**Metabolomics identifies placental dysfunction and confirms Flt-1 biomarker specificity**

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**Abstract**

Clinical end-stage parameters define the pregnancy disorders preeclampsia and fetal growth restriction (FGR) while classification of the underlying placental dysfunction is missing and urgently needed. FMS-like tyrosine kinase receptor 1 (Flt-1) is the most promising placenta derived predictive biomarker for preeclampsia. We aimed to classify placental dysfunction in preeclampsia and FGR at delivery by metabolic profiling and authenticate the biomarker Flt-1 for placental dysfunction. We studied 143 pregnancies with or without preeclampsia and/or FGR delivered by caesarean section. Metabolic placenta profiles were created by high-resolution magic angle spinning nuclear magnetic resonance spectroscopy (HR-MAS MRS) and the resulting placental phenotypes obtained by hierarchical clustering. Placental Flt-1 expression (membrane-bound and soluble isoforms combined) and maternal serum Flt-1 expression (soluble isoforms) were analyzed by immunohistochemistry and ELISA, respectively. We identified three distinct placenta groups by 21 metabolites and diagnostic outcome parameters; normal placentas, moderate placental dysfunction and severe placental dysfunction. Increased placental Flt-1 was associated with severe placental dysfunction and increased serum Flt-1 was associated with moderate and severe placental dysfunction. The preeclamptic pregnancies with and without placental dysfunction could be distinguished by five metabolites and placental Flt-1. Placental Flt-1 alone could separate normal pregnancies with and without placental dysfunction. In conclusion, metabolomics could classify placental dysfunction and provide information not identified by traditional diagnostics and metabolites with biomarker potential were identified. Flt-1 was confirmed as precision biomarker for placental dysfunction, substantiating its usefulness for identification of high-risk pregnancies for preeclampsia and FGR with placental involvement.

Keywords: Fetal growth restriction, Flt-1, metabolomics, placenta, preeclampsia

## Introduction

Preeclampsia affects 3-4% of pregnancies[1,2] and poses a serious threat to maternal and fetal health. Preeclampsia is described as excessive maternal inflammatory response to a dysfunctional placenta or the vascular load of pregnancy itself.[3,4] That some women handle placental insufficiency without developing preeclampsia, adds to the complexity of defining placental involvement.[5] Preeclamptic pregnancies are often complicated by fetal growth restriction (FGR). Both entities involve placental dysfunction, but only preeclampsia develops into maternal disease.[4] Diagnostic consensus for preeclampsia is lacking. The disease is commonly diagnosed by new onset maternal hypertension and proteinuria and if present, other severe symptoms such as liver or kidney dysfunction.[1] Preeclampsia may be subclassified as non-severe or severe, early and late onset, or by degree of FGR, signs inappropriate for identifying early pathological placental development. Still, early onset and severe preeclampsia are frequently used as proxy for preeclampsia with placental involvement.[6,7] Characterization of the underlying placental dysfunction is needed to separate preeclamptic pregnancies of fetal and maternal origin, provide better understanding of the causal relationships between preeclampsia and FGR, and improve the diagnostic and prognostic precision for preeclampsia.

Although risk factors like preexisting medical conditions or previous preeclampsia are recognized, the majority of preeclampsia occurs in primigravida and remains largely undetected until late gestation.[8] FMS-like tyrosine kinase receptor 1 (Flt-1) is the most promising placenta derived predictive biomarker for preeclampsia.[9] When released to the maternal circulation, soluble Flt-1 acts as growth factor antagonist and vasoconstrictor and sensitizes the endothelium for stress response and endothelial dysfunction.[10] Placental and serum Flt-1 levels are increased in preeclampsia and higher levels are associated with more severe disease.[11-13] Still, the predictive value of maternal serum Flt-1 for preeclampsia is suboptimal.[9] We hypothesise that Flt-1 is a specific diagnostic biomarker for placental dysfunction and not a biomarker for preeclampsia in general.[14]

Metabolites are chemical intermediates or end-products reflecting cellular processes, environmental influence and disease development. Metabolomic approaches show promising clinically potential in many diseases.[15-17] We have previously used metabolomics to identify disease specific processes in preeclampsia and FGR in serum,[18] urine[18] and placental tissue.[19] Our robust assay for profiling placental tissue by high-resolution magic angle spinning nuclear magnetic resonance spectroscopy (HR-MAS MRS), has proven useful for revealing distinct phenotypes of healthy and preeclamptic pregnancies.[19] This study aimed to classify placental dysfunction in preeclampsia and FGR at delivery by HR-MAS MRS and authenticate the biomarker Flt-1 for placental dysfunction.

## Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### Study participants, placental biopsies and serum samples

Women with singleton pregnancies delivering without labor by caesarean section were recruited at St. Olavs and Haukeland University Hospitals between 2002 and 2012. Pregnant women with preeclampsia and/or FGR and healthy pregnant women with no history of preeclampsia, gestational hypertension or FGR were included. Women with preexisting or gestational diabetes mellitus were not included. Preeclampsia was defined as persistent hypertension (blood pressure ≥140/90 mmHg) plus proteinuria (≥0.3 g/24 hours or ≥+1 by dipstick) developing after 20 weeks of gestation.[2] Women with pre-gestational hypertension developing proteinuria in the second half of pregnancy were diagnosed with superimposed preeclampsia and included as preeclamptic. Preeclampsia was classified as severe or non-severe according to presence of severe features (Table S1).[2,6] Early onset preeclampsia manifested before 34th gestational week and preterm delivery was defined as birth <37th gestational week. FGR was diagnosed by serial ultrasound measurements indicating reduction of weight gain, or birth weight <5$^{th}$ percentile of Norwegian reference curves.[20]

Maternal venous blood was collected prior to delivery, clotted for ≤30 minutes, centrifuged at 1800G for 10 minutes, and serum stored at -80°C. A tangential, central section (100 mg) from the maternal side of the placenta was processed within 90 minutes after delivery and visible blood was carefully wiped off. Samples were frozen (in liquid nitrogen or -80°C) for metabolomics and fixed (10% neutral-buffered formalin) and paraffin embedded for immunohistochemistry.

The Norwegian Regional Committee for Medical and Health Research Ethics (REC 2012/1040) approved the study and written informed consent was obtained from participants. Procedures were in accordance with institutional guidelines.

### Metabolomics analyses

Placental biopsies (9.8±2.2 mg) were analyzed by a previously developed HR-MAS MRS based assay for profiling placental variants of metabolic expression. The analysis was performed randomly and blinded to pregnancy outcomes. The method was previously performed and validated in a subset of pregnancies ($n$=43) included in the current study.[19]

### Flt-1 detection and quantification

Tissue sections of 3 µm were pre-treated in PT link (#PT101, Dako) using Target Retrieval Solution (#K8004) at 97°C for 20 minutes. Sections were incubated with peroxidase blocking solution for 10 minutes (#K4011, Dako), Flt-1 primary antibody (detecting membrane-bound and soluble isoforms combined) (1:175, # ab32152, Abcam) for 40 minutes at room temperature, and HRP-labeled polymer (#4011, Dako) for 30 minutes. DAB+ (1:50, #K4011, Dako) was used as chromogen with two five-minute incubations. Staining was performed using

Autostainer Plus (#S3800, Dako) and slides counterstained with hematoxylin. Tissue sections stained for negative isotype control (#3900, Cell Signaling) were included. Microscopic images (bright field) at two locations per placental tissue section were taken of randomly selected areas with well-preserved placenta morphology at 20x magnification with an Eclipse E400 microscope with a DS-Fi1 camera (Nikon). The syncytiotrophoblast covering villous trees was delineated in a binary layer by manual adjustment. This binary layer defined the area included in automatic assessment of intensity values (statistical mean of intensity pixel values). The staining intensity score and protein expression level were inversely proportional. Staining intensity of Flt-1 in the syncytiotrophoblast layer of mature villi was quantified in 35 normal and 29 preeclamptic pregnancies blinded for pregnancy outcome by NIS-Elements BR 4.0 (Nikon).

Soluble Flt-1 in maternal serum was measured in duplicate by quantitative sandwich ELISA according to the manufacturer's instructions (#DVR100B, R&D Systems, Abingdon, UK).

## Statistical methods

Spectra were preprocessed and metabolites assigned as previously described[19] and quantified by integrating metabolite spectral regions. Multivariate and cluster analysis was performed in PLS-Toolbox 7.3.1 (Eigenvector Research Inc., WA, USA) and quantitative enrichment analysis in MetaboAnalyst.[21] Hierarchical clustering of metabolic profiles was performed using standard Euclidian distance measure and Wards clustering. Further information on preprocessing of metabolite data and multivariate statistical analyses are available in the online-only Data Supplement.

Clinical characteristics were compared between placenta groups and diagnostic groups with Fisher's exact test or Kruskal-Wallis tests. Linear regression models were used to correct for gestational age differences. Placental Flt-1 expression was affected by inclusion location and evaluated by a linear regression model with location as random effect. Prediction of placenta groups by serum Flt-1 levels was carried out by binary logistic regression and receiver-operator characteristic (ROC) analysis. All *P*-values were adjusted for multiple comparisons using Benjamini-Hochberg false discovery rate $q \leq 0.05$.

## Results

Pregnancies with preeclampsia or FGR were delivered earlier and with reduced placenta and birthweights compared to normal pregnancies as expected ($P \leq 0.001$) (Table 1). Characteristics of the traditional preeclampsia subclasses (presence of FGR, severity of maternal symptoms and time of onset) are shown (Table S2).

Hierarchical clustering of the metabolic profiles revealed three distinct groups that could be phenotypically evaluated by diagnostic presentation and metabolic expression profiles. The placenta groups were termed: the normal placenta group, the moderate placental dysfunction group, and the severe placental dysfunction group (Figure 1A and 1B). The novel placenta groups were assessed for placental Flt-1 expression (Figure 2), maternal serum Flt-1 expression, and distribution of preeclampsia and FGR diagnoses (Figure 1C-F). The normal placenta group was dominated by normal pregnancies (87%), and comparison with the two placental dysfunction groups (Figure 1) revealed significantly lower number of preeclamptic and FGR pregnancies, lower systolic blood pressure, and higher placenta weight and gestational age at delivery (Table 1). Six of the 47 pregnancies (13%) in the normal placenta group were complicated with preeclampsia and/or FGR, and their clinical characteristics did not differ from the rest of the preeclamptic and/or FGR pregnancies. The moderate placental dysfunction group contained almost all the late onset preeclamptic pregnancies and a substantial number of normal pregnancies (34%). Compared to the moderate placental dysfunction group, the severe placental dysfunction group included more women with early onset preeclampsia (62% vs 41%, $P$=0.010), FGR (73% vs 42%, $P$=0.006), preterm delivery (91% vs 58%, $P$=0.003) and lower placenta weight ($P$=0.007) (Figure 1 and Table 1). Importantly, the moderate and severe placental dysfunction groups could not be distinguished by the diagnosis severe preeclampsia (45% vs 51%, $P$=0.533). Furthermore, normal pregnancies in the moderate placental dysfunction group had higher diastolic blood pressure compared to the normal pregnancies in the severe placental dysfunction group ($P$=0.044) (Table 1).

Six percent ($n$=4) of all preeclampsia cases ($n$=62) were in the normal placenta group, while 94% of the preeclampsia cases showed placental dysfunction (Table 1). Likewise, 7% ($n$=4) of all FGR pregnancies ($n$=56) had normal placentas, while 93% of the FGR pregnancies were associated with moderate or severe placental dysfunction. Interestingly, the majority of the less severe preeclampsia diagnoses presented with placental dysfunction (91% of the late onset preeclamptic cases, 93% of the non-severe preeclamptic cases, and 91% of the preeclamptic pregnancies without FGR).

Metabolite levels in the three placenta groups are shown in Figure S1 and Table 3, and in relation to traditional preeclampsia and/or FGR diagnoses in Table S3. Tissue processing time did not differ between normal and preeclamptic pregnancies ($P$=0.278) or between the three placenta groups ($P$=0.936). Quantitative enrichment analysis was performed to assess the biological differences between placenta groups. Samples in the normal placenta group were significantly different from the placental dysfunction groups in 21 metabolites. These metabolites are involved in several relevant metabolic pathways including valine, leucine and isoleucine (branched-chain amino acids, BCAA) degradation; the glycine, serine and threonine metabolism; and the phospholipid biosynthesis (Table S4), directly confirming the metabolic profile of normal pregnancies from our previous establishment of the HR-MAS MRS assay.[19] Affected pathways in the moderate placental dysfunction group included: valine, leucine and isoleucine, the glucose-alanine cycle, urea cycle, glycolysis, gluconeogenesis and insulin signaling (Table S5). Pathways distinct to severe placental dysfunction were phospholipid

biosynthesis, arginine and proline metabolism, glycine, serine and threonine metabolism, and bile acid biosynthesis (Table S6).

Severe placental dysfunction was strongly associated with increased placental Flt-1 expression, and serum Flt-1 was increased in both placental dysfunction groups compared to normal placentas (Figure 1 and Table 4). Assessing preeclamptic pregnancies only, high placental Flt-1 expression was still highly specific for severe placental dysfunction (Table 4). Logistic regression analysis revealed that preeclampsia with severe placental dysfunction could be identified with 100% sensitivity and 81% specificity for serum Flt-1 ≥945 pg/mL. Five metabolite biomarkers could distinguish between preeclamptic pregnancies with normal placentas and preeclamptic pregnancies with placental dysfunction (Table 3). Normal pregnancies with placental dysfunction showed lower placental and serum Flt-1 expression compared to normal pregnancies with normal placentas. In the traditional diagnostic groups, placental Flt-1 showed a mixed pattern by being significantly increased in some preeclampsia groups (overall, non-severe, with FGR and early onset), but not in others (severe, without FGR and late onset) (Table 4). This pattern could be explained by significantly higher placental Flt-1 expression only in the subgroups containing more than 40% pregnancies with severe placental dysfunction (not shown), underpinning the importance of assessing placental dysfunction in the classification of preeclampsia and FGR.

## Discussion

Reliable classification of placental dysfunction in preeclampsia and FGR are lacking, making biomarker discovery for early identification of high-risk women nearly impossible. We present NMR-based metabolic profiling as a sensitive tool for placental classification into three groups: normal placentas, moderate placental dysfunction with higher fraction of late onset preeclampsia and fewer FGR pregnancies, and severe placental dysfunction with higher fraction of early onset preeclampsia and FGR pregnancies. Placental function was defined by 21 novel metabolite biomarkers, five specific for preeclamptic pregnancies with normal placentas. Severe placental dysfunction displayed higher placental Flt-1 levels, confirming placental Flt-1 as sensitive marker for severe placental dysfunction, and serum Flt-1 as marker for moderate and severe placental dysfunction. The ability to identify preeclampsia with severe placental dysfunction by serum Flt-1 substantiates the potential of using circulating biomarkers to identify placental dysfunction.

This novel placenta classification provides information about pregnancies that cannot be identified by traditional diagnostics. Most normal pregnancies presented with normal placentas, while a few showed placental dysfunction. These few verify the clinical observation that some women manage vascular stress better than others.[14] Reduced placental Flt-1 expression in these pregnancies may reflect compensatory mechanisms preventing escalation to maternal disease. To our knowledge this is the first proof of distinct placenta phenotypes in preeclamptic pregnancies and identification of preeclamptic pregnancies with normal placentas. While undetected by established diagnostic tools, we provide five placental metabolite biomarkers specific for preeclampsia with normal placentas, but due to low case numbers this needs further validation. The data confirms presence of placental dysfunction in a large portion of late onset and non-severe preeclampsia. Identification of severe placental dysfunction in early onset and severe preeclampsia confirms the current understanding of the underlying pathogenesis. Still, the data warrants caution for using severe or early onset preeclampsia as a proxy for severe placental dysfunction.[6,7] A role for placental dysfunction in FGR is agreed but far from defined,[22] and it is particularly interesting that the FGR cases mainly presented with severe placental dysfunction.

Placental Flt-1 expression provided strong specificity for severe placental dysfunction, although serum Flt-1 identifying overall placental dysfunction has stronger diagnostic value.[23] The main placental source of Flt-1 is activated syncytiotrophoblasts and soluble isoforms of Flt-1 are released to the maternal circulation.[24,25] Different ratios of placental to serum Flt-1 may point to variation in placental expression of membrane-bound and soluble isoforms of Flt-1[11] between the two placental dysfunction groups, or contribution to serum Flt-1 from other sources in the moderate placental dysfunction group. Placental Flt-1 was measured as a combination of the Flt-1 isoforms and the specific isoform distribution still needs to be determined.

The specificity of placental Flt-1 for severe placental dysfunction and not for the classical diagnosis severe preeclampsia, reinforces the inadequacy of presently used diagnostic tools. Until now, serum Flt-1 has been considered a suboptimal predictive marker for preeclampsia, although the serum Flt-1 to placental growth factor ratio has shown promising results even in primigravidas.[26] Our study recognizes the usefulness of serum Flt-1 detection for identifying pregnancies with placental dysfunction and for separating these from pregnancies without placental dysfunction among normal and preeclamptic pregnancies. This addresses a need for clinical studies separately targeting prediction of preeclampsia with and without placental dysfunction.

Flt-1 and metabolite expression in the placenta groups may help elucidate underlying pathological processes. Taurine depletion is a marker of placental dysfunction,[27] and for preeclamptic pregnancies with normal placentas, taurine, threonine and lysine remained elevated compared to the placental dysfunction groups, supporting a normal placental function.

Compared to the severe placental dysfunction group, the moderate placental dysfunction group included women with later onset preeclampsia, higher placenta weight and birthweight, and the normal pregnancies in this group showed higher maternal systolic blood pressure. Likewise, moderate placental dysfunction was characterized by dysregulation of BCAA and alanine, metabolites associated with obesity, insulin resistance and preeclampsia.[28,29] From this, we hypothesize that moderate placental dysfunction is associated with a strong maternal disease component. Importantly, a maternal systemic immune activation directly affects the placenta by inflammatory activation at the syncytium[30] and may contribute to the altered placental metabolite profile in this group. A moderate placental dysfunction at delivery may therefore reflect harmful interaction between placental tissue and maternal blood and not merely insufficient placental development. This is further supported by the relatively higher serum to placental Flt-1 ratio in this group, suggesting added maternal contribution to serum Flt-1 levels.

Severe placental dysfunction was characterized by increased placental creatine and phosphocholine, and decreased ethanolamine. Phosphocreatine breakdown generates creatine and adenosine triphosphate (ATP).[31] Increased placental ATP likely generated in response to preeclamptic hypoxia, reflects the severity of placental stress and provides a biologic correlation to the strong placental Flt-1 expression in this group.[32] Increased phosphocholine and decreased ethanolamine represent changes in the phospholipid metabolism, previously linked to placental complications in preeclampsia.[19] Consequently, we hypothesize that preeclamptic pregnancies classified with severe placental dysfunction show a stronger placental contribution to disease development.

HR-MAS MR spectroscopy gives robust qualitative and semi-quantitative information with minimal sample handling and objective profiling of disease-related processes. Although five placenta groups have previously been revealed by transcriptomics, one with severe disease and increased Flt-1 gene expression,[33] our data represent the first use of metabolomics for placental dysfunction classification. The similarities from different “omics” techniques indicate biological reliability.[34] The robustness of HR-MAS MR analysis supports clinical usefulness for assessment of placental dysfunction. A potential influence on the placental metabolome cannot be excluded for traces of blood from sampling or from antenatal corticosteroids administered to women delivering preterm. Placental dysfunction can only be determined at delivery due to safety concerns and lack of placental sampling protocols during pregnancy, but may have implications for future pregnancies and maternal vascular disorders.

## Perspectives

We have established a metabolomics-based novel classification of placental dysfunction. The moderate placental dysfunction group was characterized by BCAA deficiency and high serum to placental Flt-1 ratio indicating added maternal contribution to the disease, while the severe placental dysfunction group showed a major placental burden by strong placental Flt-1 expression and altered placental energy and phospholipid metabolism. This targeted identification of placental phenotypes provides novel pathologic information not identified by the clinical signs used for preeclampsia and FGR diagnostics today. This new classification of

placental dysfunction can be used to identify biomarkers, increase knowledge on pathophysiological processes, and perform targeted research on underlying disease mechanisms. Importantly, this study shows that the severe preeclampsia diagnosis does not identify severe placental dysfunction and proves that placental and serum Flt-1 are strong biomarkers for placental dysfunction. This study further provides rationale for inclusion of maternal serum Flt-1 in prediction of high-risk pregnancies for preeclampsia and/or FGR with placental involvement.

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**Disclosures**

None.

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## Novelty and Significance

What is New?

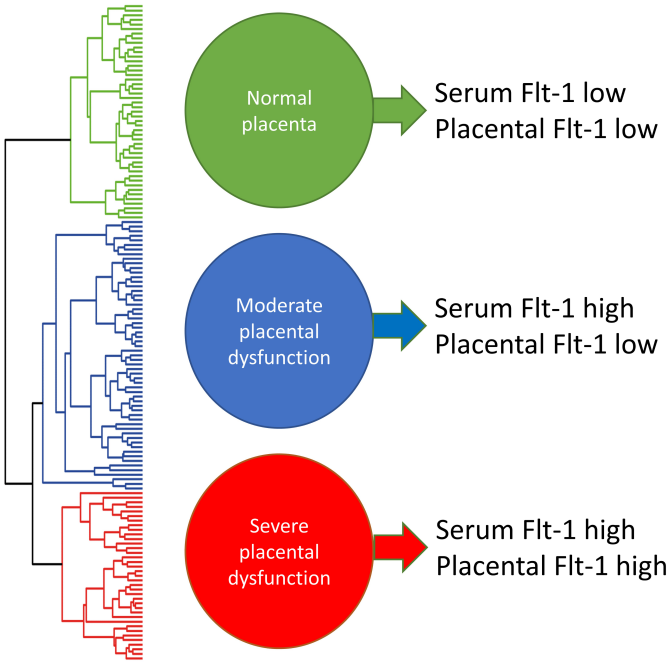
- This is the first use of metabolomics for classification of placental dysfunction.
- Metabolic placenta profiling by magnetic resonance spectroscopy differentiates normal placentas from dysfunctional placentas across traditional diagnostic groups.
- Placental function can be defined by 21 novel metabolite biomarkers, five specific for preeclamptic pregnancies with normal placentas.
- The anti-angiogenic protein Flt-1 is found to be a specific diagnostic biomarker for placental dysfunction and not a biomarker for preeclampsia in general.

What is Relevant?

- There is urgent need for classification of distinct placenta phenotypes in preeclampsia and fetal growth restriction (FGR).
- This sensitive classification system can improve diagnostic subtyping in pathophysiological studies and authenticate diagnostic biomarkers for preeclampsia and FGR.
- Characterization of placental dysfunction is needed to separate preeclamptic pregnancies of fetal and maternal origin and provide better understanding of the causal relationship between preeclampsia and FGR.

Summary
Metabolic profiling could classify placental dysfunction and provide information not identified by traditional diagnostics. Metabolites with biomarker potential in subgroups of preeclampsia and FGR were identified. Flt-1 was confirmed as precision biomarker for placental dysfunction, substantiating its usefulness for identification of high-risk pregnancies for preeclampsia and FGR with placental involvement.

## Graphical abstract



## Figures

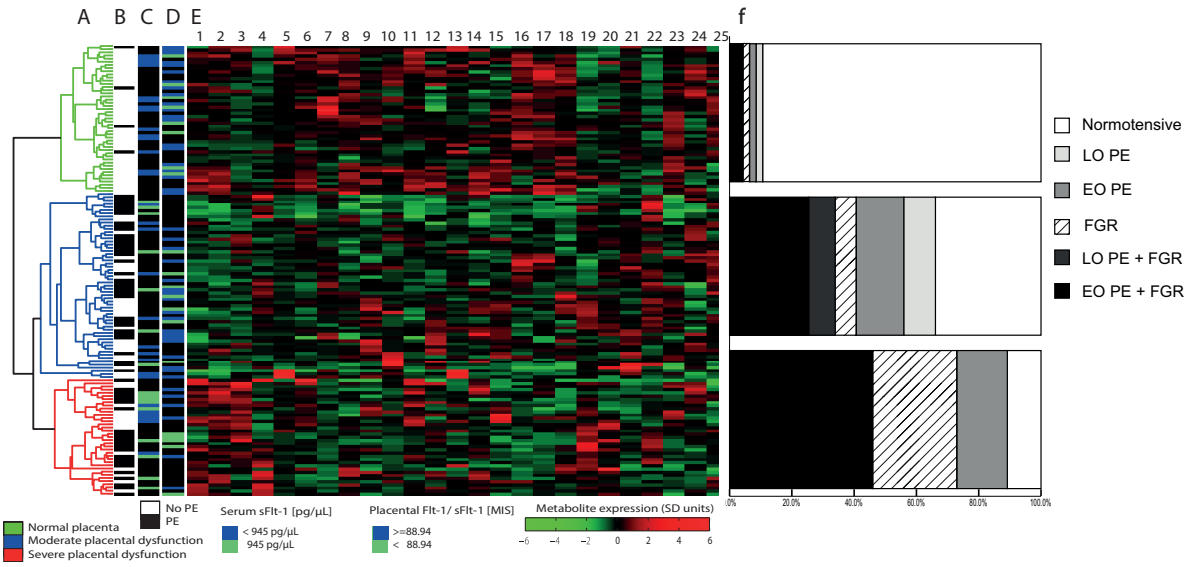



**Figure 1.** Three distinct metabolic placenta groups were revealed by hierarchical clustering and termed the normal placenta group, moderate placental dysfunction group and severe placental dysfunction group. **A**, Dendrogram from the clustering shows splitting into three metabolic subgroups with 47, 59 and 37 samples, respectively. The distribution in metabolic placenta groups is shown for pregnancy diagnosis (**B**), maternal serum Flt-1 levels (sFlt-1) (**C**), and placental Flt-1 levels (**D**). **E**, Metabolite levels are shown scaled by standard deviation (SD). Metabolites listed: 1, Leucine; 2, Alanine; 3, Creatine; 4, Aspartate; 5, Lysine; 6, Isoleucine; 7, Dihydroxyacetone; 8, Threonine; 9, Ascorbic acid; 10, 3-hydroxybutyrate; 11, Valine; 12, Succinate; 13, Acetate; 14, Glutamine; 15, Glucose; 16, Ethanolamine; 17, Glycine; 18, Glutamate; 19, Phosphocholine; 20, Myo-inositol; 21, Glycerol; 22, Glycerophosphocholine; 23, Taurine; 24, Lactate; 25, Choline. **F**, Bar charts illustrating distribution of the classical subdiagnoses of preeclampsia (EO, early onset; FGR, fetal growth restriction; LO, late onset; Normal, normal pregnancies; PE, preeclampsia). The normal placenta group was dominated by normal pregnancies and the two placental dysfunction groups revealed a higher number of preeclamptic and FGR pregnancies, with presence of more severe diagnostic subgroups in the severe placental dysfunction group.

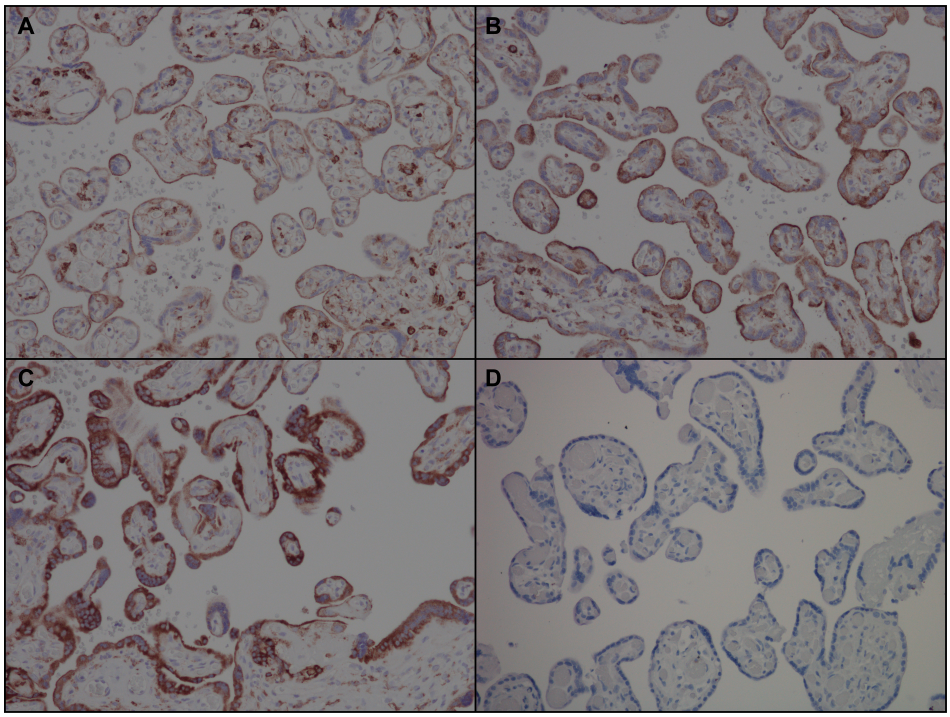



**Figure 2.** FMS-like tyrosine kinase-1 (Flt-1) is expressed in the placenta of normal and preeclamptic pregnancies with or without fetal growth restriction (FGR). Flt-1 protein was detected in third trimester placentas by immunohistochemistry and representative images shown for 35 normal pregnancies (**A**), 10 preeclamptic pregnancies without FGR (**B**), and 19 pregnancies with preeclampsia and FGR (**C**). Negative isotype control was included (**D**).

## Tables

### Table 1. Clinical characteristics of the diagnostic groups

| Characteristic | Normal pregnancies (n=65) | Preeclampsia (n=62) | FGR (n=16) |
|---|---|---|---|
| BMI, kg/m$^2$* | 24.8 (17.9-37.3) | 25.4 (19.8-41.18) | 26.6 (18.0-39.7) |
| SBP, mm Hg† | 120 (92-150) | 150 (118-220)‡ | 130 (100-170) |
| DBP, mm Hg† | 78.5 (56-113) | 94.5 (60-160)‡ | 72.5 (57-121) |
| FGR, n (%) | n.a. | 40 (64.5) | 16 (100) |
| Severe PE, n (%) | n.a. | 49 (79) | n.a. |
| Late onset PE, n (%) | n.a. | 11 (18) | n.a. |
| Superimposed PE, n (%) | n.a. | 4 (7) | n.a. |
| Characteristic at delivery | | | |
| Maternal age, y | 33 (21-43) | 29 (20-40) | 34 (21-41) |
| Gestational age, wk | 39 (37-42) | 32 (23-38)‡ | 33 (24-39)‡ |
| Placenta weight, g | 620 (450-950) | 285 (100-750)‡ | 300 (88-312)‡ |
| Birthweight, g | 3610 (2690-4700) | 1304 (410-3470)‡ | 1330 (290-2720)‡ |
| Fetal sex, female, n (%) | 35 (54) | 33 (53) | 6 (38) |

BMI indicates body mass index; DBP, diastolic blood pressure; FGR, fetal growth restriction; n.a., not applicable; PE, preeclampsia; SBP, systolic blood pressure.

Categorical variables listed as number (percent in column), assessed for differences between groups by Fisher's exact test. Continuous variables listed as median (min-max), assessed for differences between groups by Mann-Whitney U tests.

*Maternal BMI in first trimester, missing from 10 women (7%)

†Blood pressure from last healthcare visit before delivery, missing from 13 women (9.1%)

‡Significantly different from normal pregnancies ($P$<0.001)

**Table 2. Clinical characteristics of the three metabolic placenta groups**

| Characteristic | Normal placenta (n=47) | Moderate placental dysfunction (n=59) | Severe placental dysfunction (n=37) |
|---|---|---|---|
| BMI, kg/m$^2$* | 24.9 (19.0-37.3) | 24.7 (18.0-40.1) | 25.5 (18.0-41.2) |
| SBP, mm Hg† | 125 (92-186) | 140 (100-200)‡ | 142 (101-220)‡ |
| DBP, mm Hg† | 78 (56-113) | 80 (57-160) | 79 (57-160) |
| Normal pregnancy, n (%) | 41 (87) | 20 (34) | 4 (11) |
| PE, n (%) | 4 (9) | 35 (59)‡ | 23 (62)‡ |
| FGR, n (%) | 4 (7) | 25 (42)‡ | 27 (73)‡§ |
| Non-severe PE, n (%) | 1 (2) | 8 (14) | 4 (11) |
| Severe PE, n (%) | 3 (6) | 27 (46)‡ | 19 (51)‡ |
| Early onset PE, n (%) | 3 (6) | 24 (41)‡ | 23 (62)‡§ |
| Late onset PE, n (%) | 1 (2) | 10 (17)‡ | 0 (0)§ |
| PE without FGR, n (%) | 2 (4) | 14 (24) | 6 (16) |
| PE with FGR, n (%) | 2 (4) | 21 (36)‡ | 17 (46)‡ |
| Characteristic at delivery | | | |
| Maternal age, y | 33 (23-39) | 28 (21-43) | 33 (20-41) |
| Gestational age, wk | 38 (24-42) | 35 (24-41)‡ | 31 (23-39)‡§ |
| Placenta weight, g | 600 (520-900) | 430 (88-950)‡ | 275 (150-900)‡§ |
| Birthweight, g | 2720 (410-4700) | 2610 (430-3950)‡ | 2410 (290-4500)‡§ |
| Fetal sex, female, n (%) | 26 (55) | 30 (51) | 18 (49) |

BMI indicates body mass index; DBP, diastolic blood pressure; FGR, fetal growth restriction; PE, preeclampsia; SBP, systolic blood pressure.

Categorical variables listed as number (percentage in placenta group), assessed for differences by Fisher's exact test. Continuous variables listed as median (min-max), assessed for differences by Mann-Whitney U tests (two way comparisons).

*BMI calculated from first trimester maternal weight, missing from 10 women (7%)

†Blood pressures from last healthcare visit before delivery, missing from 13 women (9%)

‡Significantly different from normal placentas ($P$<0.05)

§Significantly different from moderate placental dysfunction ($P$<0.05)

**Table 3. Comparison of metabolite levels in placenta groups**

| Metabolite | Normal placenta (n=47) | Moderate placental dysfunction (n=59) | Severe placental dysfunction (n=37) |
|---|---|---|---|
| Phospholipid metabolism, glycerolipid metabolism | | | |
| Ethanolamine | 9.8 (2.5)* | 7.3 (2.8)† | 5.7 (1.6)† |
| Glycerophosphocholine | 27.4 (11.3)* | 51.1 (19.3)† | 41.4 (17.5)† |
| Phosphocholine | 15.0 (3.9)* | 18.3 (4.9)† | 22.3 (5.9)† |
| Choline | 92.8 (15.4) | 92.0 (14.8) | 80.6 (16.8)† |
| Glycerol | 22.8 (3.4) | 24.8 (8.3) | 18.3 (3.6)† |
| Dihydroxyacetone | 2.0 (1.3)* | 1.4 (0.7) | 0.7 (0.4) |
| Taurine metabolism, urea cycle, bile acid biosynthesis | | | |
| Glutamine | 6.5 (1.5) | 5.9 (1.6)† | 6.7 (1.4)† |
| Aspartate | 7.3 (1.9)* | 8.8 (3.5)† | 10.3 (5.3)† |
| Taurine | 39.2 (4.7)*‡ | 30.7 (7.5)† | 31.4 (7.2)† |
| Acetate | 4.6 (1.4)*‡ | 4.4 (1.8) | 3.9 (1.4)† |
| Branched-chain amino acid degradation, lysine degradation | | | |
| Isoleucine | 2.3 (0.4)* | 1.7 (0.5)† | 2.1 (0.8)† |
| Lysine | 12.6 (2.9)*‡ | 10.7 (6.3)† | 10.3 (1.9)† |
| Leucine | 13.7 (1.4)* | 11.8 (1.3)† | 14.3 (1.9) |
| Valine | 4.5 (0.7)* | 3.4 (0.7)† | 4.4 (1.0) |
| Glucose-alanine cycle, glycolysis, insulin signaling | | | |
| Alanine | 11.2 (1.9)* | 10.3 (1.9)† | 11.9 (2.3) |
| Glucose | 6.0 (2.1)* | 5.0 (2.0)† | 4.9 (3.4)† |
| Myoinositol | 16.7 (2.9)* | 18.4 (3.0)† | 19.6 (6.8)† |
| 3-Hydroxybutyrate | 2.8 (0.2)* | 2.8 (2.6) | 1.7 (1.1)† |
| Ascorbate | 3.4 (0.5)* | 3.1 (0.9) | 2.9 (0.8)† |
| Glutamate | 23.9 (2.3)* | 20.4 (3.6) | 20.5 (3.1) |
| Lactate | 47.6 (7.4) | 45.0 (8.9) | 45.4 (7.2) |
| Succinate | 5.0 (1.1) | 4.8 (1.2) | 4.9 (0.9) |
| Glycine, serine and threonine metabolism | | | |
| Threonine | 3.3 (0.5)*‡ | 2.6 (0.5)† | 2.8 (0.6)† |
| Glycine | 9.5 (1.8)*‡ | 7.3 (1.7)† | 7.0 (1.3)† |
| Creatine | 8.1 (2.9)* | 8.1 (3.1) | 12.9 (5.1)† |

Metabolite concentrations, mean (standard deviation) of normalized area under the spectrum curve. Differences between groups (normal placenta to placental dysfunction combined, and normal placenta to moderate or severe placental dysfunction) were assessed with Mann-Whitney U tests, adjusted for multiple comparisons. Metabolites groups are based on the Small Molecule Pathway Database (www.smpd.ca).

*Significantly different between normal placenta and the combined placental dysfunction groups ($P$<0.05). Aspartate, glucose, 3-hydroxybutyrate and creatine were not significantly different after correction for gestational age

†Significantly different to normal placenta ($P$<0.05). Aspartate, glucose, lysine (moderate placental dysfunction), myoinositol, isoleucine, 3-hydroxybutyrate (severe placental dysfunction) were not significantly different after correction for gestational age

‡Significantly different for preeclamptic pregnancies with normal placenta compared to preeclamptic pregnancies with placental dysfunction ($P$<0.05)

**Table 4. Preeclampsia biomarker Flt-1 levels related to metabolic placenta groups, diagnoses and preeclampsia subgroups**

| Group (n placenta / n serum) | Placental Flt-1 (MIS) | Serum Flt-1 (pg/mL) |
|---|---|---|
| Metabolic placenta groups | | |
| Normal placenta (n=28/14) | 94.8 (14) | 317 (365) |
| Moderate placental dysfunction (n=25/17) | 96.1 (1.5) | 909 (1410)§ |
| Severe placental dysfunction (n=11/15) | 84.5 (2.3)* | 949 (1433)§ |
| Diagnosis with placenta groups | | |
| Normal pregnancies with normal placentas (n=26/12) | 94.3 (1.1) | 357 (318) |
| Normal pregnancies with placental dysfunction (n=9/5) | 100.6 (1.8)† | 272 (167) |
| PE with moderate placental dysfunction (n=18/13) | 93.9 (1.2) | 1752 (895)† |
| PE with severe placental dysfunction (n=9/9) | 83.9 (2.7)† | 1752 (1290)† |
| PE with either placental dysfunction (n=27/22) | 90.4 (1.5) | 1752 (881)† |
| Diagnosis | | |
| Normal pregnancies (n=35/17) | 95.9 (1.3) | 314 (250) |
| PE (n=29/23) | 91.3 (1.5)‡ | 1752 (893)‡ |
| FGR (n=0/6) | -- | 304 (337) |
| Traditional PE subgroups | | |
| Non-severe PE (n=7/2) | 85.3 (3.8)‡ | 2245 (-)‡ |
| Severe PE (n=22/21) | 93.1 (2.2) | 1744 (858)‡ |
| PE without FGR (n=10/8) | 95.2 (3.1) | 948 (707)‡ |
| PE with FGR (n=19/15) | 88.7 (2.3)‡ | 1752 (916)‡ |
| Early onset PE (n=21/21) | 89.8 (2.2)‡ | 1752 (885)‡ |
| Late onset PE (n=8/2) | 94.4 (3.6) | 894 (-) |

FGR indicates fetal growth restriction; Flt-1, FMS-like tyrosine kinase receptor 1; MIS, mean intensity score (higher intensity corresponds to lower concentrations); PE, preeclampsia. Placental Flt-1 shows estimated means (std. error) and *P*-values from general linear model with placental Flt-1 as dependent variable, grouping as fixed factor, and recruitment location as random factor. Serum Flt-1 shows medians and interquartile ranges and *P*-values from Mann-Whitney U or Kruskal-Wallis tests.

*Significantly different from normal placenta and moderate placental dysfunction ($P<0.05$)

†Significantly different from normal pregnancies with normal placentas ($P<0.05$)

‡Significantly different from normal pregnancies ($P<0.05$)

§Significantly different from normal placenta ($P<0.05$)

**ONLINE SUPPLEMENT**

## Supplementary Methods

### Preprocessing of metabolite data

Spectra were Fourier transformed into 65.5k points following 0.3Hz line broadening, automatically phased and baseline corrected, and referenced to the alanine peak at δ1.485 (Topspin 3.1, Bruker Biospin GmbH). Further processing of the spectra was performed in Matlab r2013b (The Mathworks Inc., Natick, MA, USA). The spectral region of interest was selected as δ4.7-0.5 ppm. Signals from contaminants (polyethyleneglycol δ3.73-3.70, acetone δ2.26-2.20 and methanol δ3.37-3.35) were removed. The baseline was corrected by asymmetric least squares with parameters $\lambda=10^7$ and $p=10^{-4}$, and by subtracting the mean value of the non-signal regions δ4.50-4.46, 2.62-2.60, 1.88-1.84, and 0.80-0.75 to set baseline variation around zero. Finally, the spectra were divided by the total area under the spectrum curve to account for variation in sample weight. The metabolites were measured by integrating the metabolite spectral regions.

### Multivariate statistical analyses

Metabolite levels were mean centered, autoscaled, and evaluated by principal component analysis (PCA). Three spectra were removed as outliers due to low metabolite levels, excess lipids and ethanol contamination. Hierarchical clustering of the placental tissue samples based on the metabolic profiles was performed using standard Euclidian distance measure and Wards clustering. The dendrogram was cut to give three equivalent clusters or placenta groups to assess the overall metabolic variation within the heterogeneous pregnancy group. To confirm that the placenta groups were characterized by significantly different metabolic profiles, a classification model was constructed using partial least squares discriminant analysis (PLS-DA) where 20% of the samples were repeatedly left out of the model building and then their placental group predicted (Figure S1). Prediction accuracies were 87.6%, 82.6%, and 91.4% respectively for the three placenta groups ($P<0.001$).

## References


1. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. *Lancet*. 2005;365:785-799. doi: 10.1016/S0140-6736(05)17987-2.
2. Jewison T, Su Y, Disfany FM, Liang Y, Knox C, Maciejewski A, Poelzer J, Huynh J, Zhou Y, Arndt D, Djoumbou Y, Liu Y, Deng L, Guo AC, Han B, Pon A, Wilson M, Rafatnia S, Liu P, Wishart DS. Smpdb 2.0: Big improvements to the small molecule pathway database. *Nucleic Acids Res*. 2014;42:D478-484. doi: 10.1093/nar/gkt1067.

## Supplementary Tables

**Table S1.** Diagnostic criteria for severe preeclampsia[1]

| Sign or Symptom | Details |
|---|---|
| Persistent severe hypertension | ≥160/110 mm Hg |
| Severe proteinuria | ≥3g protein excretion into urine/day |
| Pulmonary symptoms | Pulmonary edema, dyspnea, cyanosis |
| Seizures/eclampsia | |
| Oliguria | <500mL urine/day |
| Thrombocytopenia | Platelet count <100 000 per μL |
| Abnormal liver enzymes | Increased serum aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase |
| Hemolysis | Low serum haptoglobin (<0.2g/L) |
| Microangiopathic hemolytic anemia | |
| Epigastric or right upper quadrant pain | |
| Central nervous system symptoms | Altered mental status, headaches, blurred vision, blindness |
| HELLP syndrome | Hemolysis, elevated liver enzymes, low platelets |

**Table S2.** Clinical characteristics of subgroups of preeclampsia

| Characteristic | PE+FGR (n=40) | Severe PE (n=49) | Early onset PE (n=51) |
|---|---|---|---|
| BMI, kg/m$^2$* | 25.3 (19.8-41.2) | 25.3 (19.8-41.2) | 25,8 (19.8-41.2) |
| SBP, mm Hg† | 155 (118-186)§ | 157 (118-220) § | 152 (120-220) § |
| DBP, mm Hg† | 91 (60-160)‡ | 90 (60-160) § | 100 (60-160) § |
| FGR, n (%) | 40 (100) | 29 (59) | 35 (69) |
| Severe PE, n (%) | 29 (72.5) | 49 (100) | 41 (80) |
| Characteristic at delivery | | | |
| Maternal age, y | 29.0 (20-40) | 31.2 (20-40) | 29 (20-40) |
| Gestational age, wk | 30.3 (23.6-36.9) § | 31.9 (23.6-38.4) § | 31.1 (23.6-36.14) § |
| Placenta weight, g | 274 (100-525) § | 285 (150-600) § | 275 (100-600) § |
| Birthweight, g | 1133 (410-2330) § | 1322 (410-3090) § | 1154 (410-2610) § |
| Fetal sex, female, n (%) | 23 (57.5) | 27 (55) | 26 (49) |
| | | | |
| Characteristic | PE÷FGR (n=22) | Non-severe PE (n=13) | Late onset PE (n=11) |
| BMI, kg/m$^2$* | 27 (21-40) | 27.6 (20.7-39.3) | 24.4 (20.7-40.1) |
| SBP, mm Hg† | 157 (118-186)§ | 140 (120-151) | 147 (118-182)§ |
| DBP, mm Hg† | 95 (60-125)§ | 95 (62-110) | 82 (60-105)§ |
| FGR, n (%) | 0(0) | 11 (84.6)§ | 5 (45) |
| Severe PE, n (%) | 20 (91) | 0 (0) | 8 (73) |
| Characteristic at delivery | | | |
| Maternal age, y | 29.6 (20-40) | 30.0 (22-37)‡ | 29 (27-40) |
| Gestational age, wk | 34 (25.6-38.4)§ | 32.0 (24.9-37.0)§ | 36.9 (34.7-38.4)‡ |
| Placenta weight, g | 452 (200-750)§ | 275 (100-750)§ | 450 (290-750) |
| Birthweight, g | 2105 (690-3470)§ | 1240 (470-3470)§ | 2330 (1800-3470)‡ |
| Fetal sex, female, n (%) | 10 (45) | 6 (46.2) | 8 (73) |

BMI indicates body mass index; DBP, diastolic blood pressure; FGR, fetal growth restriction; PE, preeclampsia; SBP, systolic blood pressure.
Categorical variables assessed for differences between placenta groups by Fisher's exact test. Continuous variables listed as median (min-max), assessed for differences between groups by Mann-Whitney U tests (two way comparisons).
*BMI from first trimester maternal weight. Information is missing from 10 women (7%)
†Blood pressure from last healthcare visit before delivery, missing from 13 women (9%)
‡ Significantly different from the normal pregnancies (Table 1)($P$<0.05)
§ Significantly different from the normal pregnancies (Table 1)($P$<0.001)

**Table S3.** Comparison of metabolite levels between the diagnostic groups

| Metabolite | Normal pregnancies (n=65) | Preeclampsia (n=62) | Fetal growth restriction (n=16) |
|---|---|---|---|
| Phospholipid, glycerolipid metabolism | | | |
| Ethanolamine | 9.3 (2.7) | 6.2 (2.2)* | 7.2 (3.2)* |
| Choline | 89.2 (15.3) | 91.1 (15.8) | 82.6 (20.7) |
| Glycerophosphocholine | 32.4 (15.3) | 50.0 (19.9)* | 39.3 (17.1) |
| Phosphocholine | 16.6 (5.0) | 19.7 (6.0)* | 19.3 (4.6) |
| Dihydroxyacetone | 1.8 (1.2) | 1.1 (0.7)* | 0.8 (0.5)* |
| Glycerol | 23.6 (7.1) | 22.0 (6.0) | 19.4 (4.4) |
| Myoinositol | 17.5 (3.8) | 18.0 (3.2) | 20.9 (8.4)*† |
| Ammonia recycling, urea cycle, bile acid biosynthesis | | | |
| Glutamine | 6.3 (1.4) | 6.3 (1.6) | 6.6 (1.8) |
| Aspartate | 7.4 (2.2) | 10.1 (4.4)* | 9.6 (4.6) |
| Glutamate | 23.5 (2.9) | 19.7 (3.2)* | 21.4 (2.3)* |
| Acetate | 4.5 (1.8) | 4.3 (1.9) | 3.8 (1.1) |
| Alanine | 10.5 (2.3) | 9.8 (2.6) | 11.1 (2.4) |
| Taurine | 37.8 (5.8) | 29.1 (6.4)* | 34.5 (9.1)† |
| Protein biosynthesis, valine, leucine and isoleucine metabolism | | | |
| Leucine | 13.2 (1.6) | 12.8 (2.1) | 13.6 (2.03) |
| Isoleucine | 2.2 (0.4) | 1.8 (0.7)* | 1.9 (0.8) |
| Valine | 4.2 (0.9) | 3.8 (0.9)* | 4.1 (1.2) |
| Threonine | 3.2 (0.5) | 2.6 (0.6)* | 2.9 (0.7) |
| Lysine | 12.7 (5.3) | 10.0 (3.7)* | 10.1 (1.7) |
| Glycolysis, ketone body metabolism | | | |
| Lactate | 46.7 (7.7) | 45.5 (8.7) | 44.6 (7.0) |
| Glucose | 6.1 (2.3) | 4.3 (1.9)* | 5.9 (3.8)† |
| Succinate | 5.0 (1.1) | 4.7 (1.1) | 5.2 (1.1) |
| 3-Hydroxybutyrate | 2.7 (1.8) | 2.0 (2.3)* | 2.5 (1.4) |
| Catecholamine biosynthesis | | | |
| Ascorbate | 3.5 (0.7) | 2.8 (0.8)* | 3.1 (0.8) |
| Glycine and serine metabolism | | | |
| Glycine | 8.9 (2.1) | 7.0 (1.5)* | 7.5 (1.2)* |

| | | | |
|---|---|---|---|
| Creatine | 8.1 (3.2) | 12.0 (6.7)* | 10.0 (4.0)* |

Metabolite concentrations given as mean (SD). *P*-values from one-way ANOVA with Tukey post-hoc. All *P*-values are adjusted for multiple comparisons. The metabolites are grouped by metabolic pathways described in the Small Molecule Pathway Database.[2]

*Significantly different from normal pregnancies

†Significantly different from preeclamptic pregnancies

**Table S4.** Results from Quantitative Metabolite Set enrichment analysis, normal placenta vs placental dysfunction

| Pathway | Total Cmpd | Hits | Statistic Q | Exp. Q | Raw p | Holm p | FDR |
|---|---|---|---|---|---|---|---|
| Valine, leucine and isoleucine degradation | 36 | 3 | 8.1 | 0.7 | 4.8e-06 | 0.00016 | 0.00016 |
| Glycine, serine and threonine metabolism | 26 | 3 | 5.1 | 0.7 | 8.3e-05 | 0.0027 | 0.0014 |
| Propanoate metabolism | 18 | 1 | 9.9 | 0.7 | 0.0001 | 0.0040 | 0.0014 |
| Phospholipid biosynthesis | 19 | 2 | 6.0 | 0.7 | 0.0001 | 0.0052 | 0.0014 |
| Taurine and hypotaurine metabolism | 7 | 1 | 8.1 | 0.7 | 0.0006 | 0.0167 | 0.0038 |
| Bile acid biosynthesis | 49 | 2 | 4.6 | 0.7 | 0.0013 | 0.0353 | 0.0069 |
| Glucose-alanine cycle | 12 | 2 | 4.3 | 0.7 | 0.0021 | 0.0579 | 0.0101 |
| Urea cycle | 20 | 3 | 2.9 | 0.7 | 0.0062 | 0.1620 | 0.0209 |
| Selenoaminoacid metabolism | 15 | 1 | 5.0 | 0.7 | 0.0073 | 0.1823 | 0.0209 |
| Alanine metabolism | 6 | 1 | 5.0 | 0.7 | 0.0073 | 0.1823 | 0.0209 |
| Lysine degradation | 13 | 1 | 4.9 | 0.7 | 0.0076 | 0.1823 | 0.0209 |
| Biotin metabolism | 4 | 1 | 4.9 | 0.7 | 0.0076 | 0.1823 | 0.0209 |
| Arginine and proline metabolism | 26 | 2 | 3.2 | 0.7 | 0.0105 | 0.2200 | 0.0266 |
| Beta-alanine metabolism | 13 | 1 | 3.7 | 0.7 | 0.0210 | 0.4207 | 0.0402 |
| Aspartate metabolism | 12 | 1 | 3.7 | 0.7 | 0.0210 | 0.4207 | 0.0402 |
| Malate-aspartate shuttle | 8 | 1 | 3.7 | 0.7 | 0.0210 | 0.4207 | 0.0402 |
| Glycolysis | 21 | 1 | 3.7 | 0.7 | 0.0219 | 0.4207 | 0.0402 |
| Insulin signaling | 19 | 1 | 3.7 | 0.7 | 0.0219 | 0.4207 | 0.0402 |
| Galactose metabolism | 25 | 3 | 1.8 | 0.7 | 0.0491 | 0.7367 | 0.0854 |
| Gluconeogenesis | 27 | 2 | 2.0 | 0.7 | 0.0539 | 0.7541 | 0.0889 |
| Ammonia recycling | 18 | 3 | 1.6 | 0.7 | 0.0769 | 0.9993 | 0.1208 |
| Inositol metabolism | 19 | 1 | 1.4 | 0.7 | 0.1610 | 1 | 0.2446 |
| Glutathione metabolism | 10 | 1 | 1.0 | 0.7 | 0.2271 | 1 | 0.3123 |
| Porphyrin metabolism | 22 | 1 | 1.0 | 0.7 | 0.2271 | 1 | 0.3123 |
| Glycerolipid metabolism | 13 | 1 | 0.4 | 0.7 | 0.4300 | 1 | 0.5536 |
| Methionine metabolism | 24 | 2 | 0.6 | 0.7 | 0.4362 | 1 | 0.5536 |
| Pyruvate metabolism | 20 | 2 | 0.5 | 0.7 | 0.5009 | 1 | 0.6122 |
| Citric acid cycle | 23 | 1 | 0.2 | 0.7 | 0.5546 | 1 | 0.6311 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Mitochondrial electron transport chain | 15 | 1 | 0.2 | 0.7 | 0.5546 | 1 | 0.6311 |
| Betaine metabolism | 10 | 1 | 0.1 | 0.7 | 0.6546 | 1 | 0.7201 |
| Pyrimidine metabolism | 36 | 1 | 0.1 | 0.7 | 0.7754 | 1 | 0.7996 |
| Purine metabolism | 45 | 1 | 0.1 | 0.7 | 0.7754 | 1 | 0.7996 |
| Glutamate metabolism | 18 | 2 | 0.1 | 0.7 | 0.8035 | 1 | 0.8035 |

Cmpd indicates compounds; Exp, expected; FDR, false discovery rate.

**Table S5.** Results from Quantitative Metabolite Set Enrichment Analysis, moderate placental dysfunction vs normal placenta

| Pathway | Total Cmpd | Hits | Statistic Q | Exp. Q | Raw p | Holm p | FDR |
|---|---|---|---|---|---|---|---|
| Valine, leucine and isoleucine degradation | 36 | 3 | 15.7 | 1.0 | 2.4e-08 | 7.8e-07 | 7.8e-07 |
| Propanoate metabolism | 18 | 1 | 23.3 | 1.0 | 1.6-07 | 5.1e-06 | 2.6e-06 |
| Glucose-alanine cycle | 12 | 2 | 12.0 | 1.0 | 2.1e-06 | 6.4e-05 | 2.3e-05 |
| Selenoaminoacid metabolism | 15 | 1 | 17.4 | 1.0 | 8.6e-06 | 0.0003 | 5.7e-05 |
| Alanine metabolism | 6 | 1 | 17.4 | 1.0 | 8.6e-06 | 0.0003 | 5.7e-05 |
| Urea cycle | 20 | 3 | 7.3 | 1.0 | 4.3e-05 | 0.0012 | 0.0002 |
| Glycine, serine and threonine metabolism | 26 | 3 | 5.7 | 1.0 | 0.0003 | 0.0091 | 0.0016 |
| Taurine and hypotaurine metabolism | 7 | 1 | 8.7 | 1.0 | 0.0021 | 0.0544 | 0.0086 |
| Bile acid biosynthesis | 49 | 2 | 4.64 | 1.0 | 0.0067 | 0.1663 | 0.0238 |
| Glycolysis | 21 | 1 | 6.58 | 1.0 | 0.0080 | 0.1908 | 0.0238 |
| Insulin signaling | 19 | 1 | 6.58 | 1.0 | 0.0080 | 0.1908 | 0.0238 |
| Phospholipid biosynthesis | 19 | 2 | 4.02 | 1.0 | 0.0142 | 0.3121 | 0.0390 |
| Gluconeogenesis | 27 | 2 | 3.8 | 1.0 | 0.0177 | 0.3718 | 0.0449 |
| Lysine degradation | 13 | 1 | 4.5 | 1.0 | 0.0282 | 0.5643 | 0.0621 |
| Biotin metabolism | 4 | 1 | 4.5 | 1.0 | 0.0282 | 0.5643 | 0.0621 |
| Galactose metabolism | 25 | 3 | 2.7 | 1.0 | 0.0353 | 0.6354 | 0.0728 |
| Beta-alanine metabolism | 13 | 1 | 3.3 | 1.0 | 0.0607 | 1 | 0.1054 |
| Aspartate metabolism | 12 | 1 | 3.3 | 1.0 | 0.0607 | 1 | 0.1054 |
| Malate-aspartate shuttle | 8 | 1 | 3.3 | 1.0 | 0.0607 | 1 | 0.1054 |
| Ammonia recycling | 18 | 3 | 1.7 | 1.0 | 0.1513 | 1 | 0.2496 |
| Arginine and proline metabolism | 26 | 2 | 1.7 | 1.0 | 0.1724 | 1 | 0.2710 |
| Inositol metabolism | 19 | 1 | 1.5 | 1.0 | 0.2076 | 1 | 0.3114 |
| Pyrimidine metabolism | 36 | 1 | 1.2 | 1.0 | 0.2716 | 1 | 0.3734 |
| Purine metabolism | 45 | 1 | 1.2 | 1.0 | 0.2716 | 1 | 0.3734 |
| Citric acid cycle | 23 | 1 | 1.0 | 1.0 | 0.3050 | 1 | 0.3872 |
| Mitochondrial electron transport chain | 15 | 1 | 1.0 | 1.0 | 0.3050 | 1 | 0.3872 |
| Glutamate metabolism | 18 | 2 | 1.1 | 1.0 | 0.3180 | 1 | 0.3886 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Betaine metabolism | 10 | 1 | 0.9 | 1.0 | 0.3444 | 1 | 0.4060 |
| Glutathione metabolism | 10 | 1 | 0.5 | 1.0 | 0.4550 | 1 | 0.5010 |
| Porphyrin metabolism | 22 | 1 | 0.5 | 1.0 | 0.4550 | 1 | 0.5010 |
| Methionine metabolism | 24 | 2 | 0.7 | 1.0 | 0.4833 | 1 | 0.5145 |
| Pyruvate metabolism | 20 | 2 | 0.6 | 1.0 | 0.5602 | 1 | 0.5777 |
| Glycerolipid metabolism | 13 | 1 | 0.1 | 1.0 | 0.7901 | 1 | 0.7901 |

Cmpd indicates compounds; Exp, expected; FDR, false discovery rate.

**Table S6.** Results from Quantitative Metabolite Set Enrichment Analysis, severe placental dysfunction vs normal placenta

| Pathway | Total Cmpd | Hits | Statistic Q | Exp. Q | Raw p | Holm p | FDR |
|---|---|---|---|---|---|---|---|
| Phospholipid biosynthesis | 19 | 2 | 12.3 | 1.2 | 1.3e-05 | 0.0004 | 0.0004 |
| Arginine and proline metabolism | 26 | 2 | 12.9 | 1.2 | 3.3e-05 | 0.0010 | 0.0005 |
| Glycine, serine and threonine metabolism | 26 | 3 | 9.1 | 1.2 | 5.7e-05 | 0.0018 | 0.0006 |
| Lysine degradation | 13 | 1 | 10.9 | 1.2 | 0.0022 | 0.0654 | 0.0144 |
| Biotin metabolism | 4 | 1 | 10.9 | 1.2 | 0.0022 | 0.0654 | 0.0144 |
| Glycerolipid metabolism | 13 | 1 | 9.0 | 1.2 | 0.0056 | 0.1555 | 0.0254 |
| Taurine and hypotaurine metabolism | 7 | 1 | 8.8 | 1.2 | 0.0060 | 0.1628 | 0.0254 |
| Beta-alanine metabolism | 13 | 1 | 8.3 | 1.2 | 0.0077 | 0.2000 | 0.0254 |
| Aspartate metabolism | 12 | 1 | 8.3 | 1.2 | 0.0077 | 0.2000 | 0.0254 |
| Malate-aspartate shuttle | 8 | 1 | 8.3 | 1.2 | 0.0077 | 0.2000 | 0.0254 |
| Bile acid biosynthesis | 49 | 2 | 5.3 | 1.2 | 0.0108 | 0.2477 | 0.0296 |
| Galactose metabolism | 25 | 3 | 4.4 | 1.2 | 0.0108 | 0.2477 | 0.0296 |
| Valine, leucine and isoleucine metabolism | 36 | 3 | 4.3 | 1.2 | 0.0178 | 0.3730 | 0.0451 |
| Ammonia recycling | 18 | 3 | 3.8 | 1.2 | 0.0233 | 0.4668 | 0.0550 |
| Urea cycle | 20 | 3 | 3.2 | 1.2 | 0.0468 | 0.8889 | 0.1029 |
| Propanoate metabolism | 18 | 1 | 2.3 | 1.2 | 0.1714 | 1 | 0.3314 |
| Glycolysis | 21 | 1 | 2.2 | 1.2 | 0.1808 | 1 | 0.3314 |
| Insulin signaling | 19 | 1 | 2.2 | 1.2 | 0.1808 | 1 | 0.3314 |
| Inositol metabolism | 19 | 1 | 2.1 | 1.2 | 0.1911 | 1 | 0.3319 |
| Glutathione metabolism | 10 | 1 | 1.8 | 1.2 | 0.2289 | 1 | 0.3597 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Porphyrin metabolism | 22 | 1 | 1.8 | 1.2 | 0.2289 | 1 | 0.3597 |
| Pyrimidine metabolism | 36 | 1 | 1.2 | 1.2 | 0.3183 | 1 | 0.4567 |
| Purine metabolism | 45 | 1 | 1.2 | 1.2 | 0.3183 | 1 | 0.4567 |
| Pyruvate metabolism | 20 | 2 | 1.223 | 1.2048 | 0.36668 | 1 | 0.5042 |
| Glucose-alanine cycle | 12 | 2 | 1.0882 | 1.2048 | 0.40611 | 1 | 0.5171 |
| Gluconeogenesis | 27 | 2 | 1.0935 | 1.2048 | 0.40739 | 1 | 0.5171 |
| Methionine metabolism | 24 | 2 | 1.0062 | 1.2048 | 0.43469 | 1 | 0.5313 |
| Glutamate metabolism | 18 | 2 | 0.65222 | 1.2048 | 0.58127 | 1 | 0.6851 |
| Betaine metabolism | 10 | 1 | 0.25174 | 1.2048 | 0.65037 | 1 | 0.7401 |
| Citric acid cycle | 23 | 1 | 0.089835 | 1.2048 | 0.78666 | 1 | 0.8374 |
| Mitochondrial electron transport chain | 15 | 1 | 0.089835 | 1.2048 | 0.78666 | 1 | 0.8374 |
| Selenoamino acid metabolism | 15 | 1 | 0.0024036 | 1.2048 | 0.9647 | 1 | 0.9647 |
| Alanine metabolism | 6 | 1 | 0.0024036 | 1.2048 | 0.9647 | 1 | 0.9647 |

Cmpd indicates compounds; Exp, expected; FDR, false discovery rate.

## Supplementary Figure

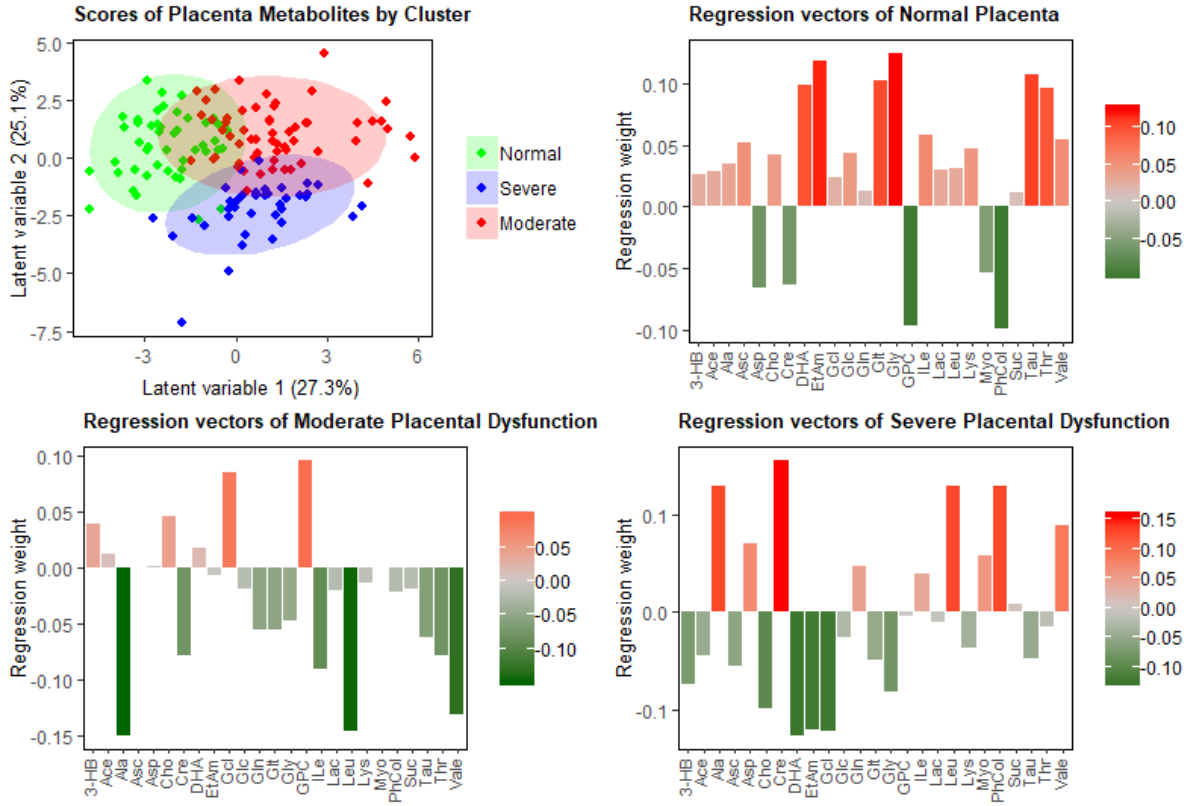



**Figure S1.** Partial least squares discriminant analysis (PLS-DA) of clusters of placental metabolic expression. Upper left: Score plot of the PLS-DA, showing grouping of the three placental clusters. Clockwise from upper right: Regression vectors for the three placenta groups identifying the metabolites important for discriminating each group, with bars colored and sized according to importance (weight) in the regression model.