Maternal immune profiles of abnormal prenatal ultrasonographic findings following SARS-CoV-2 infection

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UCLA Health | David Geffen School of Medicine

Background

Background

- Clinical spectrum of SARS-CoV-2 infection in pregnancy is variable[1]
  - Asymptomatic to severe disease
- Possible increased risk of adverse pregnancy and neonatal outcomes with SARS-CoV-2 infection in pregnancy
  - Fetal death, preterm birth, preeclampsia, low birthweight infants, emergency cesarean birth[2]
  - Greatest risk for severe or critical disease[3]

1. Allotey et al, BMJ 2020
2. Gurol-Urganci I et al, Am J Obstet Gynecol 2021
3. Metz et al, Obstet Gynecol 2021

Background

- **Dysregulated immune response** induced by SARS-CoV-2 suggested as the primary driver for severe disease and associated adverse outcomes[1]
- Changes in maternal immunity following infections may be associated with adverse perinatal outcomes
  - Limited data on the immunological consequences following SARS-CoV-2 infection in pregnancy

1. Del Valle et al, Nat Med 2020

Background

- Pregnancies recovering from COVID-19 → at least one ultrasonographic assessment after symptom resolution suggested
  - Evaluate for fetal growth and morphologic changes
- Potential effects of SARS-CoV-2 infection on prenatal imaging and its association with pregnancy outcomes is not well-described

Objective

- To **characterize the maternal immune profiles** following SARS-CoV-2 infection in pregnancy

- To evaluate the **correlation of COVID-19 induced alteration of maternal immunity** with **prenatal ultrasonographic abnormalities**

Methods

Study Design

- Prospective observational cohort study
- Single quaternary care center
- Pregnancies with SARS-CoV-2
  - Diagnosed by NP RT-PCR between March 2020 and February 2021
- Healthy pregnant controls concurrently recruited
  - Confirmed negative SARS-CoV-2 by NP RT-PCR (universal screening on admission to Labor & Delivery)

Procedures and Sample Collection

- Peripheral blood specimens
  - Collected at two time points:
    1. Initial diagnosis (time of enrollment)
    2. Time of delivery (admission to Labor & Delivery)
  - Isolated sera subjected to high-throughput Next Generation Sequencing-based proteomics profiling
    - **Olink Explore 1536 panel** (PEA technology + NGS) → detect 1472 proteins

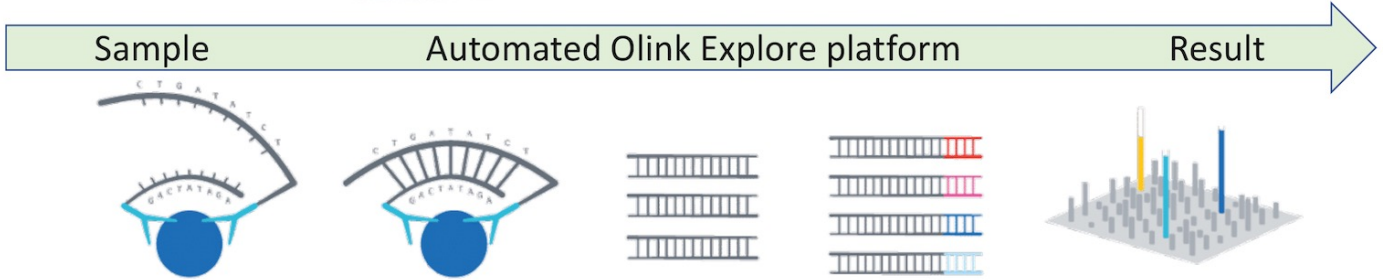
Procedures and Sample Collection

- Serial prenatal ultrasonographic examination following infection
  - Standard fetal biometry
    - Biparietal diameter, head circumference, abdominal circumference, femur length
    - Estimated fetal weight (grams) by Hadlock formula
  - General morphological evaluation for signs of congenital infection
  - Amniotic fluid volume assessment
  - Placental thickness measurement
  - Doppler evaluation
    - Umbilical artery (UA)
    - Middle cerebral artery (MCA)

Data Analysis

- Categorized as **abnormal ultrasound** if any of the following were identified at least once:
  - Fetal growth restriction
  - Placentomegaly
  - Infection-associated structural anomaly
    - Intracranial calcifications, ventriculomegaly, microcephaly, cardiomegaly, intraabdominal calcifications, echogenic bowel, ascites, pleural effusion, pericardial effusion, hydrops
  - Abnormal UA or MCA Doppler measurement
    - UA Doppler – elevated S/D ratio, absent end diastolic flow, or reverse end diastolic flow
    - MCA Dopplers – elevated PSV (> 1.5 MoM)
  - Abnormal amniotic fluid volume
    - Oligohydramnios, polyhydramnios
  - Other structural anomaly

Data Analysis



Comparison of sera proteome profiles

SARS-CoV-2 positive with abnormal ultrasound

vs

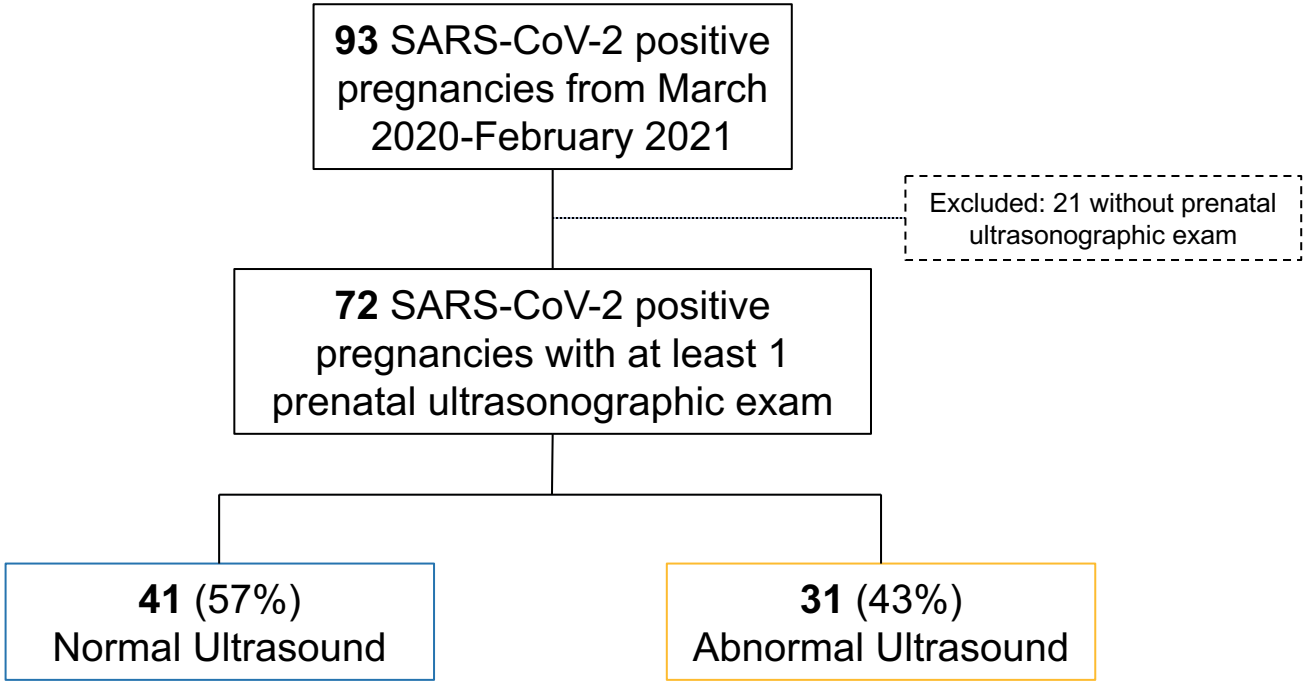
Gestational age-matched healthy controls

Statistical Analysis

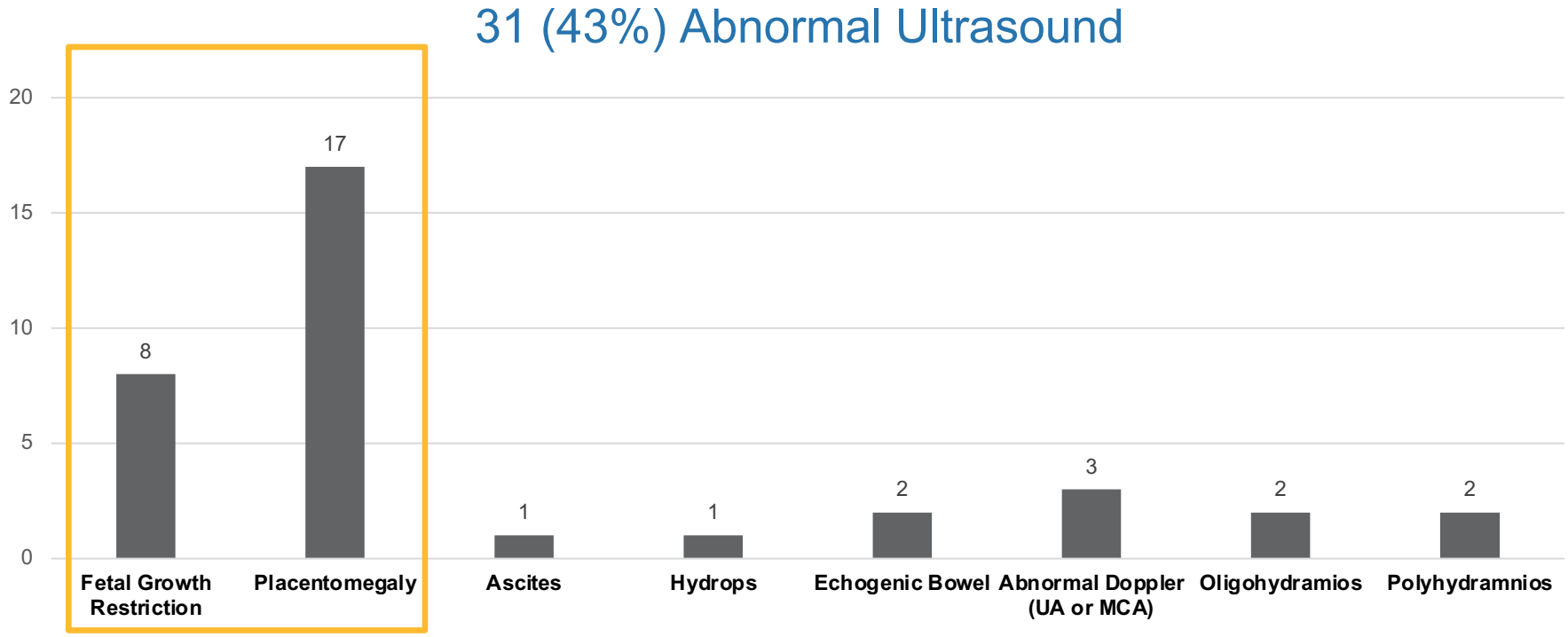
- Normalized protein expression (NPX) values of proteins and samples
  - Compared against limits of detection (LOD) for each protein
  - Proteins with > 80% of NPX values lower than LOD excluded
- Gene expression Fold Change (FC) calculated using ΔΔCt Method
  - FC for each gene calculated as $2^{-\Delta\Delta Ct}$
- Maternal characteristics and obstetric outcomes compared by prenatal ultrasonographic findings
  - Chi-square or Fisher's exact – categorical variables
  - T-test or Wilcoxan rank-sum test – continuous variables
- Analysis performed on R

Results

Study Population



Abnormal Ultrasonographic Findings



Maternal Demographics

No significant differences in maternal demographics or clinical characteristics in pregnancies with COVID-19 that did or did not have an abnormal ultrasound

	Abnormal Ultrasound N=31	Normal Ultrasound N=41	p-value
Age (years), median (range)	34 (22-44)	33 (18-41)	0.64
Race/Ethnicity			0.57
Black	1 (3.23)	3 (7.32)	
Hispanic	13 (41.94)	21 (51.22)	
White	10 (32.26)	8 (19.51)	
Other	7 (22.58)	9 (21.95)	
Gravida, median (range)	2 (1-7)	3 (1-10)	0.25
Maternal comorbidity	13 (41.94)	20 (48.78)	0.48
Obesity	8 (25.81)	15 (36.59)	0.45
Diabetes mellitus	0	3 (7.32)	0.25
Asthma	2 (6.45)	7 (17.07)	0.28
Trimester of Infection			0.36
First	5 (16.13)	10 (24.39)	
Second	14 (45.16)	12 (29.27)	
Third	12 (38.71)	19 (46.34)	
COVID Severity			0.69
Asymptomatic	2 (6.45)	4 (9.76)	
Mild/Moderate	24 (77.42)	28 (68.29)	
Severe/Critical	5 (16.13)	9 (21.95)	

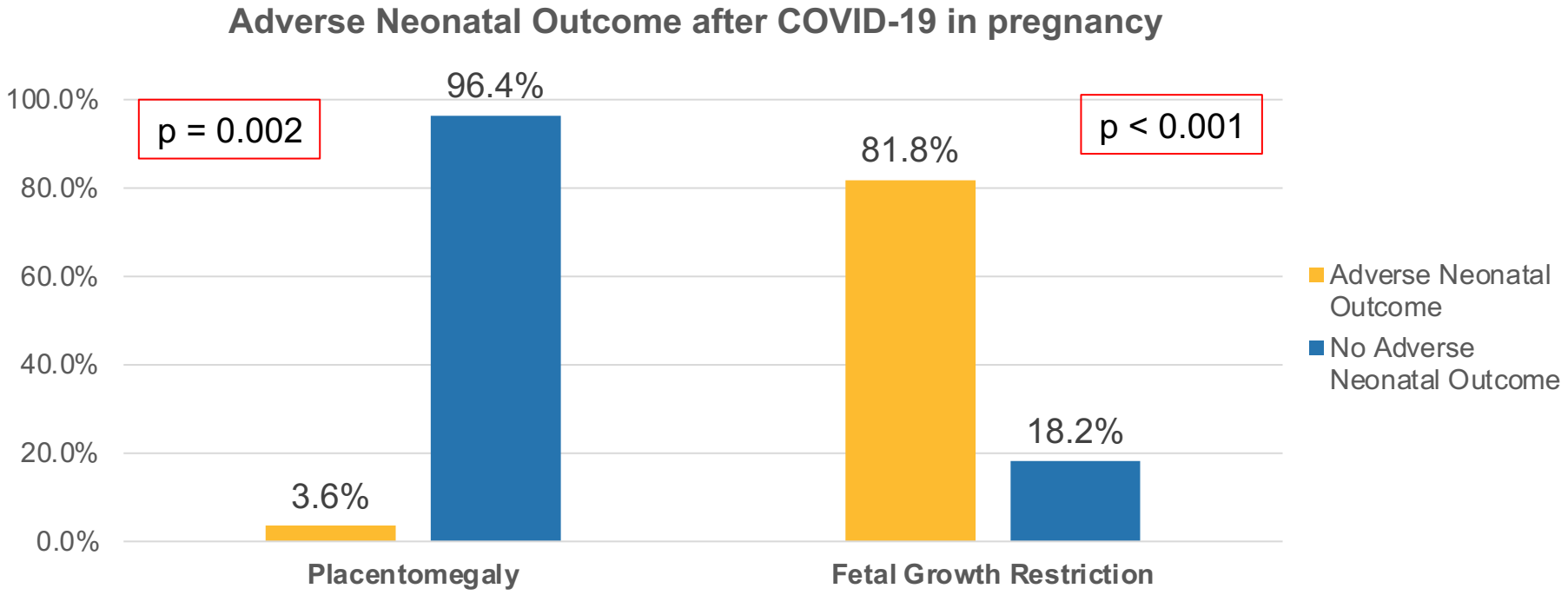
Obstetric and Neonatal Outcomes

Neonatal outcomes available for infants delivered before March 1, 2021 (N = 65)

	Abnormal Ultrasound N=29	Normal Ultrasound N=36	p-value
Mode of Delivery	N=29 (45%)	N=36 (55%)	0.58
Vaginal birth	22 (75.86)	23 (63.89)	
Cesarean birth	6 (20.69)	11 (30.56)	
Operative vaginal birth	1 (3.45)	2 (5.56)	
Gestational age at birth (weeks), median (range)	39 (21-41)	39 (27-41)	0.34
Preterm birth	6 (20.69)	5 (13.89)	0.74
Birthweight (grams), median (range)	2914 (309-4252)	3108 (939-4780)	0.03
Low birthweight	10 (31.25)	2 (5.56)	<0.01
Small for gestational age	5 (15.63)	1 (2.78)	0.09
NICU admission	6 (18.75)	27 (19.44)	1
Neonatal respiratory distress	8 (25)	5 (13.89)	0.17
Any neonatal complication	14 (43.75)	10 (27.78)	0.21

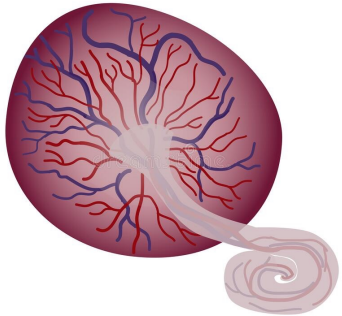
Abnormal ultrasound associated with **lower birthweight** and **birthweight < 2500 g**

Placentomegaly vs Fetal Growth Restriction



Maternal Serum Samples

Abnormal Ultrasonographic Findings



Placentomegaly
(N = 17)

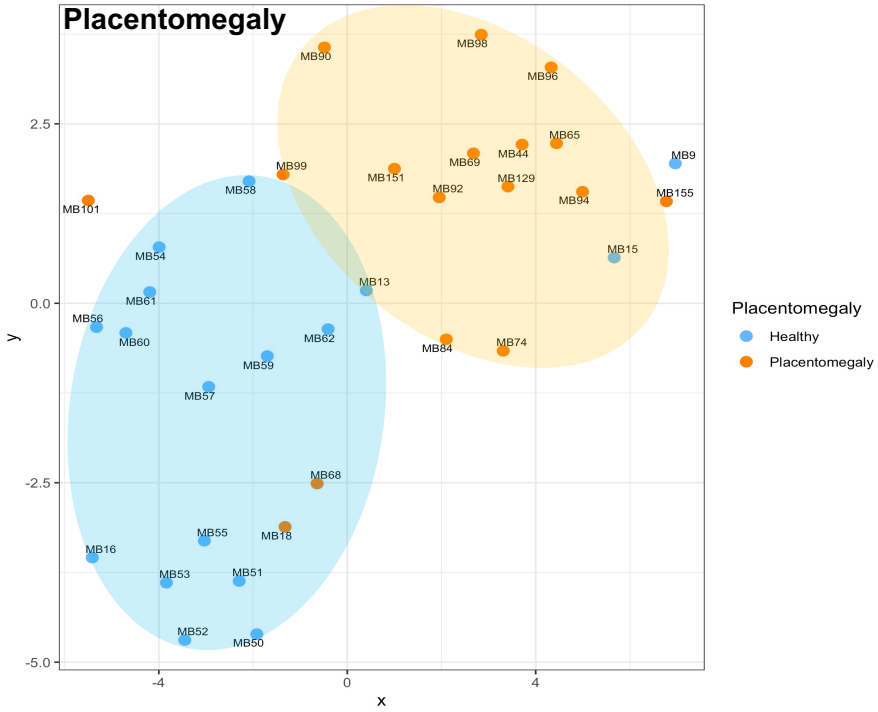
Fetal Growth Restriction
(N = 8)

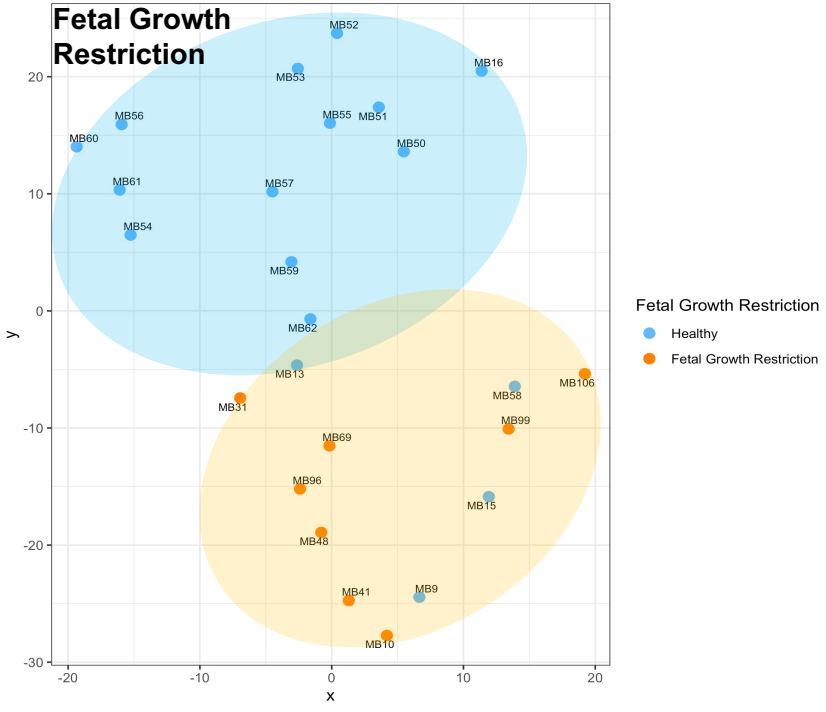
VS

GA-Matched Controls

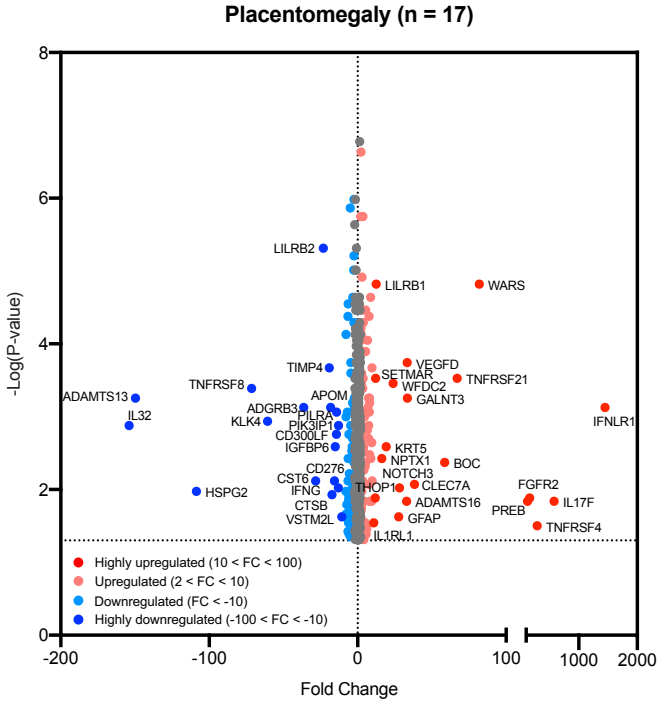
Healthy Controls
(N = 17)

Serum Proteomic Profiles: Abnormal Ultrasound after SARS-CoV-2 Infection



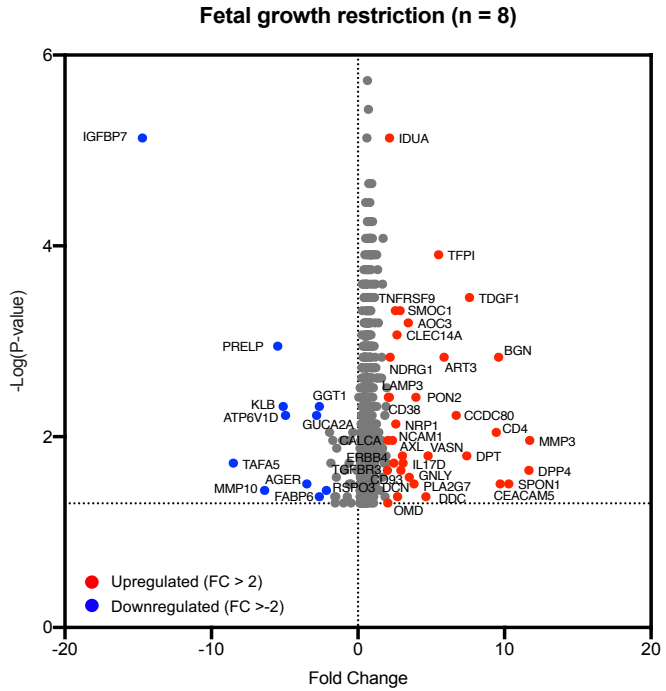


Serum Proteomic Profiles: Placentomegaly



Protein	Function
Upregulated	
IFNLR1	Class II cytokine receptor, activation of JAK/STAT pathway
IL17F	Cytokine expressed by activated T cells, production of other cytokines, inhibits angiogenesis of endothelial cells
TNFRSF4	TNF-receptor superfamily, activates NF-κB, suppresses apoptosis, role in CD4+ T cell response and T cell-dependent B-cell proliferation and differentiation
FGFR2	Fibroblast growth factor receptor, mitogenesis and differentiation, bone growth
Downregulated	
ADAMTS13	Cleaves vWF multimers in plasma, controlling vWF-mediated platelet thrombus formation
IL32	Proinflammatory cytokine, induces production of TNFα from macrophage cells

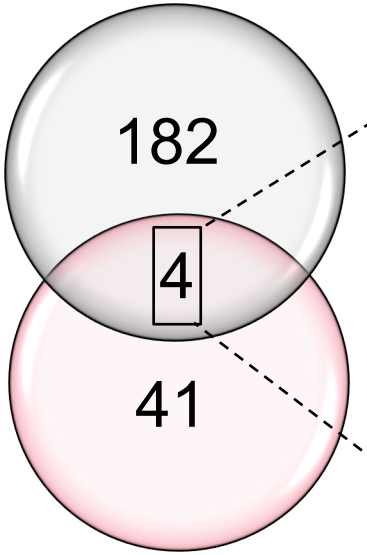
Serum Proteomic Profiles: Fetal Growth Restriction



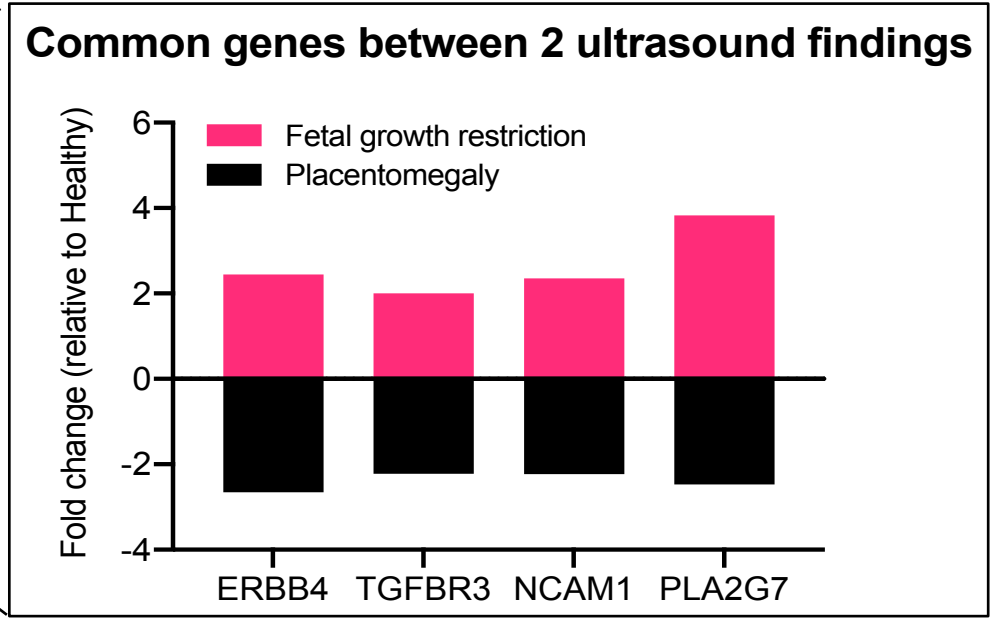
Protein	Function
Upregulated	
MMP3	Matrix metalloproteinase family, breakdown of extracellular matrix in embryonic development, reproduction, and tissue remodeling
DPP4	Dipeptidyl peptidase, glucose and insulin metabolism, immune regulation, functional receptor of MERS-CoV
Downregulated	
IGFBP7	Insulin-like growth factor-binding protein family, regulates IGF availability and modulates IGF binding to receptors, stimulates prostacyclin production and cell adhesion

Placentomegaly vs Fetal Growth Restriction

Placentomegaly



Fetal Growth Restriction



Conclusion

Summary

- SARS-CoV-2 positive pregnancies with abnormal ultrasonographic findings had highly inflammatory immune profiles
- Placentomegaly and fetal growth restriction most common ultrasound finding
  - Adverse neonatal outcomes: ↑ fetal growth restriction, ↓ placentomegaly
- 182 cytokines significantly altered in placentomegaly
  - ↑ IFNLR1, IL17F, TFNFRSF4, FGFR2
  - ↓ ADAMTS13, IL32
- 41 cytokines significantly altered in fetal growth restriction
  - ↑ MMP3, DPP4
  - ↓ IGFB7
- Placentomegaly and fetal growth restriction both demonstrated dysregulated cytokine expression but with a distinct pattern
  - 4 overlapping cytokines but inverse responses - ERBB4, TGFBR3, NCAM1, PLA2G7

Strengths and Limitations

- Strengths
  - Quaternary care center - heterogenic population
  - Prospective cohort
  - Comprehensive specimen and data collection
- Limitations
  - Exploratory study
    - Does not propose conclusive underlying biologic mechanism (further investigation required)
  - Observational study
    - Confounding factors and reverse causality

Conclusions

- SARS-CoV-2 infected pregnancies that develop abnormal ultrasound findings have immune profiles consistent with a **pronounced inflammatory response**
- **Placentomegaly** and **fetal growth restriction** both have dysregulated but **unique immune profiles**
  - Insight into **pathogenesis of COVID-19** and **changes in maternal immunity after infection**
  - **Potential biomarkers** to predict perinatal complications after SARS-CoV-2 infection in pregnancy

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