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FINAL REPORT

Project / Program Title		Novel treatment for obesity and maternal-obesity related chronic kidney disease
Name		Dr Benjamin Larkin
Award Received		2019 Jacquot Research Entry Scholarship
Report Date		May 2021
Chief Investigator / Supervisor		A/Prof Sonia Saad
Administering Institution		The University of Sydney
Funding Period	Start Date:	February 2019
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PROJECT SUMMARY

Obesity is health condition which significantly increases the risk of chronic kidney disease (CKD). Maternal obesity during pregnancy also predisposes offspring to CKD in later life. Given the significant morbidity, mortality and enormous healthcare costs associated with CKD, novel strategies to prevent its development and progression are urgently required. Hydralazine is a drug which has been used clinically for decades to reduce blood pressure. It is safe when used in pregnancy. In recent animal studies, hydralazine has been shown to reduce scarring in the kidneys when administered at low doses which do not reduce blood pressure.

This study assessed whether low-dose hydralazine can prevent CKD related to obesity or maternal obesity. Several protective effects were observed. Administering low-dose hydralazine to obese mice protected against kidney damage due to obesity. Further, treating obese mothers during pregnancy prevented their offspring from developing kidney scarring in later life. Interestingly, treating lean mothers with low-dose hydralazine during pregnancy prevented CKD in offspring who subsequently developed obesity. These data support the repurposing of hydralazine as a strategy to prevent obesity and maternal obesity-induced CKD.

This project also identified gene signatures in the blood and kidney due to obesity and maternal obesity. Differences in gene signatures may help explain the mechanisms by which hydralazine protects against obesity-related CKD. Alternatively, these gene signatures may have utility as potential diagnostic tests for CKD due to obesity and maternal obesity.

PROJECT AIMS / OBJECTIVES

1. **To determine if the use of a demethylating agent (hydralazine) prevents the development of obesity-related chronic kidney disease**

A mouse model of diet-induced obesity was used. Male mice were fed either high fat diet (HFD) or chow from 8 weeks of age. From the same timepoint, animals received low-dose hydralazine in the drinking water (25 mg/L) or normal drinking water. Mice were sacrificed in mid-adulthood at 32 weeks of age. Biometric parameters, metabolic markers, and markers of renal function were evaluated. Renal markers of fibrosis, injury, inflammation and oxidative stress were also assessed. At endpoint, renal global DNA methylation was performed. Reduced representation bisulfite sequencing (RRBS) was used to identify genes in the kidney which were differentially methylated due to obesity and/or hydralazine treatment.

2. **Use a mouse model of maternal obesity to determine whether low-dose hydralazine administration to mothers during gestation interrupts the fetal programming effects of maternal obesity on offspring CKD.**

   Maternal obesity was modelled using a HFD mouse model. Dams were fed HFD from 6 weeks prior to breeding, during gestation and lactation. Female mice were administered either hydralazine or saline during gestation. Male offspring were weaned to chow and were harvested at either 9 weeks or 32 weeks of age. At endpoint, biometric parameters and metabolic markers were measured. Markers of renal function, renal fibrosis, renal injury, inflammation and oxidative stress were assessed. Renal global DNA methylation was assessed in offspring at endpoint.

3. **Determine whether low-dose hydralazine administration to obese or lean dams during gestation can protect obese offspring from developing CKD.**

   Maternal obesity was modelled as above. Dams received either low-dose hydralazine or saline during the gestational period. Male offspring were weaned on postnatal day 20 to either HFD or chow. Offspring were harvested at 32 weeks of age. Biometric markers, metabolic markers, and renal function, fibrosis, injury, inflammation, oxidative stress and DNA global methylation were assessed.

4. **In kidney tissue and blood, identify differentially methylated genes at adulthood between offspring exposed to maternal obesity and controls, as these may have utility as biomarkers.**

   Maternal obesity was modelled as described above. Male offspring were weaned to chow diet on postnatal day 20. Genomic DNA was extracted from blood and kidneys at 32 weeks of age. RRBS was performed to determine significantly differentially methylated genes between offspring exposed to maternal obesity and controls.

5. **Identify differentially methylated genes in the fetal cord blood of obese versus lean mothers.**

   Women undergoing elective Caesarean section were recruited for the study. The study group were obese women, whereas women of normal weight were used as controls. At Caesarean section, fetal cord blood was collected, from which genomic DNA was extracted. The Illumina Infinium MethylationEPIC BeadChip was used to identify highly significant genes which were differentially methylated between the obese and control groups.

SIGNIFICANCE AND OUTCOMES

Obesity is a deleterious health condition associated with the development and progression of chronic kidney disease (CKD). Further, maternal obesity predisposes offspring to CKD later in life. Therefore, strategies are required to mitigate the development and progression of CKD due to obesity or maternal obesity. When administered at low doses, the antihypertensive agent hydralazine was shown to exert several renoprotective effects when given either directly to obese animals or to mothers during gestation. As hydralazine has been used clinically for decades and has demonstrated safety during pregnancy, these data support the repurposing of hydralazine as a strategy to prevent obesity and maternal obesity-related CKD.

PUBLICATIONS / PRESENTATIONS

Publications

1. Larkin BP, Glastras SJ, Chen H, Pollock CA, Saad S. DNA methylation and the potential role of demethylating agents in prevention of progressive chronic kidney disease. FASEB J. 2018;32(10):5215-26.

2. Larkin BP, Ngyuen LT, Glastras SJ, Gangadharan Komala M, Pollock CA, Saad S. Epigenetic regulation of ILDR2 in the cord blood of obese mothers. Translational Metabolic Syndrome Research. 2020; 3:6-8.

3. Larkin BP, Saad S, Glastras SJ, Nguyen LT, Hou M, Chen H, et al. Low-dose hydralazine during gestation reduces renal fibrosis in rodent offspring exposed to maternal high fat diet. PLoS One. 2021;16(3):e0248854.

Abstracts/ conference presentations

1. Benjamin P. Larkin, Long T. Nguyen, Sarah J. Glastras, Hui Chen, Rosy Wang, Carol A. Pollock and Sonia Saad. The effects of gestational hydralazine on obesity-related chronic kidney disease in offspring. Presented in Young Investigator category of Australian and New Zealand Society of Nephrology Annual Scientific Meeting (ANZSN ASM) November 2020. Awarded Runner-up prize.

2. Benjamin P. Larkin, Long T. Nguyen, Sarah J. Glastras, Hui Chen, Carol A. Pollock and Sonia Saad. Maternal obesity induces DNA methylation changes in the kidneys and blood of rodent offspring at adulthood. Poster presentation, ANZSN November 2020.

3. Benjamin P. Larkin, Sarah J. Glastras, Long T. Nguyen, Miao Hou, Hui Chen, Jason Chen, Rosy Wang, Carol A. Pollock and Sonia Saad**.** Obesity-related chronic kidney disease is ameliorated by low-dose hydralazine independent of a blood pressure-lowering effect. Presentation at Australasian Diabetes Congress, November 2020.

4. Benjamin P. Larkin, Sarah J. Glastras, Long T. Nguyen, Miao Hou, Hui Chen, Jason Chen, Rosy Wang, Carol A. Pollock and Sonia Saad. The effect of low-dose hydralazine on obesity-related chronic kidney disease. Presentation at Austral-Asia Obesity Research Update, October 2020.

5. Benjamin Larkin, Sarah Glastras, Hui Chen, Jason Chen, Carol Pollock, Sonia Saad, Repurposing hydralazine for the treatment of obesity-related kidney disease. New Horizons 2019, University of Technology, Sydney.

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1. Larkin BP, Nguyen LT, Glastras SJ, Gangadharan Komala M, Pollock CA, Saad S. Epigenetic regulation of ILDR2 in the cord blood of obese mothers. Translational Metabolic Syndrome Research. 2020; 3:6-8.

2. Larkin BP, Saad S, Glastras SJ, Nguyen LT, Hou M, Chen H, et al. Low-dose hydralazine during gestation reduces renal fibrosis in rodent offspring exposed to maternal high fat diet. PLoS One. 2021;16(3):e0248854.

The award will also be acknowledged in future manuscripts when these are published.