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

By

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A thesis submitted for the degree of

Doctor of Philosophy, Food Science

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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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<thead>
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<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>( \bar{x} )</td>
<td>Mean</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer's disease</td>
</tr>
<tr>
<td>ADHD</td>
<td>Attention Deficit Hyperactivity Disorder</td>
</tr>
<tr>
<td>AIHW</td>
<td>Australian Institute of Health and Welfare</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AUD</td>
<td>Australian Dollars</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>bp</td>
<td>Base pairs</td>
</tr>
<tr>
<td>COMT</td>
<td>Catechol-O-methyltransferase</td>
</tr>
<tr>
<td>CpG</td>
<td>Cytosine-Guanine</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>CβS</td>
<td>Cystathionine β-Synthase</td>
</tr>
<tr>
<td>CγL</td>
<td>Cystathionine-γ-lyase</td>
</tr>
<tr>
<td>DHFR</td>
<td>Dihydrofolate Reductase</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>dNTPs</td>
<td>deoxyribonucleoside triphosphate</td>
</tr>
<tr>
<td>dTMP</td>
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</tr>
<tr>
<td>dUMP</td>
<td>deoxyuridine monophosphate</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetra-acetic acid</td>
</tr>
<tr>
<td>eNOS</td>
<td>endothelial nitric oxide synthase</td>
</tr>
<tr>
<td>FAD</td>
<td>Flavin adenine dinucleotide</td>
</tr>
<tr>
<td>FFQ</td>
<td>Food Frequency Questionnaire</td>
</tr>
<tr>
<td>FMN</td>
<td>Flavin mononucleotide</td>
</tr>
<tr>
<td>FSANZ</td>
<td>Food Standards Australia New Zealand</td>
</tr>
<tr>
<td>GWAS</td>
<td>Genome-wide association studies</td>
</tr>
<tr>
<td>GCPII</td>
<td>Glutamate carboxypeptidase II</td>
</tr>
<tr>
<td>H₂PteGlu</td>
<td>Dihydrofolate</td>
</tr>
<tr>
<td>H₄PteGlu</td>
<td>Tetrahydrofolate</td>
</tr>
<tr>
<td>HADS</td>
<td>Hospital Anxiety Depression Scale/Score</td>
</tr>
<tr>
<td>HDL</td>
<td>High Density Lipoprotein</td>
</tr>
<tr>
<td>Het</td>
<td>Heterozygote</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>ICPMR</td>
<td>Institute of Clinical Pathology and Medical Research</td>
</tr>
<tr>
<td>IVF</td>
<td>In vitro fertilisation</td>
</tr>
<tr>
<td>LDL</td>
<td>Low Density Lipoprotein</td>
</tr>
<tr>
<td>MMSE</td>
<td>Mini mental State Examination</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger Ribonucleic Acid</td>
</tr>
<tr>
<td>MTHFR</td>
<td>Methylene-tetrahydrofolate Reductase</td>
</tr>
<tr>
<td>MTR</td>
<td>Methionine Synthase</td>
</tr>
<tr>
<td>MTRR</td>
<td>Methionine Synthase Reductase</td>
</tr>
<tr>
<td>( n )</td>
<td>Number</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide Adenine Dinucleotide Phosphate</td>
</tr>
<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>NHMRC</td>
<td>National Health and Medical Research Council</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-d-aspartate</td>
</tr>
<tr>
<td>NSW</td>
<td>New South Wales, Australia</td>
</tr>
<tr>
<td>NTD</td>
<td>Neural Tube Defects</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>PCFT</td>
<td>Proton Coupled Folate Transporter</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PLP</td>
<td>Pyridoxal 5’ Phosphate</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated Fatty Acid</td>
</tr>
<tr>
<td>RDI</td>
<td>Recommended Daily Intake</td>
</tr>
<tr>
<td>Rec</td>
<td>Recessive</td>
</tr>
<tr>
<td>RFC</td>
<td>Reduced Folate Carrier</td>
</tr>
<tr>
<td>RFLP</td>
<td>Restriction Fragment Length Polymorphism</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
</tr>
<tr>
<td>SAH</td>
<td>S-adenosylhomocysteine</td>
</tr>
<tr>
<td>SAM</td>
<td>S-adenosylmethionine</td>
</tr>
<tr>
<td>SBDF</td>
<td>7-Fluorobenzo-2-oxa-1,3-diazole-4-sulfonic acid ammonium salt</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SHMT</td>
<td>Serine hydroxymethyltransferase</td>
</tr>
<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
</tr>
<tr>
<td>TBE</td>
<td>Tris/Borate/EDTA</td>
</tr>
<tr>
<td>TCEP</td>
<td>Tris(2-carboxyethyl)phosphine</td>
</tr>
<tr>
<td>TS</td>
<td>Thymidylate synthase</td>
</tr>
<tr>
<td>TSER</td>
<td>Thymidylate synthase enhancer region</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
</tr>
<tr>
<td>USD</td>
<td>United States Dollars</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra Violet</td>
</tr>
<tr>
<td>VIC</td>
<td>Victoria, Australia</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>Wt</td>
<td>Wild-type</td>
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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; $p=0.0001$, $r^2=0.01246$, slope estimate=-0.232; $p=0.0008$, $r^2=0.01246$, 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 \((p=0.0443, \text{slope estimate}=0.009)\), the 19 bp deletion DHFR \((p=0.0157, \text{slope estimate}=0.009)\) and the 1561C>T GCPII \((p=0.0397, \text{slope estimate}=-0.021)\) 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 \((p=0.0046, r^2=0.0348, \text{slope estimate}=-6.127)\).

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 \((\text{OR}=0.60:95\%\text{CI};0.39-0.92, p=0.0260)\). 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 \((\text{OR}=2.50:95\%\text{CI};1.23-5.10, p=0.0163, \text{ordinal logistic regression}, p=0.0149, r^2=0.2744)\). This latter SNP was also associated with adenomatous polyp occurrence in subjects with low folate status \((\text{below median red cell folate, OR}=3.40:95\%\text{CI};\ 1.32-8.75, p=0.0164, \text{ordinal logistic regression}, p=0.0261, r^2=0.5799)\). In subjects with a high folate status, the 1420C>T SHMT SNP was a positive risk factor \((\text{OR}=4.56:95\%\text{CI};\ 1.38-15.03, p=0.0225)\). Individuals with a low folate status were also found to have red cell folate levels that predicted adenomatous polyp occurrence \((\text{ordinal logistic regression } p=0.0331, r^2=0.0548)\). 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.

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.

PhD Candidate  Principal Supervisor

Journal Papers


