Changes in buccal cytome biomarkers in relation to ageing and Alzheimer's disease.

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DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or 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 my consent to this thesis, when deposited in the University library, being available for photocopy or loan.

Philip Thomas

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ABSTRACT

The aim of this thesis was to investigate the possibility of using buccal cells derived from a multi layered epithelial tissue from the oral mucosa as a model to identify potential biomarkers of genomic instability in relation to normal ageing and premature ageing syndromes such as AD and DS. A buccal micronucleus cytome assay was developed and used to investigate biomarkers for DNA damage, cell proliferation and cell death in healthy young, healthy old and young Down's syndrome cohorts. Cells with micronuclei, karyorrhectic cells, condensed chromatin cells and basal cells increased significantly with normal ageing ($P<0.0001$). Cells with micronuclei and binucleated cells increased ($P<0.0001$) and condensed chromatin, karyorrhectic, karyolytic and pyknotic cells decreased ($P<0.002$) significantly in Down's syndrome relative to young controls.

The buccal micronucleus cytome assay was used to measure ratios of buccal cell populations and micronuclei in clinically diagnosed Alzheimer's patients compared to age and gender matched controls. Frequencies of basal cells ($P<0.0001$), condensed chromatin cells ($P<0.0001$) and karyorrhectic cells ($P<0.0001$) were found to be significantly lower in Alzheimer's patients, possibly reflecting changes in the cellular kinetics or structural profile of the buccal mucosa.

Changes in telomere length were investigated using a quantitative RTm-PCR method to measure absolute telomere length (in Kb per diploid genome) and show age-related changes in white blood cells and buccal cell telomere length (in kb per diploid genome) in normal healthy individuals and Alzheimer's patients. We observed a

significantly lower telomere length in white blood cells (P<0.0001) and buccal cells (P<0.01) in Alzheimer's patients relative to healthy age-matched controls (31.4% and 32.3% respectively). However, there was a significantly greater telomere length in hippocampus cells of Alzheimer's brains (P=0.01) compared to control samples (49.0

Buccal cells were also used to investigate chromosome 17 and 21 aneuploidy. A 1.5 fold increase in trisomy 21 (P<0.001) and a 1.2 fold increase in trisomy 17 (P<0.001) was observed in buccal cells of Alzheimer's patients compared to age and gender matched controls. Chromosome 17 and chromosome 21 monosomy and trisomy increase significantly with age (P<0.001). Down's syndrome, which exhibits similar neuropathological features to those observed in Alzheimer's disease also showed a strong increase in chromosome 17 monosomy and trisomy compared to matched controls (P<0.001). However, aneuploidy rate for chromosome 17 and 21 in the nuclei of hippocampus cells of brains from Alzheimer's patients and controls were not significantly different.

Observations that AD individuals have altered plasma folate, B12 and Hcy levels compared to age-matched controls who have not been clinically diagnosed with AD were investigated. Genotyping studies were undertaken to determine whether polymorphisms within particular genes of the folate methionine pathway contributed to AD pathogenesis. Correlations between folate, B12 and Hcy status with previously determined buccal micronucleus assay cytome biomarkers for DNA damage, cell proliferation and cell death markers was investigated.

Lastly, the potential protective effects of phytonutrient polyphenols on genomic instability events in a transgenic mouse model for AD were investigated. We

determined the effects of curcumin and GSE polyphenols on DNA damage by testing the mice over a 9 month period utilizing a buccal micronucleus cytome assay, an erythrocyte micronucleus assay and measuring telomere length in both buccal cells and olfactory lobe brain tissue.

PUBLICATIONS ARISING FROM THESIS

1. **Chapter 1**: Philip Thomas and Michael Fenech
   A review of genome mutation and Alzheimer's disease.
   Mutagenesis vol. 22 no. 1 pp. 15–33, 2007.

2. **Chapter 2**: Philip Thomas, Sarah Harvey, Tini Gruner and Michael Fenech
   The buccal cytome and micronucleus frequency is substantially altered in Down's syndrome and normal ageing compared to young healthy controls
   Mutation Research Fundamental and Molecular Mechanisms of Mutagenesis 2007; doi:10.1016/j.mrfmmm.2007.08.012MUT10526 (*In press*)

3. **Chapter 3**: Philip Thomas, Jane Hecker, Jeffrey Faunt and Michael Fenech
   Buccal cytome biomarkers may be associated with Alzheimer's disease
   Mutagenesis 2007; doi: 10.1093/mutage/gem029Mutagenesis (*In press*)

4. **Chapter 4**: Philip Thomas, Nathan O'Callaghan and Michael Fenech
   Telomere length in white blood cells, buccal cells and brain tissue and it's variation with ageing and Alzheimer's disease
   Mechanisms of ageing and development (*In press)*

5. **Chapter 5**: Philip Thomas and Michael Fenech
   Chromosome 17 and 21 aneuploidy in buccal cells is increased with ageing and in Alzheimer's disease.
   Mutagenesis *(In press)*

ABBREVIATIONS

ACT	Alpha-1-antichymotrypsin
ALT	Alternative lengthening of telomere
AD	Alzheimer's disease
ANOVA	Analysis of variance
APOE	Apolipoprotein E
APOE4	Apolipoprotein E allele 4
APP	Amyloid precursor protein
β amyloid 42	42 amino acid β amyloid peptide
BACE	Beta site APP cleaving enzyme
BFB	Breakage fusion bridge
BC	Buccal cell
BSA	Bovine serum albumin
CAT	Catalase
CBS	Cystathionine β synthase
CSIRO	Commonwealth Scientific and Industrial Research organisation
C_T	Cycle threshold
Cu^{2+}	Copper
DABCO	1, 4-diazabicyclo-(222) octane
DAPI	4, 6 diamidino-2-phenylindole
dATP	2'-deoxyadenosine 5'-triphosphate
dCTP	2'-deoxycytidine 5'-triphosphate
dGTP	2'-deoxyguanosine 5'-triphosphate
dNTP	Deoxyribonucleotide triphosphate
DNA	Deoxyribo nucleic acid

DS	Down’s syndrome
dTMP	Deoxythymidine monophosphate
DTT	Dithiothreitol
dTTP	2’-deoxythymidine 5’-triphosphate
dUMP	Deoxyuracil monophosphate
EDTA	Ethylenediamine tetraacetic acid
EGCG	Epigallocatechin-3-gallate
FAD	Familial Alzheimer’s disease
Fe^{2+}	Iron
FBP	Folate binding protein
FISH	Fluorescence in situ hydridisation
FITC	Fluorescein isothiocyanate
GPx	Glutathione peroxidase
GSE	Grape Seed extract
HCL	Hydrochloric acid
Hcy	Homocysteine
HO-1	Heme oxygenase-1
8-OHdG	8 hydroxy-2-deoxyguanosine
Kb	kilobase
KOH	Potassium hydroxide
MCI	Mild cognitive impairment
MDS	Mothers of Down’s syndrome
$MgCL_2$	Magnesium chloride
MGSE	Microencapsulated Grape Seed Extract
MPO	Myeloperoxidase

MMSE	Mini Mental State Exam
MN	Micronuclei
MN-NCE	Micronucleated non polychromatic erythrocyte
MN-PCE	Micronucleated polychromatic erythrocyte
Mo/HuAPP695	transgenic mouse secreting 695 amino acid APP peptide
MRI	Magnetic Resonance Imaging
MTHFR	Methylenetetrahydrofolate reductase
MTR	Methionine synthase
MTRR	Methionine synthase reductase
NAD	Nicotinamide adenine dinucleotide
NINCDS-AD&DA	National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's disease and related disorders association
PBS	Phosphate buffered saline
PCE	Polychromatic erythrocyte
PSEN1	Presenilin 1
PSEN1-dE9	Deletion in exon 9 of presenilin gene
PSEN2	Presenilin 2
ROS	Reactive oxygen species
RLU	Relative light units
RTm-PCR	Real time polymerase chain reaction
SAH	S-Adenosylhomocysteine
SAM	S-Adenosyl Methionine
SNP	Single nucleotide polymorphism
SOD	Superoxide Dismutase
SSC	Standard saline citrate

TERT	Telomerase
WBC	White blood cells
Zn^{2+}	Zinc