EFFECTS OF PRENATAL DIETARY METHYL DONOR DEFICIENCY ON DEVELOPMENT AND EPIGENETIC MECHANISMS IN OFFSPRING – STUDIES IN THE RAT

GALINA KONYCHEVA

A thesis submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy in Biological Sciences,
The University of Auckland, 2010

ABSTRACT

Human epidemiological and animal studies suggest that unbalanced maternal diet during pregnancy leads to low birth weight and predisposes offspring to the development of a number of pathological conditions like metabolic and cardiovascular diseases. This effect is referred to as fetal or developmental programming, however the underlying mechanism is yet unknown. An epigenetic mechanism of gene regulation, DNA methylation, has been suggested as a strong candidate for the underling mechanism of developmental programming due to the fact that establishment of DNA methylation patterns begins during early development and requires a constant supply of methyl donors. The main purpose of the present study was to determine whether a deficiency of methyl donors in the maternal diet during prenatal development would result in phenotypic changes and abnormal epigenetic gene regulation in the offspring. The study was designed to help understand whether DNA methylation provides an epigenetic basis to developmental programming.

A new rat model of prenatal dietary methyl donor deficiency was established, whereby female rats were fed a diet deficient in methyl donors choline, folate and methionine two weeks prior to mating and throughout pregnancy. The effect of prenatal exposure to methyl donor deficiency on developmental programming was investigated by measuring systolic blood pressure, glucose metabolism, endocrine pancreas structure and behavioral changes in the adult offspring. To investigate immediate and delayed effects of maternal methyl donor deficiency on molecular processes in the offspring, liver, pancreas, kidney, lung and hippocampus tissues were being used for specific DNA methylation measurements by means of Matrix assisted laser desorption/ionization time-of-flight mass spectrometry. Expression of target genes, suggested to be regulated by DNA methylation, was tested using a real time PCR-based technique.

The offspring of methyl deficient mothers (MD) had low weight at birth. Young adult MD males had a transient increase in systolic blood pressure and altered pancreatic structure, but no changes in glucose metabolism. Aged female and male MD offspring demonstrated traits of anxiety-like behavior. MD females had improved learning abilities. At the molecular level, the offspring of MD mothers showed age, DNA region and tissue-specific changes in DNA methylation. The MD offspring also demonstrated differences in gene expression, which were not associated with changes in DNA methylation.

In conclusion, the present study demonstrated that maternal methyl donor deficiency had a mild selective effect on development of phenotypic changes associated with developmental programming in the adult offspring. This study indicated that prenatal deficiency in methyl donors programmed changes in DNA methylation in the adult offspring, but its' effect was rather complex and largely unrelated to the observed changes in gene expression.

DEDICATION

This thesis is dedicated to my wonderful husband, Sergei, who has been with me every step of the way, through good times and bad. Thank you for all the unconditional love and support that you have always given me.

ACKNOWLEDGEMENTS

I would like to express my gratitude to the people who made this thesis possible.

I would like to thank my supervisors Professor Marie Dziadek for her support and guidance during the experimental part of this work and Associate Professor Bernhard H. Breier who rescued this study with his invaluable advice at the very beginning of the experiments. I would also like to say thanks Dr Lorna Johnstone who bravely took over my supervision at later stages of the project.

I am especially grateful to my supervisor Professor Lynnette Ferguson for her support and confidence in me during the writing process. Her insightful comments and guidance made it possible to complete this work.

Very special thanks go to people who helped me with their expert advice and assistance on different technical aspects of this study and who taught me valuable research skills. I am thankful to Dr Pierre van Zijl and Eric Thorstensen for their expert advice and assistance with high performance liquid chromatography and mass spectrometry assays. I would like to thank Dr Susan Ravelich for her expert advice on allelic discrimination assay. I want to express my gratitude to Dr Chris Krageloh who selflessly helped me out with operant conditioning tests. I would like to thank Dr Marcel Coolen for his assistance with running Sequenom's quantitative DNA methylation assay. I am thankful to Greg Smith for providing his expert advice and assistance with glucose metabolism and pancreas histology assays. I would also like to thank Dug Yeo Han and Dr Kathy Ruggiero for their expert advice on statistical analysis of DNA methylation data.
I am forever in debt to Dr Susan Ravelich, Selina Patel and Greg Smith for their help with tissue collections.

On a personal note my special thanks go to Susan Ravelich, Selina Patel, Greg Smith and Stefan Krechowec for their help, support, laughs and chats. I have been fortunate to come across these people, without whom these years would be bleak.

I am grateful to my parents and my husband for their endless support and encouragement.

In conclusion, I recognize that this research would not have been possible without the financial assistance of the Royal Society of New Zealand (Marsden fund) and the University of Auckland's Graduate Research fund.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACTH	adrenocorticotropic hormone
A^{vy}	viable yellow agouti allele
BP	blood pressure
Cdkn1a	cyclin-dependent kinase inhibitor 1a
Cdkn2a	cyclin-dependent kinase inhibitor 2a
cDNA	complementary DNA
CHD	coronary heart disease
CNS	central nervous system
CON	control group
CR	calorie restriction
CREB	cyclic AMP responsive element
CRH	corticotropin-releasing hormone
C_t	cycle threshold
CTCF	CCTC-binding factor
DBP	diastolic blood pressure
DMR	differentially methylated region
DNA	deoxyribonucleic acid
DNMT1	DNA methyl transferase 1
DNMT3a	DNA methyl transferase 3a
DNMT3b	DNA methyl transferase 3b
DOHaD	developmental origins of health and disease

Dusp5	dual specificity phosphatase 5
En	endodermal enhancer
EPM	elevated plus maze
Erk	extracellular signal-regulated kinase
Gcr	glucocorticoid receptor
HBW	high birth weight
hMC	homogenous MassCLEAVE base-specific cleavage
HPA	hypothalamic-pituitary-adrenal
Hprt	hypoxanthine guanine phosphoribosyl transferase
Hsd11β1	11β-hydroxysteroid dehydrogenase type 1
Hsd11β2	11β-hydroxysteroid dehydrogenase type 2
Igf2	insulin-like growth factor 2
IPGTT	intraperitoneal glucose tolerance test
IUGR	intrauterine growth restriction
LBW	low birth weight
LC	liquid chromatography
LDP	long term depression
LPD	low protein diet
LTP	long term potentiation
MALDI TOF	matrix assisted laser desorption/ionization time-of-flight
MAPK	mitogen-activated protein kinase
MAR3	matrix attachment region 3

MD	methyl deficient
MLP	maternal low protein
mRNA	messenger RNA
MS	mass spectrometry
NBW	normal birth weight
ncRNA	non-coding RNA
Nnat	neuronatin
PCR	polymerase chain reaction
PPAR-α	peroxisome proliferator activated receptor-α
PPAR-γ	peroxisome proliferator activated receptor-γ
QGE	quantitative gene expression
QPCR	quantitative reverse transcriptase real-time PCR
RAS	renin-angiotensin-system
RELN	reelin
RNA	ribonucleic acid
RT	room temperature
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
SBP	systolic blood pressure
SGA	small for gestational age
TFEB	transcription factor EB