0001	0.011
GO:0001944	vasculature development	7	447	7.4	0.0001	0.011
GO:0001525	angiogenesis	6	311	8.9	0.0001	0.011
GO:0001666	response to hypoxia	5	197	11.5	0.0001	0.011
GO:0009887	organ morphogenesis	8	631	6.1	0.0002	0.012
GO:0070482	response to oxygen levels	5	212	10.6	0.0002	0.014
GO:0009653	anatomical structure morphogenesis	13	1711	4.1	0.0002	0.014
GO:0010038	response to metal ion	5	223	10.1	0.0003	0.016
GO:0045064	T-helper 2 cell differentiation	2	10	101.1	0.0003	0.016
GO:0071294	cellular response to zinc ion	2	10	101.1	0.0003	0.016
GO:0035295	tube development	6	359	7.6	0.0003	0.016

GO ID	GO Name	DE genes	GO Size	Odds Ratio	p-value	q-value
GO:0048514	blood vessel morphogenesis	6	374	7.3	0.0004	0.019
GO:0043066	negative regulation of apoptotic process	7	535	6.1	0.0004	0.020
GO:0043069	negative regulation of programmed cell death	7	539	6.0	0.0004	0.020
GO:0006952	defense response	9	911	4.8	0.0004	0.020
GO:0070098	chemokine-mediated signaling pathway	2	12	80.8	0.0004	0.020
GO:0051093	negative regulation of developmental process	6	394	6.9	0.0005	0.021
GO:0006954	inflammatory response	6	396	6.9	0.0005	0.021
GO:0070555	response to interleukin-1	3	60	21.9	0.0005	0.021
GO:0060548	negative regulation of cell death	7	560	5.8	0.0005	0.021
GO:0006879	cellular iron ion homeostasis	3	63	20.8	0.0006	0.023
GO:0001936	regulation of endothelial cell proliferation	3	66	19.8	0.0007	0.024
GO:0009408	response to heat	3	66	19.8	0.0007	0.024
GO:0046631	alpha-beta T cell activation	3	66	19.8	0.0007	0.024
GO:0002467	germinal center formation	2	15	62.2	0.0007	0.024

Table 4

Pathways enriched in an arrest of dilatation as determined by Metacore pathway

Pathway	Ratio		p-value	q-value
Immune response-CCL2 signaling	3	38	0.0003	0.001
Regulation of metabolism_Triiodothyronine and Thyroxine signaling	2	28	0.005	0.009
Muscle contraction-Regulation of eNOS activity in endothelial cells	2	36	0.0078	0.01
Development VEGF signaling via VEGFR2 - generic cascades	2	51	0.015	0.015

CCL2 = chemokine (C-C motif) ligand 2;
eNOS = endothelial nitric oxide synthase;
VEGF=Vascular endothelial growth factor;
VEGFR2 = Vascular endothelial growth factor receptor 2

Table 5

Comparison of qRT-PCR and microarray analysis of select genes*

	Microarray			qRT-PCR		
SYMBOL	**p-value**	**Fold Change**	**Direction**	**P value**	**Fold Change**	**Direction**
NOS3	0.0000	1.74	Up	0.000317	2.70	Up
ANGPTL4	0.0022	2.07	Up	0.000701	3.02	Up
ADAMTS9	0.0001	2.19	Up	0.003901	3.02	Up
CCL2	0.0020	2.32	Up	0.005847	1.82	Up
GPR4	0.0000	1.68	Up	0.006322	2.34	Up
MT1A	0.0005	2.19	Up	0.006342	3.66	Up
MT2A	0.0006	2.11	Up	0.008314	3.01	Up
SELE	0.0022	2.13	Up	0.011038	4.66	Up
HIF1A	0.0006	1.60	Up	0.019147	1.72	Up

*Genes with significant results by microarray analysis and confirmed differential expression by qRT-PCR

Direction of change denotes changes of gene expression in arrest of dilatation compared to the term labor group