Journal of Reproductive Immunology 159 (2023) 104136

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jri

Can single-cell and spatial omics unravel the pathophysiology of pre-eclampsia?

Sunhild Hartmann [a,b,c,d,e,f,1], Stefan Marc Botha [a,b,c,d,e,1], Clive M. Gray [g], Daniela S. Valdes [a,d,e], Stephen Tong [b,c], Tu'uhevaha J. Kaitu'u-Lino [b,c], Florian Herse [a,d,e], Lina Bergman [h,i,j], Catherine A. Cluver [b,c,h], Ralf Dechend [a,d,e,f,k,2], Olivia Nonn [a,d,e,f,l,*,2]

[a] Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität, Berlin, Germany
[b] Mercy Perinatal, Mercy Hospital for Women, Heidelberg, Victoria 3084, Australia
[c] Translational Obstetrics Group, The Department of Obstetrics and Gynaecology, Mercy Hospital for Women, University of Melbourne, Heidelberg, Victoria 3084, Australia
[d] Experimental and Clinical Research Center, a cooperation between the Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association and the Charité - Universitätsmedizin Berlin, Berlin, Germany
[e] Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany
[f] DZHK (German Center for Cardiovascular Research), partner site Berlin, Germany
[g] Division of Molecular Biology and Human Genetics, Biomedical Research Institute, Stellenbosch University, Cape Town 7505, South Africa
[h] Department of Obstetrics and Gynaecology, Stellenbosch University, Cape Town 7505, South Africa
[i] Department of Women's and Children's Health, Uppsala University, Uppsala 751 85, Sweden,
[j] Department of Obstetrics and Gynaecology, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg 405 30, Sweden
[k] HELIOS Clinic, Department of Cardiology and Nephrology, Berlin, Germany
[l] Division of Cell Biology, Histology and Embryology, Gottfried Schatz Research Center, Medical University of Graz, Graz, Austria

ARTICLE INFO

Keywords:
Pre-eclampsia
Single-cell RNA-seq
Multi-omics
Spatial omics
Placenta

ABSTRACT

Pre-eclampsia is a leading cause of maternal and fetal morbidity and mortality. Characterised by the onset of hypertension and proteinuria in the second half of pregnancy, it can lead to maternal end-organ injury such as cerebral ischemia and oedema, pulmonary oedema and renal failure, and potentially fatal outcomes for both mother and fetus. The causes of the different maternal end-organ phenotypes of pre-eclampsia and why some women develop pre-eclampsia condition early in pregnancy have yet to be elucidated. Omics methods include proteomics, genomics, metabolomics, transcriptomics. These omics techniques, previously mostly used on bulk tissue and individually, are increasingly available at a single cellular level and can be combined with each other. Multi-omics techniques on a single-cell or spatial level provide us with a powerful tool to understand the pathophysiology of pre-eclampsia. This review will explore the status of omics methods and how they can and could contribute to understanding the pathophysiology of pre-eclampsia.

1. Introduction

Pre-eclampsia is a pregnancy specific multisystem disorder that develops in the second half of pregnancy. Pre-eclampsia is commonly diagnosed by new-onset hypertension in pregnancy and significant proteinuria (Chappell et al., 2021). The natural course of pre-eclampsia, if untreated, results in maternal end-organ complications and therefore it is one of the major causes for maternal mortality. These maternal deaths are caused by pulmonary oedema, cerebral complications, acute renal failure, haemolysis elevated liver enzymes low platelet syndrome (HELLP) or eclampsia (generalised seizures) (Babazhanova et al., 2021). Additionally, stillbirth and neonatal deaths, caused by placental abruption, are significantly more common in women with pre-eclampsia.

Pre-eclampsia can be categorised on the timing of disease onset as it can develop at different time points in pregnancy. Classically, the

* Corresponding author at: Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität, Berlin, Germany.
E-mail address: olivia.nonn@charite.de (O. Nonn).
[1] Equal contribution
[2] Equal contribution

https://doi.org/10.1016/j.jri.2023.104136
Received 3 August 2023; Accepted 16 August 2023
Available online 17 August 2023

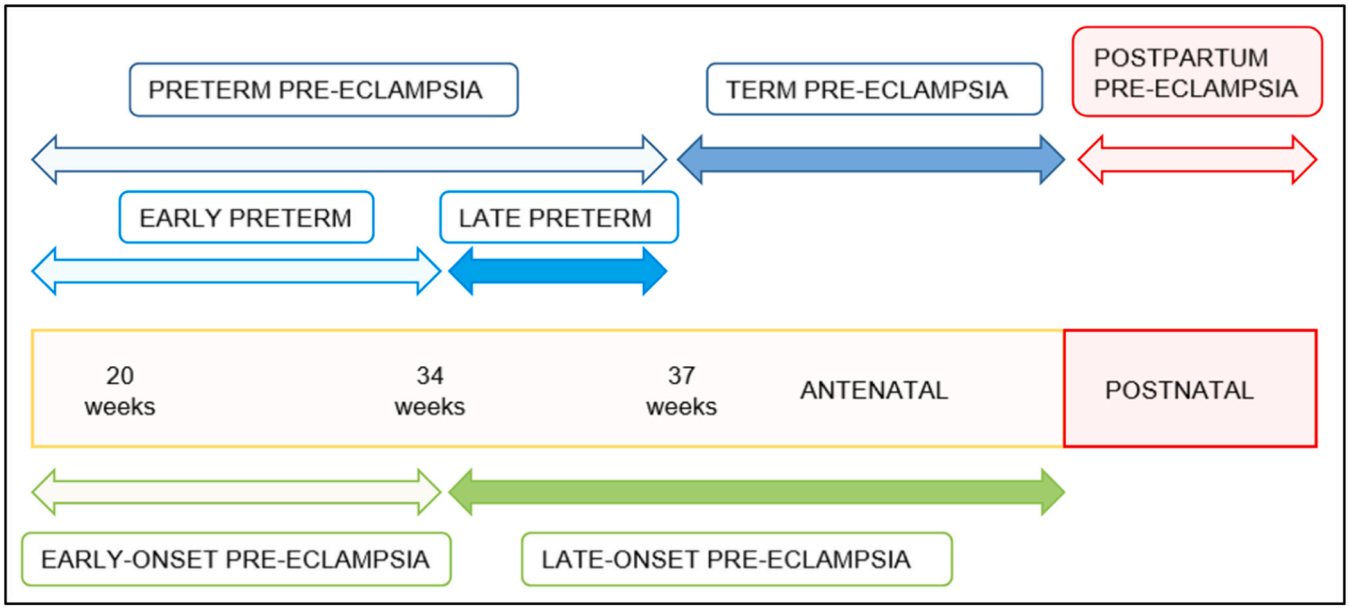

Fig. 1. Timeline of different onsets of pre-eclampsia.

disease has been categorised as term pre-eclampsia (delivery after 37 weeks' gestation) or preterm pre-eclampsia (delivery before 37 weeks). Preterm pre-eclampsia is then further divided into early preterm (delivery before 34 weeks' gestation) and late preterm pre-eclampsia (delivery after 34 weeks but before 37 weeks' gestation) (Duhig et al., 2021). Another classification is early versus late onset pre-eclampsia. Here, early-onset is defined as the occurrence before 34 weeks' gestation and late-onset is defined as the development of pre-eclampsia after 34 weeks' gestation (Fig. 1).

Some severe features of pre-eclampsia have a higher incidence in early-onset disease, specifically heavier proteinuria, eclampsia and HELLP syndrome (Kucukgoz Gulec et al., 2013). Despite this the numbers of complications are relatively greater among women with late-onset disease as early onset is disease is rarer (Kucukgoz Gulec et al., 2013). There are also significant differences in the epidemiology of early- and late-onset pre-eclampsia. In most high-income countries late-onset pre-eclampsia represents 80–90% of cases, whilst in some low-and-middle-income countries early-onset pre-eclampsia represents up to 35% of cases (Robillard et al., 2022; Teka et al., 2023). This disparity highlights possible differences in genetics or potential underlying immunity to environmental and infectious pathophysiological processes that have an impact on timing and severity of disease (Aimée, 2022, WHO, 2023).

Women with pre-eclampsia produce greater amounts of anti-angiogenic factors like soluble Fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin. sFlt-1 binds to circulating vascular endothelial growth factor and placental growth factor leading to lower concentrations in the bloodstream. This disturbed equilibrium results in endothelial dysfunction with multi-organ involvement (Maynard et al., 2003; Tong et al., 2022). Interestingly, women with eclampsia, HELLP syndrome and severe hypertension have even higher levels of these biomarkers. Women with pre-eclampsia with pulmonary oedema did not show significant changes in sFlt-1 levels (Hastie et al., 2022). Further investigation into why certain women develop severe complications such as HELLP syndrome, pulmonary oedema or eclampsia are needed.

2. Unravelling pre-eclampsia with omics techniques

To investigate the pathogenesis of preeclampsia, a detailed analysis of processes occurring on a cellular level may be important. With a combination of different targeted and untargeted omics methods, crucial pathways may be discovered. Multi-omics is a state-of-the-art technique that enables the simultaneous analysis of multiple molecular compartments and changes therein at a high resolution. Together, data generated from genomics, epigenomics, transcriptomics, proteomics and metabolomics may be combined. The resulting data has led to a revolution in the field of biology and medicine, allowing an integrative analysis for a deeper understanding of the interplay of molecules and therefore improving the prognosis of diseases finally leading to enhanced treatment and prevention (Hasin et al., 2017). Omics techniques are becoming increasingly feasible on a single-cell level. This high resolution allows for a change in perspective, allowing the discovery of cell-specific pathways and cell interactions. These techniques can now be used to explore underlying pathophysiological mechanisms. Molecular signatures may be identified rather than target molecules.

3. Genomics and epigenomics

Genomics is the study of a person's genetic make-up. Several genomics studies have investigated the maternal and fetal genome. Genomics is widely used to investigate disease and has led to a greater understanding of underlying mechanisms. Evolving technologies have made genomics more affordable and cost-efficient by allowing high-throughput analysis.

In pre-eclampsia, genomic studies have identified the gene *TIMP3* as a potential marker for early-onset disease (Yuen et al., 2010) and a polymorphism in the maternal genome (*HLA-DRB5*) has been associated with the development of pre-eclampsia (Xu et al., 2023). Table 1 details the genomic studies that have been performed on different tissue types to investigate pre-eclampsia.

Genomic methods are ideal to investigate genes and their potentially altered expression in pre-eclampsia but are not able to provide information on functional alterations. Epigenomics can show reversible modifications of the DNA or DNA-associated proteins but have not yet been able to describe pathophysiological pathways in pre-eclampsia.

4. Transcriptomics: single-cell RNA sequencing and spatial transcriptomics

Single-cell transcriptomics allows the detection of heterogeneity in single cells. By analysing transcribed genes in a single cell, the cell state, differentiation potential, cell to cell interaction and the disease related

Table 1
Genomic and transcriptomic studies to investigate pregnancy and pre-eclampsia.

Study type/ method	Tissue	Finding	Reference
DNA methylation profiling	Placenta (early-onset pre-eclampsia)	Hypomethylation of 4 loci, *TIMP3* potential marker for early-onset pre-eclampsia	Yuen et al., 2010
Genome-wide analysis of expression profiles	Placenta	63 genes are significantly expressed in pre-eclampsia	Trifonova et al., 2022
GWAS	Blood samples	Polymorphism in maternal *HLA-DRB5* is associated with pre-eclampsia	Xu et al., 2023
GWAS	Fetal genome	SNPs in *sFlt1* are associated with pre-eclampsia	Mcginnis et al., 2017
GWAS	Maternal genome	No SNPs associated with pre-eclampsia	Zhao et al., 2013
GSEA	Placenta and serum	miR-34a-5p and miR-106a-5p are associated with pre-eclampsia	Zhang et al., 2022
miRNA and RNA expression analysis	Placenta, trophoblasts	miRNAs targeting the TGF-β pathway are altered	Brooks et al., 2016
miRNA expression analysis	Maternal plasma exosomes	Increased miR-885–5p levels in pre-eclampsia	Sandrim et al., 2016
RNA-seq, GSEA	Plasma	cfRNA signature of pre-eclampsia is linked to the pathophysiology	Rasmussen et al., 2022
scRNA-seq	Fetal tissue, decidua and blood samples	Single-cell reconstruction of the maternal-fetal interface	Vento-Tormo et al., 2018
scRNA-seq	Placenta and decidua	Cell-type specific transcription factors	Suryawanshi et al., 2018
scRNA-seq	Placental villi, basal plate, chorionamniotic membrane	Cell-specific transcriptomic differences between pre-term and term delivered placental tissues	Pique-Regi et al., 2019
scRNA-seq	Trophoblasts	Trophoblast subtypes, patterns of differentiation in the placenta	Liu et al., 2018
Transcriptome analysis	Decidua basalis	Apoptosis and cell signalling genes are altered in pre-eclampsia	Yong et al., 2015

cfRNA = circulating free-RNA, GWAS = Genome wide association studies, GSEA = Gene set enrichment analysis, miRNA = micro-RNA, scRNA-seq = single-cell RNA sequencing, SNP = Single nucleotide polymorphism

changes in gene expression can be ascertained (Tang et al., 2019). There are several steps that need to take place when performing single-cell sequencing. Firstly, one may need to perform pre-processing. Here, certain cells of interest may need to be enriched if they are underrepresented (Vento-Tormo et al., 2018). Processing then entails converting RNA into complementary DNA. Polymerase chain reaction amplification is then performed as a single cell only contains 1–50 pg RNA, and 0.1–1 μg complementary DNA is needed to detect a signal with RNA-seq (Svensson et al., 2018). The single-cell RNA sequencing data is then analysed using computational methods such as unsupervised clustering where cell populations are identified based on their transcriptomic profiles using marker genes (Zhang et al., 2019). This information is then connected with cellular descriptions such as location and morphology to finally create a reference map of the molecular state of cells (Regev et al., 2017).

Single-cell RNA-sequencing has been used to study pregnancy. Examples include studies investigating the decidua of the first trimester. Several subpopulations with enrichment of cell type specific transcription factors and trophoblast subtypes with different patterns of differentiation were found (Suryawanshi et al., 2018; Liu et al., 2018). Others have reconstructed the early maternal-fetal interface to investigate the immunological crosstalk of decidual immune cells (Vento-Tormo et al., 2018).

Transcriptomic changes of genes related to pre-eclampsia have been investigated in global RNA sequencing studies. Identified genes include *SERPIN1*, *PRG2*, *FSTL3*, *FABP4*, *RDH13*, *PLAC1*, *GSTA3* in STBs (Gormley et al., 2017; Trifonova et al., 2022) together with several miRNAs (Yuen et al., 2010; Brooks et al., 2016; Tsai et al., 2011; Sandrim et al., 2016; Zhang et al., 2022). Differentially expressed genes have also been described in the decidua basalis of the placenta (*CD72*, *PER3* or *PDK4)* (Yong et al., 2015). Additionally, a recent large multi-national transcriptomics study showed that plasma cell-free RNA signatures, with a moderate predictive accuracy, could identify women who were at risk of developing pre-eclampsia. Interestingly, transcriptomics was able to track pregnancy progression and define gestational length (Rasmussen et al., 2022) (Table 1).

Spatial transcriptomics connects tissue histology and expression changes based on two approaches, namely targeted and untargeted spatial omics. Targeted methods include fluorescence *in situ* hybridisation (FISH) based methods. Untargeted approaches often use methods where transcripts are directly labelled to tissue sections which enables the atlas mapping of their location after sequencing (Burgess, 2019). A non-spatial approach is the use of Drop-seq beads (Binan et al., 2019). Droplets with barcoded beads encapsulate single cells, labelling the transcripts with specific barcodes. Spatial adaption techniques like Slide-seq (Rodriques et al., 2019) are then used and the barcoded beads are arrayed on a solid surface. This is then sequenced to identify the position of each barcode. When a tissue section is now placed on the slide and RNA is released through tissue digestion, RNA sequenced libraries can be generated containing the position specific barcodes (Rodriques et al., 2019). This technique combines the general omics tools, yielding measurements of different biomolecules together with the analysis of a smaller number of molecules within intact tissues or cells obtained by immunostaining or *in situ* hybridisation (Tian et al., 2023).

These spatial transcriptomic methods may be important in understanding the pathophysiology of pre-eclampsia. In a recent study, digital spatial transcriptomic profiles using *in situ* sequencing enabled the visualisation of gene expression within trophoblast structures. In the pre-eclamptic placentas there was an increased senescent expression profile and in the villous wall consisting of trophoblasts there were more senescent areas closer to fetal vessels. This spatial dysregulation may be associated with disrupted transplacental transport (Nonn et al., 2022).

5. Proteomics

Proteomics is the study of the interactions, function, composition, and structures of proteins and their cellular activities (Wilkins et al., 1996). Proteomics can provide a better understanding of the structure and function of the organism than genomics. Pathophysiological changes may be missed on a transcriptomic level making multi-omics with proteomics a better option.

Techniques include mass-spectrometry with targeted and untargeted approaches like matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF), liquid chromatography mass spectrometry (LC-MS) and Olink®, a proximity extension assay screen for small proteins (Carlyle et al., 2022). More advanced methods include MALDI-TOF imaging (MALDI IMS) which enables the spatial visualisation of proteins (Leinweber et al., 2009). Here, enzyme activities are traced by applying marked substrates to tissues and subsequently analysing their conversion (Klein et al., 2020). Further studies using techniques such as SOMAmer, which are aptamer like single stranded

Table 2
Proteomic approaches to study pregnancy and pre-eclampsia.

Method	Tissue	Finding	Reference
LC-MS	Plasma	Apolipoprotein-A1, hemoplaxin and haptoglobin are differentially expressed in pre-eclampsia	Mary et al., 2017
2D-LC-MS/MS	Serum	Increased fibronectin expression in pre-eclampsia	Rasanen et al., 2010
2D-LC-MS/MS	Trophoblasts	Laminin expression is downregulated in pre-eclampsia	Ma et al., 2014
LC-MS/MS	Serum	RBP4 concentration is lower in pre-eclampsia	Lu et al., 2016
MALDI-TOF	Placenta/chorion	Altered expression of proteins involved in apoptosis and mitochondrial dysfunction in pre-eclampsia	Yang et al., 2015
Olink®	Plasma	CCL20 is a novel predictive and inflammatory marker for pre-eclampsia	Wang et al., 2022
SOMAmer	Plasma	PlGF and MMP-7 are markers for late-onset pre-eclampsia	Erez et al., 2017
SOMAmer	Plasma	VEGF-121, PlGF and activin are predictors for late-onset pre-eclampsia	Tarca et al., 2019
SOMAmer	Plasma	4 distinct clusters are either linked to early-onset pre-eclampsia, anti-fetal rejection, extracellular matrix regulation or angiogenic imbalance	Than et al., 2023
UPLC-UDMS^{E}	Urine	Pre-pregnancy BMI is a predictor for pre-eclampsia	Joenväärä et al., 2022

MS = Mass spectrometry, LC = Liquid chromatography, MALDI-TOF = Matrix assisted laser desorption ionisation – time of flight, SOMAmer = Slow Off-rate Modified Aptamers, UPLC-UDMS^{E} = ultra performance liquid chromatography-ultra definition mass spectrometry

deoxyoligonucleotides which bind specific molecular targets such as proteins, aimed to understand differences in the proteome of women with pre-eclampsia and find molecular subclasses (Than et al., 2023). Deep visual proteomics also has recently been described. Deep visual proteomics combines artificial-intelligence-driven image analysis of cellular phenotypes with automated single-cell or single-nucleus laser microdissection and ultra-high-sensitivity mass spectrometry. One is therefore able to obtain precise information about spatial distribution of proteins in the context of tissues (Mund et al., 2022). Some of the earlier techniques have been modified for high-throughput investigations of thousands of proteins in cells or body fluids (Hein et al., 2013).

Several studies have aimed to understand differences in the proteome of women with pre-eclampsia and are summarised in Table 2. No proteomic studies have been performed for the different severe phenotypes of preeclampsia like eclampsia, pulmonary oedema and HELLP syndrome.

6. Metabolomics

Metabolomics can quantify amino acids, fatty acids, carbohydrates, or other by-products of cellular metabolic processes. Metabolite concentrations and ratios represent metabolic activity, and abnormal changes are often a sign of disease. With the development of microfluidic systems, high-throughput single-cell metabolomics has allowed the analysis of the metabolome of a single cell (Côrte-Real et al., 2023). This can then be incorporated with spatial proteomic techniques (Ali et al., 2019; Ong et al., 2015). Spatial metabolomics enables one to localise metabolites, lipids and drugs in tissue sections (Alexandrov, 2020).

7. Multi-omics for unravelling subtypes of pre-eclampsia

Only a few studies have used multi-omics in pre-eclampsia (Table 3).

Integrated single-cell RNA sequencing with spatially resolved proteo- and transcriptomics may prove beneficial in understanding pre-eclampsia pathophysiology and subtypes. By combining several omics techniques, an enhanced understanding of cell-cell interaction and cell specific dysregulation can be explored. More research is urgently needed.

In the future, fundamental questions regarding pre-eclampsia research need to be addressed to improve the outcome of the disease. In the context of single-cell and spatial multi-omics approaches it must be investigated, how differences in pre-eclampsia subtypes lead to variations in the onset of pre-eclampsia. Furthermore, it is still unknown why some women are more likely to develop severe complications of pre-eclampsia than others. Lastly, one of the major gaps in knowledge is why pre-eclampsia and its severe forms differ geographically and ethnically. Here, single-cell and spatial multi-omics techniques offer a promising approach to answer those questions.

8. Conclusion

Multi-omics techniques have the potential to help unravel the puzzle of pre-eclampsia. By employing novel single-cell and spatial multi-omics techniques one may be able to better understand the pathophysiology of pre-eclampsia and its different phenotypes. These innovative approaches allow for the comprehensive analysis of multiple biological layers, encompassing genomics, transcriptomics, proteomics, and other molecular facets. By integrating data from various omics disciplines, a deeper probe into underlying molecular mechanisms of pre-eclampsia will be obtained. This will shed light on the complex interplay between genes, proteins, and cellular processes that contribute to the development and progression of the disease. Such multi-omics investigations hold tremendous promise in unravelling the intricate web of molecular events associated with pre-eclampsia, opening new avenues for targeted therapeutic interventions and improved management strategies.

Table 3
Multi-omics approaches to study pregnancy and pre-eclampsia.

Omics study	Tissue	Gestational Age	Finding	Reference
Spatial proteomics, spatial transcriptomics	Placenta, decidua, and vessels	6 – 20 weeks	Spiral artery remodelling progression is preferentially correlated with extravillous trophoblast invasion	Greenbaum et al., 2023
Spatial transcriptomics, spatial proteomics, snRNA-seq	Placenta, decidual tissue, maternal serum	5–10 weeks, 27–33 weeks, > 38 weeks	Trophoblast differentiation causes disturbed placental senescence profile	Nonn et al., 2022
Metabolomics, Transcriptomics	Blood, urine, vaginal swabs	35–36 weeks	Early prediction of pre-eclampsia	Marić et al., 2022
Proteome and lipidome analysis	Plasma	38–39 weeks	Visualisation of the end-stage molecular state in pre-eclampsia	Odenkirk et al., 2020
scRNA-seq, snRNA-seq, spatial multi-omics	Placenta and decidua	4–13 weeks	Trophoblast development in early pregnancy	Arutyunyan et al., 2023

scRNA-seq = single-cell RNA-sequencing, snRNA-seq = single-nucleus RNA-sequencing

Declaration of Competing Interest

None.

References

Aimée, N. 2022. DAC List of ODA Recipients.

Alexandrov, T., 2020. Spatial metabolomics and imaging mass spectrometry in the age of artificial intelligence. Annu. Rev. Biomed. Data Sci. 3, 61–87.

Ali, A., Abouleila, Y., Shimizu, Y., Hiyama, E., Emara, S., Mashaghi, A., Hankemeier, T., 2019. Single-cell metabolomics by mass spectrometry: advances, challenges, and future applications. TrAC Trends Anal. Chem. 120, 115436.

Arutyunyan, A., Roberts, K., Troulé, K., Wong, F.C., Sheridan, M.A., Kats, I., Garcia-Alonso, L., Velten, B., Hoo, R., Ruiz-Morales, E.R., 2023. Spatial multiomics map of trophoblast development in early pregnancy. Nature 616, 143–151.

Babazhanova, S., Lyubchich, A., Jabbarova, Y.K., 2021. Risk factors of maternal death in preeclampsia. Fundam. Clin. Med. 6, 27–31.

Binan, L., Drobetsky, E.A., Costantino, S., 2019. Exploiting molecular barcodes in high-throughput cellular assays. SLAS Technol.: Transl. Life Sci. Innov. 24, 298–307.

Brooks, S.A., Martin, E., Smeester, L., Grace, M.R., Boggess, K., Fry, R.C., 2016. miRNAs as common regulators of the transforming growth factor (TGF)-β pathway in the preeclamptic placenta and cadmium-treated trophoblasts: links between the environment, the epigenome and preeclampsia. Food Chem. Toxicol. 98, 50–57.

Burgess, D.J., 2019. Spatial transcriptomics coming of age. Nat. Rev. Genet. 20, 317-317.

Carlyle, B.C., Kitchen, R.R., Mattingly, Z., Celia, A.M., Trombetta, B.A., Das, S., Hyman, B.T., Kivisäkk, P., Arnold, S.E., 2022. Technical performance evaluation of olink proximity extension assay for blood-based biomarker discovery in longitudinal studies of Alzheimer's Disease. Front. Neurol. 13, 889647.

Chappell, L.C., Cluver, C.A., Tong, S., 2021. Pre-eclampsia. Lancet 398, 341–354.

Côrte-Real, B.F., Hamad, I., Hornero, R.A., Geisberger, S., Roels, J., V.A.N. Zeebroeck, L., Dyczko, A., VAN GISBERGEN, M. W., KURNIAWAN, H. & WAGNER, A. 2023. Sodium perturbs mitochondrial respiration and induces dysfunctional Tregs. Cell Metabolism, 35, 299–315. e8.

Duhig, K.E., Seed, P.T., Placzek, A., Sparkes, J., Hendy, E., Gill, C., Brockbank, A., Shennan, A.H., Thangaratinam, S., Chappell, L.C., 2021. Prognostic indicators of severe disease in late preterm pre-eclampsia to guide decision making on timing of delivery: the PEACOCK study. Pregnancy Hypertens. 24, 90–95.

Erez, O., Romero, R., Maymon, E., Chaemsaithong, P., Done, B., Pacora, P., Panaitescu, B., Chaiworapongsa, T., Hassan, S.S., Tarca, A.L., 2017. The prediction of late-onset preeclampsia: Results from a longitudinal proteomics study. PloS One 12, e0181468.

Gormley, M., Ona, K., Kapidzic, M., Garrido-Gomez, T., Zdravkovic, T., Fisher, S.J., 2017. Preeclampsia: novel insights from global RNA profiling of trophoblast subpopulations. Am. J. Obstet. Gynecol. 217, 200. e1-200. e17.

Greenbaum, S., Averbukh, I., Soon, E., Rizzuto, G., Baranski, A., Greenwald, N.F., Kagel, A., Bosse, M., Jaswa, E.G., Khair, Z., 2023. A spatially resolved timeline of the human maternal–fetal interface. Nature 619, 595.

Hasin, Y., Seldin, M., Lusis, A., 2017. Multi-omics approaches to disease. Genome Biol. 18, 1–15.

Hastie, R., Bergman, L., Walker, S.P., Kaitu'u-Lino, T.U., Hannan, N.J., Brownfoot, F., Schell, S., Harper, A., Cannon, P., Cluver, C.A., 2022. Associations between soluble fms-like tyrosine kinase-1 and placental growth factor and disease severity among women with preterm eclampsia and preeclampsia. J. Am. Heart Assoc. 11, e024395.

Hein, M.Y., Sharma, K., Cox, J. & Mann, M. 2013. Proteomic analysis of cellular systems. *Handbook of systems biology: concepts and insights.* Academic Press.

Joenväärä, S., Holm, M., Saraswat, M., Agarwal, R., Tohmola, T., Kajantie, E., Räikkönen, K., Laivuori, H., Villa, P.M., Hämäläinen, E., 2022. Quantitative urine proteomics in pregnant women for the identification of predictive biomarkers for preeclampsia. Transl. Med. Commun. 7, 1.

Klein, O., Haeckel, A., Reimer, U., Nebrich, G., Schellenberger, E., 2020. Multiplex enzyme activity imaging by MALDI-IMS of substrate library conversions. Sci. Rep. 10, 15522.

Kucukgoz Gulec, U., Ozgunen, F.T., Buyukkurt, S., Guzel, A.B., Urunsak, I.F., Demir, S.C., Evruke, I.C., 2013. Comparison of clinical and laboratory findings in early-and late-onset preeclampsia. J. Matern. -Fetal Neonatal Med. 26, 1228–1233.

Leinweber, B.D., Tsaprailis, G., Monks, T.J., Lau, S.S., 2009. Improved MALDI-TOF imaging yields increased protein signals at high molecular mass. J. Am. Soc. Mass Spectrom. 20, 89–95.

Liu, Y., Fan, X., Wang, R., Lu, X., Dang, Y.-L., Wang, H., Lin, H.-Y., Zhu, C., Ge, H., Cross, J.C., 2018. Single-cell RNA-seq reveals the diversity of trophoblast subtypes and patterns of differentiation in the human placenta. Cell Res. 28, 819–832.

Lu, Q., Liu, C., Liu, Y., Zhang, N., Deng, H., Zhang, Z., 2016. Serum markers of pre-eclampsia identified on proteomics. J. Obstet. Gynaecol. Res. 42, 1111–1118.

Ma, K., Jin, H., Hu, R., Xiong, Y., Zhou, S., Ting, P., Cheng, Y., Yang, Y., Yang, P., Li, X., 2014. A proteomic analysis of placental trophoblastic cells in preeclampsia–eclampsia. Cell Biochem. Biophys. 69, 247–258.

Marić, I., Contrepois, K., Moufarrej, M.N., Stelzer, I.A., Feyaerts, D., Han, X., Tang, A., Stanley, N., Wong, R.J., Traber, G.M., 2022. Early prediction and longitudinal modeling of preeclampsia from multiomics. Patterns 3.

Mary, S., Kulkarni, M.J., Malakar, D., Joshi, S.R., Mehendale, S.S., Giri, A.P., 2017. Placental proteomics provides insights into pathophysiology of pre-eclampsia and predicts possible markers in plasma. J. Proteome Res. 16, 1050–1060.

Maynard, S.E., Min, J.-Y., Merchan, J., Lim, K.-H., Li, J., Mondal, S., Libermann, T.A., Morgan, J.P., Sellke, F.W., Stillman, I.E., 2003. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J. Clin. Investig. 111, 649–658.

Mcginnis, R., Steinthorsdottir, V., Williams, N.O., Thorleifsson, G., Shooter, S., Hjartardottir, S., Bumpstead, S., Stefansdottir, L., Hildyard, L., Sigurdsson, J.K., 2017. Variants in the fetal genome near FLT1 are associated with risk of preeclampsia. Nat. Genet. 49, 1255–1260.

Mund, A., Coscia, F., Kriston, A., Hollandi, R., Kovács, F., Brunner, A.-D., Migh, E., Schweizer, L., Santos, A., Bzorek, M., 2022. Deep visual proteomics defines single-cell identity and heterogeneity. Nat. Biotechnol. 40, 1231–1240.

Nonn, O., Debnath, O., Valdes, D.S., Sallinger, K., Secener, A.K., Haider, S., Fischer, C., Tiesmeyer, S., Nimo, J., Kuenzer, T., 2022. Disturbed trophoblast transition links preeclampsia progression from placenta to the maternal syndrome. *bioRxiv*, 2022 10 (10), 511539.

Odenkirk, M.T., Stratton, K.G., Gritsenko, M.A., Bramer, L.M., Webb-Robertson, B.-J.M., Bloodsworth, K.J., Weitz, K.K., Lipton, A.K., Monroe, M.E., Ash, J.R., 2020. Unveiling molecular signatures of preeclampsia and gestational diabetes mellitus with multi-omics and innovative cheminformatics visualisation tools. Mol. omics 16, 521–532.

Ong, T.-H., Kissick, D.J., Jansson, E.T., Comi, T.J., Romanova, E.V., Rubakhin, S.S., Sweedler, J.V., 2015. Classification of large cellular populations and discovery of rare cells using single cell matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. Anal. Chem. 87, 7036–7042.

Pique-Regi, R., Romero, R., Tarca, A.L., Sendler, E.D., Xu, Y., Garcia-Flores, V., Leng, Y., Luca, F., Hassan, S.S., Gomez-Lopez, N., 2019. Single cell transcriptional signatures of the human placenta in term and preterm parturition. Elife 8.

Rasanen, J., Girsen, A., Lu, X., Lapidus, J.A., Standley, M., Reddy, A., Dasari, S., Thomas, A., Jacob, T., Pouta, A., 2010. Comprehensive maternal serum proteomic profiles of preclinical and clinical preeclampsia. J. Proteome Res. 9, 4274–4281.

Rasmussen, M., Reddy, M., Nolan, R., Camunas-Soler, J., Khodursky, A., Scheller, N.M., Cantonwine, D.E., Engelbrechtsen, L., Mi, J.D., Dutta, A., 2022. RNA profiles reveal signatures of future health and disease in pregnancy. Nature 601, 422–427.

Regev, A., Teichmann, S.A., Lander, E.S., Amit, I., Benoist, C., Birney, E., Bodenmiller, B., Campbell, P., Carninci, P., Clatworthy, M., 2017. The human cell atlas. elife 6, e27041.

Robillard, P.-Y., Dekker, G., Scioscia, M., Saito, S., 2022. Progress in the understanding of the pathophysiology of immunologic maladaptation related to early-onset preeclampsia and metabolic syndrome related to late-onset preeclampsia. Am. J. Obstet. Gynecol. 226, S867–S875.

Rodriques, S.G., Stickels, R.R., Goeva, A., Martin, C.A., Murray, E., Vanderburg, C.R., Welch, J., Chen, L.M., Chen, F., Macosko, E.Z., 2019. Slide-seq: A scalable technology for measuring genome-wide expression at high spatial resolution. Science 363, 1463–1467.

Sandrim, V., Luizon, M., Palei, A., Tanus-Santos, J.E., Cavalli, R.D.C., 2016. Circulating micro RNA expression profiles in pre-eclampsia: evidence of increased miR-885–5p levels. BJOG: Int. J. Obstet. Gynaecol. 123, 2120–2128.

Suryawanshi, H., Morozov, P., Straus, A., Sahasrabudhe, N., Max, K.E., Garzia, A., Kustagi, M., Tuschl, T., Williams, Z., 2018. A single-cell survey of the human first-trimester placenta and decidua. Sci. Adv. 4 eaau4788.

Svensson, V., Vento-Tormo, R., Teichmann, S.A., 2018. Exponential scaling of single-cell RNA-seq in the past decade. Nat. Protoc. 13, 599–604.

Tang, X., Huang, Y., Lei, J., Luo, H., Zhu, X., 2019. The single-cell sequencing: new developments and medical applications. Cell Biosci. 9, 1–9.

Tarca, A.L., Romero, R., Benshalom-Tirosh, N., Than, N.G., Gudicha, D.W., Done, B., Pacora, P., Chaiworapongsa, T., Panaitescu, B., Tirosh, D., 2019. The prediction of early preeclampsia: Results from a longitudinal proteomics study. PloS One 14, e0217273.

Teka, H., Yemane, A., Abraha, H.E., Berhe, E., Tadesse, H., Gebru, F., Yahya, M., Tadesse, Y., Gebre, D., Abrha, M., 2023. Clinical presentation, maternal-fetal, and neonatal outcomes of early-onset versus late onset preeclampsia-eclampsia syndrome in a teaching hospital in a low-resource setting: A retrospective cohort study. Plos One 18, e0281952.

Than, N.G., Romero, R., Györffy, D., Posta, M., Bhatti, G., Done, B., Chaemsaithong, P., Jung, E., Suksai, M., Gotsch, F., 2023. Molecular subclasses of preeclampsia characterized by a longitudinal maternal proteomics study: distinct biomarkers, disease pathways and options for prevention. J. Perinat. Med. 51, 51–68.

Tian, L., Chen, F., Macosko, E.Z., 2023. The expanding vistas of spatial transcriptomics. Nat. Biotechnol. 41, 773–782.

Tong, S., Tu'uhevaha, J., Hastie, R., Brownfoot, F., Cluver, C., Hannan, N., 2022. Pravastatin, proton-pump inhibitors, metformin, micronutrients, and biologics: new horizons for the prevention or treatment of preeclampsia. Am. J. Obstet. Gynecol. 226, S1157–S1170.

Trifonova, E., Svarovskaya, M., Gabidullina, T., Serebrova, V., Babovskaya, A., Gavrilenko, M. & Stepanov, V. Genomics and transcriptomics of preeclampsia. Bioinformatics of Genome Regulation and Structure/Systems Biology (BGRS/SB-2022), 2022. 453–453.

Tsai, S., Hardison, N.E., James, A.H., Motsinger-Reif, A.A., Bischoff, S.R., Thames, B.H., Piedrahita, J.A., 2011. Transcriptional profiling of human placentas from pregnancies complicated by preeclampsia reveals disregulation of sialic acid acetylesterase and immune signalling pathways. Placenta 32, 175–182.

Vento-Tormo, R., Efremova, M., Botting, R.A., Turco, M.Y., Vento-Tormo, M., Meyer, K. B., Park, J.-E., Stephenson, E., Polański, K., Goncalves, A., 2018. Single-cell reconstruction of the early maternal–fetal interface in humans. Nature 563, 347–353.

Wang, X., Yip, K.C., He, A., Tang, J., Liu, S., Yan, R., Zhang, Q., Li, R., 2022. Plasma olink proteomics identifies CCL20 as a novel predictive and diagnostic inflammatory marker for preeclampsia. J. Proteome Res. 21, 2998–3006.

Who 2023. Trends in Maternal Mortality 2000 to 2020: Estimates by WHO, UNICEF, UNFPA, World Bank Group and UNDESA/Population Division. WHO.
Wilkins, M.R., Sanchez, J.-C., Gooley, A.A., Appel, R.D., Humphery-Smith, I., Hochstrasser, D.F., Williams, K.L., 1996. Progress with proteome projects: why all proteins expressed by a genome should be identified and how to do it. Biotechnol. Genet. Eng. Rev. 13, 19–50.
Xu, C., Zhou, L., Fang, Q., Liu, Y., Yang, J., Luo, L., Li, S., Guo, P., Niu, Y. & Deng, W. 2023. Polymorphism in maternal HLA-DRB5 is associated with the risk of preeclampsia in Chinese population.
Yang, J.I., Kong, T.W., Kim, H.S., Kim, H.Y., 2015. The proteomic analysis of human placenta with pre-eclampsia and normal pregnancy. J. Korean Med. Sci. 30, 770–778.
Yong, H.E., Melton, P.E., Johnson, M.P., Freed, K.A., Kalionis, B., Murthi, P., Brennecke, S.P., Keogh, R.J., Moses, E.K., 2015. Genome-wide transcriptome directed pathway analysis of maternal pre-eclampsia susceptibility genes. PLoS One 10, e0128230.
Yuen, R.K., Penaherrera, M.S., Dadelszen, V.O.N., P., Mcfadden, D.E., Robinson, W.P., 2010. DNA methylation profiling of human placentas reveals promoter hypomethylation of multiple genes in early-onset preeclampsia. Eur. J. Hum. Genet. 18, 1006–1012.
Zhang, M., Deng, X., Jiang, Z., Ge, Z., 2022. Identification of underlying mechanisms and hub gene-miRNA networks of the genomic subgroups in preeclampsia development. Medicine 101.
Zhang, X., Li, T., Liu, F., Chen, Y., Yao, J., Li, Z., Huang, Y., Wang, J., 2019. Comparative analysis of droplet-based ultra-high-throughput single-cell RNA-seq systems. Mol. Cell 73, 130-142. e5.
Zhao, L., Bracken, M.B., Dewan, A.T., 2013. Genome-wide association study of pre-eclampsia detects novel maternal single nucleotide polymorphisms and copy-number variants in subsets of the hyperglycemia and adverse pregnancy outcome (HAPO) Study Cohort. Ann. Hum. Genet. 77, 277–287.