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Microarray profiling reveals that placental transcriptomes of early-onset HELLP syndrome and preeclampsia are similar

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Abstract

Background—The involvement of the placenta in the pathogenesis of preeclampsia and HELLP syndrome is well established, and placental lesions also similar in these two syndromes. Here we aimed to examine the placental transcriptome and to identify candidate biomarkers in early-onset preeclampsia and HELLP syndrome.

Methods—Placental specimens were obtained at C-sections from women with early-onset preeclampsia and HELLP syndrome, and from controls who delivered preterm or at term. After histopathological examination, fresh-frozen placental specimens were used for microarray profiling and validation by qRT-PCR. Differential expression was analysed using log-linear models while adjusting for gestational age. Gene ontology and pathway analyses were used to interpret gene expression changes. Tissue microarrays were constructed from paraffin-embedded placental specimens and immunostained.

Results—Placental gene expression was gestational age-dependent among preterm and term controls. Out of the 350 differentially expressed genes in preeclampsia and 554 genes in HELLP syndrome, 224 genes (including *LEP*, *CGB*, *LHB*, *INHA*, *SIGLEC6*, *PAPPA2*, *TREMI*, and *FLT1*) changed in the same direction (elevated or reduced) in both syndromes. Many of these encode proteins that have been implicated as biomarkers for preeclampsia. Enrichment analyses revealed similar biological processes, cellular compartments and biological pathways enriched in early-onset preeclampsia and HELLP syndrome; however, some processes and pathways (e.g., cytokine-cytokine receptor interaction) were over-represented only in HELLP syndrome.

Conclusion—High-throughput transcriptional and tissue microarray expression profiling revealed that placental transcriptomes of early-onset preeclampsia and HELLP syndrome largely overlap, underlying a potential common cause and pathophysiologic processes in these syndromes. However, gene expression changes may also suggest a more severe placental pathology and pronounced inflammatory response in HELLP syndrome than in preeclampsia.

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Conflict of interest statement

The authors have no conflict of interest.

Keywords

Differential expression; high-dimensional biology; syncytiotrophoblast; systemic inflammation; virtual microscopy

INTRODUCTION

Preeclampsia, one of the great obstetrical syndromes, results from heterogeneous causes, and its underlying pathomechanisms may lead to clinical manifestation at different time points. Late-onset preeclampsia is often mild, while early-onset preeclampsia necessitating delivery before 34–35 weeks of gestation is more severe and often associated with HELLP syndrome [1–8]. Whether HELLP syndrome is a severe form of preeclampsia is controversial. The majority of patients with HELLP syndrome develop preeclampsia; however, ~15–20% of them do not have hypertension and/or proteinuria. Moreover, laboratory findings in HELLP syndrome usually do not correlate with the severity of hypertension or proteinuria. Preeclampsia typically develops gradually, while the onset of HELLP syndrome is frequently rapid [9–12].

Preeclampsia is associated with abnormal placentation, utero-placental vascular insufficiency and altered intervillous haemodynamics, placental nitrative-, endoplasmic reticulum- and oxidative stress, and increased placental release of syncytiotrophoblast debris and anti-angiogenic molecules, which cause maternal generalized endothelial cell dysfunction and an exaggerated systemic inflammatory response. Although abnormal histopathological findings can be detected in placental morphological abnormalities are mainly found in early-onset preeclampsia [4,6–8,13–19]. Differences between early- and late-onset preeclampsia have been identified in placental gene expression signatures [20]. When comparing preeclampsia with HELLP syndrome, the frequency of placental histopathological lesions was found to be similar in these two syndromes in a patient group with early-onset diseases [21]. It is possible that a common cause and pathophysiologic processes will lead to shared placental and clinical features in early-onset preeclampsia and HELLP syndrome.

We found similar morphological and gene expression changes in placentas taken from women with preterm preeclampsia complicated with or without HELLP syndrome [22]. The aim of the present study was to reveal the similarities and differences in placental transcriptome in early-onset preeclampsia and HELLP syndrome and to find potential markers for their differentiation.

MATERIALS AND METHODS

Patients, clinical definitions

Samples were collected at the First Department of Obstetrics and Gynecology, Semmelweis University (Federalwide Assurance: FWA00002527) (2007–2009). Pregnancies were dated according to ultrasound scans between 8 and 12 weeks. Patients with multiple pregnancies or fetuses having congenital or chromosomal abnormalities were excluded. The research was approved by the health Science Board of Hungary and the Human Investigation Committee of Wayne State University. Informed consent was obtained from women prior to sample collection. Specimens and data were stored anonymously.

A meaningful sample size determination for microarray studies requires the existence of a pilot dataset using samples from the same population run on the same microarray platform as the one to be used in the main experiment. As such dataset was not available, we used

recommendations from the literature regarding the minimum number of samples per group ($n = 5$) [23]. Women were enrolled in the following groups: (1) early-onset preeclampsia ($n = 6$; < 35 weeks), (2) early-onset HELLP syndrome ($n = 6$; < 35 weeks), and (3) controls ($n = 10$) who consisted of term ($n = 5$; > 37 weeks) and preterm controls ($n = 5$; < 35 weeks). Term controls had no medical or obstetrical complications and delivered a neonate with a birth-weight appropriate for gestational age [24]. Preterm controls delivered preterm without clinical or histological signs of chorioamnionitis. Preeclampsia was defined according to the criteria set by the American College of Obstetricians and Gynecologists [6,25]. HELLP syndrome was defined according to the criteria set by Barton and Sibai [11]. C-section was performed in all cases due to severe symptoms as well as in all controls due to previous C-section or malpresentation.

Sample collection

Tissues samples were obtained immediately after delivery. For gene expression studies, villous tissue samples were excised from central cotyledons close to the umbilical cord in order to reduce the possible bias due to regional differences in gene expression, dissected from the choriodecidua on dry ice and stored at -80°C . For tissue microarray, five representative histological blocks were taken from each formalin-fixed placenta to include central and peripheral cotyledons and the maternal side of the placenta with the fetal membranes, and were paraffin-embedded.

Histopathology

Placental specimens were examined according to a standard protocol, describing the topography and size of macroscopic lesions. Four micrometer sections were cut from 5 tissue blocks in case of all placentas ($n = 110$ blocks in total) and mounted on SuperFrost/Plus slides (Fisherbrand, UK). After deparaffination, slides were rehydrated, stained with haematoxylin-eosin and evaluated in 10 randomly chosen microscopic fields. Macroscopic and microscopic lesions were defined according to published criteria [26–28].

RNA isolation

Villous tissues were homogenized using a Thermo Savant FastPrep FP120 Homogenizer (Thermo Scientific, USA) with Lysing Matrix D (MP Biomedicals, France). Total RNA was isolated using RNeasy Fibrous Tissue Mini Kit (Qiagen, Germany), quantified with NanoDrop 1000 (Thermo Scientific) and assessed by Agilent 2100 Bioanalyzer (Matrix, Norway).

Microarray analysis

Total RNAs (800 ng) were reverse transcribed; cDNAs were transcribed into antisense cRNAs, which were labeled with One-Color Quick Amp Labeling Kit (Agilent Technologies Inc., USA) and purified with RNeasy Mini Kit (Qiagen). Cy3-RNAs (1650 ng) were fragmented and hybridized on Agilent 44K Whole Human Genome Oligo Microarray Chips using Agilent Gene Expression Hybridization Kit. Microarrays were washed, scanned, array images were quantified, and data were normalized.

Expression levels were background corrected followed by the removal of low intensity probes. Log_2 transformed data were quantile normalized between arrays [29]. Term and preterm controls were combined and differential expression was tested using a linear model that included gestational age and array processing date as covariates. Model coefficients were evaluated by a moderated t -test [30]. Genes were selected as differentially expressed upon a False Discovery Rate (FDR) [31] adjusted p -value < 0.25 and fold-change ≥ 2 . Analyses were performed using the *R* statistical environment (www.r-project.org) and

additional Bioconductor (www.bioconductor.org) packages such as *limma* and *preprocessCore*.

An enrichment analysis was performed to identify gene ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway over-represented in the list of differentially expressed genes using the *GOstats* [32] package. The Signaling Pathway Impact Analysis (SPIA) [33,34] was used to better exploit gene-gene interactions and the magnitude and direction of gene expression changes in a given pathway. Enrichment and impact analyses results were considered significant using a 0.10 threshold on the FDR-corrected *p*-values.

Quantitative real-time RT-PCR

Total RNAs (2 µg) were revers transcribed using High Capacity RNA-to-cDNA Kit (Applied Biosystems, USA). TaqMan® Gene Expression Assays (for 5 genes differentially expressed in both preeclampsia and HELLP syndrome, and for 6 genes with the largest fold-change difference between preeclampsia and HELLP syndrome on microarray; Suppl. Table 1), endogenous control (4333761T) and Universal PCR Master Mix were used for gene expression quantification on an ABI 7300 RT-PCR instrument (Applied Biosystems). Ct values for each target gene and for the within-plate endogenous control were averaged over three technical replicates. For each target gene, the $-DCt$ value (surrogate of $\log_2$ gene concentration) was obtained for each sample as $-DCt_{(target)} - Ct_{(RPLPO)} - Ct_{(target)}$, and linearly fitted to the group factor while adjusting for gestational age. The significance of the group effect was evaluated via a one-tailed *t*-test based on the microarray-identified directions of changes. A *p*-value < 0.05 was considered significant.

Placental “tissue microarray” construction and immunostainings

Cores of 2 mm were collected from all tissue blocks from each specimen and inserted into recipient blocks; 5 µm sections were cut, mounted on SuperFrost/Plus slides, deparaffinated and rehydrated. Endogenous peroxidases were inhibited with 10% H₂O₂. Slides were incubated with Tris (10 mM, pH = 9.1, 1 mM EDTA, 45 min, 100 °C) for antigen retrieval. After washing, unspecific binding was blocked with NovoLink buffer (Novocastra Laboratories, UK; 10 min, RT). Slides were then incubated with anti-hCGβ (Dako North America Inc., USA; 1:3) or anti-LHβ (BioGenex Laboratories Inc., USA; 1:1) rabbit polyclonal antibodies in PBS (1% BSA, overnight, 4 °C). After washing, NovoLink kit was used for post-primary antibody blocking (30 min, RT). After washing, incubation was performed with NovoLink polymer (rabbit/mouse; 30 min, RT). Slides were washed and developed with DAB Substrate Kit (Vector Laboratories, USA), followed by haematoxylin counterstaining. The primary antibody was omitted in negative controls.

Evaluation of immunostaining

Visual evaluation of immunostainings was performed microscopically (Carl Zeiss MicroImaging GmbH, Germany) by two examiners (THEREFORE, NGT). TMAs were then digitally scanned by Mirax Scan high-resolution scanner (Carl Zeiss MicroImaging GmbH), deposited to a virtual laboratory (www.pathonet.org) and evaluated by additional examiners (KK, TV) using Mirax Viewer 1.8.3.0 (Carl Zeiss MicroImaging GmbH and 3DHistech Ltd., Hungary). Each slide was evaluated by at least two examiners blinded to clinical outcome according to a semi-quantitative score [22], and the average score was used as representative data.

Data other than on microarray and qRT-PCR were analysed by Statistica 8 (StatSoft Inc., USA). Comparisons among the groups were performed using Chi-square test and Fisher's

exact test for proportions and Kruskal-Wallis test followed by Mann-Whitney test for non-normally distributed continuous variables. A p -value < 0.05 was considered significant.

RESULTS

Demographic and clinical data, histopathological findings

Demographics and clinical characteristics are displayed in Table 1. Maternal age was lower in patients with HELLP syndrome than in controls. Severe preeclampsia was diagnosed in 66.7% of women with preeclampsia and in all patients with HELLP syndrome. Out of 6 HELLP patients, one had Mississippi class I, four had class II and one had class III syndrome. Placental and neonatal weights were lower in both disease groups than in controls. From the investigated placental lesion types, villous dysmaturity and syncytial knots were more frequent in preeclampsia and HELLP syndrome than in controls.

Placental gene expression is gestational age-dependent

Microarray analysis demonstrated that 189 probes corresponding to 181 genes were differentially expressed between preterm and term controls. Due to this finding, we developed a linear statistical model to evaluate gene expression between cases and controls, which included gestational age as a covariate.

Early-onset preeclampsia has a unique placental gene expression signature

When comparing placentas taken from patients with preeclampsia and those from term and preterm controls combined, 379 probes corresponding to 350 genes were found to be differentially expressed (Fig. 1A). As a good validation of our results, from the 20 most up- or down-regulated genes, 7 were published by microarray studies as having the same direction of placental expression change in preeclampsia (Table 2), including those encoding for leptin, hCG β , LH β , siglec-6, PAPP-A2, and TREM1.

GO analysis revealed the over-representation of 73 biological processes; the top 20 are presented in Table 3. Over-represented molecular functions and cellular components are shown in Suppl. Table 2. SPIA analysis showed the disturbance of “neuroactive ligand-receptor interaction”.

Early-onset HELLP syndrome has similar gene expression signature to early-onset preeclampsia

Microarray analysis revealed 605 probes corresponding to 554 genes that were differentially expressed in HELLP syndrome compared to term and preterm controls combined (Fig. 1B). From these, 224 genes had also significant expressional change in the same direction in preeclampsia (Fig. 1C). Indeed, from the top 20 most up- or down-regulated genes in HELLP syndrome, 16 were also differentially regulated in preeclampsia (Table 4).

GO analyses revealed mostly the same biological processes over-represented in HELLP syndrome as in preeclampsia. From the top 20 (Table 5), “extracellular matrix/structure organization” was only over-represented in HELLP syndrome. Molecular functions and cellular components over-represented in HELLP syndrome are shown in Suppl. Table 3. In addition to “neuroactive ligand-receptor interaction” altered in preeclampsia, SPIA analysis revealed “cytokine-cytokine receptor interactions” uniquely over-represented in HELLP syndrome.

Validation of microarray data revealed differences between preeclampsia and HELLP syndrome

In order to validate microarray results, we performed qRT-PCR and immunostainings of TMAs. The expression of 11 genes was quantified by qRT-PCR: (1) from the top five genes differentially expressed on microarray in the same direction in both syndromes, we could confirm the differential expression of four of these genes in both syndromes: *LEP*, *CGB*, *LHB*, and *SIGLEC6*, from which *LEP* had a highly elevated (124–225 fold) expression. (2) Six genes with the largest – yet not statistically significant – fold-change difference between preeclampsia and HELLP syndrome on microarray were tested by qRT-PCR. This showed the same direction of change for all of these six genes, and the significant differential expression of *ARHGEF4* (Rho guanine nucleotide exchange factor-4) and *MGST1* (microsomal glutathione S-transferase-1) between preeclampsia and HELLP syndrome (Fig. 2). These data show a good agreement between microarray and qRT-PCR results; however, a perfect agreement between the two platforms cannot be typically achieved due to differences in the probe sequences, variations in the targeted transcripts, and differences in the accuracy and precision of the two techniques [35].

Immunostainings of TMAs, which allowed high-throughput examination of the expression of molecular markers at the protein level [36], showed increased syncytiotrophoblastic staining for LH β and hCG β in both preeclampsia and HELLP syndrome compared to gestational age-matched controls. The staining intensity for hCG β was non-significantly higher in HELLP syndrome than in preeclampsia (Fig. 3). There was a similar extent of increase in staining intensity of the syncytiotrophoblast for LH β in both syndromes (Suppl. Figure 1).

DISCUSSION

Principal findings of the study

(1) This is the first study to present the placental transcriptome in early-onset HELLP syndrome; (2) microarray analysis revealed 350 placental differentially expressed genes in preeclampsia and 554 in HELLP syndrome; (3) out of these genes, 224 had significant change in its expression in the same direction in both syndromes; (4) biological processes “multicellular”, “developmental”, “differentiation” and “signaling”, as well as the “neuroactive ligand-receptor interaction” pathway were enriched in both syndromes; (5) among the over-represented biological processes and pathways, “extracellular matrix/structure organization” and the “cytokine-cytokine receptor interaction” were unique to HELLP syndrome; (6) *ARHGEF4* and *MGST1* had significantly higher expression in HELLP syndrome than in preeclampsia; and (7) LH β and hCG β had increased staining intensity in the syncytiotrophoblast both in early-onset preeclampsia and HELLP syndrome.

Placental morphological changes in preeclampsia and HELLP syndrome

The pathogenesis of preeclampsia and HELLP syndrome is related to abnormal placentation [4,6–8,17,18]. In spite of histological findings in term preeclampsia, more pronounced placental morphological changes are found in early-onset preeclampsia [13,14], in accord with differences in placental gene expression between these two subtypes of preeclampsia [20]. Of interest, placental morphological changes in HELLP syndrome are consistent with those in preeclampsia in early-onset cases [21]. Indeed, previously we also observed a similar degree of histopathological abnormalities in early-onset preeclampsia and HELLP syndrome [22]. It is possible that a common noxa initiates pathophysiologic processes in the early-onset forms of these two syndromes, which will lead to the activation of shared clinical pathophysiologic pathways.

Placental gene expression changes in preeclampsia

Wyatt et al. [37] showed that the expression of hypoxia-related genes in the placental disk depends on the sampling site, reflecting differences in villous perfusion. Cindrova-Davies et al. [38] showed that uterine contractions during labor leads to placental oxidative stress, NF- κ B pathway activation, proinflammatory cytokine expression and apoptosis, and the magnitude of these changes are related to the duration of labor. Taking into account these findings, we only collected central cotyledons from C-sections.

Not only sampling site and labor status, but also the gestational age at disease onset has an impact on placental gene expression. In our gestational age-matched sample set, 350 genes were differentially expressed in preeclampsia, many of which were published by microarray studies to have the same direction of change (Table 2). Of importance, from the increasing number of studies on placental transcriptome in preeclampsia [20,39–47], most of the overlapping findings come from those focusing on early-onset preeclampsia and including patient- and gestational age-matched control groups with clinical characteristics similar to those we had in our study [39,46,47]. The resemblance between the results studies underlines the importance of the inclusion of homogenous patient populations and the distinction between different subforms of preeclampsia.

In accordance with the altered trophoblast physiology in early-onset preeclampsia, many of the most differentially regulated genes in preeclampsia (*LEP*, *CGB*, *LHB*, *INHA*, *SIGLEC6*, *PAPPA2*, *FLT1*) have predominant or unique expression in the syncytiotrophoblast. Of note, as leptin stimulates gonadotropin (hCG) production through GnRH in first trimester placental explants [48], our results suggest that trophoblastic metabolic signals overactivate the reproductive axis in early-onset preeclampsia and HELLP syndrome. Moreover, enrichment analyses also revealed the over-representation of biological processes, molecular functions and pathways that are related to uteroplacental vascular insufficiency and placental oxidative stress, known to play key roles in the pathophysiology of early-onset preeclampsia [4,7,18].

Placental gene expression changes in HELLP syndrome

Two previous publications presented placental gene expression data in HELLP syndrome obtained by serial analysis of gene expression and by inflammatory cytokine & receptor gene array [44,49]. Here we first describe the placental transcriptome in early-onset HELLP syndrome and the differential expression of 554 genes, the majority of which also had a significant change in the same direction in preeclampsia. This phenomenon may be (1) only the representation of preeclamptic changes in the placenta in both disease groups as all our HELLP syndrome patients had also severe preeclampsia; or (2) the outcome of common pathophysiologic processes leading to shared placental and clinical features in the two syndromes. This latter can only be confirmed by future studies on placental gene expression in patients with HELLP syndrome who do not develop preeclampsia.

There were more genes differentially regulated, and these mostly had a larger extent of fold-change in HELLP syndrome than in preeclampsia, underlining a more severe placental pathology in the latter. The “cytokine-cytokine receptor interaction” pathway was significantly changed only in HELLP syndrome. This contains genes that were differentially regulated in both syndromes, such as *LEP*, *INHBA*, *FLT1* and *ACVRL1*, and were reported to be changed in preeclampsia. Beyond these, *KIT*, *CD70*, *GHR*, *CCL24*, *OSMR*, *CS3CR1*, and *PDGFRFA* were also differentially regulated in this pathway in HELLP syndrome. The up-regulation of *CD70* (cytokine that induces the proliferation of activated T cells and enhances the generation of cytolytic T cells) and *OSMR* (cytokine receptor that transduces oncostatin M and interleukin 31 signaling), and the down-regulation of *CCL24* (cytokine

that has a negative effect on monocytes and activated T lymphocytes) may also reflect a more pronounced inflammatory response in HELLP syndrome than in preeclampsia.

Among the six genes with the largest – yet not statistically significant – fold-change difference between preeclampsia and HELLP syndrome on microarray, we showed the differential expression of *ARHGEF4* and *MGST1* by qRT-PCR. The higher expression of *MGST1* (microsomal glutathione –S-transferase-1) may be compensatory to a larger placental stress in HELLP syndrome. The overexpression of *ARHGEF4* (Rho GTPase) is interesting as this gene was known to be uniquely expressed in the brain [50]. On the other hand, Fig. 1C depicts that most of the differentially regulated genes in HELLP syndrome were down-regulated, while it was not the case among genes differentially expressed in preeclampsia. This indicates a more severe placental insufficiency in HELLP syndrome than in preeclampsia.

Expression of known biomarkers in the placenta in early-onset preeclampsia and HELLP syndrome

Microarray analysis of first trimester chorionic villous samples revealed mostly down-regulated genes in women who subsequently developed preeclampsia when compared to controls [45]. Indeed, most first trimester placental biomarkers had lower blood concentrations in women who later developed preeclampsia (e.g., PP13, PIGF, PAPP-A, ADAM12) [51–53] than in controls, which may also reflect this down-regulation of placental gene expression.

As we cannot extrapolate gene expression differences from the third trimester to the first trimester, it is difficult to derive any conclusion regarding potential new biomarkers early in pregnancy from our results. Nevertheless, from known biomarkers for preeclampsia, we detected seven to have gene expression change both in preeclampsia and HELLP syndrome in our specimens, and all of these changes were in accord with the detected alterations in maternal blood concentrations of the corresponding protein products in preeclampsia in third trimester maternal blood specimen [51–53]: leptin, inhibin A, activin A, sFlt1, hCG, and CRF were overexpressed, while IGF-I was underexpressed. Our results suggest that these biomarkers behave similarly in preeclampsia and HELLP syndrome and may be useful markers for the latter syndrome as well.

Strengths and limitations of the study

This study describes the placental transcriptome of early-onset preeclampsia and HELLP syndrome. The strengths of the study are the following: (1) strict clinical definitions and homogenous patient groups; (2) sample collection from a standardized location on placentas taken from C-sections; and (3) gene and protein expression profiling with different array techniques. A limitation of the study is the number of cases in each group due to the strict clinical and histopathological inclusion criteria used for patient and sample collection, which on the other hand, strengthens the study and its results.

Conclusions

High-throughput transcriptional and tissue microarray expression profiling revealed that the placental transcriptomes in early-onset preeclampsia and HELLP syndrome largely overlap. We propose that a common cause and pathophysiologic processes may lead to shared placental and clinical features in these life-threatening syndromes. However, the gene expression changes between the two may also suggest a more severe placental pathology and a more pronounced inflammatory response in HELLP syndrome than in preeclampsia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Differentially expressed genes in early-onset preeclampsia and HELLP syndrome. Volcano plots show FDR-corrected p -values ($\log_{10}$) of all probe sets plotted against gene expression ($\log_2$) ratios between controls and (A) preeclampsia or (B) HELLP syndrome. y-Axis: values higher than the grey-line threshold represent genes with FDR adjusted p -values < 0.25 . x-Axis: values outside the red lines represent gene expression fold-changes > 2 . (C) Venn diagram shows a large set of genes ($n = 224$) differentially expressed in both preeclampsia and HELLP syndrome. (D) Among these overlapping genes, there is a strong correlation between the direction (83 up- and 141 down-regulated) and the extent of gene expression changes in the two syndromes.

Figure 2.

qRT-PCR validation of differential gene expression. qRT-PCR confirmed the differential expression of four genes in both (A) HELLP syndrome and (B) preeclampsia. (C) From six genes with top fold-change expression difference between preeclampsia and HELLP syndrome on microarray, qRT-PCR validated 2 genes as significantly differentially expressed.

Figure 3. hCG β immunostaining on placental TMAs. hCG β immunostaining on TMAs constructed from placentas taken from women with (A) preterm delivery (31 weeks), (B) early-onset preeclampsia (31 weeks) or (C) HELLP syndrome (30 weeks). Hematoxylin counterstain, 400 $\times$ magnification. (D) Semi-quantitative staining intensity distribution for hCG β in placental microvilli. Figures and statistics show the significant increase $9^*p < 0.05$ in hCG β staining in preeclampsia and HELLP syndrome compared to gestational age-matched controls.

Table 1

Demographic, clinical and histopathological characteristics.

Groups	Term controls	Preterm controls	Controls combined	Early-onset preeclampsia	Early-onset HELLP syndrome
Number of cases ^d	5	5	10	6	6
Maternal age (years) ^b	30.8 (30.6–31.5)	31.6 (31.5–34.3)	31.5 (30.8–34.3)	34.3 (30.2–35.4)	28.7 (24.1–30.4)*
Gestational age at delivery (weeks) ^b	38.9 (38.7–39.0)	31.0 (30.85–34.0)	36.9 (31–38.9)	32.4 (30.3–34.9)	30.7 (29.3–33.1)
Primiparity ^c	40	40	40	66.7	66.7
Smoking ^c	0	20	10	0	0
Systolic blood pressure (mmHg) ^b	130 (120–135)	120 (120–120)	120 (120–133)	160 (160–160)**##	170 (140–170)**##
Diastolic blood pressure (mmHg) ^b	80 (78–89)	80 (70–80)	80 (76–80)	100 (95–105)**##	100 (90–110)**##
Proteinuria ^c	0	0	0	100	100
Maternal BMI (kg/m ²) ^b	26.7 (25.2–28.0)	23.4 (31.6–24.6)	24.9 (21.8–28.0)	24.3 (22.6–24.9)	23.7 (21.2–27.2)
Neonatal birth-weight (g) ^b	3440 (3400–4030)	1990 (1640–2210)	2545 (1990–3440)	1065 (990–1200)##	1235 (930–1610)##
Caesarean section ^c	100	100	100	100	100
Placental weight (g) ^b	518 (458–665)	301 (294–322)	454 (301–518)	214 (210–224)##	255 (161–302)#
Thrombocytes (M/mm ³) ^b	178 (173–238)	227 (170–298)	203 (170–298)	250 (187–360)	77 (71–93)**##
Hgb (g/100 ml)	10 (10–11)	11 (11–11.2)	11 (10–11)	13 (12–13)**##	13 (12–14)
SGOT (U/L) ^{b,d}				39 (25–80)	382 (318–439)
SGTP (U/L) ^{b,d}				39 (23–80)	337 (315–448)
LDH (U/L) ^{b,d}				261 (217–350)	609 (510–1012)
Bilirubin (μmol/l) ^{b,d}				8 (4–14)	21 (18–21)
Small placenta ^c	0	80	40	100	100
Increased syncytial knots ^e	8	6.8	7.3	12.6	12.1
Villous dysmaturity ^c	0	0	0	66	66

All women were Caucasian.

** $p < 0.01$;

* $p < 0.05$ compared to gestational age-matched, preterm controls.

$p < 0.01$;

$p < 0.05$ compared to combined controls.

^a Values are presented as number.

^b Values are presented as median (interquartile (IQR) range).

^c Values are presented as percentage.

^d Routinely not examined in normal pregnant women.

^e Counts per field.

Table 2

The top 20 differentially expressed genes (by fold-change) in the placenta in early-onset preeclampsia.

Gene symbol	Protein name	Adjusted <i>p</i> -value	Fold-change In preeclampsia	References
<i>LEP</i>	<i>Leptin</i>	<i><0.001</i>	<i>108.9</i>	[20,39,42,43,46,47]
<i>CGB</i>	<i>Chorionic gonadotropin, beta polypeptide</i>	<i>0.067</i>	<i>10.2</i>	[20,40,46,47]
<i>TREM1</i>	<i>Triggering receptor expressed on myeloid cells 1</i>	<i>0.015</i>	<i>9.4</i>	[46]
LHB	<i>Luteinizing hormone beta polypeptide</i>	<i>0.085</i>	<i>7.9</i>	[46,47]
SBEM	Small breast epithelial mucin	0.088	7.7	
OPRK1	Opioid receptor, kappa 1	0.022	−7.3	
LTF	Lactotransferrin	0.102	6.5	
HTRA4	HtrA serine peptidase 4	0.131	6.3	
<i>ARHGEF4</i>	<i>Rho guanine nucleotide exchange factor (GEF) 4</i>	<i>0.100</i>	<i>6.0</i>	[46]
KRT81	Keratin 81	0.183	−6.0	
UCA1	Urothelial cancer associated 1	0.085	6.0	
MGST1	Microsomal glutathione S-transferase 1	0.102	−5.9	
SLAMF1	Signaling lymphocytic activation molecule family member 1	0.002	−5.9	
MIG7	Mig-7	0.079	4.9	
WFDC10B	WAP four-disulfide core domain 10B	0.236	−4.9	
<i>PAPPA2</i>	<i>Pappalysin 2</i>	<i>0.073</i>	<i>4.9</i>	[46,47]
BTBD16	<i>BTB (POZ) domain containing 16</i>	0.061	4.8	
SLC14A2	Solute carrier family 14 (urea transporter), member 2	0.144	−4.7	
TMEM63B	Transmembrane protein 63B	0.138	−4.6	
<i>SIBLEC6</i>	<i>Sialic acid binding Ig-like lectin 6</i>	<i>0.019</i>	<i>4.5</i>	[43,46,47]

Genes with the same direction of placental expression change in preeclampsia published by other microarray studies are depicted in italic.

Table 3

Top 20 biological processes over-represented in early-onset preeclampsia.

Rank	Biological process category	Genes in differentially expressed list	Genes in reference array	Adjusted <i>p</i> -value
1	<i>Multicellular organismal process</i>	86	2448	<0.001
2	<i>Anatomical structure morphogenesis</i>	43	951	<0.001
3	<i>Organ development</i>	45	1036	<0.001
4	<i>System development</i>	54	1424	0.003
5	<i>Regulation of biological quality</i>	40	932	0.003
6	<i>Cell-cell signaling</i>	23	415	0.004
7	<i>Multicellular organismal development</i>	63	1827	0.004
8	<i>Anatomical structure development</i>	60	1710	0.004
9	<i>Organ morphogenesis</i>	23	419	0.004
10	<i>Response to external stimulus</i>	28	581	0.005
11	<i>System process</i>	33	748	0.005
12	<i>Developmental process</i>	81	2635	0.008
13	<i>Negative regulation of lymphocyte differentiation</i>	3	5	0.01
14	<i>Regulation of growth</i>	14	203	0.01
15	<i>Keratinization</i>	5	25	0.01
16	<i>Cellular developmental process</i>	43	1171	0.01
17	<i>Angiogenesis</i>	11	139	0.01
18	<i>Blood vessel morphogenesis</i>	12	165	0.02
19	<i>Positive regulation of hormone secretion</i>	4	16	0.02
20	<i>Regulation of hormone secretion</i>	5	29	0.02

Biological processes also over-represented in HELLP syndrome are depicted in italic.

Table 4

The top 20 differentially expressed genes (by fold-change in the placenta in early onset HELLP syndrome).

Gene symbol	Protein name	Adjusted <i>p</i> -value	Fold-change in HELLP syndrome
<i>LEP</i>	<i>Leptin</i>	0.002	56.9
<i>MIG7</i>	<i>Mig-7</i>	0.010	13.0
<i>CGB</i>	<i>Chorionic gonadotropin, beta polypeptide</i>	0.037	12.7
<i>LHB</i>	<i>Luteinizing hormone beta polypeptide</i>	0.051	9.6
<i>ARHGEF4</i>	<i>Rho guanine nucleotide exchange factor (GEF) 4</i>	0.046	9.1
<i>SIGLEC6</i>	<i>Sialic acid binding Ig-like lectin 6</i>	0.004	7.6
<i>HTRA4</i>	<i>HtrA serine peptidase 4</i>	0.114	7.0
<i>TREM1</i>	<i>Triggering receptor expressed on myeloid cells 1</i>	0.022	6.5
<i>SLC16A6</i>	<i>Solute carrier family 16, member 6 (monocarboxylic acid transporter 7)</i>	0.230	6.5
<i>DNAH11</i>	<i>Dynein, axonemal, heavy chain 11</i>	0.025	6.1
<i>KRT81</i>	<i>Keratin 81</i>	0.204	-6.0
<i>NPAS3</i>	<i>Neuronal PAS domain protein 3</i>	0.065	-5.9
<i>PAPPA2</i>	<i>Pappalysin 2</i>	0.039	5.8
<i>LOC650392</i>	<i>Hypothetical protein LOC650392</i>	0.032	-5.6
<i>CEBPA</i>	<i>CCAAT/enhancer binding protein (C/EBP), alpha</i>	0.076	-5.6
<i>SPPL2B</i>	<i>Signal peptide peptidase-like 2B</i>	0.021	-5.3
<i>GREM2</i>	<i>Gremlin 2, cysteine knot superfamily, homolog (Xenopus laevis)</i>	0.021	5.2
<i>BHLHB2</i>	<i>Basic helix-loop-helix domain containing, class B, 2</i>	0.021	5.2
<i>PRSS36</i>	<i>Protease, serine, 36</i>	0.070	-5.2
<i>LOC390413</i>	<i>Similar to 60S ribosomal protein L7</i>	0.022	-5.2

Genes with the same direction of placental expression change in preeclampsia are depicted in italic.

Table 5

Top 20 biological processes over-represented in early-onset HELLP syndrome.

Rank	Biological process category	Genes in differentially expressed list (n)	Genes in reference array (n)	Adjusted <i>p</i> -value
1	<i>Multicellular organismal process</i>	123	2448	<0.0001
2	Regulation of multicellular organismal process	33	471	<0.01
3	<i>Multicellular organismal development</i>	87	1827	<0.01
4	<i>System development</i>	71	1424	0.01
5	<i>System process</i>	44	748	0.01
6	<i>Development process</i>	113	2635	0.02
7	<i>Organ development</i>	54	1036	0.02
8	Extracellular matrix organization	8	48	0.02
9	<i>Anatomical structure development</i>	79	1710	0.03
10	<i>Cell differentiation</i>	50	973	0.04
11	Regulation of system process	13	134	0.04
12	<i>Negative regulation of lymphocyte differentiation</i>	3	5	0.04
13	<i>Response to stimulus</i>	86	1950	0.04
14	<i>Cell-cell signaling</i>	26	415	0.06
15	Extracellular structure organization	9	76	0.06
16	Multicellular organism reproduction	7	47	0.06
17	Reproductive process in a multicellular organism	7	47	0.06
18	Muscle system process	12	131	0.07
19	cAMP biosynthetic process	8	65	0.07
20	<i>Keratinization</i>	5	25	0.07

Biological processes also over-represented in preeclampsia are depicted in italic.