THE ROLE OF FOLIC ACID RELATED NUTRITIONAL GENETICS IN COMMON CHRONIC DEGENERATIVE DISORDERS

By

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Statement of Originality

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

However, I acknowledge that the work embodied in this thesis has been done in collaboration with other researchers and has been carried out in part at other institutions. Where necessary, I have indicated within the thesis the extent and type of collaboration, and acknowledged the contributing parties.

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Abbreviations

^{0}C	Degree Celsius
$\overline{x}$	Mean
AD	Alzheimer's disease
ADHD	Attention Deficit Hyperactivity Disorder
AIHW	Australian Institute of Health and Welfare
ANOVA	Analysis of variance
AUD	Australian Dollars
BMI	Body Mass Index
bp	Base pairs
COMT	Catechol-*O*-methyltransferase
CpG	Cytosine-Guanine
CV	Coefficient of variation
CVD	Cardiovascular disease
CβS	Cystathionine β-Synthase
CγL	Cystathionine-γ-lyase
DHFR	Dihydrofolate Reductase
DNA	Deoxyribonucleic acid
dNTPs	deoxyribonucleoside triphosphate
dTMP	deoxythymidine monophosphate
dUMP	deoxyuridine monophosphate
EDTA	Ethylenediaminetetra-acetic acid
eNOS	endothelial nitric oxide synthase
FAD	Flavin adenine dinucleotide
FFQ	Food Frequency Questionnaire
FMN	Flavin mononucleotide
FSANZ	Food Standards Australia New Zealand
GWAS	Genome-wide association studies
GCPII	Glutamate carboxypeptidase II
H_2PteGlu	Dihydrofolate
H_4PteGlu	Tetrahydrofolate
HADS	Hospital Anxiety Depression Scale/Score
HDL	High Density Lipoprotein
Het	Heterozygote
HPLC	High-performance liquid chromatography
ICPMR	Institute of Clinical Pathology and Medical Research
IVF	*In vitro* fertilisation
LDL	Low Density Lipoprotein
MMSE	Mini mental State Examination
mRNA	Messenger Ribonucleic Acid
MTHFR	Methylenetetrahydrofolate Reductase
MTR	Methionine Synthase
MTRR	Methionine Synthase Reductase
n	Number

NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NHANES	National Health and Nutrition Examination Survey
NHMRC	National Health and Medical Research Council
NMDA	*N*-methyl-d-aspartate
NSW	New South Wales, Australia
NTD	Neural Tube Defects
OR	Odds Ratio
PCFT	Proton Coupled Folate Transporter
PCR	Polymerase Chain Reaction
PLP	Pyridoxal 5' Phosphate
PUFA	Polyunsaturated Fatty Acid
RDI	Recommended Daily Intake
Rec	Recessive
RFC	Reduced Folate Carrier
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
SAH	*S*-adenosylhomocysteine
SAM	S-adenosylmethionine
SBDF	7-Fluorobenzo-2-oxa-1,3-diazole-4-sulfonic acid ammonium salt
SD	Standard deviation
SHMT	Serine hydroxymethyltransferase
SNP	Single Nucleotide Polymorphism
TBE	Tris/Borate/EDTA
TCEP	Tris(2-carboxyethyl)phosphine
TS	Thymidylate synthase
TSER	Thymidylate synthase enhancer region
UK	United Kingdom
US	United States
USD	United States Dollars
UV	Ultra Violet
VIC	Victoria, Australia
WHO	World Health Organisation
Wt	Wild-type

Synopsis

Nutrition has long been recognised as having a significant impact on health. In developed countries, there has been a shift away from prevention of overt nutrient deficiency diseases to emphasis on preventing the health complications of nutritional excess. The contemporary burden of chronic disease, in both developed and developing nations, is increasing as society ages and is linked to dietary elements, genetic susceptibility and environmental change. Today's populations largely consume energy-dense nutrient-poor foods, an important component in our contemporary obesogenic environment. This type of diet is often low in essential micronutrients, particularly important B-group vitamins linked to the prevention of a range of chronic diseases.

Folic acid nutritional genetics, the subject of this thesis, influences a broad sphere of clinical conditions. Folic acid has a central role in one-carbon metabolism, a complex nexus responsible for donating methyl units vital for both nucleotide synthesis and provision of *S*-adenosylmethionine. Moderate folate deficiency induces DNA hypomethylation, and via uracil misincorporation, DNA instability; both events are linked to increased cancer risk. Folate deficiency is also associated with potentially vasculo-toxic homocysteine, which accumulates when there is a limited pool of folic acid derived methyl groups. Elevated homocysteine is associated with a range of disorders, most notably increased CVD risk and NTDs. Folate-related one-carbon metabolism contains various polymorphic proteins that modify metabolism and therefore influence disease risk. This dissertation examines four different, common, chronic degenerative disorders that predominately affect ageing populations, with the aim of exploring the relationship between eleven common folate polymorphisms, important indices of folate status, and transsulphuration pathway thiols. This approach employed regression models based on the *a priori* understanding of possible biochemical, genetic and physiologic relationships. The following reflects what are considered to be the major findings of this study.

An examination of hypertension in an elderly retirement village population (n=229) demonstrated that red cell folate, cysteine and cysteinyl-glycine were predictive of recumbent diastolic blood pressure (*p=0.0326*, r^2*=0.0202, slope estimate=-0.040; p=0.0001*, r^2*=01246, slope estimate=-0.232; p=0.0008*, r^2*=01246, slope estimate=0.141* respectively). As a component within a model containing key genetic factors, the 677C>T MTHFR SNP was associated with recumbent diastolic blood pressure (*p=0.0397*, r^2*=0.0650, slope estimate=-0.011*). Several folate-related SNPs were associated with standing systolic blood pressure (*r^2=0.0868* for whole model); these were the 677C>T MTHFR (*p=0.0443, slope estimate=-0.009*), the 19 bp deletion DHFR (*p=0.0157, slope estimate=0.009)* and the 1561C>T GCPII *(p=0.0397, slope estimate=-0.021*) variants. An examination of the depression phenotype was undertaken in this same population. It was shown that a novel relationship exists with the amino-thiol, cysteinyl-glycine, which was negatively associated with depression (*p=0.0046, r^2=0.0348, slope estimate=-6.127*).

The third clinical phenotype examined involved a cohort of AD patients (n=93), which was compared to the former retirement village population as a control after selecting subjects whose MMSE score reflected a specified threshold for cognitive function (n=229). The 2756A>G MTR SNP was associated with AD (*p=0.0419, r^2=0.0512*), with the G allele considered to be protective (OR=0.60:95%CI;0.39-0.92, *p=0.0260*). An ordinal logistic regression model containing all thiols (*r^2=0.1885*) indicated that higher homocysteine (*p=<0.0001*), higher glutathione (*p=0.0003*) but lower cysteinyl-glycine (*p=<0.0001*) was significantly associated with AD. Ordinal logistic regression also supported the association of AD with lower serum folate (*p=0.0097*, *r^2=0.0181*), lower total dietary folate intake (*p=0.0054, r^2=0.0231,*) and lower natural methylfolate intake (*p=<0.0001, r^2=0.0581*).

The final phenotype examined involved a cohort of subjects screened for colorectal polyps (n=203). The study had a specific focus on adenomatous polyp occurrence and its possible relationship to folate intake. The 3'UTR 6 deletion TS SNP indicated an association with increased risk for an adenomatous polyp occurrence (*p=0.0073*, *r^2=0.2744*). The 66A>G MTRR SNP was also found to be a positive risk factor for an adenomatous polyp (OR=2.50:95%CI;1.23-5.10, *p=0.0163,* ordinal logistic regression, *p=0.0149*, *r^2=0.2744*). This latter SNP was also associated with adenomatous polyp occurrence in subjects with low folate status (below median red cell folate, OR=3.40:95%CI; 1.32-8.75, *p=0.0164*, ordinal logistic regression, *p=0.0261*, *r^2=0.5799*). In subjects with a high folate status, the 1420C>T SHMT SNP was a positive risk factor (OR=4.56:95%CI; 1.38-15.03, *p=0.0225*). Individuals with a low folate status were also found to have red cell folate levels that predicted adenomatous polyp occurrence (ordinal logistic regression *p=0.0331, r^2=0.0548*). Whilst this study has identified various potential associations, the nature of the data and associations found, advocates further examination in larger populations.

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Acknowledgment of collaboration

I hereby certify that the work embodied in this thesis has been done in collaboration with other researchers, or carried out in other institutions. I have included as part of the thesis this statement clearly outlining the extent of collaboration, with whom and under what auspices.

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PhD Candidate

CHAPTER 3: B-VITAMIN NUTRITIONAL GENETICS IN THE ELDERLY - A DETAILED STUDY OF HYPERTENSIVE AND DEPRESSIVE PHENOTYPES

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Acknowledgment of Authorship

I hereby certify that the work embodied in this thesis contains published paper/s/scholarly work of which I am a joint author. I have included this written statement as part of the thesis, which attests to my contribution to the joint publication/s/scholarly work and is endorsed by my supervisor

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PhD Candidate Principal Supervisor

Journal Papers

LUCOCK, M., YATES, Z., MARTIN, C., CHOI, J. H., **BOYD, L**., TANG, S., NAUMOVSKI, N., ROACH, P. & VEYSEY, M. 2013. Hydrogen sulphide-related thiol metabolism and nutrigenetics in relation to hypertension in an elderly population. *Genes & Nutrition***,** Vol 8, 2:221-229.

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