

# An Analysis of Fluidigm Biomark™ Array Data for Placental Inflammasomes in Human Idiopathic Fetal Growth Restriction Affected Pregnancies



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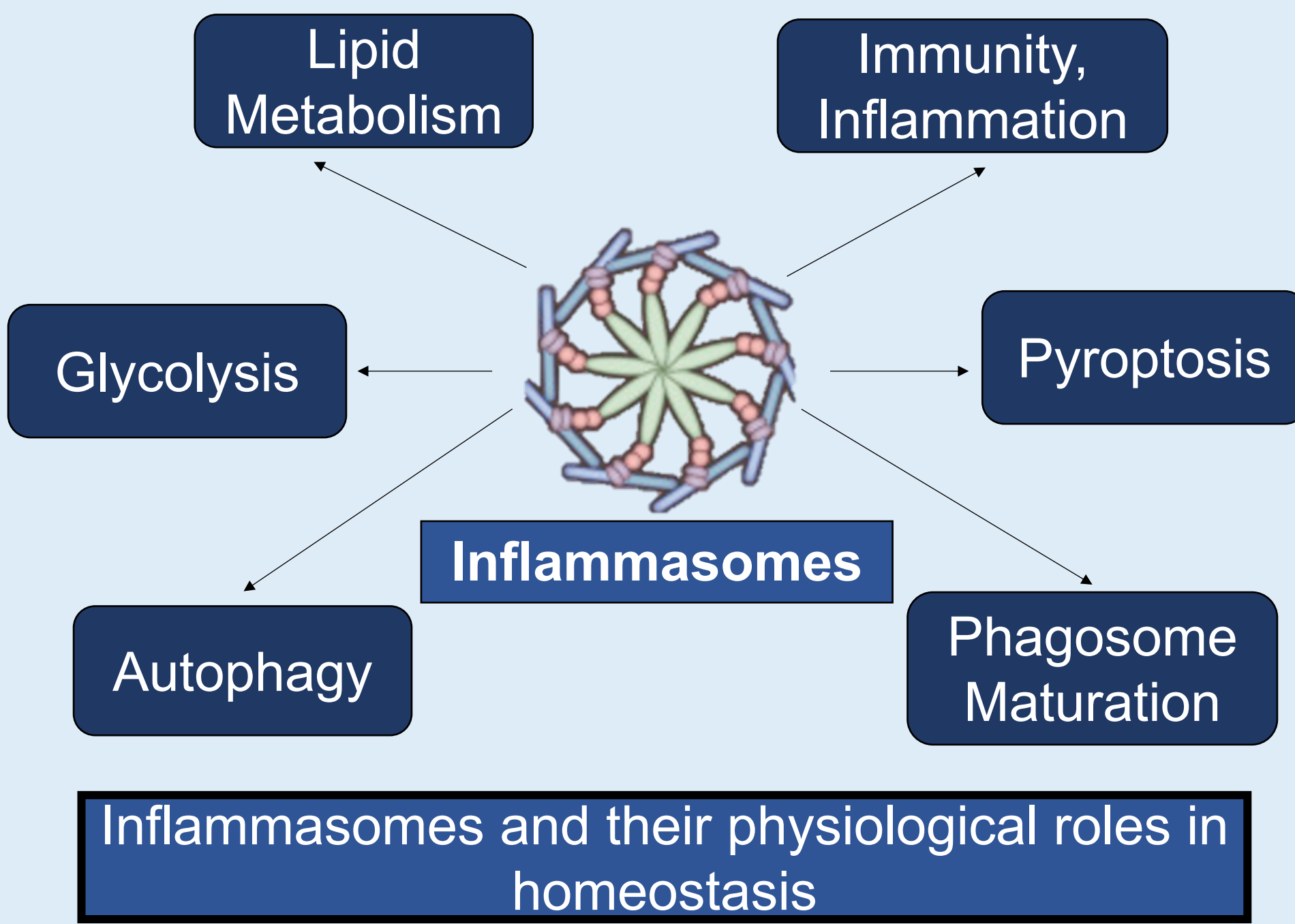
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## 1 INTRODUCTION

- Fetal growth restriction (FGR) is **clinically defined as fetal birth weight <10th percentile** contributing to perinatal mortality.
- FGR is frequently associated with **placental insufficiency** which has been reported to be exacerbated by **inflammation**.
- Inflammasomes** are the novel multimeric proteins that **regulate inflammation** in **normal pregnancies**, however their expression in **FGR** are **largely unknown**.



## 2 HYPOTHESIS

- The gene expression profile for the components of the placental inflammasome machinery will be **differentially expressed** in idiopathic FGR compared with gestation-matched uncomplicated control pregnancies.

## 3 AIMS

- To analyse data on placental gene expression profiles for the components of the inflammasome machinery that was obtained from a custom-designed Fluidigm Biomark™ Array and independent validation using Real-Time PCR.
- To correlate the inflammasome genes expression profiles with maternal age, gestational age (pre-term vs term differences), fetal birth weight, placental weight, and birth weight to placental weight ratio.

## 4 METHODS

- Fluidigm Biomark™ Array and Real-Time PCR**
- Data Analysis: **2<sup>-ΔΔCT</sup> method** – is for relative (average of 18S rRNA and GAPDH) quantitation of gene expression
- Statistical analysis
  - Patient characteristics: **Fisher's exact test, Chi-square test, unpaired t-test**
  - Fluidigm Biomark™ Array: **presence and absence of gene expression** detected by **CT values (median ± IQR)**
  - Independent validation data: **Kolmogorov-Smirnov test + Mann-Whitney U test + scatterplot**
  - Correlations : **Spearman's correlation test**

## 5 RESULTS

### A. Patient characteristics for control and FGR placental tissue samples

Characteristics	Control (n=25)	FGR (n=25)	P value
Gestational age (weeks)	34.44 ± 3.959	36.12 ± 3.232	P = 0.107
Maternal age (years)	34 ± 5.323	31.2 ± 5.008	P = 0.061
Placental weight (g)	506 ± 144.1	395.9 ± 124	<b>P = 0.006</b>
Birth weight (g)	2474 ± 875.9	1968 ± 662.9	<b>P = 0.026</b>

There was a **significant decrease** in placental and fetal birth weight in FGR compared with control

### B. Fluidigm Biomark™ Array DAMPs-involved gene expression profiles.

Genes	Control (n=25)	FGR (n=25)
<i>NLRP3</i>	1.213 ± 0.851	1.099 ± 1.204
<i>CASP1</i>	1.001 ± 1.305	1.552 ± 10.474
<i>NFκB1</i>	1.303 ± 0.905	0.937 ± 1.189
<i>CASP3</i>	1.384 ± 1.423	0.529 ± 0.845
<i>CASP8</i>	1.256 ± 0.982	0.549 ± 0.397
<i>NLR5</i>	1.197 ± 1.427	1.508 ± 2.138
<i>NOD2</i>	1.259 ± 1.117	1.767 ± 6.981

### C. Fluidigm Biomark™ Array PAMPs-involved gene expression profiles.

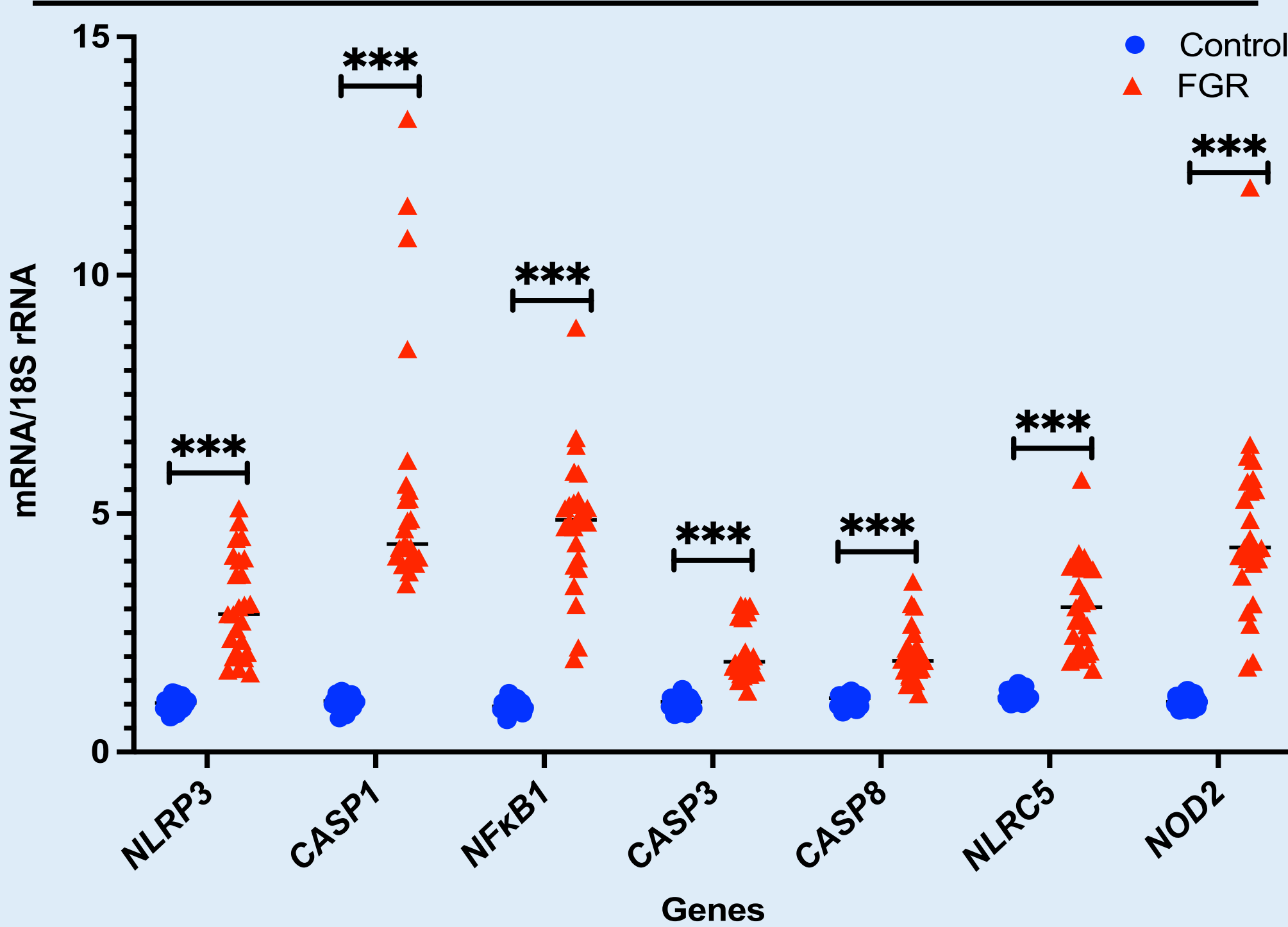
Genes	Control (n=25)	FGR (n=25)
<i>TLR2</i>	0.958 ± 2.138	1.093 ± 4.707
<i>TLR5</i>	0.685 ± 1.180	0.397 ± 4.318
<i>TLR6</i>	0.620 ± 1.469	0.135 ± 0.658

### D. Fluidigm Biomark™ Array cytokine gene expression profiles.

Genes	Control (n=25)	FGR (n=25)
<i>IL-1β</i>	0.822 ± 1.208	2.724 ± 15.483
<i>IL-6</i>	1.204 ± 0.579	0.900 ± 4.033
<i>IFNγ</i>	0.958 ± 1.596	11.7 ± 119.944
<i>IL-10</i>	0.969 ± 0.950	1.163 ± 28.943

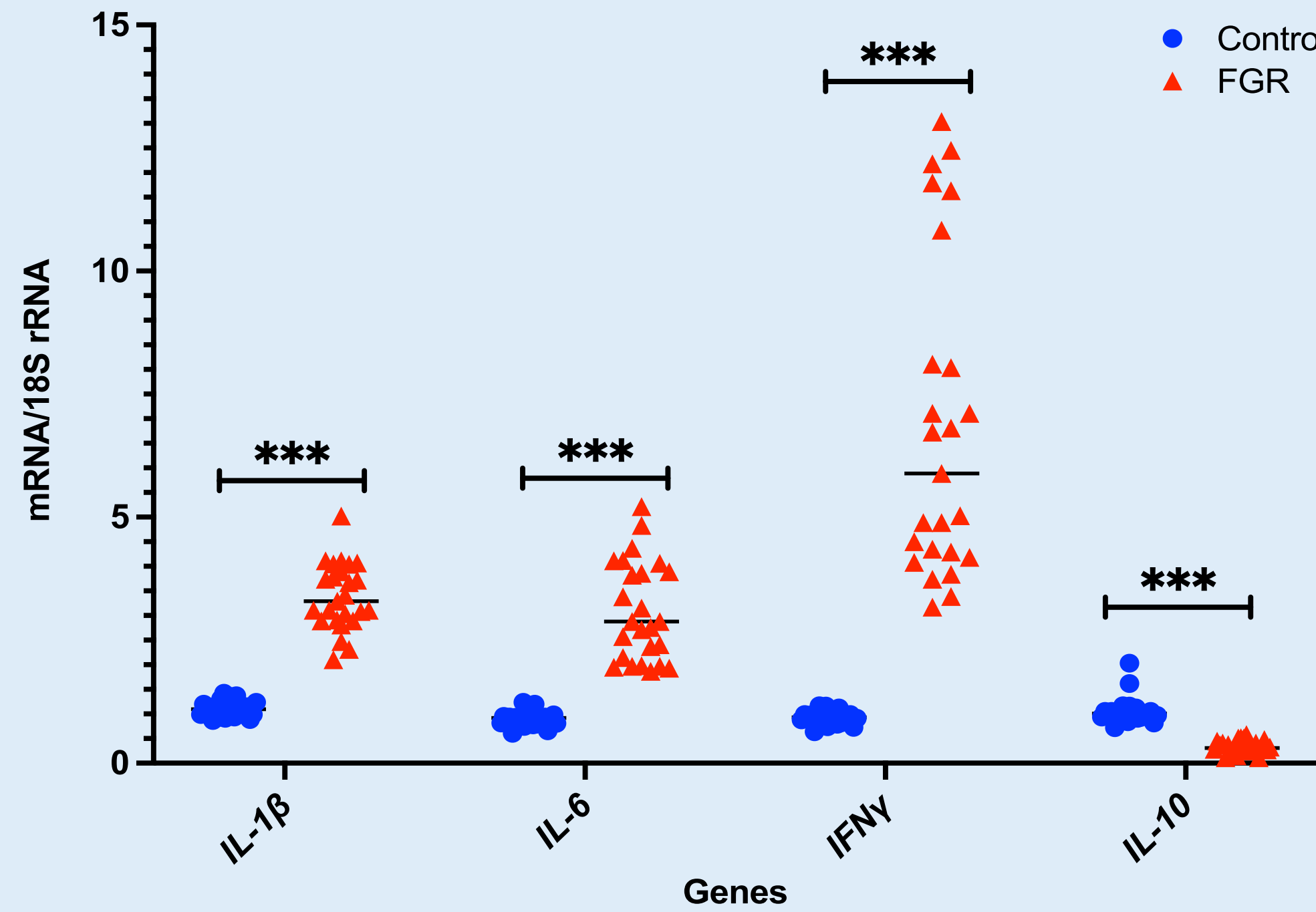
All genes tested showed expression in both groups

### E. Independent validation of DAMPs-involved mRNA expression relative to 18S rRNA.



There was a **significant increase** in all DAMPs mRNA expression in FGR compared with control

### F. Independent validation of cytokine mRNA gene expression relative to 18S rRNA.



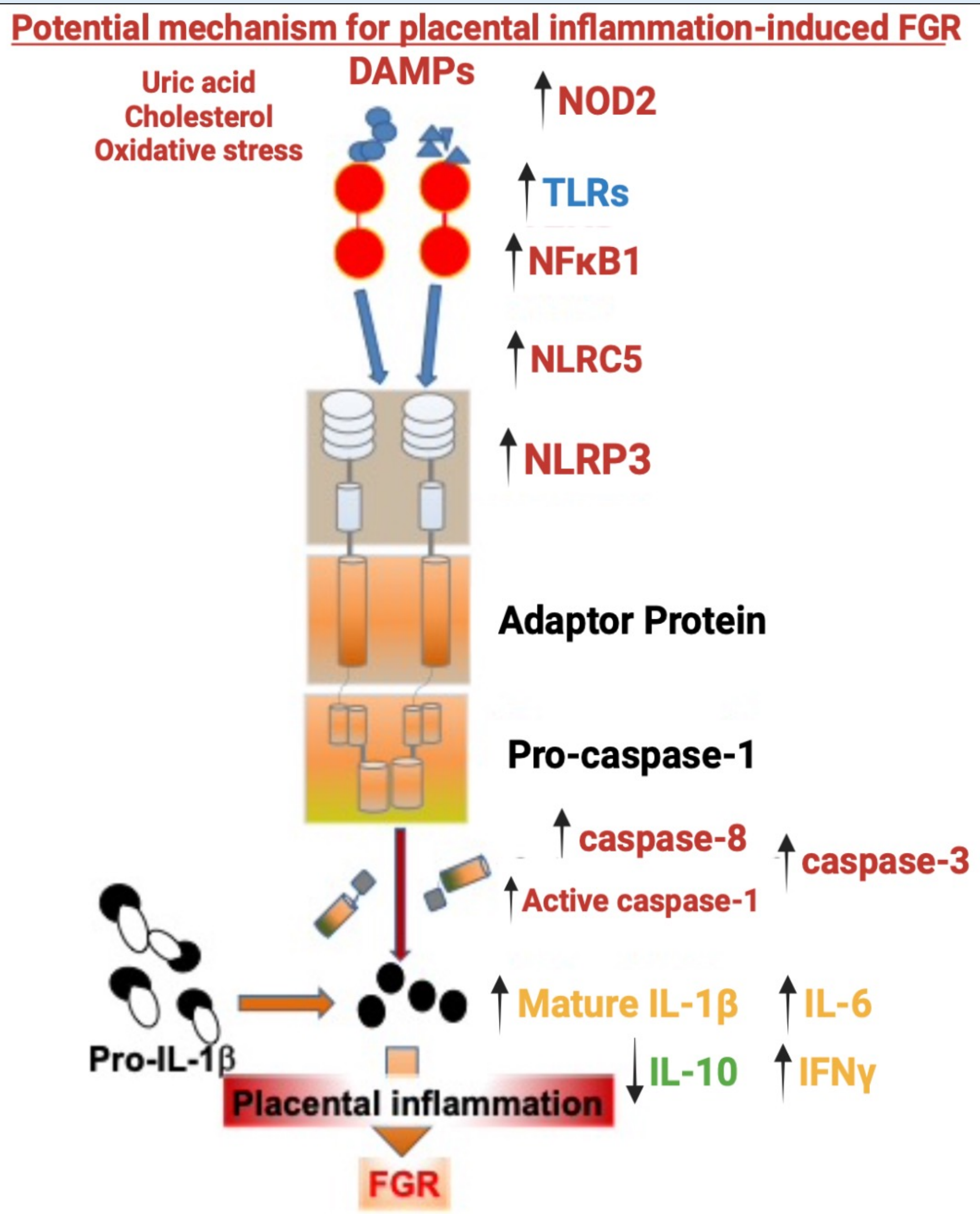
There was a **significant increase** in *IL-1β*, *IL-6*, and *IFNγ* mRNA expression in FGR compared with control

There was a **significant decrease** in anti-inflammatory, *IL-10* mRNA expression in FGR compared with control

### G. Correlations between mRNA gene expression and patient characteristics

Patient characteristics	Control	FGR
Gestational age	<i>CASP1</i>	<i>NLRP3</i>
Birth weight	<i>CASP1</i>	-
Placental weight	<i>CASP1</i> , <i>NOD2</i>	-
Birth weight - placental weight ratio	<i>NLRP3</i>	-

## 6 SUMMARY



## 7 CONCLUSION

- Placentas from FGR pregnancies showed:
  - Increased expression** for key genes that are associated with **DAMPs, PAMPs, and pro-inflammatory cytokines**.
  - Decreased expression** for anti-inflammatory cytokine, *IL-10*.

## 8 FUTURE DIRECTIONS

- Protein quantitation
- Immunohistochemistry
- Identification of functional significances

References:  
1. Murki S, Sharma D. Intrauterine growth retardation. J Neonatal Biol. 2014;3:3.  
2. Rathinam VA, Fitzgerald KA. Inflammasome Complexes: Emerging Mechanisms and Effector Functions. Cell. 2016;165(4):792-800.  
3. Murthi P, Pinar AA, Dimitriadis E, Samuel CS. Inflammasomes - a molecular link for altered immunoregulation and inflammation mediated vascular dysfunction in preeclampsia. Int J Mol Sci. 2020;21(4):1406.  
4. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402-8.