Placenta 154 (2024) 38–41

Contents lists available at ScienceDirect

Placenta

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

Gastroschisis associated changes in the placental transcriptome

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ARTICLE INFO

Keywords:
Gastroschisis
Fetal growth restriction
Prematurity

ABSTRACT

The congenital condition gastroschisis is associated with delayed villous development and placental malperfusion, suggesting placental involvement. This study uses RNA sequencing to compare the placental transcriptome in pregnancies with and without gastroschisis. 180 coding genes were differentially expressed, mapping to multiple gene ontology pathways. Altered placental gene expression may represent fetal signalling to the placenta, and these changes could contribute to the pathogenesis of gastroschisis and associated morbidities, including fetal growth restriction.

1. Introduction

Gastroschisis is a congenital condition affecting 1/2500 births where the infant's intestines extend outside the abdomen and often become inflamed [1,2]. After birth, the eviscerated intestine is returned to the abdominal cavity and the defect closed. Gastroschisis results in a higher risk of preterm birth, fetal growth restriction (FGR) and late fetal death, with potential longer-term gastrointestinal and neurodevelopmental problems [3,4]. The aetiology of gastroschisis is unclear but includes genetic, environmental and maternal causes [5,6].

Placental delayed villous development, malperfusion and vascular defects may occur as a result of gastroschisis, or be a cause of the developmental abnormalities and associated conditions such as FGR [7–9]. Indeed, there are higher rates of placental chorangiosis in placentas from growth-restricted babies with gastroschisis [10]. Evidence that placental-specific gene knockout can affect fetal cardiac development highlights the role of the placenta in fetal development [11].

Comparing the placental transcriptome from babies with and without gastroschisis will help establish whether placental defects could contribute to the development of gastroschisis or gastroschisis-associated pathologies and whether there is evidence of fetal signalling to the placenta.

2. Methods

Women known to be carrying a fetus with gastroschisis and women in whom there was no known fetal abnormality were approached and provided informed consent (South Central – Hampshire B research ethics committee, 15/SC/0702). Term placental villous tissue was collected from each placenta and stored in RNAlater. RNA extraction (Qiagen miRNeasy kit). Library preparation was performed using the TruSeq Stranded mRNA Library Prep kit (Illumina). The final library was quantified using a Roche KAPA library quantification kit (Illumina) and an Agilent 2100 Bioanalyser. Paired-end RNA sequencing (2 × 150 bp) was carried out on an Illumina NextSeq 550 as described previously [12].

FASTQ reads underwent QC with FastQC (v0.11.3), trimmed using Trimmomatic, and aligned to GRCh38 using STAR (v2.7.3a) with the –quantMode parameter set to "GeneCounts". Differential gene expression was performed in R (4.0.3) using read counts with DESeq2 (v1.30.1). Significance was calculated using Wald test and adjusted for multiple testing at 5 % using Benjamini-Hochberg. Differentially expressed genes were considered significant if adjusted p-value ≤ 0.05 and log2 fold change (log2fc) ≥ 1.2. Pathway analysis was performed on these genes using Metascape [13]. Custom analysis was performed for gene ontology (GO) biological processes pathways with a p-value cut-off

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https://doi.org/10.1016/j.placenta.2024.06.001
Received 2 March 2024; Received in revised form 16 May 2024; Accepted 2 June 2024
Available online 4 June 2024

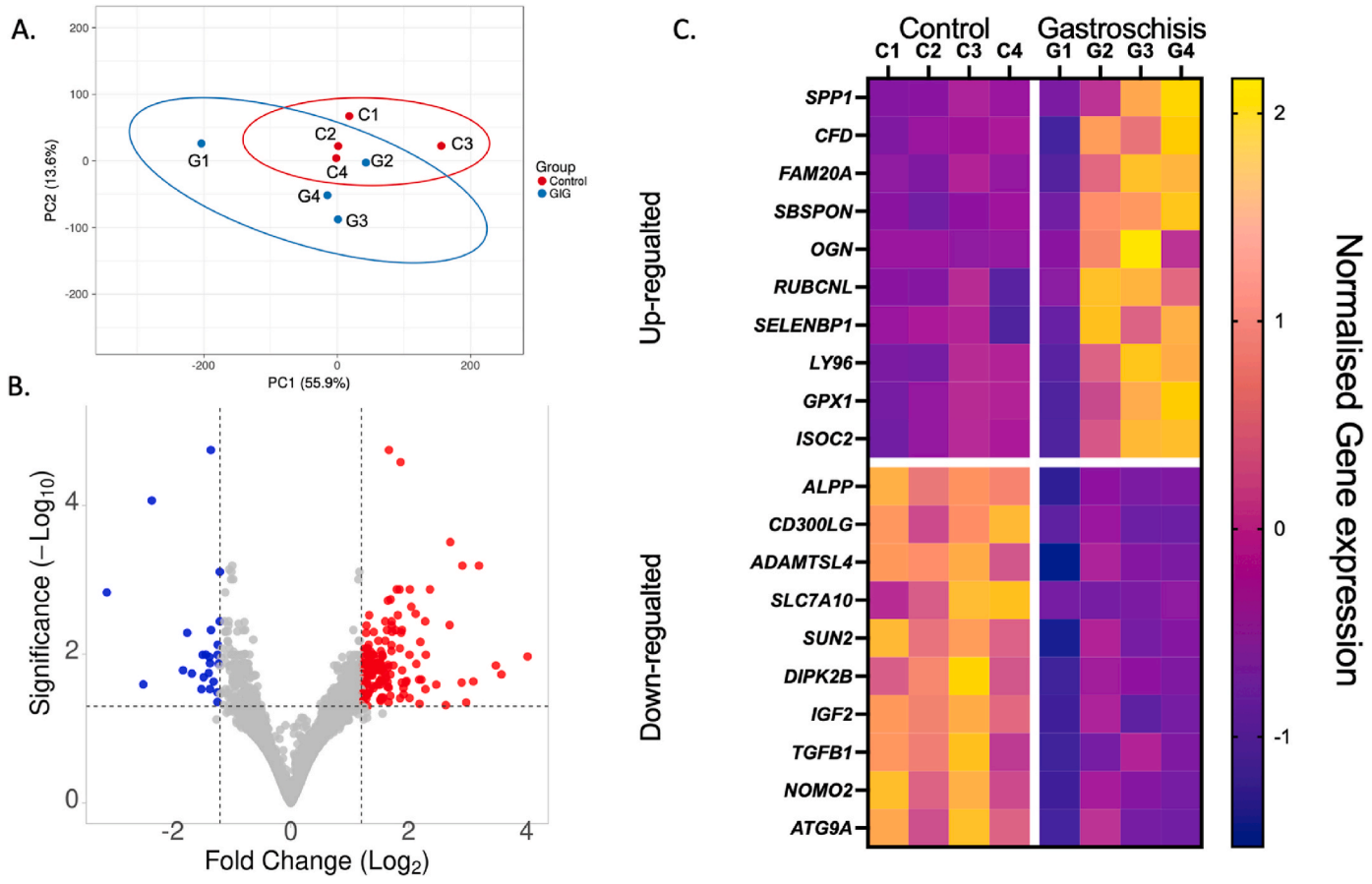

Fig. 1. Gene expression in placentas with gastroschisis vs control. **A.** A PCA analysis comparing control and gastroschisis placentas. Prediction ellipses indicate a 0.95 probability that a new observation from the same group will fall inside the ellipse. **B.** A volcano plot showing all protein coding genes highlighting those with a significant FDR adjusted p value and with a 1.2 log2 fold change in expression. **C.** a heatmap showing normalised gene expression in the top 10 most significant up and down regulated genes with a log2 fold change over 1.2 in placentas with gastroschisis.

of 0.01 and enrichment ≥1.5.

Genes with significantly altered placental expression were compared to a list of 961 genes shown to be differentially regulated in premature placentas from Paquette et al. [14].

PCA analysis was performed on all genes using ClustVis https://biit.cs.ut.ee/clustvis/[15]. Volcano plots were plotted using VocaNoseR ht tps: //huygens.science.uva.nl/VolcaNoseR/[16]. Venn diagrams were constructed InteractiVenn www.interactivenn.net [17].

3. Results

Maternal and infant data are presented as mean and 95 % CI (control, gastroschisis, P value). Maternal age (years) (33.5 ± 5.0, 25 ± 7, 0.10), Gestational age (days) (263 ± 5, 253 ± 8, 0.07), Elective section: vaginal delivery (4:0, 3:1), Birth weight (kg) (3.50 ± 0.87, 2.70 ± 0.35, 0.14), 5 min APGAR score (9.3 ± 0.5, 9.8 ± 0.5, NS), Head circumference (cm) (34.9 ± 1.9, 32.8 ± 1.1, 0.10). Each group contained 2 placentas of each sex.

180 coding genes were differentially expressed (156 upregulated, 24 downregulated) in gastroschisis placentas with a log2fc > 1.2 (Fig. 1). All 585 differentially expressed genes are reported in Supplementary Table 1.

None of these genes were observed in a dataset from placental gene expression in preterm birth. Without the log2fc cut-off, 585 genes were differentially expressed in the gastroschisis placentas, with eight of these downregulated and four upregulated in the premature placenta cohort (Supplementary Fig. 1).

Pathway analysis performed on the significantly up and downregulated genes with log2fc > 1.2 identified up and downregulated pathways (Fig. 2). The differentially regulated genes in these pathways are reported in Supplementary Table 2. For comparison, GSEA based on all 585 differentially regulated genes is presented in Supplementary Table 3.

4. Discussion

To our knowledge, this is the first study to investigate the placental transcriptome in pregnancies with gastroschisis. The differences in gene expression may represent a response to altered metabolic or endocrine signals from the fetus. However, it is also possible that placental gene expression changes could predispose to gastroschisis or associated morbidities, such as FGR.

This study cannot demonstrate the cause of gastroschisis-associated changes in placental gene expression. However, it seems likely that gastroschisis associated signals from the fetus may mediate these changes. Changes in gene pathways such as 'inflammatory response' and 'response to external stimuli' are consistent with altered signalling. Future studies could match the analysis of cord blood with placental gene expression in infants with gastroschisis to identify putative signalling pathways.

Changes in placental gene expression in the gastroschisis placentas may provide putative mechanisms by which placental function and fetal growth could be affected. For instance, there is good evidence that placental IGF2 levels are associated with fetal growth in humans and rodents [18]. So decreased placental expression of IGF2 in gastroschisis is consistent with a placental role in gastroschisis-associated morbidities

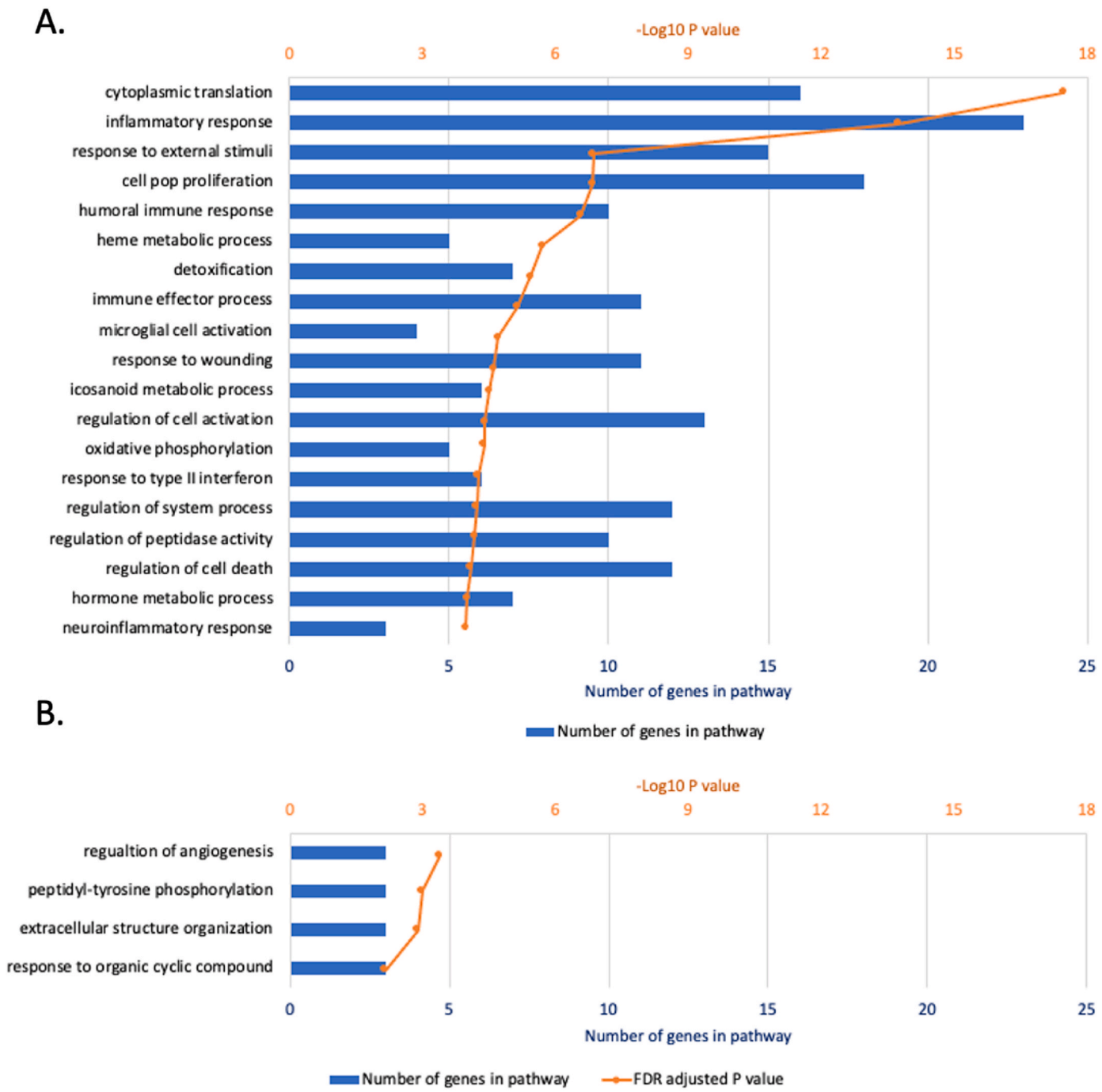

Fig. 2. Go analysis comparing control and gastroschisis placentas. **A**) The significantly upregulated GO processes. **B**) The significantly downregulated GO processes.

such as FGR.

None of the differentially expressed genes identified in this study are associated with gastroschisis-associated SNPs [5]. However, four genes identified in this study (*ALPP*, *RASGRP1*, *CDYL*, *RPS3*) were also identified in a whole exome sequencing study in a family with familial gastroschisis as co-segregating with gastroschisis [19]. Genetic or environmental factors affecting these genes could contribute to the development of gastroschisis.

Limitations of this study need to be acknowledged, and future larger studies should seek to achieve better matching between control and gastroschisis pregnancies, maternal and gestational age, mode of delivery and to include both simple and complex presentations of gastroschisis. An effect of prematurity cannot be excluded, but the fact that few of the differentially regulated genes overlapped with genes altered in prematurity supports these findings [14]. RNA sequencing on whole placental tissue does allow us to address cell specific responses within the placenta and single nuclei RNA sequencing would be a highly informative next step.

This study provides evidence for an association between gastroschisis and the placental transcriptome. An interesting aspect of this work is that gastroschisis may represent a natural model to study fetal signalling to the placenta in humans.

CRediT authorship contribution statement

Maaike Jongen: Writing – review & editing, Methodology,

Investigation, Formal analysis, Data curation. **Ian Reddin:** Writing – review & editing, Formal analysis. **Sharon Cave:** Project administration, Investigation. **Lianne Cashmore:** Project administration, Investigation. **Jenny Pond:** Project administration, Investigation. **Jane K. Cleal:** Writing – review & editing, Supervision, Funding acquisition. **Nigel J. Hall:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization. **Rohan M. Lewis:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Funding acquisition, Formal analysis, Conceptualization.

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 article.

Acknowledgements

This project was supported by the NIHR Southampton Biomedical Research Centre (IS-BRC-1215-20004). MJ was supported by a Well-being of Women project grant (RG2147).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2024.06.001.

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