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Gene expression profiles in placenta and their association with anesthesia, delivery mode and maternal diabetes

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ABSTRACT

Introduction: Fetal development is dependent on placenta and affected by multiple factors including maternal diabetes. Here we aimed to identify maternal diabetes-associated changes in placentas and analyzed placental gene expression to understand its modulation by maternal diabetes and birth mode.

Methods: Placental RNAseq transcriptome analyses were performed on maternally-derived decidua and fetal-derived villous tissue from pregnancies of mothers with type 1 diabetes (n = 14), gestational diabetes (n = 6) and without diabetes (n = 14). Information on delivery mode and anesthesia were included as covariables. Analyses were performed separately for decidua and fetal tissues and adjusted for sex.

Results: Substantial placenta gene expression variation was associated with factors other than maternal diabetes, including site, sex, anesthesia type and delivery mode. Two dominant gene expression clusters aligned to anesthesia and delivery mode were observed for decidua and villous tissue. Upregulation of genes within pathways related to organ morphogenesis and downregulation of immune response to steroid- and hypoxia pathway genes was characteristic of placentas from primary cesarean section deliveries with spinal anesthesia. Opposite profiles were observed for placentas from secondary cesarean and epidural anesthesia deliveries. Placentas from vaginal delivery had intermediate gene expression profiles. More subtle changes were associated with maternal diabetes: upregulation of ribosome activity, down-regulation of maternally-derived decidua chemokine signaling pathways and for gestational diabetes, alteration in hypoxia response genes.

Discussion: The findings reveal suppression of immune pathways and upregulation of ribosome activity in the placenta by maternal diabetes highlighting the importance of confounding factors when examining cell and tissue expression profiles. Further studies should determine whether the observed gene expression differences are related to underlying causes for cesarean section deliveries.

1. Introduction

The placenta is a complex organ that serves multiple vital functions in supporting fetal growth [1]. In humans, the placenta is a discoid structure primarily composed of trophoblasts immersed in maternal blood [2]. At term, villi are vascular projections of fetal origin surrounded by a monolayer of syncytiotrophoblasts, acting as the interface between fetal and maternal blood. Columns of cytotrophoblasts anchor the villi to extravillous trophoblasts in the maternal decidua [2–4].

As a crucial interface between the mother and the fetus, the placenta

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is susceptible to modulation by maternal diseases such as preeclampsia (PE), diabetes, obesity, or substance abuse. These changes can influence molecular processes, subsequently impacting the mother or the fetus [5–8]. Glucose serves as the primary nutritional source for the fetus, crossing the placental barrier via facilitated diffusion through glucose transporters (*GLUT*) [9]. Maternal diabetes upregulates GLUT expression density on placental villi, leading to increased glucose transport and hyperinsulinemia [10]. This, in turn, results in fetal overgrowth and an elevated risk of T2D later in life[11–13]. Maternal Type 1 Diabetes (T1D) also influences glucose import and fetal insulin levels but also provides a relative protection against the development of islet autoantibodies and T1D in offspring [14–17]. Maternal diabetes is also associated with increased frequencies of cesarean section delivery, which may be linked to underlying conditions such as pre-eclampsia [18,18,19].

In this study, we utilized RNA-seq of fetal placental villi and maternal decidua samples to investigate whether maternal T1D and other maternal factors are associated with changes in placental gene expression.

2. Materials and methods

2.1. Samples

Tissue samples were collected from placental villi (villous) and maternal decidua (decidua) of 34 mothers and their newborns, immediately frozen within 3 h after delivery. The deliveries were either vaginal (N = 17) or by cesarean section (n = 17), comprising primary planned section (N = 9) or secondary emergency section (N = 8) as detailed in Table 1. Anesthesia methods during delivery included spinal (N = 11), epidural (N = 11), or local anesthesia (N = 6), with unknown anesthesia information for six cases (Table 1). Of the 34 mothers, 6 had gestational diabetes (GDM), 14 had type 1 diabetes (T1D), and 14 had no diabetes history. Additional clinical characteristics of pregnancies for placentas studied are available on the clinical characteristics table in Supplemental Data [20] and childbirth risks and indications for section in Supplemental Table 1. Delivery mode (vaginal, primary or secondary section) and anesthesia applied during parturition were also recorded and analyzed. The distribution of these factors in relation to diabetes is shown in the contingency table (Supplemental Table 2). The study was approved by the ethics committee of the Technische Universität München (Ethikkommission der Fakultät für Medizin, no. 5293/12. All participating women provided informed consent.

Table 1
Maternal, delivery and offspring characteristics of cohort.

	No Diabetes	Maternal type 1 diabetes	Gestational diabetes	*P* value
Sex of offspring, male/female	7/7	10/4	3/3	0.46
Age of mother, median (IQR)	33 (31–35)	33 (32–34)	31 (26–34)	0.51
Delivery mode				0.023
Vaginal (%)	8 (47.1)	4 (23.6)	5 (29.4)	
Primary section (%)	4 (44.5)	4 (44.4)	1 (11.1)	
Secondary section (%)	2 (25.0)	6 (75.0)	0 (0.0)	
Anesthesia				0.84
Local (%)	3 (50.0)	2 (33.3)	1 (16.7)	
Epidural (%)	4 (36.4)	4 (36.4)	3 (27.3)	
Spinal (%)	5 (45.5)	5 (45.5)	1 (9.1)	
Unknown (%)	2 (33.3)	3 (50.0)	1 (16.7)	

Table shows counts of samples used and accompanying metadata. Columns show maternal diabetes state, rows show sex of the offspring, median age of the mother, delivery mode and anesthesia used ("Unknown" might include cases without anesthesia). The number of samples in each category is shown. P values comparing the variables for the 3 groups (No diabetes, Maternal type 1 Diabetes and Gestational Diabetes) are shown in the column on the right. Kruskall-Wallis test was used for age; Fishers exact test or Chi squared test for the categorical factors. % of sample per group for each category are shown in brackets for delivery mode and anesthesia.

2.2. Placental samples isolation and library preparation

Placentae were kept at 4 °C after delivery or caesarean section until further processing and during sample preparation. Time between placenta delivery and processing never exceeded 3.5 h, as gene expression had been shown to be stable over this time (E.-M. Sedlmeir, personal communication). Placentae were weighed and four samples dissected according to a standardized sampling protocol, avoiding regions with large vessels or calcifications [21]. Samples were further sectioned into 3 parts: one part facing the fetal side, containing the fetal layer (not further used here), the middle part containing the fetal villous chorionic plate (referred to as "villous") and one part, facing the maternal side, containing the maternal basal plate (referred to as "decidua). For each layer, one piece of each of four quadrants obtained when partitioning the placenta into four parts were dissected, and pestled in liquid nitrogen. The obtained powders were joined in equal weights, stored in Trizol and whole RNA was extracted from the tissue samples using the RNeasy® Midi Kit (Qiagen). Median RIN was 7.5 (IQR, 7.4–8.1) and 7.6 (IQR, 7.3–8.1) for villous and decidua. 500 ng RNA were subjected to the workflow for strand specific RNA-seq library preparation (Ultra II Directional RNA Library Prep, NEB) followed by and PCR enrichment. After AMPure XP bead purification (in a ratio of 1:0.9) and quantification using the Fragment Analyzer (Agilent), libraries were subjected to RNA sequencing on an Illumina NovaSeq 6000 with 2 × 100 bp reads using a S4 flowcell to an average depth of 50 million read pairs.

2.3. Bioinformatical analysis

The characteristics between groups were compared using the Kruskal-Wallis H test or, for categorical variables, Fishers exact test or Chi squared test. FastQC (http://www.bioinformatics.babraham.ac.uk/) and MultiQC were used to quality control the resulting sequencing data [22]. Fragments were aligned to the human reference genome hg38 with support of the Ensembl 104 splice sites using the aligner star (2.7.10b) [23]. Counts per gene and sample were obtained based on the overlap of the uniquely mapped fragments with the same Ensembl annotation using featureCounts (v2.0.1) [24]. Differential gene expression was analyzed using the DESeq2 v(1.41.10) package in R [25]. Data was normalised using the median of ratios method (default in DESeq2). For visualization and principal component analysis a regularized log` transformation was applied using the DESeq2 function rlog. Formulas used were: `ddsData < − DESeqDataSetFromMatrix(countData = data, colData = meta, design = ~ factor)` followed by: `ddsData < - DESeq (ddsData)`; with correction for sex as a confounding factor: `dds_Data < - DESeqDataSetFromMatrix(countData = data, colData = meta, design = ~ Sex + factor)` followed by: `dds_Data < - DESeq(dds_Data)`. To identify the factors contributing to gene expression variation, we utilized the variancePartition package [26]. For the likelihood ratio test (LRT), we first combined the birth mode and anesthesia factors into a single factor; we then controlled for this factor (BirthMode_Anesthesia) and for Diabetes. Formulas used were: `ddsData < - DESeqDataSetFromMatrix(countData = data, colData = meta, design = ~ Sex + BirthMode_Anesthesia + Diabetes)` followed by: `ddsData < - DESeq (ddsData, test = "LRT", reduced = ~Sex + Diabetes`). Significant genes were then used for hierarchical clustering with complete linkage in pheatmap (hclust) using default parameters [27].Thresholds for fold-changes and adjusted p values (padjust) were 2 and 0.05, respectively. The clusterProfiler [28] package was used to identify and visualize enriched pathways among differentially expressed genes (DEG) based on the Gene Ontology (GO) or Kyoto Encyclopedia of Genes and

Genomes (KEGG) collections. Terms were considered as statistically significant when their corresponding q-values were less than 0.05.

3. Results

3.1. Placental tissue origin and offspring sex influence gene expression of placental cells

Placenta samples derived from villous or decidua tissue of 34 mothers and their offspring were subjected to RNA-seq analysis. Principal Component Analysis (PCA) on expression data revealed distinct groupings based on offspring sex and placental site of origin (Fig. 1). A total of 98 DEGs were identified comparing samples between male and female offspring, across both placental sides, including 32 genes on the Y-Chromosome, 12 on the X-Chromosome, and 54 on non-sex chromosomes (Supplemental Table 3, Supplemental Fig. 1). Additionally, 714 DEG were found between villous and decidua placental tissue samples. Decidua samples, which originate from maternal tissue, exhibited enrichment in pathways related to immune activation, immune regulation and cell migration (Supplemental Table 4, Fig. 2). Due to the strong confounding effects, subsequent analyses were performed separately for villous- and decidua tissue and included adjustment for child sex.

3.2. Placental gene expression clusters

We first searched for variables that contributed to placenta gene expression variation using variance partitioning analysis (Supplemental Fig. 2). Variables included were maternal diabetes status (T1D, GDM, no diabetes), anesthesia at delivery (spinal, epidural, local) and delivery mode (vaginal, primary cesarean section, secondary cesarean section) and sex. As expected, for the villous tissue, sex was the major factor determining variance in a minority of genes. For both villous and decidua, the largest contribution to the gene expression variation of all genes was the type of anesthesia used at delivery, followed by delivery mode, maternal diabetes, and sex. Since anesthesia and delivery mode were strongly associated with each other, they were combined into a single factor for subsequent analysis of DEG in relation to these factors.

The likelihood ratio test on the sex- and diabetes regressed data identified 1111 DEG in relation to anesthesia/delivery mode for samples from villous and 641 DEG for samples from decidua (Supplemental Table 5). Clustering the 500 most significant DEG yielded two major gene clusters and three major sample clusters for both decidua and villous samples (Fig. 3A and B). Sample clusters 1 and 2 had opposing expression levels for genes with the two gene clusters, and sample cluster 3 had a mixed gene expression phenotype. Sample cluster 1 comprised all primary section samples (n = 9) for both villous and decidua samples. Additionally, this cluster included 9 of 11 placentas from deliveries where spinal anesthesia was used. Sample cluster 2 included 10 of 11 villous samples and all 11 decidua samples from deliveries where epidural anesthesia was administered. Sample cluster 2 also encompassed all 6 secondary section deliveries, all of which involved epidural or spinal anesthesia. Furthermore, sample cluster 2 incorporated samples from vaginal deliveries (6 out of 13 villous samples; 7 out of 13 decidua samples). DEG expression profiles in sample cluster 2 closely mirrored those in cluster 1. All placentas within sample cluster 3 were derived from vaginal delivery and almost all were delivered with local anesthesia.

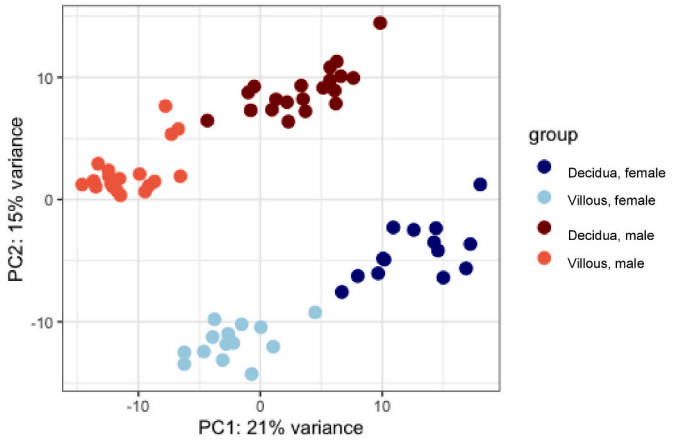

Fig. 1. Grouping of samples from same sex offspring or same tissue origin. Separation of all 34 samples by PCA. Samples, represented by dots, are coloured according to sex and tissue origin. Samples from females are shown in blueish- and samples from males in reddish colours, darker shading indicates origin from decidua and lighter shading from villous.

The DEG within gene cluster 1 were enriched in pathways related to organ morphogenesis, particularly in heart, vessel, bone, and cartilage development. The PI3K-Akt pathway was also prominent (Fig. 3E). Similar pathway enrichments were identified in both villous and decidua samples within gene cluster 1. Up-regulation of these genes was observed in the sample cluster 1, enriched with primary section delivery and spinal anesthesia, while they were down-regulated in sample cluster 2, enriched with secondary section delivery and epidural anesthesia. The DEG within gene cluster 2 were enriched in pathways involving immune response, and responses to steroids or hypoxia (Fig. 3F). Sample cluster 1 was associated with the down-regulation of these genes, whereas sample cluster 2 exhibited their up-regulation. Sample cluster 3 generally displayed an up-regulation of gene cluster 1 genes and variable expression of gene cluster 2 genes. Taken together, the findings show that placental gene expression variation was associated with the type of anesthesia and delivery mode with two major gene expression clusters.

3.3. Maternal diabetes and placental gene expression

In view of the association between diabetes in pregnancy with anesthesia and/or delivery mode (Supplemental Table 2), the sex-regressed data from villous and decidua samples were further stratified based on anesthesia category and delivery mode. Following this stratification, comparisons between matched groups were separately conducted. First, births from mothers with T1D and mothers without diabetes who received spinal anesthesia and underwent primary section delivery were compared and second, births from mothers with GDM and mothers without diabetes who received epidural anesthesia and had a vaginal delivery (Fig. 4). Other comparisons were not made because of very low sample numbers.

In total, 24 DEGs were identified between samples from mothers with T1D and samples from mothers without diabetes. This included 3 DEGs in both villous and decidua samples, 9 DEGs in villous samples only and 12 DEGs in decidua samples only (Fig. 4A). No enriched GO terms were found for those genes. However, KEGG analysis revealed an upregulation of ribosome-associated activity in both decidua and villous samples and down-regulation of the chemokine-signaling pathway in decidua samples (Fig. 4B).

In comparisons between samples from children of mothers with GDM and those without diabetes, 25 DEGs were identified. This encompassed 6 DEGs common to both villous and decidua samples, 10 DEGs exclusive to villous samples, and 9 DEGs exclusive to decidua samples (Fig. 4C). None of these overlapped with the DEGs in offspring of mothers with type 1 diabetes. There was a striking downregulation of interferon gamma inducible chemokine ligand genes (*CXCL9*, *CXCL10*, *CXCL11*) in GDM decidua samples. This was also reflected by the suppression of the chemokine signaling and response pathways, similar to the pathway analysis for T1D (Fig. 4D and E). Also of interest were genes responsive to hypoxia observed in villous samples, including *CXCL12* and *EGR1*, which were downregulated, and *ARNT2*, which was upregulated in GDM samples. Pathway analysis was consistent with an impaired response to

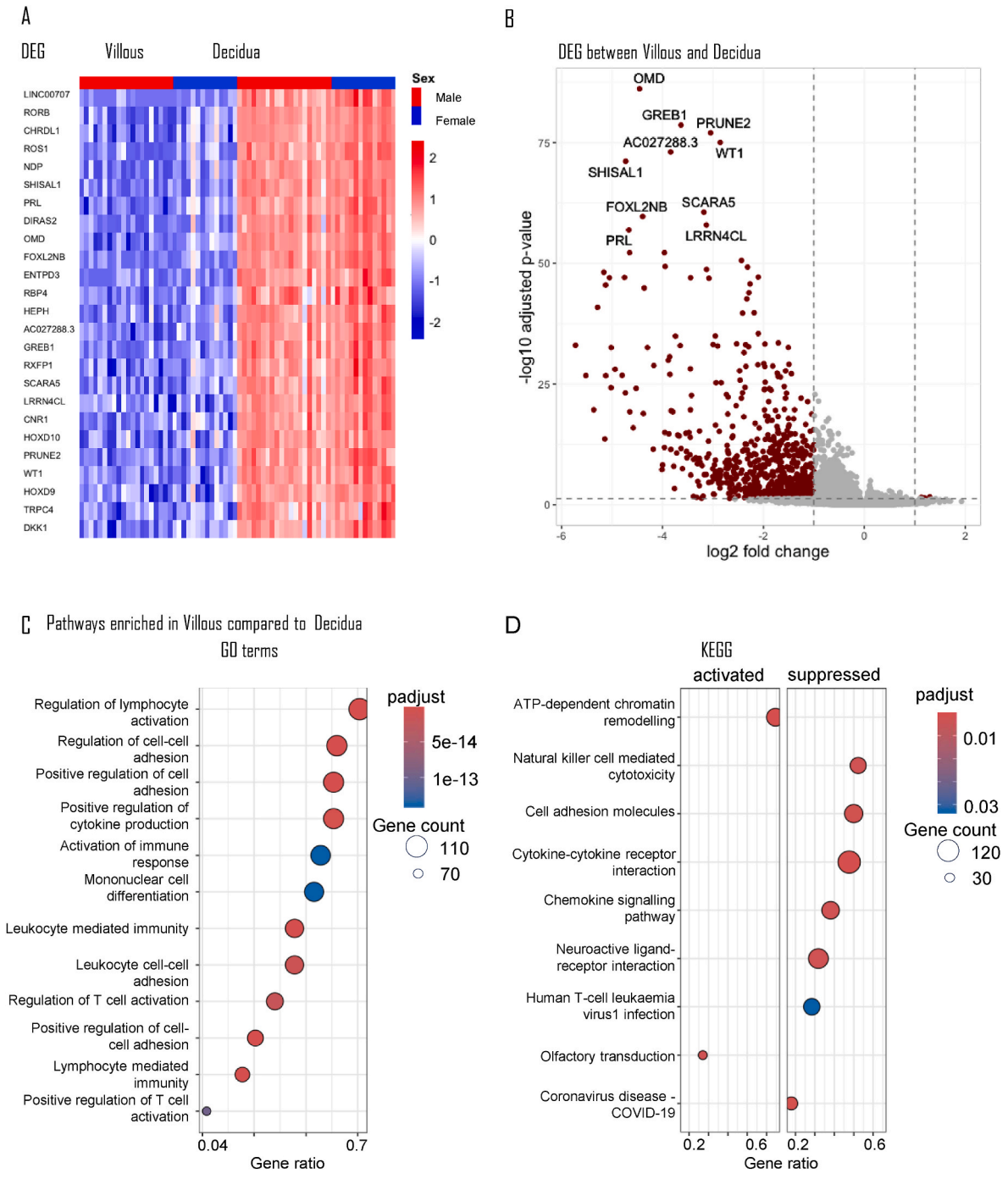

Fig. 2. Transcriptome difference between samples from different placental tissue origin. **A** Heatmap showing normalised counts of the 25 most significant DEG between tissue from villous and from decidua. DEG are arranged after padj, with the lowest one at the top. Samples from villous are shown on the left, and from decidua on the right side of the heatmap. Samples from female are in blue and from male in red, as shown on top of the heatmap. Normalised counts are colour-coded as shown in the legend (blue: low expression; red: high expression). **B** Volcano plot shows all transcripts, highlighting in dark red the 714 DEG between villous- and decidua tissue (3 upregulated in villous (right part) and 711 down regulated in villous, thus upregulated in decidua, left part). The $\log_2$ fold change is presented against the $-\log_{10}$ adjusted p-value. Each dot corresponds to a transcript; red dots represent significant DEG (fc > 2; padj<0.05). Gene names for the 10 transcripts with the lowest padj and fc > 2 are shown (all downregulated in villous and thus upregulated in decidua). **C** Dotplot showing the significance of the enriched biologic terms (Gene Ontology term). Gene count, the number of genes involved in the term is reflected by the dot size. The x-axis shows the gene ratio (the ratio of significant genes involved in each term to the total number of genes in the term). *p* adjust is the adjusted *p*-value and is reflected by colouring of the dots. **D** Dotplot showing the significance of the pathways enriched in villous compared to decidua samples (KEGG pathway). Pathways activated in villous are shown on the left and suppressed ones (thus activated in decidua) on the right.

hypoxia in the placental villous tissue from mothers with GDM. In summary, stratification allowed to find subtle transcriptomic differences between placenta samples from mothers with diabetes and from healthy mothers including a suppression of chemokine pathways common to both T1D and GDM.

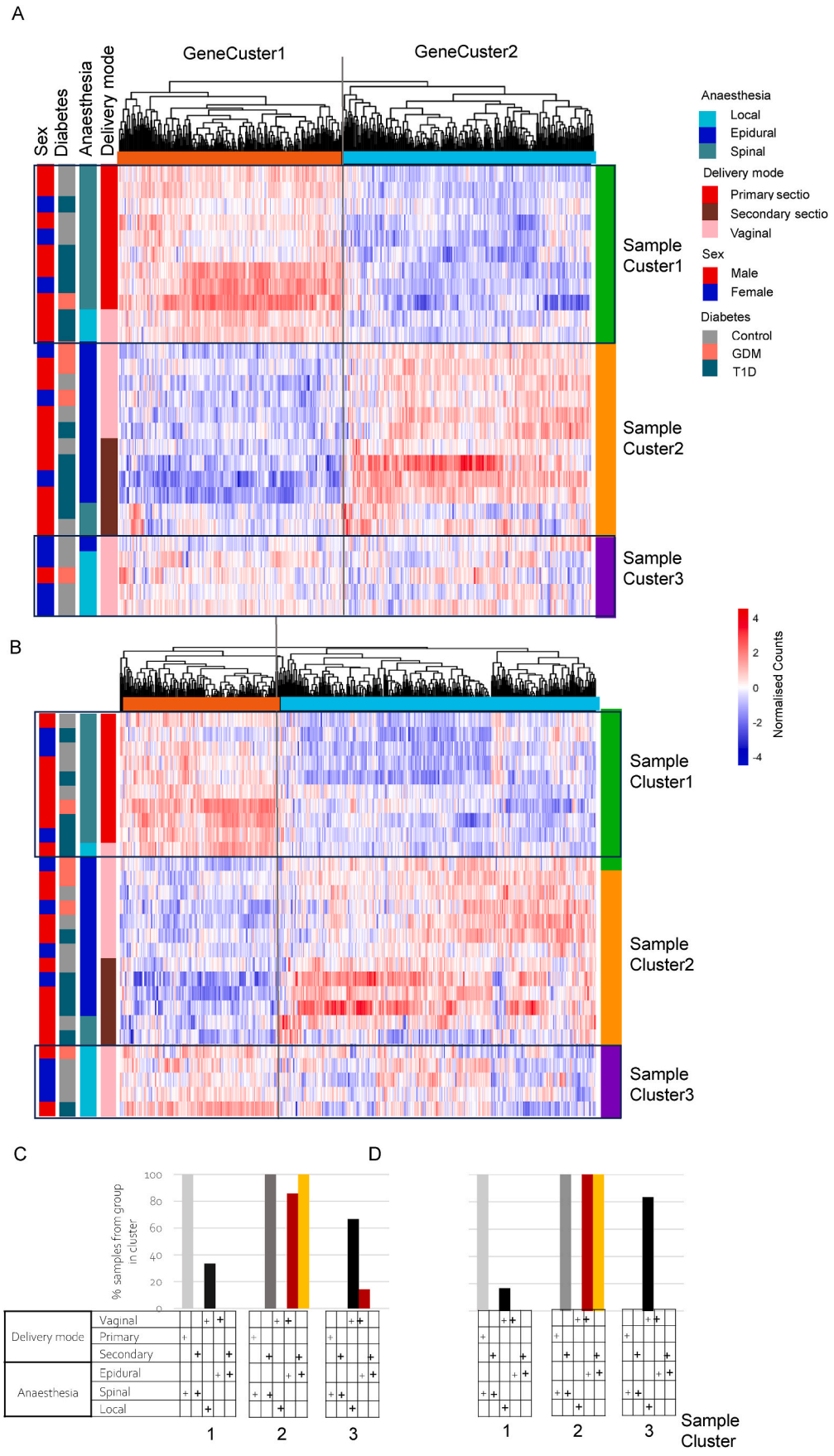

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Fig. 3. Delivery mode and Anesthesia given during delivery have an impact on transcriptome. A and **B** Heatmaps show clustering (complete linkage method for hierarchical clustering) of normalised counts of the top 500 DEG obtained by likelihood ratio test (LRT) (each column corresponds to one gene) (**A**: villous samples; **B**: decidua samples). On the x-axis, two gene clusters are shown, GeneCluster1 (dark orange) and GeneCluster2 (turquoise); on the y-axis three sample clusters are shown: SampleCluster1 (green), SampleCluster2 (orange) and SampleCluster3 (purple). Colour-coding of the samples according to delivery mode (red: primary sectio, light pink: vaginal, dark red: secondary sectio), anesthesia (blue-grey: spinal-, dark blue: epidural-, turquoise: local anesthesia), sex (blue: samples from female, red: samples from male) and Diabetes (mother with T1D: green, mother with GDM: salmon, healthy mother: grey) is shown on the left. Normalised counts are colour-coded as shown in the legend (blue: low expression; red: high expression). Shown are 29 samples, including only samples with information on all variables. **C** and **D** Bar plots show the sample distribution over the three samples clusters (SampleCluster1, SampleCluster2 and SampleCluster3) for samples from villous- (C) and from decidua (D) tissue. The frequency of samples from each group found in the cluster is shown. Sample groups are described in the grids below (delivery mode and anesthesia applied). **E** and **F** Dotplot shows Gene Ontology term analysis of significant genes from gene cluster 1 (E) or gene cluster 2 (F) as determined by Gene Ontology term analysis (E top plot and F) or by KEGG enrichment analysis (E bottom plots; F: no KEGG enrichment terms found for cluster 2 in villous samples, 2 terms only found for decidua samples, not shown). Left plots: villous samples and right plots decidua samples. Legends are as in Fig. 2.

4. Discussion

The placenta serves as a critical interface between the mother and the fetus, modulated by the maternal environment, thereby influencing fetal development and the development of chronic diseases later in life [2]. This study aimed to investigate the effect maternal diabetes has on placental function. A hierarchical order of factors associated with placental gene expression was observed, with placental site (villous or decidua) and offspring sex as important determinants. Variation was also associated with the type of delivery, including the type and mode of anesthesia administration. These associations may reflect the underlying causes for cesarean section delivery, which were not investigated. More subtle changes were associated with maternal diabetes. These included the down-regulation of maternally-derived decidua chemokine signaling pathways and an upregulation of ribosome activity in both maternal and fetal tissue.

Offspring sex was a factor dictating gene expression differences. This is consistent with previous findings highlighting functional disparities between male and female placentae and contributing to sex-biased preterm vulnerability [29–35]. Female fetuses display adaptability by slowing down placental growth, resulting in reduced fetal growth, while male fetuses exhibit fewer placental changes, maintaining normal fetal growth. The most profound and earliest gene expression differences between sexes are linked to sex chromosomes [36]. In our analysis, Y-linked genes were among the top DEG, including *DDX3Y*, *NLGN4Y*, *TMSB4Y*, *PCDH11Y*, *RPS4Y1*, *USP9Y*, *UTY*, *EIF1AY*, and *ZFY*, consistent with previous findings in male and female placenta samples [29].

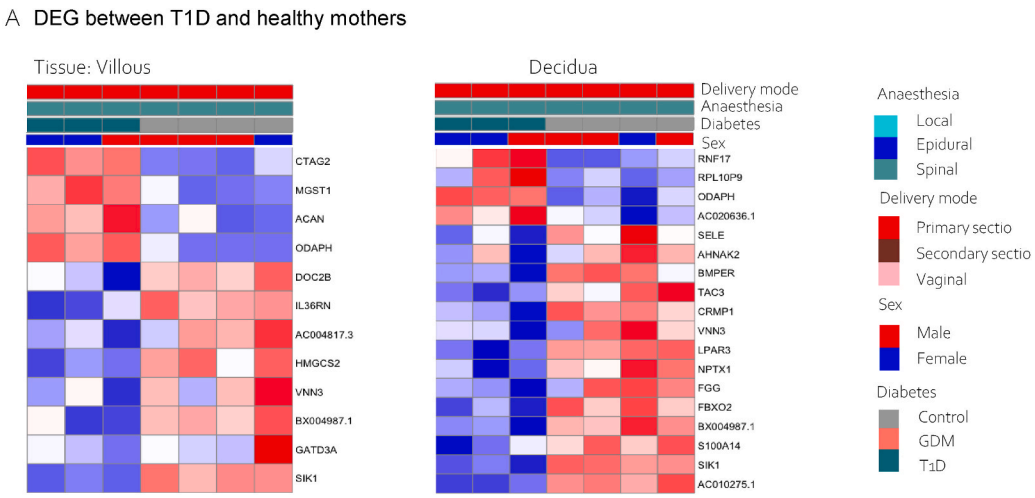
Placental gene expression variation was associated with the type of anesthesia administered to the mother. Anesthesia was tied to delivery mode and delivery mode is often determined by underlying maternal factors. Mechanistic studies are, therefore, required to elucidate whether there is a causal relationship between anesthesia and gene expression patterns. Gene expression in placentas coming from spinal anesthesia together with primary section delivery was associated with down-regulated genes of immune pathways and up-regulated genes important for morphogenesis and development of heart, vessels, bone, and cartilage, as well as the extracellular matrix-receptor interaction. Additionally, genes related to the PI3K-Akt pathway, regulating cell cycle and stress response, were upregulated. Akt promotes survival after cell injury and the PI3K-Akt pathway has been implicated in recovery from general anesthesia [37,38]. Mothers who received epidural anesthesia had all undergone labor, either resulting in vaginal birth or secondary section. Genes found up-regulated in placental samples from these mothers were associated with immune responses, cytokine production, responses to oxygen levels, and the reaction to corticosteroid hormones. Notably, labor induces a pro-inflammatory state and the release of cytokines [39]. Additionally, epidural-related maternal fever is observed exclusively in the context of labor following epidural anesthesia. The interplay between the inflammatory response during labor and epidural anesthesia has been postulated to impact immune cells, potentially contributing to the occurrence of fever.

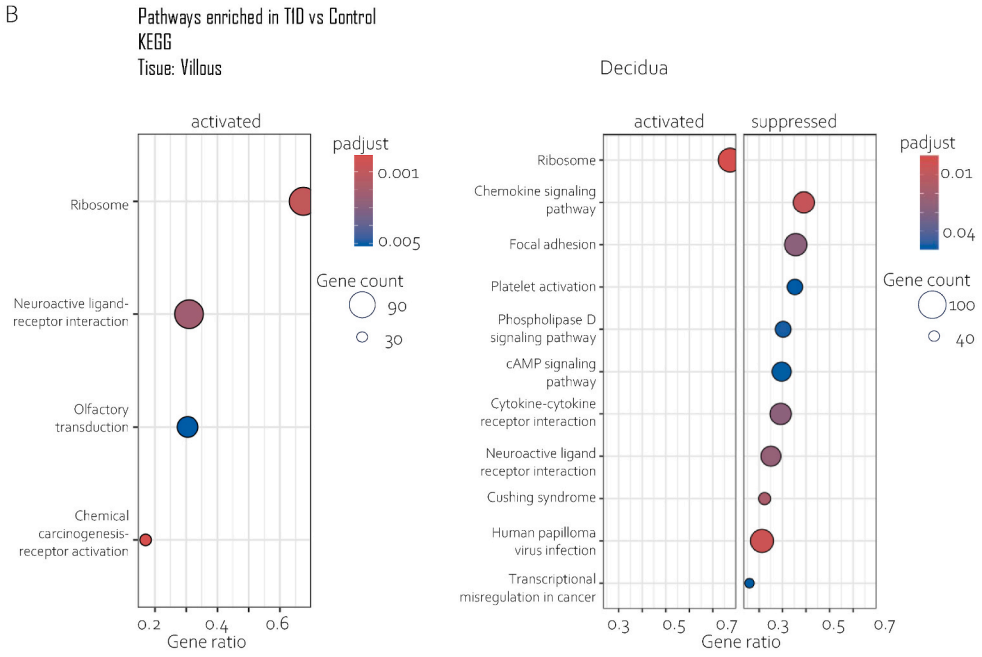
Such an association with anesthesia for gene expression in human tissue has not been described previously. Levka et al. analyzed gene expression in placenta samples from mothers with pre-eclampsia or GDM and analyzed other maternal factors accountable for DEG, including anesthesia [40]. Although they also found effects associated with anesthesia, they did not stratify or adjust for the sex and placental site influences and, therefore, their analysis is incomplete. Surgically removed tissue or autopsy tissue is a wealthy source of patient material for understanding disease and treatment effects [29,36]. However, the influence of anesthesia or of intensive care therapy as well as the underlying cause of death for autopsy material is not often considered in analyses. Our data indicates that these are important and necessary in interpreting transcriptomic findings from human tissues.

Despite the challenges in dissecting the impact of T1D and GDM on placental tissue transcriptomics, stratification revealed several differentially expressed genes (DEGs). Pathway analysis suggested that T1D pregnancies are associated with increased ribosomal activity and, therefore, protein production in the placenta. This is consistent with increased nutrient availability and cell growth, which is associated with fetal development in offspring of mothers with diabetes. Interestingly, the suppression of chemokine signaling in the decidua was observed in both T1D and GDM pregnancies. Parturition is associated with signals from the fetus to promote a proinflammatory environment [41]. Moreover, the response to pathogens during pregnancy is the result of signals from the maternal immune system and the placenta. Therefore, the reduction of immune responsiveness at the maternal side of the placenta in T1D and GDM may be associated with an increased risk for complications during pregnancy and delivery [42]. It is unknown whether the observed suppression of placental immune pathways is linked to the reduced the risk of islet autoimmunity and type 1 diabetes in the offspring of mothers with T1D. GDM was also associated with a down-regulation of responses to hypoxia and pathways regulating cell proliferation.

The study has several important limitations. The number of births in each group is small and, although not significant, there is potential bias in the delivery mode or anesthesia between groups. This limits the study power to discern differences and to confidently associate differences to birth mode or anesthesia. Birth mode is likely to be influenced by underlying conditions and some of the gene expression variation associated with birth mode and anesthesia are likely to reflect such underlying conditions. Moreover, the strong influence of birth mode and/or anesthesia led to a stratified analysis, which limited the ability to address the influence of maternal diabetes on the placenta. While our analysis revealed a limited number of DEGs, indicating potential alterations in stress-related responses and intrinsic control due to maternal GDM and T1D, we acknowledge the likelihood of other maternal factors influencing or obscuring these effects.

In conclusion, our study has demonstrated variation in placental gene expression associated with sex, placental site, anesthesia, and delivery mode. Through stratification and adjustment for these factors, we successfully identified a set of genes associated with maternal diabetes and which suggest a down-regulation of chemokine signaling and upregulation of ribosome activity. Moving forward, investigations involving human placenta and other tissues must be large enough to account for the dominant effects associated with multiple factors,





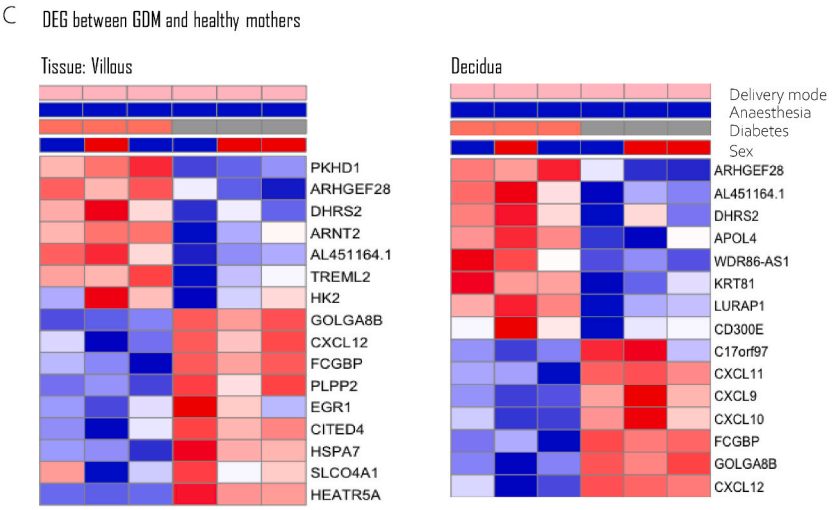


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Fig. 4. DEG between samples form mothers with differing diabetes condition. A and **C** Heatmaps showing normalised counts of the significant DEG between tissue from mothers with T1D and healthy mothers (A) or between tissue from mothers with GDM and healthy mothers (C) that had received the same anesthesia and went through the same delivery mode (primary section and spinal in A and vaginal birth and epidural in C). DEG are arranged after padj, with the lowest one at the top. Samples from villous are shown on the left, and from decidua on the right. Samples from female are in blue and from male in red, samples from maternal T1D are in green, from maternal GDM in dark pink and from healthy mothers in grey, as shown on top of the heatmap. Normalised counts are colour-coded as shown in the legend (blue: low expression; red: high expression). **B** Dotplots show enrichment analysis of DEG found between maternal T1D and controls (KEGG). **D** and **E** Dotplots show Gene Ontology term analysis (D) or KEGG analysis (E) of DEG found between maternal GDM and controls. Legends are as in Fig. 2.

including short term duration effects such as anesthesia and hospital procedures and the underlying causes for these, when analyzing transcriptomic and other omics data.

CRediT authorship contribution statement

Bassam Aljani: Writing – review & editing, Formal analysis. **Annette I. Garbe:** Writing – review & editing, Project administration, Conceptualization. **Eva-Maria Sedlmeier:** Writing – review & editing, Methodology, Data curation. **Ramona Lickert:** Writing – review & editing, Methodology, Data curation. **Fabian Rost:** Writing – review & editing, Formal analysis. **Anette-Gabriele Ziegler:** Writing – review & editing, Project administration, Investigation, Conceptualization. **Ezio Bonifacio:** Writing – original draft, Resources, Project administration, Investigation, Conceptualization. **Anne Eugster:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Conceptualization.

Data and code availability

Raw sequencing files have been deposited in the European Genome-phenome Archive (EGA) und the accession number (EGAD50000000701).

Code has been deposited on Zenodoo.org (DOI: 10.5281/zenodo.10809829).

Declaration of interest summary

B.A has nothing to declare; A.I.G has nothing to declare; E.M.S has nothing to declare; R.L has nothing to declare; F.R. has nothing to declare; A.G.Z. has nothing to declare. E.B. has nothing to declare; A.E. has nothing to declare.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix. BSupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2024.10.008.

Appendix

Table describing Clinical characteristics of pregnancies of placentas studied

Parameter	No diabetes	Maternal Diabetes	Gestational diabetes
Gravidity	Median = 2; 25–75 % = 0.5; Range = 1–4; NA = 2	Median = 1; 25–75 % = 2; Range = 1-1; NA = 0	Median = 1; 25–75 % = 0; Range = 1–2; NA = 1
Parity	Median = 2; 25–75 % = 0.5; Range = 1–3; NA = 2	Median = 1; 25–75 % = 1.75; Range = 1–3; NA = 0	Median = 1; 25–75 % = 0; Range = 1; NA = 1
Gestational age (weeks)	Average = 38.75; SD = 1.48; NA = 2	Average = 37.57; SD = 1.55; NA = 0	Average = 38.8; SD = 1.30; NA = 1
Maternal age (years)	Average = 33.46; SD = 3.04; Range = 31–40; NA = 1	Average = 33.28; SD = 2.89; Range = 29–40; NA = 0	Average = 29.6; SD = 5.50; Range = 22–35; NA = 1
Race	Unknown	Unknown	Unknown
Ethnicity	Unknown	Unknown	Unknown
Prenatal medications	Unknown	Unknown	Unknown
Drugs	Unknown	Unknown	Unknown
Previous prenatal admission (s)	Unknown	Unknown	Unknown
Blood pressures <140/90 mm Hg	Unknown	Unknown	Unknown
Screened for diabetes	Yes, no Diabetes	Yes, T1D	Yes, GDM
Antibiotics in labor	Unknown	Unknown	Unknown
Beta strep status	Unknown	Unknown	Unknown
Antenatal steroids:	Unknown	Unknown	Unknown
Magnesium sulfate	Unknown	Unknown	Unknown

(*continued on next page*)

(*continued*)

Parameter	No diabetes	Maternal Diabetes	Gestational diabetes
Anesthesia	Epidural	Narcotics	General
Cervical ripening agent (N = # patients)	None: N = 3; NA: N = 6; prostaglandin E: N = 1; others*: N = 3	None: N = 2; NA: N = 2; prostaglandin E: N = 0; others**: N = 2	None: N = 6; NA: N = 5; prostaglandin E: N = 1; others***: N = 2
Labor	See Delivery mode	See Delivery mode	See Delivery mode
Delivery mode (N = # patients)	Vaginal: N = 8 (with labor); C-section, Primary: N = 4 (no labor); C-section Secondary: N = 2 (with labor)	Vaginal: N = 4 (with labor); C-section, Primary: N = 4 (no labor); C-section Secondary: N = 6 (with labor)	Vaginal: N = 5 (with labor); C-section, Primary: N = 5 (no labor); C-section Secondary: N = 0 (with labor)
Maternal Oxygen given at delivery?	Unknown	Unknown	Unknown
Birth weight (grams)	Average = 3493.92; SD = 466.95; NA = 0	Average = 3378.92; SD = 542.46; NA = 0	Average = 3220.00; SD = 252.58; NA = 1
Placental weight (grams)	Average = 491.25; SD = 104.55; NA = 0	Average = 482.76; SD = 91.19; NA = 0	Average = 453.60; SD = 78.08; NA = 0
Baby's sex	Female: N = 7; Male: N = 7	Female: N = 10; Male: N = 4	Female: N = 3; Male: N = 3
Delivery to processing (mins)	Average = 159.5; SD = 26.99; NA = 0	Average = 160.64; SD = 37.20; NA = 0	Average = 153.8; SD = 33.36; NA = 1

*Miprostin; Cytotec; Oxytocine| ** Cytotec| *** Cytotec; Propess
NA= (count of) non available (samples).

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