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Characterization of the transcriptome of chorioamniotic membranes at the site of rupture in spontaneous labor at term

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Abstract

OBJECTIVE—The purpose of this study was to compare the transcriptome between the site of membrane rupture and the chorioamniotic membranes away from the site of rupture.

STUDY DESIGN—The transcriptome of amnion and chorion (n =20 each) from and distal to the site of rupture from women with spontaneous labor and vaginal delivery at term after spontaneous rupture of membranes was profiled with Illumina HumanHT-12 microarrays. Selected genes were validated with the use of quantitative reverse transcription–polymerase chain reaction.

RESULTS—Six hundred seventy-seven genes were differentially expressed in the chorion between the rupture and nonrupture sites (false discovery rate <0.1; fold change >1.5). Quantitative reverse transcription–polymerase chain reaction confirmed the differential expression in 10 of 14 genes. Enriched biological processes included anatomic structure development, cell adhesion and signal transduction. Extracellular matrix–receptor interaction was the most impacted signaling pathway.

CONCLUSION—The transcriptome of fetal membranes after spontaneous rupture of membranes in term labor is characterized by region-and tissue-specific differential expression of genes that are involved in signature pathways, which include extracellular matrix–receptor interactions.

Keywords

chorion; extracellular matrix; inflammation; labor; microarray; parturition; spontaneous rupture of membranes

The common pathway of human parturition is characterized by anatomic, immunologic, and physiologic changes that occur in the mother and/or fetus in both term and preterm

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parturition.¹ In normal pregnancy, the chorioamniotic membranes have important anatomic,² biochemical,^{3–5} and antibacterial^{6,7} properties that serve to protect the fetus and pregnancy. The membranes (chorion and amnion) are intact during gestation and rupture without intervention close to the beginning of the second stage of labor.⁸

The fetal membranes undergo complex physical and biochemical changes before the onset of labor at term.^{8–11} In patients at term without labor, the portion of the fetal membranes overlying the cervix contains characteristic histologic and biochemical changes and is thought to be the site at which eventual spontaneous rupture of membranes occurs.^{2,5,10,12,13} This area has been termed the “zone of altered morphology” (ZAM).^{2,10} Defined by histologic criteria, the membranes in the ZAM are thinner than in the membranes away from the ZAM. The features of the ZAM include an increase in the thickness of connective tissue of amnion and chorion and decreased thickness of the cytotrophoblast and decidua layers.^{10,12} Moreover, El Khwad et al¹³ demonstrated that the ZAM is a weak zone by using biophysical testing. Several mechanisms have been proposed to contribute to the weakening of the chorioamniotic membranes in the ZAM; these mechanisms include spontaneous separation of fetal membrane layers,¹⁴ the exposure of the membranes to cytokines and matrix-degrading enzymes that are present in the vagina^{15–17} and in amniotic fluid^{18–25} that are known to increase in normal pregnancy at term and in labor.

The use of high-dimensional biology technology has led to the identification of novel genes, proteins, and pathways that are involved in both term and pre-term labor.^{26–28} Specifically, transcriptomics (the description of the entire set of genes that are transcribed by a particular tissue at a specific point in time) has been used to examine the differences in global gene expression profiles in intact fetal membranes of women at term with and without labor,²⁹ women in term and preterm labor,³⁰ and in pregnancies that are complicated by preterm labor with and without preterm prelabor rupture of membranes (PROM).³¹ Gene expression profiling of the chorioamniotic membranes in the context of spontaneous rupture of membranes (SROM) at the site of membrane rupture and away from the site of rupture has not been previously reported.

This study was undertaken to examine the differences in the transcriptome between the site of membrane rupture and a section of the intact chorioamniotic membranes away from the site of rupture in patients at term with spontaneous labor and to gain further insight into the biochemical changes that are associated with the phenomenon of membrane rupture.

Methods

Study group

A prospective study was designed to examine differential gene expression of the chorioamniotic membranes at the gross site of rupture and distant from the site of rupture; samples were obtained from women who were admitted with spontaneous labor at term with SROM (n = 30). The inclusion criteria were (1) gestational age of ≥ 37 weeks, (2) absence of medical or obstetric complications of pregnancy, (3) no antibiotic administration in labor for group beta streptococcus (GBS) prophylaxis, (4) absence of meconium staining of the amniotic fluid, (5) no prostaglandin administration, (6) no use of amnioinfusion, (7) vaginal birth, and (8) normal pregnancy outcome, including an infant who was of appropriate weight for gestational age without congenital anomalies and Apgar scores >7 at 1 and 5 minutes. All patients provided written informed consent for the collection and use of samples for research purposes under the protocols approved by the institutional review boards of Wayne State University and the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services.

Clinical definitions

Labor was defined as the presence of regular uterine contractions accompanied by progressive cervical dilation that led to delivery. *SROM* was defined as rupture of membranes in the presence of labor and confirmed with sterile speculum examination and a combination of pooling, ferning, and Nitrazine tests. An *appropriate-for-gestational-age neonate* was defined as a birthweight between the 10th and 90th percentile for the gestational age at birth.³² Histologic chorio-amnionitis was diagnosed on the basis of the presence of inflammatory cells in the chorionic plate and/or chorioamniotic membranes.^{33,34} Acute funisitis was diagnosed by the presence of neutrophils in the wall of the umbilical vessels and/or Wharton's jelly.

Collection of the fetal membranes

Fetal membranes were processed within 30 minutes of delivery. After the identification of the site of rupture, a linear piece of membrane that measured 1 × 4 cm and spanned the gross rupture site was dissected sharply (Figure 1). Similarly, a 1 × 4 cm strip of reflected extraplacental membranes was removed at a site distal to the site of rupture that was approximately one-half the distance between the site of rupture and the placental disk.^{35,36} The amnion was separated from the chorion, and each tissue was flash-frozen with liquid nitrogen. This method yielded a set of 4 specimens from each patient: (1) amnion from the rupture site, (2) chorion from the rupture site, (3) amnion from the nonrupture site, and (4) chorion from the non-rupture site. In addition, a section of membranes from each patient was obtained for histologic examination with the use of hematoxylineosin-stained histologic sections. The cases with chorioamnionitis, funisitis, meconium-staining, or severe amnion degeneration were excluded from the study.^{33,34} The sets of specimens were collected prospectively until 30 total sets of fetal membranes were obtained that met all inclusion and histologic criteria. The RNA was extracted and stored at −70°C until it was assayed. The materials were housed at the bank of biologic specimens that was held at the Perinatology Research Branch, Eunice Kennedy Shriver National Institute of Child Health and Human Development.

Microarray analysis and real-time quantitative polymerase chain reaction

For microarray analysis, complete sets of chorioamniotic membranes were used (n = 20 matched sets of amnion from the rupture site, chorion from the rupture site, amnion from the nonrupture site, and chorion from the nonrupture site from each patient). The Illumina HumanHT-12 expression microarray (version 3; Illumina, San Diego, CA) platform was used to measure the gene expression levels in each specimen, per manufacturer's instructions. To verify the results from microarray, 14 genes were selected for quantitative polymerase chain reaction (qRT-PCR) assays in a subset of the original sample set (n = 10) and in a new set of samples from patients at term with SROM (n = 10). The selection criteria for the new specimens were the same as those used to select patients for the microarray analysis, with the exception of 2 patients with mild intermittent asthma without asthma exacerbations or exposure to steroids during the index pregnancy. A detailed description of the method and analysis is available as supplementary material (Supplement 1).

Statistical analyses

The raw gene expression data were normalized by a quantile normalization approach.³⁷ A paired moderated *t* test was used to test for differential expression with a false discovery rate (FDR)³⁸ threshold of 0.1 in conjunction with a threshold of 1.5 on the fold change to assign gene significance.³⁹ Gene ontology analysis was performed with algorithms that were described previously.⁴⁰ Pathway analysis was performed on the Kyoto Encyclopedia of Genes and Genomes (KEGG)⁴¹ pathway database with an overrepresentation analysis⁴² and

the signaling pathway impact analysis.^{43,44} The signaling pathway impact analysis differs from the overrepresentation approaches by taking into account the gene-gene signaling interactions and the magnitude and direction of gene expression changes to determine significantly impacted pathways.

Differential expression between experimental regions from qRT-PCR data was performed with a paired *t* test on $-\Delta\text{Ct}$ values. The Student *t*, Mann-Whitney *U*, and χ^2 tests were used to identify significant differences in patient demographics between women in the microarray and qRT-PCR groups. SPSS software (version 12.0; SPSS Inc, Chi-cago, IL) was used for statistical analysis of demographic data. A probability value of $<.05$ was considered statistically significant.

Results

Demographics

Table 1 displays the demographic characteristics of patients who were included in the microarray and qRT-PCR analyses.

Results of the microarray analysis

Rupture site chorion vs nonrupture site chorion—Microarray analysis demonstrated 815 probes that corresponded with 677 unique genes that were expressed differentially in the chorion between the rupture and nonrupture sites (pFDR <0.1 ; fold change >1.5). A total of 453 genes had decreased expression, and 224 genes had increased expression in the rupture site, compared with the nonrupture site. A “volcano plot” shows the differential expression of all the annotated probes on the Illumina HumanHT-12 v3 array with the log (base 10) of the FDR-adjusted probability values (y-axis) plotted against the log (base 2) fold changes (x-axis) between the rupture site chorion and nonrupture site chorion (Figure 2). A heat map that displays the hierarchical clustering of all samples and 200 probes whose expression had the largest variance across all amnion and chorion samples (both rupture and nonrupture sites) is shown in Figure 3, A. Two main clusters are seen in the heat map that predominantly represent amnion from the rupture and non-rupture site in 1 cluster and chorion from the rupture and nonrupture site in the other cluster. A smaller cluster of mixed samples (both amnion and chorion and rupture vs nonrupture site specimens) is also seen in the heat map and represents specimens that did not fit into the 2 main clusters of specimens. Figure 3, B, shows a hierarchical clustering of the 200 probes with the highest variance across only chorion specimens (both rupture and nonrupture site). A list of the top 100 differentially expressed genes between the rupture site chorion and nonrupture site chorion is presented in Table 2; the complete list of differentially expressed probes is available as supplementary material (Supplement 2).

Gene ontology metaanalysis of the significantly up- and down-regulated genes was performed to identify gene ontology terms that were represented by the differentially expressed genes. In this analysis, 130 biologic processes were enriched (pFDR, <0.05); the top 20 processes are presented in Table 3.

Pathway analysis of the significant genes was undertaken with an overrepresentation method and a signaling pathway impact analysis method. Using the overrepresentation method, 14 KEGG pathways were significant (pFDR, <0.05) in the comparison between rupture site chorion and nonrupture site chorion (Table 4). The 5 most significant pathways were (1) cell junctions, (2) graft-vs-host disease, (3) complement and coagulation cascades, (4) extracellular matrix–receptor interaction, and (5) allograft rejection. Impact analysis of the KEGG signaling pathways also identified 14 significant pathways (Figure 4), although the

ranking of the most impacted pathways were different than the ranking of the pathways from the overrepresentation method. The most impacted pathway was the extracellular matrix–receptor interaction. Moreover, the signaling pathway impact method identified 4 pathways that were not identified by the overrepresentation method: cytokine-cytokine receptor interaction, gap junction, pathogenic *Escherichia coli* infection, and adipocytokine signaling.

Rupture site chorion vs rupture site amnion—In the comparison between rupture site chorion and rupture site amnion, transcriptional profiling demonstrated 2279 differentially expressed probes that corresponded with 1981 unique genes (pFDR <0.1; fold change >1.5). Gene ontology metaanalysis of the significant genes revealed the enrichment of 172 biological processes that include developmental process, anatomic structure development, cell adhesion, biologic adhesion, and multicellular organismal development. The over-representation analysis of genes identified 6 significant KEGG pathways: (1) cell junctions, (2) cell adhesion molecules, (3) complement and coagulation cascades, (4) extracellular matrix–receptor interaction, (5) focal adhesion, and (6) asthma. In contrast, signaling pathway impact analysis identified 12 significantly impacted pathways. The data are available as supplementary material (Supplement 3).

Nonrupture site chorion vs nonrupture site amnion—In the comparison between chorion and amnion from the nonruptured site, 3427 probes that represented 2849 genes were differentially expressed (pFDR <0.1; fold change >1.5, data not shown). There were 217 biological processes that were enriched; the top 5 biological processes were anatomic structure development, developmental process, cell adhesion, biologic adhesion, and organ development. Twelve overrepresented KEGG pathways and 17 significantly impacted pathways were identified in this comparison and are available as supplementary material (Supplement 3).

Rupture site amnion vs nonrupture site amnion—There were no significant regional differences in the amnion transcriptome (rupture vs nonrupture).

Augmentation of spontaneous labor—In spontaneous labor at term, there was no difference in the transcriptome of the chorion of patients who underwent augmentation with oxytocin, when compared with patients without augmentation. In contrast, 4 genes were expressed differentially when amnion was compared with and without exposure to oxytocin augmentation: (1) *AGA* (1.5-fold decrease; pFDR <0.01); (2) *RRAGD* (1.8-fold increase; pFDR <0.01); (3) *LGALS7B* (4.2-fold increase; pFDR <0.01); and (4) *SORD2* (1.8-fold increase; pFDR <0.01). No significant biological processes or pathways were identified between the amnion groups. These data were not validated with qRT-PCR.

qRT-PCR analysis

Rupture site chorion vs nonrupture site chorion—The results of qRT-PCR of a random subset of the original specimens (n =10) confirmed the differential expression of 11 of 14 genes that were found to be significant on the microarray analysis (Table 5). In a new set of specimens (n =10), qRT-PCR validated the microarray results in 10 of 14 genes (Figure 5). Specifically, qRT-PCR confirmed increased expression of *IL6* and *PTGS2*. Consistent with the microarray analysis, qRT-PCR revealed decreased expression of *PAEP*, *GNLY*, *CXCL12*, *CXCL14*, *IGFBP2*, *IGFBP4*, *CNR1*, and *ADAMTS5* in the rupture site, compared with the nonrupture site. Combining the 2 sets of patients (n =20) for analysis, qRT-PCR validated both the direction of fold change and the significance of 13 of 14 of the differentially expressed genes selected from the microarray analysis.

Nonrupture site chorion vs nonrupture site amnion—In the comparison between nonrupture site chorion and nonrupture site amnion, gene expression was found to correspond with the microarray data for *PTGS2* (9.4-fold decrease; $P=.0044$), *IL1RN* (69.9-fold decrease; $P=.0039$), and *CXCL14* (76.4-fold increase; $P=.002$).

Rupture site chorion vs rupture site amnion—In the comparison between rupture site chorion and rupture site amnion, significant fold changes in messenger RNA (mRNA) expression were detected for *PTGS2* (2.9-fold decrease; $P=.011$), *IL1RN* (30.2-fold decrease; $P=.002$), and *CXCL14* (88-fold increase; $P=.002$).

Rupture site amnion vs nonrupture site amnion—Corresponding with the microarray analysis, *PTGS2*, *IL1RN*, and *CXCL14* PCR results were not statistically significant in the comparisons between rupture site amnion and nonrupture site amnion.

Comment

Microarray analysis revealed 677 differentially expressed genes in the chorion of the fetal membranes between the site of rupture and the site away from rupture in patients with term labor and SROM. Biological processes with enrichment in the chorion included anatomic structure development, cell adhesion, signal transduction, and cell communication. The extracellular matrix–receptor interaction, graft vs host disease, and complement and coagulation cascades were among the most impacted signaling pathways by the presence of rupture in the chorion. There were no significant regional gene expression differences (rupture vs nonrupture) in the amnion transcriptome. Stereotypic impacted pathways and biological processes with enrichment were represented in the comparisons between amnion and chorion in the rupture and nonrupture sites.

This study describes, for the first time, the transcriptome of the amnion and chorion at the site of SROM and at the site distal to the site of rupture in patients with spontaneous labor at term. Previous investigations about gene expression,^{45–47} protein immunolocalization and quantification,^{4,12,13,45–50} and enzymatic activity⁴ at the site of rupture have used a targeted approach. The development of high-throughput technology and computational biology makes possible the use of high-dimensional biological techniques to characterize the global changes that are associated with a particular phenomenon.^{26,28} This is the first study to use transcriptomics to identify changes in gene expression in amnion and chorion at the site of membrane rupture.

Malak, Bell, McLaren, McParland, and Taylor made classic contributions by demonstrating that the fetal membranes overlying the cervix undergo morphologic^{2,10,12} and biochemical^{4,12,48} changes before labor. They referred to this area as the ZAM.¹⁰ The morphologic changes include decreased thickness of the trophoblast and decidua and increased thickness of the connective tissue of amnion and chorion.^{2,10,12,13} The biochemical changes that were observed in this area included an increase in pro–matrix metalloproteinase (MMP)-9 (92 kD) protein concentration and enzymatic activity determined by enzyme-linked immunosorbent assay and zymography, respectively; no significant regional differences of pro–MMP-2 (72 kD) activity were detected in prelabor specimens.⁴ In the same study, increased MMP-9 protein concentration was found in specimens that were obtained after labor. Subsequently, El Khwad et al¹³ reported a zone of weakness on biophysical testing; this weak zone had increased MMP-9 and decreased tissue metalloproteinase inhibitor-3 (TIMP-3) protein by Western blot analysis. This study found that TIMP-1 was barely detectable in the membranes; TIMP-2 and TIMP-4 were detectable but did not change as a function of membrane strength. Moreover, another study from the same group demonstrated that the incubation of membranes in vitro with interleukin (IL)-1 β

and tumor necrosis factor alpha (TNF α) was able to induce the biophysical changes, increase MMP-9 protein (both 92 kd and 83 kd bands), and decrease TIMP-3.²⁵ These observations are consistent with an increase in amniotic fluid MMPs (MMP-1, MMP-3, MMP-7, MMP-8, MMP-9),^{19,20,22–24,51–56} neutrophil elastase,⁵⁷ IL-1 β , and TNF α ^{18,20,58–61} in spontaneous labor at term and/or preterm PROM.

In a study of chorioamniotic membranes from patients with SROM or artificial rupture of membranes who delivered vaginally at term, El Khwad et al⁴⁹ used topographic mapping to identify a 10-cm (diameter) zone of weakness that surrounded the site of rupture where a decreased rupture threshold was observed. Moreover, Strohl et al¹⁴ recently demonstrated the increased separation and decreased adherence of the amnion and choriodecidua in specimens that were obtained from term deliveries, when compared with preterm membranes, which was an effect that was more pronounced in specimens from women in labor.⁶² However, there is a paucity of evidence about whether the gene expression profile of the chorion and amnion is different at the site of rupture or away from the site of rupture.

One study has examined the change in the transcriptome of the chorioamniotic membranes that were obtained from women not in labor at term and in women at term.²⁹ Importantly, membranes of women with subclinical inflammation (histologic chorioamnionitis) were excluded. The key finding of the study was that labor was associated with up-regulation of genes that are involved in the inflammatory response. These findings, which were derived originally from microarray studies, were confirmed with quantitative PCR. Therefore, spontaneous labor is associated with a signature gene expression that is consistent with inflammation of the chorioamniotic membranes, even in the absence of histopathologic evidence of such processes. Targeted studies have yielded findings that are consistent with these observations. For example, Osman et al⁴⁷ reported a higher mRNA expression for IL-1 β , -6, and -8 in women in labor than in women not in labor. Such findings are consistent with previous observations that the concentrations of these cytokines are increased in the amniotic fluid of women in labor (term and preterm).^{18,58,59,61,63–71} Of interest, Osman et al did not find regional differences in cytokine expression in the ZAM compared with specimens of membranes that were obtained distal to this site.

Our microarray results demonstrated higher expression of *IL-6* and *IL-8* in the chorion at the site of rupture than in the chorion from the nonrupture site. These results were confirmed by qRT-PCR for IL-6 (13.2-fold increase). The difference in study design may explain the different results between these studies: Osman et al⁴⁷ examined specimens from the ZAM in women (n = 8) in labor with a cervical dilation that ranged from 4–9 cm who underwent cesarean delivery for fetal distress (no information was provided regarding the rupture status or mode of membrane rupture at the time of cesarean delivery). In contrast, the specimens that were used in our study were collected from 20 patients with confirmed SROM who underwent successful spontaneous vaginal delivery.

It is possible that the inflammatory changes at the site of rupture are due to exposure to vaginal fluid and its content, which includes microbial products, microorganisms, and inflammatory mediators. Microbial invasion of the amniotic cavity is identified with standard culture techniques in 18% of patients at term in labor with intact membranes⁷² and in 30% of patients with PROM at term.⁷³ Because bacteria can cross the chorioamniotic membranes,⁷⁴ it has been proposed that the source of the organisms is from ascending microbial invasion from the vagina. In the absence of microbial invasion of the amniotic cavity, fetal membranes that are exposed to the vagina have been thought to respond to stimuli that is present in the vaginal fluid with the production of proinflammatory cytokines and prostaglandins.⁷⁵ Concentrations of IL-1 β secreted by regional decidua and the prostaglandins PGE2 and PGF2 α were higher in cervical/vaginal lavage specimens from

women in labor than that of women not in labor.⁷⁶ Moreover, in vitro studies with fetal membranes demonstrate reduction of elasticity and bursting tension after exposure to bacteria and products of activated neutrophils.⁷⁷

The changes in the gene expression at the rupture site may be associated with alterations in the concentration of inflammatory mediators in the amniotic fluid adjacent to the site of impending rupture. In humans, amniotic fluid prostaglandin concentrations increase before the onset of spontaneous term labor and may play a role in the initiation of labor.^{78–84} Moreover, rupture of membranes at term is accompanied by increased concentrations of prostaglandins in amniotic fluid in patients with and without labor, when compared with women with intact membranes without labor.⁸⁵ The concentrations of eicosanoids, protein-degrading enzymes, and chemokines, which include tissue plasminogen activator, urokinase plasminogen activator, plasminogen activator inhibitor-2,⁸⁶ MMP-9,²¹ MMP-9/TIMP-1 ratios,²¹ and the prostaglandins PGF2 α , PGFM, and PGE2,^{87,88} are higher in the fluid in direct contact with the ZAM (fore-water) than in fluid from the upper compartment (hindwater) in women with term labor. The changes of the concentrations of serine proteases (tissue plasminogen activator and urokinase plasminogen activator) were thought to be a result of exposure to cytokines that are present in the vagina. Moreover, in the case of prostaglandins, the concentrations increase with advancing cervical dilation.⁸⁸ Further evidence of topographic differences in amniotic fluid markers is the increased concentration of IL-6 concentration in the forewater than in the hindwater in both term⁸⁹ and preterm¹⁸ labor. The source for the higher regional concentrations of prostaglandins and IL-6 in the forewater is unclear but may also be related to exposure to cervical and vaginal stimuli or a result of increased production by regional fetal membranes or decidua.

The results of the microarray reported herein support the concept that the fetal membranes at the site of rupture have a different pattern of gene expression than the chorioamniotic membranes away from the site of rupture. The expression of prostaglandin endoperoxidase synthase 2 gene (*PTGS2*), also known as cyclooxygenase 2 (*COX2*), was also increased in chorion from the site of membrane rupture when compared with chorion from the site away from rupture (validated by qRT-PCR). *PTGS2* may be regulated by nuclear factor kappa-B (NF- κ B) and is produced predominantly in amnion at term^{90–92} and significantly increased in labor.⁹³ Our microarray results do not demonstrate increased *PTGS2* expression in the amnion at the site of rupture when compared with amnion at the site away from rupture; however, the increase in the chorion at the site of rupture suggests that the prostaglandin biosynthesis pathway may be subject to a spatial regulation (amnion vs chorion).

ZAM is associated with a reduction in the cellularity of the cytotrophoblast and decidual layers. Apoptotic bodies (detected by electron microscopy) are increased in all regions of the fetal membranes, but specifically in the ZAM rather than in the distal regions of the membranes.⁴⁸ In addition, there was no substantial immunoreactivity for B-cell lymphoma protein 2 (BCL2), an anti-apoptotic protein, but strong for BCL2-associated X protein, a proapoptotic protein, in the decidual layer. This is consistent with observations that apoptosis occurs in the rat amnion.^{94,95}

Further evidence in support of this concept is the observation that there is increased cleaved caspase-3 and caspase-9 (apoptotic markers) and decreased BCL2 in the ZAM.⁵⁰ Immunohistochemical staining of BCL2 in earlier work by McLaren et al. did not demonstrate a significant difference in BCL2 expression between the ZAM and a site distal to this site.⁴⁸ Interestingly, *BCL2* was one of the most significant differentially expressed genes in our microarray experiments and was decreased in the chorion at the site of rupture, compared with the nonruptured site. Further studies are required to validate these findings at the mRNA and protein levels.

As discussed earlier, there is extensive evidence of extracellular matrix remodeling at the site of rupture that is demonstrated by the increased expression of MMP-9 in the membranes before spontaneous labor. We did not find a significant difference in MMP-9 mRNA expression at the site of rupture in either the chorion or amnion, when compared with the nonrupture site. The difference in these findings may be due to the different assays. Microarrays assess changes in mRNA expression; immunoassays detect protein, and zymography detects enzymatic activity.

The *PAEP* gene encodes for glycodelin, which previously was also known as progestagen-associated endometrial protein or placental protein-14, which is a major progesterone-regulated lipocalin that has high carbohydrate content.^{96–98} Three differentially glycosylated isoforms of glycodelin are expressed in different tissues. Glycodelin-A is the most abundant glycoprotein in the uterus in early pregnancy. It has angiogenic,⁹⁹ immunosuppressive,¹⁰⁰ and paracrine effects on gestational tissues and has been detected in human serum,^{101,102} amniotic fluid,^{101,103–105} endometrium (mainly secretory),¹⁰⁶ decidua,¹⁰⁷ and cervix.¹⁰⁸ Glycodelin-A concentrations in maternal serum and decidua increase from conception to 6–10 weeks and then decrease with advancing gestational age.¹⁰¹ Moreover, glycodelin-A concentrations in amniotic fluid are detected at 10 weeks, peak at 15–16 weeks, and decline with advancing gestational age.^{101,104,105}

Perturbations of glycodelin-A expression have been identified in abnormal pregnancies. It has been found to be increased in the decidua of molar pregnancies¹⁰⁹ and decreased in decidua from pregnancies that are complicated by spontaneous abortion,¹⁰⁹ ectopic implantation,¹¹⁰ intrauterine growth restriction,¹⁰⁷ and HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome.¹⁰⁷ In vitro studies have demonstrated that recombinant or immunopurified glycodelin-A induces apoptosis in monocytes and inhibits their NF- κ B activation and inhibits T-cell proliferation and IL-2 secretion, which are roles that support immunotolerance during pregnancy.^{100,111} Moreover, glycodelin-A suppresses MMP-2 mRNA and protein expression and MMP-2, MMP-9, and urokinase plasminogen activator enzyme activity in a first-trimester extravillous cytotrophoblast cell line.¹¹²

We report the decreased expression of *PAEP* in microarray experiments (3.9-fold) in the chorion at the rupture site, which was confirmed with qRT-PCR (31-fold). It is possible that the decreased *PAEP* expression is associated with the release of inhibition of the NF- κ B signaling pathway or MMP activity. Indeed, increases in NF- κ B signaling proteins⁵ and MMP-9 activity^{4,49} have been described previously in supracervical membranes before the onset of labor, when compared with membranes distal to the cervix.

A disintegrin and metalloproteinase with thrombospondin type-1 motif-5 (ADAMTS-5), also known as aggrecanase-2, ADAMTS11, and implantin, is a proteinase that is known to cleave aggrecan, which is a large proteoglycan that is found in the extracellular matrix of the cartilage and cervix.¹¹³ Members of the ADAM metalloproteinase with thrombospondin motif (ADAMTS) family are secreted molecules that can interact with the extracellular matrix. The ADAMTS-5 aggrecanase is expressed in the uterus, cervix, ovary, and placenta.¹¹⁴ In mice, ADAMTS-5 mRNA expression has been detected in the decidua in the dam in the periimplantation period, although its highest expression was detected in the placenta and is thought to contribute to extracellular matrix remodeling at the maternal-fetal interface.^{115,116} The role of ADAMTS-5 is unknown in humans. In vitro studies have described that ADAMTS-5 expression is altered by cytokines; IL-1 β increases and TGF- β 1 decreases the expression of ADAMTS-5 in human decidual stromal cell cultures.¹¹⁶ Other members of the ADAMTS family of aggrecanases have similar functions, although the distribution varies. For instance, in the cervix after spontaneous labor and delivery at term, ADAMTS-9 (an aggrecanase that cleaves both aggrecan and versican) is up-regulated.¹¹⁷

In both the microarray and qRT-PCR analysis, *ADAMTS-5* was found to have decreased expression at the rupture site chorion, when compared with the non-rupture site chorion. We propose that, at the ZAM and the subsequent site of rupture, the maintenance of the maternal-fetal interface is no longer required and that *ADAMTS-5* is rendered inconsequential. In support of this view, examination of the microarray data reveals that *ADAMTS-9* is also decreased significantly in the rupture site chorion. Furthermore, our results identify the activation of the graft-vs-host disease and the allograft rejection pathways, both of which are among the top 5 most impacted and significant pathways.

Microarray analysis did not reveal a significant difference in gene expression profile of the amnion samples that were obtained from the rupture site and non-rupture site. This finding was unexpected. We expected differences in gene expression in this fetal membrane based on its role in membrane integrity and previous observations in the literature.^{5,10,36,118–120}

The lack of detectable difference may be a consequence of the large variance among patients. It is also possible that the anatomic location of the amnion precludes regional differences in the amnion at the rupture site and the nonrupture site. Indeed, the thinning of the fetal membranes in the ZAM is mostly a consequence of thinning of decidual and trophoblast layers and not the amnion.^{2,10} In addition, the studies that have evaluated membrane strength indicate that the weakness in fetal membranes is predominantly due to the changes that have occurred in the chorion and less because of the amnion.¹³ Recently, our group has reported that the amnion overlying the placenta is distinctly different from the reflected amnion of the extraplacental membranes.³⁶ Using microarray analysis and tissue culture and immunoblotting techniques, we have reported that, in term fetal membranes, labor-associated switching to a proinflammatory signature is localized to placental amnion and not reflected amnion. Indeed, regional perturbations of gene expression and regulation in the amnion may occur between reflected and placental amnion but may not be evident at the site of membrane rupture.

Global analyses of mRNA expression of a particular tissue in a particular condition have been useful in the understanding of the physiologic and pathologic evidence of parturition. In spontaneous term labor, the transcriptome of the chorioamniotic membranes has an acute inflammatory gene expression signature, even in the absence of histologic chorioamnionitis.²⁹ Indeed, inflammation plays an important role in normal term labor,¹²¹ as evidenced by activation of the inflammatory pathways described in the cervix,^{117,122} myometrium,¹²² and fetal membranes^{29,122} of women in labor. Although different genes and specific pathways are represented in each different gestational tissue type, the core response is the activation of the inflammatory pathway. The transcriptome of the chorion at the site of rupture reported herein also supports the role of inflammation in SROM. The graft-vs-host disease, complement and coagulation cascades, and allograft rejection pathways are participants in inflammation.

The inflammatory pathway is not the only pathway of interest. In this study, the most significant and impacted pathways represented by SROM were the extracellular matrix–receptor interaction pathway and other pathways that involve cell adhesion, angiogenesis, apoptosis, and immunotolerance. Two of the top 5 most impacted pathways that have been identified in the chorion are graft-vs-host disease and allograft rejection, which sustains the role of regional withdrawal of pregnancy support at the site of rupture. Moreover, the signature pathways that have been identified were both stereotypic and novel and support the notion that human parturition and SROM are complex interactions of many biological processes.

This study describes the transcriptome of a set of matched amnion and chorion from both the site of rupture and the site away from rupture. The strengths of the study are its prospective design, inclusion of a large sample number of normal pregnant women (n =20) with SROM and vaginal delivery, exclusion of maternal diseases and specimens with documented GBS colonization of the genital tract, clinical or histologic chorioamnionitis, and meconium staining. A limitation of the study is that the differential expression of some of the genes reported herein may be due to the mechanical stress and biochemical changes that are associated with the act of rupture of membranes. To characterize the genes that are expressed differentially as a result of exposure to labor processes and/or mechanical/ physiologic stress of rupture of membranes, the gene expression profile of the ZAM should be investigated. Future studies of fetal membranes may include the validation of gene regulation and the processes and pathways that have been identified to be significant and were impacted in this study.

The transcriptome of fetal membranes in spontaneous rupture in term labor is characterized by region- and tissue-specific differential expression of genes that are involved in signal transduction, cell communication, the complement and coagulation cascades, and graft-vs-host disease. In accordance with previous global studies of the transcriptome of other tissues (such as the uterine cervix) after spontaneous term parturition, genes from the extracellular matrix–receptor interaction pathway and those involved in inflammation appear to be involved in parturition. These regional differences were detected in the chorion but not in the amnion. Our findings suggest that the site of rupture in chorionic membranes has a region- and tissue-specific gene expression signature in spontaneous labor at term.

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References

1. Romero R, Espinoza J, Kusanovic JP, et al. The preterm parturition syndrome. *BJOG*. 2006; 113(suppl 3):17–42. [PubMed: 17206962]
2. McLaren J, Malak TM, Bell SC. Structural characteristics of term human fetal membranes prior to labour: identification of an area of altered morphology overlying the cervix. *Hum Reprod*. 1999; 14:237–41. [PubMed: 10374127]
3. Sawdy RJ, Dennes WJ, Allport V, et al. Region and labour-dependent synthesis of prostaglandin E2 by human fetal membranes. *Placenta*. 1999; 20:181–4. [PubMed: 10195739]
4. McLaren J, Taylor DJ, Bell SC. Increased concentration of pro-matrix metalloproteinase 9 in term fetal membranes overlying the cervix before labor: implications for membrane remodeling and rupture. *Am J Obstet Gynecol*. 2000; 182:409–16. [PubMed: 10694345]
5. Lappas M, Odumetse TL, Riley C, et al. Pre-labour fetal membranes overlying the cervix display alterations in inflammation and NF-kappaB signaling pathways. *Placenta*. 2008; 29:995–1002. [PubMed: 18952281]
6. Talmi YP, Sigler L, Inge E, Finkelstein Y, Zohar Y. Antibacterial properties of human amniotic membranes. *Placenta*. 1991; 12:285–8. [PubMed: 1754577]
7. Kjaergaard N, Hein M, Hyttel L, et al. Antibacterial properties of human amnion and chorion in vitro. *Eur J Obstet Gynecol Reprod Biol*. 2001; 94:224–9. [PubMed: 11165729]
8. Santolaya-Forgas J, Romero R, Espinoza J, et al. Prelabor rupture of the membranes. In: Reece, EA.; Hobbins, JC., editors. *Clinical obstetrics: the fetus and mother*. Malden, (MA): Black-well Publishing; 2007. p. 1130-88.

9. Artal R, Sokol RJ, Neuman M, Burstein AH, Stojkov J. The mechanical properties of prematurely and non-prematurely ruptured membranes: methods and preliminary results. *Am J Obstet Gynecol.* 1976; 125:655–9. [PubMed: 937390]
10. Malak TM, Bell SC. Structural characteristics of term human fetal membranes: a novel zone of extreme morphological alteration within the rupture site. *BJOG.* 1994; 101:375–86.
11. Bell, SC.; McParland, PC. Fetal membrane rupture. In: Critchley, HO.; Bennett, P.; Tibshirani, R., editors. *Preterm birth.* London: Royal College of Obstetricians and Gynaecologists Press; 2004. p. 195-212.
12. McParland PC, Taylor DJ, Bell SC. Mapping of zones of altered morphology and chorionic connective tissue cellular phenotype in human fetal membranes (amniochorion and decidua) overlying the lower uterine pole and cervix before labor at term. *Am J Obstet Gynecol.* 2003; 189:1481–8. [PubMed: 14634589]
13. El Khwad M, Stetzer B, Moore RM, et al. Term human fetal membranes have a weak zone overlying the lower uterine pole and cervix before onset of labor. *Biol Reprod.* 2005; 72:720–6. [PubMed: 15548732]
14. Strohl A, Kumar D, Novince R, et al. Decreased adherence and spontaneous separation of fetal membrane layers—amnion and choriodecidua—a possible part of the normal weakening process. *Placenta.* 2010; 31:18–24. [PubMed: 19922998]
15. McGregor JA, Lawellin D, Franco-Buff A, Todd JK, Makowski EL. Protease production by microorganisms associated with reproductive tract infection. *Am J Obstet Gynecol.* 1986; 154:109–14. [PubMed: 3004213]
16. McGregor JA, French JI, Lawellin D, Franco-Buff A, Smith C, Todd JK. Bacterial protease-induced reduction of chorioamniotic membrane strength and elasticity. *Obstet Gynecol.* 1987; 69:167–74. [PubMed: 3027636]
17. McGregor JA, Schoonmaker JN, Lunt BD, Lawellin DW. Antibiotic inhibition of bacterially induced fetal membrane weakening. *Obstet Gynecol.* 1990; 76:124–8. [PubMed: 2193262]
18. Cox SM, Casey ML, MacDonald PC. Accumulation of interleukin-1beta and interleukin-6 in amniotic fluid: a sequela of labour at term and preterm. *Hum Reprod Update.* 1997; 3:517–27. [PubMed: 9528914]
19. Athayde N, Edwin SS, Romero R, et al. A role for matrix metalloproteinase-9 in spontaneous rupture of the fetal membranes. *Am J Obstet Gynecol.* 1998; 179:1248–53.
20. Maymon E, Ghezzi F, Edwin SS, et al. The tumor necrosis factor alpha and its soluble receptor profile in term and preterm parturition. *Am J Obstet Gynecol.* 1999; 181:1142–8. [PubMed: 10561634]
21. Maymon E, Edwin S, Gonzalez R, et al. Topographical differences in the bioavailability of matrix metalloproteinases within the uterine cavity: a possible mechanism or the development of a regional area of membrane weakness predisposing to rupture. *Am J Obstet Gynecol.* 1999; 180(suppl):S1444.
22. Maymon E, Romero R, Pacora P, et al. Human neutrophil collagenase (matrix metalloproteinase 8) in parturition, premature rupture of the membranes, and intrauterine infection. *Am J Obstet Gynecol.* 2000; 183:94–9. [PubMed: 10920315]
23. Maymon E, Romero R, Pacora P, et al. A role for the 72 kDa gelatinase (MMP-2) and its inhibitor (TIMP-2) in human parturition, premature rupture of membranes and intraamniotic infection. *J Perinat Med.* 2001; 29:308–16. [PubMed: 11565199]
24. Park KH, Chaiworapongsa T, Kim YM, et al. Matrix metalloproteinase 3 in parturition, premature rupture of the membranes, and microbial invasion of the amniotic cavity. *J Perinat Med.* 2003; 31:12–22. [PubMed: 12661139]
25. Kumar D, Fung W, Moore RM, et al. Proinflammatory cytokines found in amniotic fluid induce collagen remodeling, apoptosis, and biophysical weakening of cultured human fetal membranes. *Biol Reprod.* 2006; 74:29–34. [PubMed: 16148217]
26. Romero R, Espinoza J, Gotsch F, et al. The use of high-dimensional biology (genomics, transcriptomics, proteomics, and metabolomics) to understand the preterm parturition syndrome. *BJOG.* 2006; 113(suppl 3):118–35. [PubMed: 17206980]

27. Romero R, Tromp G. High-dimensional biology in obstetrics and gynecology: functional genomics in microarray studies. *Am J Obstet Gynecol.* 2006; 195:360–3. [PubMed: 16890547]
28. Romero R, Tarca AL, Tromp G. Insights into the physiology of childbirth using transcriptomics. *PLoS Med.* 2006; 3:e276. [PubMed: 16752954]
29. Haddad R, Tromp G, Kuivaniemi H, et al. Human spontaneous labor without histologic chorioamnionitis is characterized by an acute inflammation gene expression signature. *Am J Obstet Gynecol.* 2006; 195:394. e1–24. [PubMed: 16890549]
30. Marvin KW, Keelan JA, Eykholt RL, Sato TA, Mitchell MD. Use of cDNA arrays to generate differential expression profiles for inflammatory genes in human gestational membranes delivered at term and preterm. *Mol Hum Reprod.* 2002; 8:399–408. [PubMed: 11912289]
31. Tromp G, Kuivaniemi H, Romero R, et al. Genome-wide expression profiling of fetal membranes reveals a deficient expression of proteinase inhibitor 3 in premature rupture of membranes. *Am J Obstet Gynecol.* 2004; 191:1331–8. [PubMed: 15507962]
32. Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. *Obstet Gynecol.* 1996; 87:163–8. [PubMed: 8559516]
33. Redline RW, Faye-Petersen O, Heller D, Qureshi F, Savell V, Vogler C. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. *Pediatr Dev Pathol.* 2003; 6:435–48. [PubMed: 14708737]
34. Redline RW, Heller D, Keating S, Kingdom J. Placental diagnostic criteria and clinical correlation: a workshop report. *Placenta.* 2005; 26(suppl A):S114–7. [PubMed: 15837060]
35. Benirschke, K.; Kaufmann, P.; Baergen, R. Anatomy and pathology of the placental membranes. In: Benirschke, K.; Kaufmann, P.; Baergen, R., editors. *Pathology of the human placenta.* New York: Springer; 2006. p. 321–79.
36. Han YM, Romero R, Kim JS, et al. Region-specific gene expression profiling: novel evidence for biological heterogeneity of the human amnion. *Biol Reprod.* 2008; 79:954–61. [PubMed: 18685129]
37. Irizarry RA, Hobbs B, Collin F, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics.* 2003; 4:249–64. [PubMed: 12925520]
38. Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behavior genetics research. *Behav Brain Res.* 2001; 125:279–84. [PubMed: 11682119]
39. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci U S A.* 2001; 98:5116–21. [PubMed: 11309499]
40. Gentleman RC, Carey VJ, Bates DM, et al. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol.* 2004; 5:R80. [PubMed: 15461798]
41. Draghici S, Khatri P, Tarca AL, et al. A systems biology approach for pathway level analysis. *Genome Res.* 2007; 17:1537–45. [PubMed: 17785539]
42. Khatri P, Draghici S, Ostermeier GC, Krawetz SA. Profiling gene expression using onto-express. *Genomics.* 2002; 79:266–70. [PubMed: 11829497]
43. Tarca AL, Romero R, Draghici S. Analysis of microarray experiments of gene expression profiling. *Am J Obstet Gynecol.* 2006; 195:373–88. [PubMed: 16890548]
44. Tarca AL, Draghici S, Khatri P, et al. A novel signaling pathway impact analysis. *Bioinformatics.* 2009; 25:75–82. [PubMed: 18990722]
45. Osman I, Young A, Ledingham MA, et al. Leukocyte density and proinflammatory cytokine expression in human fetal membranes, decidua, cervix and myometrium before and during labour at term. *Mol Hum Reprod.* 2003; 9:41–5. [PubMed: 12529419]
46. Osman I, Crawford M, Jordan F, Young A, Norman J, Thomson A. Expression and localization of cell adhesion molecules in human fetal membranes during parturition. *J Reprod Immunol.* 2004; 63:11–21. [PubMed: 15284000]
47. Osman I, Young A, Jordan F, Greer IA, Norman JE. Leukocyte density and proinflammatory mediator expression in regional human fetal membranes and decidua before and during labor at term. *J Soc Gynecol Investig.* 2006; 13:97–103.
48. McLaren J, Taylor DJ, Bell SC. Increased incidence of apoptosis in non-labour-affected cytotrophoblast cells in term fetal membranes overlying the cervix. *Hum Reprod.* 1999; 14:2895–900. [PubMed: 10548644]

49. El Khwad M, Pandey V, Stetzer B, et al. Fetal membranes from term vaginal deliveries have a zone of weakness exhibiting characteristics of apoptosis and remodeling. *J Soc Gynecol Investig.* 2006; 13:191–5.
50. Reti NG, Lappas M, Riley C, et al. Why do membranes rupture at term? Evidence of increased cellular apoptosis in the supracervical fetal membranes. *Am J Obstet Gynecol.* 2007; 196:484–10. [PubMed: 17466714]
51. Athayde N, Romero R, Gomez R, et al. Matrix metalloproteinases-9 in preterm and term human parturition. *J Matern Fetal Med.* 1999; 8:213–9. [PubMed: 10475503]
52. Fortunato SJ, Menon R, Lombardi SJ. MMP/TIMP imbalance in amniotic fluid during PROM: an indirect support for endogenous pathway to membrane rupture. *J Perinat Med.* 1999; 27:362–8. [PubMed: 10642956]
53. Maymon E, Romero R, Pacora P, et al. Evidence for the participation of interstitial collagenase (matrix metalloproteinase 1) in preterm premature rupture of membranes. *Am J Obstet Gynecol.* 2000; 183:914–20. [PubMed: 11035337]
54. Maymon E, Romero R, Pacora P, et al. Evidence of in vivo differential bioavailability of the active forms of matrix metalloproteinases 9 and 2 in parturition, spontaneous rupture of membranes, and intra-amniotic infection. *Am J Obstet Gynecol.* 2000; 183:887–94.
55. Maymon E, Romero R, Pacora P, et al. Matrilysin (matrix metalloproteinase 7) in parturition, premature rupture of membranes, and intrauterine infection. *Am J Obstet Gynecol.* 2000; 182:1545–53. [PubMed: 10871477]
56. Maymon E, Romero R, Chaiworapongsa T, et al. Amniotic fluid matrix metalloproteinase-8 in preterm labor with intact membranes. *Am J Obstet Gynecol.* 2001; 185:1149–55. [PubMed: 11717649]
57. Helmig BR, Romero R, Espinoza J, et al. Neutrophil elastase and secretory leukocyte protease inhibitor in prelabor rupture of membranes, parturition and intra-amniotic infection. *J Matern Fetal Neonatal Med.* 2002; 12:237–46. [PubMed: 12572592]
58. Romero R, Brody DT, Oyarzun E, et al. Infection and labor. III. Interleukin-1: a signal for the onset of parturition. *Am J Obstet Gynecol.* 1989; 160:1117–23. [PubMed: 2786341]
59. Romero R, Parvizi ST, Oyarzun E, et al. Amniotic fluid interleukin-1 in spontaneous labor at term. *J Reprod Med.* 1990; 35:235–8. [PubMed: 2325034]
60. Romero R, Mazor M, Sepulveda W, Avila C, Copeland D, Williams J. Tumor necrosis factor in preterm and term labor. *Am J Obstet Gynecol.* 1992; 166:1576–87. [PubMed: 1595815]
61. Opsjln SL, Wathen NC, Tingulstad S, et al. Tumor necrosis factor, interleukin-1, and interleukin-6 in normal human pregnancy. *Am J Obstet Gynecol.* 1993; 169:397–404. [PubMed: 8362955]
62. Kumar D, Novince R, Strohl A, et al. A new methodology to measure strength of adherence of the fetal membrane components, amnion and the chorion. *Placenta.* 2009; 30:560–3. [PubMed: 19410292]
63. Romero R, Avila C, Santhanam U, Sehgal PB. Amniotic fluid interleukin 6 in preterm labor: association with infection. *J Clin Invest.* 1990; 85:1392–400. [PubMed: 2332497]
64. Romero R, Ceska M, Avila C, Mazor M, Behnke E, Lindley I. Neutrophil attractant/activating peptide-1/interleukin-8 in term and preterm parturition. *Am J Obstet Gynecol.* 1991; 165:813–20. [PubMed: 1951537]
65. Santhanam U, Avila C, Romero R, et al. Cytokines in normal and abnormal parturition: elevated amniotic fluid interleukin-6 levels in women with premature rupture of membranes associated with intrauterine infection. *Cytokine.* 1991; 3:155–63. [PubMed: 1888885]
66. Cherouny PH, Pankuch GA, Romero R, et al. Neutrophil attractant/activating peptide-1/interleukin-8: association with histologic chorioamnionitis, preterm delivery, and bioactive amniotic fluid leukoattractants. *Am J Obstet Gynecol.* 1993; 169:1299–303. [PubMed: 8238198]
67. Romero R, Yoon BH, Kenney JS, Gomez R, Allison AC, Sehgal PB. Amniotic fluid interleukin-6 determinations are of diagnostic and prognostic value in preterm labor. *Am J Reprod Immunol.* 1993; 30:167–83. [PubMed: 8311926]
68. Romero R, Yoon BH, Mazor M, et al. A comparative study of the diagnostic performance of amniotic fluid glucose, white blood cell count, interleukin-6, and gram stain in the detection of

- microbial invasion in patients with preterm premature rupture of membranes. *Am J Obstet Gynecol.* 1993; 169:839–51. [PubMed: 7694463]
69. Romero R, Yoon BH, Mazor M, et al. The diagnostic and prognostic value of amniotic fluid white blood cell count, glucose, interleukin-6, and gram stain in patients with preterm labor and intact membranes. *Am J Obstet Gynecol.* 1993; 169:805–16. [PubMed: 7694461]
 70. Gomez R, Romero R, Galasso M, Behnke E, Insunza A, Cotton DB. The value of amniotic fluid interleukin-6, white blood cell count, and gram stain in the diagnosis of microbial invasion of the amniotic cavity in patients at term. *Am J Reprod Immunol.* 1994; 32:200–10. [PubMed: 7533501]
 71. Figueroa R, Garry D, Elimian A, Patel K, Sehgal PB, Tejani N. Evaluation of amniotic fluid cytokines in preterm labor and intact membranes. *J Matern Fetal Neonatal Med.* 2005; 18:241–7. [PubMed: 16318974]
 72. Romero R, Norez J, Mazor M, et al. Microbial invasion of the amniotic cavity during term labor. Prevalence and clinical significance. *J Reprod Med.* 1993; 38:543–8. [PubMed: 8410850]
 73. Romero R, Mazor M, Morrotti R, Avila C, Oyarzun E, Insunza A, et al. Infection and labor: VII, microbial invasion of the amniotic cavity in spontaneous rupture of membranes at term. *Am J Obstet Gynecol.* 1992; 166:129–33. [PubMed: 1301006]
 74. Galask RP, Varner MW, Petzold CR, Wilbur SL. Bacterial attachment to the chorioamniotic membranes. *Am J Obstet Gynecol.* 1984; 148:915–28. [PubMed: 6424476]
 75. MacDonald PC, Koga S, Casey ML. Decidual activation in parturition: examination of amniotic fluid for mediators of the inflammatory response. *Ann N Y Acad Sci.* 1991; 622:315–30. [PubMed: 2064192]
 76. Cox SM, King MR, Casey ML, MacDonald PC. Interleukin-1 beta, -1 alpha, and -6 and prostaglandins in vaginal/cervical fluids of pregnant women before and during labor. *J Clin Endocrinol Metab.* 1993; 77:805–15. [PubMed: 8370702]
 77. Schoonmaker JN, Lawellin DW, Lunt B, McGregor JA. Bacteria and inflammatory cells reduce chorioamniotic membrane integrity and tensile strength. *Obstet Gynecol.* 1989; 74:590–6. [PubMed: 2552366]
 78. Keirse MJ, Mitchell MD, Turnbull AC. Changes in prostaglandin F and 13,14-dihydro-15-ketoprostaglandin F concentrations in amniotic fluid at the onset of and during labour. *BJOG.* 1977; 84:743–6.
 79. Romero R, Emamian M, Wan M, Quintero R, Hobbins JC, Mitchell MD. Prostaglandin concentrations in amniotic fluid of women with intra-amniotic infection and preterm labor. *Am J Obstet Gynecol.* 1987; 157:1461–7. [PubMed: 3480691]
 80. Romero R, Wu YK, Mazor M, Hobbins JC, Mitchell MD. Increased amniotic fluid leukotriene C4 concentration in term human parturition. *Am J Obstet Gynecol.* 1988; 159:655–7. [PubMed: 3421265]
 81. Romero R, Wu YK, Mazor M, Hobbins JC, Mitchell MD. Amniotic fluid prostaglandin E2 in preterm labor. *Prostaglandins Leukot Essent Fatty Acids.* 1988; 34:141–5. [PubMed: 3222272]
 82. Romero R, Wu YK, Sirtori M, et al. Amniotic fluid concentrations of prostaglandin F2 alpha, 13,14-dihydro-15-keto-prostaglandin F2 alpha (PGFM) and 11-deoxy-13,14-dihydro-15-keto-11, 16-cyclo-prostaglandin E2 (PGEM-LL) in pre-term labor. *Prostaglandins.* 1989; 37:149–61. [PubMed: 2717777]
 83. Romero R, Munoz H, Gomez R, et al. Increase in prostaglandin bioavailability precedes the onset of human parturition. *Prostaglandins Leukot Essent Fatty Acids.* 1996; 54:187–91. [PubMed: 8860106]
 84. Lee SE, Romero R, Park IS, Seong HS, Park CW, Yoon BH. Amniotic fluid prostaglandin concentrations increase before the onset of spontaneous labor at term. *J Matern Fetal Neonatal Med.* 2008; 21:89–94. [PubMed: 18240075]
 85. Romero R, Baumann P, Gomez R, et al. The relationship between spontaneous rupture of membranes, labor, and microbial invasion of the amniotic cavity and amniotic fluid concentrations of prostaglandins and thromboxane B2 in term pregnancy. *Am J Obstet Gynecol.* 1993; 168:1654–64. [PubMed: 8317506]

86. Baumann P, Romero R, Gonzelez R, Cotton DB, Mammen E. Evidence of topographic differences in amniotic fluid plasminogen activators/plasminogen activator inhibitor concentrations during spontaneous active labor at term. *Am J Obstet Gynecol.* 1994; 170:270.
87. MacDonald PC, Casey ML. The accumulation of prostaglandins (PG) in amniotic fluid is an aftereffect of labor and not indicative of a role for PGE2 or PGF2 alpha in the initiation of human parturition. *J Clin Endocrinol Metab.* 1993; 76:1332–9. [PubMed: 8496326]
88. Romero R, Gonzalez R, Baumann P, et al. Topographic differences in amniotic fluid concentrations of prostanoids in women in spontaneous labor at term. *Prostaglandins Leukot Essent Fatty Acids.* 1994; 50:97–104. [PubMed: 8171074]
89. Olah KS, Neilson JP, Johnson PM. Interleukin-6 in amniotic fluid obtained at forewater amniotomy compared with hindwater samples in women in spontaneous labour. *Eur J Obstet Gynecol Reprod Biol.* 1995; 60:65–7. [PubMed: 7635234]
90. Slater DM, Berger LC, Newton R, Moore GE, Bennett PR. Expression of cyclooxygenase types 1 and 2 in human fetal membranes at term. *Am J Obstet Gynecol.* 1995; 172:77–82. [PubMed: 7531399]
91. Slater D, Dennes W, Sawdy R, Allport V, Bennett P. Expression of cyclooxygenase types-1 and -2 in human fetal membranes throughout pregnancy. *J Mol Endocrinol.* 1999; 22:125–30. [PubMed: 10194515]
92. Allport VC, Pieber D, Slater DM, Newton R, White JO, Bennett PR. Human labour is associated with nuclear factor-kappaB activity which mediates cyclooxygenase-2 expression and is involved with the 'functional progesterone withdrawal'. *Mol Hum Reprod.* 2001; 7:581–6. [PubMed: 11385114]
93. Ackerman WE, Summerfield TL, Vandre DD, Robinson JM, Kniss DA. Nuclear factor-kappa B regulates inducible prostaglandin E synthase expression in human amnion mesenchymal cells. *Biol Reprod.* 2008; 78:68–76. [PubMed: 17928629]
94. Paavola LG, Furth EE, Delgado V, et al. Striking changes in the structure and organization of rat fetal membranes precede parturition. *Biol Reprod.* 1995; 53:321–38. [PubMed: 7492684]
95. Lei H, Kalluri R, Furth EE, Baker AH, Strauss JF III. Rat amnion type IV collagen composition and metabolism: implications for membrane breakdown. *Biol Reprod.* 1999; 60:176–82. [PubMed: 9858503]
96. Than, GN.; Bohn, H.; Szabo, DG. *Advances in pregnancy-related protein research.* Boca Raton: CRC Press; 1993. p. 1-333.
97. Seppala M, Taylor RN, Koistinen H, Koistinen R, Milgrom E. Glycodelin: a major lipocalin protein of the reproductive axis with diverse actions in cell recognition and differentiation. *Endocr Rev.* 2002; 23:401–30. [PubMed: 12202458]
98. Seppala M. Advances in uterine protein research: reproduction and cancer. *Int J Gynaecol Obstet.* 2004; 85:105–18. [PubMed: 15099771]
99. Park JK, Song M, Dominguez CE, et al. Glycodelin mediates the increase in vascular endothelial growth factor in response to oxidative stress in the endometrium. *Am J Obstet Gynecol.* 2006; 195:1772–7. [PubMed: 17132480]
100. Rachmilewitz J, Riely GJ, Tykocinski ML. Placental protein 14 functions as a direct T-cell inhibitor. *Cell Immunol.* 1999; 191:26–33. [PubMed: 9918684]
101. Julkunen M, Rutanen EM, Koskimies A, Ranta T, Bohn H, Seppala M. Distribution of placental protein 14 in tissues and body fluids during pregnancy. *BJOG.* 1985; 92:1145–51.
102. Than GN, Tatra G, Szabo DG, Csaba IF, Bohn H. Beta lactoglobulin homologue placental protein 14 (PP14) in serum of patients with trophoblastic disease and non-trophoblastic gynecologic malignancy. *Arch Gynecol Obstet.* 1988; 243:131–7. [PubMed: 2844125]
103. Joshi SG, Smith RA, Stokes DK. A progestagen-dependent endometrial protein in human amniotic fluid. *J Reprod Fertil.* 1980; 60:317–21. [PubMed: 6776278]
104. Riittinen L, Julkunen M, Seppala M, Koistinen R, Huhtala ML. Purification and characterization of endometrial protein PP14 from midtrimester amniotic fluid. *Clin Chim Acta.* 1989; 184:19–29. [PubMed: 2688994]

105. Chatzakis K, Wathen N, Campbell J, Iles R, Dawney A, Chard T. Dramatic increase in levels of placental protein 14 in amniotic fluid at 10–15 weeks' pregnancy. *Early Hum Dev.* 1994; 36:113–6. [PubMed: 8200319]
106. Julkunen M, Koistinen R, Sjoberg J, Rutanen EM, Wahlstrom T, Seppala M. Secretory endometrium synthesizes placental protein 14. *Endocrinology.* 1986; 118:1782–6. [PubMed: 3516653]
107. Jeschke U, Kunert-Keil C, Mylonas I, et al. Expression of glycodeclin A in decidual tissue of preeclamptic, HELLP and intrauterine growth-restricted pregnancies. *Virchows Arch.* 2005; 446:360–8. [PubMed: 15756591]
108. Connor JP, Brudney A, Ferrer K, Fazleabas AT. Glycodeclin-A expression in the uterine cervix. *Gynecol Oncol.* 2000; 79:216–9. [PubMed: 11063647]
109. Toth B, Roth K, Kunert-Keil C, et al. Glycodeclin protein and mRNA is downregulated in human first trimester abortion and partially up-regulated in mole pregnancy. *J Histochem Cytochem.* 2008; 56:477–85. [PubMed: 18256018]
110. Foth D, Romer T. Glycodeclin serum levels in women with ectopic pregnancy. *Eur J Obstet Gynecol Reprod Biol.* 2003; 108:199–202. [PubMed: 12781411]
111. Tee MK, Vigne JL, Yu J, Taylor RN. Natural and recombinant human glycodeclin activate a proapoptotic gene cascade in monocyte cells. *J Leukoc Biol.* 2008; 83:843–52. [PubMed: 18203874]
112. Lam KK, Chiu PC, Chung MK, et al. Glycodeclin-A as a modulator of trophoblast invasion. *Hum Reprod.* 2009; 24:2093–103. [PubMed: 19520712]
113. Porter S, Clark IM, Kevorkian L, Edwards DR. The ADAMTS metalloproteinases. *Biochem J.* 2005; 386:15–27. [PubMed: 15554875]
114. Abbaszade I, Liu RQ, Yang F, et al. Cloning and characterization of ADAMTS11, an aggrecanase from the ADAMTS family. *J Biol Chem.* 1999; 274:23443–50. [PubMed: 10438522]
115. Hurskainen TL, Hirohata S, Seldin MF, Apte SS. ADAM-TS5, ADAM-TS6, and ADAM-TS7, novel members of a new family of zinc metalloproteases. General features and genomic distribution of the ADAM-TS family. *J Biol Chem.* 1999; 274:25555–63. [PubMed: 10464288]
116. Zhu H, Leung PC, MacCalman CD. Expression of ADAMTS-5/implantin in human decidual stromal cells: regulatory effects of cytokines. *Hum Reprod.* 2007; 22:63–74. [PubMed: 17067994]
117. Hassan SS, Romero R, Haddad R, et al. The transcriptome of the uterine cervix before and after spontaneous term parturition. *Am J Obstet Gynecol.* 2006; 195:778–86. [PubMed: 16949412]
118. Meinert M, Malmstrom A, Tufvesson E, et al. Labour induces increased concentrations of biglycan and hyaluronan in human fetal membranes. *Placenta.* 2007; 28:482–6. [PubMed: 17125833]
119. Lappas M, Riley C, Rice GE, Permezel M. Increased expression of ac-FoxO1 protein in prelabor fetal membranes overlying the cervix: possible role in human fetal membrane rupture. *Reprod Sci.* 2009; 16:635–41. [PubMed: 19293131]
120. Moore RM, Redline RW, Kumar D, et al. Differential expression of fibulin family proteins in the paracervical weak zone and other areas of human fetal membranes. *Placenta.* 2009; 30:335–41. [PubMed: 19230968]
121. Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel LA, Nien JK. Inflammation in preterm and term labour and delivery. *Semin Fetal Neonatal Med.* 2006; 11:317–26. [PubMed: 16839830]
122. Bollopragada S, Youssef R, Jordan F, Greer I, Norman J, Nelson S. Term labor is associated with a core inflammatory response in human fetal membranes, myometrium, and cervix. *Am J Obstet Gynecol.* 2009; 200:104–11. [PubMed: 19121663]

FIGURE 1. Sampling sites of the fetal membranes

The image of the placenta demonstrates the representative sampling sites of the gross site of rupture (*yellow box*) and the site away from rupture (*white box*).

FIGURE 2. Transcriptomic analysis of chorion specimens

The volcano plot shows probability values of all probes in the microarray plotted against the fold changes. In this Figure, the log (base 10) of the false discovery rate-adjusted probability values are plotted against the log (base 2) ratio of fold changes between ruptured site chorion and nonruptured site chorion. On the *Y-axis*, values higher than the *grey-line* threshold represent significant probes with an adjusted probability value of $< .1$. On the *X-axis*, values outside the *red lines* represent fold-changes of ≥ 1.5 between ruptured site chorion and nonruptured site chorion. The 815 differentially expressed probes are labeled *blue* in the upper outer quadrants.

FIGURE 3. Heat maps of the clustering of genes

A, A heat map displays the hierarchical clustering of the 200 probes whose expression had the largest variance across all amnion and chorion specimens. The *red* and *green* colors in the heat map correspond with high and low gene expression, respectively. The *labels* at the top indicate individual patient samples and their membrane region designation. Two main clusters are seen that predominantly represent amnion from the rupture site and nonrupture site on the left and chorion from the rupture site and nonrupture site in the middle. A smaller cluster of mixed samples are on the right. **B**, A heat map displays the hierarchical clustering of the 200 probes whose expression had the largest variance across chorion specimens. Two separate cluster regions represent the rupture site on the right and non-rupture site on the left.

FIGURE 4. Significantly impacted pathways in chorion specimens

Two-dimensional plot illustrates the relationship between the 2 types of evidence considered by signaling pathway impact analysis. The *X-axis* shows the overrepresentation evidence ($-\log[P\text{ value}]$); the *Y-axis* shows the perturbation evidence ($-\log[P\text{ value}]$). Each pathway is represented by a *point*. Pathways above the oblique red line (*red dots*) are significant at 5% after Bonferroni correction; the pathways (*blue dots* and *red dots*) above the oblique blue line are significant at 5% after false discovery rate correction. The *vertical* and *horizontal* thresholds represent the same corrections for the 2 types of evidence considered individually.

FIGURE 5. Quantitative reverse transcriptase–polymerase chain reaction results

Results of quantitative reverse transcriptase–polymerase chain reaction assay of selected genes in a new set of specimens. The *shaded boxes* represent genes that are statistically significant ($P < .05$); the *white boxes* represent nonsignificant genes. The data are presented as the difference between the $-\Delta Ct$ of rupture site chorion and the $-\Delta Ct$ of non-rupture site chorion: positive values correlate with increased expression and negative values correlate with decreased expression of the gene in the rupture site chorion compared with the nonrupture site chorion. The boxes encompass 50% of the data from the 1st quartile to the 3rd quartile. The *middle line* represents the median value (50%) quartile. The *whiskers*

extend to the most extreme data point but do not exceed values of >1.5 times the inter-quartile range from the box. The *circles* represent outliers.

TABLE 1
Patient demographics

Demographic	Microarray (n =20)	Quantitative reverse transcriptase–polymerase chain reaction (n = 10)	P value
Maternal age, y ^a	24.5 (20.3–27.8)	26 (20–30)	NS ^b
Gravidity, n ^a	3 (2–4)	2.5 (2–4.5)	NS ^c
Parity, n ^a	1 (0–2)	1 (0–2.8)	NS ^c
Maternal race, %			NS ^d
African American	80	70	
White	10	10	
Other	10	20	
Gestational age at delivery, wk ^a	38.6 (38.2–40.1)	39 (38.2–40.3)	NS ^b
Rupture of membranes-to-delivery interval, min ^a	259 (104–624)	121 (59–189)	NS ^c
Birthweight, g ^a	3285 (3097–3360)	3335 (3203–3409)	NS ^b

NS, not significant.

^aValues expressed as medians (interquartile range);

^bStudent *t* test;

^cMann-Whitney *U* test;

^d χ^2 test.

TABLE 2

List of the top 100 differentially expressed probes in chorion between the rupture site and nonruptured site

Rank	Name	Entrez ^a	Symbol ^b	Fold change ^c	P value
1	Methyltransferase like 7A	25840	METTL7A	-1.9	.000023
2	Epoxide hydrolase 1, microsomal (xenobiotic)	2052	EPHX1	-1.7	.000048
3	Arylamide deacetylase-like 2	344752	AADACL2	-4.5	.000057
4	KIT ligand	4254	KITLG	-1.6	.000057
5	Glutathione S-transferase alpha 4	2941	GSTA4	-1.6	.000057
6	Killer cell lectin-like receptor subfamily C, member 1	3821	KLRC1	-1.7	.000069
7	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	6387	CXCL12	-1.7	.000100
8	Basic helix-loop-helix family, member e40	8553	BHLHE40	1.6	.000100
9	Aldehyde oxidase 1	316	AOX1	-3.3	.000100
10	Meis homeobox 1	4211	MEIS1	-1.8	.000100
11	Complement factor 1	3426	CFI	-1.7	.000100
12	Tetraspanin 7	7102	TSPAN7	-2.2	.000110
13	Phosphoglucomutase 5	5239	PGM5	-3.3	.000120
14	G protein-coupled receptor, family C, group 5, member A	9052	GPRC5A	1.8	.000140
15	Arylamide deacetylase (esterase)	13	AADAC	-5.5	.000140
16	EGF-containing fibulin-like extracellular matrix protein 1	2202	EFEMP1	-2.2	.000140
17	Chromosome 12 open reading frame 48	55010	C12orf48	-1.8	.000140
18	B-cell CLL/lymphoma 2	596	BCL2	-2.3	.000140
19	Regulator of G-protein signaling 5	8490	RGS5	-2.0	.000140
20	BTB (POZ) domain containing 3	22903	BTBD3	-2.2	.000140
21	Phosphoribosyl pyrophosphate synthetase 2	5634	PRPS2	-1.7	.000140
22	Phosphoglucomutase 5	5239	PGM5	-3.3	.000140
23	Primary ciliary dyskinesia protein 1	200373	hCG_17324	-1.6	.000150
24	Killer cell lectin-like receptor subfamily C, member 1	3821	KLRC1	-2.1	.000150
25	Gtpase, IMAP family member 6	474344	GIMAP6	-2.2	.000150
26	Transmembrane 4 L 6 family member 18	116441	TM4SF18	-1.8	.000150
27	Interleukin 15	3600	IL15	-1.8	.000160

Rank	Name	Entrez ^d	Symbol ^b	Fold change ^c	P value
28	Lectin, galactoside-binding, soluble, 9	3965	LGALS9	-1.8	.000170
29	Plasticity related gene 1	9890	LPPR4	-1.6	.000180
30	Endo/exonuclease (5'-3'), endonuclease G-like	9941	EXOG	2.0	.000180
31	CD34 molecule	947	CD34	-3.3	.000180
32	MAP6 domain containing 1	79929	MAP6D1	1.6	.000180
33	Calcitonin receptor-like	10203	CALCRL	-1.9	.000180
34	Cathepsin W	1521	CTSW	-2.2	.000180
35	Toll-like receptor 7	51284	TLR7	-1.5	.000190
36	G protein-coupled receptor 34	2857	GPR34	-1.8	.000190
37	Keratin 14	3861	KRT14	3.0	.000190
38	Acetyl-coenzyme A acetyltransferase 1	38	ACAT1	-1.6	.000190
39	C-type lectin domain family 14, member A	161198	CLEC14A	-2.2	.000190
40	Similar to Complement C3 precursor	653879	LOC653879	-2.6	.000190
41	Phosphoglucomutase 5	5239	PGM5	-2.2	.000190
42	Chemokine (C-C motif) ligand 18 (pulmonary and activation- regulated)	6362	CCL18	-2.2	.000190
43	LIM domain binding 2	9079	LDB2	-2.1	.000190
44	GRB2-related adaptor protein	10750	GRAP	-1.9	.000190
45	B-cell CLL/lymphoma 2	596	BCL2	-1.9	.000190
46	Olfactomedin-like 1	283298	OLFML1	-1.6	.000190
47	CHK2 checkpoint homolog (S. Pombe)	11200	CHEK2	-1.6	.000190
48	Gtpase, IMAP family member 7	168537	GIMAP7	-2.4	.000190
49	Chromosome 5 open reading frame 4	10826	C5orf4	-1.9	.000190
50	Sterile alpha motif domain containing 3	154075	SAMD3	-1.6	.000190
51	Src kinase associated phosphoprotein 1	8631	SKAP1	-1.7	.000190
52	Tetraspanin 7	7102	TSPAN7	-2.5	.000190
53	Teashirt zinc finger homeobox 2	128553	TSHZ2	-2.1	.000190
54	Transcobalamin II; macrocytic anemia	6948	TCN2	-1.9	.000190
55	Purinergic receptor P2Y, G-protein coupled, 14	9934	P2RY14	-2.7	.000190
56	Family with sequence similarity 150, member B	285016	FAM150B	-3.1	.000200

Rank	Name	Entrez ^d	Symbol ^b	Fold change ^c	P value
57	Carboxypeptidase B1 (tissue)	1360	CPB1	-2.8	.000200
58	Regulator of G-protein signaling 22	26166	RGS22	-2.8	.000200
59	Uracil-DNA glycosylase	7374	UNG	-1.6	.000210
60	Phospholipid scramblase 4	57088	PLSCR4	-1.7	.000210
61	Dynein, cytoplasmic 2, light intermediate chain 1	51626	DYNC2L1	-1.6	.000220
62	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	6387	CXCL12	-1.9	.000220
63	Uridine phosphorylase 1	7378	UPP1	1.6	.000220
64	Pyruvate dehydrogenase kinase, isozyme 4	5166	PDK4	-2.2	.000230
65	Nuclear factor I/B	4781	NFIB	-2.0	.000230
66	Regulator of G-protein signaling 5	8490	RGS5	-1.9	.000250
67	Chromosome 21 open reading frame 34	388815	C21orf34	-1.8	.000250
68	Pannexin 1	24145	PANX1	1.6	.000250
69	Acetyl-coenzyme A carboxylase beta	32	ACACB	-1.6	.000270
70	CHK2 checkpoint homolog (S. Pombe)	11200	CHEK2	-1.6	.000280
71	Family with sequence similarity 5, member B	57795	FAM5B	-2.0	.000280
72	Integral membrane protein 2A	9452	ITM2A	-2.3	.000280
73	Chemokine (C-C motif) ligand 15	6359	CCL15	-2.2	.000290
74	ATP-binding cassette, sub-family A (ABC1), member 8	10351	ABCA8	-2.2	.000290
75	Lectin, galactoside-binding, soluble, 9	3965	LGALS9	-2.0	.000290
76	Phosphoinositide-3-kinase, regulatory subunit 1 (alpha)	5295	PIK3R1	-1.7	.000290
77	Gap junction protein, alpha 4, 37 kd	2701	GJA4	-1.6	.000290
78	Sorting nexin family member 21	90203	SNX21	-1.6	.000300
79	Phosphoribosyl pyrophosphate synthetase 2	5634	PRPS2	-1.5	.000300
80	LRRN4 C-terminal like	221091	LRRN4CL	-1.8	.000310
81	Transmembrane protein 27	57393	TMEM27	-2.0	.000340
82	CD34 molecule	947	CD34	-3.2	.000340
83	Von Willebrand factor	7450	VWF	-4.3	.000350
84	Major histocompatibility complex, class II, DO alpha	3111	HLA-DOA	-1.9	.000360
85	Granzyme A (granzyme 1)	3001	GZMA	-2.9	.000380

Rank	Name	Entrez ^d	Symbol ^b	Fold change ^c	P value
86	Transforming growth factor, beta-induced, 68 kd	7045	TGFB1	1.6	.000390
87	Nuclear factor I/B	4781	NFIB	-2.0	.000390
88	Galactosidase, beta 1-like 2	89944	GLB1L2	-2.3	.000390
89	Endoplasmic reticulum metalloproteinase 1	79956	ERMP1	-1.5	.000400
90	Serum deprivation response (phosphatidylserine binding protein)	8436	SDPR	-2.2	.000410
91	ABI family, member 3	51225	ABI3	-1.6	.000420
92	Sterol-C4-methyl oxidase-like	6307	SC4MOL	1.6	.000430
93	Solute carrier family 7 (cationic amino acid transporter, y3 system), member 5	8140	SLC7A5	1.9	.000430
94	Src homology 2 domain containing E	126669	SHE	-1.5	.000440
95	Similar to Complement C3 precursor	653879	LOC653879	-2.2	.000440
96	Ras homolog gene family, member J	57381	RHOJ	-1.9	.000440
97	Membrane-spanning 4-domains, subfamily A, member 4	51338	MS4A4A	-1.7	.000440
98	Hypothetical protein LOC100133666	100133666	LOC100133666	-1.6	.000440
99	Frizzled homolog 8 (<i>Drosophila</i>)	8325	FZD8	1.7	.000440
100	Osteoglycin	4969	OGN	-2.7	.000440

ABI, Abelson-interactor; ATP, adenosine triphosphate; BTB, Broad-complex, Tramtrack and Bric-a-brac; CD34, cluster of differentiation 34; CHK2, checkpoint kinase 2; CLL, chronic lymphocytic leukemia; EGF, epidermal growth factor; GRB2, growth factor receptor-bound protein 2; IMAF, immunity-associated protein; KIT, c-Kit; LRRN4, leucine rich repeat neuronal 4; MAP6, microtubule-associated protein 6; POZ, poxvirus and zinc finger.

^dEntrez gene identification;

^bSymbol was taken from the gene database and corresponds to official Human Genome Organization Gene Nomenclature Committee symbols;

^cfold change (the number of times the average expression level in the ruptured sites is different from the average expression level in the nonruptured sites) refers to probe expression change in ruptured chorion relative to nonruptured chorion; positive values (no sign) indicate increased expression, and negative values (minus sign) indicate decreased expression.

TABLE 3

Top 20 biological processes with enrichment in chorion between the rupture site and the nonrupture site

Rank	Biological process	Genes in differentially expressed list, n	Genes in reference array, n	Adjusted <i>P</i> value
1	Anatomic structure development	151	1785	< .0001
2	Multicellular organismal process	202	2681	< .0001
3	Immune response	61	520	< .0001
4	Multicellular organismal development	152	1919	< .0001
5	Response to external stimulus	66	596	< .0001
6	Epidermis development	25	118	< .0001
7	Antigen processing and presentation of peptide or polysaccharide antigen via major histocompatibility complex class II	10	16	< .0001
8	Cell adhesion	65	591	< .0001
9	Biologic adhesion	65	591	< .0001
10	Developmental process	196	2720	< .0001
11	Organ development	98	1074	< .0001
12	Cell communication	211	3006	< .0001
13	Ectoderm development	26	132	< .0001
14	Signal transduction	194	2739	< .0001
15	Anatomic structure morphogenesis	89	971	< .0001
16	System development	122	1496	< .0001
17	Response to wounding	47	381	< .0001
18	Response to stimulus	157	2100	< .0001
19	Immune system process	72	753	< .0001
20	Locomotion	42	346	< .0001

TABLE 4

Significant pathways with overrepresentation analysis in chorion between rupture site and nonrupture site

Rank	Map name	Kyoto Encyclopedia of Genes and Genomes identification number	Genes in differentially expressed list, n	Genes in reference array, n	Adjusted P value
1	Cell junctions	1430	30	108	< .0001
2	Graft-vs-host disease	5332	17	38	< .0001
3	Complement and coagulation cascades	4610	17	53	< .0001
4	Extracellular matrix–receptor interaction	4512	20	73	< .0001
5	Allograft rejection	5330	13	32	< .0001
6	Cell adhesion molecules	4514	25	115	< .0001
7	Type I diabetes mellitus	4940	13	38	< .0001
8	Asthma	5310	10	23	< .0001
9	Focal adhesion	4510	30	184	< .0001
10	Autoimmune thyroid disease	5320	12	40	.0001
11	Antigen processing and presentation	4612	16	74	.0002
12	Hematopoietic cell lineage	4640	15	70	.0003
13	Systemic lupus erythematosus	5322	16	101	.0064
14	Metabolism of xenobiotics by cytochrome P450	980	9	43	.0130

TABLE 5

Comparison of microarray data to quantitative reverse transcriptase–polymerase chain reaction results of selected genes in original study samples and in a new replication set in chorion between rupture site and nonrupture site

Gene symbol	Quantitative reverse transcriptase–polymerase chain reaction					
	Original sample set (n = 20)		Original sample set (n = 10) ^a		New replication set (n = 10)	
	Fold change	P value	Fold change	P value	Fold change	P value
ADAMTS5	-3.17	.0239	-2.81	.0239	-2.20	.0816
BCL2	-2.26	.0190	-5.47	.0190	-2.48	NS
CNR1	-2.26	.0681	-2.64	.0681	-2.77	.0824
IL1RN	3.05	.0965	2.37	.0965	3.71	NS
CXCL12	-1.72	.0009	-15.60	.0009	-10.58	.0139
IL-6	2.71	NS	2.66	NS	13.25	.0233
CXCL14	-2.32	.0205	-6.14	.0205	-2.83	.0545
PTGS2	2.10	NS	1.61	NS	3.45	.0213
GNLY	-4.40	.0050	-6.27	.0050	-6.31	.0123
PAEP	-3.93	.0072	-24.15	.0072	-30.97	.0218
IGF2	1.57	NA	NA	NA	-1.00	NS
IGFBP2	-2.97	.0156	-3.51	.0156	-3.29	.0323
IGFBP4	-1.50	.0420	-2.71	.0420	-3.07	.0166
IGFBP6	-2.30	.0394	-3.13	.0394	-1.09	NS
					-1.74	.0711

NA, not available; NS, not significant.

^aOf the 20 original specimens, 10 specimens were selected randomly for quantitative reverse transcriptase–polymerase chain reaction.