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Laeverin protein expression in normal and preeclamptic placentas using tissue microarray analysis

Running headline: Laeverin - a trophoblast biomarker

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Disclosure statement

The authors report no conflict of interest.

Abstract

Introduction: Laeverin is a placenta-specific protein that is normally expressed in the plasma membrane of human trophoblasts. In previous studies, we showed higher expression levels of laeverin gene in preeclamptic compared to normal placentas and found that laeverin protein was ectopically expressed in the cytoplasma of the preeclamptic placentas. Our objective was to investigate laeverin protein expression in normal and preeclamptic placentas combining immunohistochemistry and immunofluorescence. *Material and methods:* Tissue microarray analysis of 72 placentas, obtained from 33 preeclamptic and 39 uncomplicated pregnancies was performed. Laeverin was labelled with a specific antibody for immunohistochemistry and immunofluorescence studies. *Results:* Immunohistochemistry showed that laeverin was expressed in syncytiotrophoblasts, cytotrophoblasts and extravillous trophoblasts in all placentas examined. In preeclamptic placentas (n=33) compared to normal placentas (n=39), laeverin was expressed in the cell membrane in 21 (64%) vs 21 (54%) of samples (p=0.726), in the cytoplasm in 3 (9%) vs 2 (5%) of samples (p=0.795) and in both the cytoplasm and membrane in 9 (27%) vs 16 (41%) of samples (p=0.0522). All placental samples that showed cytoplasmic expression of laeverin were obtained from women delivered before 34 weeks of gestation (early-onset preeclampsia). Further, immunofluorescence studies showed laeverin expression in the cytoplasm of six preeclamptic (three early-onset and three late-onset) and one normal placenta but did not reveal any simultaneous cell membrane and cytoplasmic expression of laeverin. *Conclusion:* Laeverin is expressed in all trophoblast cell types of normal and preeclamptic placentas. Expression pattern of laeverin in trophoblast cells is heterogeneous and not necessarily membrane-bound.

Key Words

Preeclampsia, laeverin, aminopeptidase-Q, placenta, tissue microarray, cytokeratin 7, CK7

Abbreviations

EVT extravillous trophoblasts

CK7 cytokeratin 7

IHC immunohistochemistry

IF immunofluorescence

TMA tissue microarray

HRP horse raddish peroxidase

FFPE formalin fixed paraffin embedded

Key message

Expression and localization of laeverin in trophoblast cells is heterogeneous and not necessarily membrane-bound.

Introduction

Preeclampsia is a pregnancy specific syndrome associated with defective placentation (1). The placenta is a heterogeneous organ containing several types of trophoblastic cells, mainly comprising cytotrophoblasts - that are the progenitor cells of the syncytiotrophoblasts - and extravillous trophoblasts (EVT) which invade the maternal endometrium to establish the feto-maternal circulation (2). EVTs are also found in the chorionic plate, the smooth chorion (chorion leave), the cell columns, the basal plate and in the placental septa (3). The syncytiotrophoblasts form the outer lining of the chorionic villi, which are the functional units of the placenta and create the feto-maternal interface (2, 4). Syncytiotrophoblast fragments, syncytial knots (5, 6) and

syncytiotrophoblast derived extracellular vesicles (7, 8) are shed into the maternal circulation during normal pregnancy and in pregnancy related diseases. This phenomenon is thought to be especially pronounced in preeclampsia (9).

Laeverin, also called Aminopeptidase Q, is a cell-surface marker of human EVT (10) and might play a regulatory role in EVT migration (11). We showed previously on individual placentas obtained from women with severe preeclampsia that laeverin might be ectopically expressed in the cytoplasma of villous trophoblasts (12).

Tissue microarray (TMA) is based on assembling many tissue samples on a single slide allowing simultaneous microscopic histological examination (13). TMA is a high-throughput and cheap method investigating the expression of proteins, RNA or DNA using immunohistochemistry (IHC), immunofluorescence (IF) and fluorescence/chromogenic *in situ* hybridization methods (ISH). The method was initially developed and designed for molecular profiling of tumor specimens (14), but may also be used for other purposes. Indeed recent studies have shown that TMA is feasible for placental investigation (15, 16).

In this study we used a series of placental tissue samples obtained from clinically well-characterized patients with severe preeclampsia and healthy controls to construct TMA blocks. We hypothesized that laeverin protein is differently expressed in placentas obtained from healthy and preeclamptic pregnancies. Our objective was to investigate laeverin protein expression in a series of normal and preeclamptic placentas combining IHC and IF methods.

Material and methods

TMA slides were prepared from 77 placental tissue samples obtained from 36 women with severe preeclampsia and 41 women with uncomplicated pregnancies. Severe preeclampsia was defined as blood pressure ≥160/110mmHg and proteinuria ≥300mg/24 hour urine or ≥2+ in spot urine after 20 weeks of gestation in previously normotensive women according to International Society for the study of Hypertension in Pregnancy (ISSHP) criteria (17). All the preeclamptic women included in our study received antihypertensive therapy. Doppler ultrasonography was performed ≤48 hours before delivery in each case to assess utero-placental and umbilical circulation.

Placentas were collected immediately after delivery and submerged in formaldehyde (4%) solution. Hematoxylin/eosin staining (18) was performed on individual formalin fixed paraffin embedded (FFPE) placental tissue slides (4 µm thick tissue sections) of all participants according to standard protocols.

The area of interest, consisting of macroscopically normal villous parenchyma, was localized by inspection of hematoxylin/eosin stained slides. Separate tissue cores (0.6 mm in diameter) of each individual paraffin block were punched out from primary blocks and mounted in a Manual Tissue Arrayer MTA-1 (Beecher Instruments, Sun Prairie, WI, USA) (Figure 1). Duplicates of each donor block to paraffin recipient blocks were used in each array. In addition, technical quadruplicates were performed in 4 samples. Two different array blocks (A and B) were used in the experiments; with different quantity of negative controls (normal liver samples; 4 in A- and 2 in B-block). Each TMA-block was then incubated at 37°C for 10 minutes to make a homogeneous block. These blocks were then cut (4 µm thick tissue sections) and mounted on Superfrost™ Plus glass slides (Thermo Fischer Scientific, Boston, MA, USA) according to standard procedures. TMA-sections on slides were mounted in paraffin in order to protect epitopes of the proteins and stored at 4°C until staining.

The following antibodies were used: Laeverin (rabbit polyclonal anti-Laeverin ab185345; AbCam, Cambridge, UK) and cytokeratin 7 (CK7) (rabbit monoclonal anti-CK7, SP52, code 05986818001; Roche, Tucson, USA) antibodies were used for IHC and IF on the TMA-slides.

IHC studies were performed on two separate tissue sections of the individual A- and B-TMA FFPE blocks with antibodies against laeverin and CK7 using the Ventana Discovery Ultra research Instrument (Ventana Medical Systems, Inc., Roche, Tucson, USA). Separate labeling protocols for laeverin and CK7 were used. Slides were incubated at 56-60°C for minimum 4 hours to dissolve most of the paraffin before IHC-staining and analysis.

The TMA slide was deparaffinized in EZ-Prep™ using heat at 68°C in three different cycles of 12 min each. Antigen unmasking and epitope-retrieval was done using heat treatment at 95°C in EDTA-buffer (pH 8.0-8.5) for repeated steps of 4 minutes, in total 32 minutes. Inhibitor CM was added and incubated for 12 minutes. Primary anti-laeverin antibody (1:50 dilution in PBS; final concentration 6 ng/µl) or anti-CK7 antibody (pre-diluted) and incubated at 36°C for 60 minutes for laeverin and 44 minutes for CK7. OmniMap (OMAP)-anti rabbit multimer horse raddish peroxidase (HRP) (code 5269679001; Roche, Tucson, USA) was added and incubated for 16 minutes for laeverin and 20 minutes for CK7. Laeverin was also amplified with Discovery Amplification AMP- HQ kit and

Discovery anti-HQ HRP both 8 minutes to give multimere Tyramide Signal Amplification (TSA™). ChromoMap 3,3'-Diaminobenzidine (DAB) and H_2O_2 was used for the last step of detection. Ultra liquid coverslip (oil) was used to prevent evaporation in each step. Details of the protocols are presented in Supporting Information Appendices S1 and S2.

Pictures were taken on an inverted light microscope (Leica DMI6000B; Leica microsystems, Wetzlar, Germany) with LAS v4.4 imaging software (Leica microsystems, Wetzlar, Germany).

IF studies were performed on two separate sections of the individual A- and B-TMA FFPE blocks with antibodies against laeverin and CK7 using the Ventana Discovery Ultra research Instrument (Ventana Medical Systems, Inc., Roche, Tucson, USA). Slides were incubated at 56-60°C for minimum 4 hours to dissolve most of the paraffin before IF-staining and analysis.

The TMA-slide was deparaffinized in EZ-Prep™ using heat at 68°C in three different cycles of 12 min each. Antigen unmasking and epitope-retrieval was done using heat treatment at 95°C in EDTA-buffer (pH 8.0-8.5) for repeated steps of 4 minutes, in total 40 minutes. Inhibitor CM was added and incubated for 12 minutes. Antibody block was added and incubated for 16 minutes at 37°C. Primary added rabbit anti-laeverin antibody was manually added (ab185345; AbCam, Cambridge, UK)(1:50 dilution in PBS; final concentration 6 ng/µl) and the slide was then incubated at 36°C for 60 minutes. Multimer HRP-blocking was added and incubated for 16 minutes before OmniMap (OMAP)-anti rabbit multimer HRP (code 5269679001; Roche, Tucson, USA) was added and incubated for 20 minutes. Rhodamine labelled secondary antibody (Discovery Rhodamine kit, code 07259883001; Roche, Tucson, USA) was added and incubated for 1 hour and 20 minutes. The antibodies were then denaturated using heat at 90°C for 8 minutes. Antibody block was added and incubated for 16 minutes for 37°C. Primary rabbit anti-CK7 antibody (Rabbit monoclonal (SP52), code 05986818001; Roche, Tucson, USA) was manually added and the slide was then incubated at 36°C for 60 minutes. Multimer HRP-blocking was added and incubated for 16 minutes before OmniMap (OMAP)-anti rabbit multimer HRP (code 5269679001; Roche, Tucson, USA) was added and incubated for 20 minutes. FITC labelled secondary antibody (Discovery FITC kit, code 07259212001; Roche, Tucson, USA) was added to the slide and incubated for 32 minutes before the slide was cleaned. Manual washing in Elix water (Elix Advantage Water Purification System, Massachusetts, USA) and colorless soap (Pantastic Clean, ECOLAB Etterstad, Norge) was performed to get rid of oil. Slides were then air dried and counterstained with DAPI (4',6-diamidino-2-phenylindole) II (Abbott Molecular Inc., Des Plaines, IL 60018, USA). Details of the protocol is presented in Supporting Information Appendix S3.

Imaging analysis was performed in a Nicon Eclipse E800 fluorescence microscope (Nikon Instruments Inc., Melville, NY, USA). Images were obtained using a CytoVision (Applied Imaging, San Jose, CA, USA) digital system equipped with a CCD camera (Cohu Inc., San Diego, USA).

Pictures from the most intensively laeverin and CK7 stained areas of all cores were taken and scored independently by two scientists [one pathologist (CN) and one cytogeneticist (MN)], before inter-observer correlation was done. IF studies were performed by an experienced cytogeneticist (MN). The following scoring criteria for laeverin protein expression were used:

- Membrane staining
- Cytoplasmic staining
- Membrane and cytoplasmic staining
- Negative; not expressed
- Co-localization of laeverin with CK7 (only for IF studies)

Statistical analyses

Differences between groups were tested using Student's t-test for parametric variables and Mann-Whitney U test for non-parametric variables as appropriate. A p-value of <0.05 was considered significant. Kappa value (19, 20) for inter-rater reliability was calculated for the IHC studies. The kappa value was based on analysis of 35 placentas obtained from uncomplicated pregnancies and 28 placentas from women with severe preeclampsia. The two raters used three criteria for rating: Laeverin protein localization in membrane of trophoblast cells, in cytoplasm of trophoblast cells or expression in both membrane and cytoplasm of trophoblasts. Data were analyzed with IBM SPSS Statistics 23 software (SPSS Inc, Chicago, IL).

Ethical approval

The study was approved by the Regional Committee for Medical and Health Research Ethics-North Norway (REK Nord ref. number 2010/2058-4) and informed written consent was obtained from all the participants.

Results

The baseline demographic characteristics and clinical phenotype of the study population are shown in Table 1. There were significant differences in mean arterial pressure, uterine artery pulsatility index, uterine artery pulsatility index, umbilical artery pulsatility index, gestational age at delivery, cesarean section rate, neonatal birth weight, placental weight, 5 min Apgar score and venous cord blood pH between preeclamptic and uncomplicated pregnancies.

Tissue microarray slides were of high quality and the histological details were clear in both IHC (Figure 2) and IF studies (Figure 3) for each core. Two scientists scored the IHC TMA slides blindly to evaluate the technical performance of IHC studies. We found 5.6% discordance (in 4 of 72 duplicates) in scoring. The inter-rater reliability (Cohen's kappa) was 0.890 (SE 0.047).

Laeverin was expressed in syncytiotrophoblasts, cytotrophoblasts and EVT in all placentas. Expression of laeverin in cell membranes, cytoplasm or both in preeclamptic compared to normal placentas is presented in Table 2. Laeverin was not expressed in stromal cells. The differences regarding the subcellular localization of laeverin protein were not statistically significant between healthy and preeclamptic placentas in the IHC studies (Table 2A). However, all placentas with only cytoplasmic expression of laeverin had early onset disease i.e. required delivery before 34 weeks of gestation (Table 2B). Laeverin was also expressed in syncytial knots; more pronounced in preeclamptic compared to normal placentas (Figure 2D).

IF studies were performed to validate the IHC results, using co-labeling of CK7 specific antibody to identify trophoblast cell populations (21, 22) and laeverin specific antibody.

Immunofluorescence studies did not reveal any membrane and cytoplasmic expression of laeverin protein simultaneously. Laeverin was expressed in the cytoplasm of 6 preeclamptic placentas (3 early- and 3 late-onset preeclampsia) and in only one normal placenta.

Discussion

In this study we investigated the expression of laeverin protein in a series of normal and preeclamptic placentas. We found laeverin to be expressed in syncytiotrophoblasts, cytotrophoblasts and EVT in all placentas. In a previous study (12) we found that laeverin might be ectopically expressed in the cytoplasm and microvesicles in cytotrophoblasts of preeclamptic placentas, rather than in the cell membrane. Therefore, we hypothesized that it might be possible to histologically differentiate preeclamptic from healthy placentas based on cytoplasmatic laeverin

expression. However, in the present study we discovered that some healthy placentas also have cytoplasmic laeverin expression, indicating that laeverin cannot be used as a specific diagnostic biomarker of preeclampsia in pathological specimens using IHC. However, in IHC, there were more preeclamptic placentas with only cytoplasmic expression of laeverin (9%) compared to normal placentas (5%), indicating that laeverin might play a role in the molecular pathophysiology of preeclampsia. The differences between the groups were not statistically significant, but our study was underpowered to demonstrate significant differences regarding dissimilar subcellular localization of laeverin between healthy and preeclamptic placentas. Another possible reason for the discordance with our previous study could be the use of different antibodies; one antibody binds to epitopes located to amino acid position 960-990 on the outer surface of the protein, including the COOH-end of laeverin and was house-made by rabbit immunization (12). On the other hand, the antibody used in this study is a commercial (AbCam) recombinant fragment of human laeverin, which is longer, targeting amino acids 818-905 of the laeverin protein. Interestingly all of the preeclamptic placentas expressing laeverin in the cytoplasm were delivered before 34 weeks gestation. This is in line with our previous finding of higher laeverin expression in the maternal circulation in early pregnancy (23), and laeverin's role in migration and invasion capacities of first trimester trophoblasts (24).

The IF co-localization studies of laeverin and CK-7 showed either membrane or cytoplasmic expression; no simultaneous membrane and cytoplasmic expression of laeverin was detected (Figure 2). The advantage of using the two markers together in the same IF study, is that cytoplasma expression could easily be detected (as a yellow signal), where both CK7 and laeverin are co-expressed. Moreover, the company producing the antibody provided pictures of healthy human placenta showing membrane expression. Further, the presence of a transmembrane motif of laeverin protein further argues for a membrane-only expression in normal placenta (10).

We found that only a subset of preeclamptic placentas with early onset disease showed intracytoplasmatic expression of laeverin, together with a placenta obtained from a woman with uncomplicated pregnancy, but with histologic signs of chorioamnionitis, which was not included in the analysis (data not shown). This is an interesting finding indicating that laeverin might be involved and/or disarranged in the inflammatory process, which is excessive in preeclampsia (23). Moreover, the results of the present study challenge the knowledge that laeverin is a membrane bound aminopeptidase, indicating other possible enzymatic functions of this protein during normal pregnancy. In fact, it is shown that laeverin has several enzymatic substrates in the normal human

placenta (24). We did not have sufficient samples to answer whether all preeclamptic placentas delivered at term lack laeverin expression intracytoplasmatically.

The limitations of our study are mainly related to pre-TMA sample preparation, i.e. size of tissue used in fixation, different exposure time in air before fixation and different exposure time of the individual placentas to formalin. Moreover, the placenta is a relatively heterogeneous organ containing different cell types. Therefore, the cores for the construction of the TMA should be carefully chosen to get a representative area of interest for investigation. A TMA has the advantage of including many samples lowering the costs are providing similar experimental conditions for all samples. However, the scoring procedure demands experienced personnel and provides a large amount of data to handle. Nevertheless, we found an inter-rater reliability (Kappa) value of 0.890 (standard error 0.047) suggesting a strong level of agreement between the raters (19). Another limitation of our study is that we did not include cases of mild preeclampsia and we were therefore not able to perform a subgroup analysis for this population. Furthermore, in the TMA, there was a mixture of placentas delivered vaginally and by emergency or elective cesarean sections. In a previous study (25) we compared gene expression profile of placentas delivered vaginally following normal labor with placentas delivered by elective cesarean section before the onset of labor, and found that global placental gene expression profile is not affected by labor. We specifically measured the expression profile of laeverin in these placentas (data not shown) and we did not find any differences. Therefore, we do not have any indication that the expression of laeverin protein might be influenced by mode of delivery, but possible influence of laeverin expression by induced or prolonged labor cannot be excluded.

Regarding the performance of the techniques used, IHC of single samples was proven to be a good tool for initial screening of placenta tissue, while antibody-labeled TMA sections from many placentas was shown to be a versatile and cheap method for screening a series of samples simultaneously. Moreover, placental TMA IHC and IF seem to complement each other. In particular, IHC gives better morphological overview and easy recognition of histological features characterizing the preeclamptic placenta, such as syncytical knots. For example, we found by IHC that laeverin appears to be polarized to the plasma membrane facing the intervillous space. On the other hand, IF is better for detecting the subcellular structures and localization of the protein within them.

Quantitative measurements of laeverin protein expression would have been preferable to do, especially since we noticed differences in expression patterns of laeverin regarding the intensity of membrane staining between normal and preeclamptic placentas (Figure 2A compared to Figure 2C). However, standard IHC and IF are not suitable for quantitative measurements, whereas automated quantitative IF or mass spectrometry may be used for this purpose.

In conclusion, we demonstrated laeverin expression in EVT, villous cytotrophoblasts and syncytiotrophoblasts in all placentas. Laeverin is expressed both in the cell membrane and in the cytoplasm in normal as well as in preterm delivered preeclamptic placentas, indicating possible activities in the fetal-maternal interface that may be important for placental dysfunction in preterm preeclampsia.

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Supporting Information legends

Appendix S1: Ventana Discovery automatic immunohistochemistry protocol for laeverin labelling. (Ventana Discovery automatic IHC RUO DISCOVERY Universal (v0.00.0265) Protocol name 561 Laeverin 185345 AMP.)

Appendix S2: Ventana Discovery automatic immunohistochemistry protocol for CK7 labelling. (Ventana Discovery automatic IHC RUO DISCOVERY Universal (v0.00.0265) Protocol name 483 R CK7).

Appendix S3: Ventana Discovery automatic immunofluorescence protocol for co-labelling of Laeverin and CK7. (Ventana Discovery automatic immunofluorescence RUO DISCOVERY Universal (v0.00.0265) Protocol name 586 Laev-CK7 RHOD-FITC 120).

Table and Figure Legends

Table 1. Clinical data of study population at delivery (healthy pregnant women and women with preeclampsia participating in the study). Continuous variables are presented as mean±SE or median (range) and categorical variables as n (%) as appropriate.

Table 2. Summary of results from the tissue microarray immunohistochemistry analysis according to subcellular localization of laeverin protein between (A) Normal and preeclamptic placentas and (B) early- and late-onset preeclampsia. Note that 2 of 41 normal and 3 of 36 preeclamptic placentas could not be scored because of weak or no-expression of laeverin due to technical issues.

Figure 1. Tissue microarray (TMA) protocol. The tissue area of interest was identified on the individual specimen paraffin block and a small part was microdissected out with a tissue-arrayer

(pink). The microarray was assembled in a paraffin recipient block precut with a tissue-arrayer (blue) with a slightly bigger diameter. After inserting all different patient cores in the recipient block, the paraffin block was heated to make an uniform TMA-block. Immunohistochemistry (IHC) or immunofluorescence (IF) were then performed on different slides from the same TMA-block. Slides were analyzed in microscopes, databases were made and SPSS-statistics done.

Figure 2. Tissue microarray immunohistochemistry of healthy- (A-C) and preeclamptic placentas (D-E) labelled with laeverin antibody (brown). A) Healthy placenta showing laeverin staining in syncytiotrophoblast membranes of chorion villi. B) Healthy placenta showing laeverin membrane staining in syncytiotrophoblasts and in extravillous trophoblasts. C) Healthy placenta showing membrane- and cytoplasma staining of syncytio- and cytrotrophoblast cells in chorion villi. D) Placenta from preeclamptic patient showing cytoplasmic staining of syncytio- and cytrotrophoblast cells in chorion villi. In fetal capillaries (FC) red blood cells are depicted. Several syncytial knots are seen in this patient, one of these is highlighted in the right column labelled SK. E) Placenta from preeclamptic patient showing cytoplasmic staining of syncytio- and cytrotrophoblast cells in chorion villi. In fetal capillaries (FC) red blood cells are depicted. F) Negative control (liver tissue) stained with laeverin shows no membrane nor cytoplasmic staining, but some areas of pigment staining. In the left column 10x magnification of tissue microarray cores are shown. Areas further magnified (40x magnification) are depicted in the right column. EVT=extravillous trophoblasts, FC=fetal capillaries, SK=syncytial knot and ST=stroma. Nuclei are counterstained (blue). Scale bars are 200 μm (low resolution pictures) or 50 μm (high resolution pictures).

Figure 3. Tissue microarray immunofluorescence of healthy- (A) and preeclamptic (B) placentas labelled with laeverin antibody (red) and cytokeratin 7 (CK7)(green). A) Laeverin (red) is expressed in the plasma membrane of syncytiotrophoblast cells in healthy placenta (this is the same placenta as in Figure 2A). CK7 (green) is localized in the cytoplasm of trophoblastic cells. B) Preeclamptic placenta shows partly co-localized laeverin and cytokeratin in the cytoplasm. Fetal capillaries (FC) inside chorionic villi containing red blood cells (pink and blue) are shown. C) Liver cells stained with laeverin and CK7 showed no staining. Nuclei were counterstained with DAPI II (blue). FC=fetal capillaries. Red blood cells are depicted pink in the pictures. Scale bars are 50 μm.

Table 1. Clinical data of study population at delivery (healthy pregnant women and women with preeclampsia participating in the study). Continuous variables are presented as mean±SE or median (range) and categorical variables as n (%) as appropriate.

	Healthy women (*n*=41)	Preeclampsia (*n*=36)	*P* value
Maternal age, median (range), years	31 (17-40)	31 (21-43)	0.741
Body mass index before delivery, (kg/m^2)	30.2±0.71	29.8±0.64	0.846
Primipara, n (%)	19 (46)	20 (56)	0.420
Mean arterial pressure, mmHg	88.8±2.1	123.5±2.1	p<0.0001
Uterine artery pulsatility index (mean of the left and right side)	0.74±0.0	1.32±0.23	p<0.0001
Middle cerebral artery pulsatility index	1.30±0.06	1.37±0.08	0.433
Umbilical artery pulsatility index	0.77±0.03	1.35±0.13	p<0.0001
Gestational age at delivery, median (range), weeks	39 (35-42)	35 (26-42)	p<0.0001
Cesarean section (emergency/elective), n (%)	21 (5/16) (51)	28 (28/0)(78)	0.016
Neonatal birth weight, g	3621±90	2067±161	p<0.0001
Placental weight, g	679±31	253±43	0.001
5 min APGAR score median (range)	10 (7-10)	9 (0-10)	0.006
Arterial cord blood pH	7.27±0.01	7.26±0.02	0.376
Arterial cord blood Base Excess	-3.23±0.56	-2.87±0.72	0.691
Venous cord blood pH	7.34±0.01	7.28±0.02	0.011
Venous cord blood Base Excess	-2.93±0.94	-3.00±1.14	0.965

Table 2. Summary of results from the TMA immunohistochemistry analysis according to subcellular localization of laeverin protein between (A) Normal and preeclamptic placentas and (B) early- and late-onset preeclampsia. Note that 2 of 41 normal and 3 of 36 preeclamptic placentas could not be scored because of weak or no-expression of laeverin due to technical issues.

A

	Normal (n=39)	Preeclampsia (n=33)	*P* value
Plasma membrane	21 (54%)	21 (64%)	0.726
Plasma membrane and cytoplasma	16 (41%)	9 (27%)	0.522
Cytoplasma	2 (5%)	3 (9%)	0.795

B

	Early-onset (n=15)	Late-onset (n=18)	*P* value
Plasma membrane	10 (30.3%)	11 (33.3%)	0.703
Plasma membrane and cytoplasma	3 (9.1%)	6 (18.2%)	0.408
Cytoplasma	3 (9.1%)	0	0.048