Labor-Associated Gene Expression in the Human Uterine Fundus, Lower Segment, and Cervix

Radek Bukowski[1]*, Gary D. V. Hankins[1], George R. Saade[1], Garland D. Anderson[1], Steven Thornton[2]

1 Department of Obstetrics and Gynecology, University of Texas Medical Branch at Galveston, Galveston, Texas, **2** Warwick Medical School, University of Warwick, Coventry, United Kingdom

Abbreviations: ERA, estrogen receptor alpha; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; REA, repressor of estrogen receptor activity; RXR, retinoid X receptor alpha

* To whom correspondence should be addressed. E-mail: rkbukows@ utmb.edu

A B S T R A C T

Background

Preterm labor, failure to progress, and postpartum hemorrhage are the common causes of maternal and neonatal mortality or morbidity. All result from defects in the complex mechanisms controlling labor, which coordinate changes in the uterine fundus, lower segment, and cervix. We aimed to assess labor-associated gene expression profiles in these functionally distinct areas of the human uterus by using microarrays.

Methods and Findings

Samples of uterine fundus, lower segment, and cervix were obtained from patients at term (mean $\pm$ SD = 39.1 $\pm$ 0.5 wk) prior to the onset of labor ($n = 6$), or in active phase of labor with spontaneous onset ($n = 7$). Expression of 12,626 genes was evaluated using microarrays (Human Genome U95A; Affymetrix) and compared between labor and non-labor samples. Genes with the largest labor-associated change and the lowest variability in expression are likely to be fundamental for parturition, so gene expression was ranked accordingly. From 500 genes with the highest rank we identified genes with similar expression profiles using two independent clustering techniques. Sets of genes with a probability of chance grouping by both techniques less than 0.01 represented 71.2%, 81.8%, and 79.8% of the 500 genes in the fundus, lower segment, and cervix, respectively. We identified 14, 14, and 12 those sets of genes in the fundus, lower segment, and cervix, respectively. This enabled networks of co-regulated and co-expressed genes to be discovered. Many genes within the same cluster shared similar functions or had functions pertinent to the process of labor.

Conclusions

Our results provide support for many of the established processes of parturition and also describe novel-to-labor genes not previously associated with this process. The elucidation of these mechanisms likely to be fundamental for controlling labor is an important prerequisite to the development of effective treatments for major obstetric problems—including prematurity, with its long-term consequences to the health of mother and offspring.

The Editors' Summary of this article follows the references.

Introduction

The onset and progression of normal labor involves complex maternal and fetal interactions leading to dilation of the cervix and coordinated uterine contractions.

Temporal disruption of this process can lead to preterm delivery, and ineffective uterine contractility can cause failure to progress in labor or postpartum hemorrhage. These problems have important consequences. Preterm delivery is a major cause of neonatal mortality and morbidity, including long-term neurological impairment [1]. Failure to progress in labor may lead to maternal morbidity and/or caesarean section [2] with its inherent risks, and postpartum hemorrhage is one of the main causes of maternal mortality worldwide [3].

Pregnancy is maintained by myometrial quiescence and cervical resistance. Toward term, there is a progressive activation of the myometrium and the cervix ripens in preparation for labor. Labor is associated with dramatic changes in myometrial contractions and cervical dilation resulting from increased stimulatory and reduced inhibitory processes [4]. These effects are due to simultaneous and interdependent changes in cellular proteins initiated by a multitude of genes. The molecular processes are spatially coordinated to result in uterine contractions with simulta-

neous cervical dilation. Additional spatial organization of contractile processes within the myometrium results in increased contractility of the fundus compared to the lower segment [4–7].

The specific changes in gene expression that cause these temporal and spatial effects are largely unknown. Our hypothesis is that labor results from the simultaneous change in expression of a large number of genes that are organized into co-regulated networks. We examined the labor-associated gene expression changes in the human fundus, lower segment, and cervix using Affymetrix genome DNA microarrays.

Methods

Sample Collection

Tissue was obtained from patients undergoing cesarean section and sterilization without medical or obstetrical complications of pregnancy and who were not exposed to medications immediately before enrollment. The procedure was approved by the Institutional Review Board and Coventry Research Ethics Committee (IRB 00-022, CREC 062/05/01), and informed consent was obtained from all eligible patients. Samples were obtained from patients at term (mean $\pm$ SD = 39.1 $\pm$ 0.5 wk) prior to the onset of labor ($n = 6$), or in active phase of labor with spontaneous onset ($n = 7$). Labor was
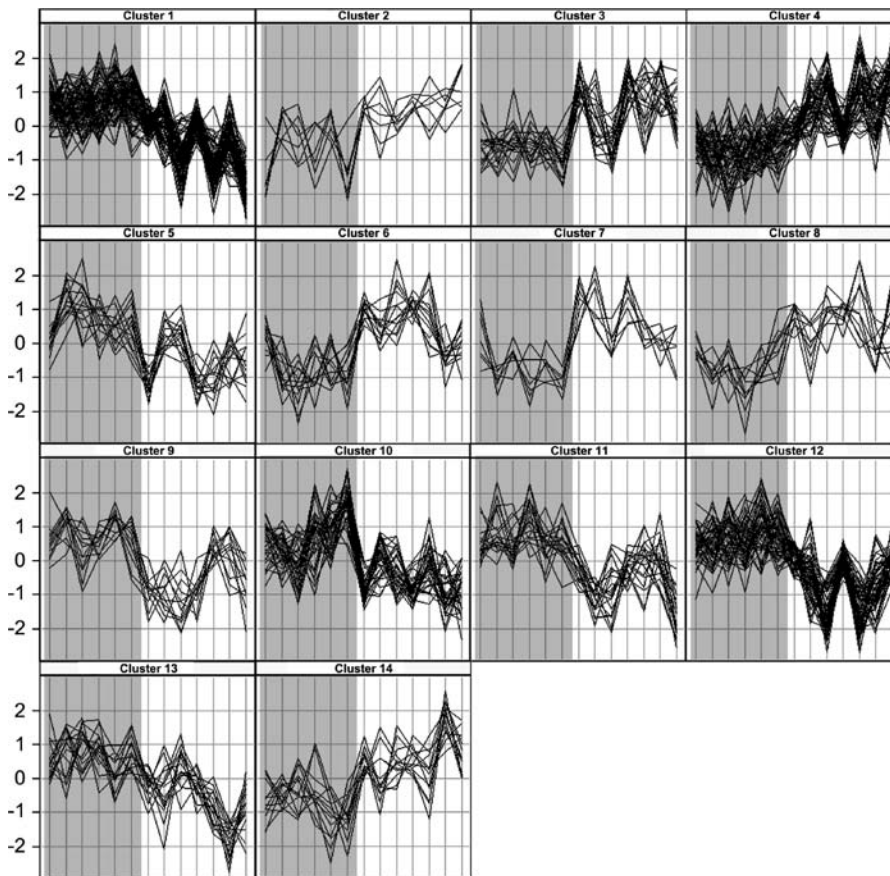
Figure 1. Profiles of Gene Expression in the Uterine Fundus from Women before or after the Onset of Labor

Each panel shows profiles of the genes within one of the clusters determined jointly by K-means and hierarchical clustering. On the x-axis, samples from individual patients are arranged and represented by vertical lines. Non-labor samples (gray background) are shown on the left and labor (white background) on the right. The y-axis represents the level of gene expression as a number of standard deviations from the mean of all observations for each gene (z-score).

DOI: 10.1371/journal.pmed.0030169.g001

defined as cervical dilatation of >3 cm or progressive dilation accompanied by regular uterine contractions. Patients not in labor were delivered by cesarean section on maternal request following counseling by obstetrician because of previous cesarean section or because an abnormal fetal presentation in the index pregnancy made vaginal delivery unsafe. Patients in labor had failure to progress despite adequate contractility or fetal intolerance of labor.

For each patient, samples (approximately 1 cm^3) were taken from the uterine fundus (the outside surface of the uterus that does not include decidua), the lower segment at the upper edge of the incision, and the anterior lip of the cervix, through the vagina. We have previously shown that our lower-segment samples are more than 98% myometrial smooth muscle [8]. In one patient, a biopsy of the cervix could not be obtained. Samples were immediately snap-frozen in liquid nitrogen and stored at −80 °C.

Microarray Analysis

All samples were analyzed separately without pooling of extracted RNA. RNA isolation was performed using TRIzol Reagent (Gibco BRL Life Technologies, San Diego, California, United States) followed by phenol extraction and ethanol precipitation. Genomic contamination was removed by on-column treatment of RNA samples with DNAse (27 Kunitz units) for 20 min at 20 °C (Qiagen, Valencia, California, United States).

Isolated total RNA was quantified by spectrophotometry. Double-stranded cDNA was synthesized from total RNA using T7-(dT)$_{24}$ oligomer primer (Genset Corp., La Jolla, California, United States) and Superscript II Reverse Transcriptase (Gibco BRL Life Technologies). For complete recovery of the cDNA, samples were subjected to phase-lock gel phenol-chloroform extraction and ethanol precipitation. 1 μg of cDNA was used for an in vitro transcription reaction, which involved the synthesis of the biotin-labeled cRNA from the cDNA with biotinylated CTP and UTP (Enzo Life Sciences, Farmingdale, New York, United States). The biotin-labeled RNA fragments were then hybridized to microarray chips (Human Genome U95A; Affymetrix, Santa Clara, California, United States). Microarrays from several different lots were used to analyze samples. Different lots of microarrays will
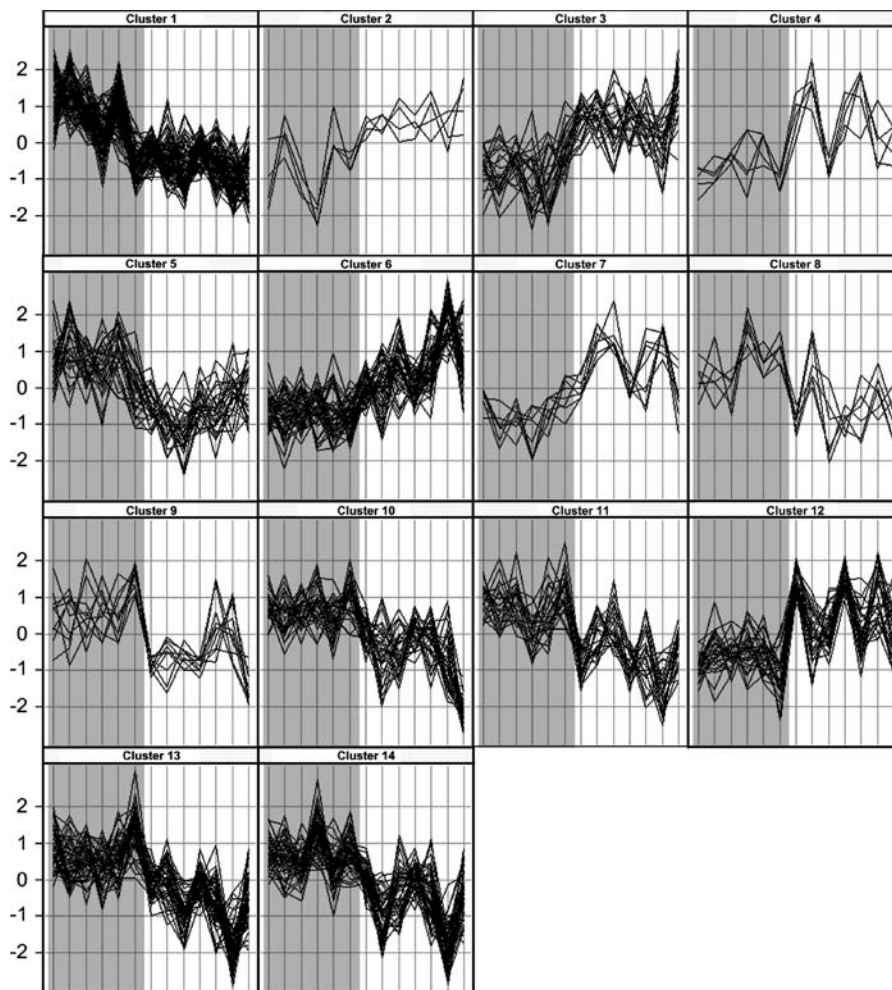
Figure 2. Profiles of Gene Expression in the Uterine Lower Segment from Women before or after the Onset of Labor

Each panel shows profiles of the genes within one of the clusters determined jointly by K-means and hierarchical clustering. On the *x*-axis, samples from individual patients are arranged and represented by vertical lines. Non-labor samples (gray background) are shown on the left and labor (white background) on the right. The *y*-axis represents the level of gene expression as a number of standard deviations from the mean of all observations for each gene (*z*-score).
DOI: 10.1371/journal.pmed.0030169.g002

increase variability of findings but will minimize the chance of a bias or systematic error associated with a certain lot and resulting in false positive and negative findings. The chips were washed, stained on a fluidic station, and scanned by confocal microscope. Each chip was used only once. The average difference intensity was calculated and describes the difference between the intensities of emitted light from hybridized matched probes and their mismatched controls. The average for the 20 probes and their controls are calculated for each gene.

To allow comparison between genes and patients, average difference intensities were converted into percentiles and z-scores. To allow comparison between genes, the differences in RNA hybridizations between probes and controls were normalized by conversion into percentiles. To allow comparison of samples (chip to chip), the percentile values were converted into z-scores for a given gene expression across all samples (expression value – mean/standard deviation).

Identification of the putative gene functions used NetAffx (Affymetrix), an integrated online resource from the Gen-Bank, UniGene, and Gene Ontology databases, and the Ingenuity database (http://www.ingenuity.com).

Statistical Methods

All samples were analyzed separately. To identify genes demonstrating a maximal labor-associated change in expression, the p-value was calculated by Student's t-test. This p-value was used as a measure of the magnitude of the change and inter-subject variability rather than to determine significance. Genes were ordered according to the p-value. The 500 genes in each of the fundus, lower segment, and cervix with the lowest p-values were selected for further analysis.

These genes were clustered using two different techniques: K-means and hierarchical. K-means is a non-hierarchical clustering method that groups data points into a predetermined number of clusters. It is an iterative process in which each gene profile is assigned to the closest centroid, which is the center point of a cluster. The centroid is then recomputed until a steady state has been reached. Euclidian distance was used as a similarity measure for gene profiles. Centroids were initialized using a data-based centroid search. The number of clusters was selected to provide a wide range of genes per cluster without uninformative clusters containing no or single genes.

Hierarchical clustering arranges the genes on a treelike system. Clusters are merged if the expression profiles are similar. The similarity between gene expression profiles was calculated using Euclidian distance and between clusters using unweighted pair-group method with arithmetic mean. Genes clustered together by both techniques were identified. Coincidence testing [9] was used to determine whether co-clustering was likely to have arisen by chance. Figures 1–3 depict clusters of genes grouped together by both methods where probability of chance co-clustering was $p < 0.01$.

Within each of the coincidence clusters, we identified genes with functions similar to other genes within the same cluster or functions pertinent to the process of labor. For this purpose we used an interactive database of gene functions and interactions (Ingenuity pathway analysis) and biological knowledge database (http://www.ingenuity.com).
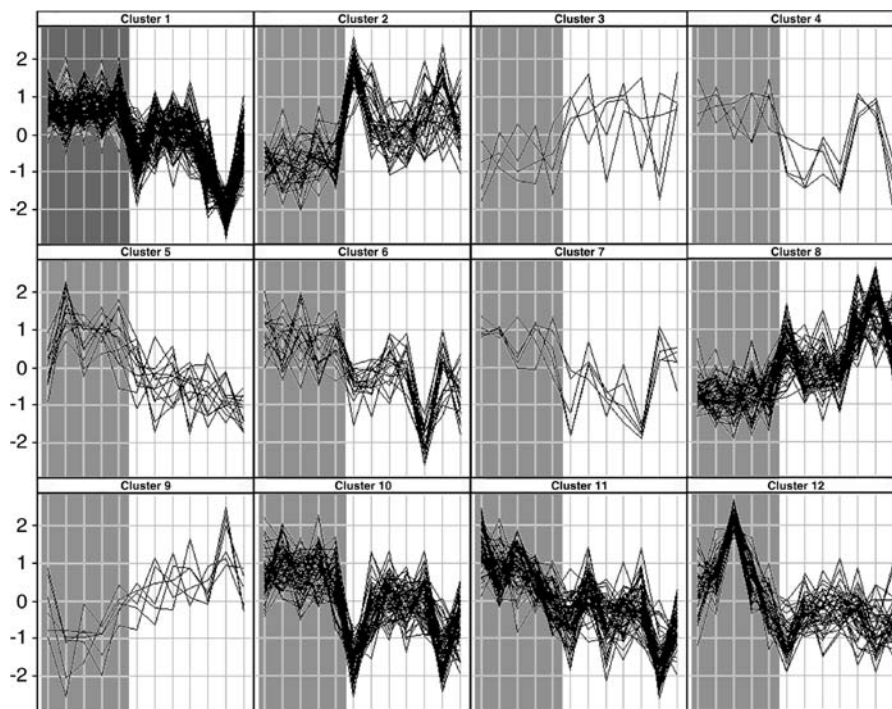
Figure 3. Profiles of Gene Expression in the Uterine Cervix from Women before or after the Onset of Labor

Each panel shows profiles of the genes within one of the clusters determined jointly by K-means and hierarchical clustering. On the x-axis, samples from individual patients are arranged and represented by vertical lines. Non-labor samples (gray background) are shown on the left and labor (white background) on the right. The y-axis represents the level of gene expression as a number of standard deviations from the mean of all observations for each gene (z-score).

DOI: 10.1371/journal.pmed.0030169.g003

Table 1. Fundus: Selected Genes within Each of the Coincidence Clusters with Functions Similar to Other Genes within the Same Cluster or Functions Pertinent to the Process of Labor

Cluster	GenBank ID	Gene Name	Change	Fold	p	Function
1	U72511	Repressor of estrogen receptor activity	−	1.72	0.0120	Inhibits activity of liganded estrogen receptors a and b
1	X52773	Retinoid X receptor alpha	−	1.22	0.0412	Binds estrogen receptor a (ERA) and estrogen response element (ERE)
1	X89416	Protein phosphatase 5 catalytic subunit	−	1.46	0.0197	Estrogen inducible, binds ERA and ERE, binds protein phosphatase 2A
1	M65254	Protein phosphatase 2 regulatory subunit A beta isoform	−	1.10	0.0039	Activates MARCKS-actin cross-linking proteins
1	D10495	Protein kinase C, delta	−	1.61	0.0015	Inhibits P/Q Ca channels and releases nitric oxide
1	M60165	G protein alpha polypeptide O	−	0.90	0.0399	Inhibits voltage-gated and L-type Ca channels, and adenylate cyclase
1	U95626	Chemokine receptor 5	−	1.54	0.0116	Progesterone stimulated chemokine receptor binding cytokines (e.g., IL-8)
1	AF024578	Protein phosphatase 1, inhibitory subunit 3A	−	1.02	0.0424	Increases concentration of glycogen in muscle cells; is Ca inhibited
2	X16302	Insulin-like growth factor binding protein 2	+	1.89	0.0162	Inhibited by EGF; stimulated by estrogens
2	AF040723	Huntington-associated protein 1—neuroan 1	+	1.58	0.0233	Decreases degradation of EGF receptor; involved in synaptic transmission
2	AF009624	Kinesin 17	+	3.15	0.0308	Increases expression of a channel involved in potentiation of synaptic transmission
2	D63485	Inhibitor of NFkB-inducing kinase epsilon	+	1.83	0.0201	NFKB-inducing kinase
3	X89066	Transient potential cation channel C 1	+	2.50	0.0333	Increases influx of calcium into cell, store-operated calcium channel
3	L31584	Chemokine receptor 7	+	2.67	0.0288	Increases intracellular calcium and actin polymerization; is PGE2 induced
3	AF055033	Insulin-like growth factor (IGF) binding protein 5	+	1.25	0.0355	PGE2 and estradiol induced; stimulates IGF1 in smooth muscle cells
3	U50748	Leptin receptor	+	2.55	0.0430	Increases lipolysis and glucose uptake in the muscle cells
4	U26742	Dystrobrevin alpha	+	1.91	0.0003	Neuromuscular junction function
4	AF011406	Corticotropin releasing hormone receptor 2	+	1.45	0.0022	Increases phosphorylation of myosin light chain
4	X66141	Regulatory light chain of myosin	+	2.41	0.0242	Ca-stimulated phosphorylation triggers muscle contraction
4	M60459	Erythropoietin receptor	+	1.80	0.0027	Ca channel activator; present in the muscle cells
4	L25119	Opioid receptor mu 1	+	2.55	0.0291	Increases intracellular Ca, stimulates PLA2, and inhibits adenylate cyclase
4	M17017	Interleukin 8	+	1.31	0.0144	Increases intracellular calcium, activated by NFKB
4	M16441	Tumor necrosis factor b	+	1.16	0.0227	Activates NFKB
4	J02625	Cytochrome P450 IIE 1	+	2.55	0.0054	Increases concentration of PGE2
5	X68149	Burkitt lymphoma receptor 1, G protein–coupled chemokine receptor 5	−	3.23	0.0279	Chemokine receptor regulated by NFKB
5	U53003	Chromosome 21 open reading frame 33	−	1.43	0.0111	Expression regulated by NFKBIA and TNF
5	X62055	Protein tyrosine phosphatase, non-receptor type 6	−	2.99	0.0119	Inhibits Ca mobilization, regulated by TNF
5	Y08110	Sortilin-related receptor 1	−	2.05	0.0264	Lipid and protein transport, regulated by TNF receptor-TNFRSF6
6	D78586	Carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase	+	2.00	0.0158	Pyrimidine synthesis; expression increased by estrogen
6	U96876	Insulin induced gene 1	+	2.06	0.0054	Transcription regulator; present in muscle cells
6	M60618	Nuclear transcription regulator Sp100	+	2.25	0.0252	Transcription repressor
6	X89750	TGFB-induced factor	+	2.05	0.0316	Transcription co-repressor
6	X76091	Regulatory factor X 2	+	1.92	0.0069	Transcription regulator
7	M24486	2-oxoglutarate 4-dioxygenase alpha	+	1.15	0.0178	Expression increased by hypoxia and inhibited by PGI2
7	D11466	Phosphatidylinositol glycan A	+	2.51	0.0276	Transferase of glycosyl groups; role in cell damage

Table 1. Continued

Cluster	GenBank ID	Gene Name	Change	Fold	p	Function
8	M68941	Protein tyrosine phosphatase, non-receptor type 4	+	1.87	0.0136	Signal transduction downstream of the glutamate receptor
8	X90530	GTP-binding protein ragB	+	2.13	0.0302	Signal transduction
8	X86019	Wiskott-Aldrich syndrome protein interacting protein	+	1.27	0.0365	Signal transduction from cell surface receptors to actin cytoskeleton
8	AL030996	THO complex 2	+	1.67	0.0078	Transcription complex element
9	L22475	BCL2-associated X protein	−	1.87	0.0024	Decreases intracellular Ca storage
9	M80563	S100 calcium-binding protein A4	−	1.85	0.0440	Ca-binding protein decreasing ATPase activity of myosin
9	Y12336	RAS guanyl releasing protein 2—guanine nucleotide exchange factor	−	2.13	0.0364	Stimulates RhoA Ca sensitization–dependent maintenance of contraction
9	M13981	Inhibin alpha	−	2.21	0.0345	Stimulated by antiprogestins, synergistic with progesterone, and inhibited by indomethacin
10	M27318	Interferon alpha 4	−	1.75	0.0188	Increases expression of IFNG (gamma)
10	U11870	Interleukin 8 receptor alpha	−	2.26	0.0393	Upregulated by INFG
10	D14838	Fibroblast growth factor 9	−	1.75	0.0220	Activated by INFG
10	W27605	Cone-rod homeobox gene	−	3.02	0.0226	Increases PDE6-cGMP-specific phosphodiesterase
10	X67594	Melanocortin 1 receptor	−	1.72	0.0195	Activates adenylate cyclase; binds POMC
10	AA846749	Apolipoprotein M	−	1.76	0.0003	Lipid transporter in energy pathway; inhibited by POMC
11	U80017	Baculoviral IAP repeat-containing 1	−	1.72	0.0006	Apoptosis inhibitor
11	D29013	Polymerase beta	−	1.38	0.0367	Apoptosis inhibitor
11	M15024	v-myb myeloblastosis viral oncogene	−	1.20	0.0432	Apoptosis inhibitor
11	U76388	Steroid hormone nuclear receptor 5 A1	−	1.96	0.0054	Increases cAMP-mediated progesterone synthesis
11	U15422	Protamine 2	−	0.75	0.0290	Dephosphorylates glycogen synthase
12	U96781	Ca++ transporting ATPase in sarcoplasmic reticulum	−	1.82	0.0021	Ca-ransporting ATPase in sarcoplasmic reticulum; K activated
12	X63575	Ca++ transporting ATPase in plasma membrane	−	1.58	0.0256	Ca-transporting ATPase in plasma membrane; K activated
12	D49919	Chemokine receptor 8	−	1.04	0.0311	Increases intracellular Ca; inhibited by E2
12	X83228	Cadherin 17	−	2.07	0.0100	Ca-dependent protein transporter and cell-adhesion molecule
12	D78011	Dihydropyrimidinase	−	1.69	0.0096	Inhibits L-type voltage-gated Ca channel
12	AL034562	Prodynorphin	−	1.48	0.0268	Ca stimulated, induces release of CGRP, which increases cAMP and Ca
12	AF035594	Protein kinase C-alpha	−	0.99	0.0357	Ca-dependent regulation of Na/K ATPase in sarcolemma
12	J05428	UDP glycosyltransferase 2	−	1.24	0.0166	Increases glucuronidation of E but not of P
12	D32202	Adrenergic alpha-1A receptor	−	1.84	0.0254	Increases smooth muscle tone via RhoGEF activation
12	AB008430	FERM, RhoGEF-Rho guanine nucleotide exchange factor	−	1.08	0.0432	Stimulates RhoA Ca sensitization–dependent maintenance of contraction
12	L26584	Ras protein-specific guanine nucleotide-releasing/exchange factor 1	−	1.07	0.0238	Stimulates RhoA Ca sensitization–dependent maintenance of contraction
12	W27674	Guanylate cyclase activator 1A	−	1.15	0.0262	Ca-sensitive guanylate cyclase activator

Cluster number identifies a coincidence cluster of genes grouped together by K-means and hierarchical clustering methods where probability of chance co-clustering was $p < 0.01$. The fold change is a ratio of the difference between labor and non-labor medians of gene expression to the non-labor median level of gene expression. The change sign indicates the direction of the expression change in the labor samples compared to non-labor. The p-value is given for the difference in gene expression in samples taken before and after labor. Gene function is derived from the database of gene functions and interactions Ingenuity Pathway Analysis and Biological Knowledge Database.
DOI: 10.1371/journal.pmed.0030169.t001

Validation of the Microarray Findings

Labor-associated changes in the expression of selected genes were examined using RT-PCR. Reaction products were separated, detected, and quantified with chip-based gel electrophoresis (Agilent 2100 bioanalyzer; Agilent Technologies, Palo Alto, California, United States) as described previously [10]. The number of PCR cycles (35) was selected from the linear portions of the dynamic ranges of amplification. The quantification and sizing coefficients of variation are <6.7% and <2.1%, respectively [11]. All mRNA abundance data were expressed relative to constitutively expressed 18S rRNA. The non-labor and labor samples were compared using the Mann-Whitney U test.

Results

We analyzed the expression of 12,626 known genes in biopsies taken from the fundus, lower segment, and cervix either before ($n = 6$) or after the onset of labor ($n = 7$). The expression of each gene was quantified using an Affymetrix gene microarray. Student's t-test was used to determine the p-value for the difference in gene expression in samples taken before or after labor. This test identifies those genes with the

Table 2. Lower Segment: Selected Genes within Each of the Coincidence Clusters with Functions Similar to Other Genes within the Same Cluster or Functions Pertinent to the Process of Labor

Cluster	GenBank ID	Gene Name	Change	Fold	p	Function
1	AB015228	Aldehyde dehydrogenase 1 A2	−	1.62	0.0048	Apoptosis
1	M15059	Fc fragment of IgE	−	1.44	0.0231	Apoptosis
1	AL080218	Transcription activator STAT 5	−	2.63	0.0274	Apoptosis
1	U09759	Mitogen-activated protein kinase 9	−	1.73	0.0143	Apoptosis and cell growth
1	X75621	Tuberin GTPase activator	−	1.89	0.0112	Cell growth
1	M30448	Casein kinase-fibrillarin	−	1.70	0.0257	Cell growth
1	U50939	Amyloid beta precursor protein–binding protein	−	2.08	0.0267	Apoptosis
1	AJ132917	Methyl CpG–binding protein 2	−	1.63	0.0353	Cell size
1	M28211	Rho GTPase	−	1.67	0.0016	Cell size
2	D88827	Zinc finger protein 263	+	1.56	0.0351	Regulation of transcription
2	AF053944	AE binding protein 1	+	2.01	0.0027	Transcription factor in muscle development
3	AL022329	Beta-adrenergic receptor kinase 2	+	2.53	0.0254	Inhibits voltage-gated Ca channels and increases cAMP
3	U56998	Cytokine-inducible kinase	+	1.87	0.0080	cAMP-dependent and ATP-binding kinase involved in apoptosis
3	X63575	ATPase, Ca++ transporting, plasma membrane 2	+	2.48	0.0055	Calmodulin-binding Ca-transporting ATPase
3	X60201	Brain-derived neurotrophic factor	+	2.21	0.0133	Cell proliferation
3	M60828	Fibroblast growth factor 7 (keratinocyte growth factor)	+	1.98	0.0076	Cell proliferation
3	U96876	Insulin-induced gene 1	+	1.53	0.0073	Cell proliferation
4	Z22555	Scavenger receptor class B 1	+	2.17	0.0273	Increases uptake of LDL and triacyl glycerol
4	L13939	Adaptor-related protein complex 1, beta 1 subunit	+	1.14	0.0349	Involved in endocytosis and vesicle transport
5	U77914	Jagged 1 gene	−	1.66	0.0115	Cell differentiation, growth and apoptosis
5	L76517	Presenilin 1	−	1.69	0.0139	Cell differentiation, growth and apoptosis
5	X56687	RNA polymerase I transcription factor	−	2.41	0.0140	Cell growth and death
5	X05608	Neurofilament light polypeptide	−	1.54	0.0084	Cell growth and death
5	U11791	Cyclin H	−	1.80	0.0297	Temporal coordination of mitosis
6	L76380	Calcitonin-like receptor	+	1.71	0.0211	Increased production of cAMP and mobilization of Ca binds CGRP
6	M22430	Phospholipase A2, IIA	+	1.83	0.0056	Increases expression of INOS and PGE2
6	AA004795	Amyloid beta precursor protein 2–binding protein	+	2.03	0.0291	Ca-binding protein in the heart
6	J00068	Actin alpha 1	+	1.37	0.0198	Muscle contractility
6	L76571	Nuclear receptor 0 B 2	+	1.18	0.0087	Orphan receptor inhibiting activity of estrogen and thyroid hormone receptors
6	X13967	Leukemia inhibitory factor	+	1.27	0.0193	Cell proliferation and growth
6	X62055	Protein tyrosine phosphatase, non-receptor type 6	+	1.48	0.0174	Cell proliferation and growth
6	X82240	T-cell leukemia	+	1.62	0.0026	Cell proliferation and growth
7	X66363	PCTAIRE protein tyrosine kinase 1	+	1.00	0.0227	Protein phosphorylation in sarcolemma
7	N36295	Dolichyl-mannosyltransferase regulatory subunit 2	+	2.51	0.0015	Protein glycosylation; regulates glycosylphosphatidylinositol synthesis
7	M16750	Pim-1 protein tyrosine kinase	+	1.48	0.0325	Protein phosphorylation; involved in cardiac hypertrophy
8	L13463	Regulator of G protein signaling 2	−	2.78	0.0343	Decreases Ca release and response to cAMP
8	U19261	TNF receptor-associated factor 1	−	1.75	0.0166	Mediates TNF-a activation of NFkB and MAPK8/JAK and cell apoptosis
8	AF041381	E2F transcription factor 6	−	2.24	0.0258	Suppressor of transcription; regulated by TNFRS5 a TRAF1 receptor
9	U95626	Chemokine receptor 5	−	1.99	0.0227	Regulated by IL-4 and TNF; involved in cell apoptosis
9	Y14737	Immunoglobulin heavy constant gamma 3	−	2.13	0.0206	Regulated by IL-4; regulates TNF; involved in cell apoptosis
9	X76079	Platelet-derived growth factor receptor, alpha subunit	−	1.97	0.0205	Cell apoptosis
9	AJ001366	Potassium voltage-gated channel H 1	−	2.49	0.0198	Activated by membrane depolarization and inhibited by intracellular Ca^{2+}
10	AF015950	Telomerase reverse transcriptase	−	1.37	0.0180	Cell apoptosis
10	U58334	Tumor protein p53 binding protein, 2	−	1.24	0.0068	Cell apoptosis
10	S69369	Paired box gene 3	−	1.94	0.0099	Cell apoptosis
10	X87176	17-beta hydroxysteroid dehydrogenase 4	−	1.75	0.0065	Progesterone-stimulated; facilitates conversion of E2 to E1
11	AJ001015	Calcitonin receptor activity–modifying protein 2	−	1.28	0.0296	Facilitates effect of adrenomedullin
11	U18760	Nuclear transcription factor I/X	−	1.30	0.0192	CCAAT-binding transcription factor regulated by ADRA1
11	D50929	Eukaryotic translation initiation factor 3	−	1.55	0.0173	Translation regulator
11	AF049703	E74-like factor 5	−	1.48	0.0090	Transcription regulator
12	M14083	Plasminogen activator inhibitor type 1, member 1	+	1.49	0.0098	Cell migration and tissue formation
12	AI743134	Plasminogen activator inhibitor type 1, member 2	+	1.42	0.0308	Cell migration and tissue formation
12	X57766	Matrix metalloproteinase 11 (stromelysin 3)	+	1.08	0.0208	Cell migration and tissue formation
12	M23379	RAS p21 GTPase activator 1	+	1.06	0.0240	Cell migration and tissue formation
12	X12451	Cathepsin L	+	2.49	0.0313	Cell migration and tissue formation
13	U34584	BCL2-interacting killer (apoptosis-inducing)	−	1.81	0.0148	Cell apoptosis
13	U05340	CDC20 cell division cycle 20 gene	−	1.96	0.0056	Cell apoptosis

Table 2. Continued

Cluster	GenBank ID	Gene Name	Change	Fold	p	Function
13	U58334	Tumor protein p53–binding protein, 2	−	1.42	0.0306	Cell apoptosis
13	AF001383	Bridging integrator 1	−	1.56	0.0038	Cell apoptosis
13	AF018253	Tumor necrosis factor receptor 11a	−	1.72	0.0228	Cell apoptosis
13	U83600	Tumor necrosis factor receptor 25	−	1.12	0.0202	Cell apoptosis
13	AI193606	Potassium channel K 3	−	2.57	0.0050	Decreases depolarization and excitation
13	U39196	Potassium inwardly rectifying channel J 3	−	1.22	0.0322	Increase of K+ efflux; shortening of action potential; activated by ADRA1A
13	D25235	Adrenergic alpha-1A receptor	−	2.22	0.0256	Activates KCNJ3
13	AJ224874	Voltage-gated calcium channel alpha 1F subunit	−	1.38	0.0301	Tonically active over large range of voltage
14	U60521	Caspase 9, apoptosis-related cysteine protease	−	3.14	0.0350	Cell apoptosis
14	M89470	Paired box gene 2	−	2.01	0.0229	Cell apoptosis
14	M22976	Cytochrome b-5	−	2.30	0.0047	Cell apoptosis
14	Y10659	Interleukin 13 receptor, alpha 1	−	2.46	0.0010	Regulated by IL4 and IL13
14	L41067	Nuclear transcription factor calcineurin-dependent 3	−	1.73	0.0213	Regulates IL4 and IL13

Cluster number identifies a coincidence cluster of genes grouped together by K-means and hierarchical clustering methods where probability of chance co-clustering was $p < 0.01$. The fold change is a ratio of the difference between labor and non-labor medians of gene expression to the non-labor median level of gene expression. The change sign indicates the direction of the expression change in the labor samples comparing to non-labor. The p-value is given for the difference in gene expression in samples taken before and after labor. Gene function is derived from the database of gene functions and interactions Ingenuity Pathway Analysis and Biological Knowledge Database.
DOI: 10.1371/journal.pmed.0030169.t002

largest labor-associated change in expression and the lowest variability. The 500 genes with the lowest p-values were selected from fundus (Dataset S1), lower segment (Dataset S2), and cervix (Dataset S3). Of the 500 genes with the largest change in expression, 28 were common to both the fundus and lower segment. This finding suggests that a small core of genes is associated with labor in both the upper and lower segments of the uterus. Most changes in gene expression however, are not common, supporting the hypothesis of differential spatial regulation [12]. In both areas of the uterus, labor was associated with an overall reduction, rather than increase in gene expression. Expression was reduced in 71.4%, 72.4%, and 79.2% of the 500 genes after the onset of labor in the fundus, lower segment, and cervix, respectively.

Since many genes in reproductive tissues may be co-regulated or interdependent, we identified groups of genes with similar expression profiles. We placed the selected 500 genes into one of ten clusters. Two different techniques were used: K-means and hierarchical. The number of genes per cluster determined by K-means ranged between 31–83, 26–93, and 115–102 for fundus, lower segment, and cervix, respectively. The corresponding number of genes for each cluster by hierarchical clustering was 3–239, 3–181, and 2–333, respectively. To further refine the gene groups we determined those genes which were co-clustered using both techniques. Coincidence testing was done to determine the probability that each set of genes was co-clustered using both techniques by chance. Sets of genes with a probability of chance grouping less than 0.01 were analyzed further. These sets represented 71.2%, 81.8%, and 79.8% of the 500 genes in the fundus, lower segment, and cervix, respectively. Since genes grouped by one technique can also be grouped in any of the ten groups from the second technique, there are 100 possible co-clusters. We found only 14, 14, and 12 clusters in the fundus, lower segment, and cervix, respectively, suggesting that these co-clusters are likely to represent interdependent or co-regulated genes. Examples of genes clustered together by both techniques are shown in Tables 1–3. (Complete data

are available online and can be accessed at http://www.ebi.ac.uk/arrayexpress, accession number E-MEXP-106).

Examination of the data raises some interesting hypotheses. For example, in the lower segment, expression of the genes for the nuclear binding protein C/EBP, TNF receptor, alpha 1A–adrenergic receptor, phospholipase A2 (group IIA), and G protein–coupled receptor 18 have similar expression profiles. In the fundus, the expression of repressor of estrogen receptor activity (REA) is reduced, while prothymosin alpha remains unchanged with labor. The two genes constitute one of the reported regulatory pathways of estrogen receptor alpha (ERA) activity [13].

Numerous genes have been reported to change in expression dramatically in reproductive tissues at the onset of labor. Our results are consistent with these previous results and demonstrate in the lower segment a marked increase in expression of the genes for beta-adrenergic receptor kinase 2 [14], phospholipase A2 IIA [15], and calcium ion–transporting ATPase 2 [16]. Furthermore, there was a reduction in expression of regulator of G protein signaling 2 [17], calcitonin receptor activity–modifying protein 2 [18], and protein kinase C [19]. Nevertheless, some genes that would be expected to demonstrate a marked labor-associated increase, such as prostaglandin receptor EP 4 [20], were not selected by our technique, possibly due to a large inter-patient variability in expression. Since other genes' expression patterns were consistent with prior findings, this variability may reflect gene polymorphism.

Expression changes of REA, retinoid X receptor alpha (RXR), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in association with labor in the uterine fundus corresponded to the findings of microarray experiments. Both REA and RXR expressions decreased in labor, while expression of GAPDH remained unchanged (Figure 4).

Discussion

Our results demonstrate labor-associated changes in gene expression in three functionally important areas of the

Table 3. Cervix: Selected Genes within Each of the Coincidence Clusters with Functions Similar to Other Genes within the Same Cluster or Functions Pertinent to the Process of Labor

Cluster	GenBank ID	Gene Name	Change	Fold	p	Function
1	L32976	Mitogen-activated protein kinase 11	−	1.89	0.0386	Cell apoptosis
1	U07418	DNA mismatch repair gene	−	1.95	0.0057	Cell apoptosis
1	X87843	Transcription regulator factor gene MNAT1	−	1.89	0.0412	Cell apoptosis
1	AF015950	Telomerase reverse transcriptase	−	1.48	0.0335	Cell growth; cell cycle progression
1	AF034780	Sphingolipid G protein–coupled receptor 5	−	1.50	0.0347	Cell growth; cell cycle progression
1	U82130	Tumor susceptibility gene 101-transcription regulator gene	−	2.16	0.0491	Cell growth; cell cycle progression
1	AL049386	Transcription regulator gene Notch2	−	1.61	0.0069	Cell growth
1	M34423	Beta 1 galactosidase	−	1.45	0.0427	Lysosomal enzyme
1	X76488	Lipase A	−	1.91	0.0140	Lysosomal enzyme
1	AF046888	Tumor necrosis factor (TNF) 13	−	1.09	0.0413	Activates NFKB and cell apoptosis
1	U59863	TNF receptor–associated (TRAF) NFKB activator	−	1.70	0.0265	Increases IL-8 and TRAF2–mediated activation of NFKB
1	S74720	Orphan nuclear steroid hormone receptor B1	−	0.88	0.0465	Decreases activity of ERA and ERB
1	U51903	GTPase-activating protein 2	−	2.03	0.0070	Cross-linkage of actin filaments
2	AL022310	Tumor necrosis factor 4	+	1.14	0.0220	Increases activation of NFKB
2	U48730	Signal transducer and activator of transcription 5B	+	1.41	0.0289	Transcription factor activated by TNF and other cytokines
2	M33684	Protein tyrosine phosphatase, non-receptor type 1	+	1.41	0.0366	Signal transduction gene regulated by TNF
2	AA292277	Nuclear transcription co-activator 4	+	1.41	0.0441	Inhibits expression of TNF and IL8; NFKB decreases its nuclear import
2	AI961040	Tubulin, gamma complex–associated protein 2	+	2.10	0.0113	Protein binding to IL8 receptors A and B
2	M36653	POU domain 2 transcription factor 2	+	1.64	0.0372	Increases activity of CCR5 chemokine receptor for IL8
2	X07109	Protein kinase C beta 1	+	1.35	0.0077	Increases activity of NFKB and is activated by IL8 and TNF
3	D43945	Transcription factor EC	+	1.93	0.0407	GTP-binding translation initiating factor
3	L19161	Translation initiation factor 2, subunit 3	+	2.12	0.0309	Transcription regulating factor
3	U78575	Phosphatidylinositol-4-phosphate 5-kinase alpha I	+	1.96	0.0354	Increases activity of EGFR, which stimulates interstitial collagenase MMP1
3	X90579	Cytochrome P450 IIIA, polypeptide 5 pseudogene 2	+	2.16	0.0312	Metabolizes progesterone
4	L37042	Casein kinase 1, alpha 1	−	2.14	0.0375	Cell apoptosis
4	U59435	Proliferation-associated metallopeptidase 2G4	−	1.51	0.0420	Proteo and peptidolysis
5	M36803	Hemopexin	−	2.07	0.0273	Enhances TIMP inhibition of MMP degradation of extracellular matrix
5	U33821	Tax1-binding protein 1	−	1.74	0.0324	Apoptosis inhibitor
5	M11186	Prepro-oxytocin (neurophysin I)	−	1.76	0.0018	Increases prostaglandin E2 synthesis
5	U07620	Mitogen-activated protein kinase 10	−	1.25	0.0053	Mediates cytokine-induced prostaglandin synthesis
6	M18079	Fatty acid–binding protein 2, intestinal	−	1.23	0.0254	Increases fat oxidation and insulin resistance; is inhibited by collagen
6	D31766	Glucosamine-6-phosphate isomerase	−	1.72	0.0326	Energy pathway
6	X13916	Low-density lipoprotein-related protein 1	−	1.80	0.0121	Mediates catabolism of MMP-9
7	X65293	Protein kinase C, epsilon	−	1.35	0.0033	Activates NFKB and cell apoptosis
7	AF053977	Anaphase-promoting complex protein member gene	−	1.50	0.0384	Decreases cell proliferation
8	X72308	Chemokine ligand 7	+	1.31	0.0240	Chemotaxis of monocytes and leukocytes
8	U03905	Chemokine receptor 2	+	1.14	0.0217	Receptor for CCL7; chemotaxis of monocytes and leukocytes
8	X80343	Cyclin-dependent protein kinase 5 activator—regulatory subunit	+	1.14	0.0489	Cell growth and differentiation
8	W28588	Neurofilament light polypeptide	+	1.57	0.0273	Cell growth and differentiation
8	L41827	Neuregulin 1	+	1.16	0.0268	Cell growth and differentiation
8	X07024	TAF1 RNA polymerase II transcription co-activator	+	2.13	0.0426	Cell growth
8	M25269	ELK1 transcription regulator	+	1.62	0.0121	Cell growth
8	U94902	Transmembrane receptor–binding collagen and hyaluronic acid	+	2.03	0.0387	Cell growth and proliferation of connective tissue cells
8	M69136	Mast cell's chymase 1	+	2.00	0.0291	Peptidase degrading extracellular matrix
8	AC002366	Amelogenin	+	1.37	0.0036	Extracellular matrix glycoprotein
8	M31153	Cytochrome P450 XVII—steroid 17-alpha-hydroxylase	+	1.73	0.0486	Key enzyme in steroidogenesis of estrogens and androgens
9	U69108	TNF receptor–associated factor 5	+	0.95	0.0372	Mediates TNF-induced NFKB activation and protection from cell death
9	U40705	Telomeric repeat binding factor 1	+	1.68	0.0198	Induces mitotic entry and apoptosis
10	X84709	Adaptor protein for TNF receptors—FAS associated	−	4.68	0.0419	Apoptosis of fibroblasts
10	X91648	Purine-rich element binding protein A	−	1.29	0.0108	Apoptosis of fibroblasts
10	M29870	Rho small GTP binding protein Rac1	−	2.18	0.0094	Apoptosis of fibroblasts; regulates various MAPK
10	U07620	Mitogen-activated protein kinase 10	−	2.26	0.0474	Cell death and apoptosis
10	U33052	Protein kinase C–like 2	−	1.98	0.0112	Cell death and apoptosis regulated by rho proteins
10	Z75311	Single-stranded specific endoDNAase RAD50	−	1.27	0.0116	Cell death and apoptosis
10	AL046322	Karyopherin alpha 6	−	1.56	0.0374	Cell apoptosis
10	Y12670	Leptin receptor	−	1.57	0.0498	Cell apoptosis
11	U52960	RNA polymerase II transcription regulator	−	1.09	0.0419	Transcription regulator binding CREBBP
11	J02621	High-mobility group nucleosome binding domain 1	−	1.35	0.0255	Transcription factor that is regulated by CREBBP
11	X96924	Mitochondrial carrier protein 25-1	−	1.29	0.0466	Expression is increased by HNF4A activated by CREBBP

Table 3. Continued

Cluster	GenBank ID	Gene Name	Change	Fold	*p*	Function
11	Z46606	Matrix-associated, actin-dependent regulator of chromatin a3	−	1.93	0.0233	Transcription regulator that binds CA4, which in turn binds SURB7, known to bind CREBBP
12	U65093	Cbp/p300-interacting transactivator 2	−	2.42	0.0514	Transcription regulator binding CREBBP
12	U30246	Solute carrier 12 - sodium/potassium/chloride transporter 2	−	2.51	0.0496	Expression increased by MYOD1 regulated by CREBBP
12	X75252	Prostatic binding protein	−	1.59	0.0316	Decreases activity of chymotrypsin; binds RAF1 regulating TP53, also regulated by CREBBP
12	U35139	Necdin	−	1.59	0.0287	Binds CREBBP; colony formation of mast, connective tissue, and blood cells
12	AB006909	Microphthalmia-associated transcription factor	−	1.42	0.0502	Colony formation of mast, connective tissue, and blood cells
12	AB028972	Colony-stimulating factor 2 receptor alpha	−	2.31	0.0231	Colony formation of mast, connective tissue, and blood cells

Cluster number identifies a coincidence cluster of genes grouped together by K-means and hierarchical clustering methods where probability of chance co-clustering was $p < 0.01$. The fold change is a ratio of the difference between labor and non-labor medians of gene expression to the non-labor median level of gene expression. The change sign indicates the direction of the expression change in the labor samples comparing to non-labor. The p-value is given for the difference in gene expression in samples taken before and after labor. Gene function is derived from the database of gene functions and interactions Ingenuity Pathway Analysis and Biological Knowledge Database.
DOI: 10.1371/journal.pmed.0030169.t003

human uterus. The primary objective of this study was to identify novel-to-labor genes important for the process of parturition. The second main objective was to identify groups of genes with similar expression profiles in order to recognize those with common regulatory mechanisms. Rather than providing a list of genes, this results provides a map of gene interactions in labor. We postulated that the onset of labor is likely to be caused by a reduction in inhibitory and an increase in stimulatory processes, and our data support this theory; for example, we demonstrated that in the lower segment in labor expression of genes for the stimulatory tumor necrosis factor receptor is increased, whereas that of the relaxatory potassium channel is reduced.

The study was specifically designed to investigate gene expression in human labor because the mechanisms of labor vary between species. Previous gene array studies have documented changes in expression in a rodent model [21,22]. Such animal models are useful since variability is reduced because of the animals' similar genotypes and exposure to a controlled environment. Expression data from such studies can be compared and contrasted with those from human tissue, thus providing an insight into the similarities and differences between species. However, we consider that data from human studies are the most important for understanding human physiology.

Previous human gene array data [22,23], has marked differences in methodology from our study. Bethin and colleagues [22] determined the expression profile in human extracts obtained either preterm, prior to labor or preterm, and at term following the onset of labor. In contrast, we designed our study to specifically determine labor-associated alterations and to exclude the marked changes in expression at the end of pregnancy. A further difference in our study was that we analyzed human uterine samples from all three functionally distinct areas of the uterus in the same women. It is the cooperation of these components of the uterus (contraction of the fundus, relaxation of the lower segment, and dilation of the cervix) that result in the process of labor. Our study also differed in that the method of analysis and sample size enabled the individual variation between women to be taken into account—i.e., to preserve these character-

istics samples were not pooled. This individual analysis enabled the expression of each gene to be identified in each sample. The genes were then grouped into clusters based on their similarity of expression across individual samples. This similarity of genes' expressions in different samples dramatically increases the power of the cluster analysis and is possible only because the individual sample characteristics are maintained. However, one limitation of an individual analysis is that individual variation in expression in human tissue is likely to be high, not only because there are marked genetic and environmental effects but also because the time to the onset of spontaneous labor in non-labor samples is not known.

Aguan and colleagues utilized a different experimental design and methodology to investigate gene expression in the lower uterine segment before and after the onset of labor [23]. The type of array, number of investigated genes (588), and normalization procedures make valid comparison with our study difficult. The studies differ also in how the fold change in the gene expression calculation was done. However, there are several consistent changes in gene expression. For example, we demonstrated a 91% decrease in G protein–coupled receptor 161 in lower-segment samples, which is consistent with the 84% reduction reported by Aguan et al. We also demonstrated consistent changes in guanine nucleotide binding protein alpha expression.

Chan and colleagues [24] studied uterine samples in labor using a subtractive hybridization technique. Although this study used a different technique from ours, and the number of genes upregulated in labor was small, their findings have shown a consistent with our results, significant increase in the expression of interleukin-8.

Gene array data provide a wealth of information, which presents unique analytical challenges. We determined expression in six samples taken before and seven after the onset of labor at term. In order to compare the differences in samples taken before and after labor, the *t*-test for the difference in expression was performed and the *p*-value was calculated. The genes were ranked according to this value, not to determine significance, which would be inappropriate for this number of comparisons, but to determine genes that

demonstrated the greatest and most consistent change in labor. We did not correct for multiple comparisons since the expression of the different genes is not independently regulated. This method of analysis is likely to provide more consistent data than techniques using fewer samples, duplicate arrays on the same samples, or identification of an arbitrary change in expression [21,22]. By this method, any difference in expression during labor of those genes with the smallest p-values is unlikely to have arisen by chance due to observer and instrument variability. Hence, the genes with the most consistent change in expression during labor are most likely to have an important function, although current methodology does not allow primary changes in expression to be distinguished from those secondary to increased contractility.

Although we cannot exclude false positives and negatives, the lower the p-value the smaller the probability of a false positive results. However, as the number of genes selected increases, so does the chance of inclusion of a false positive result, while the chance of a false negative one decreases. False negative results may also occur due to wide inter-patient variability. There are many potential causes of inter-patient variability. Particularly important is that it cannot be determined in non-laboring patients when parturition would otherwise commence—that is, how close to the onset of labor a non-laboring patient is.
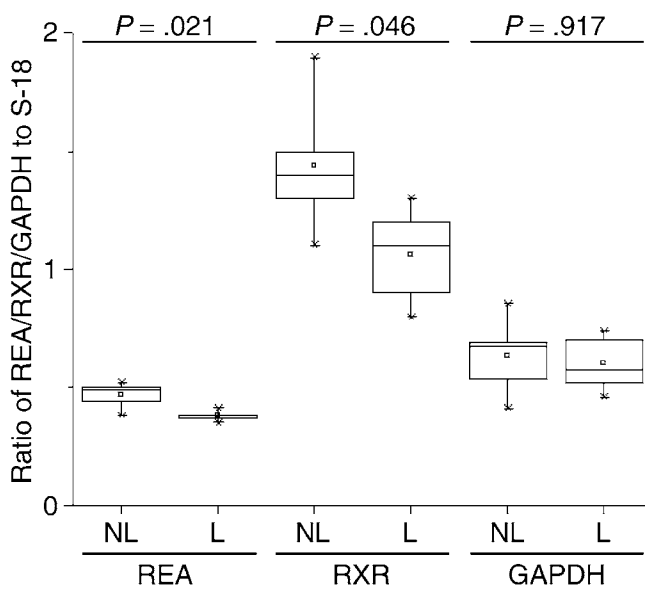
It is likely that many changes in gene expression precede the clinical signs of labor: for example, the steroid hormones estrogen and progesterone are fundamentally important for the maintenance of pregnancy and the onset of labor [4,7]. In some species, such as the sheep, pregnancy is maintained by progesterone and labor is caused by a dramatic fall in progesterone. The decrease in progesterone concentration increases the estrogen/progesterone ratio leading to contrac-

Figure 4. Validation of the Microarray Findings by RT-PCR
Relative abundance of mRNA, normalized to S-18, encoding the gene for REA, RXR, and GAPDH in the myometrium obtained from the uterine fundus in five non-laboring (NL) and five spontaneously laboring (L) patients. Box limits represent 25th and 75th quartiles, line within the box represents median, and whiskers represent 5th and 95th percentiles.
DOI: 10.1371/journal.pmed.0030169.g004

tions [25]. A fall in plasma progesterone has not been demonstrated in women, although administration of anti-progestins can induce labor [26]. This suggests that the mechanism may be slightly different in women.

Our demonstration of a fall in the expression of a modulator of estrogen receptor activity provides a mechanism whereby the functional estrogen/progesterone ratio could be increased without a change in plasma concentration of either. REA is a protein that competitively and selectively binds to the nuclear receptor reducing its function [13]. Although identified in breast cancer and placental cells, this modulator has not been described in the human myometrium. REA and RXR (which also inhibits estrogen activity) were both clustered into one group based on their decreased expression pattern in labor. The expression of prothymosin alpha (an antagonist of REA) is unchanged in labor and further supports this hypothesis. Jointly, they demonstrate existence of a pathway that may represent a novel mechanism of uterine control [13].

During labor there are concomitant physiological changes in the fundus, lower uterine segment, and cervix. The fundus generates coordinated forceful uterine contractions while the contractile lower segment elongates over the presenting part. The cervix undergoes softening in late pregnancy with a dramatic shortening and dilation during labor. Our data demonstrate related marked spatial differences in gene expression, consistent with previous publications using alternative techniques for quantification [12]. Some of these differences in gene expression may, however, be due to cell type. We have previously demonstrated that more than 98% of cells in our lower segment biopsies are myometrial [8], and fundal samples were taken from the peritoneal (outer) surface to prevent decidual contamination. It is therefore unlikely that changes in gene expression in the fundus and lower segment were derived from non-myometrial cells. In contrast, the cellular composition of the cervix is more heterogeneous, and expression within the different cell types cannot be discerned. Nevertheless, we considered that maintenance of the physiological cellular environment was more important than a homogenous cell population.

Oxytocin and prostaglandins are known to have a fundamental role in human parturition [6]. Our gene array data are consistent with existing evidence on these oxytocics. Oxytocin is produced by the choriodecidua during human labor [8] and acts on myometrial oxytocin receptors to cause contraction. Since oxytocin is not produced in the myometrium but in other gestational tissues, it is reassuring that there was no increase in myometrial expression of oxytocin in our study. The increase in myometrial oxytocin receptor formation precedes the onset of labor, and uterine expression increases from mid-pregnancy to term rather than at the onset of labor [27]. Consistent with these data, we did not demonstrate an increase in oxytocin expression in labor. In contrast, we have previously demonstrated that expression of myometrial secretory phospholipase A2 is increased in samples taken after the onset of labor [15]. This enzyme catalyzes mobilization of arachidonic acid from membrane phospholipids for the synthesis of prostaglandins. Our gene array results confirm an increase in secretory phospholipase A2 expression in myometrial samples taken after the onset of labor and are consistent with a regulatory role for prostaglandins

There is no generally accepted statistical method to analyze differences in gene expressions between groups, due to correlation of expressions of individual genes. To validate our findings, we confirmed expression of genes with different technique and demonstrated functional relationship of co-expressed genes. We validated a proportion of our micro-array findings by RT-PCR, and we were reassured that the results using both techniques were consistent among all tested genes. We also analyzed the patterns of expression by two techniques: K-means and hierarchical clustering. Although these techniques may not be completely independent, the method of gene clustering is different and hence the combination provides additional confidence for the identification of networks of co-regulated genes. Prior studies have shown that co-expressed genes have been demonstrated to be functionally related and to participate in common biological processes defined by the Gene Ontology database. These relationships are identified across species and functional categories [28–31]. The identification within each cluster of genes with similar functions pertinent to labor strengthens our hypothesis that these genes are co-regulated. It is likely that expression of a particular gene can regulate expression of a second, which may itself influence a third. In this way, a single controlling mechanism may induce a multitude of phenotypic alterations leading to a change in function. Furthermore, transcription regulating factors (such as CAAT enhancer binding protein, CEBP, which is increased after labor in the lower segment) may promote transcription for numerous contraction-associated genes. Indeed, CEBP binding to the oxytocin receptor promoter has recently been demonstrated [32]. We anticipate that elucidating the networks of genes associated with labor will enable a more holistic understanding of the process, leading to more rational methods for manipulating uterine contraction.

In summary, we have demonstrated consistent changes in gene expression in the human lower segment, fundus, and cervix in association with labor. A number of novel-to-labor genes have been identified in addition to networks of co-expressed genes. There are marked tissue and spatial differences in gene expression in the uterus during parturition.

Supporting Information

Dataset S1. Cervix: Selected Genes

Found at DOI: 10.1371/journal.pmed.0030169.sd001 (565 KB XLS).

Dataset S2. Fundus: Selected Genes

Found at DOI: 10.1371/journal.pmed.0030169.sd002 (558 KB XLS).

Dataset S3. Lower Segment: Selected

Found at DOI: 10.1371/journal.pmed.0030169.sd003 (574 KB XLS).

Accession Numbers

The GenBank (http://www.ncbi.nlm.nih.gov/Genbank) accession numbers for the genes and gene products discussed in this paper are: alpha 1D–adrenergic receptor (M76446), beta-adrenergic receptor kinase 2 (AL022329), CEBP (M83667), calcitonin receptor activity–modifying protein 2 (AJ001015), calcium ion–transporting ATPase 2 (X63575), G protein–coupled receptor 161 (AI703188), G protein–coupled receptor 18 (L42324), GAPDH (U34995), guanine nucleotide-binding protein alpha (AC002077), interleukin-8 (M28130), oxytocin (NM__000915), phospholipase A2 IIA (M22430), prostaglandin receptor EP 4 (L28175), protein kinase C beta1(X07109), prothymosin alpha (M26708), REA (U72511), regulator of G protein signaling 2 (L13463), RXR (X52773), and tumor necrosis factor receptor (X60592).

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Editors' Summary

Background. Childbirth, or labor, although a basic event in life, is actually a complex process that involves three parts of the uterus (womb) working together to expel the baby. One particularly important part of the process, which is poorly understood, is how labor begins. The actual changes that occur in the uterus once labor has begun are well known, and include contractions in the muscle of the uterus wall (the myometrium) and dilation of the cervix (the neck of the womb). Some of the triggers for these changes are also known: for example, in non-primate animals changes in the blood levels of the hormones estrogen and progesterone and changes in the membranes that surround the fetus. Previous studies have suggested that these effects are likely, in turn, to be triggered by changes in many genes, but exactly which ones is not clear.

Why Was This Study Done? Learning more about which genes are important in the various stages of labor may help to design treatments for the various problems that occur in labor (such as failure of labor to begin, or, alternatively, preterm labor). Little is known about the genes that trigger, or are necessary for, labor to start and to continue in a coordinated fashion. A technology known as DNA microarrays allows researchers to take a sample from any part of the body and use it to look at how active many thousands of genes are, all at the same time. By analyzing these results, it is possible to suggest either single genes or groups of genes that may be important in a particular process.

What Did the Researchers Do and Find? The authors took samples from the uterus top, lower part, and cervix of six women before their labor started, and seven from those whose labor had started. All women were having cesarean sections either for medically indicated reasons, or for choice. Then, in each of the samples in each woman, they looked at 12,626 known genes to see how active they were (scientists call these active genes "expressed"). They found that the changes in gene expression were not, generally, the same across the three parts of the uterus. Of the 500 genes with the largest change in expression, 28 were common to both the upper and lower parts of the uterus, and this small group of genes may be important in labor in both the upper and lower parts of the uterus. The authors also classified the 500 genes into related groups, and they believe that these relationships may be important in controlling how labor happens.

What Do These Findings Mean? Identifying new genes or groups of genes involved in labor is important for understanding how labor occurs. One limitation of this study is the small number of women who were studied—which is understandable, given the difficulty of obtaining such samples—and the differences between the women studied. Another difficulty with such studies is that the methods used to analyze the expression patterns can affect the results. However, as is the custom with these types of studies, all the results were placed in a public database so anyone can look at them and, if they wish, do further analyses. In a related Perspective article that was commissioned to comment on this paper, Roberto Romero, one of the original reviewers of the paper, has done just that. He finds that there were differences in the results of his analyses and those of the authors'. He goes on to discuss the question of how hard it is to use these techniques to look at complex problems, such as how labor starts. Clearly, much more work needs to be done before it is clear what all these results really mean. Nonetheless, these studies have the potential to help to understand more about the basic science behind labor.

Additional Information. Please access these Web sites via the online version of this summary at http://dx.doi.org/10.1371/journal.pmed.0030169.

• Medline Plus has a page of links on childbirth