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

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

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ABSTRACT

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 coregulated 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 simultaneous 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 ± 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).

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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 cm³) 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

0 -1 -2

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 oncolumn 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 phenolchloroform extraction and ethanol precipitation. 1 μ g 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



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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 coclustering 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).

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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
7	D11466	Phosphatidylinositol glycan A	+	2 51	0.0276	inhibited by PGI2 Transferase of glycosyl groups: role in
	2	······································		2.0 .	1.02.0	cell damage

Table 1. Continued

Cluster	GenBank	Gene	Change	Fold	p	Function
	U	Name				
8	M680/1	Protein tyrosine phosphatase pop-recentor	L	1 87	0.0136	Signal transduction downstream of the
0	1000941	type 4	Ŧ	1.07	0.0150	dutamate recentor
8	X90530	GTP-binding protein ragB	+	2 13	0.0302	Signal transduction
8	X86019	Wiskott-Aldrich syndrome protein interacting	+	1 27	0.0365	Signal transduction from cell surface
U	100017	nrotein	'	1.27	0.0505	recentors to actin cytoskeleton
8	AI 030996	THO complex 2	+	1 67	0.0078	Transcription complex element
9	122475	BCI 2-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
,	11100505	stoo calciant sinang protein An		1.05	0.0110	activity of myosin
9	Y12336	RAS quanyl releasing protein 2—	_	2.13	0.0364	Stimulates RhoA Ca sensitization-dependent
-		guanine nucleotide exchange factor				maintenance of contraction
9	M13981	Inhibin alpha	_	2.21	0.0345	Stimulated by antiprogesting, synergistic with
2					0100 10	progesterone, and inhibited by indomethacin
10	M27318	Interferon alpha 4	_	1.75	0.0188	Increases expression of IENG (gamma)
10	U11870	Interleukin 8 receptor alpha	_	2.26	0.0393	Upregulated by INEG
10	D14838	Fibroblast growth factor 9	_	1.75	0.0220	Activated by INFG
10	W27605	Cone-rod homeobox gene	_	3.02	0.0226	Increases PDF6-cGMP-specific phosphodiesterase
10	X67594	Melanocortin 1 receptor	_	1.72	0.0195	Activates adenvlate cyclase: binds POMC
10	AA846749	Apolipoprotein M	_	1.76	0.0003	Lipid transporter in energy pathway: inhibited
	/				0.0000	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	_	1.82	0.0021	Ca-ransporting ATPase in sarcoplasmic reticulum:
		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	-	1.08	0.0432	Stimulates RhoA Ca sensitization-dependent
		factor				maintenance of contraction
12	L26584	Ras protein-specific guanine nucleotide-	_	1.07	0.0238	Stimulates RhoA Ca sensitization-dependent
		releasing/exchange factor 1				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

18S rRNA. The non-labor and labor samples were compared using the Mann-Whitney U test.

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

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 pvalue 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
6	M22430	Phospholipase A2, IIA	+	1.83	0.0056	Increases expression of INOS and PGE2
6	AA004795	Amyloid beta precursor protein 2-binding	+	2.03	0.0291	Ca-binding protein in the heart
6	100068	Actin alpha 1	<u>т</u>	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
б	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	+	2.51	0.0015	Protein glycosylation; regulates glycosylphosphatidylinositol
7	M167E0	Suburiit 2	1	1 40	0.0225	Synthesis Distain phasehendation involved in cardias hypertraphy
/	10/50	Pim-1 protein tyrosine kinase	+	1.48	0.0325	Protein phosphorylation; involved in cardiac hypertrophy
ð	L13403	Regulator of G protein signaling 2	-	2.78	0.0343	Madiates TNE a activation of NEUD and MADKO (IAK and call
8	019261		_	1.75	0.0166	apoptosis
8	AF041381	E2F transcription factor 6	-	2.24	0.0258	Suppressor of transcription; regulated by INFRS5 a IRAF1 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	р	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 coregulated 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

1 U2070 Mitory matrixed provide instart 11 - 1.8 A016 Cell apoptois 1 U2070 Transcription regulator factor gene WAR1 - 1.89 0.012 Cell apoptois 1 APD1590 Telemerse retranscription equiption factor gene WAR1 - 1.80 0.0347 Cell growth: cell cycle progression 1 APD1590 Tumor succeptibility gene 104-transprint - 1.40 0.0347 Cell growth: cell cycle progression 1 APD1490 Tumor succeptibility gene 104-transprint - 1.40 0.040 U200-transprint 1 APD44988 Unprove factor (TNF 13 - 1.90 0.025 Increases. III- and TRA2 method and cell apoptois 1 SPR020 Orphan indrise and activation of fastor fas	Cluster	GenBank ID	Gene Name	Change	Fold	р	Function
1 UV/14 UV/14 <thu 14<="" th=""> UV/14 UV/14</thu>	1	122076	Mitogon activated protain kinaco 11		1 00	0.0296	Call apoptacia
1 XXXVVX Transcription regulator frame gene WMA1 - 1.89 0.012 Cill apportsic 1 AF71590 Splingalpid G protein-coupled receptor 5 - 1.80 0.037 Cill growth: cell cycle progression 1 AU33780 Splingalpid G protein-coupled receptor 5 - 1.80 0.037 Cill growth: cell cycle progression 1 AU43780 Francingtion regulator gene Noch2 - 1.16 0.009 Cill growth: cell cycle progression 1 AU43780 Francingtion RoyLator gene Noch2 - 1.16 0.009 Cill growth: cell cycle progression 1 AU43780 Francingtion RoyLator gene Noch2 - 1.16 0.009 Cill growth: cell cycle progression 1 AU43780 Francingtion RoyLator gene Noch2 - 1.00 Cill growth: cell cycle progression 1 M35040 Francingtion RoyLator	1	L32970	DNA microstch ropair gono	_	1.09	0.0360	
1 AP317500 Telemonae meanse tensergione - 14.8 0335 Cell growth: cell cycle progression 1 AP317500 Telemonae meanse tensergione - 1.30 0327 Cell growth: cell cycle progression 1 AP317500 Telemonae meanse tensergione - 1.30 0327 Cell growth: cell cycle progression 1 AP437800 Telemonae meanse tensergione - 1.30 0427 Cell growth: cell cycle progression 1 AP439880 Tennor recroix factor A - 1.30 0427 Cyclotenet etypes	1	V87843	Transcription regulator factor gene MNAT1	_	1.95	0.0037	
1 AP334399 Splingelisid C gratemic-scopied receptors 5 - 1.50 0.0347 Cell growth: cell cycle progression 1 AU43930 Turnor successibility gene 101 transcription - 1.60 0.0497 Cell growth: cell cycle progression 1 AU4393 Eta 1 galacticatiose - 1.40 0.0497 Cyclessimal approximation 1 AV4433 Beta 1 galacticatiose - 1.40 0.0497 Cyclessimal approximation 1 AV4433 Beta 1 galacticatiose - 1.40 0.0497 Cyclessimal approximation 1 AV4433 Beta 1 galactication - 1.40 0.0497 Cyclessimal approximation 1 Mayson Orphan nuclessimate receiptor YPa1 - 2.00 Orphan nuclessimation NFR8 2 AU43720 Orphan nuclessimate receiptor YPa1 1.41 0.0390 First and the receiptor Applan tapproximation NFR8 2 AU30727 Nucleat transcription focor 4 + 1.41 0.041 Imbinition tegrate NTA 2	1	ΔΕ015950	Telomerase reverse transcriptase	_	1.09	0.0412	Cell growth: cell cycle progression
1 UB2110 Tume succeptibility gene 101-transcription - 2.16 0.009 Cell growth 1 M4428 Transcription regulator gene Not.h2 - 1.56 0.009 Cell growth 1 M4428 Bet 1 glasticolisate - 1.56 0.009 Cell growth 1 M4428 Etal glasticolisate - 1.00 0.0014 Avesoreal enzyme 1 M4428 Etal glasticolisate - 1.00 0.0014 Avesoreal NB and OM2-protein Statuton of NFK8 1 US9505 Third encode integrate explore flasticolisate	1	AF034780	Sphingolipid G protein-coupled receptor 5	_	1.50	0.0347	Cell growth; cell cycle progression
enclose enclose <t< td=""><td>1</td><td>U82130</td><td>Tumor susceptibility gene 101-transcription</td><td>_</td><td>2.16</td><td>0.0317</td><td>Cell growth; cell cycle progression</td></t<>	1	U82130	Tumor susceptibility gene 101-transcription	_	2.16	0.0317	Cell growth; cell cycle progression
1 M4429 Transcription regulator gene Natch2 - 1.61 0.0000 Cell growth 1 M4429 Lipse A - 1.50 0.047 Lysosomal enzyme 1 A74688 Lipse A - 1.90 0.0140 Lysosomal enzyme 1 0.9563 Thir receptor-associated (TMA) NRB achieves - L - 0.0205 Increases LB and TMA2-served TAR and EBS 2 0.0203 Dirphan Inclocate aread Norme encaptor Bio Inclocate Action Interves LB and TMA2-served TR And EBS - 0.0205 Increases LB and TMA2-served Norme Action Interves LB and TMA2-served TR And EBS - 0.0205 Increases LB and TMA2-served TR And EBS - 0.0205 Increases activity of LB and the cytokines 2 M43044 Protein tyrosine phosphatuse, non-receptor type I + 1.41 0.0305 Protein Indring to LB arceptors And B - 1.20 0.0113 Protein Activity of MRIA and the cytokines - 2.10 0.0113 Protein Activity of MRIA and the St Band Call approach - 1.10 0.0113 Protein Activity of MRIA and the St Band Call approach - 1.10 0.0113 P	•	002100	regulator gene		20	010 12 1	
1 M34423 Bet 1 gate Condition - 145 0.0427 Lysoornal enzyme 1 M76688 Turor necrosis factor (TM) 13 - 100 0.0431 Activates NFRI and cell approxis 1 US980 Turor necrosis factor (TM) 13 - 100 0.0255 Intersector sector (MR) NFR activator - 100 0.0255 Intersector (MR) TRI and Cell approxis 2 AU32210 Turor necrosis factor 4 - 100 0.0255 Intersector (MR) TRI and Cell approxis 2 AU32210 Turor necrosis factor 4 - 1.41 0.0210 Constrained activation of MFR and Like NFR decreases its nuclear import 2 AU36104 Tutaling amma complex-associated protein 2 + 1.40 0.0113 Protein binding to Like activation of MFR and Like NFR decreases its nuclear impact instantion initiating factor 2 2 M3653 POU demain factor 22 + 1.50 0.027 Circlestending instantion initiating factor 2 3 UJ357 Protechinding protein 1 + 1.50 0.027 Circlestending instantin inininitiatin factor 2	1	AL049386	Transcription regulator gene Notch2	_	1.61	0.0069	Cell growth
1 X74988 Upsach - 191 0.0140 Lysochal enzyme 1 X74988 Timer receptor-associated (TRAF) NK8 activator - 1.00 0.025 Increase Like and TRAC-mediated activator of NK88 1 US9803 Timer receptor associated (TRAF) NK8 activator - 0.080 Decrease-activator of FRAC (NFR) 2 AL02210 Timera excitation of NK88 - 0.080 Decrease-activator of FRAC (NFR) 2 AL02210 Timera excitation of NK88 - 0.026 Decrease-activator of NK88 2 AL02210 Timera excitated by TNF and activator of transcription factor (Sectorese) is nuclear import 1.11 0.026 Signal transcription factor (Sectorese) is nuclear import 1.01011 Protein import 1.010111	1	M34423	Beta 1 galactosidase	_	1.45	0.0427	Lysosomal enzyme
1 AP06888 Time recosts factor (NF) 13 - 1.09 0.001 Activates NF46 and ell appross 1 US960 TRF receptor-associated TRAF/S MR8 activator - 0.026 foreases activity of FR4-activation of NF48 2 AL02210 Tumor neccosis factor 4 + 1.14 0.026 foreases activity of RA and CR8 2 AL02210 Tumor neccosis factor 4 + 1.41 0.026 foreases activity of NF48 2 AL02210 Tumor neccosis factor 4 + 1.41 0.026 foreases activity of NF48 Ansettor 4 2 AL02210 Tumor neccosis factor 4 + 1.41 0.026 foreases activity of NF48 Ansettor 4 2 AL0210 Tumor neccosis factor 4 + 1.41 0.026 foreases activity of NF48 Ansettor 4 3 Liny 1 Transcription factor 2 + 1.45 0.007 foreases activity of CGR, which simulates interestription factor 2 3 Liny 1 Transcription factor 2, suburit 3 + 1.45 0.024 forease activity of CGR,	1	X76488	Lipase A	_	1.91	0.0140	Lysosomal enzyme
1 US9863 Thif receptor-associated (TRAF) NPR8 activator – 1.70 0.025 Increases Like and TRAF-mediated activation of NFR8 1 US1903 GTPsex-activating protein 2 – 0.80 0.0050 Cross-linkage of activity of ENA and ER8 2 AL02210 Trunser receives factor 4 + 1.41 0.028 Trunser receives factor 4 2 M3304 Protein trunscription no cactivator 4 + 1.41 0.028 Trunscription factor 2014 2 M306104 Tubulin, gamma complex-associated protein 2 + 1.44 0.028 Trunscription factor 2014 + 1.41 0.041 Trunscription factor 2014 + 1.41 0.041 Trunscription factor 2014 + 1.33 0.0027 Trunscription receptor 1.8 + 1.20 0.0027 Trunscription receptor 1.8 + 1.21 0.0021 Trunscruscription receptor 1.8 +	1	AF046888	Tumor necrosis factor (TNF) 13	-	1.09	0.0413	Activates NFKB and cell apoptosis
1 5/4720 Opthan incident steroid hormone receptor PI - 208 0.0465 Berease activity of EAP and ERB 2 44.02210 Tumor necrosis factor 4 + 1.14 0.020 Torsiting of KFB and ERB 2 44.02210 Tumor necrosis factor 4 + 1.14 0.028 Turnicipion activity and other cytoking 2 44.02210 Tumor necrosis factor 4 + 1.41 0.026 Signal Turnicipion activity and other cytoking 2 A4.02210 Turbulin gamma complex-associated protein 2 + 1.41 0.026 Signal Turnicipion activity and the cytoking 3 D43845 Transcription factor 3/, abunit 3 + 1.40 0.0407 GFB-banding transfactor and and TKF 3 U3575 Phosphathidylinoticl-phosphate 5-kinase alpha 1 + 1.20 0.0407 GFB-banding transfactor and tra	1	U59863	TNF receptor-associated (TRAF) NFKB activator	-	1.70	0.0265	Increases IL-8 and TRAF2-mediated activation of NFKB
1 US1903 GT2sea-citivating protein 2 - 2.03 0.007 Cross-Indage Acit Inflaments 2 V48720 Signal transducer and acitvator of transcription 58 + 1.44 0.0226 Transcription factor 44 + 1.44 0.0285 Transcription factor 44 + 1.44 0.0285 Transcription factor 44 + 1.44 0.0245 Transcription factor 44 + 1.44 0.0245 Transcription factor 44 + 1.44 0.0241 Inhibits expression 51 (Record 44) Bail 44 1.44 0.0241 Inhibits expression 51 (Record 44) Bail 44 1.35 0.0277 Increases activity of CCR5 chemokine receptor for 1.8 2 M3663 POU domain 2 transcription factor 2 + 1.46 0.0234 Increases activity of CCR5 chemokine receptor for 1.8 0.0245 Protein binding factor 3 U3979 Transcription factor 24 - 2.16 0.0334 Increase activity of CCR5 chemokine receptor 67 1.84 4 U3943 Translation initiation factor 24 - 2.16 0.0334 Indamares 1.040 1.74<	1	S74720	Orphan nuclear steroid hormone receptor B1	-	0.88	0.0465	Decreases activity of ERA and ERB
2 H40230 Tumor necrois factor 4 + 1.14 0.202 Increase activation of NF8 2 H43044 Protein tryosine phosphatase, non-receptor type 1 + 1.41 0.0208 Tignal transduction of NF8 2 AA322277 Nackest transcription co-activator 4 - 1.41 0.0208 Tignal transduction of NF8 2 AA3663 POU domain 2 transcription factor 2 + 2.10 0.0111 Protein binding to 164 transcription factor 2 + 1.40 0.0111 Protein binding to 164 transcription factor 2 3 U3957 Transcription factor C + 1.50 0.0071 Increases activity of NF8 and the activated by UA and TNF 3 U39570 Crochrome P450 IIA, polypaptide 5 paeudogen 2 + 2.16 0.0131 Protein fixed activation of ALB 4 U3902 Crochrome P450 IIA, polypaptide 5 paeudogen 2 - 2.16 0.0131 Netabolizas prosestrence 4 U3902 Crochrome P450 IIA, polypaptide 5 paeudogen 2 - 2.16 0.0131 Netabolizas prosestrence 4 U3902 Crochrome P450 IIA, polypaptide 5 paeudogen 2 - 2.16 0.0131	1	U51903	GTPase-activating protein 2	-	2.03	0.0070	Cross-linkage of actin filaments
2 M4320 Signal transducer and activator of transcription fasts + 1.41 0.0280 Transcription fasts Final other cytokines 2 M33640 Protein tryosine phosphazes, non-receptor type 1 + 1.41 0.0386 Signal transcription cachicated by TNF and other cytokines 2 M36053 POU domain 2 transcription cachicated protein 2 + 1.44 0.0341 Inhibits expression of TNF and LB, NKR decreases is transcription factor 2 2 M36053 POU domain 2 transcription factor 2 + 1.46 0.0371 Increases activity of CCRS chemokine receptor for ILB 2 M36053 POU domain factor 2, suburit 3 + 2.10 0.0391 Transcription factor 12 + 1.46 0.0391 Increases activity of ECRF, Wich's trimulates interstitial calignama MMP1 3 UV875 Phosphatibilinositol -thybolybilinositol -thybolybi	2	AL022310	Tumor necrosis factor 4	+	1.14	0.0220	Increases activation of NFKB
2 M3364 Protein tyrosine phosphatase, non-neceptor type 1 + 1.41 0.366 Signal transfution gene regulated by TWE 2 M3661 Tubulin, gamma complex-associated protein + 1.41 0.0246 inclear import 2 M3663 POU domin 2 transfription factor 2 + 1.64 0.0317 increases activity of NRE and is activated by UB and TWE 3 D3785 Phorophatikismas C beta 1 + 1.80 0.0071 increases activity of NRE and is activated by UB and TWE 3 L19161 Transfription factor EC + 1.98 0.0072 increases activity of NRE and is activated by UB and TWE 3 U38357 Phorophatikityinositot-4-phosphate 5-kinase alpha 1 + 1.96 0.033 increases activity of NRE and is activated by UB and TWE 3 V30579 Cytochrome P450 IIA, polypaptide 5 pseudogene 2 + 1.56 0.033 increases activity of NRE and isos 1. alpha 1 - 1.64 0.033 increases activity of NRE and isos 1. alpha 1 - 1.64 0.033 increases activity of NRE and isos 1. alpha 1 - 1.64 0.033 increases activity of NRE and isos 1. alpha 1 - 1.64 0.033	2	U48730	Signal transducer and activator of transcription 5B	+	1.41	0.0289	Transcription factor activated by TNF and other cytokines
2 Av22227 Nucker transcription co-activator 4 + 1.41 0.041 Inhibits expression of TNF and ILS; NFB decreases its muclear import 2 M3603 POUd omina complex-associated protein 2 + 2.10 0.0113 Protein kinase C beta 1 + 1.54 0.032 Transcription factor 2 + 1.64 0.032 CFBs hiding translation initiating factor 3 L19161 Transcription factor 2 + 1.35 0.0077 Increases activity of CFRS hiding translation initiating factor 3 L29767 Photein kinase C beta 1 + 2.12 0.039 Transcription regulating factor 4 L37042 Casein kinase 1, alpha 1 - 2.16 0.031 Vertabolizes progestore 5 M3803 Hemopexin - 2.16 0.032 Korale Alpha 1 - 1.77 0.032 Apoptosis in Interases prostaglandin 2 synthesis 5 M31805 Fault binding protein 1 - 1.78 0.033 Mediates cynthe-induced prostaglandin 3 synthesis 6 M18026 Glucosamine-6-phosphate isomerase - 1.72 0.034 Apoptosin-induced prostaglandin 3 synthesis	2	M33684	Protein tyrosine phosphatase, non-receptor type 1	+	1.41	0.0366	Signal transduction gene regulated by TNF
vickstrimport nuckstrimport 2 Adjobis Tubulin, gamma complex-associated proten 2 + 1.64 0.037 Increases activity of CR5 chemokine receptor for IL8 2 X07109 Protein kinase C beta + 1.64 0.037 Increases activity of CR5 shemokine receptor for IL8 3 L19161 Transcription factor 2 + 1.98 0.0407 GTP-binding translation initiation factor 3 L19161 Transcription regulatorin initiation factor 2, subunit 3 + 2.10 0.0031 Transcription regulatorin factor 2 - 1.00 0.032 Microses activity of EGR, which situalizes interstrial collagenase MMP 3 X0579 Cytuchrome P450 ILA, polypeptide 5 pseudogene 2 + 2.16 0.032 Metabolizes progestrome 4 L37942 Casei fixase lapha 1 - 1.76 0.0324 Metabolizes progestrome 5 M36031 Hemopen - 1.76 0.0324 Metabolizes cryotisni ribubitor 6 W3385 Proliferation-associated metallopeptide interse - 1.72 0.0324 Metaboliz	2	AA292277	Nuclear transcription co-activator 4	+	1.41	0.0441	Inhibits expression of TNF and IL8; NFKB decreases its
2 M960100 Tubulin, gamma complex-associated protein 2 + 2.10 0.0113 Protein kinase C bata 2 M36053 POUd omina 12 transcription factor 2 + 1.36 0.0077 Increases activity of CFGS chemokine meceptor for IL3 3 L19161 Translation initiation factor 2, subunt 3 + 2.18 0.0399 Transcription factor 5C 3 U18755 Phosphatidylinositol-4phosphate 5-kinase alpha 1 + 2.18 0.0399 Transcription initiating factor 4 U39835 Politorium sociatid metallopeptides 2 geudegene 2 + 2.18 0.0325 Cell appotsis 5 U33821 Trant-binding protein 1 - - 2.17 0.0324 Apoptosis inhibitor of MMP degradation of extracellular matrix 5 U33821 Trant-binding protein 1 - - 1.26 0.0324 Apoptosis inhibitor 5 U138201 Trant-binding protein 1 - - 1.26 0.0334 Apoptosis inhibitor 6 M118079 Fatty acid-binding protein 2, luteximal - 1.26 0.0334 Mediates cychine-induced prosalpandin synthesis 6 M18079 Fatty acid-binding protein 2, luteximal - 1.26 0.0334 Mediates cychine-induced prosalpandin in synthes							nuclear import
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2 X07109 Protein Kinase C beta 1 + 1.35 0.0077 Increases activity of NFK8 and is activated by I&B and TNF 3 L19161 Translation initiation factor 2, subunt 3 + 2.12 0.309 Transcription regulating factor 3 U78575 Phosphatidylinasitol-4-phosphate 5-kinase alpha 1 + 1.16 0.0357 Casein kinase 1, alpha 1 - 2.16 0.037 Cell adolts in initiation factor 4 U59435 Proliferation-associated metallopeptidas 2C4 - 2.16 0.0327 Enhances IMP inhibition of MMP degradation of extracellular matrix 5 M1186 Perto-axyotion incurophysin 1 - 1.76 0.0038 Hortesses prostalgandin E2 synthesis 6 M18079 Fast-binding protein 1 - 1.23 0.033 Moltase catabilism of MMP-0 7 K62397 Katabilism of MAP-0 - 1.23 0.033 Activates WFK8 and e10 and prosts 7 X62397 Fortein kinase C apoin 0 - 1.35 0.0033 Activates WFK8 and e10 and prosts 7 X62397 Anaphase-promoting complex protein member gene - 1.50 0.0342 Ch	2	M36653	POU domain 2 transcription factor 2	+	1.64	0.0372	Increases activity of CCR5 chemokine receptor for IL8
3 D43945 Transcription factor EC + 19.3 0.0407 GTP-binding translation initiation factor 2, subunt 3 + 19.0 0.037 GTP-binding translation initiation factor 2, subunt 3 + 12.0 0.039 Transcription regulating factor 3 UV8575 Phosphatidylinositol-+phosphate 5-kinase alpha 1 + 19.0 0.0312 Metabolics progesterone 4 L37042 Casen finase 1, alpha 1 - 2.14 0.0325 Cell apoptosis 4 U59435 Proliferation-associated metallopeptidase 2G4 - 1.51 0.0420 Proteo and peptidolysis 5 U38201 Tast-honding protein 1 - 1.74 0.033 Medges prostaglandin 22 synthesis 6 M1186 Prepro-axytocin fneurophysin 1 - 1.74 0.035 Honders cytokine-induced prostaglandin synthesis 6 M18079 Fatty acid-binding protein 2, intestinal - 1.72 0.035 Horease sci to xidation and insulin resistance; is inhibited by collagen 7 X65293 Protein insaes C, epsion - 1.80 0.031 Medases acid proliferation 7 X65288 Protein in	2	X07109	Protein kinase C beta 1	+	1.35	0.0077	Increases activity of NFKB and is activated by IL8 and TNF
3 U/9161 Translation initiation factor 2, subunit 3 + 2.12 0.339 Transcription regulating factor 3 U/8575 Phosphatidylinositol-4-phosphate 5-kinase alpha 1 + 196 0.334 Increases activity of EGR, which stimulates interstitial collagements. MMP1 4 L37042 Casein kinase 1, alpha 1 - 2.14 0.0357 Cell approximation 5 M36803 Hemopexin - 2.14 0.0357 Cell approximation 5 U3821 Taxi-binding protein 1 - 1.74 0.0324 Approximation 6 M11186 Prepro-oxytocin (neurophysin 1) - 1.76 0.0013 Mediates cytokine-induced prostaglandin synthesis 6 M10079 Fatty acid-binding protein 2, intestinal - 1.23 0.0234 Increases prostaglandin E2 synthesis 6 X13971 Approxyntacin (neurophysin 1) - 1.72 0.0336 Increases fat oxidation and insulin resistance; is inhibitor 7 K63377 Arphase-promoting complex protein in member gene - 1.72 0.0334 Increases cell protein insulin resistance; is inhibitor 8 X20343	3	D43945	Transcription factor EC	+	1.93	0.0407	GTP-binding translation initiating factor
3 U78575 Phosphatidylinositel-4-phosphate 5-kinase alphal + 1,90 0.0354 Increases activity of EGR, which stimulates interstitial collageness with of EGR, which stimulates interstitial collageness (MPI) 3 X90579 Cytochrome P450 IIIA, polypeptide 5 pseudogene 2 + - 2,16 0.0312 Metabolizes progesterone 4 L37042 Casein kinase 1, alpha 1 - 2,16 0.0312 Metabolizes progesterone 5 M35803 Hemopexin - 1,24 0.0420 Prote and peptidolysis 5 M35803 Hemopexin - 1,74 0.018 Increases prostaglandin of MMP degradation of extracturation of kinks 110 5 U37630 Mitogen-activated protein kinks 10 - 1,76 0.0018 Increases prostaglandin E2 synthesis 6 M118679 Fatty acid-binding protein 2, intestinal - 1,20 0.0254 Increases fat oxidation and insulin resistance; is inhibited by collagen 6 N13076 Giucosamine-6-phosphate isomerase - 1,30 0.0334 Activates NFKB and cell apoptosis 7 Ar633977 Anaphase-promoting complex protein member gene - 1,30 0.0334 C	3	L19161	Translation initiation factor 2, subunit 3	+	2.12	0.0309	Transcription regulating factor
3 X90579 Cytochrome P450 IIIA, polypeptide 5 pseudogene 2 + 2.16 0.212 Metabolizes progesterone 4 L537042 Casein kinase 1, ajpha 1 - 2.14 0.0375 Cell apoptosis 5 M36803 Hemopexin - 2.14 0.0375 Cell apoptosis 5 U33821 Taxi-binding protein 1 - 1.74 0.0324 Apoptosis inhibitor 5 U13821 Taxi-binding protein 1 - 1.74 0.0324 Apoptosis inhibitor 5 U07630 Mitogen-activated protein kinase 10 - 1.25 0.0038 Increases fac oxidation and insulin resistance; is inhibited by collagen 6 D31766 Glucosamine-6-phosphate isomerase - 1.20 0.0324 Percesses fac oxidation and insulin resistance; is inhibited by collagen 7 Ar65337 Apohase-promoting complex protein member gene - 1.30 0.034 Octraves NFK8 and cell apoptosis 8 W23905 Chemokine legand 7 + 1.14 0.0496 Cell growth and differentiation 8 W23938 Cyclin-dependent protein kinase 5 activator- + 1.14<	3	U78575	Phosphatidylinositol-4-phosphate 5-kinase alpha I	+	1.96	0.0354	Increases activity of EGFR, which stimulates interstitial
3 X90579 Cytochrome PAS0 IIM, polypeptide 5 pseudogen 2 + 2,16 0.0312 Metabolizes progestrone 4 L37042 Casein kinase 1, alpha 1 - 2,16 0.0375 Cell apoptosis 4 U59435 Proliferation-associated metallopeptidase 264 - 1,51 0.0420 Price and peptidolysis 5 W38003 Hemopexin - 1,74 0.0324 Apoptosis inhibitor 5 U3321 Tax1-binding protein 1 - 1,76 0.0018 Increases prostaglandin E2 synthesis 5 U07620 Mitogen-activated protein kinase 10 - 1,23 0.0254 Increases far oxidation and insulin resistance; is inhibited by 2045 6 M1079 Fatty acid-binding protein 2, intestinal - 1,23 0.0324 Increases far oxidation and insulin resistance; is inhibited by 2045 6 N13966 Gluco-asmine-6-phosphate isomerase - 1,72 0.0324 Increase far oxidation and insulin resistance; is inhibited by 2045 7 K62397 Anaphase-promoting complex protein member gene - 1,50 0.0334 Chivates NRAS and cell apoptosis 7 K62397 Chemokine ligand							collagenase MMP1
4 L3/042 Castern kinase 1, alpha 1 - 2.14 0.0375 Protein and peptidolysis 5 M36803 Hemopexin - 2.07 0.022 Proteo and peptidolysis 5 M38803 Hemopexin - 2.07 0.022 Proteo and peptidolysis 5 M38821 Tax1-binding protein 1 - 1.74 0.0324 Apoptosis inhibitor 5 M1180 Prepro-oxytocin (neurophysin 1) - 1.76 0.0318 Increases prostaglandin E2 synthesis 6 M31807 Fatty add-binding protein 2, intestinal - 1.23 0.0034 Increases fat oxidation and insulin resistance; is inhibited by collagen 6 M31766 Glucosamine-6-phosphate isomerase - 1.23 0.0324 Increases fat oxidation and insulin resistance; is inhibited by collagen 7 Ar65397 Anphase-promoting complex protein member gene - 1.30 0.0384 Decreases ell proliferation 8 X2038 Chemokine receptor 2 + 1.14 0.0217 Receptor for CCL?, chemotaxis of monocytes and leukocytes 8 X8033 Cyclin-dependent protein kinase 5 activator—	3	X90579	Cytochrome P450 IIIA, polypeptide 5 pseudogene 2	+	2.16	0.0312	Metabolizes progesterone
4 059435 Proliferation-associated metallopptidase dod - 1.51 00279 Enhances TIMP inhibition of MMP degradation of extracellular matrix 5 U33821 Taxt-binding protein 1 - 1.74 0.0275 Enhances TIMP inhibition of MMP degradation of extracellular matrix 5 U3760 Mitogen-activated protein kinase 10 - 1.76 0.0031 Meciates cytokne-induced prostagiandin synthesis 6 M11080 Prepro-exytocin (neurophysin I) - 1.23 0.0054 Meciates cytokne-induced prostagiandin synthesis 6 D31766 Glucosamine-6-phosphate isomerase - 1.23 0.0034 Meciates cytokne-induced prostagiandin synthesis 7 K65293 Protein kinase C, esplon - 1.30 0.0121 Meciates catabolism of MMP-9 8 V2338 Chemokine ligand 7 + 1.30 0.0240 Chemokis of monocytes and leukocytes 8 V23383 Cyclin-dependent protein kinase 5 activator— + 1.14 0.0273 Cell growth and differentiation 8 V28388 Neurofilament light polypeptide + 1.57 0.0234 Cell growth and differentiation	4	L37042	Casein kinase 1, alpha 1	-	2.14	0.0375	Cell apoptosis
S M36803 Hemopexin - 2.07 0.027 bit and the set of the	4	059435	Proliferation-associated metallopeptidase 2G4	-	1.51	0.0420	Proteo and peptidolysis
 U33821 Tax1-binding protein 1 U33821 Tax1-binding protein 1 U33821 Tax1-binding protein 1 U4 0.0324 Apoptoxis inhibitor U7620 Mittogen-activated protein kinase 10 1.25 0.0053 Mediates cytokine-induced prostaglandin synthesis U7620 Mittogen-activated protein kinase 10 1.23 0.0254 Increases fat oxidation and insulin resistance; is inhibited by collagen U3766 Glucosamine-6-phosphate isomerase 1.23 0.0254 Increases fat oxidation and insulin resistance; is inhibited by collagen U3766 Glucosamine-6-phosphate isomerase 1.20 0.0124 Mediates catabolism of MMP-9 K13916 Low-density lipoprotein-related protein 1 1.20 0.033 Activates NRS and cell apoptosis Arbos3977 Anaphase-promoting complex protein member gene 1.50 0.034 Decreases cell proliferation K72308 Chemokine fagand 7 1.14 0.0217 Receptor for CCL7; chemotaxis of monocytes and leukocytes U03905 Chemokine fagand 7 1.14 0.0217 Receptor for CCL7; chemotaxis of monocytes and leukocytes W80348 Cyclin-dependent protein kinase 5 activator— regulatory subunit W2838 Neurofilament light polypeptide 1.15 0.0223 Cell growth and differentiation W2856 ELK1 transcription regulator 1.16 0.0268 Cell growth and differentiation W2856 ELK1 transcription regulator 1.23 0.0324 Cell growth and proliferation of connective tissue cells W39153 Cytochrome P450 XVII—steroid 17-alpha-hydroxylase 1.37 0.036 Extracellular matrix glycoprotein M31133 Cytochrome P450 XVII—steroid 17-alpha-hydroxylase 1.37 0.036 Extracellular matrix glycoprotein M39270 Rho small GTP binding protein Rac1 2.18 0.0094 Apoptosis of fibroblasts W40705 Telomeric repeat binding	5	M36803	Hemopexin	-	2.07	0.02/3	Enhances TIMP inhibition of MMP degradation of
5 M1180 Prepro-oxytocin (neurophysin 1) - 1.74 0.0324 Apptosis inhibitor 5 M1180 Prepro-oxytocin (neurophysin 1) - 1.76 0.0018 Increases prostaglandin E2 synthesis 6 M18079 Fatty acid-binding protein 2, intestinal - 1.23 0.0024 Increases fat oxidation and insulin resistance; is inhibited by collagen 6 D31766 Glucosamine-6-phosphate isomerase - 1.30 0.0121 Mediates catabolism of MMP-9 7 A65293 Protein kinase C, epsilon - 1.35 0.0334 Chemotaxis of monocytes and leukocytes 8 V23905 Chemokine ligand 7 + 1.31 0.0211 Mediates catabolism of MMP-9 8 V23905 Chemokine ligand 7 + 1.31 0.0218 Celeptor for CCL7; chemotaxis of monocytes and leukocytes 8 V28588 Neurofilament light polypeptide + 1.14 0.0217 Celegrowth and differentiation 8 V20524 TAFI RNA polymerase II transcription co-activator + 1.16 0.0268 Cell growth 8 M69136 Mast cell's chymase 1 +	5	1122021	The All Market second second		1 7 4	0.0224	extracellular matrix
3 Win Nob Preprior by Could interception in the under provide inter provide interprovide inthe under provide interprovide interprovide interprovide	5	U33821 M11196	Tax 1-binding protein T	-	1.74	0.0324	Apoptosis innibitor
3 00/20 Introgenerativated protein number of the number of	5	10111100	Mitogon activated protein kinase 10	-	1.70	0.0018	Modiates suteking induced prostaglandin synthesis
0 Interses in Codation and insum restance, is initiated by collagen 6 D31766 Glucosamine-6-phosphate isomerase - 1.72 Vocalagen 6 X1376 Collacosamine-6-phosphate isomerase - 1.72 Vocalagen 7 AF053977 Anohensiv [logoratin-related protein - 1.80 0.0121 Mediates catabolism of MMP-9 7 AF053977 Anaphase-promoting complex protein member gene - 1.50 0.0384 Decreases cell proliferation 8 X2308 Chemokine ligand 7 + 1.31 0.0217 Receptor for CCL2; chemotaxis of monocytes and leukocytes 8 W28588 Neurogliament light polypeptide + 1.77 0.0273 Cell growth and differentiation 8 W28588 Neurogliament light polypeptide + 1.57 0.0273 Cell growth and differentiation 8 W205269 ELK1 transcription regulator + 1.62 0.0387 Cell growth 8 W205269 ELK1 transcription co-activator + 2.03 0.0387 Cell growth 8 W03020 Transmerbrane receptor-binding collagen and	5	M18079	Eatty acid_binding protein 2 intestinal	_	1.23	0.0055	Increases fat ovidation and insulin resistance; is inhibited
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 X65397 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 W203905 Chemokine receptor 2 + 1.14 0.0489 Cell growth and differentiation 8 W203905 Cyclin-dependent protein kinase 5 activator— + 1.16 0.0273 Cell growth and differentiation 8 L41827 Neurogliament light polypeptide + 1.57 0.0273 Cell growth 8 M205269 ELKI transcription regulator + 1.62 0.0121 Cell growth 8 M69136 Mast cell's chymase 1 + 2.00 0.0291 Peptidase degrading extracellular matrix 8 AC002366 Amelogenin + 1.37 0.0036 Extracellular matrix	0	1110075	ratty acid billaring protein 2, intestinar		1.25	0.0234	by collagen
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 8 X72308 Chemokine ligand 7 + 1.31 0.0240 Chemotaxis of monocytes and leukocytes 8 W03905 Chemokine receptor 2 + 1.14 0.017 Receptor for CCL7; chemotaxis of monocytes and leukocytes 8 X80343 Cyclin-dependent protein kinase 5 activator— + 1.14 0.0489 Cell growth and differentiation 8 W28588 Neurofilament light polypeptide + 1.57 0.0273 Cell growth and differentiation 8 W28588 Neurofilament light polypeptide + 1.57 0.0273 Cell growth 8 W25269 ELK1 transcription regulator + 1.62 0.0121 Cell growth 8 M69136 Mast cell's chymase 1 + 1.62 0.0121 Cell growth 8 M69136 Mast cell's chymase 1 + 0.0307 Cell growth Cell growth </td <td>6</td> <td>D31766</td> <td>Glucosamine-6-phosphate isomerase</td> <td>_</td> <td>1.72</td> <td>0.0326</td> <td>Energy pathway</td>	6	D31766	Glucosamine-6-phosphate isomerase	_	1.72	0.0326	Energy pathway
7 X65293 Protein kinsse C, epsilon – 1.35 0.0033 Activates NFKB and cell apoptosis 7 AF053977 Anaphase-promoting complex protein member gene – 1.50 0.0340 Decreases cell proliferation 8 X7208 Chemokine ligand 7 + 1.31 0.0240 Chemotxis of monocytes and leukocytes 8 W03905 Chemokine receptor 2 + 1.14 0.0217 Receptor for CCL7; chemotaxis of monocytes and leukocytes 8 W28588 Neurofilament light polypeptide + 1.57 0.0233 Cell growth and differentiation 8 W28588 Neurofilament light polypeptide + 1.56 0.0273 Cell growth and differentiation 8 W28586 L41827 Neurofilament light polypeptide + 1.62 0.0217 Cell growth 8 M25269 ELK1 transcription regulator + 1.62 0.0217 Cell growth 8 M69136 Mast cell's chymase 1 + 2.03 0.0387 Cell growth 8 M69136 Mast cell's chymase 1 + 2.00 0.0291 Peptidase degra	6	X13916	Low-density lipoprotein-related protein 1	_	1.80	0.0121	Mediates catabolism of MMP-9
7 AF053977 Anaphase-promoting complex protein member gene - 1.50 0.0384 Decreases cell proliferation 8 V2308 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— + 1.14 0.0497 Cell growth and differentiation 8 W28588 Neurofilament light polypeptide + 1.57 0.0273 Cell growth and differentiation 8 W28588 Neurofilament light polymerse II transcription co-activator + 1.62 0.0426 Cell growth and differentiation 8 M25269 ELK1 transcription regulator + 1.62 0.0121 Cell growth and store connective tissue cells 8 M69136 Mast cell's chymase 1 + 2.03 0.0365 Kety enzyme in steroidogenesis of estrogens and androgens 9 U69108 TNF receptor-associated factor 5 + 0.95 0.0322 Mediates TNF-induced NKB activation and protection from TNF r	7	X65293	Protein kinase C, epsilon	-	1.35	0.0033	Activates NFKB and cell apoptosis
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 CL7; chemotaxis of monocytes and leukocytes 8 W28588 Neurofilament light polypeptide + 1.57 0.0273 Cell growth and differentiation 8 W28588 Neurofilament light polypeptide + 1.57 0.0273 Cell growth and differentiation 8 W28588 Neurofilament light polypeptide + 1.57 0.0273 Cell growth and differentiation 8 W28589 Lk1 transcription regulator + 1.62 0.0217 Cell growth 8 M25269 ELK1 transcription co-activator + 2.03 0.0387 Cell growth and proliferation of connective tissue cells hyaluronic acid 8 M69136 Mast cell's chymase 1 + 2.03 0.0387 Cell growth and proliferation of connective tissue cells hyaluronic acid 9 U69108 TNF receptor-associated factor 5 + 0.0321 Peptidase degrading extracellular matrix 9 U40705 Telomeric repeat binding factor	7	AF053977	Anaphase-promoting complex protein member gene	_	1.50	0.0384	Decreases cell proliferation
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— + 1.14 0.0489 Cell growth and differentiation 8 W28588 Neuroglin1 + 1.16 0.0268 Cell growth and differentiation 8 L41827 Neuroglin1 + 1.16 0.0268 Cell growth and differentiation 8 M25269 ELK1 transcription regulator + 2.13 0.0426 Cell growth 8 M25269 ELK1 transcription regulator + 2.03 0.387 Cell growth and proliferation of connective tissue cells 8 M4902 Transmembrane receptor-binding collagen and hyaluronic acid + 2.00 0.0291 Peptidase degrading extracellular matrix 8 AC002366 Amelogenin + 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 racif death 9 U40705 Telomeric repeat b	8	X72308	Chemokine ligand 7	+	1.31	0.0240	Chemotaxis of monocytes and leukocytes
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8 X80343 Cyclin-dependent protein kinase 5 activator— + 1.14 0.0489 Cell growth and differentiation 8 W28588 Neurofilament light polypeptide + 1.57 0.0273 Cell growth and differentiation 8 L41827 Neurogiliament light polypeptide + 1.56 0.0273 Cell growth and differentiation 8 L41827 Neurogiliament light polypeptide + 1.56 0.0273 Cell growth and differentiation 8 M20202 Transmembrane receptor-binding collagen and hyaluronic acid + 2.03 0.0387 Cell growth and proliferation of connective tissue cells hyaluronic acid 8 M69136 Mast cell's chymase 1 + 2.03 0.0387 Cell growth and proliferation of connective tissue cells hyaluronic acid 8 M69136 Mast cell's chymase 1 + 2.03 0.0372 Ketracellular matrix glycoprotein 8 M69136 Mast cell's chymase 1 + 1.68 0.019 Induces TNF-induced NFKB activation and protection from cell death 9 U69108 Treceptor -associated factor 5 + 0.95 0.0372 Mediater TNF-induced NFKB activation and protection							leukocytes
regulatory subunit regulatory subunit 8 W28588 Neurofilament light polypetide + 1.57 0.0273 Cell growth and differentiation 8 L41827 Neuregulin 1 + 1.16 0.0268 Cell growth and differentiation 8 M25269 ELK1 transcription regulator + 1.62 0.0121 Cell growth 8 M25269 ELK1 transcription regulator + 1.62 0.0121 Cell growth 8 M99136 Mast cell's chymase 1 + 2.03 0.0387 Cell growth and differentiation of connective tissue cells invaluence acid 8 A609136 Mast cell's chymase 1 + 2.00 0.0291 Peptidase degrading extracellular matrix 8 A6002366 Amelogenin + 1.37 0.0466 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 10 X84709 Adaptor protein for TNF receptors—FAS associated - 4.68 0.019 Apoptosis of fibroblasts 10	8	X80343	Cyclin-dependent protein kinase 5 activator—	+	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 M20024 TAF1 RNA polymerase II transcription co-activator + 1.32 0.0246 Cell growth 8 M25269 ELK1 transcription regulator + 1.62 0.0121 Cell growth and proliferation of connective tissue cells 8 M69136 Mast cell's chymase 1 + 2.00 0.0291 Peptidase degrading extracellular matrix 8 M60136 Mast cell's chymase 1 + 2.00 0.0367 Cell growth and proliferation of connective tissue cells 8 M31153 Cytochrome P450 XVII—steroid 17-alpha-hydroxylase + 1.73 0.0466 Key enzyme in steroidogenesis of estrogens and androgens 9 U69108 Tresceptor-associated factor 5 + 0.75 0.0116 Cell death 9 U40705 Telomeric repeat binding factor 1 + 1.68 0.0198 Induces mitotic entry and apoptosis 10 X84709 Adator protein for TNF receptors—F			regulatory subunit				
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 and proliferation of connective tissue cells hylaluronic acid 8 M69136 Mast cell's chymase 1 + 2.03 0.0387 Cell growth and proliferation of connective tissue cells hylaluronic acid 8 M69136 Mast cell's chymase 1 + 2.00 0.0291 Peptidase degrading extracellular matrix 8 AC002366 Amelogenin + 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	8	W28588	Neurofilament light polypeptide	+	1.57	0.0273	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 hyaluronic acid 8 M69136 Mast cell's chymase 1 + 2.00 0.0291 Peptidase degrading extracellular matrix 8 M202366 Amelogenin + 1.37 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 M29870 Rho small GTP binding protein Rac1 - 2.18 0.0094 Apoptosis of fibroblasts; regulates various MAPK	8	L41827	Neuregulin 1	+	1.16	0.0268	Cell growth and differentiation
8 M25269 ELKI transcription regulator + 1.62 0.0121 Cell growth 8 U94902 Transmembrane receptor-binding collagen and hyluronic acid + 2.03 0.0387 Cell growth and proliferation of connective tissue cells hyluronic acid 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 The 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 W29870 Rho small GTP binding protein Rac1 - 2.18 0.0094 Apoptosis of fibroblasts; regulates various MAPK	8	X07024	TAF1 RNA polymerase II transcription co-activator	+	2.13	0.0426	Cell growth
8 094902 Transmemorane receptor-binding collagen and hyaluronic acid + 2.03 0.0387 Cell growth and proliferation of connective tissue cells hyaluronic acid 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 M29870 Rho small GTP binding protein Rac1 - 2.18 0.0094 Apoptosis of fibroblasts 10 U3052 Protein kinase C-like 2 - 1.98 0.0112 Cell death and apoptosis <	8	M25269	ELK1 transcription regulator	+	1.62	0.0121	Cell growth
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aMost Cell's Chymase 1+2.000.0291Peptidase degrading extracellular matrix8AC002366Amelogenin+1.370.0036Extracellular matrix glycoprotein8M31153Cytochrome P450 XVII—steroid 17-alpha-hydroxylase+1.730.0486Key enzyme in steroidogenesis of estrogens and androgens9U69108TNF receptor-associated factor 5+0.950.0372Mediates TNF-induced NFKB activation and protection from cell death9U40705Telomeric repeat binding factor 1+1.680.0198Induces mitotic entry and apoptosis10X84709Adaptor protein for TNF receptors—FAS associated-4.680.0419Apoptosis of fibroblasts10X91648Purine-rich element binding protein A-1.290.0108Apoptosis of fibroblasts10M29870Rho small GTP binding protein Rac1-2.180.0094Apoptosis of fibroblasts; regulates various MAPK10U07620Mitogen-activated protein kinase 10-2.260.0474Cell death and apoptosis10U33052Protein kinase C-like 2-1.270.0116Cell death and apoptosis10AL046322Karyopherin alpha 6-1.560.0374Cell apoptosis10Y12670Leptin receptor-1.570.0498Cell apoptosis11U52960RNA polymerase II transcription regulator-1.090.0419Transcription regulator binding CREBBP11J02621 <td< td=""><td>0</td><td>M60126</td><td>Mast coll's chumasa 1</td><td>1</td><td>2.00</td><td>0.0201</td><td>Dentidase degrading outracellular matrix</td></td<>	0	M60126	Mast coll's chumasa 1	1	2.00	0.0201	Dentidase degrading outracellular matrix
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9U69108TNF receptor-associated factor 5+0.950.0372Mediates TNF-induced NFKB activation and protection from cell death9U40705Telomeric repeat binding factor 1+1.680.0198Induces mitotic entry and apoptosis10X84709Adaptor protein for TNF receptors—FAS associated-4.680.0419Apoptosis of fibroblasts10X91648Purine-rich element binding protein A-1.290.0108Apoptosis of fibroblasts10M29870Rho small GTP binding protein Rac1-2.180.0094Apoptosis of fibroblasts; regulates various MAPK10U07620Mitogen-activated protein kinase 10-2.260.0474Cell death and apoptosis10U3052Protein kinase C-like 2-1.980.0112Cell death and apoptosis10AL046322Karyopherin alpha 6-1.570.0498Cell apoptosis10Y12670Leptin receptor-1.570.0498Cell apoptosis11J02621High-mobility group nucleosome binding domain 1-1.350.0255Transcription regulator binding CREBBP11X96924Mitochondrial carrier protein 25-1-1.290.0466Expression is increased by HNF4A activated by CREBBP	0	AC002500 M21152	Amelogenin Cutochromo P450 XV/II - storoid 17 alpha bydrowylaso	+	1.57	0.0050	Extracenular matrix glycoprotein
90.9708INF receptor associated ractor 3+0.930.0372Mediates interinduced new solution and protection from cell death9U40705Telomeric repeat binding factor 1+1.680.0198Induces mitotic entry and apoptosis10X84709Adaptor protein for TNF receptors—FAS associated-4.680.0419Apoptosis of fibroblasts10X91648Purine-rich element binding protein A-1.290.0108Apoptosis of fibroblasts10M29870Rho small GTP binding protein Rac1-2.180.0094Apoptosis of fibroblasts; regulates various MAPK10U07620Mitogen-activated protein kinase 10-2.260.0474Cell death and apoptosis10U3052Protein kinase C-like 2-1.980.0112Cell death and apoptosis10Z75311Single-stranded specific endoDNAse RAD50-1.270.0116Cell death and apoptosis10AL046322Karyopherin alpha 6-1.570.0498Cell apoptosis10Y12670Leptin receptor-1.570.0498Cell apoptosis11J02621High-mobility group nucleosome binding domain 1-1.350.0255Transcription regulator binding CREBBP11X96924Mitochondrial carrier protein 25-1-1.290.0466Expression is increased by HNF4A activated by CREBBP	0		Cytochrome P430 XVII—steroid 17-alpha-hydroxylase	+	0.05	0.0460	Mediates TNE induced NEKP activation and protection
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No. SectorNo. Sector<	9	U40705	Telomeric repeat binding factor 1	+	1.68	0.0198	Induces mitotic entry and apoptosis
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10U33052Protein kinase C–like 2–1.980.0112Cell death and apoptosis regulated by rho proteins10Z75311Single-stranded specific endoDNAase RAD50–1.270.0116Cell death and apoptosis10AL046322Karyopherin alpha 6–1.560.0374Cell apoptosis10Y12670Leptin receptor–1.570.0498Cell apoptosis11U52960RNA polymerase II transcription regulator–1.090.0419Transcription regulator binding CREBBP11J02621High-mobility group nucleosome binding domain 1–1.350.0255Transcription factor that is regulated by CREBBP11X96924Mitochondrial carrier protein 25-1–1.290.0466Expression is increased by HNF4A activated by CREBBP	10	U07620	Mitogen-activated protein kinase 10	-	2.26	0.0474	Cell death and apoptosis
Z75311Single-stranded specific endoDNAase RAD50-1.270.0116Cell death and apoptosis10AL046322Karyopherin alpha 6-1.560.0374Cell apoptosis10Y12670Leptin receptor-1.570.0498Cell apoptosis11U52960RNA polymerase II transcription regulator-1.090.0419Transcription regulator binding CREBBP11J02621High-mobility group nucleosome binding domain 1-1.350.0255Transcription factor that is regulated by CREBBP11X96924Mitochondrial carrier protein 25-1-1.290.0466Expression is increased by HNF4A activated by CREBBP	10	U33052	Protein kinase C–like 2	-	1.98	0.0112	Cell death and apoptosis regulated by rho proteins
10AL046322Karyopherin alpha 6-1.560.0374Cell apoptosis10Y12670Leptin receptor-1.570.0498Cell apoptosis11U52960RNA polymerase II transcription regulator-1.090.0419Transcription regulator binding CREBBP11J02621High-mobility group nucleosome binding domain 1-1.350.0255Transcription factor that is regulated by CREBBP11X96924Mitochondrial carrier protein 25-1-1.290.0466Expression is increased by HNF4A activated by CREBBP	10	Z75311	Single-stranded specific endoDNAase RAD50	-	1.27	0.0116	Cell death and apoptosis
Y12670Leptin receptor-1.570.0498Cell apoptosis11U52960RNA polymerase II transcription regulator-1.090.0419Transcription regulator binding CREBBP11J02621High-mobility group nucleosome binding domain 1-1.350.0255Transcription factor that is regulated by CREBBP11X96924Mitochondrial carrier protein 25-1-1.290.0466Expression is increased by HNF4A activated by CREBBP	10	AL046322	Karyopherin alpha 6	-	1.56	0.0374	Cell apoptosis
11U52960RNA polymerase II transcription regulator-1.090.0419Transcription regulator binding CREBBP11J02621High-mobility group nucleosome binding domain 1-1.350.0255Transcription factor that is regulated by CREBBP11X96924Mitochondrial carrier protein 25-1-1.290.0466Expression is increased by HNF4A activated by CREBBP	10	Y12670	Leptin receptor	-	1.57	0.0498	Cell apoptosis
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	11	U52960	RNA polymerase II transcription regulator	-	1.09	0.0419	Transcription regulator binding CREBBP
11 X96924 Mitochondrial carrier protein 25-1 – 1.29 0.0466 Expression is increased by HNF4A activated by 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 characteristics 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 nucleo-tide 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 interpatient variability. There are many potential causes of interpatient 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-





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 antiprogestins 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 coexpressed genes. We validated a proportion of our microarray 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 coexpressed 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).

Acknowledgments

Author contributions. RB, GDVH, GRS, GDA, and ST designed the study. RB analyzed the data. RB, GDVH, GRS, GDA, and ST enrolled patients. RB, GDVH, GRS, GDA and ST contributed to writing the paper.

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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.

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