GENOME DAMAGE AND FOLATE NUTRIGENOMICS IN UTEROPLACENTAL INSUFFICIENCY

Denise Lyndal Fleur Furness

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BSc (Honours)

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University of Adelaide, School of Health Sciences, Discipline of Obstetrics and Gynaecology
AND
CSIRO Human Nutrition, Genome Health and Nutrigenomics Laboratory

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This Thesis is
Dedicated to My Mother

Jann Cheryl Furness

All Religions, Arts And Sciences Are Branches Of The Same Tree. All These Aspirations Are Directed Toward Ennobling Man's Life, Lifting It From The Sphere Of Mere Physical Existence And Leading The Individual Towards Freedom.

ALBERT EINSTEIN

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1 ABSTRACT

Pregnancy complications associated with placental development affect approximately one third of all human pregnancies. Genome health is essential for placental and fetal development, as DNA damage can lead to pregnancy loss and developmental defects. During this developmental phase rapid DNA replication provides an increased opportunity for genome and epigenome damage to occur[1].

Maternal nutrition is one of the principal environmental factors supporting the high rate of cell proliferation and differentiation. Folate functions in one-carbon metabolism and regulates DNA synthesis, DNA repair and gene expression[1]. Deficiencies or defects in gene-nutrient interactions associated with one-carbon metabolism can lead to inhibition of cell division, cell cycle delay and an excessive apoptotic or necrotic cell death rate[2], which may affect placentation.

This study is the first to investigate the association between genomic damage biomarkers in late pregnancy complications associated with uteroplacental insufficiency (UPI) including preeclampsia and intrauterine growth restriction (IUGR). The results indicate that genome damage in the form of micronucleated cells in peripheral blood lymphocytes at 20 weeks gestation is significantly increased in women at risk of developing an adverse pregnancy outcome. The observed OR for the high micronuclei frequency may be the highest observed for any biomarker selected in relation to risk of pregnancy complications to date (15.6 – 33.0). In addition, reduced apoptosis was observed in association with increased micronuclei, suggesting that the cells may have escaped specific cell-cycle checkpoints allowing a cell with DNA damage to proceed through mitosis.

This study demonstrated that an increase in plasma homocysteine concentration at 20 weeks gestation is associated prospectively with the subsequent development of UPI, indicating a causal relationship. The *MTR* 2756 GG genotype was significantly associated with increased plasma homocysteine concentration and UPI. Furthermore, the *MTHFD1* 1958 single nucleotide polymorphism was associated with increased risk for IUGR.

2 DECLARATION

This work contains no material which has been submitted for the award of any other degree or diploma in any University or other tertiary institution, and to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University of Adelaide Library, being made available in all forms of media, now and hereafter known.

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Denise Furness

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4 ABBREVIATIONS

AST: Aspartate amino-transferase

B_{12}: Vitamin-B_{12} (cobalamin)
B_6: Vitamin-B_6
BN: Binucleate cell
BMI: Body mass index

CBMN: Cytokinesis-block micronucleus assay
$CH_{3:}$ Methyl group
CpG: Cytosine-guanine dinucleotide

DHF: Dihydrofolate
DNA: Deoxyribonucleic acid
Dnmt: DNA methyltransferase genes

FBP: Folate binding protein

$_d$H20: Distilled water
Hcy: Homocysteine
HUMN: The international collaborative project on micronucleus frequency in human populations

IL-8: Interleukin 8
IUGR: Intrauterine growth restriction
(ICF) Immunodeficiency centromeric region instability and facial anomalies syndrome

MCP-1: Monocyte chemoattractant protein 1
MeCP2: Methyl CpG binding protein 2
mCyt: Methylated cytosines
MN: Miconuclei
MN-BN(s): Micronucleated binucleate cell(s)
MTHFR: Methylenetetrahydrofolate reductase
MTHFD1: Methylenetetrahydrofolate dyhydrogenase

MTR: Methionine synthase
MTRR: Methionine synthase reductase

NBUD: Nuclear bud
NBUD-BN(s): binucleated cells with nuclear bud(s)
NDI: Nuclear division index
NPB(s): Nucleoplasmic bridge(s)
NPB-BN(s): Binucleated cells with nucleoplasmic bridge(s)
NTDs: Neural tube defects
NO: Nitric oxide

PBS: Phosphate buffered saline
PE: Preeclampsia
PlGF: Placental growth factor
PLP: Pyridoxal 5-phosphate

RBC: Red blood cell
RCF: Red cell folate
RDI: Recommended daily intake

SAM: S-adenosylmethionine
SF: Serum folate
SGA: Small for gestational age
sFlt-1: Soluble fms-like tyrosine kinase-1
SNPs: Single nucleotide polymorphisms

dTMP: Thymine
THF: Tetrahydrofolate;

dUMP: Uracil
UPI: Uteroplacental insufficiency

VEGF: Vascular endothelial growth factor

5 PUBLICATIONS

Furness DL., Fenech MF., Khong TY., Hague WM., Dekker GA**.** *Evaluation of the use of the CBMN assay to determine inter-individual variation in spontaneous and folate deficiency-induced genome damage in humans.* Proc Nutr Soc Aust 2004, Vol. 28. Asia Pacific Journal of Clinical Nutrition, 2004; 13 (Suppl):S56 - Abstract

Furness D., Dekker G., Khong Y., Hague B., Fenech M. *The Role of Genome Damage and Nutrigenomics in Uteroplacental Insufficiency.* American Journal of Obstetrics and Gynecology, 2006, Dec; 195(6):S14 - Abstract

Furness D., Parange N., Dekker G., Fenech M. (2006) *Role of Genome Damage and Uterine Artery Doppler in Prediction of Uteroplacental Insufficiency*. American Journal of Obstetrics and Gynecology, 2006, Dec; 195(6):S221 - Abstract

Parange N., **Furness D.,** Fenech M., Wilkinson C., Dekker G. *Role of uterine artery doppler and the folate metabolic pathway in prediction of uteroplacental insufficiency.* American Journal of Obstetrics and Gynecology, 2006, Volume 195, Issue 6, Pages S207-S207-Abstract