OBSTETRICS

Placental expression of aminopeptidase-Q (laeverin) and its role in the pathophysiology of preeclampsia

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OBJECTIVE: The purpose of this study was to investigate the expression and subcellular localization of laeverin, a placenta-specific membrane-bound aminopeptidase, in preeclamptic placentas and its role in trophoblast cell migration and invasion.

STUDY DESIGN: Expression of laeverin was investigated in 6 normal and 6 preeclamptic placentas with the use of immunofluorescence, sodium dodecylsulfate-polyacrylamide gel electrophoresis with Western blot analysis and immunoelectron microscopy. The role of laeverin in trophoblast migration and invasion was studied with the use of the xCelligence system and Boyden chambers with Matrigel in HTR-8/SVneo cells. The effect of laeverin gene-silencing on selected genes that are involved in cell transformation and tumorigenesis was evaluated by polymerase chain reaction array. The Student *t* test, Mann-Whitney *U* test, χ^2 test, or F-test was used to compare groups as appropriate.

RESULTS: Laeverin was expressed in the cell membrane of villous trophoblasts in third-trimester healthy placentas; in preeclamptic placentas, it was expressed ectopically in the cytoplasm, especially in microvesicles. Immunoelectron microscopy showed laeverin leakage into the fetal capillaries and abundant expression in microvesicles in preeclamptic placentas. Migration and invasion of HTR-8/SVneo cells were reduced by 11.5% ($P = .023$) and 56.7% ($P = .001$), respectively, by laeverin gene—silencing. Analysis of downstream pathways affected by laeverin-silencing demonstrated significant down-regulation of integrin A2 (39-fold), integrin B3 (5-fold), and matrix metalloprotease 1 (36-fold).

CONCLUSION: Expression of laeverin protein is altered in preeclamptic placentas. Its ectopic expression in the cytoplasm and microvesicles, rather than the cell membrane and leakage into the fetal capillaries, may have a role in the pathophysiologic condition of preeclampsia. Laeverin gene appears to be involved in trophoblast cell migration and invasion through interaction with integrins and matrix metalloprotease 1.

Key words: aminopeptidase, laeverin, microvesicle, placenta, preeclampsia

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Preeclampsia complicates 5-10% of pregnancies and is a major cause of maternal mortality worldwide.[1] Although it is clearly a placenta-specific disorder, its pathogenesis is not understood fully. Therefore, its prediction, timely diagnosis, and appropriate management remain challenging.

Laeverin, a membrane-bound aminopeptidase, was first reported to be expressed by human trophoblast cells in 2004 by Fujiwara et al[2] and has been suggested to cooperate with the chemokine system in the regulation of human placentation.[3] The same group recently presented some molecular evidence suggesting that laeverin is important for extravillous trophoblast invasion.[4] Laeverin is a trophoblast-specific protein; however, it has been reported to be expressed in other tissues in some inflammatory diseases, such as rheumatoid arthritis.[5] In a previous study, comparing global placental gene expression profile between preeclamptic and healthy pregnancies, we found 16 genes that were able to predict preeclampsia phenotype in our study population.[6] Laeverin was among those genes, and it was up-regulated significantly in the preeclamptic placentas. Therefore, we hypothesized that the deregulation of laeverin protein may lead

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to abnormal trophoblast function and have a role in the pathophysiologic condition of preeclampsia.

In the present study, we investigated the expression of laeverin protein and its subcellular localization in healthy and preeclamptic placentas. Additionally, we investigated the role of laeverin in trophoblast cell migration and invasion.

Materials and Methods

The study was approved by the Regional Committee for Medical and Health Research Ethics-North Norway (REK Nord reference no. 2010/2058-4), and informed written consent was obtained from all the participants.

Placental samples from a total of 12 pregnant white European women (6 healthy and 6 with severe preeclampsia) were collected. They were matched for maternal age, parity, onset of labor (spontaneous or induced), and the mode of delivery (vaginal or cesarean), and the results were compared. *Severe preeclampsia* was defined as blood pressure ≥160/110 mm Hg and proteinuria ≥300 mg/24-hour urine or ≥2+ in spot urine after 20 weeks of gestation in previously normotensive women.[7] Women with preexisting medical conditions that may have affected the course and outcome of pregnancy were excluded. Doppler ultrasonography was performed at ≤48 hours before delivery in each case to assess uteroplacental and umbilical circulation.

Collection of maternal blood and placental samples

Maternal venous blood samples were taken at ≤48 hours before delivery for the analysis of hemoglobin, hematocrit, liver function, and renal function. None of the women were in labor when blood samples were taken. Placental tissue samples were obtained immediately after delivery, as described previously.[6]

Cell line

The immortalized HTR-8/SVneo trophoblast cell line, which was obtained from primary cultures of human trophoblast cells,[8] was used for migration, invasion, and polymerase chain reaction (PCR) array studies. Cells were maintained in RPMI Medium 1640 supplemented with 5% fetal bovine serum (FBS; GIBCO, Invitrogen, Carlsbad, CA) in a 37°C water-jacketed incubator (Forma Scientific, Marietta, OH) with 5% CO_2. Trypsin-ethylenediamine tetraacetic acid (Sigma Chemical Co, St. Louis, MO) was used for harvesting and for the subculturing of cells.

Laeverin antibodies

Polyclonal antibodies against laeverin were raised (Eurogentec, Seraing, Belgium). Rabbits were immunized with synthetic oligo-peptides that contained 2 predicted epitopes (EP073418:CRVHANLQTIKNENLK and EP073419:CERAEVRGPLSPGTG). Peptide sequences for these epitopes were chosen from the amino acid sequence of laeverin (Q0P5U8; http://www.ncbi.nlm.nih.gov/protein/121946569). Immunogenic epitopes of the exposed amino acids of the laeverin 3-dimensional structure were chosen for peptide synthesis with software that was provided by Sigma Chemical Co.

A commercially available goat polyclonal antibody of laeverin was used as control (Santa Cruz Biotechnology Inc, Santa Cruz, CA).

Immunofluorescence

Tissue samples from 3 preeclamptic placentas and 3 normal healthy controls were fixed in formalin, embedded in paraffin blocks, cut (4-6 μ sections), and mounted on glass slides. Immunofluorescence cell staining was performed[9] with our laeverin antibody (2.1 μg/mL) and secondary goat anti-rabbit immunoglobulin G- fluorescein isothiocyanate (2.5 μg/mL; Santa Cruz Biotechnology Inc). Slides were counterstained with DAPI (4′,6-diamidino-2-phenylindole) II (Vysis; Abbott Diagnostics, Lake Forest, IL). Images were obtained with CytoVision digital system (Applied Imaging, Grand Rapids, MI) that was equipped with a charge-coupled device camera (Cohu Inc, Poway, CA). A total of ≥200 cells were inspected on each slide. Experiments were run in triplicate.

Protein isolation

Placental tissue was cut in small pieces, and proteins were isolated with the use of T-PER (Pierce Chemical Co, Rockford, IL) with Complete Mini ethylenediamine tetraacetic acid–free protease inhibitor cocktail in combination MagNA Lyser Green Beads for homogenizing on MagNA Lyser (Roche, Indianapolis, IN). Protein concentration was measured with the use of the DC Protein Assay kit (Bio-Rad Laboratories, Hercules, CA) in a ThermoMax Microplate Reader (Molecular Devices, Downington, PA).

Sodium dodecylsulfate-polyacrylamide gel electrophoresis and Western blot analysis

Reduced and denatured proteins (5 μg) that had been isolated from 8 placentas (4 preeclamptic and 4 normal) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 4-12% NuPAGE (Invitrogen). Electrophoresis and blotting (polyvinylidene difluoride nylon membrane, pore size 0.45 μm; Invitrogen) were run on Novex Mini Cell XCell Sure Lock (Invitrogen). Blots were cut under a 49-kDa protein band to provide 2 blots; 1 for laeverin and another for the housekeeping protein actin. Labeling was done with primary antibodies against laeverin (our antibody [0.42μg/ml] and commercial antibody [1 μg/mL; Santa Cruz Biotechnology Inc] or actin [1 μg/mL; Santa Cruz Biotechnology Inc]). Detection was performed with goat anti-mouse immunoglobulin G-alkaline phosphatase–conjugated antibody (0.2 μg/mL; Santa Cruz Biotechnology Inc) and CDP-Star (Roche). Pictures were taken on ImageQuant LAS 4000 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Experiments were run in triplicate.

Immunoelectron microscopy

Immunoelectron microscopy was performed on ultrathin tissue sections of 2 healthy placentas and 2 placentas that were obtained from women with severe preeclampsia. All experiments were run in triplicates.

Fresh placental tissue samples were dissected, mounted in membrane carriers, and frozen at high pressure (EMPACT 2 HPF; Leica Microsystems,

Vienna, Austria). Frozen samples freeze substituted (EM AFS2, Leica Microsystems) and infiltrated in Lowicryl HM20 (Electron Microscopy Sciences, Hatfield, UK).[10] Ultrathin sections of 70 nm were cut on a Leica EM UC6 ultramicrotome (Leica Microsystems) and mounted on copper grids (Agar Scientific, Stansted, UK) with Formwar and carbon.

Immunolabeling was performed with the optimal dilution of primary antibodies.[11,12] Single and double labeling experiments were performed with both locally designed (26.25 μg/mL) and commercially purchased laeverin (5 μg/mL) antibodies. For double labeling, anti-endoplasmatic reticulum (ER) mouse monoclonal antibody (RL90) to protein disulphide isomerase (ab2792; 0.1 μg/mL) and anti-Golgi apparatus (GA) mouse monoclonal antibody (AE-6) to MG160 protein (MG160; ab58826; 0.05 μg/mL; Abcam, Cambridge, UK) were used as the specific markers of ER and GA, respectively.

Microscopy was done with a JEM-1010 transmission electron microscope (JEOL, Tokyo, Japan) at 4000, 10,000, 20,000, 30,000, and 70,000 magnifications. Images were taken and processed in Morada Soft Imaging Camera system with iTEM software (Olympus, Hamburg, Germany). A total of 200 images from each experiment were processed. Image montage was done in Adobe Photoshop and Adobe Illustrator (Adobe Systems Inc, San Jose, CA).

Immunoglobulin G conjugated gold particles were used as controls in similar experiments. Possible secondary antibody cross-reactivity was excluded by the omission of primary antibodies in separate experiments.

xCelligence migration assay

HTR-8/SVneo trophoblast cells (2×10^5 cells/well) were seeded the day before small interfering RNA (siRNA) transfection with FuGENE transfection reagent (Promega Corp, Madison, WI). SiRNA (10 pmol) against laeverin or scrambled siRNA A or D (control; Santa Cruz Biotechnology Inc) were used. Plates were incubated at 37°C with 5% CO_2 for 5 hours; transfection solution was replaced with fresh RPMI Medium 1640 with 5% FBS, and cells were further incubated for 24 hours; 2×10^5 cells/well were added to each well of the CIM-Plate 16 (ACEA Biosciences Inc, San Diego, CA). Migration assays were performed (for 72 hours, with sweeps of 30 minutes each) in the xCelligence system (ACEA Biosciences Inc). Three different CIM-Plates 16 were used. Experiments were run in quadruplicate. Coefficients of variation for siRNA A, D, and laeverin were 3.5%, 1.2%, and 3.5%, respectively. Untransfected cells were used as controls and were run in duplicates on each plate. Analysis was performed in the RTCA software (version 1.2.1; ACEA Biosciences Inc).

Reverse transcription–PCR of the cell line that was used in migration assays

Total RNA was isolated from cultured cells (untransfected, siRNA silenced laeverin siRNA A and D silenced) with TRIzol Reagent (Invitrogen) 55 hours after transfection at the migration optimum. Total RNA was extracted with RNeasy Mini Kit (Qiagen, Venio, The Netherlands); the concentration of RNA was measured with NanoDrop (Saveen Werner, Malmo, Sweden), and reverse transcription was performed with the High Capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, CA). Complementary DNA samples were profiled for the relative expression of the genes of laeverin, glyceraldehyde-phosphate dehydrogenase, and actin, beta with the Taq Man Gene Expression Assays on 7900HT Fast Real-Time PCR system (Applied Biosystems).

Matrigel invasion studies in Boyden chambers

HTR-8/SVneo trophoblast cells (5×10^5cells) were grown in RPMI Medium 1640 with 10% FBS and incubated at 37°C, with 5% CO_2 overnight. Medium was replaced by RPMI Medium 1640 with 5% FBS the next day. On day 3, transfection with 50 pmol laeverin siRNA or 50 pmol of siRNA A control (Santa Cruz Biotechnology Inc) with Lipofectamine 2000 (Invitrogen) was performed in separate flasks. Cultures were incubated at 37°C with 5% CO_2 for 4 hours and washed with RPMI Medium 1640 without serum before incubation overnight. Invasion studies (1×10^5 cells/well; 5% FBS used as chemoattractant) were performed in 48 hours in BD BioCoat–BD Matrigel Invasion Chambers (24-well plate 8 μ with control inserts; BD Biosciences, San Jose, CA) and incubated at 37°C with 5% CO_2. The noninvading cells were removed from the upper part of the insert's membrane by scrubbing with cotton-tipped swabs that had been moistened with medium. Cell invasion was performed by methylthiazolyldiphenyl-tetrazolium bromide (MTT)-assay.[13] Results were monitored in Thermo Multiscan Ex (ThermoFisher Scientific Inc, Waltham, MA). Experiments were run in triplicate.

Gene expression profiling

To investigate the downstream effect of laeverin-gene silencing in HTR-8/SVneo trophoblast cells, we performed a PCR array to explore 6 biologic pathways that are involved in cell transformation and tumorigenesis (Appendix; Supplemental Table 1).

HTR-8/SVneo trophoblast cells ($4\text{-}5{,}7 \times 10^5$ cells) were transfected with Lipofectamine 2000 and 120 pmol siRNA laeverin or siRNA A (control). Cells were mixed with TRIzol Reagent and RNA isolated by RNeasy Mini kit. Complementary DNA synthesis and quantitative reverse transcription–PCR were performed with the use of RT^2 Profiler PCR Array Human Cancer Pathway Finder (PAHS-033A; SABiosciences Corporation, Frederick, MD). Actin, beta was used as housekeeping gene. Analysis of fold changes was done by the comparative Ct ($\Delta\Delta$Ct) method with the integrated web-based software package for the PCR array system.

Statistical analysis

Data were analyzed with IBM SPSS Statistics 21 software (SPSS Inc, Chicago, IL). Continuous variables are presented as mean ± SE or median (range); categoric variables are presented as number (%). Assessment of normality was performed with the Shapiro-Wilk test.

Differences between groups were tested with the Student *t* test for parametric variables and the Mann-Whitney *U* test, χ^2 test, or F-test for nonparametric variables. A probability value of $< .05$ was considered significant.

RESULTS

Phenotype of the study population

The baseline demographic and clinical characteristics of the study population that included birth outcomes are shown in the Table. The mean proteinuria level was 5.9 g/L (range, 3.9–9.0 g/L) in women with preeclampsia. None of the women who were included in the study had HELLP (hemolysis, elevated liver enzymes and low-platelets) syndrome. Two women in the preeclampsia group were delivered by cesarean section because of worsening condition. Two women in the normal group also had cesarean deliveries; one because of breech presentation and another because of placenta previa. None of them were in labor. Four women in each group had vaginal delivery; 3 women in each group had induced labor.

Laeverin ectopically expressed in the trophoblastic cytoplasm in preeclampsia

Immunofluorescence analysis demonstrated that laeverin is expressed by the villous trophoblasts (Figure 1). In normal placenta, it was membrane-bound and mainly expressed in plasma membrane (Figure 1, A). Laeverin was expressed more abundantly in the preeclamptic placenta and was localized in the cytoplasm of the villous trophoblast cells (Figure 1, B). In control placentas in which laeverin was replaced by phosphate-buffered saline solution, no specific labeling was detected in the villous trophoblasts (Figure 1, C).

Molecular mass of laeverin

We estimated the molecular mass of laeverin to be approximately 60 kDa by performing denaturing and reducing SDS-PAGE and Western blot analysis of healthy and preeclamptic placentas (Figure 2). Experiments with our locally designed antibody that was raised against the N- and C-terminal part of laeverin and commercially available antibody that was raised against a peptide mapping within an internal region of laeverin gave the same results.

Laeverin in preeclamptic placentas

Immunoelectron microscopy demonstrated that laeverin was expressed in the plasma membrane of trophoblast cells of healthy placentas. It was hardly detectable in the cytosol and was not detectable in the fetal capillaries (Figure 3, B). However, in preeclamptic placentas, laeverin was expressed strongly in the cytoplasm, especially in the microvesicles and in the fetal capillaries (Figure 3, A, C, E, and G). Laeverin was expressed abundantly in microvesicles within the cytoplasm, in the extracellular space, and in areas of focal aggregation of syncytiotrophoblasts (syncytial knots). Laeverin was not expressed in mitochondria but was expressed in ER and GA. Experiments with the use of the commercially available laeverin antibody gave same results (Figure 4).

Laeverin silencing affects trophoblast cell migration

Transfection with siRNA against laeverin showed an 11.5% ($P = .023$) reduction in the migration of HTR-8/SVneo trophoblast cells compared with cells that were transfected with scrambled siRNA (control) at the peak of migration, approximately 30 hours after

TABLE
Phenotype of the study population

Variable	Preeclampsia (n = 6)	Health control subjects (n = 6)	*P* value
Maternal age, y[a]	28 ± 2.35	32 ± 1.58	.261
Body mass index before delivery, kg/m^2[a]	28.9 ± 0.85	29.6 ± 1.85	.873
Primiparous, n (%)	4 (66.7)	2 (33.3)	.567
Mean arterial pressure, mm Hg[a]	131 ± 3.82	85 ± 4.15	< .0001
24-hour proteinuria, g/L[a]	5.92 ± 5.10	N/A	
Uterine artery pulsatility index (mean of the left and right side)[a]	1.23 ± 0.29	0.69 ± 0.9	.157
Middle cerebral artery pulsatility index[a]	1.20 ± 0.14	1.38 ± 0.12	.142
Umbilical artery pulsatility index[a]	1.19 ± 0.15	0.81 ± 0.13	.049
Gestational age at delivery, wk[a]	34 ± 1.4	39 ± 0.48	.005
Cesarean delivery, n (%)	2 (33.3)	2 (33.3)	1
Neonatal birthweight, g[a]	2390 ± 430	3328 ± 207	.055
Placental weight, g[a]	437 ± 72	623 ± 69	.065
5-minute Apgar score[b]	8 (6–9)	10 (10–10)	.002
Arterial cord blood pH[a]	7.27 ± 0.02	7.25 ± 0.03	1.0
Arterial cord blood base excess, mmol/L[a]	1.97 ± 2.06	7.67 ± 0.58	.069
Venous cord blood pH[a]	7.33 ± 0.02	7.35 ± 0.02	.343
Venous cord blood base excess, mmol/L[a]	−2.12 ± 1.66	−4.50 ± 0.84	.343

Differences between groups were tested with the use of the Student *t* test for parametric variables and with the Mann-Whitney *U* test or χ^2 test for nonparametric and categorical variables, as appropriate.

N/A, not applicable.

[a] Data are given as mean ± standard error; [b] Data are given as median (range).

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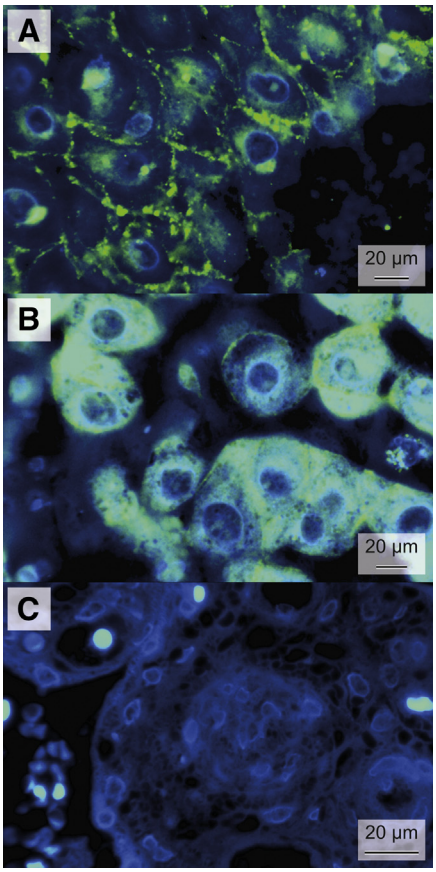
FIGURE 1
Cross-section of the terminal villi



Trophoblast cells stained with laeverin (*green*) and counterstained with DAPI II (*blue*) in **A,** normal and **B,** preeclamptic placentas. Laeverin is localized in the plasma membrane of the villous trophoblasts in the normal placenta. In the preeclamptic placenta, laeverin protein is detected in the cytoplasm of the trophoblasts and is more abundant than in normal placenta. **C,** In the negative control, no staining of laeverin is detected, but the erythrocytes showed green autofluorescence.[27] Original magnifications: A and B, ×1000; C, ×600.

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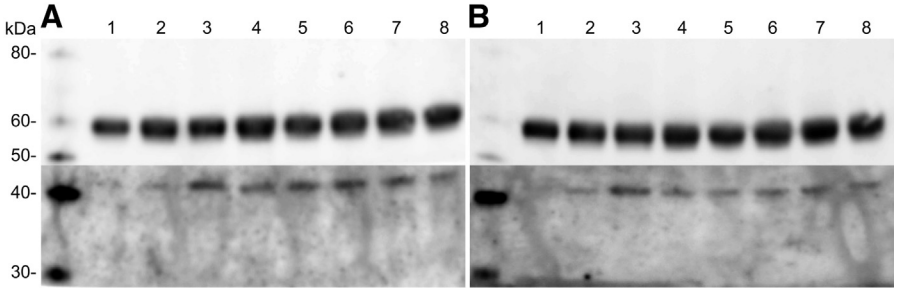
FIGURE 2
Western blot analysis of laeverin protein in normal and preeclamptic placentas



Sodium dodecylsulfate-polyacrylamide gel electrophoresis and Western blot analysis with **A,** locally designed laeverin antibody and **B,** commercially available laeverin antibody detected a 60-kDa protein. Laeverin protein detected in the placenta of 4 women with preeclampsia (lanes 1-4) and in the placenta of 4 healthy pregnant women (lanes 5-8). Molecular weight marker is shown on the *left side* of each figure. Detection of actin protein (43 kDa) was used as loading control (*lower part of the figures*). Markers used were SeeBlue Plus2 Prestained Standard and Magic Mark XP Western Standard (Invitrogen, Carlsbad, CA).

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transfection (Figure 5, A; Supplemental Table 5). Laeverin messenger RNA (mRNA) was 36% reduced in silenced cells (Figure 5, B).

Trophoblast invasion reduced in laeverin-silenced HTR-8/SVneo cells

We found that the absorbance (optical density 540) of laeverin-silenced cells was 0.16 ± 0.07 and that absorbance at 540 nm of control cells was 0.3 ± 0.11 ($P = .001$). Thus, laeverin-silenced cells had a 56.7% reduced ability to invade through Matrigel, compared with control cells (Figure 6). Laeverin mRNA was 80% reduced in silenced cells (data not shown).

Integrin alpha-2, matrix metalloproteinase 1, and integrin beta-3 genes down-regulated in laeverin-silenced HTR-8/SVneo cells

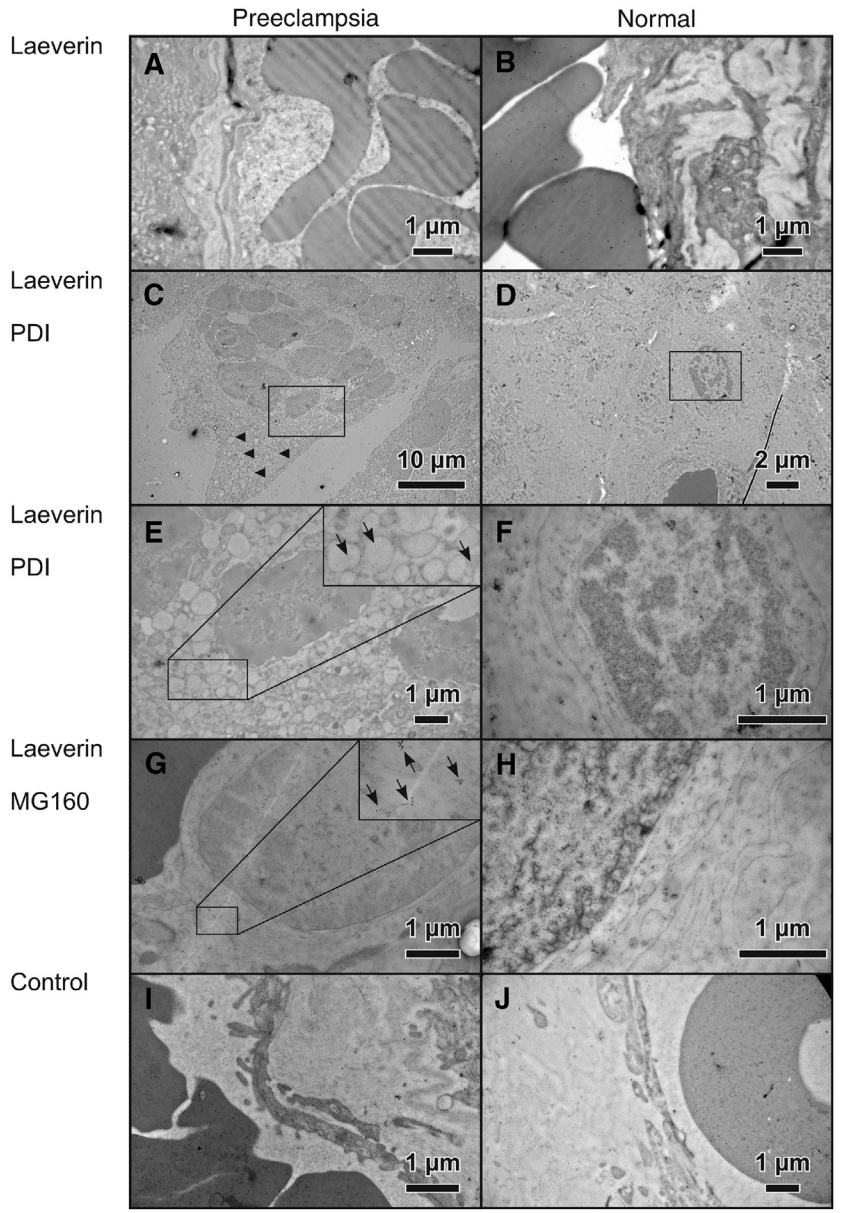
Three genes that are involved in cell transformation and tumorigenesis were shown to be down-regulated by laeverin-silencing at a significant level (>4-fold) with the PCR array. Integrin alpha-2, matrix metalloproteinase 1 (MMP1), and integrin beta-3 were down-regulated 39-fold, 36-fold, and 5-fold, respectively (Figure 7). The complete list of genes on the array together with reverse transcription–PCR results (average threshold cycle [Ct], average difference in cycle number [ΔCt], and fold-regulation) are given in Supplemental Tables 2-4.

Comment

Laeverin was first reported to be expressed in the cell surface of extravillous trophoblasts obtained from human third-trimester chorion laeve.[2] Northern blot analysis showed that laeverin is a placenta-specific protein. It contains a transmembrane domain at the N-terminus and has an amino acid sequence that is homologous with membrane-bound aminopeptidase-N.[14] However, the function of laeverin still is not understood completely. Our immunofluorescence studies on healthy placental tissues demonstrated that laeverin is expressed in the plasma membrane of trophoblast cells, which confirms previous findings.[4,15] However, in preeclamptic placentas, it was localized mainly in the cytoplasm, especially the microvesicles. To our knowledge, this has not been reported previously.

Protein modifications or cleavage of laeverin could be responsible for its altered placental expression in preeclampsia. However, results of SDS-PAGE and Western blot analysis clearly indicate that laeverin has a molecular mass of 60 kDa (Figure 2) both in preeclamptic and normal placenta. The predicted molecular mass from the amino acid sequence is 113 kDa.[11] However, this variance can be explained. Native laeverin might have cleaved during the purification that resulted in 2 identical proteins of 60 kDa, with a total mass of 120 kDa. Furthermore,

FIGURE 3
Immunoelectron microscopy of ultrathin sections of placentas



Preeclamptic placenta (*left column*) and healthy placenta (*right column*). Cross-sections of fetal capillary show red blood cells and protein debris with laeverin (*black dots of 5 nm gold particles*) in **A,** preeclamptic placenta and **B,** unspecific labeling of only red blood cells in normal placenta. **C-F,** Double labeling with laeverin (5 nm gold) and endoplasmatic reticulum protein disulphide isomerase (*PDI*) marker (10 nm gold) in preeclamptic and healthy placenta. **C,** Terminal villi of preeclamptic placenta show a syncytiotrophoblast knot and many microvesicles (*arrowheads*) and **D,** no microvesicles in trophoblast cells of healthy placenta. **E** and **F,** Magnified sections are depicted as squares. **E,** Laeverin and protein disulphide isomerase colocalize in the microvesicles (*arrows*) in preeclamptic placenta. **F,** In normal trophoblast cells, no colocalization was detected. Double labeling with laeverin (5 nm gold) and Golgi MG-160 marker (10 nm gold) in **G,** preeclamptic and **H,** healthy placenta. Colocalization was detected in preeclamptic placenta (*arrows*) but not in cytosol of healthy trophoblast cells. Trophoblast cells of both **C,** preeclamptic and **D,** normal placenta show laeverin localization in the euchromatin of the nucleus. **I** and **J,** Negative control showed no labeling.

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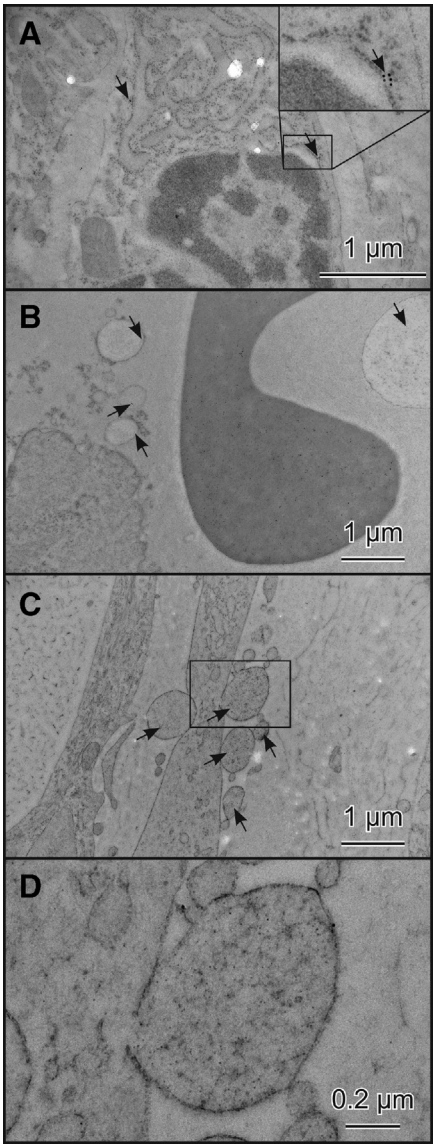
alternate splicing of the laeverin gene may produce 4 protein isoforms with different molecular masses.[11] Horie et al[4] detected 3 different bands of 200-270 kDa, 160 kDa, and 130 kDa in normal placenta instead of 1. This discrepancy could be due to the differences in antibodies and methods that were used for protein purification and analysis.

Immunoelectron microscopy demonstrated the expression of laeverin in the fetal capillaries and in the microvesicles within the cytosol of trophoblast cells in preeclamptic placentas. Microvesicles in the extracellular space and syncytial knots also expressed laeverin abundantly. Microvesicles and exosomes have been found previously in preeclamptic placentas,[16] and syncytiotrophoblast vesicles have been shown to play a role in the pathophysiologic condition of preeclampsia.[17] Trophoblastic microvesicles can also be found in the maternal circulation and release cytokines that provoke maternal inflammatory response.[18,19] Microvesicles contain fetal DNA, RNA, and proteins and play an important role in cell communication.[20] They facilitate intracellular transport of proteins and their attachment to the plasma membrane at specific sites.[21] Colocalization of laeverin together with ER and GA markers indicated aberrant processing of laeverin in preeclamptic placentas that may have resulted in massive production of microvesicles. Because the ER and GA in normal placentas did not show accumulation of laeverin, conventional exocytosis might be impaired in preeclamptic placenta.

The laeverin enzyme appears to have a broad spectrum of substrates that can affect cell migration and angiogenesis.[22] We found that laeverin silencing reduces migration and invasion of HTR-8/SVneo trophoblast cells. In line with this, Horie et al[4] have also demonstrated reduced cell invasion in laeverin-silenced human chorionic villous explants cultures.

It has been hypothesized that laeverin plays a role in extravillous trophoblast invasion in cooperation with the chemokine system in the fetomaternal interface.[3] Kisspeptin,[4] angiotensin III,

FIGURE 4
Colocalization immunoelectron microscopy of ultrathin sections of preeclamptic placenta



Commercially available antibody against laeverin (labeled with 5 nm gold) and endoplasmatic reticulum marker protein disulphide isomerase (labeled with 10 nm gold). Laeverin was detected **A,** in the endoplasmatic reticulum of trophoblasts (*arrows*) and **B,** in microvesicles within the capillaries (*arrows*). Unspecific labeling of the red blood cells was detected. **C,** Microvesicles packed with laeverin and protein disulphide isomerase (*arrows*). Part of section (*square inset*) in **C** is magnified and **D,** shows a close-up of microvesicle with laeverin.

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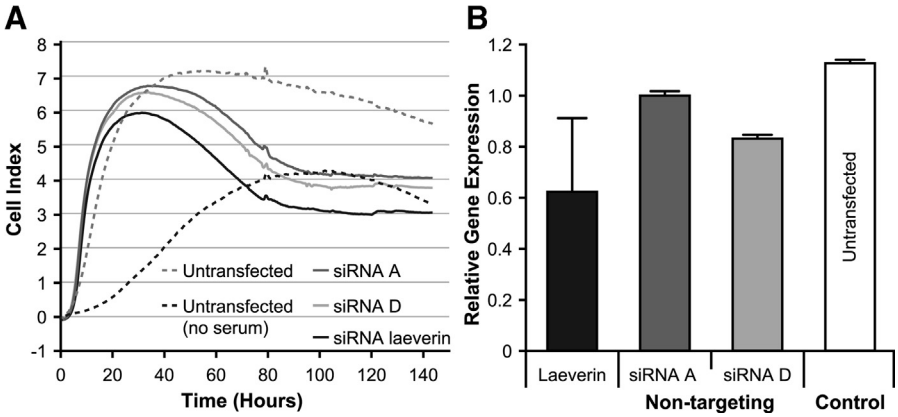
FIGURE 5
Cell migration assays



A, HTR-8/SVneo trophoblast cells in CIM-Plate 16 in the xCelligence system (ACEA Biosciences Inc, San Diego, CA). Graphs show cells that were transfected with small interfering RNA (*siRNA*) against laeverin. Controls were cells transfected with 2 different types of scrambled nontarget siRNAs (*A* and *D*), untransfected cells, and untransfected cells without serum. Laeverin siRNA silenced cells demonstrated 11.5% reduction of migration. An average of 4 parallels is shown in each graph. Time points (hours) are shown on the x-axis, and cell index is shown on the y-axis. **B,** Efficacy of laeverin silencing was evaluated with the use of real-time polymerase chain reaction. The siRNA-mediated silencing of laeverin was assessed with the comparative Ct ($\Delta\Delta$Ct) method to determine relative gene expression from quantitative polymerase chain reaction data with actin, beta as an endogenous reference gene. Cells were silenced by laeverin siRNA with messenger RNA reduction of 36%. Comparison of nontargeting controls (siRNA A and D) to untransfected cells suggests that there is no significant effect of transfection reagent plus siRNA on the cells. Target messenger RNA levels were measured and normalized against actin, beta messenger RNA from samples harvested 55 hours after siRNA transfection of cells. Different experiments are shown on the x-axis, and relative gene expression is shown on the y-axis.

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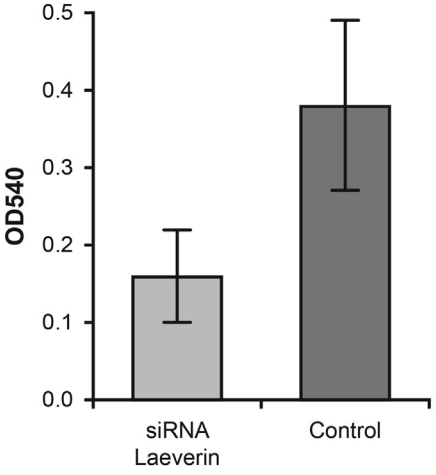
edokinin C, and dynorphin A1-8[9,22,23] are the only known proteins that can be cleaved by laeverin. We looked for other possible interaction partners by performing PCR array on 84 selected genes that are representative of the 6 biologic pathways that are involved in cell transformation and tumorigenesis. Silencing laeverin had downstream effects on the regulation of the cell adhesion system that is mediated by MMP1 and integrins. MMP1 is expressed by invasive trophoblasts in the first-, second-, and third-trimester placentas.[24] MMPs are involved in changing cell phenotype from adhesive to a migratory by degrading the extracellular matrix (ECM). They affect cell migration during physiologic processes (such as embryonic development, reproduction and tissue remodeling) and in pathologic conditions (such as cancer metastasis). Integrins are the main receptors for the ECM[25] and are involved in regulating cell adhesion and locomotion. Indeed, trophoblast interaction with the ECM has been shown to be mediated by integrins and MMPs.[26] Therefore, it is plausible that laeverin-silenced trophoblasts lose their invasiveness by interacting with the cell's integrin and MMP repertoire.

The molecular link between reduced trophoblast invasion of maternal decidua in the first trimester and the development of preeclampsia later in pregnancy is still missing. We have shown previously that laeverin mRNA is increased in preeclamptic placentas.[6] Our present study shows that laeverin is also increased at protein level and that laeverin silencing reduces trophoblast

FIGURE 6
Invasion studies in Boyden chambers



Methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay of Matrigel invasion of HTR-8/SVneo trophoblast cells that were transfected with small interfering RNA (*siRNA*) against laeverin (*gray*) in Boyden chambers demonstrated 56.7% reduced invasion compared with cells that were transfected with scrambled siRNA A (*dark grey*). The y-axis represents the absorbance (optical density) at 540 nm (*OD 540*).

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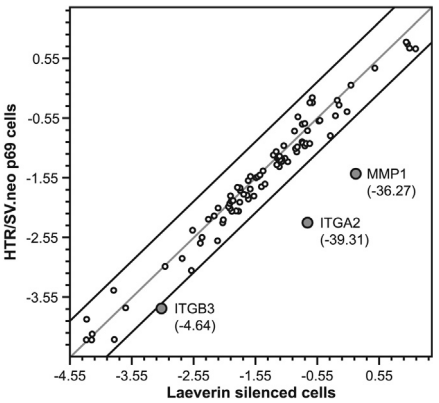
FIGURE 7
Polymerase chain reaction array



Array shows relative expression of 84 genes that were involved in cell transformation and tumorigenesis. Laeverin-silenced HTR-8/SVneo trophoblast cells (x-axis) and controls (y-axis). The log transformation plot shows relative expression (Log10 [2^- DeltaCt]) of each gene (*circles*) between laeverin-silenced cells and controls. *Black lines* indicate a 4-fold change in gene expression.

ITGA2, integrin A2 (39-fold); ITGB3, integrin B3 (5-fold); MMP1, matrix metalloprotease 1 (36-fold) are significantly down-regulated.

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cell migration and invasion in vitro. Moreover, it has been shown that endogenous laeverin on the surface of isolated extravillous trophoblastic cells acts to promote their invasion capacity.[4] We found that, in preeclampsia, laeverin is not bound to the trophoblast cell membrane, which indicates a possible deregulation of its physiologic function. Consequently, one can hypothesize that the production of a deregulated, malfunctioning protein in the preeclamptic placenta might lead to a compensatory increase of laeverin at the mRNA level.

A limitation of our study is that the preeclamptic placentas were delivered earlier compared with the control placentas. However, because term placentas express higher levels of laeverin compared with early (first-trimester) placentas,[4] one would expect to see lower levels of laeverin in preeclamptic placentas that were delivered preterm. Therefore, the observed differences in laeverin expression are likely to be real and suggest that the overexpression of laeverin in preeclampsia is associated with the disease process rather than the differences in gestational age. Another limitation of the study is the small number of placental samples that were used. However, experiments were run in triplicate, and the results were reproducible.

In summary, laeverin, a placenta-specific protein, appears to be deregulated in preeclampsia that leads to its overexpression and altered subcellular localization in the villous trophoblast. Whether it could be used potentially as a biomarker of abnormal placentation for prediction and diagnosis of preeclampsia needs further investigation. We are studying longitudinal changes in laeverin levels in maternal circulation during normal pregnancy and assessing whether first- and second-trimester serum laeverin concentration can be used to improve the prediction of preeclampsia in an unselected population. ■

REFERENCES

1. Khan KS, Wojdyla D, Say L, Gulmezoglu AM, Van Look PF. WHO analysis of causes of maternal death: a systematic review. Lancet 2006;367:1066-74.
2. Fujiwara H, Higuchi T, Yamada S, et al. Human extravillous trophoblasts express laeverin, a novel protein that belongs to membrane-bound gluzincin metallopeptidases. Biochem Biophys Res Comm 2004;313:962-8.
3. Fujiwara H. Membrane-bound peptidases regulate human extravillous trophoblast invasion. Placenta 2007;28(suppl A):S70-5.
4. Horie A, Fujiwara H, Sato Y, et al. Laeverin/aminopeptidase Q induces trophoblast invasion during human early placentation. Hum Reprod 2012;27:1267-76.
5. Haas CS, Creighton CJ, Pi X, et al. Identification of genes modulated in rheumatoid arthritis using complementary DNA microarray analysis of lymphoblastoid B cell lines from disease-discordant monozygotic twins. Arthritis Rheum 2006;54:2047-60.
6. Sitras V, Paulssen RH, Gronaas H, et al. Differential placental gene expression in severe preeclampsia. Placenta 2009;30:424-33.
7. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertens Pregnancy 2001;20:IX-XIV.
8. Graham CH, Hawley TS, Hawley RG, et al. Establishment and characterization of first trimester human trophoblast cells with extended lifespan. Exp Cell Res 1993;206:204-11.
9. Santa Cruz Biotechnology. Immunofluorescence staining protocol. Available at: https://www.scbt.com/protocol_immunofluorescence_cell_staining.html. Accessed March 3, 2014.
10. Schwarz H, Humbel BM. Influence of fixatives and embedding media on immunolabelling of freeze-substituted cells. Scanning Microsc 1989;3(suppl):57-64.
11. Griffiths GE. Fine structure immunocytochemistry. Heidelberg, Germany: Springer-Verlag; 1993.
12. Webster P, Schwarz H, Griffiths G. Preparation of cells and tissues for immuno EM. Methods Cell Biol 2008;88:45-58.
13. Zhang X, Green KE, Yallampalli C, Dong YL. Adrenomedullin enhances invasion by trophoblast cell lines. Biol Reprod 2005;73:619-26.
14. Olsen J, Cowell GM, Konigshofer E, et al. Complete amino acid sequence of human intestinal aminopeptidase N as deduced from cloned cDNA. FEBS Lett 1988;238:307-14.
15. Maruyama M, Arisaka N, Goto Y, et al. Histidine 379 of human laeverin/aminopeptidase Q, a nonconserved residue within the exopeptidase motif, defines its distinctive enzymatic properties. J Biol Chem 2009;284:34692-702.
16. Holder BS, Tower CL, Jones CJ, Aplin JD, Abrahams VM. Heightened pro-inflammatory effect of preeclamptic placental microvesicles

on peripheral blood immune cells in humans. Biol Reprod 2012;86:103.
17. Tannetta DS, Dragovic RA, Gardiner C, Redman CW, Sargent IL. Characterisation of syncytiotrophoblast vesicles in normal pregnancy and pre-eclampsia: expression of Flt-1 and endoglin. PloS One 2013;8:e56754.
18. Redman CW, Sargent IL. Circulating microparticles in normal pregnancy and pre-eclampsia. Placenta 2008;29(suppl A):S73-7.
19. Cocucci E, Racchetti G, Meldolesi J. Shedding microvesicles: artefacts no more. Trends Cell Biol 2009;19:43-51.
20. Goswami D, Tannetta DS, Magee LA, et al. Excess syncytiotrophoblast microparticle shedding is a feature of early-onset pre-eclampsia, but not normotensive intrauterine growth restriction. Placenta 2006;27:56-61.
21. Sollner T, Whiteheart SW, Brunner M, et al. SNAP receptors implicated in vesicle targeting and fusion. Nature 1993;362:318-24.
22. Maruyama M, Hattori A, Goto Y, et al. Laeverin/aminopeptidase Q, a novel bestatin-sensitive leucine aminopeptidase belonging to the M1 family of aminopeptidases. J Biol Chem 2007;282:20088-96.
23. Rawlings ND, Waller M, Barrett AJ, Bateman A. MEROPS: the database of proteolytic enzymes, their substrates and inhibitors. Nucleic Acids Res 2014;42:D503-9.
24. Huppertz B, Kertschanska S, Demir AY, Frank HG, Kaufmann P. Immunohistochemistry of matrix metalloproteinases (MMP), their substrates, and their inhibitors (TIMP) during trophoblast invasion in the human placenta. Cell Tissue Res 1998;291:133-48.
25. Giancotti FG, Ruoslahti E. Integrin signaling. Science 1999;285:1028-32.
26. Vu TH, Werb Z. Matrix metalloproteinases: effectors of development and normal physiology. Genes Dev 2000;14:2123-33.
27. Khandelwal S, Saxena RK. Age-dependent increase in green autofluorescence of blood erythrocytes. J Biosci 2007;32:1139-45.

SUPPLEMENTAL TABLE 1

Polymerase chain reaction array

PCR array catalog #:		PAHS-033				
Position	Unigene	Refseq	Symbol	Description	Gname	RT2 Catalog
A01	Hs.525622	NM_005163	AKT1	V-akt murine thymoma viral oncogene homolog 1	AKT/PKB	PPH00088A
A02	Hs.369675	NM_001146	ANGPT1	Angiopoietin 1	AGP1/AGPT	PPH00374A
A03	Hs.583870	NM_001147	ANGPT2	Angiopoietin 2	AGPT2/ANG2	PPH00377E
A04	Hs.552567	NM_001160	APAF1	Apoptotic peptidase activating factor 1	APAF-1/CED4	PPH00752A
A05	Hs.367437	NM_000051	ATM	Ataxia telangiectasia mutated	AT1/ATA	PPH00325B
A06	Hs.370254	NM_004322	BAD	BCL2-associated agonist of cell death	BBC2/BCL2L8	PPH00075B
A07	Hs.624291	NM_004324	BAX	BCL2-associated X protein	BCL2L4	PPH00078B
A08	Hs.150749	NM_000633	BCL2	B-cell CLL/lymphoma 2	Bcl-2	PPH00079B
A09	Hs.516966	NM_138578	BCL2L1	BCL2-like 1	BCL-XL/S	PPH00082B
A10	Hs.194143	NM_007294	BRCA1	Breast cancer 1, early onset	BRCAI/BRCC1	PPH00322E
A11	Hs.599762	NM_001228	CASP8	Caspase 8, apoptosis-related cysteine peptidase	ALPS2B/CAP4	PPH00359E
A12	Hs.244723	NM_001238	CCNE1	Cyclin E1	CCNE	PPH00131A
B01	Hs.437705	NM_001789	CDC25A	Cell division cycle 25 homolog A (S. pombe)	CDC25A2	PPH00930A
B02	Hs.19192	NM_001798	CDK2	Cyclin-dependent kinase 2	p33(CDK2)	PPH00117E
B03	Hs.95577	NM_000075	CDK4	Cyclin-dependent kinase 4	CMM3/PSK-J3	PPH00118E
B04	Hs.370771	NM_000389	CDKN1A	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	CAP20/CDKN1	PPH00211E
B05	Hs.512599	NM_000077	CDKN2A	Cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	ARF/CDK4I	PPH00207B
B06	Hs.390736	NM_003879	CFLAR	CASP8 and FADD-like apoptosis regulator	CASH/CASP8AP1	PPH00333A
B07	Hs.291363	NM_007194	CHEK2	CHK2 checkpoint homolog (S. pombe)	CDS1/CHK2	PPH00921B
B08	Hs.517356	NM_030582	COL18A1	Collagen, type XVIII, alpha 1	KNO/KNO1	PPH01141E
B09	Hs.654393	NM_005225	E2F1	E2F transcription factor 1	E2F-1/RBAP1	PPH00136F
B10	Hs.446352	NM_004448	ERBB2	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	CD340/HER-2	PPH00209B

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(continued)

SUPPLEMENTAL TABLE 1

Polymerase chain reaction array *(continued)*

PCR array catalog #:		PAHS-033				
Position	**Unigene**	**Refseq**	**Symbol**	**Description**	**Gname**	**RT2 Catalog**
B11	Hs.644231	NM_005239	ETS2	V-Ets erythroblastosis virus E26 oncogene homolog 2 (avian)	ETS2IT1	PPH00091B
B12	Hs.244139	NM_000043	FAS	Fas (TNF receptor superfamily, member 6)	ALPS1A/APO-1	PPH00141B
C01	Hs.533683	NM_000141	FGFR2	Fibroblast growth factor receptor 2	BEK/BFR-1	PPH00391E
C02	Hs.25647	NM_005252	FOS	V-fos FBJ murine osteosarcoma viral oncogene homolog	AP-1/C-FOS	PPH00094A
C03	Hs.90708	NM_006144	GZMA	Granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)	CTLA3/HFSP	PPH00314E
C04	Hs.90753	NM_006410	HTATIP2	HIV-1 Tat interactive protein 2, 30kDa	CC3/SDR44U1	PPH06957A
C05	Hs.37026	NM_024013	IFNA1	Interferon, alpha 1	IFL/IFN	PPH01321A
C06	Hs.93177	NM_002176	IFNB1	Interferon, beta 1, fibroblast	IFB/IFF	PPH00384E
C07	Hs.160562	NM_000618	IGF1	Insulin-like growth factor 1 (somatomedin C)	IGF1A/IGFI	PPH00167B
C08	Hs.624	NM_000584	IL8	Interleukin 8	CXCL8/GCP-1	PPH00568A
C09	Hs.644352	NM_181501	ITGA1	Integrin, alpha 1	CD49a/VLA1	PPH00627B
C10	Hs.482077	NM_002203	ITGA2	Integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)	BR/CD49B	PPH00625E
C11	Hs.265829	NM_002204	ITGA3	Integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)	CD49C/GAP-B3	PPH00175A
C12	Hs.694732	NM_000885	ITGA4	Integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)	CD49D/IA4	PPH00659E
D01	Hs.436873	NM_002210	ITGAV	Integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)	CD51/DKFZp686A08142	PPH00628B
D02	Hs.643813	NM_002211	ITGB1	Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)	CD29/FNRB	PPH00650B
D03	Hs.218040	NM_000212	ITGB3	Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	CD61/GP3A	PPH00178C
D04	Hs.536663	NM_002213	ITGB5	Integrin, beta 5	FLJ26658	PPH00634E
D05	Hs.714791	NM_002228	JUN	Jun oncogene	AP-1/AP1	PPH00095A
D06	Hs.145442	NM_002755	MAP2K1	Mitogen-activated protein kinase kinase 1	MAPKK1/MEK1	PPH00711B
D07	Hs.599039	NM_006500	MCAM	Melanoma cell adhesion molecule	CD146/MUC18	PPH00651A

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SUPPLEMENTAL TABLE 1

Polymerase chain reaction array *(continued)*

PCR array catalog #:		PAHS-033				
Position	**Unigene**	**Refseq**	**Symbol**	**Description**	**Gname**	**RT2 Catalog**
D08	Hs.484551	NM_002392	MDM2	Mdm2 p53 binding protein homolog (mouse)	HDMX/hdm2	PPH00193E
D09	Hs.132966	NM_000245	MET	Met proto-oncogene (hepatocyte growth factor receptor)	AUTS9/HGFR	PPH00194A
D10	Hs.83169	NM_002421	MMP1	Matrix metallopeptidase 1 (interstitial collagenase)	CLG/CLGN	PPH00120B
D11	Hs.513617	NM_004530	MMP2	Matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase)	CLG4/CLG4A	PPH00151B
D12	Hs.297413	NM_004994	MMP9	Matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	CLG4B/GELB	PPH00152E
E01	Hs.525629	NM_004689	MTA1	Metastasis associated 1	Mta-1	PPH01083E
E02	Hs.173043	NM_004739	MTA2	Metastasis associated 1 family, member 2	DKFZp686F2281/MTA1L1	PPH13564A
E03	Hs.700429	NM_014751	MTSS1	Metastasis suppressor 1	DKFZp781P2223/MIM	PPH10073A
E04	Hs.202453	NM_002467	MYC	V-myc myelocytomatosis viral oncogene homolog (avian)	MRTL/bHLHe39	PPH00100A
E05	Hs.654408	NM_003998	NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	DKFZp686C01211/EBP-1	PPH00204E
E06	Hs.81328	NM_020529	NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	IKBA/MAD-3	PPH00170E
E07	Hs.118638	NM_000269	NME1	Non-metastatic cells 1, protein (NM23A) expressed in	AWD/GAAD	PPH01314A
E08	Hs.9235	NM_005009	NME4	Non-metastatic cells 4, protein expressed in	NDPK-D/NM23H4	PPH01086A
E09	Hs.535898	NM_002607	PDGFA	Platelet-derived growth factor alpha polypeptide	PDGF-A/PDGF1	PPH00217B
E10	Hs.1976	NM_002608	PDGFB	Platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog)	PDGF2/SIS	PPH00488E
E11	Hs.132225	NM_181504	PIK3R1	Phosphoinositide-3-kinase, regulatory subunit 1 (alpha)	GRB1/p85	PPH00713E
E12	Hs.77274	NM_002658	PLAU	Plasminogen activator, urokinase	ATF/UPA	PPH00796B
F01	Hs.466871	NM_002659	PLAUR	Plasminogen activator, urokinase receptor	CD87/UPAR	PPH00797B
F02	Hs.409965	NM_002687	PNN	Pinin, desmosome associated protein	DRS/SDK3	PPH19485E
F03	Hs.159130	NM_002880	RAF1	V-raf-1 murine leukemia viral oncogene homolog 1	CRAF/NS5	PPH00227E
F04	Hs.408528	NM_000321	RB1	Retinoblastoma 1	OSRC/RB	PPH00228E

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SUPPLEMENTAL TABLE 1

Polymerase chain reaction array *(continued)*

PCR array catalog #:		PAHS-033				
Position	**Unigene**	**Refseq**	**Symbol**	**Description**	**Gname**	**RT2 Catalog**
F05	Hs.654444	NM_002961	S100A4	S100 calcium binding protein A4	18A2/42A	PPH01313E
F06	Hs.55279	NM_002639	SERPINB5	Serpin peptidase inhibitor, clade B (ovalbumin), member 5	PI5/maspin	PPH00695E
F07	Hs.414795	NM_000602	SERPINE1	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	PAI/PAI-1	PPH00215E
F08	Hs.349470	NM_003087	SNCG	Synuclein, gamma (breast cancer-specific protein 1)	BCSG1/SR	PPH01051E
F09	Hs.371720	NM_003177	SYK	Spleen tyrosine kinase	DKFZp313N1010	PPH01639E
F10	Hs.89640	NM_000459	TEK	TEK tyrosine kinase, endothelial	CD202B/TIE-2	PPH00795B
F11	Hs.492203	NM_198253	TERT	Telomerase reverse transcriptase	EST2/TCS1	PPH00995E
F12	Hs.645227	NM_000660	TGFB1	Transforming growth factor, beta 1	CED/DPD1	PPH00508A
G01	Hs.494622	NM_004612	TGFBR1	Transforming growth factor, beta receptor 1	AAT5/ACVRLK4	PPH00237B
G02	Hs.164226	NM_003246	THBS1	Thrombospondin 1	THBS/THBS-1	PPH00799E
G03	Hs.522632	NM_003254	TIMP1	TIMP metallopeptidase inhibitor 1	CLGI/EPA	PPH00771B
G04	Hs.644633	NM_000362	TIMP3	TIMP metallopeptidase inhibitor 3	HSMRK222/K222	PPH00762A
G05	Hs.241570	NM_000594	TNF	Tumor necrosis factor (TNF superfamily, member 2)	DIF/TNF-alpha	PPH00341E
G06	Hs.521456	NM_003842	TNFRSF10B	Tumor necrosis factor receptor superfamily, member 10b	CD262/DR5	PPH00241B
G07	Hs.279594	NM_001065	TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1A	CD120a/FPF	PPH00346B
G08	Hs.462529	NM_003790	TNFRSF25	Tumor necrosis factor receptor superfamily, member 25	APO-3/DDR3	PPH00349A
G09	Hs.654481	NM_000546	TP53	Tumor protein p53	LFS1/TRP53	PPH00213E
G10	Hs.66744	NM_000474	TWIST1	Twist homolog 1 (Drosophila)	ACS3/BPES2	PPH02132A
G11	Hs.563491	NM_017549	EPDR1	Ependymin related protein 1 (zebrafish)	EPDR/MERP-1	PPH09305E
G12	Hs.73793	NM_003376	VEGFA	Vascular endothelial growth factor A	MVCD1/VEGF	PPH00251B
H01	Hs.534255	NM_004048	B2M	Beta-2-microglobulin	B2M	PPH01094E
H02	Hs.412707	NM_000194	HPRT1	Hypoxanthine phosphoribosyltransferase 1	HGPRT/HPRT	PPH01018B
H03	Hs.523185	NM_012423	RPL13A	Ribosomal protein L13a	RPL13A	PPH01020B

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SUPPLEMENTAL TABLE 1

Polymerase chain reaction array *(continued)*

PCR array catalog #:		PAHS-033				
Position	**Unigene**	**Refseq**	**Symbol**	**Description**	**Gname**	**RT2 Catalog**
H04	Hs.592355	NM_002046	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	G3PD/GAPD	PPH00150E
H05	Hs.520640	NM_001101	ACTB	Actin, beta	PS1TP5BP1	PPH00073E
H06	N/A	SA_00105	HGDC	Human Genomic DNA Contamination	HIGX1A	
H07	N/A	SA_00104	RTC	Reverse Transcription Control	RTC	
H08	N/A	SA_00104	RTC	Reverse Transcription Control	RTC	
H09	N/A	SA_00104	RTC	Reverse Transcription Control	RTC	
H10	N/A	SA_00103	PPC	Positive PCR Control	PPC	
H11	N/A	SA_00103	PPC	Positive PCR Control	PPC	
H12	N/A	SA_00103	PPC	Positive PCR Control	PPC	

List of genes, fold-changes, and probability values.

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SUPPLEMENTAL TABLE 2

Average Ct

Well	Symbol	Laeverin knock-out cells	Control Group
A01	AKT1	24.53	24.8
A02	ANGPT1	33.86	35
A03	ANGPT2	30.9	30.78
A04	APAF1	25.93	25.84
A05	ATM	27.28	27.22
A06	BAD	24.92	24.5
A07	BAX	22.96	23.25
A08	BCL2	27.14	26.3
A09	BCL2L1	25.08	24.18
A10	BRCA1	25.24	24.77
A11	CASP8	31.14	29.34
A12	CCNE1	27.83	27.19
B01	CDC25A	26.95	26.37
B02	CDK2	23.33	23.12
B03	CDK4	22.3	20.98
B04	CDKN1A	22.79	22.43
B05	CDKN2A	22.82	22.51
B06	CFLAR	26.31	25.42
B07	CHEK2	26.46	25.57
B08	COL18A1	25.83	25.65
B09	E2F1	28.89	29.29
B10	ERBB2	26.51	26.87
B11	ETS2	25.96	25.88
B12	FAS	27.4	27.3
C01	FGFR2	33.2	32.89
C02	FOS	27.57	27.37
C03	GZMA	35	35
C04	HTATIP2	30.45	29.85
C05	IFNA1	32.23	33.53
C06	IFNB1	35	35
C07	IGF1	34.66	34.7
C08	IL8	26.55	26.74
C09	ITGA1	24.87	24.76
C10	ITGA2	28.45	23.11
C11	ITGA3	22.59	23.62
C12	ITGA4	26.88	26.54
D01	ITGAV	24.25	23.26
D02	ITGB1	22.52	21.6
D03	ITGB3	33.25	30.98

Nystad. Laeverin expression is altered in preeclampsia. Am J Obstet Gynecol 2014. *(continued)*

SUPPLEMENTAL TABLE 2

Average Ct *(continued)*

Well	Symbol	Laeverin knock-out cells	Control Group
D04	ITGB5	25.33	24.63
D05	JUN	27.23	27.12
D06	MAP2K1	26.99	25.97
D07	MCAM	24.76	24.73
D08	MDM2	23.36	23.81
D09	MET	24.55	23.69
D10	MMP1	25.75	20.52
D11	MMP2	27.63	27.9
D12	MMP9	28.31	27.64
E01	MTA1	24.19	24.39
E02	MTA2	25.9	26.16
E03	MTSS1	27.33	26.74
E04	MYC	25.6	25.5
E05	NFKB1	25.09	24.52
E06	NFKBIA	24.35	23.75
E07	NME1	21.67	21.52
E08	NME4	23.63	21.87
E09	PDGFA	26.11	26.3
E10	PDGFB	28.1	28.14
E11	PIK3R1	28.48	27.67
E12	PLAU	24.08	22.99
F01	PLAUR	24.78	24.64
F02	PNN	24.07	23.13
F03	RAF1	24.27	23.66
F04	RB1	27.83	26.8
F05	S100A4	27.79	26.94
F06	SERPINB5	35	35
F07	SERPINE1	26.99	27.26
F08	SNCG	26.71	26.72
F09	SYK	35	35
F10	TEK	29.48	27.94
F11	TERT	35	35
F12	TGFB1	23.99	23.41
G01	TGFBR1	29.31	28.76
G02	THBS1	24.89	24.32
G03	TIMP1	21.92	21.42
G04	TIMP3	35	35
G05	TNF	34.96	33.49
G06	TNFRSF10B	24.72	24.95

Nystad. Laeverin expression is altered in preeclampsia. Am J Obstet Gynecol 2014. *(continued)*

SUPPLEMENTAL TABLE 2

Average Ct *(continued)*

Well	Symbol	Laeverin knock-out cells	Control Group
G07	TNFRSF1A	28.29	28.44
G08	TNFRSF25	29.61	28.85
G09	TP53	22.98	23.37
G10	TWIST1	26.6	26.21
G11	EPDR1	27.69	27.27
G12	VEGFA	25	24.61
H01	B2M	20.83	20.77
H02	HPRT1	24.04	23.39
H03	RPL13A	29.54	19.57
H04	GAPDH	18.79	17.31
H05	ACTB	19.88	19.5
H06	HGDC	35	34.71
H07	RTC	21.78	22.99
H08	RTC	21.5	22.86
H09	RTC	21.78	22.87
H10	PPC	18.42	17.82
H11	PPC	18.52	17.73
H12	PPC	18.76	17.62

Nystad. Laeverin expression is altered in preeclampsia. Am J Obstet Gynecol 2014.

SUPPLEMENTAL TABLE 3

2^(-Avg.(Delta(Ct))

Well	Symbol	Laeverin knock-out	Control Group
A01	AKT1	0.076919	0.061462
A02	ANGPT1	0.000119	0.000052
A03	ANGPT2	0.000926	0.000972
A04	APAF1	0.029033	0.029787
A05	ATM	0.011382	0.011486
A06	BAD	0.058744	0.075732
A07	BAX	0.227406	0.18004
A08	BCL2	0.012602	0.021693
A09	BCL2L1	0.052235	0.094312
A10	BRCA1	0.047061	0.062477
A11	CASP8	0.000786	0.00263
A12	CCNE1	0.007796	0.011693
B01	CDC25A	0.01429	0.020707
B02	CDK2	0.175929	0.196621
B03	CDK4	0.359433	0.864344
B04	CDKN1A	0.256466	0.316172
B05	CDKN2A	0.250776	0.30093
B06	CFLAR	0.02228	0.03996
B07	CHEK2	0.02011	0.03586
B08	COL18A1	0.031267	0.033989
B09	E2F1	0.003724	0.002722
B10	ERBB2	0.019492	0.014569
B11	ETS2	0.02843	0.028947
B12	FAS	0.010507	0.010838
C01	FGFR2	0.000188	0.000225
C02	FOS	0.009327	0.010315
C03	GZMA	0.000054	0.000052
C04	HTATIP2	0.001266	0.001847
C05	IFNA1	0.000368	0.000145
C06	IFNB1	0.000054	0.000052
C07	IGF1	0.000068	0.000064
C08	IL8	0.018861	0.015945
C09	ITGA1	0.060808	0.063202
C10	ITGA2	0.005052	0.198592
C11	ITGA3	0.293734	0.139061
C12	ITGA4	0.015075	0.018329
D01	ITGAV	0.093453	0.177765
D02	ITGB1	0.30892	0.564292
D03	ITGB3	0.000183	0.000848
D04	ITGB5	0.044108	0.068928

Nystad. Laeverin expression is altered in preeclampsia. Am J Obstet Gynecol 2014. *(continued)*

SUPPLEMENTAL TABLE 3

2^(-Avg.(Delta(Ct)) *(continued)*

Well	Symbol	Laeverin knock-out	Control Group
D05	JUN	0.011773	0.01228
D06	MAP2K1	0.013949	0.0272
D07	MCAM	0.065449	0.064392
D08	MDM2	0.172121	0.121974
D09	MET	0.075415	0.132754
D10	MMP1	0.032851	1.191526
D11	MMP2	0.008935	0.007137
D12	MMP9	0.005577	0.008571
E01	MTA1	0.096905	0.081612
E02	MTA2	0.029606	0.023832
E03	MTSS1	0.011011	0.015931
E04	MYC	0.036459	0.037639
E05	NFKB1	0.052116	0.07473
E06	NFKBIA	0.08665	0.126951
E07	NME1	0.555393	0.594031
E08	NME4	0.143637	0.468422
E09	PDGFA	0.025604	0.021651
E10	PDGFB	0.006461	0.006059
E11	PIK3R1	0.004972	0.008382
E12	PLAU	0.10458	0.214459
F01	PLAUR	0.064538	0.06844
F02	PNN	0.105633	0.194843
F03	RAF1	0.091882	0.134911
F04	RB1	0.007782	0.015336
F05	S100A4	0.007992	0.013934
F06	SERPINB5	0.000054	0.000052
F07	SERPINE1	0.013942	0.011173
F08	SNCG	0.016954	0.016164
F09	SYK	0.000054	0.000052
F10	TEK	0.002485	0.006958
F11	TERT	0.000054	0.000052
F12	TGFB1	0.111931	0.160761
G01	TGFBR1	0.002802	0.003939
G02	THBS1	0.059905	0.085591
G03	TIMP1	0.469703	0.636935
G04	TIMP3	0.000054	0.000052
G05	TNF	0.000056	0.000148
G06	TNFRSF10B	0.067447	0.055121
G07	TNFRSF1A	0.005666	0.004922

Nystad. Laeverin expression is altered in preeclampsia. Am J Obstet Gynecol 2014. *(continued)*

SUPPLEMENTAL TABLE 3

2^(-Avg.(Delta(Ct)) *(continued)*

Well	Symbol	Laeverin knock-out	Control Group
G08	TNFRSF25	0.002268	0.003694
G09	TP53	0.224556	0.1647
G10	TWIST1	0.018237	0.023076
G11	EPDR1	0.008613	0.011097
G12	VEGFA	0.055564	0.069821
H01	B2M	1	1
H02	HPRT1	0.108052	0.163553
H03	RPL13A	0.002381	2.30887
H04	GAPDH	4.096049	11.029787
H05	ACTB	1.932241	2.420406
H06	HGDC	0.000054	0.000064
H07	RTC	0.514533	0.215528
H08	RTC	0.6251	0.235065
H09	RTC	0.514609	0.234047
H10	PPC	5.314673	7.769474
H11	PPC	4.95624	8.213203
H12	PPC	4.183476	8.865592

Nystad. Laeverin expression is altered in preeclampsia. Am J Obstet Gynecol 2014.

SUPPLEMENTAL TABLE 4

Fold reg

Well	Symbol	Laeverin knock-out cells
A01	AKT1	1.2515
A02	ANGPT1	2.2862
A03	ANGPT2	-1.049
A04	APAF1	-1.026
A05	ATM	-1.0091
A06	BAD	-1.2892
A07	BAX	1.2631
A08	BCL2	-1.7215
A09	BCL2L1	-1.8055
A10	BRCA1	-1.3276
A11	CASP8	-3.3446
A12	CCNE1	-1.4999
B01	CDC25A	-1.449
B02	CDK2	-1.1176
B03	CDK4	-2.4047
B04	CDKN1A	-1.2328
B05	CDKN2A	-1.2
B06	CFLAR	-1.7935
B07	CHEK2	-1.7832
B08	COL18A1	-1.087
B09	E2F1	1.3682
B10	ERBB2	1.3379
B11	ETS2	-1.0182
B12	FAS	-1.0315
C01	FGFR2	-1.1974
C02	FOS	-1.1059
C03	GZMA	1.0375
C04	HTATIP2	-1.4591
C05	IFNA1	2.5468
C06	IFNB1	1.0375
C07	IGF1	1.0659
C08	IL8	1.1829
C09	ITGA1	-1.0394
C10	ITGA2	-39.309
C11	ITGA3	2.1123
C12	ITGA4	-1.2159
D01	ITGAV	-1.9022
D02	ITGB1	-1.8267
D03	ITGB3	-4.6435
D04	ITGB5	-1.5627

Nystad. Laeverin expression is altered in preeclampsia. Am J Obstet Gynecol 2014. *(continued)*

SUPPLEMENTAL TABLE 4

Fold reg *(continued)*

Well	Symbol	Laeverin knock-out cells
D05	JUN	-1.0431
D06	MAP2K1	-1.95
D07	MCAM	1.0164
D08	MDM2	1.4111
D09	MET	-1.7603
D10	MMP1	-36.2703
D11	MMP2	1.252
D12	MMP9	-1.5369
E01	MTA1	1.1874
E02	MTA2	1.2423
E03	MTSS1	-1.4469
E04	MYC	-1.0324
E05	NFKB1	-1.4339
E06	NFKBIA	-1.4651
E07	NME1	-1.0696
E08	NME4	-3.2611
E09	PDGFA	1.1826
E10	PDGFB	1.0664
E11	PIK3R1	-1.6861
E12	PLAU	-2.0507
F01	PLAUR	-1.0605
F02	PNN	-1.8445
F03	RAF1	-1.4683
F04	RB1	-1.9707
F05	S100A4	-1.7435
F06	SERPINB5	1.0375
F07	SERPINE1	1.2478
F08	SNCG	1.0488
F09	SYK	1.0375
F10	TEK	-2.7997
F11	TERT	1.0375
F12	TGFB1	-1.4362
G01	TGFBR1	-1.4057
G02	THBS1	-1.4288
G03	TIMP1	-1.356
G04	TIMP3	1.0375
G05	TNF	-2.6713
G06	TNFRSF10B	1.2236
G07	TNFRSF1A	1.1513

Nystad. Laeverin expression is altered in preeclampsia. Am J Obstet Gynecol 2014. *(continued)*

SUPPLEMENTAL TABLE 4

Fold reg *(continued)*

Well	Symbol	Laeverin knock-out cells
G08	TNFRSF25	-1.6286
G09	TP53	1.3634
G10	TWIST1	-1.2653
G11	EPDR1	-1.2884
G12	VEGFA	-1.2566
H01	B2M	1
H02	HPRT1	-1.5136
H03	RPL13A	-969.6478
H04	GAPDH	-2.6928
H05	ACTB	-1.2526
H06	HGDC	-1.1759
H07	RTC	2.3873
H08	RTC	2.6593
H09	RTC	2.1987
H10	PPC	-1.4619
H11	PPC	-1.6571
H12	PPC	-2.1192

Nystad. Laeverin expression is altered in preeclampsia. Am J Obstet Gynecol 2014.

SUPPLEMENTAL TABLE 5

xCelligence system raw data

Time(Hour)	Time(hh:mm:ss)	Y (A1, A2)	Y (B1, B2)	Y (C1, C2, D1, D2)	Y (E1, E2, F1, F2)	Y (G1, G2, H1, H2)
0	0:00:00	0	0	0	0	0
0.0008	0:00:03	−0.0047	−0.0001	−0.0009	−0.0002	−0.0016
0.0925	0:05:33	−0.0968	−0.0408	−0.0741	−0.0709	−0.0644
0.5928	0:35:34	−0.0632	−0.0533	−0.0985	−0.0867	−0.0902
1.0931	1:05:35	−0.0003	0.0011	−0.0822	−0.0745	−0.0856
1.5933	1:35:36	0.0229	0.0326	−0.0665	−0.0593	−0.0751
2.0936	2:05:37	0.0358	0.0641	−0.0406	−0.0267	−0.0572
2.5939	2:35:38	0.0488	0.1036	−0.0063	0.0154	−0.0335
3.0942	3:05:39	0.0621	0.1508	0.0449	0.0782	0.0003
3.5944	3:35:40	0.0742	0.2098	0.1198	0.1684	0.0508
4.0947	4:05:41	0.0854	0.2712	0.2175	0.2943	0.1205
4.595	4:35:42	0.0925	0.3464	0.3536	0.4638	0.218
5.0953	5:05:43	0.1014	0.4335	0.5408	0.6821	0.3508
5.5956	5:35:44	0.1071	0.5313	0.7798	0.9505	0.5298
6.0958	6:05:45	0.1118	0.6435	1.0827	1.2712	0.7623
6.5961	6:35:46	0.1182	0.7645	1.4401	1.6409	1.053
7.0964	7:05:47	0.1257	0.8896	1.8572	2.0287	1.3971
7.5967	7:35:48	0.1356	1.0278	2.2893	2.4357	1.7809
8.0969	8:05:49	0.1455	1.1631	2.6848	2.789	2.1615
8.5972	8:35:50	0.155	1.3113	3.0502	3.132	2.5145
9.0975	9:05:51	0.1663	1.481	3.3768	3.4245	2.8595
9.5978	9:35:52	0.1737	1.6514	3.6743	3.6901	3.1521
10.0981	10:05:53	0.187	1.8429	3.9328	3.9223	3.4114
10.5983	10:35:54	0.2016	2.0338	4.1582	4.1094	3.6424
11.0986	11:05:55	0.2145	2.2244	4.3551	4.2898	3.8433
11.5989	11:35:56	0.2282	2.419	4.5305	4.45	4.0151
12.0992	12:05:57	0.2413	2.6097	4.6891	4.5994	4.1577
12.5994	12:35:58	0.2555	2.8009	4.8305	4.7393	4.303
13.0997	13:05:59	0.2704	2.9947	4.959	4.8751	4.4422
13.6	13:36:00	0.2905	3.2008	5.0775	4.9993	4.563
14.1003	14:06:01	0.3065	3.3772	5.2053	5.1221	4.6635
14.6006	14:36:02	0.3245	3.5445	5.3165	5.2256	4.7803
15.1008	15:06:03	0.3437	3.7175	5.418	5.3266	4.8815
15.6011	15:36:04	0.3619	3.8844	5.5248	5.409	4.9794
16.1014	16:06:05	0.3817	4.0343	5.6163	5.4913	5.0736
16.6017	16:36:06	0.4037	4.1906	5.6904	5.565	5.155
17.1019	17:06:07	0.4239	4.3228	5.7738	5.6322	5.2267
17.6022	17:36:08	0.4443	4.4642	5.8388	5.6909	5.2871
18.1025	18:06:09	0.4673	4.5847	5.9071	5.746	5.3413
18.6028	18:36:10	0.4922	4.6945	5.9651	5.7948	5.4025

Nystad. Laeverin expression is altered in preeclampsia. Am J Obstet Gynecol 2014.

(continued)

SUPPLEMENTAL TABLE 5

xCelligence system raw data *(continued)*

Time(Hour)	Time(hh:mm:ss)	Y (A1, A2)	Y (B1, B2)	Y (C1, C2, D1, D2)	Y (E1, E2, F1, F2)	Y (G1, G2, H1, H2)
19.1031	19:06:11	0.5191	4.8119	6.0249	5.8335	5.4514
19.6033	19:36:12	0.5441	4.9203	6.071	5.8832	5.4972
20.1036	20:06:13	0.5741	5.0361	6.1277	5.9162	5.5436
20.6039	20:36:14	0.6017	5.129	6.1866	5.972	5.591
21.1042	21:06:15	0.6316	5.2373	6.231	6.019	5.6184
21.6044	21:36:16	0.6658	5.3247	6.2744	6.0643	5.6461
22.1047	22:06:17	0.698	5.4261	6.307	6.119	5.6796
22.605	22:36:18	0.7282	5.526	6.3418	6.169	5.7148
23.1053	23:06:19	0.7631	5.6065	6.3716	6.205	5.7483
23.6056	23:36:20	0.8035	5.6865	6.3967	6.2437	5.7738
24.1058	24:06:21	0.8362	5.7534	6.4261	6.2765	5.7958
24.6061	24:36:22	0.8744	5.8051	6.4534	6.3047	5.8182
25.1064	25:06:23	0.9118	5.8948	6.474	6.3392	5.8413
25.6067	25:36:24	0.9427	5.9758	6.5039	6.3666	5.8488
26.1069	26:06:25	0.9814	6.0302	6.5265	6.3881	5.864
26.6072	26:36:26	1.0202	6.0725	6.5471	6.4054	5.8734
27.1075	27:06:27	1.0522	6.122	6.5701	6.4149	5.8828
27.6078	27:36:28	1.0854	6.1717	6.5835	6.4385	5.8932
28.1081	28:06:29	1.122	6.2137	6.6058	6.459	5.8973
28.6083	28:36:30	1.1546	6.247	6.6188	6.4747	5.9117
29.1086	29:06:31	1.19	6.2982	6.635	6.4864	5.9192
29.6089	29:36:32	1.2243	6.3303	6.6439	6.4946	5.9238
30.1092	30:06:33	1.2597	6.3793	6.6554	6.5056	5.9197
30.6094	30:36:34	1.2875	6.4163	6.6701	6.5009	5.9221
31.1097	31:06:35	1.3253	6.4498	6.6826	6.5079	5.9323
31.61	31:36:36	1.3608	6.4648	6.6843	6.5102	5.9342
32.1103	32:06:37	1.3986	6.4917	6.697	6.516	5.9292
32.6106	32:36:38	1.4269	6.5349	6.6942	6.5241	5.93
33.1108	33:06:39	1.4555	6.5723	6.7066	6.5165	5.9251
33.6111	33:36:40	1.4924	6.5889	6.7183	6.5263	5.9176
34.1114	34:06:41	1.5321	6.6335	6.7149	6.5263	5.9084
34.6117	34:36:42	1.5686	6.6782	6.7141	6.5113	5.8962
35.1119	35:06:43	1.6077	6.6924	6.7133	6.5036	5.8829
35.6122	35:36:44	1.6482	6.7149	6.7148	6.5019	5.8753
36.1125	36:06:45	1.6893	6.7339	6.7122	6.5014	5.8722
36.6128	36:36:46	1.7353	6.7584	6.7128	6.4903	5.8622
37.1131	37:06:47	1.7624	6.7844	6.7093	6.4819	5.853
37.6133	37:36:48	1.8052	6.8198	6.7107	6.4792	5.84
38.1136	38:06:49	1.8507	6.8441	6.7047	6.4759	5.8199
38.6139	38:36:50	1.8907	6.8657	6.6974	6.463	5.7958

Nystad. Laeverin expression is altered in preeclampsia. Am J Obstet Gynecol 2014. *(continued)*

SUPPLEMENTAL TABLE 5

xCelligence system raw data *(continued)*

Time(Hour)	Time(hh:mm:ss)	Y (A1, A2)	Y (B1, B2)	Y (C1, C2, D1, D2)	Y (E1, E2, F1, F2)	Y (G1, G2, H1, H2)
39.1142	39:06:51	1.9291	6.8985	6.6939	6.46	5.7799
39.6144	39:36:52	1.9829	6.9259	6.6944	6.4404	5.7674
40.1147	40:06:53	2.0269	6.932	6.6831	6.4321	5.7479
40.615	40:36:54	2.0607	6.941	6.6826	6.4148	5.7292
41.1153	41:06:55	2.1041	6.9695	6.6763	6.4125	5.7171
41.6156	41:36:56	2.1504	6.9865	6.6716	6.4006	5.7059
42.1158	42:06:57	2.1896	7.0079	6.6698	6.3834	5.6772
42.6161	42:36:58	2.23	7.0234	6.6685	6.3684	5.6567
43.1164	43:06:59	2.277	7.025	6.6682	6.3588	5.6258
43.6167	43:37:00	2.3197	7.0423	6.6606	6.3439	5.5958
44.1169	44:07:01	2.3531	7.0598	6.6475	6.3278	5.5777
44.6172	44:37:02	2.398	7.0704	6.6392	6.314	5.5485
45.1175	45:07:03	2.4473	7.0841	6.6241	6.301	5.5228
45.6178	45:37:04	2.4698	7.0864	6.61	6.2925	5.4923
46.1181	46:07:05	2.512	7.0963	6.6064	6.2763	5.4694
46.6183	46:37:06	2.5463	7.076	6.5973	6.2606	5.4436
47.1186	47:07:07	2.5907	7.0788	6.588	6.2514	5.4205
47.6189	47:37:08	2.6383	7.102	6.5768	6.2374	5.3884
48.1192	48:07:09	2.6791	7.1128	6.5725	6.2106	5.3607
48.6194	48:37:10	2.6993	7.1134	6.5651	6.1882	5.3325
49.1197	49:07:11	2.7273	7.1122	6.5551	6.1716	5.3076
49.62	49:37:12	2.759	7.1178	6.5401	6.1663	5.2802
50.1203	50:07:13	2.7952	7.1253	6.5223	6.1479	5.2499
50.6206	50:37:14	2.8343	7.1239	6.5095	6.1277	5.2189
51.1208	51:07:15	2.8636	7.1278	6.4959	6.1072	5.189
51.6211	51:37:16	2.8998	7.1267	6.4855	6.1055	5.1643
52.1214	52:07:17	2.9375	7.1445	6.4661	6.0755	5.1273
52.6217	52:37:18	2.9686	7.1428	6.4461	6.0524	5.0929
53.1219	53:07:19	3.0061	7.1427	6.4254	6.0246	5.0708
53.6222	53:37:20	3.0237	7.133	6.4052	5.9859	5.032
54.1225	54:07:21	3.0497	7.1486	6.3876	5.9707	5.0013
54.6228	54:37:22	3.0732	7.1477	6.3657	5.9544	4.9775
55.1231	55:07:23	3.0994	7.1386	6.3439	5.9392	4.9418
55.6233	55:37:24	3.1337	7.1494	6.3174	5.9174	4.9073
56.1236	56:07:25	3.1653	7.1448	6.2869	5.8842	4.8642
56.6239	56:37:26	3.1895	7.1451	6.2566	5.8522	4.8381
57.1242	57:07:27	3.2203	7.1465	6.2398	5.8268	4.8048
57.6244	57:37:28	3.2421	7.139	6.2166	5.8039	4.7709
58.1247	58:07:29	3.252	7.1335	6.1886	5.7864	4.7397
58.625	58:37:30	3.2673	7.1261	6.1697	5.7553	4.6924

Nystad. Laeverin expression is altered in preeclampsia. Am J Obstet Gynecol 2014. *(continued)*

SUPPLEMENTAL TABLE 5

xCelligence system raw data *(continued)*

Time(Hour)	Time(hh:mm:ss)	Y (A1, A2)	Y (B1, B2)	Y (C1, C2, D1, D2)	Y (E1, E2, F1, F2)	Y (G1, G2, H1, H2)
59.1253	59:07:31	3.2979	7.1263	6.1437	5.7234	4.6655
59.6256	59:37:32	3.3248	7.1136	6.1365	5.6968	4.628
60.1258	60:07:33	3.3426	7.1213	6.0918	5.6596	4.597
60.6261	60:37:34	3.3572	7.114	6.0623	5.6259	4.5619
61.1264	61:07:35	3.3849	7.1131	6.0407	5.5991	4.5207
61.6267	61:37:36	3.4036	7.1068	6.0005	5.5708	4.4898
62.1269	62:07:37	3.428	7.1119	5.9607	5.539	4.4524
62.6272	62:37:38	3.4528	7.0988	5.9251	5.5099	4.4182
63.1275	63:07:39	3.4765	7.098	5.9005	5.4791	4.3788
63.6278	63:37:40	3.5188	7.0803	5.8758	5.445	4.3424
64.1281	64:07:41	3.5384	7.0845	5.8508	5.4045	4.3031
64.6283	64:37:42	3.5715	7.0879	5.8213	5.3703	4.2688
65.1286	65:07:43	3.5898	7.1056	5.7828	5.3439	4.2389
65.6289	65:37:44	3.6097	7.0846	5.7509	5.31	4.2117
66.1292	66:07:45	3.6251	7.0847	5.7127	5.281	4.1708
66.6294	66:37:46	3.6368	7.0862	5.6755	5.2448	4.14
67.1297	67:07:47	3.6453	7.073	5.6488	5.1963	4.1024
67.63	67:37:48	3.6738	7.0583	5.6201	5.1585	4.0659
68.1303	68:07:49	3.6917	7.0585	5.5902	5.1273	4.0383
68.6306	68:37:50	3.7095	7.062	5.5607	5.0965	4.0046
69.1308	69:07:51	3.7402	7.0574	5.5233	5.0645	3.9676
69.6311	69:37:52	3.7569	7.0501	5.4948	5.0279	3.9299
70.1314	70:07:53	3.7556	7.0511	5.4686	4.9861	3.9012
70.6317	70:37:54	3.7633	7.0428	5.4342	4.9455	3.8661
71.1319	71:07:55	3.7799	7.0478	5.3959	4.9152	3.8365
71.6322	71:37:56	3.7957	7.09	5.3423	4.8315	3.8104
72.1325	72:07:57	3.822	7.0615	5.327	4.8559	3.7833
72.6328	72:37:58	3.8198	7.0048	5.2724	4.798	3.7338
73.1331	73:07:59	3.8326	7.0073	5.2101	4.6879	3.6855
73.6333	73:38:00	3.8564	6.9869	5.1795	4.6959	3.6656
74.1336	74:08:01	3.8749	6.9823	5.1328	4.6696	3.6284
74.6339	74:38:02	3.8973	6.9547	5.0835	4.6482	3.5991
75.1342	75:08:03	3.9111	6.9507	5.0431	4.6222	3.5683
75.6344	75:38:04	3.914	6.9381	5.0194	4.5998	3.5372
76.1347	76:08:05	3.9341	6.9545	4.992	4.5621	3.5092
76.635	76:38:06	3.9474	6.9576	4.9664	4.5326	3.4789
77.1353	77:08:07	3.9507	6.9563	4.9391	4.5005	3.4556
77.6356	77:38:08	3.9533	6.9396	4.9079	4.4721	3.4308
78.1358	78:08:09	3.9642	6.9747	4.8816	4.3583	3.4179
78.6361	78:38:10	4.0013	6.9486	4.9656	4.3475	3.4201

Nystad. Laeverin expression is altered in preeclampsia. Am J Obstet Gynecol 2014. *(continued)*

SUPPLEMENTAL TABLE 5

xCelligence system raw data *(continued)*

Time(Hour)	Time(hh:mm:ss)	Y (A1, A2)	Y (B1, B2)	Y (C1, C2, D1, D2)	Y (E1, E2, F1, F2)	Y (G1, G2, H1, H2)
79.1364	79:08:11	4.1295	7.2833	4.9261	4.4131	3.5147
79.6367	79:38:12	4.1894	7.1293	4.9164	4.4476	3.5104
80.1369	80:08:13	4.1154	6.9189	4.8	4.3056	3.4395
80.6372	80:38:14	4.0458	6.89	4.6965	4.2909	3.4124
81.1375	81:08:15	4.0177	6.8544	4.6412	4.2795	3.4196
81.6378	81:38:16	4.017	6.8289	4.6216	4.2306	3.3977
82.1381	82:08:17	4.0367	6.8282	4.6044	4.21	3.382
82.6383	82:38:18	4.0642	6.8328	4.5996	4.2017	3.377
83.1386	83:08:19	4.0885	6.8471	4.5902	4.1818	3.3477
83.6389	83:38:20	4.0982	6.8509	4.5649	4.1629	3.337
84.1392	84:08:21	4.0933	6.8294	4.5432	4.1425	3.3091
84.6394	84:38:22	4.0806	6.8184	4.515	4.1199	3.2916
85.1397	85:08:23	4.0758	6.801	4.4934	4.1069	3.2693
85.64	85:38:24	4.1047	6.7904	4.4752	4.0888	3.2616
86.1403	86:08:25	4.1088	6.7766	4.4621	4.0724	3.2408
86.6406	86:38:26	4.139	6.7545	4.4354	4.0461	3.2365
87.1408	87:08:27	4.1487	6.7495	4.4218	4.0314	3.221
87.6411	87:38:28	4.1348	6.7356	4.3959	4.016	3.2162
88.1414	88:08:29	4.1382	6.7477	4.3875	4.01	3.1967
88.6417	88:38:30	4.1395	6.7366	4.3766	4.0002	3.1963
89.1419	89:08:31	4.1494	6.7335	4.3601	3.9823	3.1903
89.6422	89:38:32	4.1548	6.7131	4.3501	3.9687	3.1947
90.1425	90:08:33	4.1533	6.7198	4.3218	3.9558	3.1931
90.6428	90:38:34	4.1666	6.7111	4.3164	3.9436	3.182
91.1431	91:08:35	4.1592	6.7064	4.2905	3.9343	3.1766
91.6433	91:38:36	4.1505	6.7014	4.2906	3.9269	3.1674
92.1436	92:08:37	4.1495	6.6812	4.2654	3.9216	3.162
92.6439	92:38:38	4.1511	6.6764	4.2621	3.9149	3.158
93.1442	93:08:39	4.1665	6.6697	4.2442	3.9011	3.1503
93.6444	93:38:40	4.1768	6.6581	4.2461	3.8897	3.1429
94.1447	94:08:41	4.1747	6.6511	4.2217	3.8828	3.1443
94.645	94:38:42	4.1719	6.6488	4.2187	3.8769	3.1381
95.1453	95:08:43	4.1597	6.6816	4.2184	3.7986	3.1413
95.6456	95:38:44	4.1805	6.6535	4.2272	3.8627	3.1526
96.1458	96:08:45	4.18	6.6078	4.1946	3.8591	3.1347
96.6461	96:38:46	4.191	6.6082	4.1582	3.8126	3.1374
97.1464	97:08:47	4.1882	6.6428	4.1615	3.7913	3.1423
97.6467	97:38:48	4.195	6.5873	4.1655	3.8314	3.1439
98.1469	98:08:49	4.1907	6.5497	4.174	3.8327	3.1259
98.6472	98:38:50	4.1831	6.533	4.1832	3.8331	3.1178

Nystad. Laeverin expression is altered in preeclampsia. Am J Obstet Gynecol 2014. *(continued)*

SUPPLEMENTAL TABLE 5

xCelligence system raw data *(continued)*

Time(Hour)	Time(hh:mm:ss)	Y (A1, A2)	Y (B1, B2)	Y (C1, C2, D1, D2)	Y (E1, E2, F1, F2)	Y (G1, G2, H1, H2)
99.1475	99:08:51	4.1853	6.5315	4.1707	3.8289	3.1112
99.6478	99:38:52	4.1885	6.5261	4.1547	3.8229	3.1034
100.1481	100:08:53	4.2104	6.5337	4.1698	3.8223	3.0928
100.6483	100:38:54	4.2181	6.5404	4.167	3.8111	3.0864
101.1486	101:08:55	4.2201	6.5294	4.154	3.7999	3.0804
101.6489	101:38:56	4.2336	6.5302	4.1418	3.7935	3.0712
102.1492	102:08:57	4.2517	6.5244	4.1496	3.7906	3.0697
102.6494	102:38:58	4.2324	6.5104	4.1358	3.784	3.0672
103.1497	103:08:59	4.2296	6.5062	4.1385	3.771	3.0668
103.65	103:39:00	4.2312	6.5004	4.1288	3.7595	3.061
104.1503	104:09:01	4.2374	6.4935	4.1132	3.7611	3.0579
104.6506	104:39:02	4.2214	6.5258	4.1244	3.7045	3.0725
105.1508	105:09:03	4.2381	6.5219	4.1838	3.7525	3.0893
105.6511	105:39:04	4.2132	6.4854	4.1814	3.748	3.0664
106.1514	106:09:05	4.2017	6.4598	4.1584	3.7656	3.0543
106.6517	106:39:06	4.2053	6.443	4.1595	3.757	3.0501
107.1519	107:09:07	4.1975	6.4428	4.1573	3.7701	3.0482
107.6522	107:39:08	4.1948	6.437	4.1577	3.7709	3.0478
108.1525	108:09:09	4.1981	6.4338	4.1576	3.7767	3.044
108.6528	108:39:10	4.1883	6.4358	4.1506	3.7666	3.0335
109.1531	109:09:11	4.1732	6.4144	4.1413	3.7681	3.027
109.6533	109:39:12	4.1658	6.4086	4.1376	3.7631	3.0241
110.1536	110:09:13	4.1562	6.4108	4.1336	3.7736	3.0191
110.6539	110:39:14	4.1541	6.4116	4.1319	3.7576	3.0125
111.1542	111:09:15	4.1529	6.4038	4.1226	3.7631	3.0047
111.6544	111:39:16	4.1485	6.3936	4.1175	3.7631	3.0035
112.1547	112:09:17	4.1426	6.3915	4.1132	3.7804	2.9984
112.655	112:39:18	4.1254	6.3846	4.1061	3.7687	2.9956
113.1553	113:09:19	4.1016	6.3787	4.1008	3.7803	2.9914
113.6556	113:39:20	4.0911	6.3802	4.1064	3.7834	2.9935
114.1558	114:09:21	4.0834	6.3683	4.112	3.7862	2.9889
114.6561	114:39:22	4.0767	6.3579	4.1098	3.7893	2.9899
115.1564	115:09:23	4.0705	6.3449	4.1191	3.7875	2.9855
115.6567	115:39:24	4.0743	6.3274	4.1126	3.79	2.9874
116.1569	116:09:25	4.0645	6.3075	4.1098	3.7947	2.9911
116.6572	116:39:26	4.0497	6.304	4.1121	3.8022	2.9877
117.1575	117:09:27	4.0419	6.2996	4.1123	3.7926	2.9818
117.6578	117:39:28	4.0313	6.2875	4.1036	3.7947	2.9842
118.1581	118:09:29	4.0159	6.2693	4.1036	3.798	2.984
118.6583	118:39:30	4.0059	6.2629	4.1032	3.7967	2.9805

Nystad. Laeverin expression is altered in preeclampsia. Am J Obstet Gynecol 2014. *(continued)*

SUPPLEMENTAL TABLE 5

xCelligence system raw data *(continued)*

Time(Hour)	Time(hh:mm:ss)	Y (A1, A2)	Y (B1, B2)	Y (C1, C2, D1, D2)	Y (E1, E2, F1, F2)	Y (G1, G2, H1, H2)
119.1586	119:09:31	3.9951	6.2476	4.0982	3.802	2.9779
119.6589	119:39:32	3.9891	6.239	4.1012	3.7992	2.9752
120.1592	120:09:33	3.9757	6.2316	4.0971	3.7959	2.9694
120.6594	120:39:34	3.9782	6.2372	4.0805	3.7966	2.9913
121.1597	121:09:35	3.9809	6.2319	4.1372	3.8361	3.012
121.66	121:39:36	3.976	6.1769	4.126	3.8715	3.0387
122.1603	122:09:37	3.9342	6.1258	4.1102	3.8724	3.0593
122.6606	122:39:38	3.9081	6.1007	4.1023	3.8884	3.0723
123.1608	123:09:39	3.8902	6.0893	4.1048	3.8972	3.0695
123.6611	123:39:40	3.8755	6.0917	4.1091	3.8943	3.0738
124.1614	124:09:41	3.8675	6.0891	4.103	3.8801	3.0696
124.6617	124:39:42	3.8669	6.0857	4.0953	3.8714	3.0722
125.1619	125:09:43	3.8549	6.0779	4.087	3.8649	3.0674
125.6622	125:39:44	3.8334	6.0711	4.0838	3.8463	3.0578
126.1625	126:09:45	3.8199	6.0587	4.0774	3.8325	3.0519
126.6628	126:39:46	3.8063	6.0495	4.0717	3.8247	3.0493
127.1631	127:09:47	3.8027	6.0263	4.0727	3.8188	3.0495
127.6633	127:39:48	3.788	6.0068	4.0697	3.8152	3.0473
128.1636	128:09:49	3.7895	5.9893	4.0697	3.8064	3.0523
128.6639	128:39:50	3.7674	6.0325	4.081	3.7755	3.0616
129.1642	129:09:51	3.7756	5.9667	4.0898	3.7944	3.0767
129.6644	129:39:52	3.755	5.9706	4.0899	3.8226	3.0851
130.1647	130:09:53	3.7356	5.9299	4.0789	3.8138	3.0811
130.665	130:39:54	3.713	5.9361	4.0676	3.8119	3.082
131.1653	131:09:55	3.6888	5.9266	4.0724	3.8136	3.0788
131.6656	131:39:56	3.6771	5.9209	4.071	3.8127	3.0783
132.1658	132:09:57	3.6639	5.9171	4.0679	3.8043	3.075
132.6661	132:39:58	3.6531	5.8848	4.056	3.7991	3.0706
133.1664	133:09:59	3.6299	5.8785	4.0541	3.7923	3.0632
133.6667	133:40:00	3.6159	5.8751	4.0532	3.7907	3.0562
134.1669	134:10:01	3.5999	5.8603	4.0522	3.7853	3.0538
134.6672	134:40:02	3.5833	5.8471	4.0524	3.7802	3.0481
135.1675	135:10:03	3.564	5.8368	4.0536	3.7755	3.0492
135.6678	135:40:04	3.5379	5.8096	4.0448	3.7723	3.045
136.1681	136:10:05	3.5264	5.81	4.0468	3.7701	3.0459
136.6683	136:40:06	3.5091	5.7913	4.0508	3.7682	3.0398
137.1686	137:10:07	3.4892	5.7661	4.0507	3.7659	3.0387
137.6689	137:40:08	3.4669	5.7616	4.0515	3.7653	3.0382
138.1692	138:10:09	3.4504	5.7516	4.0558	3.7617	3.0346
138.6694	138:40:10	3.4356	5.7347	4.0506	3.7624	3.0379

Nystad. Laeverin expression is altered in preeclampsia. Am J Obstet Gynecol 2014. *(continued)*

SUPPLEMENTAL TABLE 5

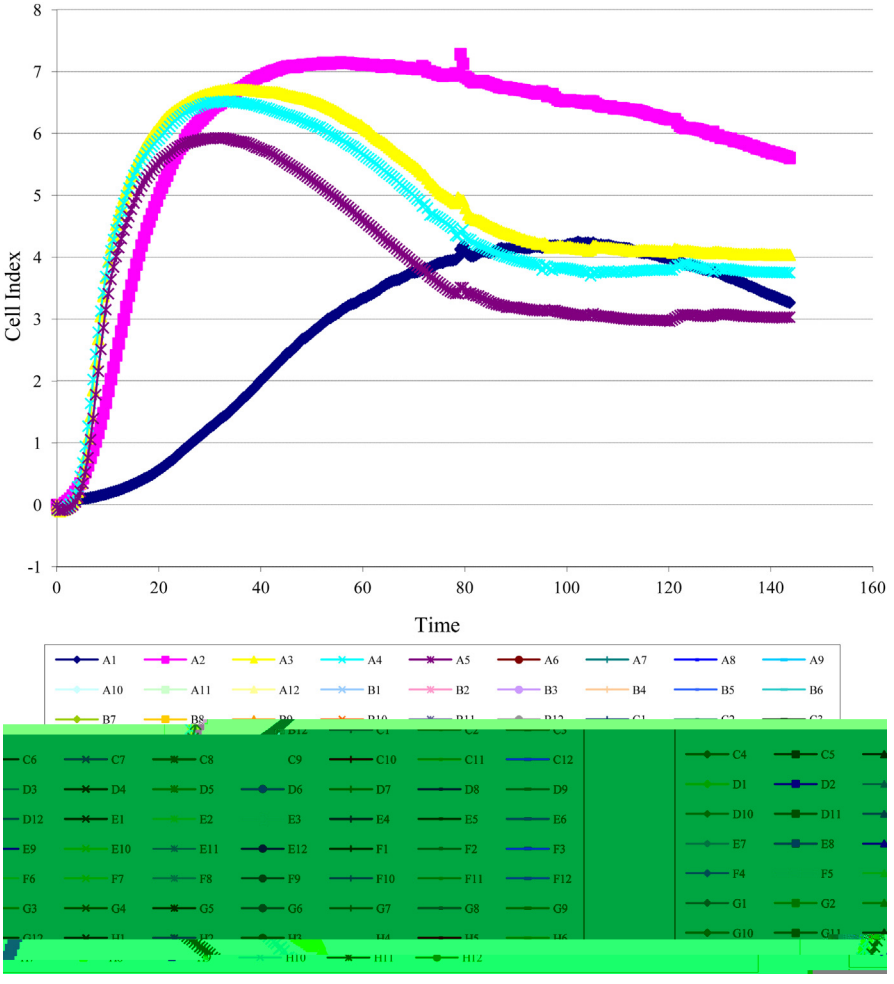
xCelligence system raw data *(continued)*

Time(Hour)	Time(hh:mm:ss)	Y (A1, A2)	Y (B1, B2)	Y (C1, C2, D1, D2)	Y (E1, E2, F1, F2)	Y (G1, G2, H1, H2)
139.1697	139:10:11	3.4136	5.7094	4.0453	3.7631	3.0341
139.67	139:40:12	3.3967	5.714	4.0411	3.7621	3.0264
140.1703	140:10:13	3.386	5.6805	4.0402	3.7615	3.0277
140.6706	140:40:14	3.3691	5.6924	4.044	3.7574	3.027
141.1708	141:10:15	3.3509	5.6743	4.0468	3.7545	3.0277
141.6711	141:40:16	3.3329	5.6621	4.0455	3.7585	3.029
142.1714	142:10:17	3.3131	5.6519	4.0439	3.7554	3.0241
142.6717	142:40:18	3.2991	5.6378	4.0409	3.7549	3.031
143.1719	143:10:19	3.2828	5.6294	4.0437	3.7522	3.0317
143.6722	143:40:20	3.2676	5.6084	4.0393	3.7525	3.0329

Experiment notes, layout, schedule, cell index, plot, and well graph.

xCelligence; ACEA Biosciences Inc, San Diego, CA.

Nystad. Laeverin expression is altered in preeclampsia. Am J Obstet Gynecol 2014.



Nystad. Laeverin expression is altered in preeclampsia. Am J Obstet Gynecol 2014.