Paternal exposure to early life adversity alters placental efficiency in offspring

Rattan Kaur[1,2], Pui-Pik Law[1], Ruben Esse[1], Lillina M Vignola[1], Vardhman K Rakyan[3], Marika Charalambous[1], Michelle L Holland[1]

[1]Department of Medical and Molecular Genetics, King's College London, UK

[2]Department of Biological Sciences, IISER Mohali, India

[3]Blizard Institute, Queen Mary University of London, UK

Introduction

Developmental programming in response to maternal pathophysiology and environmental exposures is well documented in humans and reproducibly supported by animal models. Studies show that exposure to sub-optimal conditions during pregnancy can result in phenotypic and epigenetic alterations in G1 animals [1,2]. However, it has not yet been described whether these early life adversities experienced by G1 animals can result in intergenerational effects in the G2 generation. Here, we explore this question using an inbred mouse model of protein restriction (PR).

G1 male offspring of dams fed a PR diet from conception to weaning show signs of being developmentally programmed, with altered phenotypes and epigenetic modifications [1, 2]. The present study examined whether offspring of these males have altered developmental outcomes in the absence of any further environmental exposures.

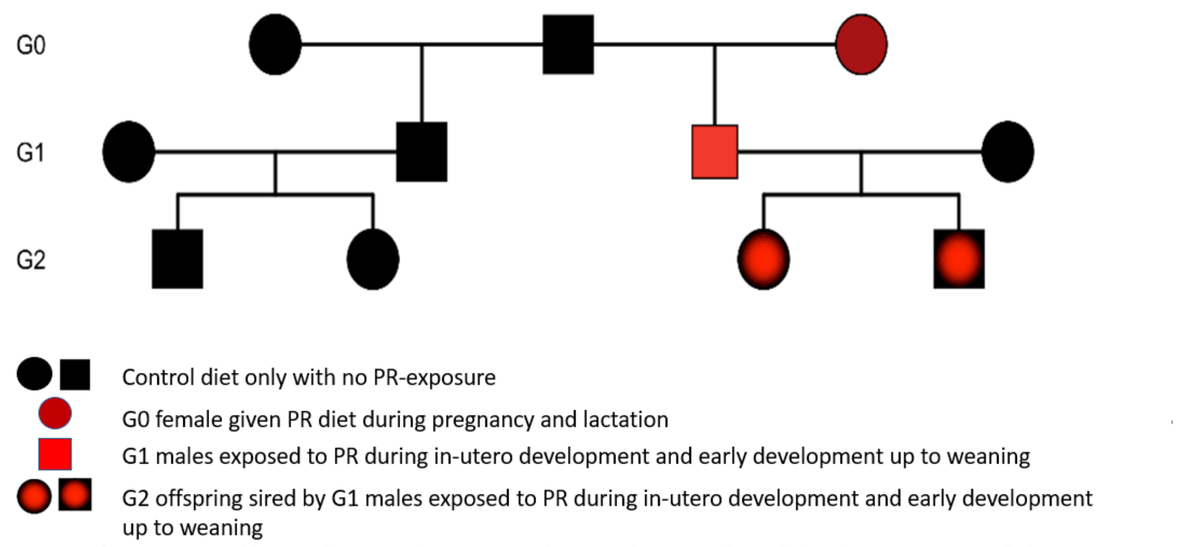


Fig. 1: Breeding scheme of maternal protein restricted (PR) mouse model

Unexposed offspring of PR exposed males show altered growth trajectories and reduced placental efficiency

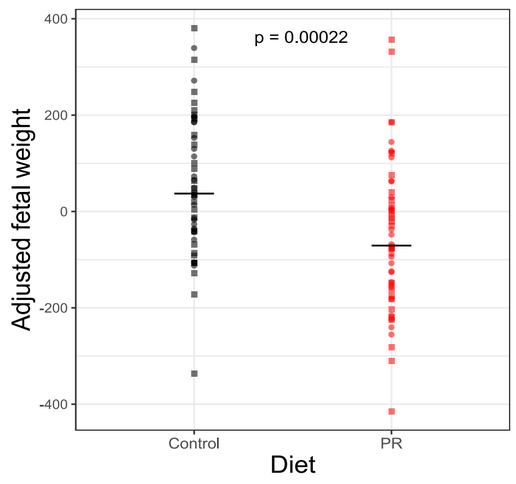


Fig. 2: G2 PR offspring have reduced fetal weights. Fetal weights of G2 offspring were measured at late gestation (17.5 dpc). Fetal weights were adjusted for sex and litter size and plotted against diet. [Welch two sample t-test, p = 0.00022, n = 108 (54 PR and 54 Control), 14 litters]

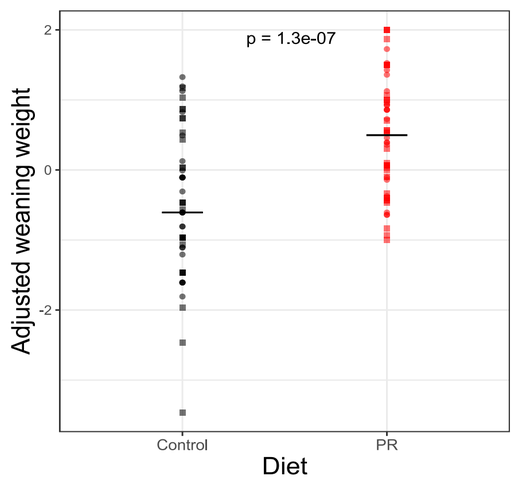


Fig. 3: G2 PR offspring have increased weights at weaning. Weights of G2 offspring were measured at weaning. Weaning weights were adjusted for sex and litter size and plotted against diet. [Welch two sample t-test, p = 1.3e-07, n = 111 (56 PR, 55 Control), 14 litters]

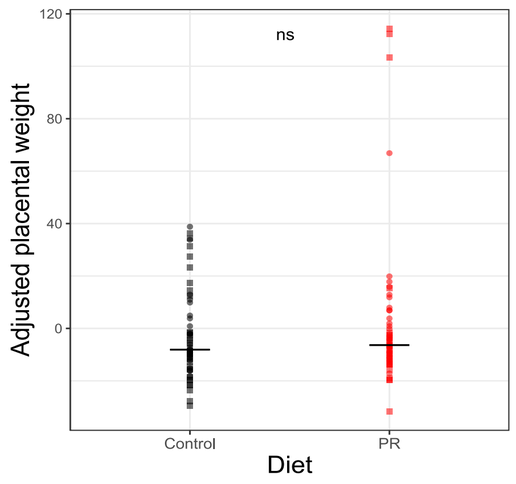


Fig. 4: G2 PR and control offspring have similar placental weights. Placental weights of G2 offspring were measured at late gestation (17.5 dpc). Placental weights were adjusted for sex and litter size and plotted against diet. [Mann-Whitney U test, p = 0.49, n = 108 (54 PR and 54 Control), 14 litters]

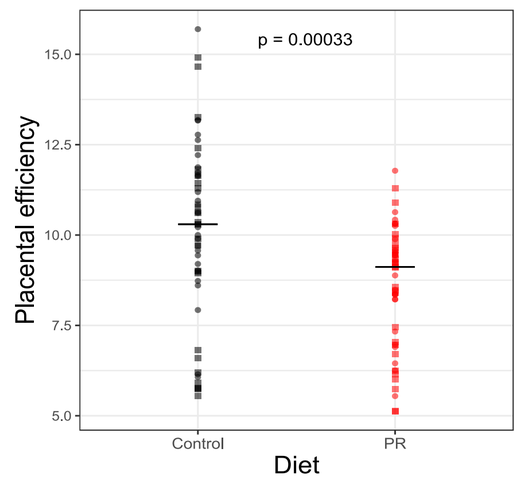


Fig. 5: Placentae of G2 PR offspring are less efficient. Observed fetal weights were divided by observed placental weights to calculate placental efficiencies and plotted against diet. . [Mann-Whitney U test, p = 0.00033, n = 108 (54 PR, and 54 Control), 14 litters]

G2 PR offspring show altered gene expression in placenta

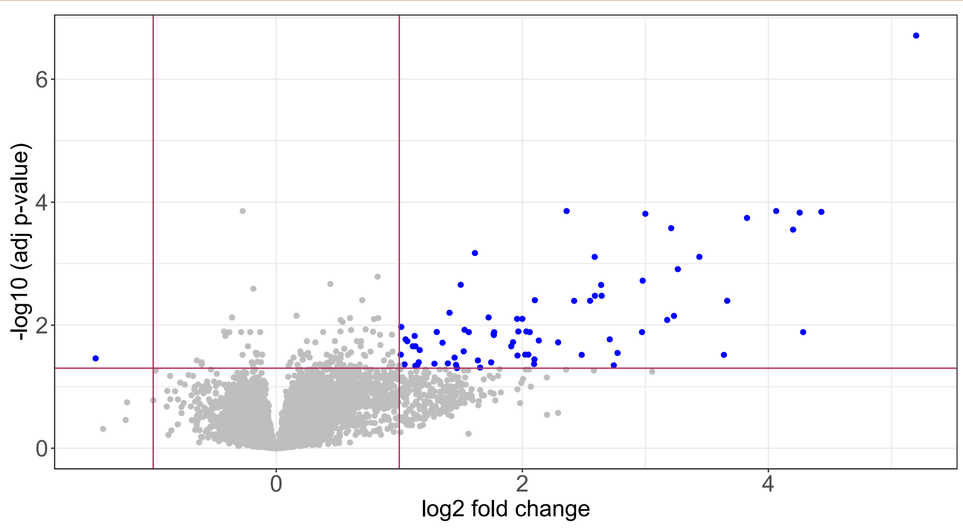


Fig. 6: Transcriptomic analysis shows differential expression of genes in placentae of G2 PR offspring. RNA-seq was performed for late gestation (17.5 dpc) placentae of G2 offspring [n = 37 (18 PR, 19 Control)]. DE-seq utilized sex and litter size as cofactors. Analysis revealed that 75 genes are upregulated and 1 gene is downregulated in placentae of G2 PR offspring. (Criteria: log2foldchange >1 or < -1, baseMean >10 and p adj value <0.05)

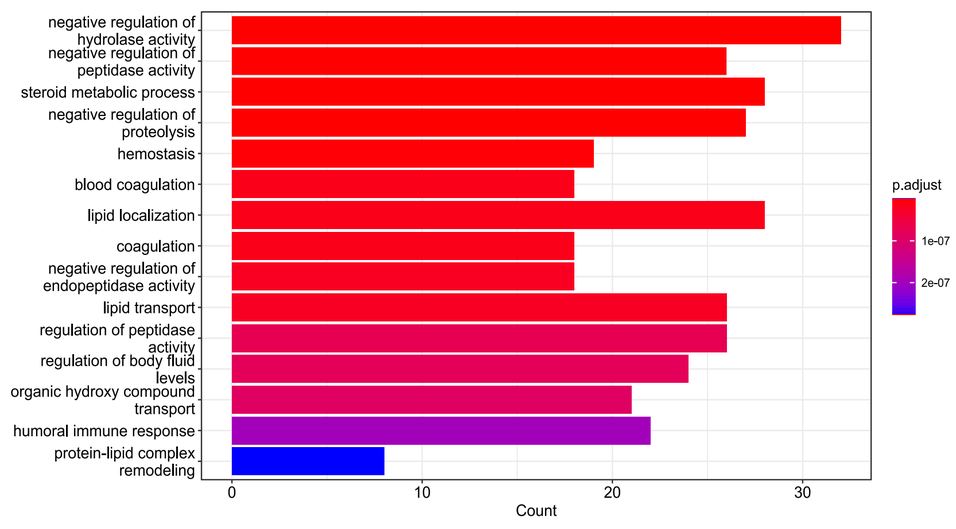


Fig. 7: Differentially expressed genes (DEGs) in placental tissues of G2 offspring are enriched for pathways involved in nutrient transport, steroid hormone metabolism and immune response. GO analysis was performed using enrichGO function from BiocManager package in R. The plot shows top 15 GO hits for the differentially expressed gene set in G2 PR placentae.

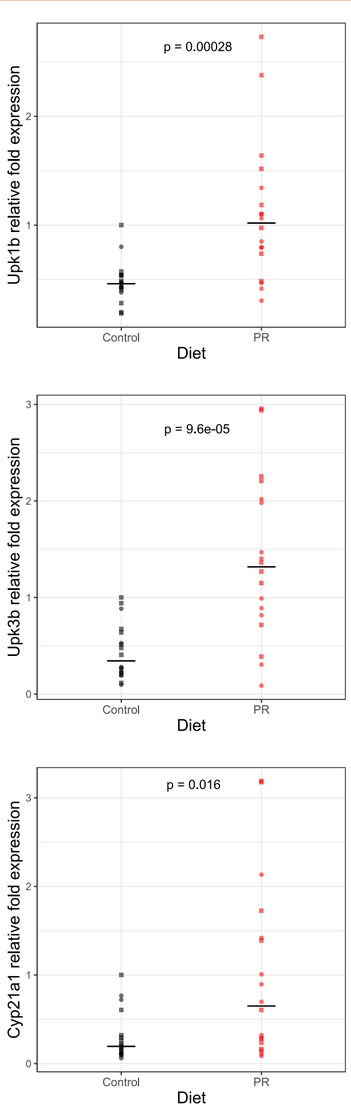
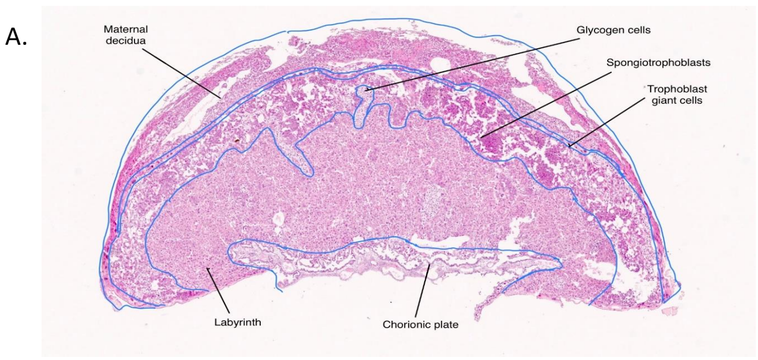


Fig. 8: RT-qPCR validation of RNA-seq results. qPCR was performed using SYBR green assay following cDNA synthesis for RNA samples used in RNA-seq analysis [n = 36 (18 PR, 18 Control)]. RNA-seq data was validated for three DEGs: Upk1b, Upk3b and Cyp21a1. These genes were found to be upregulated in placental tissues of G2 PR offspring.

Histological analysis of placental tissues



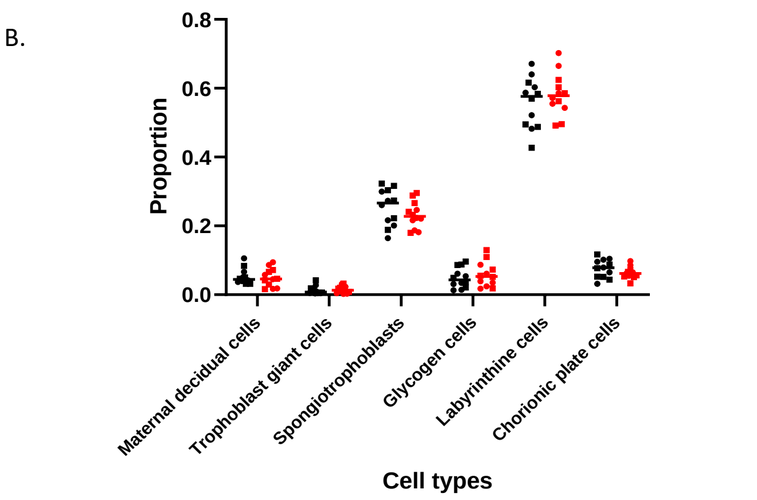


Fig. 9: Cell proportion analysis in independent 17.5 dpc placental tissues. A. The placental cell types used in stereological analysis. Stereological analysis was performed on H&E-stained placental sections [n = 24 (12 PR, 12 control)] and proportion of different cell types were calculated. B. The graph shows the results for Two-way ANOVA test performed on the cell proportion data. None of the cell types showed statistically significant changes in proportion, although there seems be a slight decrease in the proportion of spongiotrophoblasts.

Conclusions

- Exposure to early life adversity in sires results in intergenerational effects in their unexposed offspring.
- The altered growth phenotypes in G2 PR offspring may be explained by reduced placental efficiency.
- The reduced placental efficiency seems to result from functional and structural alterations in placentae of G2 PR offspring.

Acknowledgements

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References