MUTAGENIC PREDISPOSITION IN GENES IMPLICATED IN ALZHEIMER'S DISEASE

A dissertation submitted to the Faculty of Health Sciences, Technikon Witwatersrand, in fulfilment of the requirements for the degree of Magister Technologiae in the programme Biomedical Technology

By

Mandla Mlotshwa

(Student no. 9741493)

Supervisor: _____ _____

Dr H. Abrahamse Date

Co-supervisor: _____ _____

Mrs J.V. Hind Date

Johannesburg, 2004

DECLARATION

I declare that this dissertation is my own unaided work. It is submitted for the Magister Technologiae Degree at the Technikon Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other Technikon or University.

Signature_____ Date_____

ABSTRACT

Alzheimer's disease is the most common cause of late-life dementia and the fourth leading cause of death in the developed world. The aetiology of AD has not yet been resolved. It has been suggested that AD could result from multifactorial process involving both a genetic predisposition and an exposure to environmental factors modulated by the biological aging process. To date, epidemiological and molecular genetic data have led to the identification of three genes, amyloid precursor protein (APP), presenilin 1 (PS1) and presenilin 2 (PS2) genes, which, when mutated, can cause an early onset form of AD. Genetic linkage studies and association studies have also shown that the ε4 allele of the apolipoprotein E gene increases risk for AD in a dose dependent manner in both early onset and late onset AD. Recently, it has also been suggested that environmental factors may interact with a genetic predisposition to modify the risk of AD. Extensive research is underway to identify environmental and genetic risk factors for this complex disease. Over 40 genes have been tested as AD candidates yet none has been clearly established as an AD risk factor. Currently scientists are investigating the interrelationship between various gene loci and how environmental factors could affect an individual's susceptibility to AD.

This study evaluated the genotoxicity of environmental agents such as hydrogen peroxide, cadmium chloride and γ radiation induced oxidative DNA damage in lymphocytes and within specific DNA sequences of APP (exon 15-18) and PS1 (exon 3-12) genes of AD patients and age-matched control subjects. As indicators of oxidative DNA damage, the frequencies of DNA strand breaks, oxidized pyrimidines and altered purines was assessed using the alkaline Comet assay modified with lesion-specific endonucleases, endo-III and fpg; and fluorescence *in situ* hybridisation.
The number of APP and PS1 hybridisation spots per comet were used as an indicator of the extent of damage. The location of the hybridisation spots in the head or tail of the comet were recorded to further determine

whether the gene of interest lies within or in the vicinity of a damaged region of DNA. With the alkaline Comet assay modified with endo-III and fpg, it was demonstrated that patients with AD had significantly increased levels of DNA strand breaks, oxidized pyrimidines and altered purines induced by hydrogen peroxide, cadmium chloride and γ radiation compared with control subjects ($p<0.05$). This was further confirmed by the fluorescence *in situ* hybridisation modification of the alkaline Comet assay by demonstrating a significant increase in the mean number of APP and PS1 gene hybridisation spots per comet in AD patients compared with control subjects. Moreover, the gene sensitivity index of APP and PS1 to hydrogen peroxide, cadmium chloride and γ radiation were found to be higher in AD patients than in control subjects.

Taken together, our results suggest (i) that lymphocytes from patients with AD are sensitive to these environmental genotoxic agents and (ii) there was an overall increase in the mean number and sensitivity index of APP and PS1 genes to environmental genotoxic agents which might link a genetic cause to oxidative stress in peripheral cells of AD patients than in control subjects. Although the mechanisms by which these environmental agents induced oxidative DNA damage remained to be elucidated, our data suggest that increased oxidative stress is an inherent property of cells carrying genes associated with AD.

In the memory of my father

Micah Mlotshwa

1946-2003

and

My brothers

Nhlanhla Mlotshwa

1974-2002

and

Bongani Mlotshwa

1979-2004

ACKNOWLEDGEMENTS

The present study was carried out in the Department of Biomedical Technology, School of BioSciences, Faculty of Health Sciences, Technikon Witwatersrand during the years 2002-2004.

I am greatly indebted to Dr H. Abrahamse, Mrs J.V. Hind, Mr N.H. de villiers and the official reviewers of this dissertation for their friendly advice, criticism and suggestions to improve the final manuscript.

I owe my dearest thanks to my mother, Thembekile Khumalo, my sister, Bongekile and my girlfriend, Brenda Mngomezulu for their support, encouragement and understanding attitude during these years.

I also thank my colleagues, Netty Malatsi and Chantel Buikhuizen for friendship during my years of studentship.

I would also like to Nicolette Houreld for collecting blood samples for this study.

I also thank Dr M. Connor at the Neurological Units, Johannesburg Hospital for making it possible for us to gain contact with Alzheimer's disease patients.

I also thank the Alzheimer's disease patients and control subjects who participated in this study. Without them this study would not have been possible.

This study was supported in part by grants from the Technikon Witwatersrand Research Committee and the National Research Foundation (NRF).

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS

α: Alpha

β: Beta

ε: Epsilon

γ: Gamma

μ: Micro

$CdCl_2$: Cadmium chloride

^{60}Co: ^{60}Cobalt

O_2: Oxygen

$^{\circ}O_2^-$: Superoxide

$ONOO^-$: peroxynitrite anion

Fe: Iron

KCN: Potassium cyanide

NaOH: Sodium hydroxide

NaCl: Sodium chloride

Na_2EDTA: Di-sodium ethylene diamine tetra-acetic acid

KCl: Potassium chloride

KOH: Potassium hydroxide

$^{\circ}OH$: Hydroxyl radical

HO_2°: hydroperoxyl radical

H_2O_2: Hydrogen peroxide

L°: Peroxyl radical

LOO°: Lipid hydroperoxide

NO: Nitric oxide

LIST OF ABBREVIATIONS

Aβ: Amyloid beta peptide

AD: Alzheimer's disease

ADMDB: Alzheimer's disease mutation database

ACT: Alpha-1- antichymotrypsin

A2M: Alpha-2-macroglobulin

ANOVA: Analysis of variable

APP: Beta amyloid precursor protein

APEs: Apurinic/Apyrimidinic endonucleases

ApoE: Apolipoprotein E

Asp: Aspartic acid

CO: Cytochrome c oxidase

CSF: Cerebrospinal fluid

Cu/Zn-SOD: Copper/Zinc superoxide dismutase

DAPI: 4',6-diamine-2-phenylindol dihydrochloride

DBS: Double stranded breaks

DNA: Deoxyribose nucleic acid

DMSO: Dimethylsulfoxide

E. coli: *Escherichia coli*

Endo III: Endonuclease III

FAD: Familial Alzheimer's disease

Fapy-Ade: 4,6-diamino-5-formamidopyrimidine

Fapy-Gua: 2,6-diamino-4-hydroxy-5-formamidopyrimidine

FISH: Fluorescence *in situ* hybridisation

FITC: Fluorescein

Fpg: Formamidopyrimidine glycosylase

Gl: Glutamic acid

γGCS: Gamma glutamylcysteine synthetase

G-6-PD: Glucose-6-phoshate dehydrogenase

GPx: Glutathione peroxide

GRd: Glutathione reductase

GSH: Reduced glutathione

GSSG: Oxidized glutathione

GST: Glutathione-S-transferase

HLA: Human leukocyte antigen

HCHWA-D: Hereditary cerebral haemorrhage with amyloidosis-Dutch type

HNTH1: Human homologue of *E. coli* Endo III

IDE: Insulin degrading enzyme

IL: Interleukin

IFN: Interferon

Ile: Isoleucine

Leu: Leucine

LMP: Low melting point

LRP: Low-density lipoprotein receptor related gene

Lys: Lysine

Met: Methionine

Mn-SOD: Manganese-superoxide dismutase

$NADP^+$: Nicotinamide Adenine dinucleotide phosphate oxidized form

NADPH: Nicotinamide Adenine dinucleotide phosphate reduced form

NMP: Normal melting point

NOS: Nitric oxide synthetase

OGG1: 8-oxyguanine-DNA glycosylase

hOGG1: human 8-oxyguanine-DNA glycosylase

8-oxo-guanine: 8-hydroxyguanine

8-oxo-adenine: 7,8-dihydroxy-8-oxoadenine

PBS: Phosphate buffer saline

PF-EMF: Power frequency electromagnetic field

PS1: Presenilin 1

PS2: Presenilin 2

PUFA: Polyunsaturated fatty acids

RNA: Ribose nucleic acid

RNS: Reactive nitrogen species

ROS: Reactive oxygen species

SAD: Sporadic Alzheimer's disease

SCGE: Single cell gel electrophoresis

SEM: Standard error of the mean

SOD: Superoxide dismutase

SSB- Single stranded breaks

SSC: Tri-sodium citrate / sodium chloride

TNF: Tumour necrosis factor

TM: Transmembrane domain

Tris/Trizma: Tris(hydroxymethyl)aminomethane

UDS: Unschedule DNA synthesis

UTR: Untranslated region