Predictive Modelling of Stress-Related Behaviour Through Gene Expression Following Prenatal Glucocorticoid Exposure

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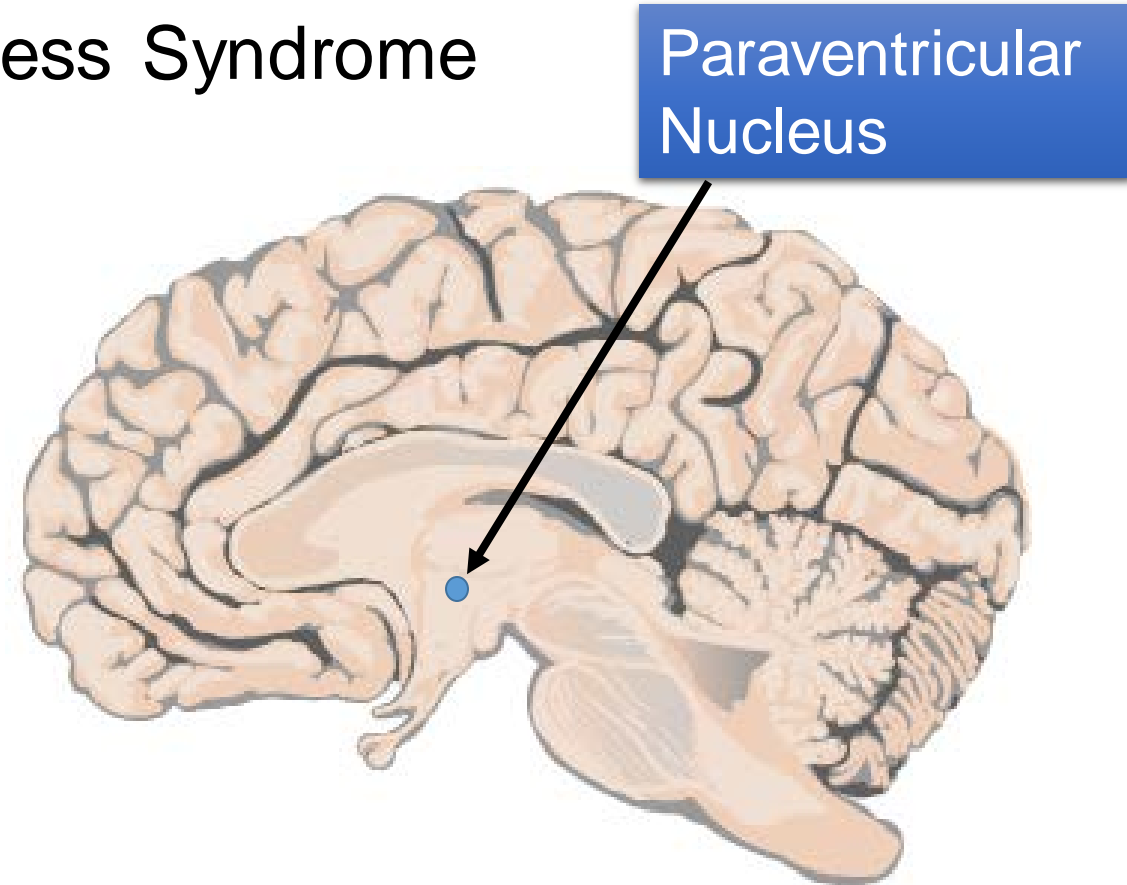
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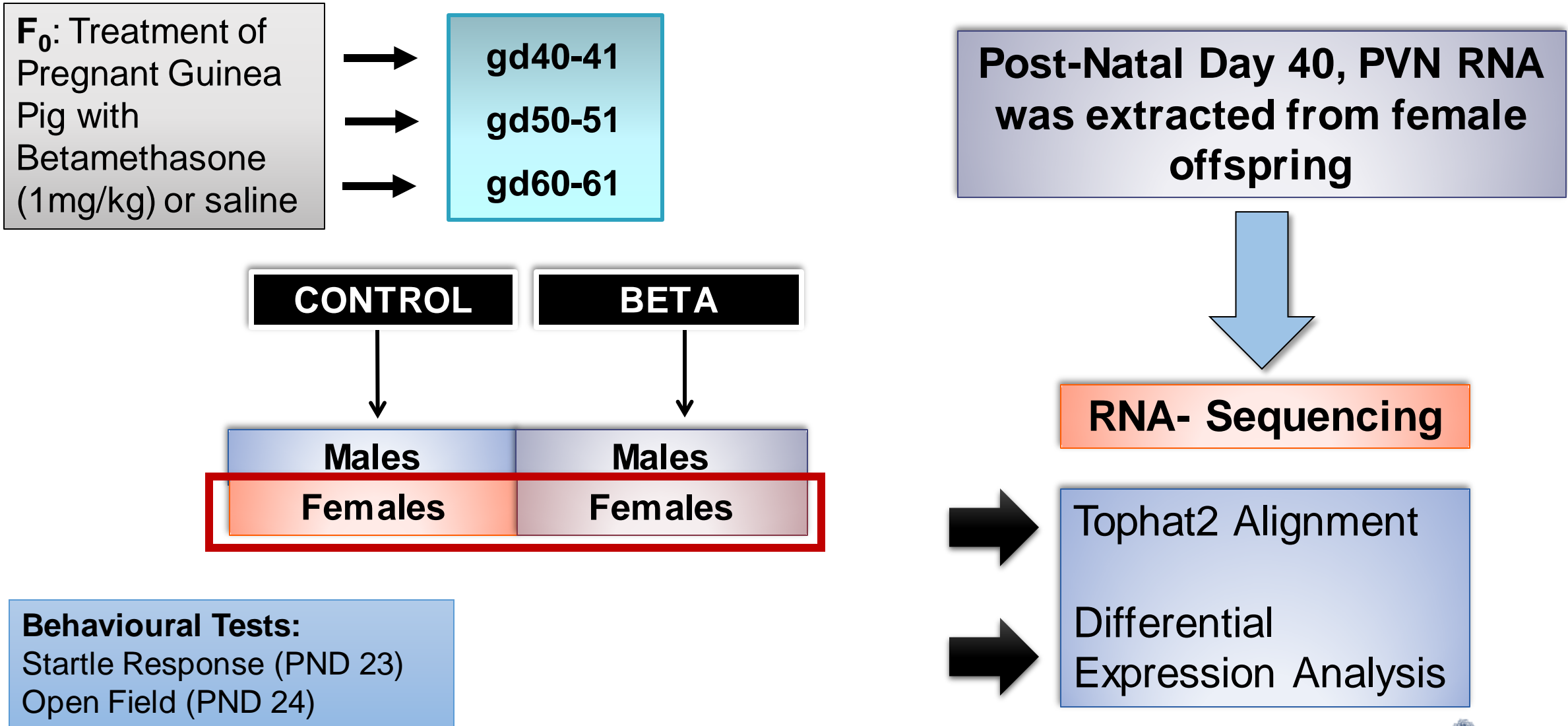
Glucocorticoids and Early Life Environment

- The early life environment has long term influences on future health

- Perinatal exposure to high levels of glucocorticoids has been linked to the development of:
  - heart disease
  - diabetes mellitus
  - depression and anxiety disorders

Synthetic Glucocorticoids (sGC)

- Administered to women at risk for pre-term labor (~10% of pregnant women)

- Reduces incidence of Respiratory Distress Syndrome

- Hypothalamic Paraventricular Nucleus (PVN) plays a central role in regulating stress response and behaviour

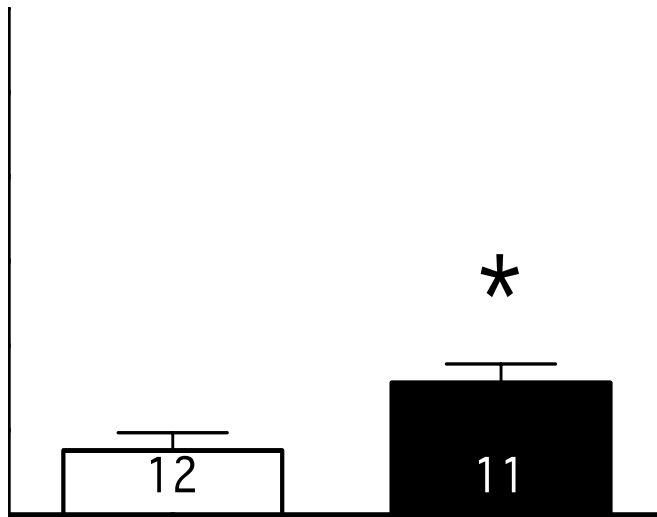- Highly affected by antenatal synthetic glucocorticoid exposure

Paraventricular Nucleus

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Experimental Method and Model

F₀: Treatment of Pregnant Guinea Pig with Betamethasone (1mg/kg) or saline

→ **gd40-41**
→ **gd50-51**
→ **gd60-61**

CONTROL   **BETA**

Males   **Males**
Females   **Females**

Behavioural Tests:
Startle Response (PND 23)
Open Field (PND 24)

Post-Natal Day 40, PVN RNA was extracted from female offspring

RNA- Sequencing

Tophat2 Alignment

Differential Expression Analysis

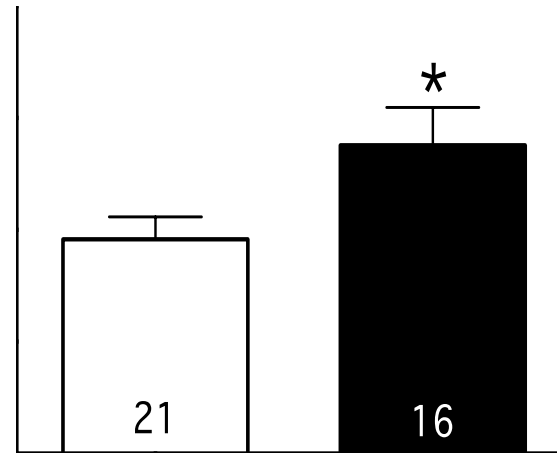Moisiadis VG, Constantinof A, *et al*. submitted, 2017

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Antenatal sGC Results in Significant Changes in Behaviour

HPA Response to Open Field Stress

Total Locomotor Activity in the Open Field

Acoustic Startle Response

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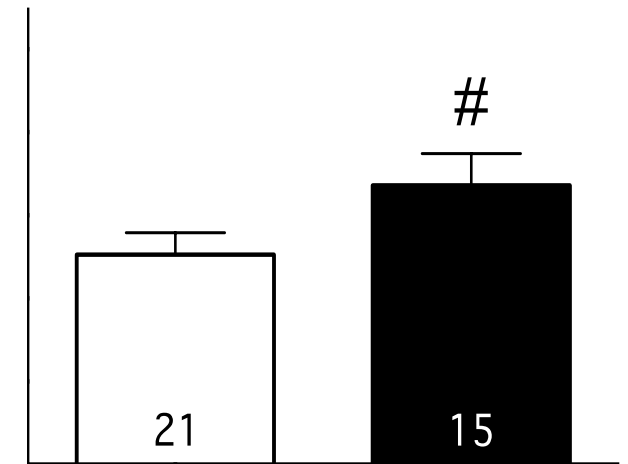
Antenatal sGC Results in Significant Changes in Gene Expression in PVN

Low **High**

Control **Beta**

161 genes ⬇

597 genes ⬆

-Log₁₀(p_value) → $-Log_{10}(p\_value)$

● Significant (FDR< 5%)
● Not Significant

Log₂(fold change) → $Log_2(fold\ change)$

Moisiadis VG, Constantinof A, *et al*. submitted, 2017
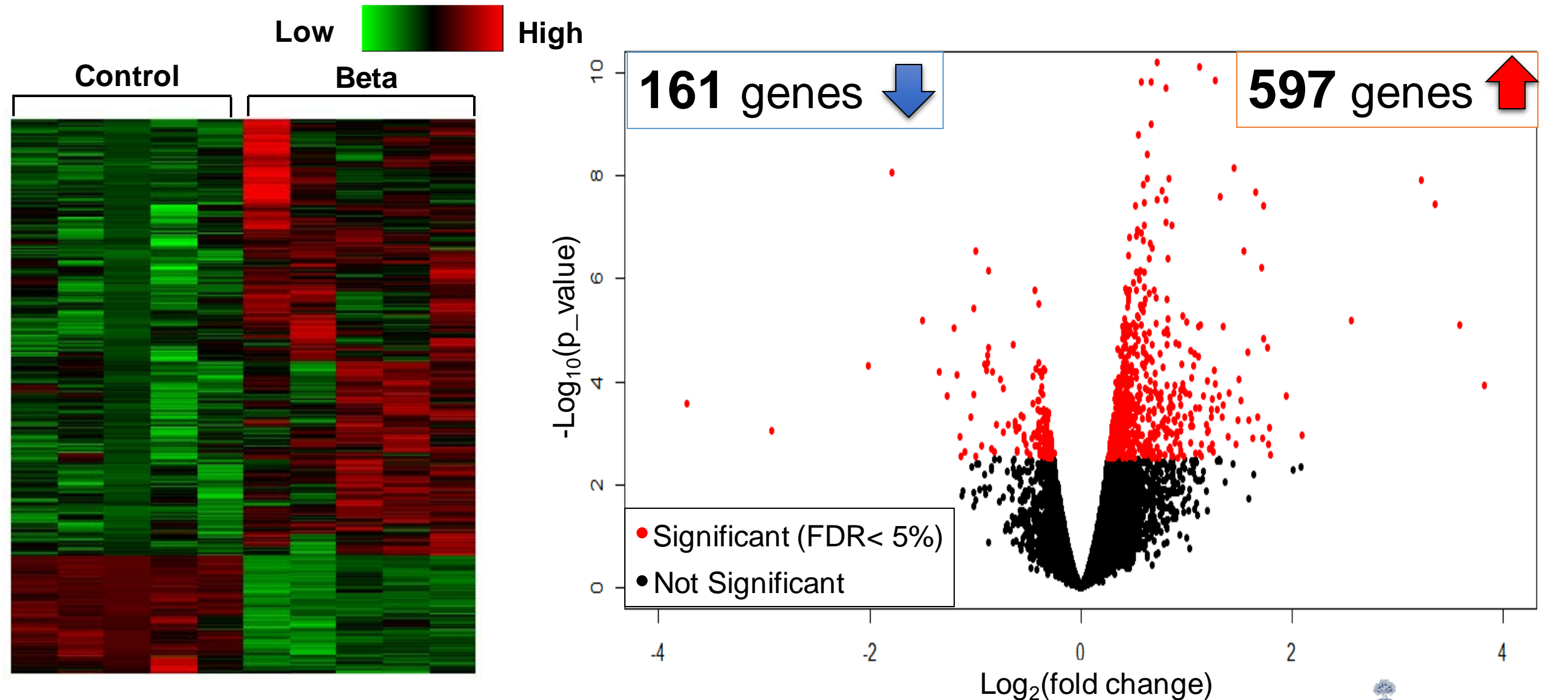
Objective

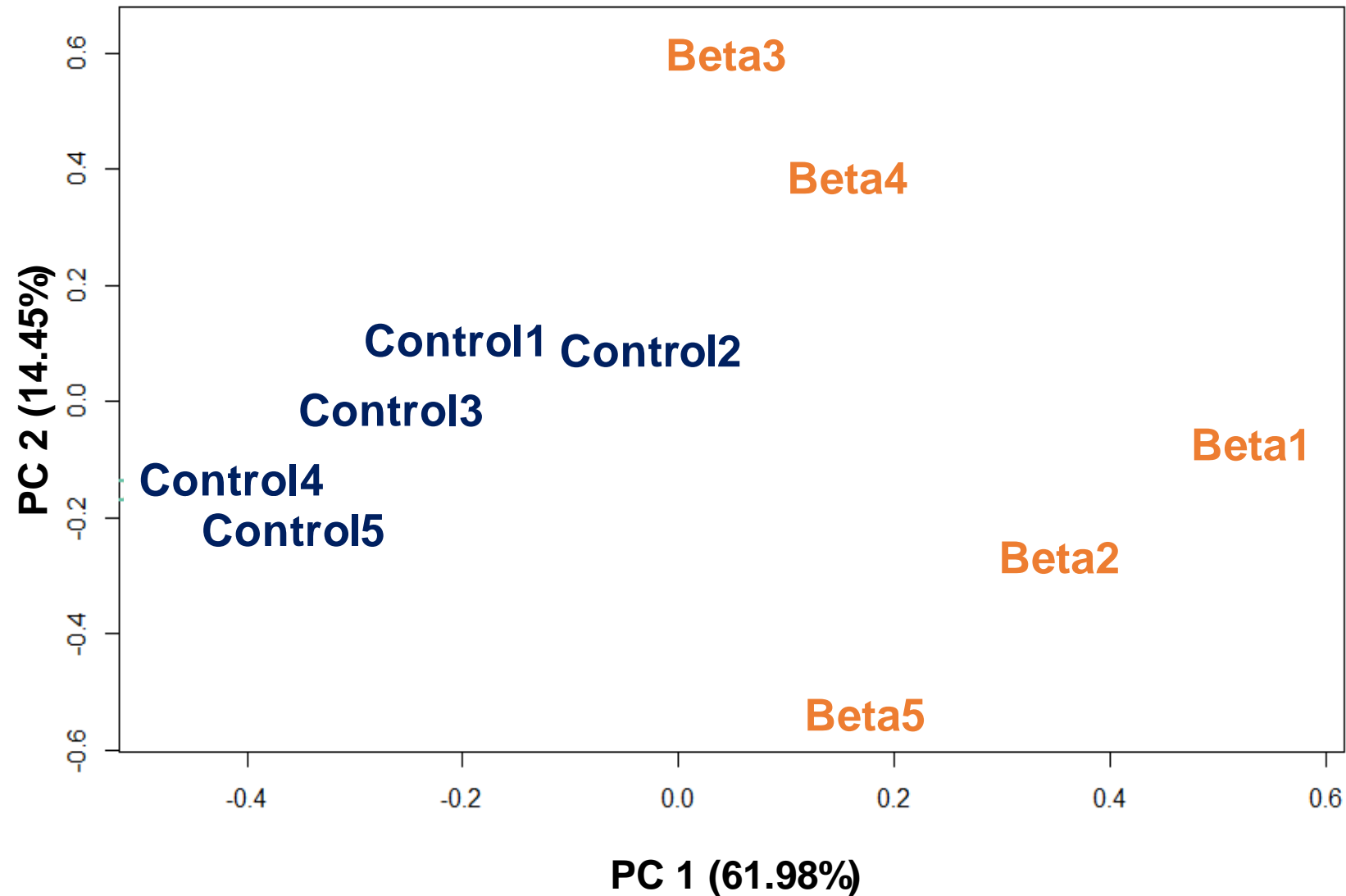To determine the relationship between gene expression in the PVN and behaviour

Hypothesis

Transcriptional programming of the PVN by sGC is correlated to behavioural outcomes and can be used in predictive modelling

Methods

- Principal Component Analysis
- Multivariate Linear Regression

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Principal Component Analysis (PCA)

- PCA is useful to describe multi-dimensional data.

- The expression of each gene = a new dimension

- We can reduce the dimensions of the data to the first two Principal Components that describe the majority of variation within the dataset.
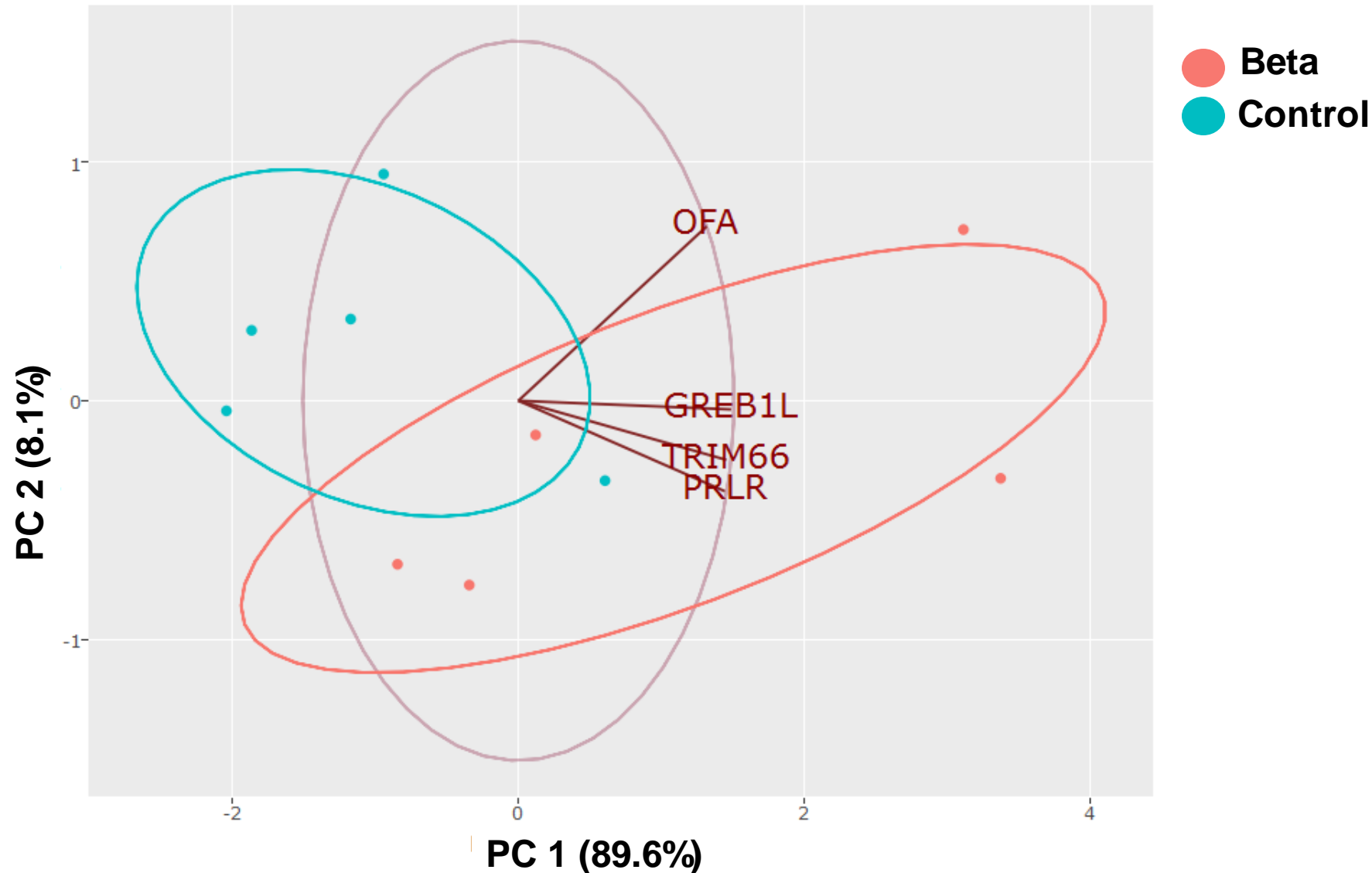
PCA of Gene Expression and Behaviour

Circle of Correlations and PCA:

Determine the relationship between gene expression and behaviour
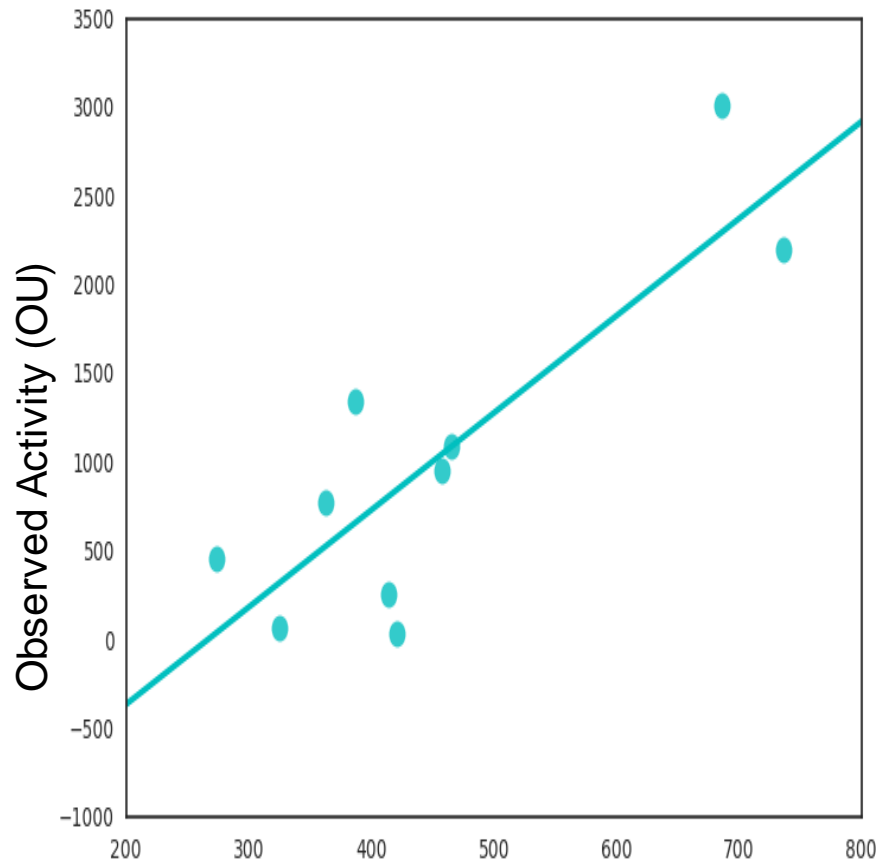
PCA of Gene Expression and Behaviour

- PCA suggests that there may exist a relationship between the expression of GREB1L, TRIM66, PRLR and Open Field Activity

- Relationship is tested with Linear Regression

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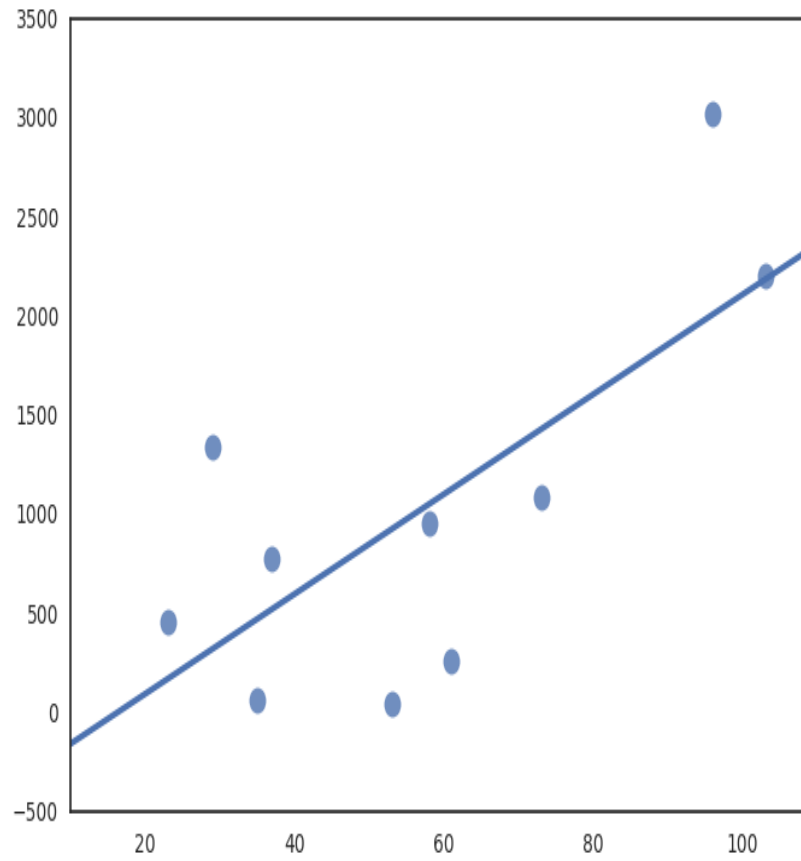
Linear Regression

GREB1L
$R^2 = 0.71$, p= 0.002

PRLR
$R^2 = 0.51$, p= 0.019

TRIM66
$R^2 = 0.58$, p=0.01

Observed Activity (OU)
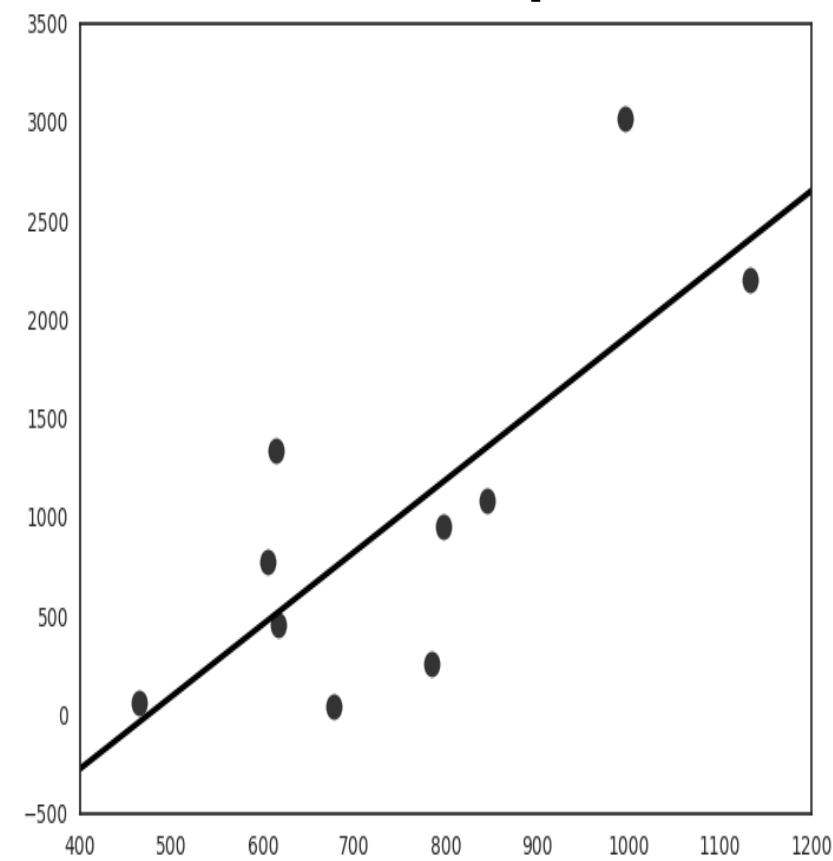
Normalized Gene Expression (Counts)

Multivariate Linear Regression Results

- Use the expression of 3 genes to model the relationship between gene expression and Open Field Activity

- Determine accuracy of model by plotting predicted value as a function of observed values and measuring R^2

$R^2 = 0.81$

pValue=0.03

Predicted Activity (OU)

Observed Activity (OU)

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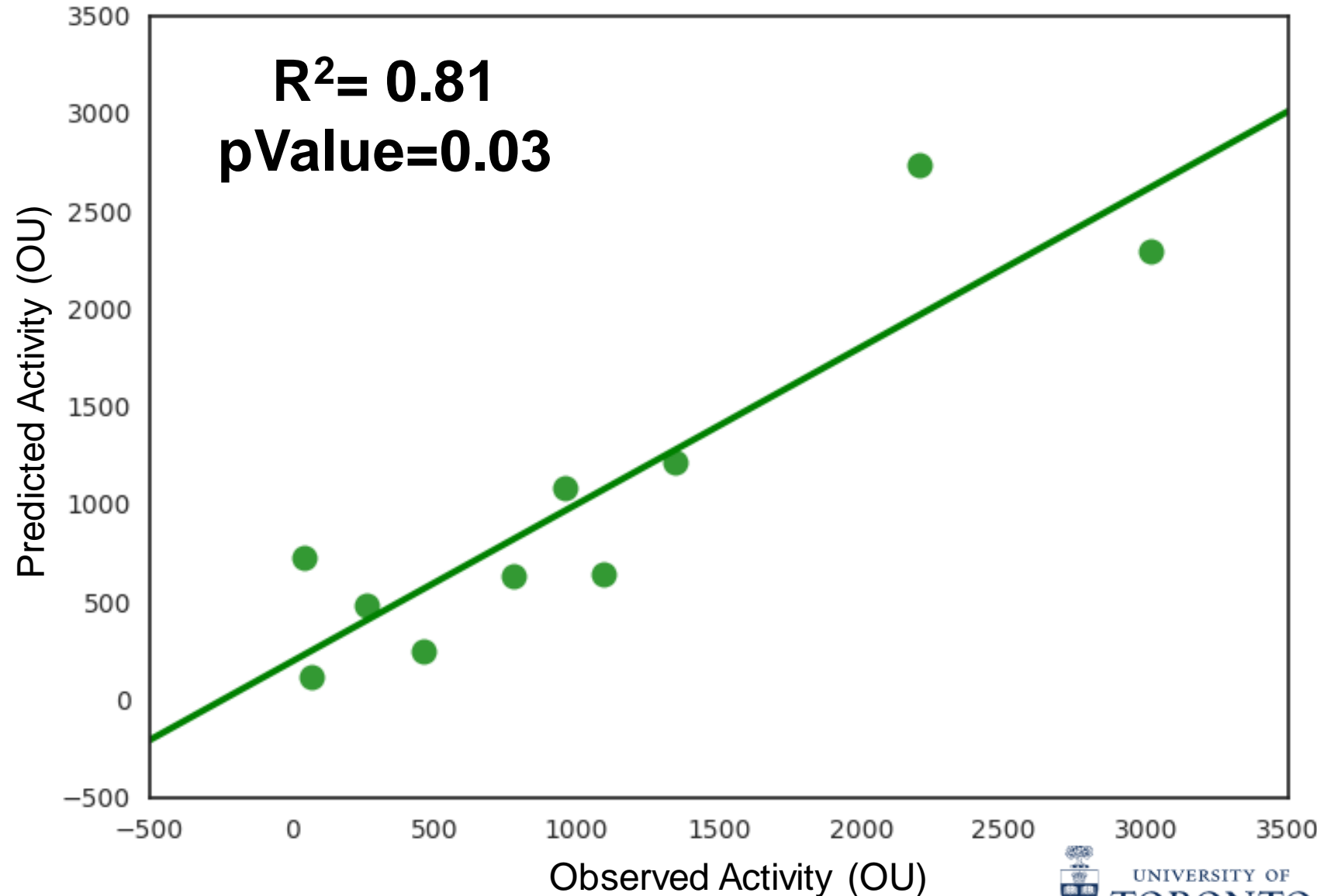
Multivariate Linear Regression Cross Validation

- **Leave One Out Technique:** remove one sample of your dataset to use as 'test' dataset. Repeat for all samples

- Obtain focused genes for further investigation

R^2= 0.51
pValue=0.02

(Axes: Predicted Activity (OU) vs Observed Activity (OU))
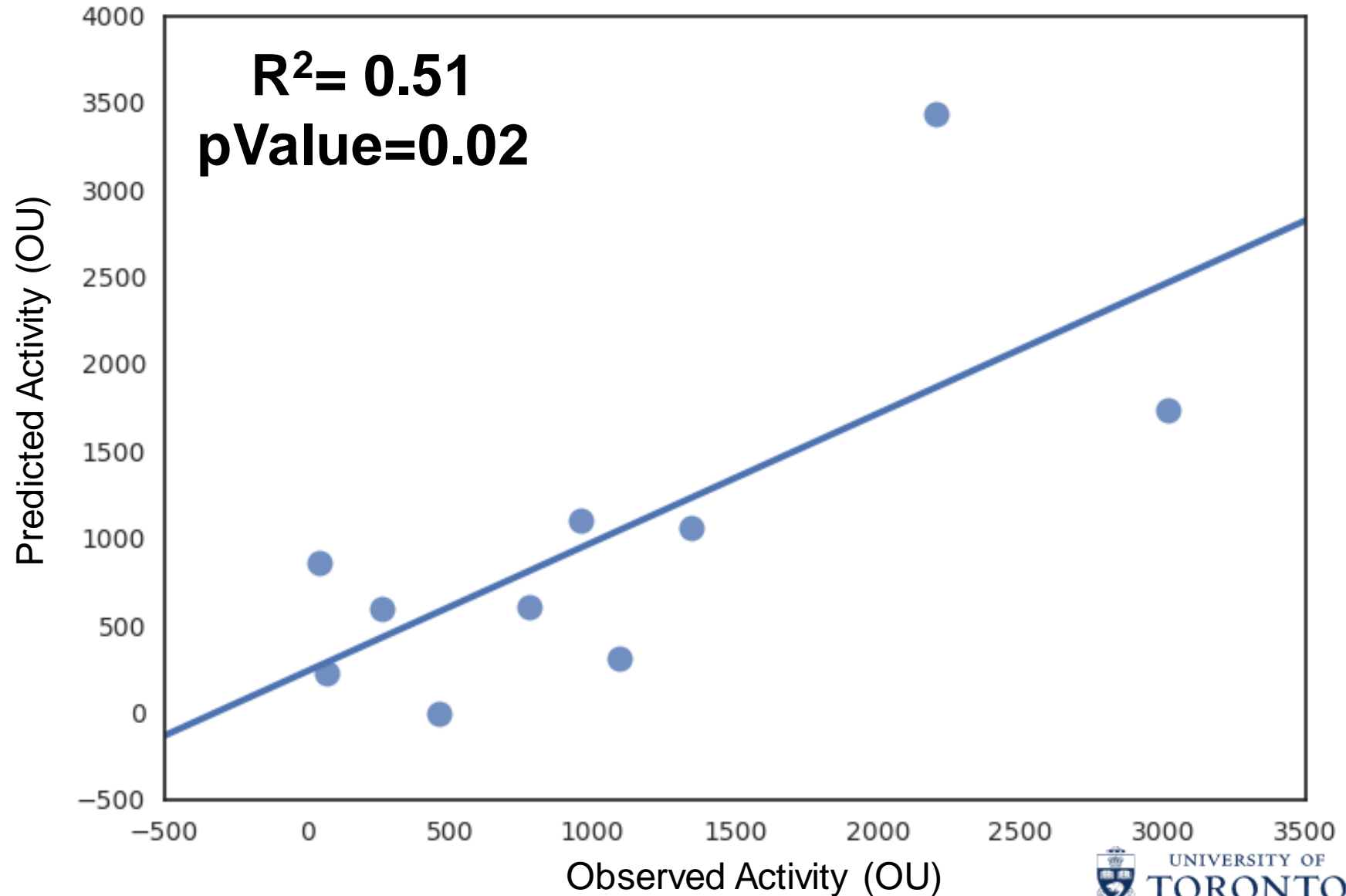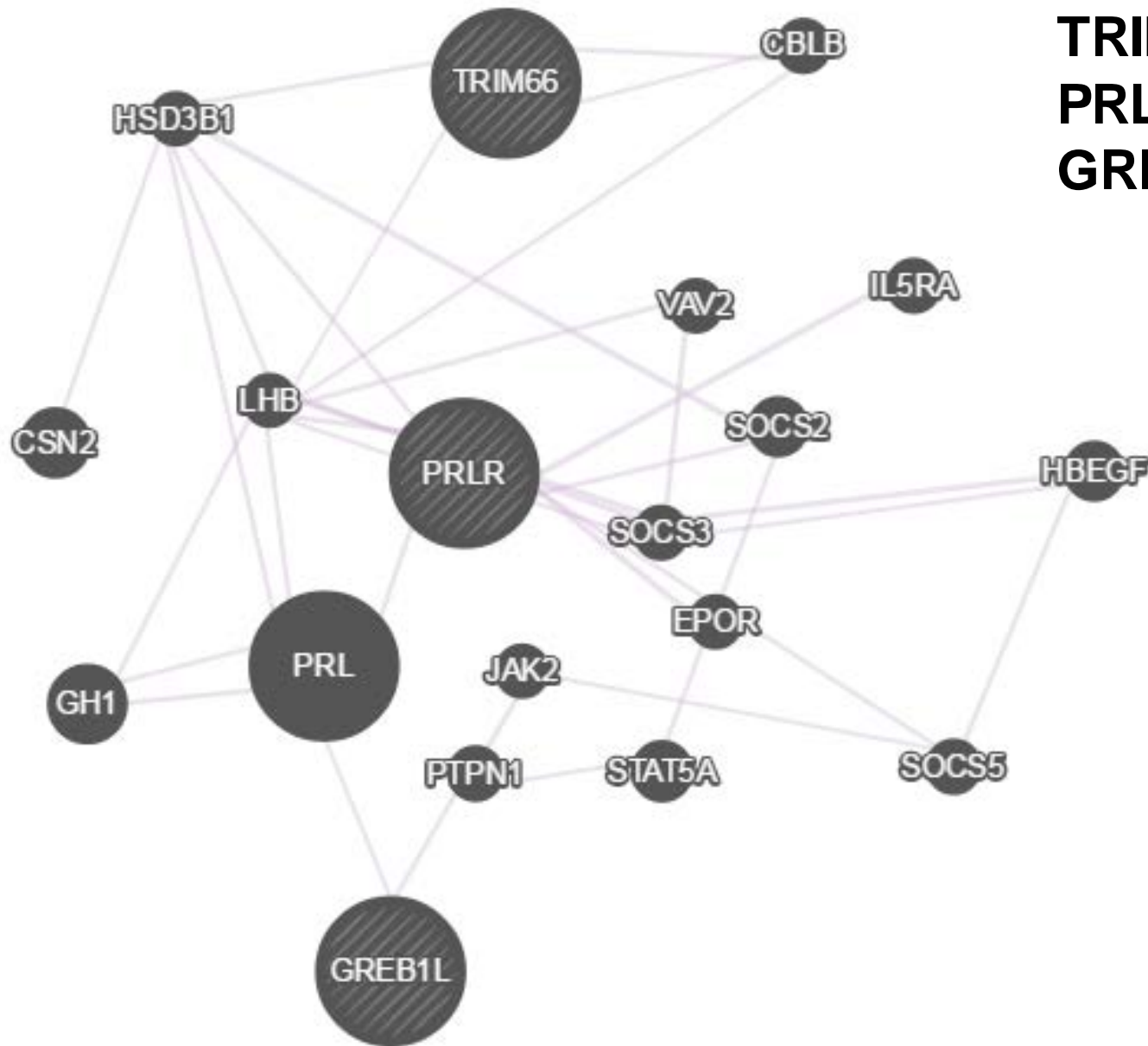
TRIM66: Transcription Regulation
PRLR: Prolactin Receptor
GREB1L: Estrogen Receptor Signaling

- These genes are co-expressed

Image from genemania.com

Conclusions and Significance

- PCA and linear regression analyses suggest that a relationship exists between the expression of *Greb1l, Prlr* and *Trim66* and behaviour in the Open Field

- Additional samples are required to fully validate the model

- Insight into the potential mechanisms of antenatal sGC and how these molecular events may relate to behavior

- Proof-of-Principal for the use of gene expression profiles in disease prediction, detection, and prevention
  - Targeted therapeutic intervention

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