Measures of onset, progression and intervention in Alzheimer's disease: the familial paradigm

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For Hannah and Elliot

I, William D. Knight confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

ABSTRACT

Familial Alzheimer's disease (FAD) is a valuable paradigm for the study of the more common sporadic AD (SAD). The two forms of the illness share many neuropathological, clinical and radiological characteristics but it is not yet possible to predict the onset of SAD or confirm its presence without histopathological analysis. Fully penetrant amyloid precursor protein (*APP)*, presenilin 1 (*PSEN1)* or presenilin 2 (*PSEN2)* gene mutations permit both, and therefore lend themselves to clinical research with results which may be applied to the study of SAD. The identification of biomarkers of onset and progression are vital in the selection of research participants and in the rapid evaluation of new therapies.

This thesis further characterizes FAD with a number of studies. These address the use of imaging biomarkers and help clarify the nature of the relationship between FAD and SAD. Two reports of novel pathogenic FAD mutations are included as well as other studies exploring the clinical and radiological (structural and molecular) phenotypes associated with FAD. Key findings are as follows: the novel *PSEN1* p. L166del and S132A mutations are both associated with FAD; the *APP*V717G mutation can be associated with pure progressive amnesia reflected in an atypical structural imaging profile; *APP* locus duplication is a significant cause of early onset dementia in the UK and the recognised phenotype should be expanded to include early seizures and

apparently sporadic disease; regional cortical thickness (CTh) decline accelerates after diagnosis in FAD mutation carriers (MC) and differences between MC and controls are detectable in presymptomatic mutation carriers more than 4 years prior to clinical diagnosis; *APP* and *PSEN1* mutations may produce different temporal and topographical patterns of cortical change; increased ^{11}C-PiB retention in a highly heterogeneous pattern may be detected in presymptomatic *PSEN1* mutation carriers.

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CHARACTERIZING FAD

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Abbreviations

AAO	Age at onset
AAOf	Mean family AAO
Aß	ß-amyloid
ACE-R	Addenbrooke's Cognitive Examination (Revised version)
ACh	Acetylcholine
AchE	Acetylcholinesterase
AChEI	Acetylcholinesterase inhibitor
AD	Alzheimer's disease
ADAS-Cog	Cognitive Subscale of the Alzheimer's disease Assessment Scale
ADCS-CGIC	AD Co-operative Study-Clinical Global Impression of Change
ADL	Activities of daily living
ALS	Amyotrophic lateral sclerosis
aMCI	Amnestic MCI
ApoE	Apolipoprotein E
ApoB	Apolipoprotein B
APP	Amyloid precursor protein
APP	Amyloid precursor protein gene
APPd	Amyloid precursor protein gene locus duplication
BACE-1	ß-site amyloid precursor protein cleaving enzyme
BBSI	Brain boundary shift integral
BuChE	Butyrylcholinesterase
bvFTLD	Behavioural variant of FTLD
CA-1	Cornu ammonis 1
CAA	Cerebral amyloid angiopathy

CADASIL	Cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy
CBS	Corticobasal syndrome
CDR	Clinical dementia rating
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
Cho	Choline
Chr	Chromosome
CMR_{glc}	Cerebral metabolic rate for glucose
CNS	Central nervous system
CNV	Copy number variants
Cr	Creatine
CSF	Cerebrospinal fluid
CT	X-ray computed tomography
DAD	Disability Assessment for Dementia
DLB	Dementia with Lewy bodies
DNA	Deoxyribonucleic acid
DRPLA	Dentatorubropallidoluysian atrophy
DWI	Diffusion-weighted imaging
DTI	Diffusion-tensor imaging
ECE	Endothelin-converting enzyme
EEG	Electroencephalography
EOAD	Early onset AD
ER	Endoplasmic reticulum
ERC	Entorhinal cortex
FAD	Familial Alzheimer's disease
^{18}FDG	^{18}Fluorodeoxyglucose

FLAIR	Fluid-attenuated inversion recovery
fm-qPCR	Fluorescent microsatellite quantitative PCR
FTLD	Frontotemporal lobar degeneration
gDNA	Genomic DNA
GMV	Grey matter volume
GSK-3	Glycogen synthase kinase-3
Hcy	Homocysteine
HA	Homocysteic acid
HD	Huntington's disease
HIV	Human immunodeficiency virus
HMG-CoA	3, 3-hydroxymethylglutaryl-Coenzyme A
HMPAO	Hexamethylpropylene amine oxime
IDE	Insulin-degrading enzyme
IFN	Interferon
IMP	Investigational medicinal product
IPD	Idiopathic Parkinson's disease
IVIG	Intravenous immunoglobulin
LDL	Low density lipoprotein
LRP-1	LDL receptor-related protein
MAPT	Microtubule-associated protein tau
MC	Mutation carrier
MCI	Mild cognitive impairment
MHC	Major histocompatibility complex
mI	Myoinositol
MIDAS	Medical information display and analysis system
MMSE	Mini-mental state examination

MCI	Mild cognitive impairment
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MS	Multiple sclerosis
MSA	Multi-system atrophy
MTL	Medial temporal lobe
MTR	Magnetization transfer ratio
NAA	N-acetyl aspartate
NEP	Neprilysin
NFT	Neurofibrillary tangle
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association
NMDA	N-methyl-D-aspartate
NSAID	Non-steroidal anti-inflammatory drug
NT	Neuropil thread
PCA	Posterior cortical atrophy
PCR	Polymerase chain reaction
PDAPP	Platelet-derived growth factor promoter-expressing *APP*
PET	Positron-emission tomography
PiB	Pittsburgh compound B
PM	Post-mortem examination
PNFA	Progressive non-fluent aphasia
PPAR-γ	Peroxisome proliferated activated receptor-γ
PS1	Presenilin 1 protein
PS2	Presenilin 2 protein
PSEN1	Presenilin 1 gene

PSEN2	Presenilin 2 gene
PSP	Progressive supranuclear palsy
RAGE	Receptor for advanced glycation end products
ROI	Region of interest
ROS	Reactive oxygen species
SNP	Single nucleotide polymorphism
SPECT	Single Photon-Emission Computed Tomography
SAD	Sporadic AD
SALA	Selective amyloid lowering agent
SD	Standard deviation
TIV	Total intracranial volume
TNF	Tumour necrosis factor
vMRI	Volumetric MRI
VaD	Vascular dementia
VBSI	Ventricular boundary shift integral
WAIS-R	Wechsler Adult Intelligence Scale (Revised version)

The Problem

Alzheimer's disease (AD) represents the most common form of dementia and, as such, is costly both in personal and macroeconomic terms (Wimo *et al.* 2003). The sporadic form (SAD) is thought to arise from the interaction of genetic and environmental factors, though the aetiology has yet to be fully elucidated. The hallmark pathological lesions of AD are the extracellular amyloid plaque and the intracellular neurofibrillary tangle (NFT), although the extracellular accumulation of oligomeric amyloid-ß (Aß) species and altered synaptic function are increasingly seen as important (Lesne *et al.* 2006). Selective regional vulnerability to the pathology determines the distribution of neuronal loss, which in turn dictates both the observable macroscopic features (atrophy) and the clinical phenotype. Such change characteristically begins in the hippocampus and entorhinal cortex (ERC), progressing to involve neocortical structures such as the posterior cingulate gyrus and temporo-parietal association cortices (Braak and Braak 1991). Accordingly, early cross-sectional imaging studies using X-ray computed tomography (CT) demonstrated that examination of mesial temporal structures is most sensitive and specific for the detection of early AD changes (Masdeu 1985).

Subsequently, magnetic resonance imaging (MRI) has permitted a more thorough exploration of structural change in the AD brain. This is most often achieved through analysis of atrophy, the downstream consequence of neuronal loss. However, the regional distribution of pathology is also reflected in measures of regional metabolism such as ^{18}fluorodeoxyglucose positron-emission tomography (^{18}FDG PET). Further, it is now possible to visualize plaque amyloid, associated activated microglia, and acetylcholinesterase (AchE) activity *in vivo* using radio-labelled ligands in combination

with PET or single photo-emission computed tomography (SPECT). The ligands concerned are ^{11}C-PiB (Klunk *et al.* 2004), ^{11}C-(R)-PK1115 (Edison *et al.* 2008; Wiley *et al.* 2009; Okello *et al.* 2009) and ^{123}I-5IA (O'Brien *et al.* 2007) respectively. Many of these techniques have yet to be fully evaluated. Both the 'amyloid cascade hypothesis' (see 1.5.) (Hardy and Allsop 1991) and early studies showing a positive association between 'senile plaques' and severity of dementia before death (Tomlinson *et al.* 1968; Tomlinson *et al.* 1970) would suggest that non-invasive amyloid imaging represents a promising development. However, more recent work has cast doubt over the strength of the clinico-pathological association (Neary *et al.* 1986; Gomez-Isla *et al.* 1997), while others have found evidence of similar amyloid plaque loads in cognitively normal individuals (Davis *et al.* 1999a).

The development of disease-modifying agents for AD is vital in the context of rapid demographic ageing, particularly in the developed world. As age is the most powerful of risk factors for SAD, a corresponding sharp increase in its prevalence is anticipated in the coming years. There is currently no agent that reproducibly modifies the natural history of AD in humans, though novel therapeutic strategies are emerging, some with encouraging data from murine models and early human trials (Bard *et al.* 2000; Gilman *et al.* 2005; Grundman and Black 2008). Ultimately, such treatment strategies must be judged against clinically meaningful functional and neuropsychological criteria. However, use of such criteria as study outcome measures necessarily requires large sample sizes over extended periods. Many are intrinsically reliant upon subjective impressions of change, have ceiling and floor effects and are unreliable in distinguishing symptomatic from genuine disease-modifying effects. Such considerations have galvanised the search for biomarkers with high predictive and diagnostic power which can reliably identify those at risk of developing AD ('trait') and those who are in the very earliest stages of the

disease ('state'). Robust markers of progression are also required ('rate'), particularly as a means of evaluating attempts to modify the disease process. The demand for biomarkers of this kind is a pervasive and topical issue in clinical research and is not unique to AD.

A number of neuroimaging modalities, notably MRI and ^{18}FDG PET, are being explored as surrogate markers of therapeutic efficacy using smaller sample sizes over shorter periods (Alexander *et al.* 2002; Zamrini *et al.* 2004; Schott *et al.* 2005). Rates of neuronal loss are known to be reflected in regional and whole brain atrophy rates (Jack *et al.* 2002) which may be measured through both semi-automated and automated MRI techniques. Where serial scans are performed on an individual, each study subject acts as their own control, an approach which may entail digital matching (co-registration) of one image to another. Global atrophy rates may then be assessed via direct volume comparison, assessment of cerebrospinal fluid (CSF) compartment expansion or by automated techniques such as the Brain Boundary Shift Integral (BBSI) (Fox and Freeborough 1997; Freeborough and Fox 1997) or Ventricle Boundary Shift Integral (VBSI) (Schott *et al* 2005). However, the use of such techniques to assess novel therapies is a developing area and has sometimes provided unexpected results: In immunotherapy trials, for example, paradoxical changes have been observed with *increased* ventricular expansion and atrophy rates accompanying administration of experimental therapy (Fox *et al.* 2005). This was not easily reconciled with the broadly positive results of neuropsychological testing in the same study.

In SAD it is known that AD neuropathology is already well established by the time symptoms occur. Accordingly, evidence from the study of autosomal dominant familial AD (FAD) has shown that regional and whole brain atrophy rates may differ significantly from controls several years before AD is clinically detectable (Schott *et al.* 2003; Ridha *et*

al. 2006). Further, the accumulation of oligomeric Aß species may precede even this (Lesne *et al* 2006). There appears, therefore, to be a very real potential for early intervention provided agents are developed which offer disease modification and techniques arise to identify preclinical disease.

In summary, we have entered an era in which the identification of potentially modifiable risk factors and early disease markers are vital to the application of new, disease-specific diagnostic tools and treatment modalities. The development of putative disease-modifying therapies will demand the facility to identify this early therapeutic window before irreversible, pathological structural change occurs. In this way preservation of residual cognitive ability may be optimized and the currently inevitable neurodegeneration arrested or prevented. We also require the facility to evaluate swiftly the impact of novel therapies, particularly in light of increasing societal disease burden and finite resources. FAD is a valuable paradigm for the study of AD, not least because it is currently the only context in which truly reliable predictions about who will be affected can be made. It permits the use of smaller study cohorts, removes diagnostic uncertainty and represents a unique opportunity for characterization of the crucial presymptomatic phase. It may, therefore, be a key to defining robust biomarkers of risk, onset and progression.

Work undertaken for this thesis was carried out with two main aims: to characterize FAD further, informing its current and future use as an AD paradigm, and to then use the FAD model to examine potential imaging biomarkers of onset, progression and intervention. Where possible, SAD cohorts were used to investigate the comparability of the two forms, and hence the generalizability of FAD data to SAD. By studying patients in both pre-symptomatic and symptomatic stages, the relationships between regional and

whole brain volumes, cortical thickness (CTh) and clinical phenotype were examined. ^{11}C-PiB (^{11}C-Pittsburgh compound B) PET has shown potential as a marker both of early disease and therapeutic efficacy so imaging profiles in FAD and SAD cohorts are also characterized.

1.

INTRODUCTION

The mind is its own place, and in itself

Can make a Heav'n of Hell, a Hell of Heav'n.

- John Milton, *Paradise Lost*

1. 1. Normal ageing

Full characterization of the anatomy and physiology of the ageing brain is essential if a pathological departure from the norm is to be detected early. Such characterization is therefore directly relevant to the central theme of this thesis. Cortical grey matter grows until the age of five and then inexorably declines in volume with age thereafter (Pfefferbaum *et al.* 1994). White matter growth accelerates throughout adolescence, plateauing in the third decade. CSF spaces expand continuously with age at the expense of grey matter with little volume change in the white matter volume (Resnick *et al.* 2003). However, different patterns of ageing are seen in different brain regions. There is, for example, selective vulnerability of the prefrontal cortex (Tang *et al.* 2001). Other grey matter structures showing age-related volume decline include the cerebellum, thalamus and insular cortex. Importantly, hippocampal volume seems to change little with healthy ageing (Sullivan *et al.* 2005), and this can be exploited where hippocampal volume is used to distinguish AD from healthy ageing (Colliot *et al.* 2008).

Quantitative diffusion tensor imaging (DTI) (see 1. 7. 4. 5.) has repeatedly demonstrated a reduction in fractional anisotropy (FA) with increased diffusivity in white matter with

ageing (Pfefferbaum *et al.* 2000; O'Sullivan *et al.* 2001). FA decline and diffusivity increase may exhibit an anterior-posterior gradient (Sullivan *et al.* 2001). This means that frontal white matter, which develops relatively late (Sowell *et al.* 1999) and is under proportionately greater environmental control than more posterior regions (Pfefferbaum *et al.* 2001), is more vulnerable to age-related deterioration.

The resting electroencephalographic (EEG) (see 1. 7. 2.) changes with physiological ageing, showing decreased α amplitude and global 'slowing' of background activity with increased theta and delta activity (Klimesch 1999). Ageing effects on parieto-occipital α rhythm are thought to reflect underlying default activity of the neural network in the resting, awake brain. This activity is modulated by thalamo-cortical and cortico-cortical interactions controlling the movement of sensorimotor information and the retrieval of semantic information from cortical stores (Brunia 1999). Regions shown by other modalities such as functional MRI (fMRI) to be active in the resting, default state may also be particularly prone to the pathological changes associated with AD (Buckner *et al.* 2005).

1. 2. Dementia

The generic term 'dementia' is not a diagnosis in itself, rather it describes a clinical syndrome of acquired, multi-domain cognitive impairment that, by definition, must involve episodic memory and is often accompanied by changes in personality, behaviour and mood. The American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders version 4 (DSM-IV) (Am.Psychiatr.Assoc. 1994) (DSM-V is expected in 2012) defines dementia as the development of cognitive dysfunction implicating memory and at least one of: aphasia, apraxia, agnosia or executive function. These

deficits must arise in the context of full consciousness and must be sufficient to impair normal social function and/or the ability to work. The list of aetiologies is extensive and includes primary degenerative diseases such as AD, dementia with Lewy bodies (DLB) and frontotemporal lobar degeneration (FTLD); potentially remediable processes such as cerebral vasculitis, hypothyroidism and vitamin B12 deficiency; and dementia secondary to other diseases such as human immunodeficiency virus (HIV) infection and multiple sclerosis (MS). Degenerative processes predominate in the elderly population and therefore make the most significant contribution to the societal burden of dementing illness.

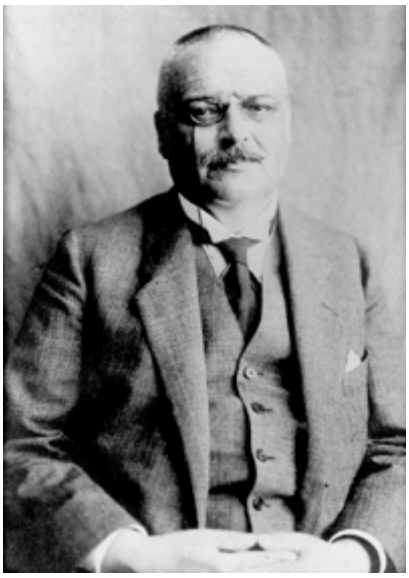
1. 3. Alzheimer's disease

1. 3. 1. History

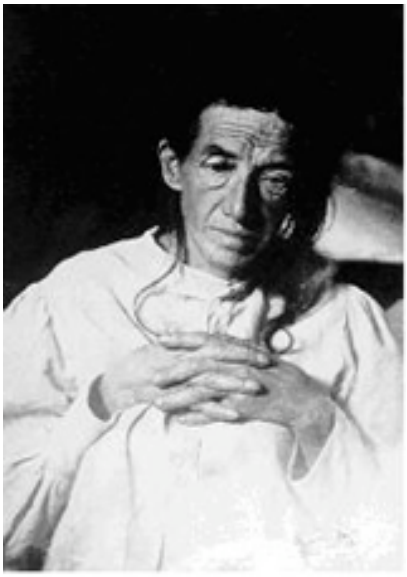
In 1906 in Tübingen, Germany the Bavarian psychiatrist and neuropathologist Aloysius 'Alois' Alzheimer reported post mortem findings in a 56 year-old patient from Frankfurt whom he had first seen in 1901 (Alzheimer 1906). In life the patient, Auguste Deter, had suffered from presenile dementia with multiple cognitive deficits including reduced comprehension and memory, aphasia, disorientation, unpredictable behaviour, paranoia, auditory hallucinations, and pronounced psychosocial impairment. Neuropathological examination of the atrophic neocortex revealed plaques of dystrophic neurites, NFT and arteriosclerotic change. Emil Kraepelin (1856-1926), the head of Alzheimer's department and himself a pioneer in neuropathology, later ensured that the illness gained its familiar eponym: 'Alzheimersche krankheit' (Alzheimer's disease).

For more than half a century thereafter the term 'Alzheimer's disease' was used exclusively to describe the rare forms of presenile dementia in which the pathological changes of plaques and tangles were evident. However, in 1968 a study demonstrated

that the neuropathology of presenile and senile forms of primary degenerative dementia were not significantly different (Blessed *et al.* 1968). This seminal observation challenged the then accepted idea that cognitive decline was an inevitable consequence of ageing. Further, it was a crucial step in defining a disease entity that has come to dominate the study of dementia.



Alois Alzheimer (1864-1915)



Auguste Deter (1850-1906)

1. 3. 2. Epidemiology

AD accounts for around 55% of degenerative dementia. The most recent estimates of prevalence in the United Kingdom and United States of America are 420,000 and 4,500,000 respectively (Hebert *et al.* 2003; Knapp and Prince 2007). However, estimated prevalence may vary with age and definition. Meta-analyses for developed nations have produced broadly similar figures in the region of 1.5% at 65 years, doubling every 4 years to reach around 30% at 80 years (Ritchie and Lovestone 2002). Indeed, all recent European epidemiological studies examining dementia prevalence on the basis of standardized diagnostic criteria have revealed an exponential increase with female preponderance after the age of 75 years (Berr *et al.* 2005). Such figures are broadly

mirrored in North American studies (Alloul *et al.* 1998). Given the well-established relationship between the incidence of dementia and increasing age, the primary factor driving this phenomenon is likely to be increased longevity evident in developed nations. The anticipated, concurrent reduction in those of working age able to offer care to elderly, impaired individuals poses a further challenge. AD and other dementias now constitute a global health and social care crisis and, as this problem has become better publicised, the requirement for large-scale strategies has gained wider appreciation (Department of Health 2009). Although the burden of the dementias in the developing world is less well known, the problem is broadly regarded to be both under-recognised and growing.

1. 3. 3. Risk factors

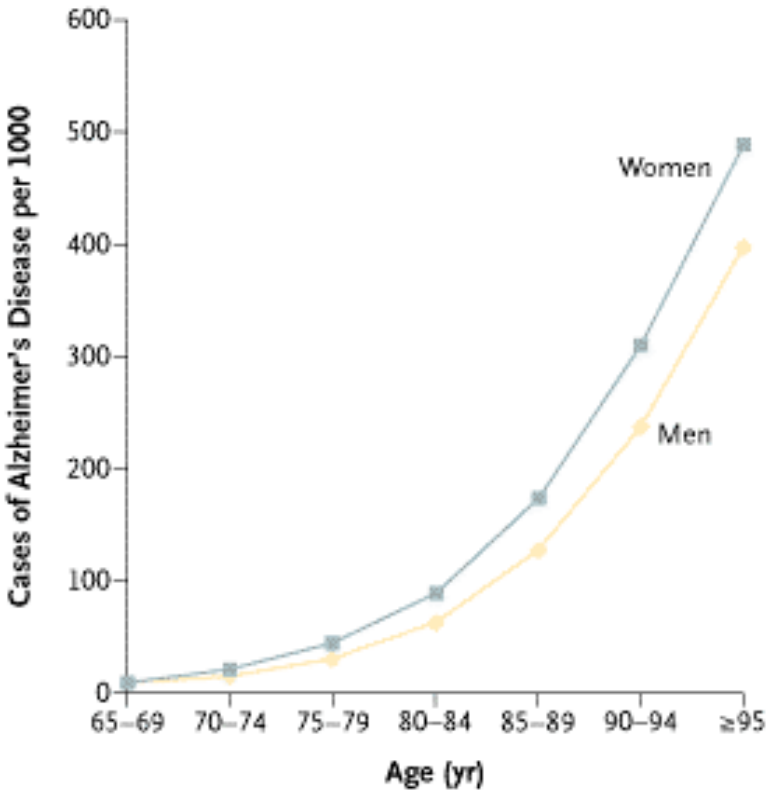
Although no single aetiology has yet been identified for SAD, the interaction of environmental and genetic variables appears to be important. The same may be said of other forms of dementia, such as vascular dementia (VaD). However, AD arguably has a unique and emotive connection to the human condition, as the most powerful of its risk factors is also the most inevitable, namely growing old.

Despite recent twin studies demonstrating heritability of up to 80% for SAD (Gatz *et al.* 2006), exogenous factors are clearly important in directly conferring risk, and in the modification of genetic variables. One idea has been that low 'cognitive reserve' associated with reduced brain size or poor educational attainment may be an independent risk factor (Mayeux 2003), or that it may act as a mechanism by which other factors, such as head injury, exert their effect upon risk (Jellinger 2004). It remains possible, however, that factors such as trauma and cerebrovascular disease (with its attendant association with diabetes, smoking, systolic hypertension, obesity and hypercholesterolaemia – each

associated with a hazard ratio of about 2 (Tyas *et al.* 2003; Luchsinger *et al.* 2005; Kivipelto *et al.* 2005; Freitag *et al.* 2006)) may have a more intimate, causative link with the pathological cascade of AD rather than simply acting to reduce cognitive reserve and hence the threshold for the appearance of symptoms. Research in this area continues, typified by a recent study suggesting a link between increased cardiorespiratory fitness and reduced brain atrophy (Burns *et al.* 2008).

Figure 1: Prevalence of AD as a function of age in men and women

(Nussbaum and Ellis 2003)



Data concerning the effects of dietary factors in AD are typically inconsistent and based on a variety of study types. Indeed, no specific dietary advice has a sound evidence base

in relation to AD risk and there is considerable overlap in the relevant biological hypotheses.

Levels of homocysteine (Hcy), vitamin B12 and folate have also been extensively studied, with variable conclusions regarding their relationship to stroke and AD risk. Hcy is a precursor of methionine and cysteine and its conversion to methionine requires folate and vitamin B12, both co-factors for methionine synthetase. Studies have suggested a link between elevated Hcy and increased AD risk (Seshadri *et al.* 2002); that homocysteic acid (HA), an oxidized metabolite of Hcy, may induce intraneuronal accumulation of $A\beta_{1\text{-}42}$ (Hasegawa *et al.* 2005) and that Hcy may increase neuronal vulnerability to Aß-mediated toxicity (Kruman *et al.* 2002).

Data linking AD pathology with oxidative stress and the association of reactive oxygen species with neuronal damage (Markesbery 1997) have led to increased interest in antioxidant supplementation. However, the results of prospective studies of antioxidants such as tocopherol (vitamin E), vitamin C and carotene are inconsistent and do not allow confident assertions of their value (Luchsinger *et al.* 2003; Grodstein *et al.* 2003).

In short, despite considerable research and a long list of putative associations, no environmental risk factor has yet been definitively linked to AD. However, many clinicians find it reasonable to offer general advice on lifestyle and diet in view of the potentially positive effect on risk for other disorders such as vascular disease. Greater knowledge of the risk factors leading to AD aids selection of subjects for pre-symptomatic studies and allows earlier, more accurate diagnosis – this is amongst the aims of studies presented in chapters 2 and 3.

1. 3. 3. 1. Apolipoprotein E

The co-ordinated expression of Apolipoprotein E (ApoE) and its receptor, the ApoE/ApoB (LDL) receptor, are important in cholesterol and phospholipid transport in the brain. Individuals may have various combinations of ApoE alleles 2, 3 and 4 on chromosome (Chr) 19: allele frequencies in the general population are 6.4%, 83.6% and 10% respectively (Carmo Martins *et al.* 2008). Changes in the balance of lipid transport, synthesis and internalization via this pathway are necessary during synaptogenesis and neuronal remodelling and, therefore, the brain's response to injury. During these reparative processes, neurones down-regulate the cholesterol-synthesising 3, 3-hydroxymethylglutaryl-Coenzyme A (HMG-CoA) reductase enzyme, while increasing cholesterol internalization.

Phospholipid homeostasis might also be important to the integrity of the cholinergic system, known to be dysfunctional in AD. The ApoE allele may modify the efficacy of cholinomimetic therapy in AD (Poirier 1999a), with E4 negative individuals deriving more benefit (interestingly the reverse appears to be true for catecholaminergic therapies (Poirier 1999b)). This may be explained by the reliance of the central nervous system (CNS) on locally available lipids for acetylcholine (Ach) synthesis and raises the possibility that genotyping may become a vital part of tailoring therapeutic regimens in future. In addition, observations arising from transgenic mouse experiments have led to a proposed role for ApoE in amyloid precursor protein (APP) processing and the clearance of extracellular Aß (Poirier 2000).

The link between SAD and ApoE4 genotype was first reported in 1993 (Corder *et al.* 1993; Poirier *et al.* 1993). Subsequent meta-analysis demonstrated an additive risk profile with three-fold and fifteen-fold risks in heterozygotes and homozygotes respectively

(Farrer *et al.* 1997). The ApoE4 polymorphism is now thought to account for the majority of genetic risk for SAD (Raber *et al.* 2004). Its predominant effect appears to be to modify age at onset (AAO) (Meyer *et al.* 1998), and there is evidence that it has a similar influence on amyloid precursor protein gene (*APP),* though not presenilin one gene *(PSEN1)* -related FAD (Hardy *et al.* 1993; Van Broeckhoven *et al.* 1994; Sorbi *et al.* 1995). It has also been suggested that the absence of the ApoE4 polymorphism may direct pathology away from the MTL towards more posterior structures (Schott *et al.* 2006), a hypothesis arising from the under-representation of ApoE4 amongst sufferers of posterior cortical atrophy (PCA). Human ApoE has since been associated with a redistribution of pathology (fibrillar amyloid) in a transgenic mouse model of the Dutch mutation (Xu *et al.* 2008). When compared with mice with an endogenous ApoE background, human ApoE 3/3 or 4/4 genotype led to a marked reduction in cerebral microvascular amyloid and the appearance of extensive, fibrillar, parenchymal plaque amyloid with strong co-localization of ApoE proteins. A corresponding shift in the distribution of activated microglia was observed. There was no difference between the groups in the total levels of Aß40, $Aß_{1-42}$ or total levels of soluble and insoluble amyloid species. Recent data have indicated an additive effect of ApoE4 allele load upon grey matter volume (GMV) in medial and anterior temporal lobes bilaterally (Filippini *et al.* 2008). However, the contrasting dominant effect of ApoE4 upon GMV in the left temporal lobe raises the possibility that ApoE exerts any influence on susceptibility, progression and lesion distribution via more than one mechanism. ApoE continues to dominate the study of genetic SAD risk, its possible epistatic influence is investigated in chapter 4.

1. 3. 3. 2. Other risk genes

A number of other genes have been investigated as potential risk factors for SAD or modifiers of FAD. *SORL-1*, which encodes a neuronal sortilin-related receptor, directs trafficking of APP into endocytic recycling pathways from the cell surface and, when under-expressed, can result in APP being sorted into Aß-generating compartments. Inherited variation in this gene has recently been associated with late onset AD (Rogaeva *et al.* 2007). Polymorphisms in the promoter region of the nicastrin gene (as described later, nicastrin is a component of the γ-secretase complex which has a fundamental role in amyloidogenesis) have not yet been excluded as a genetic risk factor for SAD, although studies have yet to demonstrate conclusively that they have a role (Orlacchio *et al.* 2004). Like nicastrin, the PEN-2 protein is a component of γ-secretase. Both mis-sense mutations and polymorphisms have been identified in the *PEN-2* gene, although the significance of these findings remains unclear (Frigerio *et al.* 2005). Genome-wide association studies show great promise in identifying new risk genes: 3 were very recently reported (see http://www.alzforum.org/new/detail.asp?id=2197): ApoJ (aka clusterin), CR1 (which encodes a complement receptor), and PICALM (which encodes an endocytic gene). Together these support key roles for innate immunity and cholesterol in AD. Genome-wide association studies continue to revolutionize the study of genetic risk, not least in AD. Related genetic screening techniques are employed in chapter 5.

1. 3. 4. Mild cognitive impairment

The gap between normal cognition and a diagnosis of AD is bridged via a heterogeneous clinical construct: mild cognitive impairment (MCI). Originally believed to represent part of a continuum of change related to normal ageing, MCI is now recognised as a pathological entity, albeit an aetiologically and clinically heterogeneous one. Consensus clinical criteria (Petersen *et al.* 1999; Winblad *et al.* 2004) require that the person is neither

normal nor demented; that there is evidence of cognitive deterioration shown by either objectively measured decline over time and/or subjective report of decline by self and/or informant in conjunction with objective cognitive deficits; and that activities of daily living are preserved and complex instrumental functions are either intact or minimally impaired.

To develop MCI does not guarantee a progression to dementia, or that any subsequent dementia will be AD, but an increased AD risk is recognised relative to an age-matched normal population with a conversion rate of 10-15% per year (Petersen *et al* 1999; Petersen 2004). MCI is often divided into amnestic (aMCI) and non-amnestic subtypes, with greater prognostic variation generally seen in the latter. Characterization of MCI is, however, ongoing and disagreement over its nosological status and the most important prognostic markers remains.

Atrophy of the hippocampus, ERC and temporal pole is greater in MCI subjects who progress to AD within 4-5 years compared to cognitively normal controls or MCI subjects who do not progress (Desikan *et al.* 2008). Microglial activation has been noted (Okello *et al* 2009) and recent evidence supports the notion that impaired episodic memory in non-demented people (including MCI) is strongly related to Aß deposition as measured by ^{11}C-PiB-PET (Pike *et al.* 2007). The relationship between these factors in established AD is, however, far less clear with some data supporting a stable amyloid load even in the face of clearly deteriorating cognition (Engler *et al.* 2006). The nature of these relationships is crucial to the timely application of anti-amyloid therapies and in defining the future role of ^{11}C-PiB -PET as a potential biomarker. A clear understanding of the nature of very early AD is a vital component of its detection at a stage amenable to

modification. Hence MCI is a pertinent concept in chapters 2 and 3, which attempt to study patients in a period which includes this phase.

1. 4. Familial Alzheimer's disease

Three genes have so far been implicated in autosomal dominant early-onset FAD: the *APP*, *PSEN-1* and the presenilin two genes (*PSEN-2)*, with the majority of known mutations found in *PSEN-1*. These may cause clinically manifest AD as early as the fourth decade, with complete penetrance the rule. Where the presence of a mutation is suspected and subsequently tested, the detection rate of sequence variations in these three genes ranges from 57% (Finckh *et al.* 2005) to 68% (Janssen *et al.* 2003), suggesting a role for as yet unidentified genes. Where the strictest criteria are applied, only around 1% of all AD is thought to fall into the autosomal dominant early onset AD (EOAD) group (Campion *et al.* 1999), although about 25% of all AD is familial in a broader sense (i.e., two or more persons in a family have AD) (Bird 2008).

FAD has served as a useful SAD paradigm for many years (Kennedy *et al.* 1995a; Fox *et al.* 1998; Fox *et al.* 2001). Prospective studies of the early stages of sporadically occurring illness have proved extremely difficult due to its insidious onset and the confounding factors associated with co-morbidity and ageing. In contrast, FAD permits an accurate ante-mortem diagnosis and the opportunity to perform targeted, longitudinal studies of clinical, radiological and neuropsychological parameters at all stages. In addition, subjects can be well matched for age, socioeconomic and other genetic factors. Such knowledge has been incorporated into cellular models of the disease process and, more recently, has permitted the development of transgenic mouse models and novel therapeutic strategies (Schenk *et al.* 1999).

It is broadly accepted that differences exist between early and late onset forms of AD. Although FAD and EOAD are strictly not equivalent entities, FAD is characterized by early onset and previous studies have proposed a higher prevalence of impairment of language or other neocortical functions (Jacobs *et al.* 1994), a relatively more rapid progression (Rogaeva 2002), differences in the pattern of cortical thinning (Frisoni *et al.* 2007) and more prominent myoclonus (Kennedy *et al.* 1995b) in early onset forms as compared to late-onset AD (LOAD). Further, in EOAD, there appears to be a strong correlation between plaque and tangle burden and severity of clinical features – a relationship not seen in the elderly (Prohovnik *et al.* 2006).

Despite these factors, the pathological and clinical similarities are clear, and the use of research subjects at risk for FAD in studying the earliest stages of AD continues to yield valuable information (Ringman 2005). Most studies show early episodic memory and executive deficits in both forms (see 1. 6. 1. and 1. 6. 3.), as well as structural neuroimaging changes 3-4 years before symptoms (Schott *et al* 2003), early cerebral hypometabolism as demonstrated by ^{18}FDG PET (Kennedy *et al* 1995a) and reduced CSF Aß (Andreasen *et al.* 1999). More recent work has shown an increase in levels of $Aß_{1-42}$ in the plasma of presymptomatic FAD mutation carriers with a fall in these levels immediately prior to symptom onset (Ringman *et al.* 2008). Further characterization of the pathological, radiological and clinical hallmarks of FAD remains important, not least because of its potential impact on our understanding of SAD. This issue is addressed in chapters 4 and 5 of this thesis.

1. 4. 1. ß-amyloid precursor protein gene

The path to our present understanding of the genetic basis for FAD has been punctuated by a number of landmarks. The first clue lay in the association of AD with trisomy 21, a link which had been observed for many years and was supported by the characterization

of Aß in 1984 in the brains of individuals with AD and Down's syndrome (Glenner and Wong 1984). Some years later, the first mis-sense mutation in *APP* (also on Chr 21) that co-segregated with autosomal dominant, early onset AD was discovered (Goate *et al.* 1991). This was the **London mutation** at position 717, significant not only for being the first known mutation of its kind, but also for its position near the site at which γ-secretase cleaves Aß.

The *APP*V717I (London) mutation changes the $Aß_{1-40}$: $Aß_{1-42}$ ratio such that there is relatively more of the fibrillogenic $Aß_{1-42}$ species. This is assumed to lead to an accumulation of neurotoxic amyloid species and the formation of plaques, thus causing neurodegeneration and clinical disease of far earlier onset than the more common SAD. With the exception of its early onset however, mutations at and around position 717 are most often associated with a canonical AD phenotype akin to that seen in SAD. This insidious, progressive impairment of episodic memory, in time leading to more widespread cognitive deficits, has been observed in the numerous reported, unrelated pedigrees with mutations around this site.

The **Swedish mutation** at positions 670 and 671 corresponds to the site of ß-secretase cleavage (see Figure 3), and pedigrees have been reported with a typical AD phenotype. There is an increase in the absolute quantities of $Aß_{1-40}$ and $Aß_{1-42}$ isoforms much like that seen in trisomy 21 (Mehta *et al.* 2007). A number of mutations have been described which cluster around the site of α -secretase cleavage within the Aß domain itself, and are associated with haemorrhagic strokes and cerebral amyloid angiopathy (CAA). The E693Q **Dutch mutation** (Levy *et al.* 1990) is known to cause hereditary CAA associated with intra-cerebral haemorrhage (ICH), as is the E693K **Italian mutation** at the same site (Tagliavini *et al.* 1999). The D694N **Iowa mutation** (Grabowski *et al.* 2001) also leads

to severe CAA and the A692G **Flemish mutation** (Hendriks *et al.* 1992b) has similar effects, albeit in association with more typical clinical features of AD and. Any generalization is, however, confounded by the presence of other rare mutations such as the E693G **Arctic mutation** (Nilsberth *et al.* 2001) which seems to cause more typical clinical AD.

Recent, significant discoveries have highlighted the importance of *APP* 'gene dose', including the role of normal variation in disomic *APP* expression (Wavrant-De Vrieze *et al.* 1999) in relation to SAD risk. Key observations so far have been that an increased quantity of γ-secretase substrate skews the $A\beta_{1\text{-}42}$: $A\beta_{1\text{-}40}$ ratio in favour of the former (Yin *et al.* 2007); that mutations in the promoter region of *APP* may cause a neurone-specific increase in *APP* transcriptional activity in patients with AD (Theuns *et al.* 2006a); and that several independent duplications of the *APP* locus have been reported in French families (Rovelet-Lecruz *et al.* 2006b) (see 1. 4. 2. and 3. 1.).

To date, 29 non-duplication *APP* mutations are known, of which 6 are not clearly pathogenic (see http://www.molgen.ua.ac.be/ADMutations). Most are associated with disease onset between 45 and 60 years and an initial amnestic syndrome progressing to more widespread cognitive impairment. However, patients have been described with a prolonged, isolated amnesia associated with the *APPV717I* (London) mutation (Gankam Kengne *et al.* 2007) (see 2. 3.). A similar prolonged, isolated amnestic phase, albeit with additional partial seizures and dysautonomia, has also been reported in association with the V714A Iranian mutation (Lindquist *et al.* 2008b) although separate reports of this mutation record a more typical AD clinical phenotype (Zekanowski *et al.* 2003). Chapter 2 includes a report of an isolated, progressive amnesia in association with a mutation at the same locus as that described by Gankam Kengne *et al.*

1. 4. 2. *APP* locus duplication

Duplications of the *APP* locus (*APP*d) causing early-onset, autosomal dominant AD have recently been described in five French families (Rovelet-Lecrux *et al* 2006; Cabrejo *et al.* 2006), confirming a much earlier report of *APP*d in France (Delabar *et al.* 1987). The prevalence of, and phenotypic spectrum associated with *APP*d are yet to be fully defined, and the issue of copy number variations (CNV) at genetic loci is potentially of great interest in the study of familial dementia *per se*. A variable phenotype with an autosomal dominant mode of inheritance was seen in the *APP*d families, recently inviting comparison with the Flemish mutation (Hendriks *et al* 1992b; Hardy 2006). Dementia and CAA were universal with a quarter also suffering ICH (Cabrejo *et al.* 2006b). Clinically it would appear that this disease falls somewhere between the canonical AD phenotype observed in most *APP* mutations and the frequent, CAA-associated ICH seen in most intra-Aß mutations. The prevalence of, and phenotypic spectrum associated with *APP* duplications are yet to be fully defined and previous Finnish reports of suspected autosomal dominant, EOAD where no causative mutation was identified (Remes *et al.* 2004a) may prove to be significant. Chapter 5 examines the prevalence and phenotype of *APP*d as it is a part of the FAD 'family' and has the potential to strengthen our understanding of the FAD: SAD relationship.

1. 4. 3. The presenilin 1 gene

PSEN1 is located on Chr 14q24.3. It has 13 exons but only 3-12 code for the PS1 integral membrane protein comprising 467 amino acids and eight transmembrane domains (Sherrington *et al.* 1995). PS1 is highly conserved in evolution and is one of four membrane proteins required to form an active γ-secretase complex (the others are PEN-2, nicastrin and APH-1 with a 1: 1: 1: 1 stoichiometry). *PSEN1* inactivation abolishes both PS1 expression and γ-secretase activity (De Strooper *et al.* 1998). Some mutations in

PSEN1 lead to a small shift in the preferred cleavage site and, therefore, the overproduction of the fibrillogenic form of beta amyloid peptide, $A\beta_{1-42}$ – an important component of the amyloid plaques seen in AD. However, some uncertainty remains over the precise mechanisms by which *PSEN1* mutations exert their pathogenic effects, with both gain and loss of function mechanisms postulated (Bentahir *et al.* 2006).

PSEN1 mutations tend to cluster in the regions coding for transmembrane domains and are the most common cause of autosomal dominant FAD with 175 reported pathogenic mutations (see http://www.molgen.ua.ac.be/ADMutations). Pathogenic mutations in the other two genes known to be associated with FAD, namely *APP* and *PSEN2*, are far less common. With the exception of their early onset, and despite evidence of phenotypic heterogeneity (Menendez 2004; Larner and Doran 2006), the majority of *PSEN1* mutations produce a clinical phenotype similar to that of SAD, with early memory impairment followed by progressive, multi-domain cognitive dysfunction. The most important exceptions are those mutations associated with early spastic paraparesis or a presentation resembling that of behavioural variant FTLD (bvFTLD).

Mutations in exons 8 and 9 are responsible for many *PSEN1*-related FAD cases. Examples of those associated with a mixture of spastic paraparesis, spastic dysarthria and seizures include Δ9, R278T, N135S and V261L (Kwok *et al.* 1997; Rudzinski *et al.* 2008; Jimenez Caballero *et al.* 2008). Spastic paraplegia has also been reported in a patient with AD-like dementia and an unusual TTATAT insertion in a highly conserved transmembrane domain 3 interface region of *PSEN1* (Rogaeva *et al.* 2001).

Four deletions in exon 9 (Δ9 mutation) have been described (Crook *et al.* 1998; Houlden *et al.* 2000; Smith *et al.* 2001; Brooks *et al.* 2003) in which the phenotype comprises memory impairment and spastic paraparesis. These mutations destroy the splice acceptor

site for exon 9 resulting in deletion of exon 9 from the mutant mRNA transcript and an amino acid substitution (S290C). This leads to deletion of residues 291-319 of the protein, including the section coding for the cleavage site of PS1. The deletion predictably has a major effect upon PS1 metabolism, although the S290C change alone has just as profound an effect upon APP metabolism (Steiner *et al.* 1999). Cellular models of such deletions show increased $A\beta_{1-42}$ production (Borchelt *et al.* 1996) and neuropathological analysis shows unusual cortical 'cotton wool' plaques which are immunoreactive for the Aß but lack a congophilic core. In these cases spastic paraparesis often precedes cognitive impairment (Verkkoniemi *et al.* 2000). Such delay in the onset of cognitive features would suggest a role for as yet unidentified modifying factors at exon 9.

At least 6 *PSEN1* mutations have been linked with a clinical phenotype more akin to bvFTLD than AD. These include a report of a behavioural presentation with relative preservation of posterior functions associated with a L113P mutation (Raux *et al.* 2000). Imaging in this case was consistent with bvFTLD. In addition, mutations such as P117R (Styczynska *et al.* 2004) and F280A (Duarte *et al.* 2004) have been linked to similar phenotypes. The M139V mutation has, in one African-American pedigree, been linked with early personality change, psychosis, rigidity, dystonia and mutism (Rippon *et al.* 2003), although this mutation can present differently between kindreds, suggesting that other factors are important in determining the phenotype. Those reports with pathological confirmation include the G183V mutation (Dermaut *et al.* 2004) in which a Pick-type tauopathy was demonstrated, with no extracellular Aß deposits, in a family with a history of FTLD-like dementia. Interestingly, the insR352 mutation (Rogaeva *et al* 2001; Tang-Wai *et al.* 2002) apparently does not increase $A\beta_{1-42}$ but rather acts as a negative presenilin, leading to a partial inhibition of γ-secretase (Amtul *et al.* 2002). This insertion

was not detected in more than 150 controls, 150 AD cases or 150 FTLD cases not linked to a tau mutation or Chr 17.

An extra-pyramidal syndrome with dementia has been described in a 52 year-old man with a family history of a similar illness (Ishikawa *et al.* 2005). This case was associated with a three base-pair deletion in exon 12 of *PSEN1* and, neuropathologically, cotton wool plaques, Lewy bodies and CAA. Another mutation – G217D in exon 8 of *PSEN1* - has been described (Takao *et al.* 2002) with an extra-pyramidal syndrome and cotton wool plaques in the cortex and striatum.

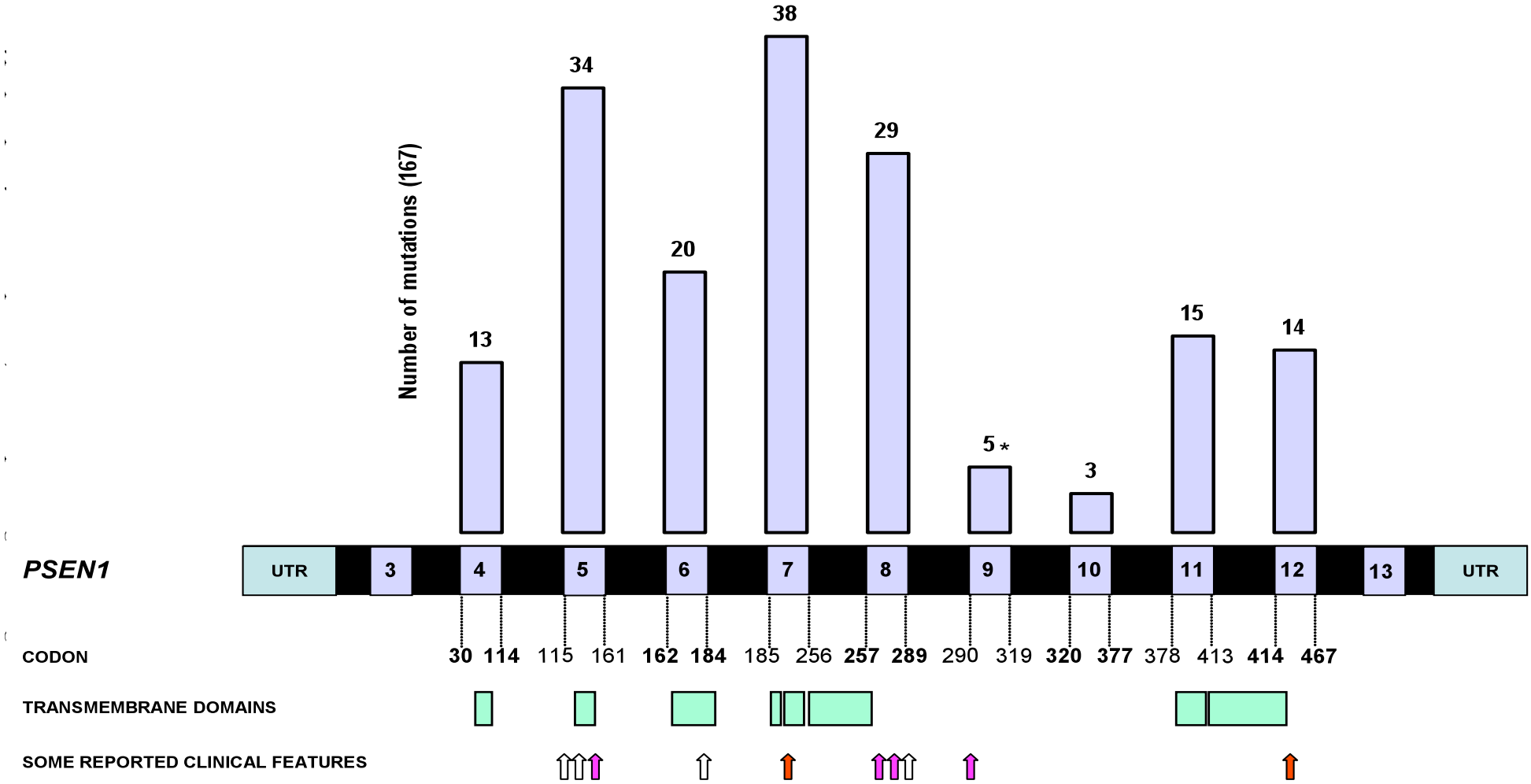
A particularly aggressive form of FAD is associated with the *PSEN1* L166P mutation which, unlike the deletion at the same site, leads to cognitive impairment beginning in adolescence. It has been found to cause a profound over-production of $A\beta_{1-42}$ and also to impair Notch signalling (Moehlmann *et al.* 2002).

1. 4. 4. The presenilin 2 gene

Pathogenic mutations in *PSEN2* on Chr 1 causing autosomal dominant EOAD are rare, with only ten so far described (see http://www.molgen.ua.ac.be/ADMutations). A further four have been identified in which pathogenicity is either unknown or absent. They were first described in Volga German families in 1995 (Rogaev *et al.* 1995) and produce an AD phenotype which typically includes older AAO, though with a relatively wide range (44-88 years). A longer disease duration has also been noted in some cases (Sherrington *et al.* 1996). Specific examples of phenotypic heterogeneity include the *PSEN2* A85V mutation which may produce a DLB-like clinical phenotype associated with synucleinopathy (widespread Lewy bodies) and AD pathology at post mortem

(Piscopo *et al.* 2008). Significant language impairment has been seen (Lindquist *et al.* 2008a), albeit in a mutation without conclusive evidence of pathogenicity (V393M). What little is known about imaging profiles suggests involvement of areas common to other forms of AD: ^{18}FDG PET in *PSEN2* N141I-associated FAD for example has shown left parietal and precuneus hypometabolism with progressive hypometabolism of both temporo-parietal and left frontal lobes (Nikisch *et al.* 2008).

FIGURE 2: *PSEN1*: KNOWN PATHOGENIC EXON MUTATIONS



*INCLUDES 4 Δ9 MUTATIONS. UTR = UNTRANSLATED REGION
2 INTRONIC MUTATIONS NOT INCLUDED

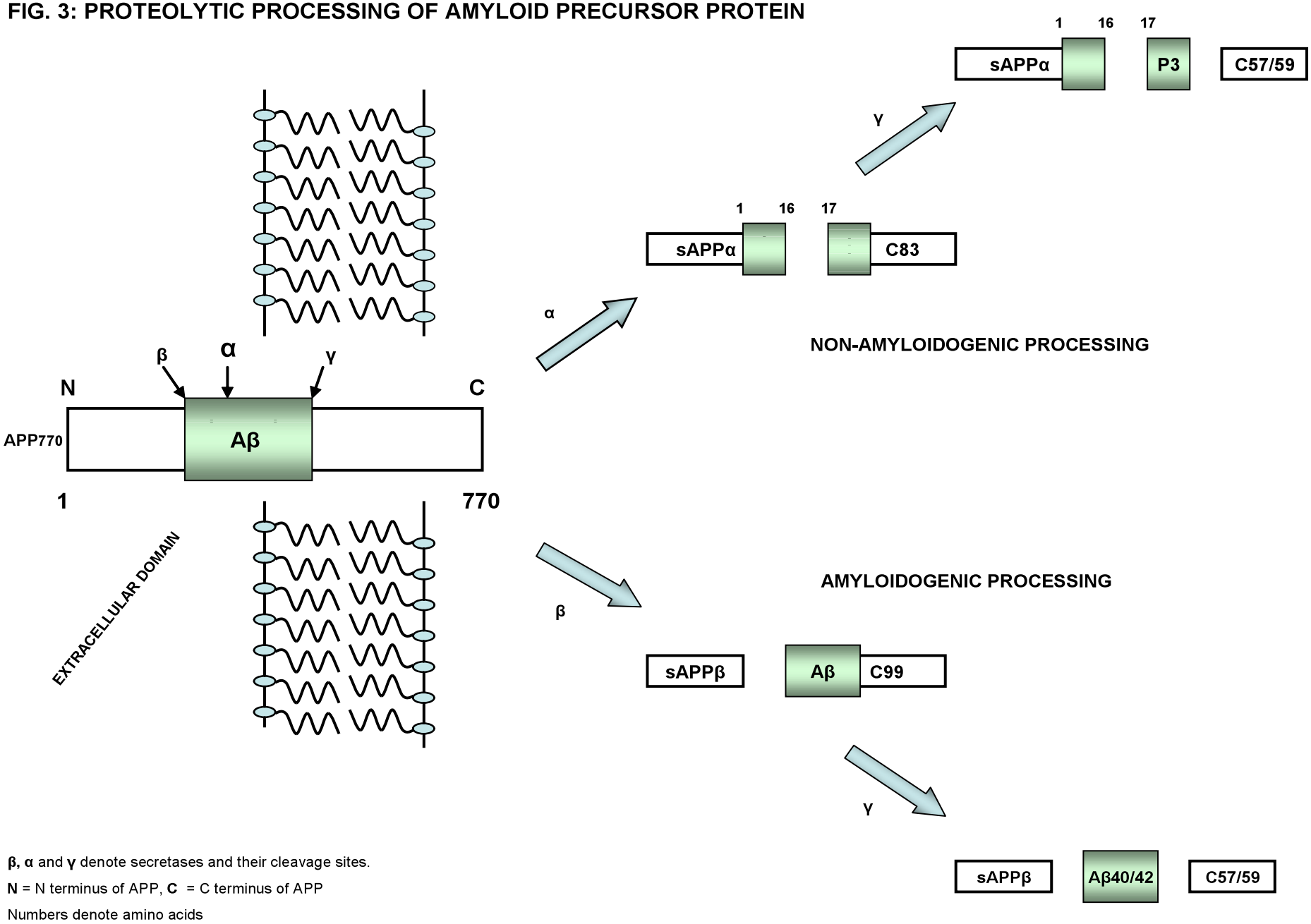
INTRON EXON BEHAVIOURAL EXTRAPYRAMIDAL SPASTIC PARAPARESIS

neuronal and synaptic degeneration, reduced synaptic plasticity, CAA and inflammatory and oxidative stress. Changes in the neurochemistry of the AD brain are also important, with neurotransmitter depletion a well-established consequence of synaptic and neuronal loss. ACh release from fibres originating in the nucleus basalis of Meynert and projecting to cortical areas is particularly affected (Francis *et al.* 1999). The main receptor types for ACh in the CNS are muscarinic (subtypes M_1 and M_2) although nicotinic receptors are also present.

Tau protein binds to microtubules, influencing their stability and the intracellular transport they facilitate. The six known isoforms of tau, which range from 352 to 441 amino acids in length, are encoded by a single gene on Chr 17. Three isoforms have three microtubule binding domains and three have four such domains (termed 'three repeat tau' and 'four repeat tau' respectively) (Wischik and Harrington 2000). The phosphorylation and dephosphorylation of tau, and hence the relative activity of kinase and phosphatase enzymes, is a key means of regulating this process and is relevant in AD as hyperphosphorylation promotes the aggregation of tau isoforms into the paired, argyrophilic helical filaments of NFT and neuropil threads (NT). One important enzyme responsible for phosphorylation of tau isoforms is glycogen synthase kinase-3 (GSK-3). As an inhibitor of GSK-3, lithium has been proposed as a neuroprotective agent in both AD and the 'tauopathies' (Engel *et al.* 2006; Engel *et al.* 2008). Mutations of the microtubule-associated protein tau (MAPT) gene and/or accumulation of three or four repeat tau are the basis for a number of other neurodegenerative diseases such as corticobasal syndrome (CBS), progressive supranuclear palsy (PSP) and some cases of FTLD.

It is the aberrant, proteolytic processing of APP that gives rise to the core component of the amyloid plaque - a 40-43 amino acid peptide (Aß) which was purified and identified only after the problem of plaque insolubility was overcome in the mid-1980s (Masters *et al.* 1985). The pathological significance of Aß has been a central, if sometimes controversial theme and the basis for the amyloid cascade hypothesis (Fig. 4) (Hardy and Allsop 1991; Hardy and Higgins 1992). This postulates that it is the cascade from peptide monomer to plaque, and altered Aß equilibrium, which drive AD pathogenesis. In this model tau pathology is regarded as a downstream process although this view has not gone unchallenged. The universality of tau pathology in AD (it forms the basis of the Braak neuropathological criteria which describe its predictable progress in the AD brain (Braak and Braak 1991)) and the poor correlation between total or extracellular fibrillar amyloid and clinical status (Giannakopoulos *et al.* 2003), means it could legitimately be considered a form of 'tau-opathy'. Some critics of the amyloid cascade hypothesis have gone further, asserting that amyloid pathology is a downstream protective response to oxidative and metabolic change (Lee *et al.* 2007) and predicting that novel anti-amyloid therapies will be ineffective or harmful (Smith *et al.* 2002). Pathological and imaging studies indicating that amyloid deposition may be associated with apparently healthy ageing (Davies *et al.* 1988; Davis *et al* 1999a; Mintun *et al.* 2006) further complicate the debate. However, perceived inconsistencies in the amyloid hypothesis are increasingly being addressed by new discoveries that tend to support the primacy of Aß. Recognition of the role of variable *APP* expression and the association between FAD mutations

FIG. 3: PROTEOLYTIC PROCESSING OF AMYLOID PRECURSOR PROTEIN



β, α and **γ** denote secretases and their cleavage sites.

N = N terminus of APP, **C** = C terminus of APP

Numbers denote amino acids

sAPP = secretory APP

and parenchymal and vascular amyloidosis have contributed in this regard, although another avenue of research has been particularly illuminating: Whether *in vitro* or *in vivo*, Aß assembles itself into an array of structures ranging from soluble oligomers to large insoluble fibrils and the latter, in the form of plaques, was historically considered to be the most important entity in neurodegeneration. However, it has become clear that various amyloid pools comprising different Aß species exist in dynamic equilibrium in the AD brain and that soluble oligomers, whether intra- or extracellular, are the main neurotoxic species with the more intimate link to clinical phenotype.

There are several candidate mechanisms for Aß oligomer toxicity, though no suggestion that they are necessarily mutually exclusive. Binding to specific excitatory post-synaptic sites has been demonstrated, with consequent impairment of long-term potentiation (a measure of synaptic plasticity) in hippocampal neurones (Lambert *et al.* 1998; Lesne *et al* 2006), providing a plausible mechanism for impairment of memory formation. They also disrupt neuronal calcium homeostasis such that cytosolic calcium is increased and cell dysfunction/apoptotic processes ensue. This probably occurs via an increase in membrane permeability with initial studies indicating that the formation of aberrant membrane channels was responsible (Pollard *et al.* 1995). However, recent work has suggested a less specific mechanism (Demuro *et al.* 2005). Increased intracellular calcium also contributes to the formation of reactive oxygen species (ROS), mitochondrial dysfunction and oxidative stress (Schubert *et al.* 1995). Aß oligomers also potentiate the release of, and prevent re-uptake of the excitatory neurotransmitter glutamate in the synapse, producing excito-toxicity (Harris *et al.* 1996; Fernandez-Tome *et al.* 2004) from which memantine may offer some protection (Song *et al.* 2008). Amongst the most important observations for the amyloid cascade hypothesis are that they also readily induce tau hyperphosphorylation, in part via up-regulation of GSK-3 (Hoshi *et al.* 2003;

De Felice *et al.* 2008), and that increased levels correlate with NFT density (McLean *et al.* 1999). The specific sites at which oligomerization occurs and the precise nature of the interactions between different Aß pools are not yet known. Also, the most important Aß assembly, or combination of assemblies, has not yet been established, although the time course of toxicity may depend upon the particular mixture of oligomers present (Deshpande *et al.* 2006).

APP itself is a single transmembrane domain protein with eight possible isoforms. Of these the 695, 751 and 770 amino acid forms are the most common in the brain. The first is produced mainly in neurones whereas 751 and 770 are predominantly found in glial cells. All three have Aß domains and are therefore potentially amyloidogenic. APP is manufactured in the endoplasmic reticulum (ER) and subsequently moved through the Golgi network to the cell surface where it is eventually re-internalized into the endosomal/lysosomal system. The Aß domain is partially embedded in the plasma membrane but also includes 28 residues outside the membrane and 14 in the transmembrane domain. The function of APP in humans is not fully understood, although its over-expression in hippocampal neurones leads to elevated Aß production and consequent depression of excitatory transmission (Ting *et al.* 2007), suggesting a possible role in modulating synaptic transmission. This effect is likely to be exerted via Aß as mutant APP incapable of producing Aß has no such effect upon excitatory transmission.

Initially thought to be an entirely abnormal protein, Aß was found to be a product of normal cellular metabolism in 1992 (Haass *et al.* 1992). APP may undergo proteolytic processing via two routes, termed amyloidogenic and non-amyloidogenic, the former preserving the Aß moiety intact. The route depends upon which of three enzymes act to

sequentially cleave the APP molecule. The non-amyloidogenic pathway involves cleavage by α -secretase (a member of the ADAM-10 disintegrin metalloprotease family) followed by γ-secretase. The former cuts APP within the Aß domain and the latter acts upon the C-terminal fragment to produce P3 and C57/59 (Figure 3). The amyloidogenic pathway entails cleavage by ß-secretase (active portion is termed BACE-1) releasing the ectodomain of APP. The membrane-bound C-terminus, which contains the intact Aß section within a 99-residue fragment (C99), is then cleaved by γ-secretase to produce $Aß_{1-40/1-42}$ and a C57/59 fragment. The variation in the size of these fragments and, therefore, the ratio of $Aβ_{1-40}$ to $Aβ_{1-42}$ is due to inherent small variations in the γ-secretase cleavage site. Point mutations in *PSEN-1* (Figure 2) may affect the cleavage site of γ-secretase, changing the $Aß_{1-40/1-42}$ ratio in favour of the more toxic, fibrillogenic species ($Aß_{1-42}$).

γ-secretase is a protease complex with four components: presenilin, nicastrin, APH-1 and PEN-2. Presenilin (and therefore γ-secretase activity) exists in both the ER/Golgi network and nearer the plasma membrane, meaning that γ-secretase-mediated APP cleavage can occur at several sites in the cell. This means that two pools of Aß are produced. Aß generated at or close to the plasma membrane (Aßsec) is secreted and aggregates in the extracellular deposits (plaques) seen in AD. $Aß_{1-42}$ is probably the initially deposited species, and indeed the predominant species in the parenchymal plaques of the AD brain (Miller *et al.* 1993; Iwatsubo *et al.* 1994). A proportion of the deposits develop from amorphous 'diffuse' plaques into more circumscribed 'immature' plaques and finally into 'mature' (cored) plaques with their well-defined amyloid core and halo of swollen neurites. As this 'maturation' proceeds they are more likely to become $Aß_{1-40}$ positive, an observation consistent with the 'seeding' hypothesis (Jarrett and Lansbury 1993) whereby immature plaques begin with a seed of $Aß_{1-42}$ and progressively incorporate $Aß_{1-40}$ - the major soluble species in extracellular fluid and CSF. Such seeding

would not however explain the striking scarcity of $A\beta_{1-40}$ positive plaques observed in some cases of *APP*V717I mutation-associated FAD (Iwatsubo *et al* 1994). An alternative explanation for the increased proportion of $A\beta_{1-40}$ observed over time is that the two or three amino acids at the C-terminal of $A\beta_{1-42}$ are removed slowly in situ by carboxypeptidases to produce $A\beta_{1-40}$. $A\beta_{1-40}$ and $A\beta_{1-42}$ appear to be equally abundant amongst vascular amyloid deposits (Roher *et al.* 1993).

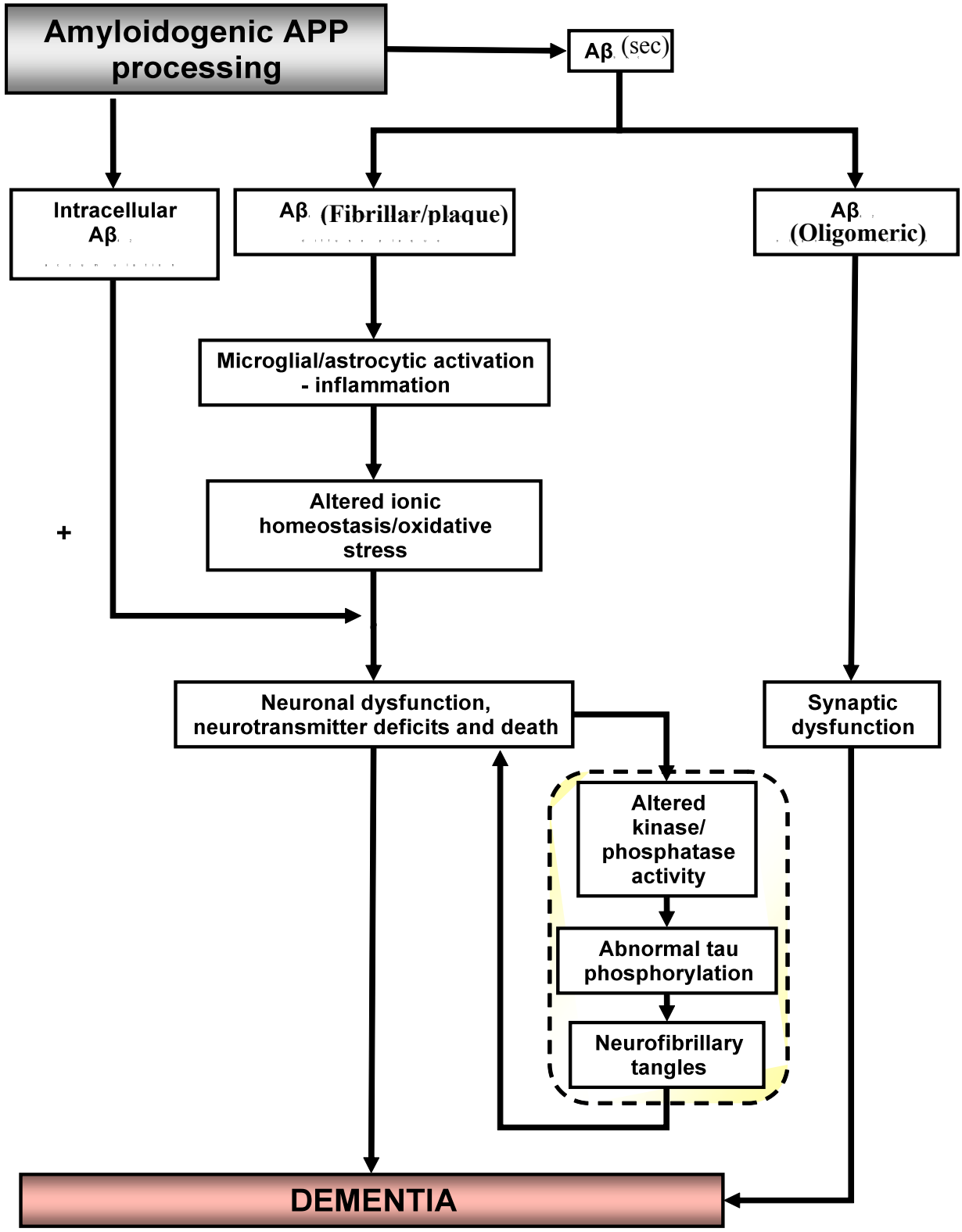
The other pool of Aß remains intracellular, may accumulate in AD and may be important in its pathogenesis, possibly by facilitating neuronal death or increasing vulnerability to neurotoxic factors (Chui *et al.* 1999). Interestingly, *PSEN-1* mutations seem to have a more profound effect upon γ-secretase cleavage site specificity when it acts near the plasma membrane to produce Aßsec than when it acts near the ER/Golgi complex to produce intracellular Aß (Grimm *et al.* 2003).

Aß is degraded by peptidases including insulin-degrading enzyme (IDE), neprilysin (NEP) and endothelin-converting enzyme (ECE) (Carson and Turner 2002). It may also be cleared from the brain across the blood brain barrier (BBB) by low density lipoprotein (LDL) receptor-related protein (LRP-1) (Tanzi *et al.* 2004). The receptor for advanced glycation end products (RAGE) contributes to the trafficking of amyloid from plasma into the CNS (Deane *et al.* 2004) and is now considered a potential therapeutic target (Sturchler *et al.* 2008). To date there is no evidence for an abnormality affecting these processes in the AD brain.

Although the MTL has an established role in episodic memory, it is clear that focus should not fall solely upon the hippocampus and MTL when considering the earliest pathological changes of AD. Rather, changes affect the whole of a distributed network

subserving episodic memory, with close links to the limbic system. This network includes the MTL, mammillary bodies, dorso-medial thalamus and retrosplenial association cortices (particularly posterior cingulate). Amyloid deposition does not appear to occur especially early in the MTL and can appear early in other areas of the network (Braak and Braak 1991; Edison *et al.* 2007). Instead, extracellular accumulation of soluble, synaptotoxic, oligomeric Aß species (Lesne *et al* 2006) or abundant NFT (Bennett *et al.* 2004) are more prominent pathological findings.

FIG 4: The Amyloid Cascade Hypothesis



1. 6. Clinical features

AD is a slowly progressive disorder of insidious onset. Its cardinal features are the early, progressive impairment of episodic memory with subsequent impairment of other cognitive domains leading to apraxia, aphasia, and agnosia. However, the spectrum of clinical features is by no means restricted to these. Dysexecutive features are common and the balance of the clinical and pathological burden may vary, causing atypical predominantly frontal or posterior (PCA) cortical syndromes. A preclinical phase of neurodegeneration is recognised during which the pathological lesion load increases before breaching a clinical threshold. This phase has been estimated to be as much as 2-3 decades in length (Davies *et al* 1988).

Clinical diagnostic criteria have been developed and include the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann *et al* 1984) (Appendix 1), and the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria (Morris *et al.* 1989). Such criteria are necessarily qualitative and require the clinical judgement of the supervising doctor in order to be successfully applied. They nonetheless form an important framework in the absence of a single diagnostic test. Revisions to the NINCDS-ADRDA criteria have recently been proposed in order to incorporate CSF and imaging biomarkers (Dubois *et al.* 2007).

Evidence for the existence of atypical AD phenotypes is growing and a variety of progressive, focal cortical syndromes have been reported in association with proven AD pathology. In one recent series (Alladi *et al.* 2007) AD pathology was found to be responsible for 100% of cases of PCA, 50% of CBS, 7.1% of bvFTLD, 44.1% of PNFA,

71.4% of mixed aphasia and 10% of semantic dementia. Atypical presentations are also well established in FAD (see 1. 4.).

1. 6. 1. Memory

A decline in episodic memory function is an early feature of AD and a necessary condition for clinical diagnosis. This is thought to reflect the earliest pathological changes occurring in the MTL. However, like pathological change, memory disruption probably precedes the full clinical syndrome necessary for an AD diagnosis (Chen *et al.* 2001). There is increasing support for the notion that, after an initial decline, memory function temporarily plateaus during the preclinical period (Smith *et al.* 2007). This is linked to the concept of cognitive reserve: imaging studies indicate recruitment of pre-frontal and posterior cortices by early AD patients during memory tasks (Grady *et al.* 2003), while neurotransmitters and brain neurotrophic factors are maintained or up-regulated in pre-clinical, though not established AD (Davis *et al.* 1999b; Mufson *et al.* 2003). Together these changes suggest that the brain may attempt to compensate for early pathological change and that further clinical decline corresponds with these compensatory mechanisms being overwhelmed.

The nature of the memory deficit associated with early AD has been well characterized. Patients exhibit problems with delayed recall of information, even when the demands of retrieving that information are reduced via recognition tests (Delis *et al.* 1991). Attenuation of the primacy effect and intrusion errors are seen, and semantic encoding is less effective in improving memory retention than in cognitively normal subjects (Backman and Small 1998). As the illness progresses, a semantic memory deficit emerges, corresponding with a loss of general knowledge, increasing language problems (see below) and a degree of retrograde amnesia Patients perform poorly on tests of

confrontational naming and recall of over-learned facts is impaired. Performance across different tests of semantic memory suggest that there is a genuine loss from the semantic store at this stage rather than a retrieval deficit alone (Chan *et al.* 1998).

1. 6. 2. Other cognitive domains

Executive function is impaired early in AD, resulting in problems with mental manipulation of information, concept formation, problem-solving, self-monitoring, sequencing and cue-directed behaviours (Perry and Hodges 1999). Neuropsychological tools for detecting these problems include the modified Wisconsin Card Sorting Task (Grant and Berg 1948; Nelson 1976), the Raven Progressive Matrices Task (Raven *et al.* 1976) and the Trail Making Test (Reitan 1958). A number of studies have indicated that executive function and **attention** may be the first non-memory domains to be affected in AD (Grady *et al.* 1988; Reid *et al.* 1996). Tasks involving the concurrent manipulation of information and/or the division of attention are particularly affected and disruption of cortico-cortical connections is probably important in producing early attentional difficulty.

Progressive impairment of **language** may occur as part of a typical AD clinical phenotype, occasionally as one of its earliest symptoms. Linguistic deficits arise at an early stage in up to 10% of cases, with impairment of word fluency and semantic access (Emery 2000). The latter is thought to result in the word finding difficulty commonly manifested during discourse and in tasks of confrontational naming as the temporal lobe semantic store deteriorates. The resulting speech is sometimes termed 'logopenic'. Articulatory and phonological impairments are relatively uncommon in these early stages (Bayles and Kazniak 1987) although can occur (Croot *et al.* 2000). Later a wider range of dysphasic deficits may emerge with a reduction in the grammatical complexity of

language, and aphasia (Kempler *et al.* 1998). The nature and degree of language deficit appears to be influenced by the extent and distribution of the pathology rather than the type of pathology *per se*. Thus, with an atypical distribution of AD neuropathology (such as where the hippocampus and ERC are relatively spared compared to the perisylvian region), phonological and articulatory disruption are, via similar mechanisms to acquired speech and language disorders, more common. Deficits in posterior cortical functions (e.g. visuospatial and visuoperceptual function) do occur in typical AD, but are usually less prominent until the later stages of the disease. PCA is perhaps the bet known exception to this.

1. 6. 3. Behavioural & neuropsychiatric features

These are an important group of symptoms and signs (Burns 2009), recognised increasingly as a component of many neurological disorders, including AD. In AD, apathy and depression are the most common and their presence is associated with caregiver distress and poor functional and therapeutic outcome (Benoit *et al.* 2007). Agitation is a relatively common problem and the poor side effect profile generally associated with pharmacological measures has led to trials of alternative treatments such as bright light therapy (Burns *et al.* 2009). Visual hallucinations are seen either as a primary manifestation in severe disease, or when intercurrent systemic illness and reduced cerebral reserve combine. They are an important cause of disability and have been linked with an accelerated progression of dementia and early institutionalization (Hauft *et al.* 1996). There is, therefore, substantial risk of morbidity, misdiagnosis and inappropriate management if behavioural and psychiatric features go unrecognised.

1. 6. 4. Olfaction

A particularly large body of work relates to the potential for olfactory testing in early AD diagnosis i.e. as a marker of onset. Olfactory dysfunction is associated with a wide variety of diseases and conditions, including a number of neurological illnesses such as amyotrophic lateral sclerosis (ALS) (Hawkes *et al.* 1998), Huntington's disease (Bylsma *et al.* 1997), multiple system atrophy (MSA) (Wenning *et al.* 1995), MS (Doty *et al.* 1999), idiopathic Parkinson's disease (IPD) (Mesholam *et al.* 1998), and AD (Corwin *et al.* 1985; Graves *et al.* 1999). Importantly, loss of olfactory function is also associated with ageing, increasing in frequency after age 65 years (Liu *et al.* 1995). Olfactory deficits are an early feature of AD (McCaffrey *et al.* 2000; Luzzi *et al.* 2007) with early pathological change occurring in the hippocampus (Hyman *et al.* 1984), piriform cortex (Reyes *et al.* 1987) and ERC (Kahn *et al.* 1987). Initial optimism surrounding the identification of AD pathology in olfactory neuroepithelium (Talamo *et al.* 1989), and its potential contribution to early diagnosis, was tempered by the subsequent realisation that similar changes were apparent in IPD and some healthy controls (Trojanowski *et al.* 1991). However, specificity is not the only issue: the relationship of AD to ageing presents further problems as neuroepithelium is replaced by respiratory epithelium with age, making the identification of olfactory neurones more difficult. Further, doubt remains over the relative contributions of central and peripheral damage (Mann *et al.* 1988) to the well established olfactory identification, recognition and threshold detection disturbances of AD (Mesholam *et al* 1998). The density of NFT in the ERC is inversely related to the ability to identify odours (Wilson *et al.* 2007) but pathological change occurs in the olfactory bulb and tract just as early i.e. there is a close temporal relationship between changes in peripheral and central regions (Christen-Zaech *et al.* 2003).

Olfactory testing may have potential as an early marker of AD risk (Doty 2003). Some tests use microencapsulated odourants that are released on scratching an impregnated strip (Doty *et al.* 1984; Doty *et al.* 1995), while others test detection threshold, discrimination or odour memory. Olfactory identification scores have been determined in individuals with MCI, with low scores associated with a greater risk of subsequently developing AD (Devanand *et al.* 2000). Olfactory assessment may also be superior to the MMSE in distinguishing AD from depression (McCaffrey *et al* 2000). Several studies seem to suggest that threshold detection is better preserved than identification (Koss *et al.* 1987; Hawkes and Shephard 1998), providing support for the view that damage to central olfactory pathways predominates i.e. that signals are able to reach the brain under the appropriate conditions but perceptual deficits prevent their correct interpretation. Studies of olfaction in non-demented individuals at genetic risk of dementia (as determined by ApoE status) suggest a higher rate of olfactory dysfunction amongst ApoE4 positive subjects (Salerno-Kennedy *et al.* 2005), while others suggest that a positive family history, rather than ApoE4 positivity *per se*, is associated with olfactory deficits (Schiffman *et al.* 2002; Handley *et al.* 2006). These studies did not, however, involve families with autosomal dominant disease and/or proven *PSEN* or *APP* mutations. Individuals at risk of developing *PSEN1*-associated FAD by virtue of a proven *PSEN1* mutation in their family have been studied, although olfactory tests did not predict mutation status or AAO (Nee and Lippa 2001).

1. 7. Investigations

1. 7. 1. Neuropsychological & functional scales

Where cognitive impairment is suspected, formal neuropsychological assessment may be valuable in assisting diagnosis. By adopting a standardized, systematic approach bias is minimised and a profile of performance across a number of cognitive domains is

produced. This profile may then be compared with age-specific control data or, to some extent, with the estimated 'premorbid' abilities of the individual concerned. It helps in confirming that a deficit genuinely exists (or that it does not), and in assessing patients longitudinally in order to demonstrate improvement (as with practice effects in normal ageing), stability or deterioration – a hallmark of neurodegenerative dementias such as AD. There are a large number of available tests, each designed to interrogate a particular cognitive domain. However, at the heart of any such assessment is the Wechsler Adult Intelligence Scale (Revised version) (WAIS-R) (Wechsler 1981) which provides a global measure of intelligence as well as two important sub-scores: the performance and verbal IQ.

Neuropsychological assessment may have a role in detecting and defining the effects of any intervention, although clinically meaningful functional outcome measures remain the ultimate test of efficacy. The limitations of this approach in evaluating therapeutic strategies have been outlined earlier, including the observation that up to ten times fewer patients are required to detect a treatment effect where MRI or ^{18}FDG PET are used rather than clinical end-points (Alexander *et al* 2002; Jack *et al.* 2003). In chapters 4 and 5, neuropsychometry is used to provide detailed information on the nature of clinical deficits in two *APP* mutation related forms of AD.

Composite scales are widely used and may be valuable in monitoring global change. Perhaps the best recognised quantitative cognitive function scale is the Mini-Mental State Examination (MMSE) (Folstein *et al.* 1975). This validated 30-point assessment can be administered rapidly, providing information on a number of cognitive domains including temporal and topographical orientation, language, verbal recall, calculation and attention. A maximum score of 30 is to be anticipated in cognitively normal subjects and a score

below 26 is considered supportive of a dementing illness. A similar bedside test sensitive to early cognitive dysfunction is the Addenbrookes Cognitive Examination (Revised version) (ACE-R) (Mioshi *et al.* 2006; Larner 2007). This has been widely adopted as a screening tool and, at least in the setting of a university hospital clinic, has been reported to be both sensitive and specific for detecting dementia. Both the MMSE and ACE-R are deployed in research and clinical settings, unlike the Cognitive Subscale of the Alzheimer's disease Assessment Scale (ADAS-Cog) (Rosen *et al.* 1984) which is used predominantly in research. The latter scale ranges from 0 (no impairment) to 70 (severe impairment) and, like the MMSE, provides information about a number of cognitive domains.

The most accurate indicator of **functional impairment** is a decline in the ability to perform activities of daily living (ADL). A number of assessment scales focus particularly upon this aspect, producing scores which help to quantify disability. One such scale is the Disability Assessment for Dementia (DAD) (Gauthier *et al.* 1997) which generates a score between 0 and 100 via a structured informant interview. The questions relate to basic self-care and ADL. Other scales provide a composite score by assessing both cognitive and functional variables together. These include the AD Co-operative Study-Clinical Global Impression of Change (ADCS-CGIC) (Schneider *et al.* 1997) and the Clinical Dementia Rating (CDR) (Morris 1993). These scales necessarily combine information from patients, informants and health professionals in generating scores.

1. 7. 2. Electroencephalography

In EEG, waveforms are described by their frequency, amplitude and location. α waves (8-13Hz) are seen in conscious adults and attenuate with attention (stress, eye opening, etc). They are normally slightly higher in amplitude on the non-dominant side and

present more posteriorly than anteriorly. α rhythm in the lower frequency range (8-10.5Hz) is associated with an individual's global attentiveness whereas, in the higher frequency range, it reflects the oscillating activity of neural systems elaborating sensorimotor and semantic information (Klimesch 1999).

Beta waves (>13Hz) are variably present in adults and may be enhanced by various drugs. Theta (3.5-7.5Hz) and delta (<3Hz) waves are collectively termed 'slow waves' and are normally seen during sleep. Both are abnormal in the conscious adult.

The hallmark changes of AD include slowing of rhythms (increased theta/delta and decreased α /beta) (Brenner *et al.* 1986) and a reduction in interaction, or 'coherence', between the neural networks of different brain regions (Hogan *et al.* 2003). These changes have been shown to correlate with severity of disease (Kowalski *et al.* 2001) and, in mixed groups of cognitively normal and affected AD subjects, EEG provides significant additional value in predicting future cognitive performance as compared with cognitive measures alone (van der Hiele *et al.* 2008).

1. 7. 3. Cerebrospinal fluid

The combination of decreased $A\beta_{1-42}$ and increased tau protein is a characteristic finding in the CSF of individuals with AD (Verbeek *et al.* 2003; Lewczuk *et al.* 2004). Normal ranges are yet to be conclusively established for these variables but an upper limit of 445 pg/mL for total tau ($_t$tau) and lower limit of 427 pg/mL for $A\beta_{1-42}$ have been proposed, giving a sensitivity of 86% and specificity of 88% for both as predictors of AD (Wallin *et al.* 2006). This CSF profile predicts the presence of AD pathology (Tapiola *et al.* 2009), may help distinguish AD from both normal controls (Verbeek *et al* 2003) and VaD (de Jong *et al.* 2006), and identifies early AD in patients with MCI (Hansson *et al.* 2006). CSF

$A\beta_{1-42}$, $_t$tau and phosphorylated tau ($_p$tau) also correlate with MTL atrophy (Herukka *et al.* 2008).

It appears that Aß equilibrium is distorted, possibly via the inhibition of soluble Aß transport between brain and CSF by insoluble plaques and/or increased clearance of Aß into plaques. Binding levels of the PiB ligand (see 1. 7. 6. 1.) to cerebral Aß have been reported to correlate inversely with CSF $A\beta_{1-42}$ levels (Fagan *et al.* 2006), although the same study found no relationship between PiB binding levels and $A\beta_{1-40}$, $_t$tau or $_p$tau, plasma $A\beta_{1-40}$ or plasma $A\beta_{1-42}$. F2 isoprostanes are markers of lipid peroxidation and are therefore also markers of inflammation. Concentrations have been shown to be elevated in the CSF of individuals with MCI or AD (Pratico *et al.* 2002) though not in FTLD (Yao *et al.* 2003).

In a recent study of 21 presymptomatic carriers of *PSEN1* and *APP* mutations a low CSF $A\beta_{1-40}$: $A\beta_{1-42}$ ratio and high CSF $_t$tau, $_p$tau and F2 isoprostanes were found compared with normal controls (Ringman *et al* 2008). In the same study concentrations of $A\beta_{1-42}$ in the plasma were raised in the presymptomatic stage, falling with disease onset and progression.

1. 7. 4. Magnetic resonance imaging

Structural imaging of the neuraxis is an important component of modern neurological diagnosis. The ability to examine the brain's structure *in vivo* represented a major advance in the diagnosis of the dementias, beginning with the emergence of CT in the early 1970s. The first MRI scan of the human body followed in 1977, and structural imaging now constitutes an essential part of the investigation of dementia (Knopman *et al.* 2001). For some time, imaging was used primarily to exclude disease amenable to surgery, such as

tumours, haematomata or hydrocephalus. This remains an important role, but MRI sequences are now increasingly used to differentiate dementia syndromes from one another and from normal ageing. MRI parameters are now amongst the most promising biomarkers for early disease detection, tracking progression and assessing intervention. Its greater tissue resolution and differentiation, the facility to manipulate the sequences used in order to emphasise the desired pathology or structure, and the absence of ionizing radiation mean that MRI is now considered superior to CT in most situations. Challenges remain however: MRI is more expensive and hence less widely available and acquisition times are longer, increasing the possibility of movement artefact.

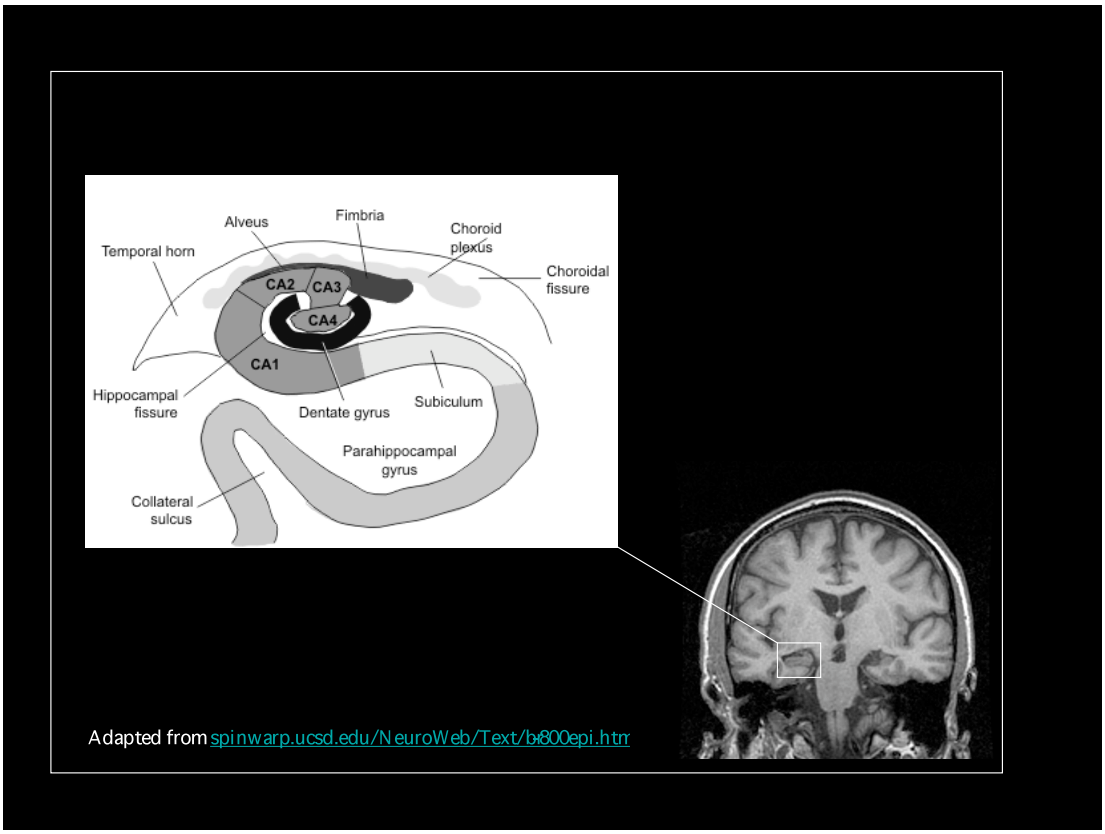
1. 7. 4. 1. Volumetric MRI

Cross-sectional MRI has been applied to the study of AD in three major ways. These comprise region of interest (ROI) analysis, examination of lobar structure and whole brain analysis. ROI studies rely upon manual delineation of the region concerned on consecutive slices of the scan, before a computer calculates the volume of the defined area by counting the voxels within it. Selection of regions *a priori* is a potential weakness of such analysis as it introduces bias, although this can be minimised through careful ROI selection. Ideal regions exhibit little anatomical variability between normal individuals, so differences between subject and control groups are more easily seen, changes can be reliably attributed to pathology rather than normal variation, and the region can be reliably delineated. Equally, it is helpful to have an idea of the magnitude of the expected difference between groups - usually achieved through neuropathological analyses. Some regions, such as the inferior temporal lobe, are particularly prone to artefact when imaged and can therefore be more challenging to assess. Neuropathological studies have indicated that MTL structures are amongst the earliest areas affected by AD pathology (Braak and Braak 1991) and hence they have provided a

focus for cross-sectional structural study. This region is known to subserve episodic memory, typically the earliest function to be affected in AD, providing a rational clinico-pathological link.

Hippocampal measurements are the best-established structural biomarker in AD whether made using time-intensive manual segmentation or automated techniques. NFT accumulate early in the hippocampus, especially in the subiculum and cornu ammonis 1 (CA1) regions (Figure 5), inducing neuronal cell death and atrophy (Convit *et al.* 1993). Hippocampal volume is reduced in MCI and AD compared with normal controls, and is a highly discriminating variable (Xu *et al.* 2000; Du *et al.* 2001) which can predict later development of AD in preclinical cases with an accuracy of approximately 80% (Wang *et al.* 2006).

Figure 5: Coronal Hippocampal Anatomy



Ante-mortem hippocampal volumes also correlate well with pathological (Jack *et al* 2002; Silbert *et al.* 2003), though probably not clinical (Ridha *et al.* 2008) severity. Frequent overlap in hippocampal volumes between AD subjects and controls can be problematic and debate over the relative merits of linear and visual rating scales compared with the more time-consuming volumetry clouds the issue still further (Pucci *et al.* 1998; Laakso *et al.* 1998; Wahlund *et al.* 2000; Scheltens *et al.* 2002). Improvements can be made by combining hippocampal measures with clinical data (Devanand *et al.* 2008) or by performing serial longitudinal imaging to establish rates of atrophy. The latter approach has been applied to both whole brain (Schott *et al* 2005) and hippocampal volumes (Barnes *et al.* 2008; Morra *et al.* 2009; Henneman *et al.* 2009). Regional fluid registration combines both automation and a longitudinal approach, and is more reliable than manual delineation in assessing hippocampal atrophy rates (van de Pol *et al.* 2007).

A key advantage of the hippocampus over the nearby parahippocampal gyrus (PHG) and ERC is that it is less laborious to evaluate. This is due to the availability of automated techniques (Colliot *et al* 2008) and the fact that these other structures are prone to artefact and relatively difficult to segment due to indistinct boundaries. The ERC, which contains major afferent pathways into the hippocampus, is nonetheless profoundly affected in the earliest stages of AD (Price *et al.* 2001) and has been studied in similar detail to the hippocampus. The two structures yield comparable levels of accuracy in distinguishing AD from normal controls (Bobinski *et al.* 1999; Juottonen *et al.* 1999; Toledo-Morrell *et al.* 2000; Pennanen *et al.* 2004). Other regions studied include the amygdala (Scott *et al.* 1991) and limbic/paralimbic structures (Callen *et al.* 2001).

1. 7. 4. 2. Cortical thickness

The ability to calculate MRI-derived cerebral CTh accurately and reproducibly is a relatively new development. Initial attempts to characterize cortical thinning in a number of disease have demonstrated that it may be complementary to existing radiological tools, and that it can provide meaningful statistical and clinical information in even small groups (Lerch and Evans 2005). Grey matter changes, often with regional specificity, are seen in normal ageing (Jack *et al.* 1997), MCI (Singh *et al.* 2006) and in a number of diseases. These include SAD (Thompson *et al.* 2003; Lerch *et al.* 2005; Frisoni *et al* 2007), FTLD (Gold *et al.* 2005), Huntington's disease (Rosas *et al.* 2002; Rosas *et al.* 2005), CBS (Boeve *et al.* 1999), MS (Sailer *et al.* 2003), amyotrophic lateral sclerosis (ALS) (Kiernan and Hudson 1994), schizophrenia (Kwon *et al.* 1999) and congenital perisylvian syndrome (Kotini *et al.* 2004). Automated CTh analysis shares many of the advantages of techniques like voxel-based morphometry (VBM), in that there is no requirement for *a priori* identification of regions of interest. However, unlike voxel density, a measurement of CTh at a given point may in itself be meaningful. The key limitation of CTh mapping is that no information is provided on sub-cortical structures.

Historically, the main difficulties in developing a robust, automated technique for measuring CTh have been the accurate delineation of the pial surface and the elaborate invagination of the neocortex. Dealing with these issues is technically demanding, particularly with current MRI resolution and the requirement for sub-millimeter accuracy (the human cortex varies in thickness between 1 and 4.5mm). The use of manual techniques with slice data has significant, inherent problems in that thickness cannot be reliably measured unless the cortical surface is orthogonal to the viewing plane. Further, partial volume effects may hinder the distinction of closely adjacent surfaces, particularly in deep sulci. Several techniques have been used in an attempt to address these two major

issues (Fischl and Dale 2000; MacDonald *et al.* 2000; Yezzi and Prince 2003). Fischl and Dale's technique is based upon a deformable model, and first seeks to define the grey/white boundary with sub-voxel accuracy before deforming outwards to find the pial surface. This produces two surfaces, each a net of 81,920 polygons, with each white matter vertex related to a grey matter equivalent. CTh is then defined as the shortest distance between these linked vertices. Sub-voxel accuracy is achieved through interpolation, using information about the intensity of each voxel, the nature of surrounding voxels (continuity information), and the ability to assume that the radius of surface curvature and thickness of tissue to be measured exceed the size of the voxels themselves. Sufficient contrast-to-noise must also exist between the different tissue classes. Because, on a millimetre scale, the cortex is smooth, representations of it at this scale should also be smooth. Many techniques exist for constraining a surface to be smooth but, by their nature, these constraints work by seeking to minimize curvature. This means that they have problems faithfully representing highly curved regions such as the fundi of sulci or crowns of gyri, and will therefore inaccurately represent them. Fischl and Dale's technique addresses this by first computing the curvature at patches of the brain surface and then altering the representation of that surface so that, on a millimetre scale, it also has this curvature.

Studies in SAD have shown a clear reduction in both regional and mean CTh with the PHG most affected (Lerch *et al* 2005). The extent and nature of clinical deficits seem to correlate with CTh in defined areas (including the MTL, left anterior cingulate and left insula) and with a well-defined sequence of grey matter loss from temporal and limbic cortices to the frontal and posterior regions, sparing sensory and motor cortices. This sequence mirrors the spread of amyloid plaques and NFT observed in post mortem studies (Braak *et al.* 1999). To date, one study has examined the different CTh patterns

associated with early and late onset forms of AD (Frisoni *et al* 2007), with AAO the sole criteria for defining the groups. Chapter 2 examines how CTh measurement can assist in the detection of cortical changes at a very early presymptomatic stage and explores the nature and distribution of such changes.

1. 7. 4. 3. ^{1}H magnetic resonance spectroscopy

Analysis of the biochemical and metabolic properties of brain tissue producing spectroscopic profiles of healthy and disease states is possible, and several metabolites have proved to be of interest in AD. N-acetyl aspartate (NAA) is located in neurones and may be a useful marker of neurone density and viability. Myoinositol (mI) is found predominantly in glial cells and is therefore found at increased levels in gliosis (Bitsch *et al.* 1999). Choline (Cho) is found mainly in membrane-bound phospholipids and creatine (Cr), being relatively stable in AD (Schuff *et al.* 2002), is often used as an internal reference.

Regional reduction in the NAA/mI ratio is seen in AD patients with the most prominent change seen in the parietal lobe grey matter (Zhu *et al.* 2006). Grey and white matter loss in AD seems to reflect known neuropathological patterns with lower NAA/Cr ratios in the posterior cingulate gyri but a normal ratio in the occipital cortex (Kantarci *et al.* 2000). MRS has been used to successfully predict cognitive decline in established AD (Kantarci *et al.* 2007), to predict progression from aMCI to AD (Metastasio *et al.* 2006) and to identify changes in the brains of *APP* and *PSEN1* mutation carriers several years before clinical onset (Godbolt *et al.* 2006). Normalization of NAA levels after treatment of AD with cholinesterase inhibitors has been reported (Modrego *et al.* 2006).

1. 7. 4. 4. Magnetization transfer ratio

Magnetization transfer ratio (MTR) imaging exploits the exchange of magnetization between two distinct groups of protons. The first pool (approximately 80%) consists of protons in intracellular or extracellular water molecules. These are relatively freely mobile, unlike the protons of the second pool that form part of macromolecules such as proteins and lipids. The two groups are connected by a constant exchange of magnetization such that a radiofrequency pulse (MT pulse) applied to the restricted pool will affect the free pool (which can be seen with MRI). The nature and extent of this exchange depends upon the restricted: free proton ratio, the strength of the RF pulse and, importantly, the physical and chemical microenvironment of the protons concerned. Deductions regarding the microstructure of the tissue in question can therefore be made.

The most widely used expression of MT characteristics is the MTR, where magnetization of the free pool is compared with and without the application of an MT pulse. Pathological processes disrupt microscopic brain architecture and hence alter (reduce) the MTR. Such microstructural change in remaining brain tissue, as measured by MTR may accompany macrostructural change (atrophy) in AD (Ridha *et al.* 2007) or may occur in the absence of atrophy in MCI (van der Flier *et al.* 2002). AD-related focal MTR changes have been noted both in the MTL (Bozzali *et al.* 2001) and outside the temporal lobes (van der Flier *et al* 2002).

1. 7. 4. 5. Diffusion-weighted & diffusion-tensor imaging

Diffusion-weighted imaging (DWI) and DTI allow visualization of the Brownian movement of water at a sub-voxel level. Physical boundaries, such as the axon sheath of a white matter fibre, both constrain diffusion (reducing diffusivity on DWI) and influence its direction such that it is greater along the axis of that boundary than across it.

This is known as anisotropy, measured as fractional anisotropy (FA), which ranges between 0 and 1 on a normalized scale, and is used in DTI to show the groups of parallel fibres which characterise white matter tracts. Regions with little or no boundaries, such as CSF have relatively unrestrained diffusion and appear isotropic on these sequences. DWI may be particularly useful in clinical practice where pathological processes such as ischaemic stroke lead to the death of tissue and the disruption of cellular boundaries to diffusion, providing a sensitive means of detecting such problems.

1. 7. 5. Positron-emission tomography

1. 7. 5. 1. ^{18}Fluorodeoxyglucose PET

^{18}F is the most widely used positron emitter, usually conjugated to form ^{18}FDG, which allows regional metabolism (expressed as cerebral metabolic rate for glucose (CMR_{glc}) in μmol glucose/100 g/min units) to be assessed. It is created by proton bombardment of ^{18}O which produces ^{18}F and a neutron. ^{18}F has a radioactive T½ of 110 minutes meaning that, unlike ^{11}C (see 1. 7. 6. 1.), ^{18}F-labelled tracers can be centrally manufactured and distributed, allowing more widespread use. Glucose is the main substrate used by the brain in producing energy for the maintenance of crucial ionic gradients, and hence neuronal function. The first step in glucose metabolism is phosphorylation by hexokinase, to which the ^{18}FDG tracer is also subject, forming the basis for its use in assessing metabolic activity. In the normal brain CMR_{glc} varies by region but is invariably higher in grey matter than in white.

Symmetrical temporoparietal, posterior cingulate and precuneus hypometabolism with later involvement of the frontal association cortices is the characteristic pattern described where ^{18}FDG PET is applied to AD (Minoshima *et al.* 1997; Herholz 2003) although, where asymmetry exists, it tends to persist. The technique is able to separate AD from

controls with as much as 93% accuracy (Herholz *et al.* 2002a) and regional posterior cingulate or hippocampal/entorhinal metabolism are of prognostic value in MCI (Minoshima *et al* 1997; de Leon *et al.* 2001). Sparing of the primary sensorimotor and visual cortices, basal ganglia and cerebellum is usual, reflecting the predominant clinical deficits associated with AD. Interestingly, metabolic deficit in the posterior association cortices can be difficult to assess as these areas have inherently higher resting metabolic rates in the normal brain meaning that, even when affected, these areas can appear much the same as the surrounding cortex may have decreased global CMR_{glc} has been observed in individuals at risk for FAD by virtue of a family history two to three years before the mean AAO for their family (Kennedy *et al* 1995a). In these cases the pattern of hypometabolism was the same as that seen in established disease.

1. 7. 6. Amyloid imaging

The earliest attempts at quantitative *in vivo* imaging of Aß were based around radio-labelling staining agents such as congo red (Klunk *et al.* 1994). A key problem was the inability of such bulky, ionized molecules to cross an intact BBB. Consequently, research became more focussed upon identifying derivatives of these substances, which maintained their affinity for fibrillar amyloid but could access the CNS. With time, progressively less ionized compounds with greater metabolic stability, brain uptake and clearance have been developed. Clinical trials in humans began with the use of ^{18}F-FDDNP (Shoghi-Jadid *et al.* 2002), a tracer which binds to both amyloid plaques and NFT. PET studies have subsequently been performed with a number of ^{11}C and ^{123}I-based compounds (Verhoeff *et al.* 2004; Opazo *et al.* 2006; Newberg *et al.* 2006). The first human studies of ^{11}C-PiB were also performed in 2002 and were reported in 2004 (Klunk *et al* 2004).

1. 7. 6. 1. Pittsburgh compound B

PET using ^{11}C-PiB represents the most successful attempt so far to visualize amyloid plaques *in vivo*. ^{11}C-PiB is a thioflavin-based radio-ligand that shows good blood-brain barrier permeability and binds with high affinity to both fibrillar and vascular amyloid (Lockhart *et al.* 2005; Johnson *et al.* 2007; Lockhart *et al.* 2007), more weakly to NFT, but not to soluble amyloid. High affinity binding sites do exist on α -synuclein filaments present in Lewy bodies, although the density/availability of these sites *in vivo* is significantly less than those associated with fibrillar amyloid and so Lewy body pathology probably contributes very little to the retention of ^{11}C-PiB (Ye *et al.* 2008).

^{11}C-PiB PET discriminates clinically probable AD patients from healthy controls, with AD cases showing two-fold greater retention in cingulate and neocortical association areas known to be targeted by amyloid deposition (Klunk *et al* 2004). ^{11}C-PiB retention correlates with rates of cerebral atrophy in AD (Archer *et al.* 2006), with decreased levels of $Aß_{1-42}$ in the CSF of demented and non-demented subjects (Fagan *et al* 2006), with post-mortem measurement of insoluble Aß levels (Ikonomovic *et al.* 2008) and inversely with rates of resting cortical glucose metabolism (Klunk *et al* 2004). The combination of ^{11}C-PiB PET and MRI seems to confer superior diagnostic accuracy to either modality alone (Jack *et al.* 2008) and retention is not influenced by anticholinesterase medication. Although ^{11}C-PiB PET localises the distribution and density of Aß plaques, these correlate inconsistently with the degree of functional or cognitive impairment (Giannakopoulos *et al* 2003). It seems more likely that the density and distribution of soluble, neurotoxic Aß oligomers are more relevant to neuronal dysfunction (Lesne *et al* 2006), but these are not detected by thioflavin based radio-ligands such as ^{11}C-PiB (Naslund *et al.* 2000).

^{11}C-PiB retention is elevated in as many as 40% of cognitively normal elderly controls (Mintun *et al* 2006; Rowe *et al.* 2007; Gomperts *et al.* 2008), in keeping with the 30% of healthy people over 75 years of age with cerebral Aß deposition at post-mortem (Price and Morris 1999). This suggests either that ^{11}C-PiB imaging is sensitive for detection of preclinical AD, or that some individuals may tolerate an amyloid burden without developing symptoms. Longitudinal study in large cohorts will be necessary to resolve this question. However, for now it seems most likely that such amyloid deposition is not a consequence of normal ageing, but rather that it represents preclinical AD (Villemagne *et al.* 2008).

^{11}C-PiB retention is increased in around 50% of MCI patients (Kemppainen *et al.* 2007) and these cases progress most rapidly to AD (Forsberg *et al.* 2008; Villemagne *et al* 2008) suggesting the potential for a prognostic/predictive role. Positive ^{11}C-PiB scans in MCI resemble those of established AD and negative scans resemble normal controls (Kemppainen *et al* 2007).

The ^{11}C of ^{11}C-PiB is produced by proton bombardment of standard nitrogen (^{14}N). The unstable ^{11}C undergoes both beta-plus decay (with positron emission) (99.77%) and electron capture (0.23%) to become the more stable boron isotope ^{11}B. Half-life (T½) is 20.38 minutes. Unfortunately ^{11}C's 20 minute radioactive T½, means that its use is restricted to sites with ^{11}C radiochemistry expertise and cyclotron facilities. It cannot be centrally produced and regionally distributed like ^{18}F ligands and so is too expensive for routine clinical use.

1. 7. 6. 2. ^{18}F BAY94-9172

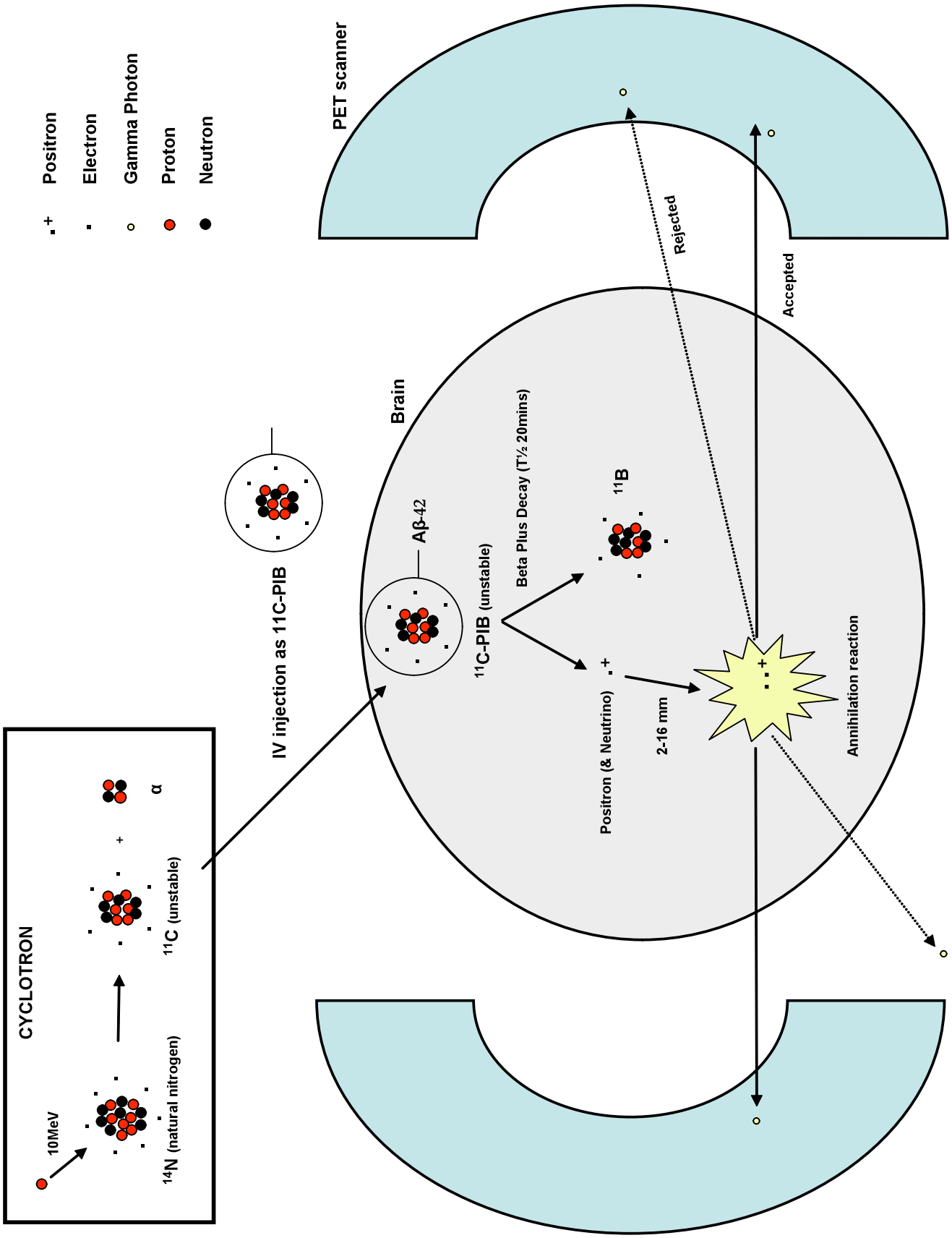
^{18}F-labelled amyloid tracers are an attractive prospect due to the practical benefits of ^{18}F over ^{11}C mentioned above but to date they have only demonstrated 9% greater uptake in AD than healthy controls (Small *et al.* 2006). This does not compare favourably to ^{11}C-PiB, which has shown up to 70% greater cortical binding in AD (Rowe *et al* 2007). Furthermore, the use of ^{11}C-PiB-based compounds requires far less scanning time. There is, therefore, a well-established need for an ^{18}F tracer with binding characteristics similar to those of ^{11}C-PiB. ^{18}F-BAY94-9172 or trans-4- (*N*-methyl-amino)-4'-{2-[2-(2-[^{18}F] fluoro-ethoxy)- ethoxy]-ethoxy}-stilbene (also known as ^{18}F-AV1/ZK) is structurally similar to PiB and competes for the same amyloid binding with similar affinity (Zhang *et al.* 2005). There is evidence to suggest that neocortical binding of ^{18}F-BAY94-9172 is similar to that of ^{11}C-PiB and that it is 57% greater than in normal controls (Rowe *et al.* 2008). This compares favourably with ^{11}C-PiB without the half-life related disadvantages discussed above.

1. 7. 7. Single photon emission computed tomography

SPECT has historically been the most widely available functional brain imaging modality. The principles are similar to those of ^{18}FDG PET, except that a γ-emitting radiopharmaceutical and a γ-camera are employed. The most commonly used γ-emitting tracer for functional SPECT brain imaging is ^{99m}Tc-HMPAO (hexamethylpropylene amine oxime) which is taken up by brain tissue in a manner proportional to cerebral blood flow. The close link between blood flow and regional metabolism makes ^{99m}Tc-HMPAO a good marker for the latter, although this link may be lost in cerebrovascular disorders, and vessel calibre/blood flow is heavily dependent upon pCO_2, making it more variable than regional glucose metabolism. Like ^{18}FDG PET and spin-labelled MRI techniques, SPECT studies have implicated temporo-parietal regions in AD (Johnson *et*

al. 2004; Pakrasi and O'Brien 2005). The evidence suggests that, in some respects, ^{18}FDG PET may be worth any extra inconvenience as it is probably superior to SPECT as a diagnostic test in AD (Herholz *et al.* 2002b). Nonetheless, accuracy of SPECT in AD diagnosis may be as high as 88% (Bonte *et al.* 2006) and meta-analysis suggests that it is superior to clinical criteria in its ability to differentiate AD from VaD, although inferior in distinguishing AD from healthy controls (Dougall *et al.* 2004). This is perhaps unsurprising if we consider that metabolic deficits are by nature more discrete in vascular disease than AD and hence more amenable to detection using regional metabolic imaging. In studies with clinico-pathological correlation, SPECT appears to be most useful when there is greatest clinical uncertainty, adding least where the clinical diagnosis is clear and most where is not (Jagust *et al.* 2001).

Figure 6: Diagrammatic ^{11}C-PiB PET process



1. 8. Alzheimer's disease therapy

A number of strategies exist for the treatment of AD, each addressing a distinct phase of the pathological process. Many are still in the very early stages of research and a truly disease-modifying agent remains elusive. In theory, it should be possible to intervene at various stages of the amyloid cascade:

1. 8. 1. Decreasing Aß production

This requires intervention at a relatively early stage of the amyloid cascade. Since the amyloidogenic pathway requires the action of ß-secretase (BACE-1) and γ-secretase, both are attractive targets for novel therapies. BACE-1 inhibitors are particularly promising as BACE-knockout mice are apparently otherwise healthy, show no detectable ß-secretase activity, and produce much less Aß from APP in primary cortical cultures (Roberds *et al.* 2001). Potent inhibitors have been developed which are effective *in vitro* and in transgenic mouse models (Rockenstein *et al.* 2001) and early data in humans are beginning to emerge (http://www.alzforum.org/new/detail.asp?id=1790). Just as BACE-1 may be up-regulated by inflammation, it may be down-regulated by NSAIDs via the stimulation of peroxisome proliferated activated receptor-γ (PPAR-γ). Treatment with specific PPAR-γ agonists (rosiglitazone) has previously been associated with significant cognitive improvement in AD albeit only in ApoE4 negative individuals (Risner *et al.* 2006). Oral administration of the GSK188909 BACE-1 inhibitor leads to significant reduction in levels of Aß in the mouse brain (Hussain *et al.* 2007).

Inhibition of γ-secretase is more problematic as the enzyme is responsible for cleaving a number of other important proteins, such as the notch-1 protein, which has an important role in normal growth. Despite this, there are currently several γ-secretase inhibitors, some 'notch-sparing', in phase II trials. A related strategy is to exploit the variability of

the γ-secretase cleavage site in an attempt to increase the $Aß_{1-40}$: $Aß_{1-42}$ ratio. This ensures that the shorter, more soluble, less toxic Aß species predominate. Agents capable of such modulation are termed 'Selective ß-Amyloid Lowering Agents' or SALAs. They include NSAIDs (Weggen *et al.* 2001) and *Flurizan*, a compound which reached phase III trials in mild AD before it failed to achieve significant benefits in primary endpoints, prompting recent discontinuation of its development.

As the action of α -secretase precludes the production of Aß, its potentiation is a further theoretical option. This is achievable, for example, by stimulating muscarinic M1 receptors. The M1 agonist talsaclidine can affect CSF $Aß_{1-42}$ (Hock *et al.* 2003), although there are presently no clinical data to support the use of such agents.

1. 8. 2. Facilitating Aß clearance

Once the Aß has been allowed to form, an alternative approach is to enhance its clearance and thus limit its toxicity. This is most effective if it is cleared prior to oligomerization and may, in theory, be achieved by several means. Immunotherapy is a promising and popular area and can facilitate the removal of Aß via a selection of mechanisms. These include opsonization and Fc-dependent phagocytosis, direct antibody-mediated dissolution and sequestration of amyloid in the plasma such that a gradient is established which favours movement of amyloid out of the CNS. Chronic, low level activation of innate immunity has been demonstrated in AD (Akiyama *et al.* 2000) , although microglia do not seem to effectively clear Aß deposits (Wisniewski *et al.* 1989). One recently tested strategy is the manipulation of cytokine signalling in peripheral macrophages in an attempt to promote cerebral Aß clearance. Blockade of TGF-ß1, for example, appears to attenuate parenchymal and vascular Aß (Town *et al.* 2008).

In 1999, Schenk *et al.* used transgenic platelet-derived growth factor promoter-expressing *APP* (PDAPP) mice which over-expressed human mutant APP to investigate active immunisation. These mice exhibit, in an age and brain region-dependent fashion, amyloid lesions (though not tau pathology) similar to that seen in human AD (Games *et al.* 1995). In younger mice, immunization with human $A\beta_{1-42}$ resulted in reduced plaque formation, neuritic dystrophy and gliosis while in older mice there was a reduction in the progression of the same. Clinical improvement was later demonstrated in a similar experiment using a standard radial arm water maze with submerged platform (Morgan *et al.* 2000). The first trial of passive immunotherapy in mice demonstrated that peripherally administered immunoglobulin entered the CNS, decorated plaques and induced microglia to clear plaque Aß via FcR-mediated phagocytosis and peptide degradation (Bard *et al* 2000). Synthetic $A\beta_{1-42}$ (AN1792) with an adjuvant T-helper (QS-21) was developed for human trials which entered phase II in October 2001 (Gilman *et al* 2005). Of the 372 AD patients randomized, 298 received AN1792. Two primary efficacy endpoints were identified relating to the whole brain volume as measured by longitudinal MRI and cognition as measured by the ADAS-cog. Only those who mounted an antibody response to vaccination (serum titre >1: 2200) were evaluated. Unfortunately, 18 cases of meningoencephalitis (Orgogozo *et al.* 2003) forced the trial to end prematurely. Blinding was maintained along with the safety and tolerability assessments for the ensuing nine months. Studies using peripheral blood mononuclear cells from AN1792-exposed patients suggest that AN1792 can induce a class II MHC restricted Th1 type response against itself. This, in combination with the neuropathological findings, suggests that an aberrant T-cell response may have been responsible for the encephalitis observed. The key efficacy signals from the AN1792 study were that antibodies selective for Aß cross the BBB, that they are specific for the N-terminus of Aß and that a number of signals are associated with an elevated titre. These signals included a significant reduction in brain

volume (Fox *et al* 2005), memory improvement at 12 months, less decline in executive function at 12 months, clearance of Aß, lower CSF tau levels and improvement in activities of daily living at 18 months. Post-mortem data indicated that neuropathological findings were similar to those of the mice with focal depletion of Aß, persistent NFT and immunoreactive macrophages showing aggregates of Aß. Encephalitis correlated with numerous T-cell infiltrates and white matter change.

Passive, as opposed to active, immunotherapy has several potential advantages. It bypasses the need for the recipient (who is usually elderly) to mount an antibody response to the vaccine, has shown similar amyloid removal benefits in animal models (Bard *et al* 2000) and allows antibody levels to be controlled by changing the dose and frequency of administration. Also, it theoretically removes the risk of inducing the aberrant T-cell response thought to be responsible for encephalitis. This strategy may, however, have its own problems: cerebral haemorrhage has been linked with passive anti-Aß therapy as clearance of Aß from vessels may damage their integrity (Pfeifer *et al.* 2002). A humanized monoclonal antibody has been tested in a phase II multiple ascending dose study in mild to moderate AD (Grundman and Black 2008), and a phase III trial is underway. PiB PET imaging techniques have been used to assess the success of this approach (see chapter 3). IVIG, IFN-α and TNF-α blockade with etanercept are currently in phase I trials (Dodel *et al.* 2004; Tobinick 2006; Yamamoto *et al.* 2007).

There are no clinical data concerning enzymatic degradation of Aß using metalloproteases such as NEP, IDE and ECE, although preclinical experience is promising. NEP knockout mice have elevated Aß levels (Hellstrom-Lindahl *et al.* 2008) and Aß levels may be reduced *in vitro* by IDE, ECE and angiotensin converting enzyme (ACE) (Eckman *et al.* 2006).

It may be possible to regulate the passage of Aß across the BBB in either direction by targeting the receptors responsible for trafficking. Two main receptors are implicated: RAGE and LRP-1. The former principally transports Aß into the brain from the systemic circulation and the latter mediates its exit from the brain. The ratio of activity of these receptors is therefore highly relevant and is raised in the AD brain. No clinical trials addressing this strategy have been performed although statins (HMG-CoA reductase inhibitors) are known to up-regulate LRP-1 (Deane *et al* 2004).

1. 8. 3. Preventing Aß aggregation

Inhibition of Aß aggregation into toxic oligomeric species is a further potential strategy. Aß has binding sites for both zinc and copper and both have a role in its aggregation. Early studies of agents such as cloquinol, which chelate metal ions, have shown promise (Ritchie *et al.* 2003). However, the only aggregation inhibitor to reach phase III trials is the synthetic glycosaminoglycan 3-amino-1-propaneosulfonic acid (3APS, tramiprosate or '*Alzhemed*') (Geerts 2004), an agent which interferes with the binding of Aß to glycosaminoglycans, a necessary stage in the assembly of oligomers and fibrils. These trials were recently discontinued due to disappointing results.

1. 8. 4. Neurotransmitter manipulation

Donepezil, galantamine and rivastigmine are currently licensed in the UK for the management of moderately severe AD (NICE 2007). They belong to a group of drugs that degrade acetylcholinesterase (AChE), the enzyme responsible for hydrolysing ACh. The earliest such drugs were physostigmine and tacrine, which proved impractical for use in AD due to poor penetration of the BBB and hepatotoxicity respectively. Amongst the newer agents, randomized, double-blind, placebo-controlled trials have demonstrated roughly equivalent efficacy in producing small but statistically significant improvements

in global function and cognition in mild to moderately severe AD (Birks 2006). There are few direct comparisons however, and no strong evidence of important differences between them: only one randomized, double blind study has compared two such agents directly (Bullock *et al.* 2005). This showed no differences at two years for measures of cognitive function and behavioural disturbance although donepezil was associated with fewer adverse events. Indeed donepezil remains the most widely prescribed drug for AD, at a maximum licensed dose of 10mg OD. Mild gastrointestinal side effects are common with all AChE inhibitors, though they may attenuate with time and/or careful titration.

Galantamine was originally extracted from the snowdrop (*Galanthus nivalis*) although is now procured from the more abundant daffodil (*Narcissus pseudonarcissus*). It has a dual mechanism of action both enhancing the effect of ACh upon nicotinic receptors and reversibly inhibiting AChE.

Rivastigmine is a reversible inhibitor of AChE and butyrylcholinesterase (BuChE) (unlike donepezil and galantamine which show no functional inhibition of the latter). In addition it displays a ten-fold greater affinity for brain AChE than for peripheral AChE. It has been suggested that such dual inhibition may confer more sustained efficacy, or even modify disease (Bullock 2002). The first transdermal AD treatment has recently been developed in the form of a rivastigmine patch (Lefevre *et al.* 2008).

Introduced in 2002, memantine is an antagonist at the N-methyl-D-aspartate (NMDA) receptor for glutamate and is thought to exert a beneficial effect in AD by ameliorating excitotoxicity. Benefits in functional and global outcome measures were established in moderate to severe AD some years ago (Reisberg *et al.* 2003) and it remains the only drug licensed for severe dementia in the UK. However, evidence for statistically significant

improvement in ADAS-cog and CIBIC-plus scores in mild to moderate disease also now exists (Bakchine and Loft 2008). Memantine may provide additional positive effects when combined with donepezil (Tariot *et al.* 2004).

Dimebolin Hydrochloride (Dimebon), originally developed as a non-selective antihistamine, is a weak inhibitor of BuChE and AChE, and a blocker of the NMDA receptor (Bachurin *et al.* 2001). A neuroprotective capability has also been suggested (Lermontova *et al.* 2001). A recent randomised, placebo-controlled study showed a significant benefit as measured by ADAS-cog (Doody *et al.* 2008). A mean difference of 4 points was seen at 26 weeks while differences ranging from 1.5 points to 3.9 points have been found in randomised clinical trials of AChE inhibitors (Kaduszkiewicz *et al.* 2005).

2.

THE FAD PARADIGM

I: Structural neuroimaging

Chapters 2 and 3 contain studies which apply the FAD paradigm to study early disease features, circumventing problems inherent in the study of SAD. They help to further characterize radiological phenotypes associated with FAD, SAD and normal ageing, while assessing the potential of two relatively new imaging techniques as biomarkers of onset, progression and intervention. The first such biomarker is CTh, a potentially more reliable marker than volume as the cytoarchitectural structure of the grey matter is less variable (Pakkenberg and Gundersen 1997; Singh *et al* 2006). Changes in the cortex are a well established consequence of AD pathology with layer II of the ERC and layer III of the neocortex preferentially affected (Pearson *et al.* 1985; Gomez-Isla *et al* 1996). Furthermore, fully automated cortical surface reconstruction is now possible, providing detailed information on the thickness of the cortical ribbon. This technique is complementary to existing radiological tools, and may provide meaningful statistical and clinical information even in small groups (Lerch and Evans 2005) (see 1. 7. 4. 2.). Regional CTh measures in established AD, including that of the MTL, left anterior cingulate, frontal and parietal lobes, correlates well with the severity and nature of clinical deficits (Thompson *et al* 2003; Du *et al.* 2007; Dickerson *et al.* 2009) and its measurement continues to show promise as a clinical and research tool (Lerch *et al.* 2008). However, the potential of CTh as a biomarker in confirmed FAD mutation carriers has not yet been fully defined. The objective of this study was to examine longitudinal patterns of CTh in a cohort of initially pre-symptomatic FAD mutation carriers (MC) in a period which included symptom onset and clinical diagnosis of AD.

2. 1. Methods

2. 1. 1. Subjects

In a previously described MRI study of atrophy progression in FAD (Ridha *et al* 2006), 9 carriers (four men) of autosomal dominant mutations associated with early-onset FAD (MC) underwent longitudinal volumetric MRI assessment (Table 1). Five had mutations in *APP* (2 V717G, 2 V717I and 1 V717L; mean 4.3 years before clinical diagnosis at baseline scan, range 1.2-7.6; mean AAO 52.7, range 48.0-61.1) and four in *PSEN1* (2 *delta* 4, 1 L250S and 1 M139V; mean 4.2 years before clinical diagnosis at baseline scan, range 2.6-5; mean AAO 42.2, range 37.7-49.8). 25 age and sex-matched controls were recruited from spouses (n=7), unaffected relatives including those with negative genetic test results (n=4), and healthy volunteers (n=14). Between 1991 and 2005, all MC (mean 4.3 years before clinical diagnosis at baseline scan, range 1.2-7.6; mean AAO 48, range 37.7-61.1) and controls had serial volumetric MRI, which spanned the time of clinical diagnosis. Written, informed consent was gained from each participant.

Table 1: Demographic & scan information

	CONTROLS	MC
n	**25**	9
Mean age at first scan (SD) (years)	**45.8 (10.4)**	43.8 (6.7)
Mean number of scans (range)	**2.2 (2-4)**	4.6 (2-8)
Mean follow-up time (range) (years)	1.5 (0.5-4.1)	4.9 (1.9-9.7)

2. 1. 2. Image acquisition

Baseline scans were performed between 1991 and 2001. Imaging between 1991 and 2000 was performed using a 1.5T GE Signa scanner (General Electric Medical Systems, Waukesha, WI, USA). Volumetric T1-weighted coronal MRI scans were obtained with a spoiled gradient echo technique (256×128 matrix, field of view 24×24 cm, TR/TE/NEX/FA=35 ms/5 ms/1/35°) yielding 124 contiguous 1.5 mm thick slices. Between 2000 and 2005 imaging was performed using a different 1.5T GE Signa scanner running software version 5.8 (General Electric Medical Systems, Waukesha, WI, USA). Volumetric T1-weighted coronal MRI scans were obtained using an inversion recovery prepared fast spoiled gradient-echo technique (256×256 matrix, field of view 24×18 cm, TR/TE/TI/NEX/FA=14 ms/5·4 ms/650 ms/1/15°) yielding 124 contiguous 1·5 mm thick slices.

Detailed cortical surface construction and thickness estimation was achieved by applying Freesurfer software (http://surfer.nmr.mgh.harvard.edu/) to the resulting images, a technique previously described and validated (Dale *et al.* 1999; Fischl *et al.* 1999; Fischl and Dale 2000). This was performed on a 64-bit Linux CentOS 4 Cluster managed by a Sun Grid Engine. Briefly, the process involves initially generating an automatic grey matter, white matter and CSF classification. The results of these segmentations were visually inspected, and if needed, manually edited by adding control points. Finally, an automatic reconstruction of the cortex was produced and CTh estimated by computing the average shortest distance between the white matter boundary and the pial surface. The standard Freesurfer processing stream was used apart from one modification: we used locally-generated brain masks for the skull-stripping process. This brain mask was produced using a semi-automated segmentation procedure that involved selection of

thresholds, followed by a series of erosions and dilations, yielding a brain region separated from surrounding CSF, skull and dura.

2. 2. Cortical thinning in FAD: a cross-sectional study

2. 2. 1. Methods

In this study, baseline scans from PMC (mean 4.3 years before symptom onset, range 1.2-7.6) and controls were compared. Further comparison was made between the baseline scans of *APP* (mean 4.3 years before symptom onset, range 1.2-7.6) and *PSEN1* (mean 4.2 years before symptom onset, range 2.6-5) mutation carriers using two approaches:

- Surface maps of all groups were generated following registration of cortical reconstructions to a common average surface and then smoothed using a surface-based Gaussian kernel of 20mm full width half-maximum. A vertex-by-vertex analysis using a general linear model was performed to examine differences in CTh between the patient group and the control group. CTh, C, was modelled as a function of group, controlling for age, sex and the scanner used by including them as nuisance covariates. $C=ß_1$ FAD + $ß_2$ controls + $ß_3$ age + $ß_4$ sex + $ß_5$ scanner + μ + ε (where μ is a constant, and ε is error) with the contrasts of interest being the two-tailed t-test between the estimates of the group parameters, i.e. $ß_1$ and $ß_2$. Thresholding the images of t-statistics at a 0.05 significance level generated maps of the differences between groups at this level of significance. We repeated these analyses after sub-stratifying for mutation.

- Regions of interest generated during the Freesurfer processing stream were combined to give mean thickness values for the frontal, temporal and parietal lobes as well as the cingulate cortex in both hemispheres in each patient, and previously reported total

hippocampal volumes from the same cohort (Ridha *et al* 2006) retrieved for comparison. Differences by patient group were investigated using regression analyses adjusted for age, sex and scanner. Robust standard errors were used to address any differences in variance between the patient groups. Mean hippocampal volumes were also compared using this method.

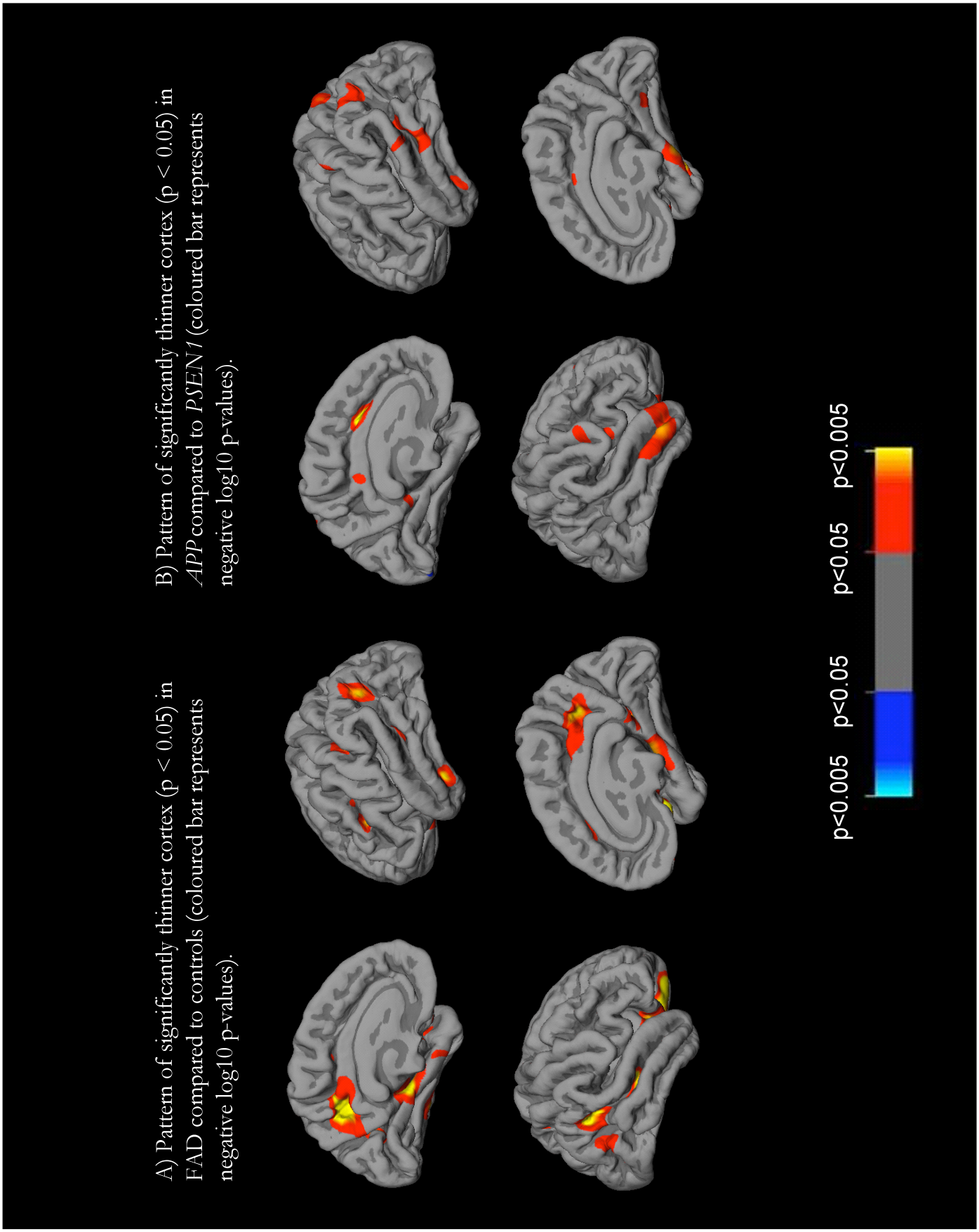
2. 2. 2. Results

CTh maps (Figure 7) revealed distinct anatomical patterns of cortical thinning in the FAD group and in each mutation subgroup. Figure 7a shows the distribution of statistically significant (p<0.05), uncorrected differences between the FAD and control groups. Compared with the healthy control group, the most prominent grey matter loss in the FAD group as a whole was seen in the inferomedial temporal lobe, precuneus, posterior cingulate, posterior superior temporal sulcus, parieto-occipital junction and orbitofrontal cortex in both hemispheres. Figure 7b shows the distribution of statistically significant (p<0.05), uncorrected differences between the *APP* and *PSEN1* mutation subgroups. The *APP* carriers had significantly thinner cortices than *PSEN1* carriers in the middle temporal, inferior temporal and parahippocampal gyri as well as the precuneus. No changes survived statistical correction for multiple comparisons.

Comparison of group CTh means by lobe, as well as total hippocampal volume (Table 2) broadly corroborated the patterns of change on CTh maps. The FAD group and *APP* subgroup had lower mean thickness than controls in frontal, temporal and parietal lobes, and in the cingulate gyrus, as well as lower mean total hippocampal volumes. Mean CTh in the *APP* group was lower than the *PSEN1* group across all regions examined, with the most prominent CTh differences seen in the temporal lobes. The *APP* subgroup was largely responsible for the differences observed between FAD and control groups,

despite the small (0.1 years) difference in the mean time before symptom onset between the subgroups. Mean hippocampal volumes were lower in the FAD, *APP* and *PSEN1* groups than in controls, and mean total hippocampal volume was lower in the *APP* than the *PSEN1* group.

Figure 7: Surface maps of CTh



2. 2. 3. Discussion

Using a fully automated method, we measured CTh across the entire brain in order to investigate cross-sectional differences between presymptomatic FAD mutation carriers and age- and sex-matched controls. The CTh profiles of carriers of the two most common FAD gene mutations were also examined individually.

Cross-sectional comparison demonstrated differences between mutation carriers and controls occurring several years before symptom onset and, although they did not survive statistical correction for multiple comparisons, our findings are in keeping with previous volumetric data in the same groups (Ridha *et al* 2006) and implicate regions which are thought to be significant in early AD. There is much imaging and pathological evidence that these regions comprise the limbic and heteromodal association cortices (Minoshima *et al* 1997; Juottonen *et al* 1999; Xu *et al* 2000; Lerch *et al* 2005; Mosconi *et al.* 2006; Dickerson *et al* 2009).

In addition, VBM and CTh analyses, including a recent study of MCI patients destined to progress to AD (Bakkour A *et al.* 2008), have confirmed that very early changes occur in the medial temporal cortex, inferior temporal gyrus, superior parietal lobule, temporal pole and precuneus with lateral temporal lobe and other areas of the neocortex affected later (Chetelat *et al.* 2002; Singh *et al* 2006; Bakkour A *et al* 2008). Many of these regions are highlighted as significant in Figure 7. A well-defined sequence of cortical thinning is recognized thereafter, progressing from temporal and limbic cortices to the frontal and posterior regions, sparing sensory and motor cortices. This spreading wave of grey matter loss mirrors the spread of amyloid and tau pathology observed in post mortem studies (Braak and Braak 1991; Braak and Braak 1996a). Such reports of early, significant change in the MTL and retrosplenial cortex are also in keeping with our findings. Further, our results are broadly consistent with observations made in established, late-onset SAD, in

which reduction of both regional and mean CTh has been documented with the PHG and cingulate of particular importance in discriminating SAD from healthy controls (Lerch *et al* 2005; Lerch *et al* 2008). Similarly, we found particular differences in the left cingulate gyrus when comparing the *PSEN1* group with controls. As mentioned earlier, EOAD and FAD are not equivalent but FAD does form part of a broader group of early onset disease. Previous contrasting descriptions of the cortical profiles of early and late onset disease (Frisoni *et al* 2007) help to further establish historical context for our results. In one study, patients were stratified by age of onset (before or after 65 years) rather than genetic status and were symptomatic at the time of analysis. None of the EOAD subjects had a history suggestive of autosomal dominant disease and groups were matched for clinical severity. Statistically significant grey matter loss was confined to the temporoparietal and retrosplenial cortices in LOAD, but was more widespread in EOAD. The cortical signature of symptomatic LOAD therefore has similarities with the pattern we have described in the presymptomatic FAD group. This may reflect more aggressive pathological change in the early-onset sporadic forms of the disease, greater functional cognitive reserve amongst EOAD subjects, or genuine differences in the onset of 'biological' (i.e. pathological) change.

Cortical thinning in the frontal lobe, albeit in the dorsolateral rather than orbitofrontal cortex, has been noted previously in symptomatic AD (Du *et al* 2007) though not, as we found, in pre-symptomatic individuals destined to develop clinical disease. Also, primate studies have previously confirmed close links between the hippocampal formation and the orbitofrontal cortex (Barbas and Blatt 1995). The distribution of cortical changes we have identified can be understood in terms of distributed but functionally connected cortical networks: a limbic/para-limbic network comprising the mesial temporal structures and their projections, and an overlapping but distinct posterior temporo-

parietal-prefrontal network. While this study does not address the issue of phenotypic correlation, preferential involvement of these cortical networks might be predicted to give rise to altered emotional and attentional processing, respectively, as early features in FAD mutation carriers. Such functions are not well captured by conventional neuropsychometric techniques, but do suggest a direction for future work.

In this study, cross-sectional differences in CTh between FAD and control groups were driven largely by the *APP* rather than the *PSEN1* carriers, as far more extensive differences were noted in the former group. Furthermore, the *APP* group had more cortical thinning than the *PSEN1* group and hippocampal volume measurements, which have previously been shown to separate presymptomatic FAD from controls (Ridha *et al* 2006), were smaller in the *APP* than in the *PSEN1* group. The largest absolute CTh differences were seen in the temporal lobes, although statistical significance at the 0.05 level selected was not demonstrated, possibly due to insufficient power associated with small group sizes. These differences between the mutation subgroups were not attributable to any discrepancy of interval from scanning to symptom onset between the groups. In light of the substantially younger mean AAO of the *PSEN1* compared with the *APP* cases, it is possible that *APP* mutations produce earlier pathological changes that subsequently progress more slowly, although larger groups would be required to confirm that the groups genuinely have different imaging profiles at equivalent stages. There are currently no data directly comparing the imaging characteristics of *APP* and *PSEN1* carriers at equivalent biological disease stage.

Table 2: Differences in Group CTh Means by Lobe (mm)

GROUPSCOMPARED / LOBE		(FAD) – (CONTROLS) (95% CI)	*(APP)* – (CONTROLS) (95% CI)	*(PSEN1)* – (CONTROLS) (95% CI)	*(APP)* – *(PSEN1)* (95% CI)
FRONTAL	**LEFT**	-0.02(-0.143, 0.11) p=0.8	-0.04(-0.23, 0.15) p=0.7	0.02(-0.13, 0.18) p=0.8	-0.06(-0.28, 0.17) p=0.6
	RIGHT	-0.04(-0.16, 0.08) p=0.5	-0.06(-0.25, 0.14) p=0.5	-0.01(-0.12, 0.10) p=0.8	-0.04(-0.26, 0.17) p=0.7
TEMPORAL	**LEFT**	-0.07(-0.19, 0.06) p=0.3	-0.13(-0.31, 0.05) p=0.1	0.02(-0.10, 0.14) p=0.7	-0.15(-0.36, 0.06) p=0.1
	RIGHT	-0.08(-0.22, 0.06) p=0.2	-0.13(-0.35, 0.08) p=0.2	-0.01(-0.11, 0.09) p=0.8	-0.12(-0.35, 0.11) p=0.3
PARIETAL	**LEFT**	-0.08(-0.20, 0.04) p=0.2	-0.10(-0.28, 0.07) p=0.2	-0.05(-0.20, 0.11) p=0.5	-0.06(-0.28, 0.17) p=0.6
	RIGHT	-0.07(-0.21, 0.07) p=0.3	-0.09(-0.29, 0.11) p=0.4	-0.05(-0.21, 0.12) p=0.6	-0.04(-0.30, 0.21) p=0.7
CINGULATE	**LEFT**	-0.02(-0.14, 0.10) p=0.7	-0.11(-0.25, 0.03) p=0.1	0.10(-0.003, 0.21) p=0.06	-0.21(-0.37, -0.08) p=0.008
	RIGHT	-0.03(-0.16, 0.09) p=0.6	-0.06(-0.75, 0.13) p=0.5	0.004(-0.14, 0.15) p=1.0	-0.07(-0.30, 0.17) p=0.6
TOTAL HIPPOCAMPAL VOLUME (mm^3)		-609(-1125, -94) p=0.02	-709(-1386, -31) p=0.04	-478(-1291, 335) p=0.2	-231(-1281, 820) p=0.7

2. 3. Cortical thinning in FAD: a longitudinal study

2. 3. 1. Methods

This longitudinal study made use of all the scans in order to examine CTh changes over a period which included symptom onset. I identified four ROI in which there is existing evidence for early structural and/or functional cortical change (see Discussion): ERC, posterior cingulate, PHG and precuneus. I selected two control regions for their relative preservation in typical AD: pericalcarine and paracentral.

For each region, a linear mixed model (developed with expert statistical support – see acknowledgements) was used to describe the association between CTh and age in the control group, adjusting for scanner. Denoting CTh and age (in years) at the j^{th} scan for the i^{th} patient by CTh_{ij} and a_{ij} respectively and with s_{ij} taking the values 0 and 1 according to scanner:

$$CTh_{ij} = (\beta_0 + b_{0i}) + \beta_1 s_{ij} + (\beta_2 + b_{2i}) a_{ij} + \varepsilon_{ij} \quad (1)$$

with $\begin{pmatrix} b_{0i} \\ b_{2i} \end{pmatrix} \sim N\left[\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \sum_b\right]$ and independently $\varepsilon_{ij} \sim N(0, \sigma_0^2)$.

A similar model was used to describe the relationship between CTh and years since clinical diagnosis in the MC group. A fixed quadratic term describing acceleration in the rate of decline over time was added. Denoting years since clinical diagnosis at the j^{th} scan for the i^{th} patient by d_{ij}:

$$CTh_{ij} = (\beta_0 + b_{0i}) + \beta_1 s_{ij} + (\beta_3 + b_{3i}) d_{ij} + \beta_4 d_{ij}^2 + \varepsilon_{ij} \quad (2)$$

with $\begin{pmatrix} b_{0i} \\ b_{3i} \end{pmatrix} \sim N\left[\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \sum_b\right]$ and independently $\varepsilon_{ij} \sim N(0, \sigma_1^2)$

For each region, a combined model for both MC and controls was fitted to describe the way in which MC deviated from normal ageing in controls. With d_{ij} taking the value zero in controls, and with g_i taking the values 0 and 1 in controls and MC respectively:

$$CTh_{ij} = (\beta_0 + b_{0i}) + \beta_1 s_{ij} + (\beta_2 + b_{2i}) a_{ij} + (\beta_3 + b_{3i}) d_{ij} + \beta_4 d_{ij}^2 + (\beta_5 + b_{5i}) g_i + \varepsilon_{ij} \quad (3)$$

with $\begin{pmatrix} b_{0i} \\ b_{2i} \\ b_{3i} \\ b_{5i} \end{pmatrix} \sim N\left[\begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}, \sum_b\right]$ and independently $\varepsilon_{ij} \sim N(0, \sigma_{g_i}^2)$.

The b_{2i} and b_{3i} random effects for age and years since clinical diagnosis respectively were only included in regions showing evidence of the need for such effects in the group-specific analysis. If the covariance between b_{0i} and b_{5i} could not be estimated, then this was set to zero. For individuals d years after diagnosis, the estimated difference in mean CTh between MC and controls is given by $CTh_{diff} = \beta_3 d + \beta_4 d^2 + \beta_5$. The statistical significance of a Wald test of this linear combination of parameter estimates was determined for a range of values of d. The value of d corresponding to a one-sided p-value of 0.05 identified the earliest point at which there was a significant difference between cases and controls, allowing for normal ageing.

2. 3. 2. Results

The nature of the decline in CTh with age was characterized using a mixed model (1) in the control group. There was no evidence that the decline varied between individuals in any region and so the random effects (b_{2i}) were omitted from the models. Table 3 shows the estimated age-related decline in CTh in controls. For example, in the posterior cingulate, the mean CTh for a 50-year old is 4.46-(0.014x(50-45)) = 4.39mm, where the

age-related decline is estimated to be 0.014mm/year. There was strong evidence for a decline in CTh with age in the posterior cingulate (change of -0.014mm/yr (95% CI -0.023 to -0.004)), and borderline evidence for a decline in the precuneus (change of -0.009mm/yr (95% CI -0.020 to 0.002)) and the paracentral region (change of -0.011mm/yr (95% CI -0.024 to 0.001)). However, there was also borderline evidence of an increase in the CTh of the ERC (change of 0.017mm/yr (95% CI -0.003 to 0.038)). There was no evidence for a change in CTh in the PHG or pericalcarine regions with age.

A similar analysis was carried out in the MC group to investigate changes in CTh with respect to time since clinical diagnosis of AD. There was no strong evidence that the rate of decline varied between individuals in any region and so the random effects (b_{3i}) were omitted from the models. There was evidence of accelerated decline in all four regions of interest, but no evidence of acceleration in either of the control regions and so quadratic acceleration terms were retained in each model. The estimated mean CTh in each region according to years since diagnosis is displayed in Figure 8. Table 4 shows the estimated change in CTh in MC in relation to years since diagnosis. For example, in the precuneus, the estimated mean CTh at diagnosis is 3.11mm. The estimated reduction in mean CTh in the final year before diagnosis is 0.10 mm, whilst that in the first year after diagnosis is 0.11mm, corresponding to acceleration in the rate of loss of 0.01mm/year^2. From these values the rate of loss in the penultimate year prior to diagnosis can be inferred to be 0.09mm/year and that in the second year post diagnosis to be 0.12mm/year etc. The mean levels of CTh at two years prior to diagnosis, one year prior to diagnosis, at diagnosis, one-year post-diagnosis and two years post-diagnosis are therefore 3.30mm, 3.21mm, 3.11, 3.00 and 2.88mm respectively.

The final analysis combined data from both MC and controls in order to adjust the results in Table 4 for normal ageing. Table 5 shows departures from normal ageing according to years since diagnosis in MC and also gives estimates of the number of years before/after clinical diagnosis of AD at which MC had significantly different CTh than controls. Since there was no strong evidence in the control/MC-specific models for random effects with either age or years since clinical diagnosis, these terms were not included in the combined model. The table reports estimated changes in mean CTh in MC over and above those seen in controls of the same age according to the number of years from diagnosis in the MC. For example, in the precuneus, the mean CTh at diagnosis is 0.50mm below that of a control of the same age. The estimated reduction in mean CTh in the final year before diagnosis is 0.09 mm greater than that in a control of the same age, whilst that in the first year after diagnosis is 0.10mm greater, corresponding to an acceleration in the rate of loss (relative to that in controls) of 0.01mm/year^2. From these values the rate of loss in the penultimate year prior to diagnosis can be inferred to be 0.08mm/year greater than that in a control of the same age and that in the second year post diagnosis to be 0.11mm/year greater etc. The mean levels of CTh at two years prior to diagnosis, one year prior to diagnosis, at diagnosis, one-year post-diagnosis and two years post-diagnosis are therefore respectively 0.33mm, 0.41mm, 0.50mm, 0.60mm and 0.71mm lower than that in a control of the same age. In both the ERC and PHG, the analysis suggested that CTh did not identify MC as significantly different from controls until after clinical diagnosis. However, in the posterior cingulate and precuneus, CTh measurements identified a significant difference between MC and controls 1.8 years and 4.1 years before clinical diagnosis respectively. Figure 8a illustrates the difference in mean CTh between cases and controls with respect to time since clinical diagnosis in the cases. The point at which the upper bound of the 90% CI indicates this difference is zero therefore corresponds to the years since diagnosis at which there is a significant

difference using a one-sided test. In contrast, in both the control regions, CTh did not differ significantly between the two groups at any point in the study period. Surface maps of regions differing early between MC and control groups are shown in Figure 9.

Table 3: Mean regional CTh in controls according to age (adjusted for scanner)

REGION	Mean CTh at age 45 (mm) (95% CI)[$]	Change in mean CTh per year of age (mm) (95% CI)* p-value
ROI		
ERC**	5.82 (5.15, 6.50)	0.017 (-0.003, 0.038), p=0.1
PHG	5.32 (4.78, 5.87)	-0.008 (-0.025, 0.009), p=0.4
Posterior cingulate	4.46 (4.16, 4.75)	-0.014 (-0.023, -0.004), p=0.005
Precuneus	3.74 (3.40, 4.08)	-0.009 (-0.020, 0.002), p=0.1
CONTROL REGION		
Paracentral	3.49 (3.11, 3.87)	-0.011 (-0.024, 0.001), p=0.08
Pericalcarine	2.59 (2.38, 2.80)	-0.003 (-0.010, 0.004), p=0.4

[$] For scanner 1

* Positive values indicate gain with age

** One individual was removed from the analysis due to probable Freesurfer error in calculating cortical thickness

Table 4: Mean regional CTh in mutation carriers according to time since clinical diagnosis of AD (adjusted for scanner)

REGION	Mean CTh at diagnosis[$] (mm) (95% CI)	Change in mean CTh in 1st year pre-diagnosis (mm) (95% CI), p-value*	Change in mean CTh in 1st year post-diagnosis (mm) (95% CI), p-value*	Acceleration in change in mean CTh (mm/ year^2) (95% CI), p-value*
ROI				
ERC**	5.93 (5.08, 6.79)	-0.11 (-0.16, -0.07) p<0.0005	-0.15 (-0.21, -0.09) p<0.0005	-0.04 (-0.06, -0.01) p=0.001
PHG	5.28 (4.71, 5.84)	-0.11 (-0.14, -0.08) p<0.0005	-0.14 (-0.19, -0.10) p<0.0005	-0.03 (-0.05, -0.02) p<0.0005
Posterior cingulate	4.05 (3.73, 4.38)	-0.08 (-0.11, -0.06) p<0.0005	-0.10 (-0.13, -0.07) p<0.0005	-0.01 (-0.02, -0.005) p=0.004
Precuneus	3.11 (2.72, 3.50)	-0.10 (-0.13, -0.07) p<0.0005	-0.11 (-0.15, -0.08) p<0.0005	-0.01 (-0.02, 0.001) p=0.07
CONTROL REGION				
Paracentral	3.20 (2.65, 3.75)	-0.05 (-0.12, 0.008) p=0.09	-0.08 (-0.16, 0.01) p=0.08	-0.02 (-0.05, 0.009) p=0.2
Pericalcarine	2.99 (2.65, 3.33)	0.02 (-0.002, 0.05) p=0.07	0.02 (-0.01, 0.05) p=0.3	-0.004 (-0.02, 0.008) p=0.5

[$] For scanner 1

* Change in each additional year post-diagnosis = change in 1st year post-diagnosis + [acceleration x (number of years post-diagnosis – 1)]. Similarly, change in each additional year pre-diagnosis = change in 1st year pre-diagnosis - [acceleration x (number of years pre-diagnosis – 1)] ** One individual was removed from analysis due to probable Freesurfer error in calculating cortical thickness

Table 5: Mean levels of and changes in regional CTh in mutation carriers relative to those in controls of the same age, according to time since clinical diagnosis of AD (adjusted for scanner)

ROI	Mean CTh in MC at diagnosis (mm) relative to controls of the same age (95% CI), p-value	Mean change in CTh (mm) in MC relative to that in controls of the same age(95% CI), p-value			Earliest time relative to clinical diagnosis where the difference between MC and controls is statistically significant**
		In 1st year pre-diagnosis (A)	In 1st year post-diagnosis (B)	Acceleration (mm/year^2) (B-A)	
ERC*	-0.46 (-1.05, 0.12) p=0.1	-0.14 (-0.22, -0.07) p<0.0005	-0.17 (-0.27, -0.07) p=0.001	-0.03 (-0.06, 0.004) p=0.09	0.1
PHG	-0.17 (-0.57, 0.24) p=0.4	-0.10 (-0.14, -0.06) p<0.0005	-0.13 (-0.18, -0.08) p<0.0005	-0.03 (-0.05, -0.02) p<0.0005	1.3
Posterior cingulate	-0.28 (-0.47, -0.08) p=0.006	-0.07 (-0.10, -0.04) p<0.0005	-0.09 (-0.12, -0.05) p<0.0005	-0.01 (-0.03, -0.002) p=0.02	-1.8
Precuneus	-0.50 (-0.73, -0.27) p<0.0005	-0.09 (-0.13, -0.06) p<0.0005	-0.10 (-0.15, -0.06) p<0.0005	-0.01 (-0.03, 0.003) p=0.1	-4.1
Control regions					
Paracentral	-0.07 (-0.30, 0.17) p=0.6	-0.04 (-0.10, 0.02) p=0.2	-0.06 (-0.15, 0.03) p=0.2	-0.02 (-0.05, 0.007) p=0.1	N/A***
Pericalcarine	-0.02 (-0.15, 0.12) p=0.8	0.01 (-0.02, 0.05) p=0.5	0.02 (-0.03, 0.06) p=0.5	0.003 (-0.01, 0.02) p=0.7	N/A***

One individual was removed from the analysis due to probable Freesurfer error in calculating cortical thickness. ** Negative values indicate that the earliest point at which differences are statistically significant (5%, 1-sided test) is prior to clinical diagnosis. *** MC do not significantly differ from controls at any point up to 30 years post-diagnosis

Figure 8: Estimated mean CTh in MC, by years since clinical diagnosis

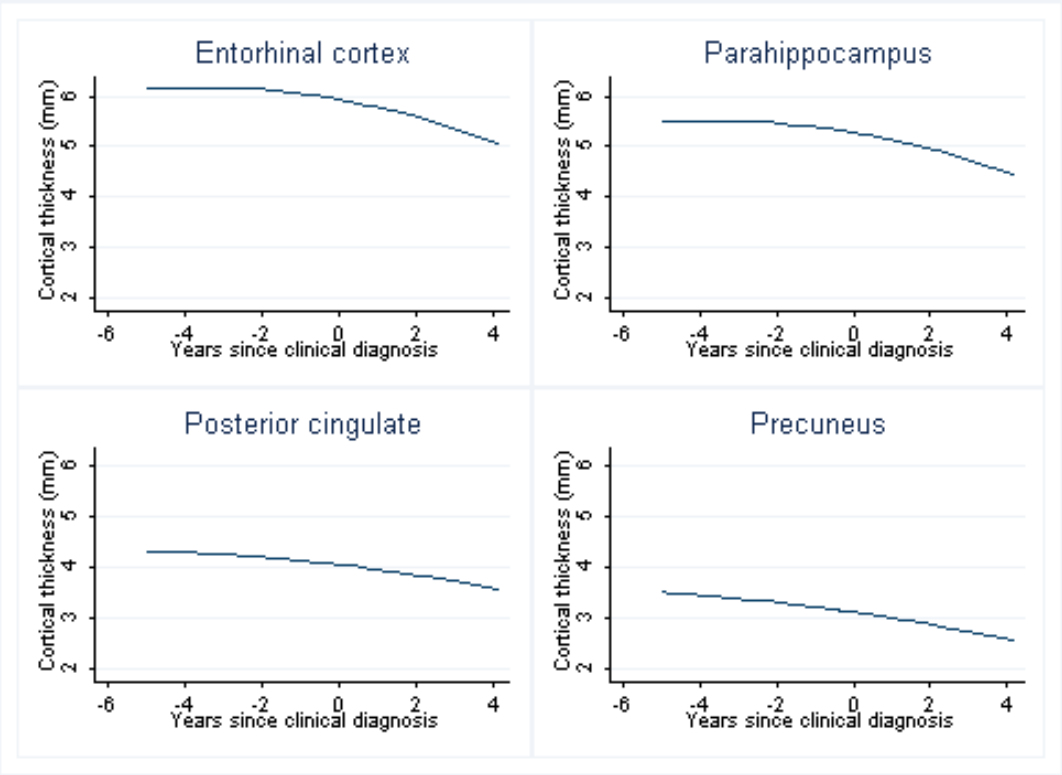


Figure 8a: Difference in mean cortical thickness between cases and controls, by years since clinical diagnosis (with 90% CI)

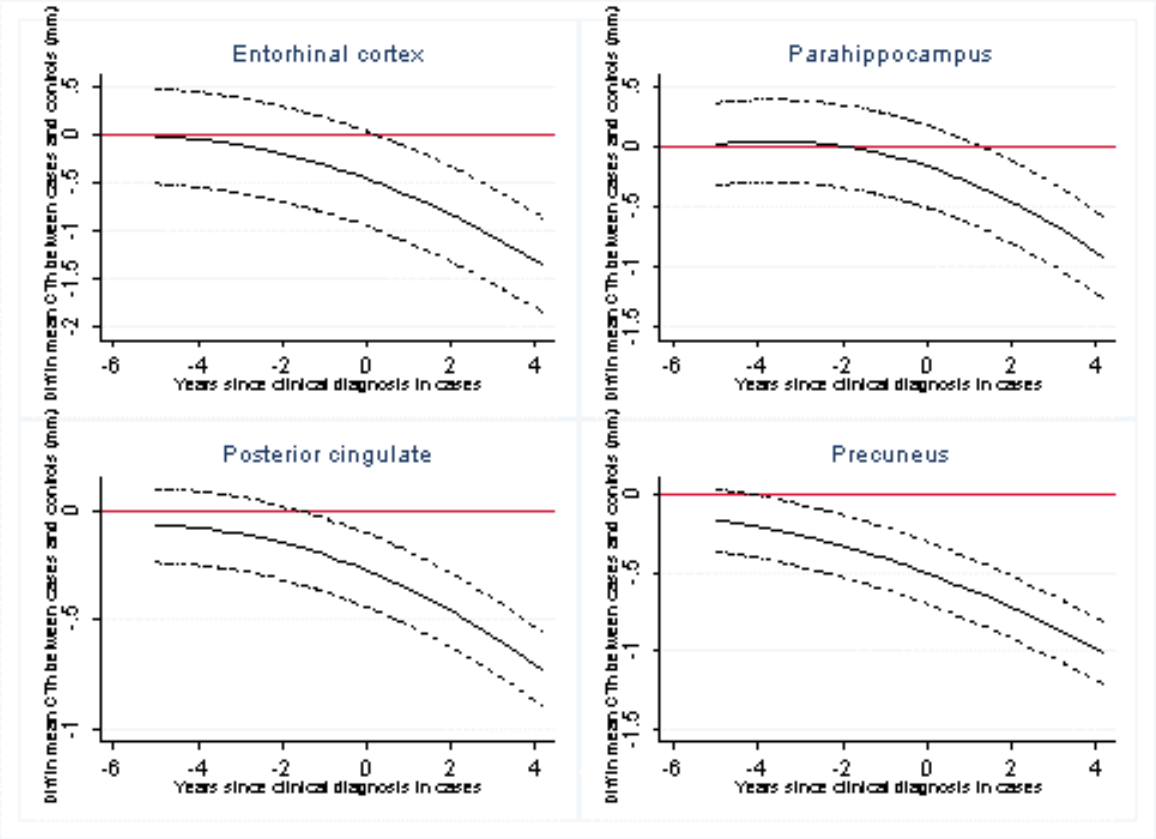
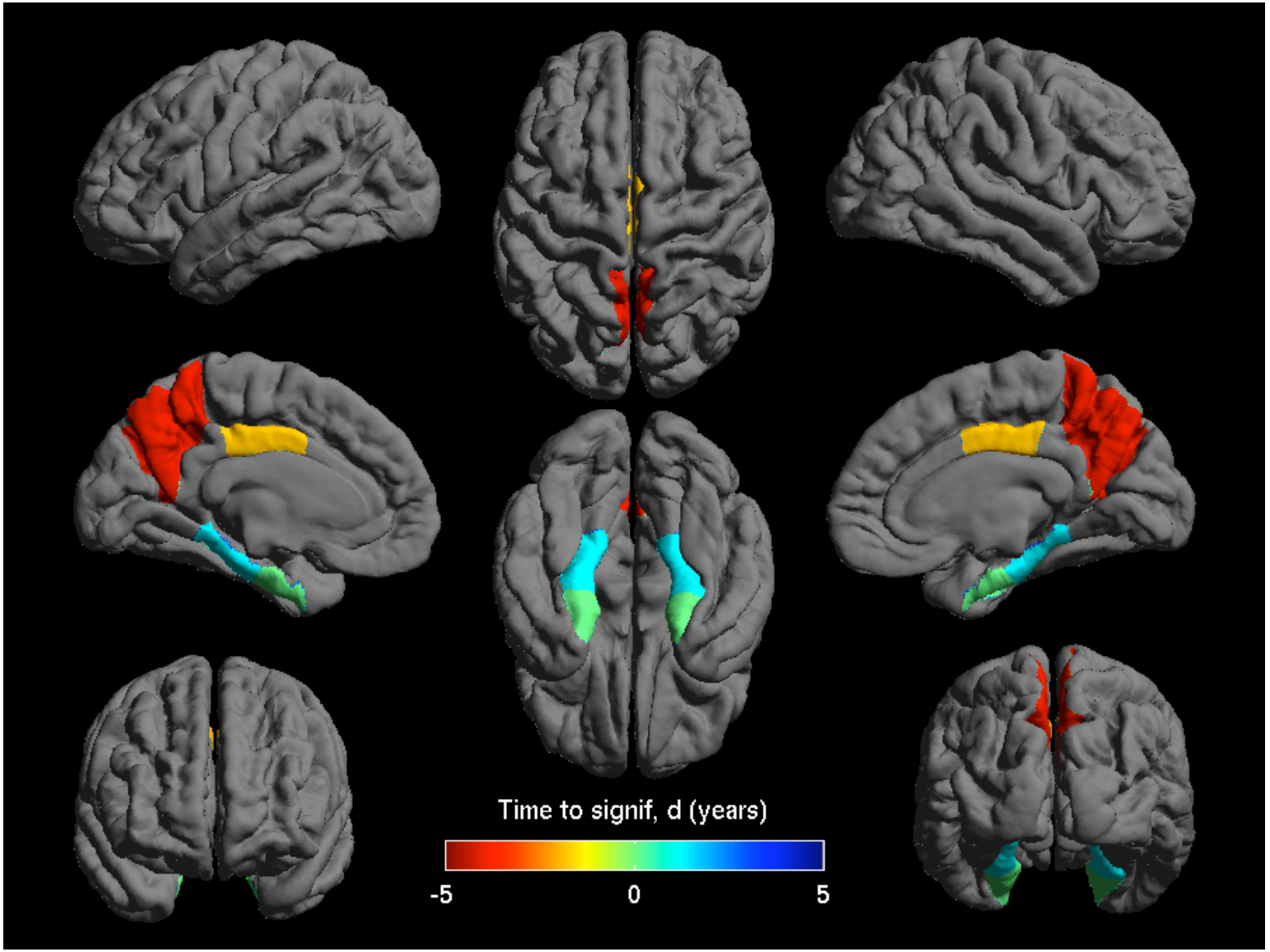


Figure 9: Earliest Time (yrs), relative to clinical diagnosis, at which regional differences emerge between MC and controls.



2. 3. 3. Discussion

Using a fully automated method, we have measured CTh in the brains of FAD mutation carriers and age/sex-matched controls. CTh change was investigated via longitudinal analysis of six regions in relation to age and time of clinical diagnosis of AD. ROI were selected on the basis of existing evidence that they are significant in early AD and may provide an early signal of disease. Histopathological and structural imaging studies indicate that limbic and heteromodal association areas fall into this group, including the MTL (particularly the PHG) and precuneus (Juottonen *et al* 1999; Xu *et al* 2000; Lerch *et al* 2005; Dickerson *et al* 2009). Functional imaging modalities have also demonstrated early change in the retrosplenial and posterior cingulate cortices (Minoshima *et al* 1997; Mosconi *et al* 2006), which have a role in episodic memory function and some visuospatial processing (Maguire 2001; Burgess *et al.* 2001). CTh measurement of sub-regions of the hippocampal formation has been previously performed (Burggren *et al.* 2008) although assessing CTh in this region is less well-established than other regions and is technically highly demanding due to the elaborate invagination of the cortex. This, the presence of other candidate MTL regions, and our desire to avoid duplication (previous hippocampal volumetric data in the same group has been reported (Ridha *et al* 2006)) led us not to select this as an ROI. Motor, sensory and primary visual cortices are relatively spared in the early stages of AD (Braak and Braak 1996b; Rizzo *et al.* 2000; Prvulovic *et al.* 2002) and were therefore selected as control regions.

Age-related grey matter loss has been well documented in all major lobes of the brain (Allen *et al.* 1991; Van Petten *et al.* 2004; Salat *et al.* 2004). There is an historically prominent view that such decline occurs with an anterior-posterior gradient although this is not universally accepted and, frequently, widespread patchy thinning implicating both primary and association cortices is found to accompany ageing (Raz and Rodrigue 2006).

In our healthy control group we demonstrated a very small degree of age-related CTh decline across all regions except the ERC, the wide distribution of which was commensurate with previously observed changes in normal ageing (Salat *et al* 2004). In particular, the relative sparing of the PHG was familiar, although the prominence of change in the posterior cingulate was unexpected. An implausible increase in ERC thickness was also unforeseen, although this should be interpreted with caution in view of well documented difficulties with surface reconstruction of this region using Freesurfer (Han *et al.* 2006). The magnitude of this increase and of the age-related decline in other regions in healthy controls was, in biological terms, exceptionally small. However, it was fully consistent with previous high-resolution MRI studies, which have demonstrated rates of global atrophy in healthy people of 0.2% per year at age 30-50 and 0.3-0.5% per year at age 70-80 (Fox and Schott Lancet 2004).

Analysis of the MC group provided insight into CTh change with respect to time since clinical diagnosis of AD. It showed strong evidence of non-linear CTh decline in all four ROI with acceleration over time. MTL structures showed the highest rates of thinning and there was no evidence for thinning or acceleration in the control regions. These findings support our initial hypothesis and choice of ROIs. They also echo previous findings of prominent early cortical thinning in the MTL and precuneus in amnestic mild cognitive impairment (aMCI) patients destined to progress to AD (Bakkour A *et al* 2008). Despite broad acceptance of an early role for the precuneus in particular, and previous observations that it may be more affected in early onset forms of AD (Karas *et al.* 2007), not all studies support a special role for the region relative to other areas of the neocortex (Nelson *et al.* 2009). The limitations of the measurement technique again demand the cautious interpretation of the effect shown in the ERC. Rates of whole brain atrophy in aMCI patients have been shown to be non-linear, accelerating as individuals

progress to typical, late-onset AD (Jack *et al* 2008), while other studies of rates of atrophy in AD also seem suggest acceleration (Chan *et al.* 2003). Our observation that CTh in FAD similarly accelerates beyond diagnosis supports the notion that FAD is a credible paradigm for sporadic, late-onset AD and that automated measurement of cortical change can reproduce the patterns described where more established neuroimaging techniques are deployed.

A combined analysis provided some evidence that CTh in the posterior cingulate and precuneus differed between the MC and control groups prior to clinical diagnosis. In the precuneus, a significant difference was apparent more than four years before diagnosis, a comparable duration to that found in a previous volumetric study of the whole brain and hippocampi in the same group (Ridha *et al* 2006). It was interesting that the PHG should not be useful in this regard in view of previous observations about its significance (Juottonen *et al* 1999; Xu *et al* 2000; Lerch *et al* 2005). Although the rate and acceleration of loss in this region were similar to those observed in the posterior cingulate and precuneus regions, the difference between the controls and cases at diagnosis was smaller. As members of the MC cohort develop established AD, any variability in the rate and acceleration of thinning between regions will lead to a change in the regions which best distinguish them from controls. Further, it is important to emphasise that failure to detect significant effects in certain regions does not necessarily imply the genuine absence of an effect in those regions. MRI-derived measurements have limitations inherent in their acquisition, and may have a lower sensitivity to detect change in certain regions because different brain structures are variably affected by artefacts such as susceptibility or inhomogeneity. The key advantage of CTh measurement over volumetric studies is that it is automated, and hence more rapid and less operator-dependent. However, as discussed above, there are important caveats to consider when

interpreting our findings, which relate to limitations of the technique and of the analysis. These include the cross-scanner analysis, which relates to the extended nature of the study. This does present a continuity problem, which we have attempted to mitigate statistically. Further, the statistical analysis of our measurements was designed to answer questions, which we felt the data were equipped to address. We cannot, for example, conclude that CTh decline in the posterior cingulate and precuneus precedes such changes in the ERC and PHG as this was not the hypothesis, and the analyses performed were not designed to establish this relationship. Caveats surrounding the differential sensitivity of MRI depending upon the size and location of brain region concerned are again relevant, as this may have obscured an effect in one or more of the ROI. The use of larger groups would certainly be helpful in further defining the utility of CTh analyses although, in this study, the small groups reflect the rarity of this form of the illness and the still greater rarity of cases whose genetic status has been established. The use of subjects at risk for autosomal dominant AD here was advantageous in at least one important sense: that we could study subjects who were certain to develop clinical AD while they were still asymptomatic. Despite the many similarities between FAD and SAD, the extent to which our findings are generalizable to the more common sporadic disease remains unknown. However, our data do contribute to a growing body of evidence that imaging biomarkers may be highly sensitive for detecting presymptomatic AD (Dickerson *et al* 2009), perhaps years before the illness declares itself clinically. While functional and structural neuroimaging techniques may be sensitive to change in different brain regions, CTh analysis in AD would appear to implicate regions previously highlighted by both. Perhaps CTh has the potential to reveal something interesting about the functionally related network of MTL and retrosplenial structures, which is so crucial in AD pathology. It certainly continues to show promise as a relatively practical, rapid and sensitive technique, although further work is needed to evaluate its role in stratifying

different molecular pathologies, and to define the phenotypic correlates of the cortical changes identified.

3.

THE FAD PARADIGM

II: Molecular neuroimaging

3. 1. A longitudinal ^{11}C-PiB PET study in FAD and SAD

Previous studies have suggested prominent, early striatal ^{11}C-PiB retention in FAD (Klunk *et al.* 2007; Remes *et al.* 2008), a pattern not yet observed in SAD. In this study, the aim was to further characterize the ^{11}C-PiB PET profiles of *PSEN1* mutation carriers in the preclinical and early stages of their illness, and to compare these with profiles of healthy controls and individuals with SAD. To achieve this early cross-sectional data from a larger prospective, longitudinal ^{11}C-PiB PET cohort study were used.

3. 1. 1. Methods

Table 6 details subject demographics. Seven presymptomatic and mildly affected (MMSE≥20) *PSEN1* mutation carriers, 10 patients with SAD and 10 healthy controls (including four members of autosomal dominant FAD families with negative predictive tests for the causative mutation) had PET. *PSEN1* mutations studied were Y115C (Cruts *et al.* 1998), M139V (Clark *et al.* 1995), M146I (Jorgensen *et al.* 1996), L171P (Ramirez-Duenas *et al.* 1998), E184D (Yasuda *et al.* 1997), R278I (Godbolt *et al.* 2004) and intron 4 (Tysoe *et al.* 1998; Janssen *et al.* 2003). Exclusion criteria comprised a current or recent history of drug or alcohol abuse/dependence, pregnancy, inability to undergo PET scanning (the most common reason envisaged was an inability to tolerate the process of

reclining supine for 90 minutes) and a history of cancer within the last 5 years (except non-melanoma skin and prostate cancer).

Presymptomatic mutation carriers (PMC) were, on average, 7.2 years younger than the documented mean AAO for their family. Mean MMSE (Folstein *et al* 1975) score for the mutation carrier group was 27.2 (range 21-30). Whole brain and regional uptake from a single, baseline ^{11}C-PiB PET scan were examined. The study received approval from the ethics committee of the National Hospital for Neurology and Neurosurgery and Institute of Neurology and the Hammersmith Hospitals Trust. Permission to administer radiation was granted by the Administration of Radioactive Substances advisory Committee (ARSAC) UK.

Patients were approached by the investigators and oral and written information provided prior to consent. All subjects and controls underwent ^{11}C-PiB PET imaging at the Hammersmith Hospital. PET scanning was performed using a Siemens ECAT EXACT HR+ scanner in 3 dimensional mode as described previously (Edison et al 2007). Each scan participant received a bolus administration of up to 370 MBq ^{11}C-PiB intravenously followed by a 90-minute dynamic PET scan.^{11}C-PiB was manufactured by Hammersmith Imanet, GE Healthcare and a 10-minute transmission scan was performed prior to the injection of ^{11}C-PiB.

All mutation carriers and controls had anatomical T1-weighted, volumetric MRI to allow co-alignment of parametric PET images of ^{11}C-PiB binding, permitting anatomical localization of regions-of-interest (ROI). Where possible, MRI was performed on the same day as the PET scanning, although an interval between the two scans of up to three months was deemed acceptable.

3. 1. 1. 1. Image analysis

Amyloid plaques have been reported to be present in the cerebellar cortex in FAD cases (Verkkoniemi et al 2001). Parametric ratio images of ^{11}C-PiB retention were therefore created using the pons as a reference region. The late (60 to 90-minute) summation images of tracer uptake were co-registered to each subject's MRI using SPM2 (Statistical Parametric Mapping; Wellcome department of Imaging Neuroscience, University College London, UK) software. Each late summation image was then normalized into Montreal Neurological Institute (MNI) space using individual MRIs as a template. Activity in the pons was sampled using an ROI placed manually on the corresponding normalized MRI image, allowing the calculation of a pontine uptake value and subsequent creation of the target region: pons ratio image. Using an individualized anatomical atlas, created using analyze software and an in-house probabilistic brain atlas (Hammers *et al.* 2003), we sampled ^{11}C-PiB uptake for each subject in the anterior and posterior cingulate, the thalamus, striatum and the frontal, temporal, parietal, occipital and cerebellar cortices. In order to allow comparison with studies that have used the cerebellum as a reference region we also calculated regional ratios relative to the cerebellum – these are given in Table 8.

Table 6: Subject Demographics

GROUP	SEX		MEAN AGE (RANGE)	MEAN MMSE (RANGE)
	F	M		
CONTROLS	7	3	47.7 (25-66)	n/a
SAD	4	6	61.9 (51-69)	21.7(12-26)
PSEN1 **- AFFECTED**	2	0	51.5 (40-63)	22(21-23)
PSEN1 **- PMC**	4	1	34.6 (31-40)	28.7(27-30)
PSEN1 **- TOTAL**	6	1	38.8 (31-63)	27.2(21-30)

3. 1. 1. 2. Statistical analyses

Statistical interrogation was performed using SPSS (release 17.0, SPSS Inc.). Mean regional ^{11}C-PiB region: pons ratios were analysed to detect significant differences. Groups were compared using the Mann-Whitney test. P values were corrected for the total number of comparisons using the Hochberg Correction combined with the p-plot estimation of the number of Null-Hypotheses in the set (Turkheimer *et al.* 2001).

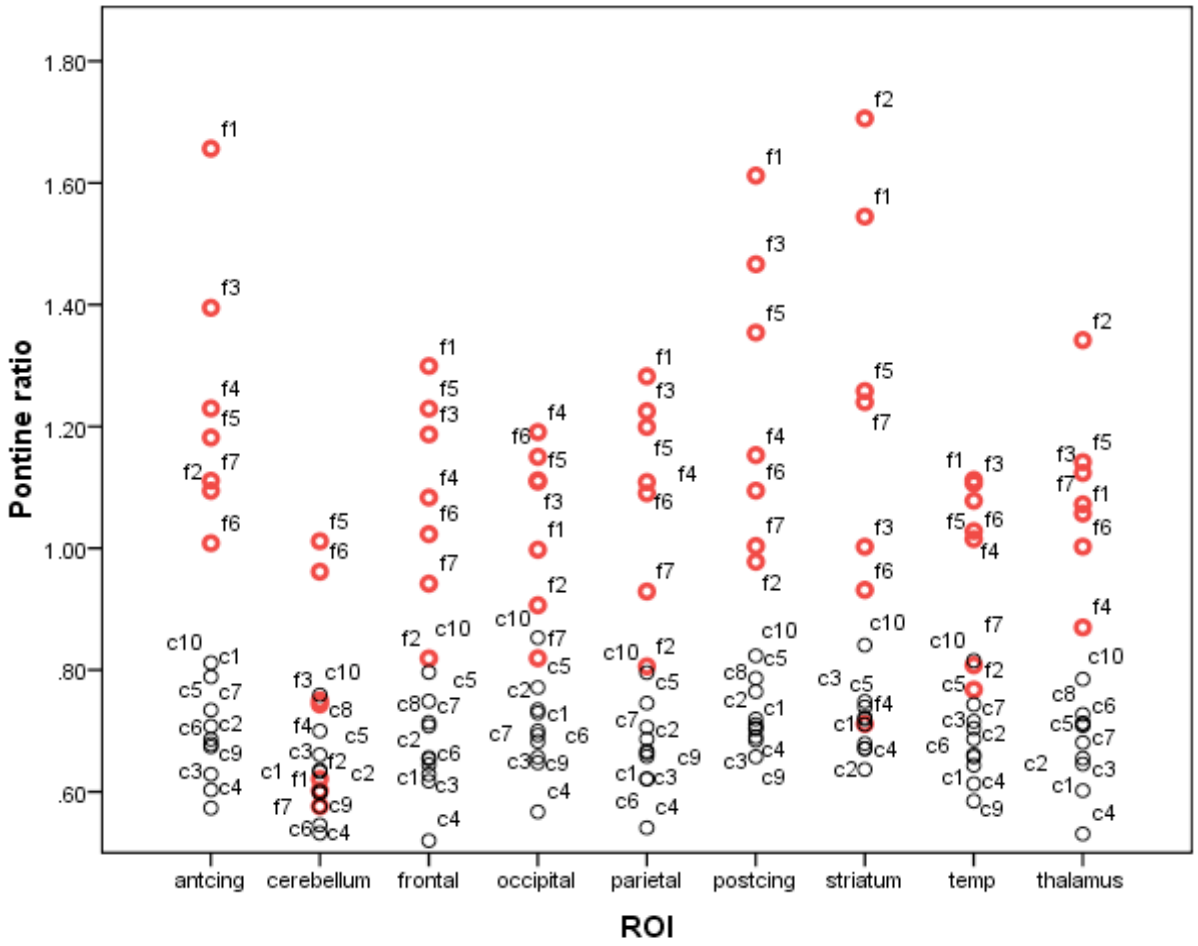
3. 1. 2. Results

Across all regions sampled, mean region: pons ratios demonstrated statistically significant differences when the SAD group was compared with controls with the exception of cerebellum. When *PSEN1* and control groups were compared, all regions except the cerebellum (p=0.133) showed significant differences that survived correction for multiple comparisons. When the same comparison was made using the cerebellum as the reference region, the temporal region was no longer significant, after correction for multiple comparisons, but all other regions remained significantly different to controls.

When *PSEN1* and SAD groups were compared, thalamus (*PSEN1*>SAD), frontal and temporal regions (SAD>*PSEN1*) demonstrated significant differences that survived multiple comparison corrections. When the same comparison was made using the cerebellum as the reference region (Table 8) the sporadic group had significantly greater PiB retention ratios in parietal and occipital as well as frontal and temporal regions.

Two mutation carriers (E184D and intron 4 mutations) had higher striatal ^{11}C-PiB retention ratios than the mean for the SAD group (1.71 and 1.54 respectively). Figures 10 and 11 demonstrate regional variation in^{11}C-PiB retention for *PSEN1* and SAD groups when compared with controls. Figure 12 shows 3 transaxial ^{11}C-PiB scans with different binding patterns in three separate *PSEN1* mutation carriers.

Figure 10: Univariate scatter plot showing region: pontine ^{11}C-PiB retention ratios for *PSEN1* subjects (f1-f7) vs. Controls



f2 and f4 = affected/symptomatic *PSEN1* mutations carriers

Figure 11: Univariate scatter plot showing pontine ^{11}C-PiB retention ratios for SAD subjects (s1-s10) vs. Controls

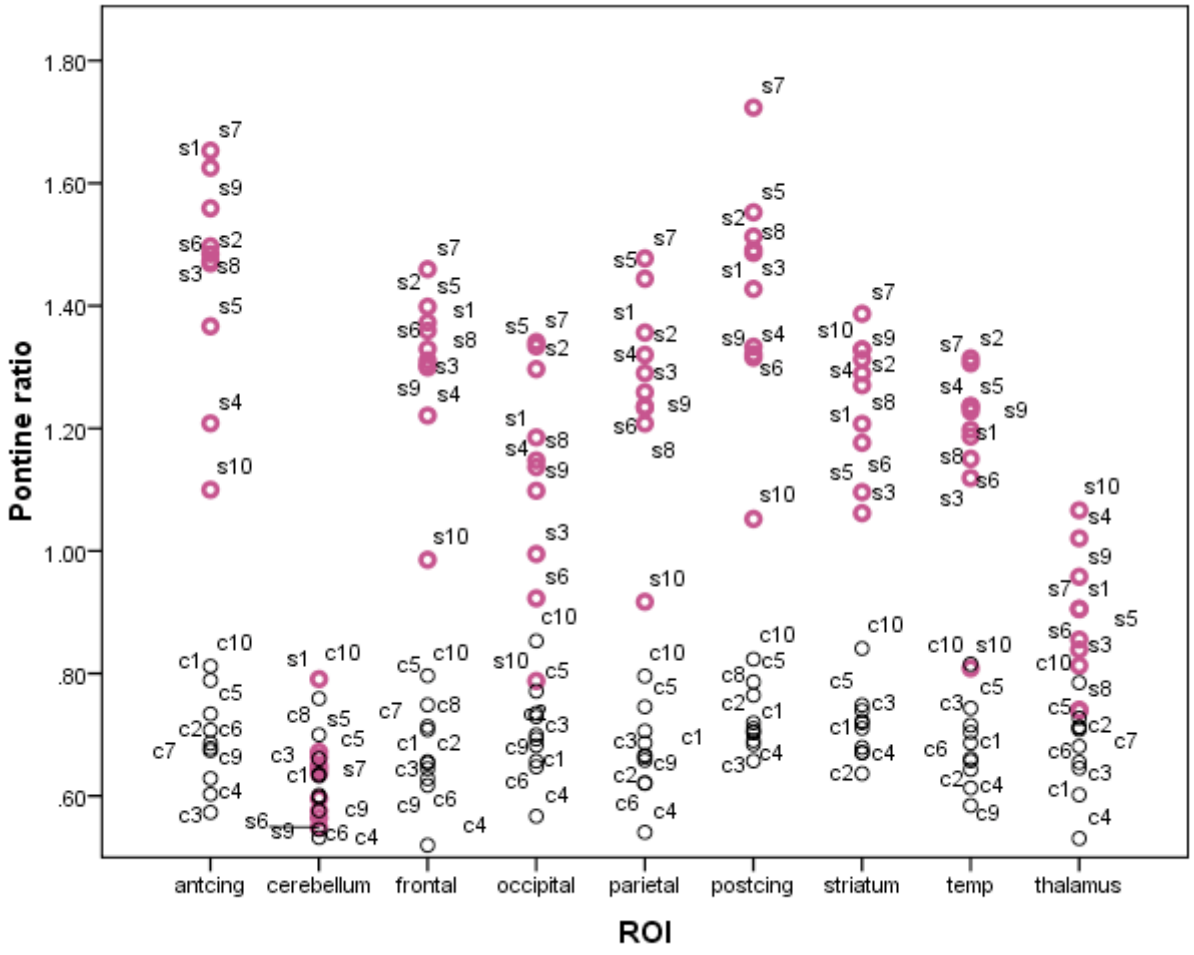


Table 7: Mean regional ^{11}C-PiB uptake by group (Pontine reference region)

ROI	^{11}C-PiB UPTAKE RATIO, ROI: PONS (SD)		
	PSEN1	SAD	CONTROLS
ANTERIOR CINGULATE	1.24 (0.22)†	1.44 (0.18)†	0.69 (0.08)
POSTERIOR CINGULATE	1.24 (0.24)†	1.42 (0.18)†	0.72 (0.05)
THALAMUS	1.09 (0.14)†*	0.88 (0.11)†	0.68 (0.07)
STRIATUM	1.20 (0.35)†	1.25 (0.11)†	0.71 (0.06)
FRONTAL	1.08 (0.17)†*	1.30 (0.13)†	0.67 (0.08)
TEMPORAL	0.99 (0.14)†*	1.18 (0.14)†	0.68 (0.07)
PARIETAL	1.09 (0.17)†	1.27 (0.15)†	0.67 (0.07)
OCCIPITAL	1.04 (0.14)†	1.12 (0.18)†	0.70 (0.08)
CEREBELLUM	0.75 (0.17)	0.63 (0.07)	0.62 (0.07)

† **p<0.05 vs. controls * p<0.05 vs. SAD**

Table 8: Mean regional ^{11}C-PiB uptake by group (Cerebellar reference region)

ROI	^{11}C-PiB UPTAKE RATIO, ROI: CEREB (SD)		
	PSEN1	SAD	CONTROLS
ANTERIOR CINGULATE	1.78 (0.56)†	2.29(0.32)†	1.09(0.07)
POSTERIOR CINGULATE	1.76 (0.50)†	2.25(0.29)†	1.17(0.08)
THALAMUS	1.55 (0.40)†	1.41(0.25)†	1.09(0.11)
STRIATUM	1.74 (0.73)†	1.98(0.26)†	1.15(0.07)
FRONTAL	1.53 (0.37)†*	2.07(0.26)†	1.07(0.05)
TEMPORAL	1.39 (0.30)*	1.87(0.28)†	1.10(0.03)
PARIETAL	1.53 (0.35)†*	2.02(0.28)†	1.08(0.05)
OCCIPITAL	1.46 (0.27)†*	1.78(0.27)†	1.13(0.05)

† **p<0.05 vs. controls * p<0.05 vs. SAD**

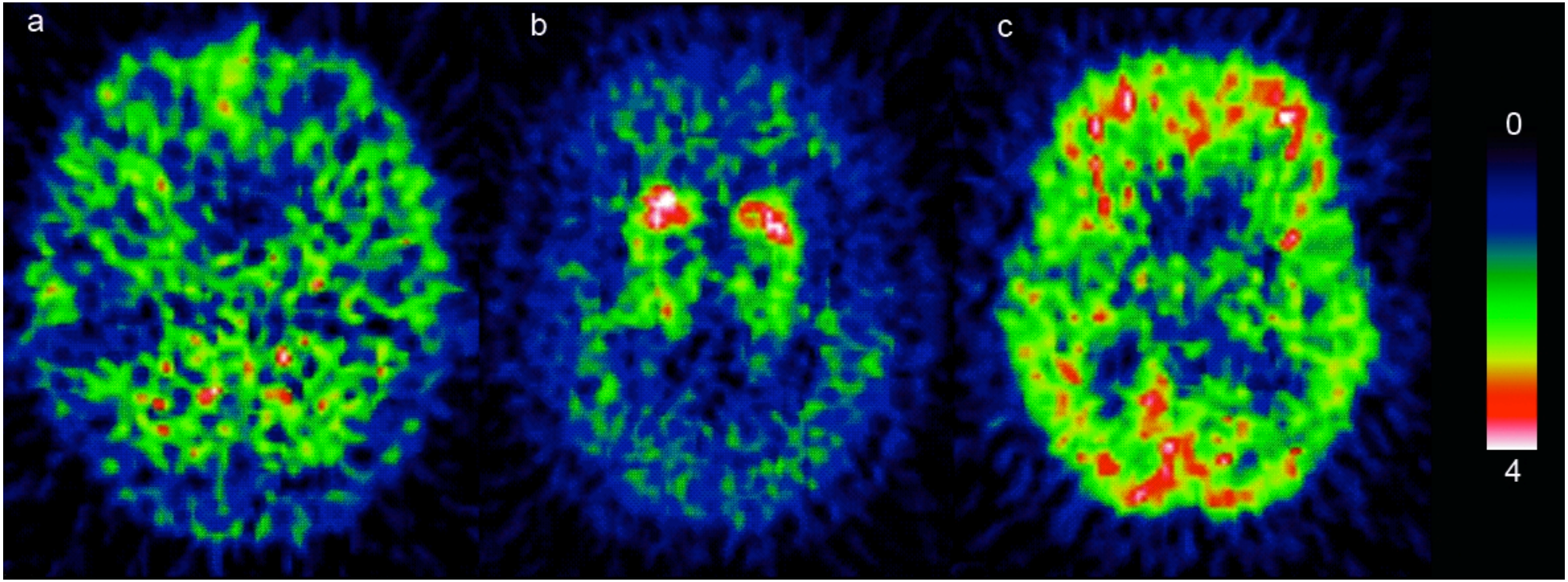


Figure 12: Transaxial ^{11}C-PiB images (pontine reference region) in *PSEN1* mutation carriers showing heterogeneity of ^{11}C-PiB binding pattern.

(a) Increased cerebellar binding in *PSEN1* M146I PMC.

(b) Increased striatal binding in an affected *PSEN1* E184D mutation carrier (f2 in figure 10).

(c) Typical AD-like pattern in an affected *PSEN1* R278I mutation carrier (f4 in figure 10).

3. 1. 3. Discussion

At a group level, we observed significantly higher global ^{11}C-PiB uptake in established SAD cases compared with the *PSEN1* group, in which the majority were presymptomatic and around seven years before the anticipated onset of symptoms. However, we have confirmed previous findings (Klunk *et al.* 2007; Remes *et al.* 2008) that it is possible to detect significantly elevated ^{11}C-PiB retention in the brains of individuals destined to develop clinical AD before symptoms arise. Structural and metabolic changes are already known to occur early in FAD patients (Matsushita *et al.* 2002; Ridha *et al.* 2006) as they are in SAD (Minoshima *et al.* 1997; Desikan *et al.* 2008) and MCI (Kemppainen *et al.* 2007). In SAD, the earliest ^{11}C-PiB retention may occur in the retrosplenial cortices, particularly the precuneus (Mintun *et al.* 2006) although early retention has been noted in the striatum as well as the frontal, parietal, temporal, and occipital cortices (Klunk *et al.* 2004).

The rarity of FAD mutations means that rather less is known about their associated ^{11}C-PiB PET profiles. One study of the *PSEN1* C410Y and *PSEN1* A426P mutations showed elevated ^{11}C-PiB retention beginning in the striatum of presymptomatic and affected mutation carriers, with neocortical areas involved later (Klunk *et al.* 2007). A similar pattern has been observed in those with early-onset disease associated with atypical clinical (spastic paraparesis) and neuropathological (cotton wool plaques) features (Koivunen *et al.* 2008) and in amyloid precursor protein (*APP*) gene duplication cases (Remes *et al.* 2008). Such studies raise important issues about the neuropathological profile of early-onset forms of AD, although the extent to which they are representative remains unknown, particularly as other investigators have found a more typical, SAD-like ^{11}C-PiB profile in symptomatic *APP* mutation-carriers (Theuns *et al.* 2006). Our study included two individuals (E184D and intron 4 mutations) with higher striatal ^{11}C-PiB

retention ratios than the mean for the SAD group (1.71 and 1.54 respectively) although, at a group level, the clear striatal prominence seen in previous reports was not evident. This finding serves to underline the value of studying a number of different mutations: it permits the observations that striatal prominence is not universal and that amyloid imaging profiles, even amongst the mutations of a single gene, may be varied. The two *PSEN1* mutations studied by Klunk and colleagues in 2007 were in exons 11 and 12, unlike our mutations, none of which was situated beyond exon 8. This may have contributed to the different profiles observed, just as clinical phenotypic heterogeneity is known to be associated with varied mutation position (Larner and Doran 2006).

In *APP* duplication cases, abundant intravascular amyloid, particularly in the leptomeningeal vessels, may contribute to increased ^{11}C-PiB signal (Remes *et al.* 2008) and it is possible that retention in our subjects was similarly influenced. Without neuropathological examination, the amount of cerebrovascular amyloid in our subjects, along with any possible influence on ^{11}C-PiB signal, cannot be precisely known. However, we can say that our study did not include any *APP* duplications and that, for *PSEN1* mutations, significant cerebral amyloid angiopathy (CAA) is more frequently associated with those beyond codon 200 (Mann *et al.* 2001), for example L282V (Dermaut *et al.* 2001), E280G (O'Riordan *et al.* 2002) and G217D (Takao *et al.* 2002). As 5 out of 6 coding region mutations in our study affected regions between codons 100 and 200, we suggest that cerebrovascular amyloid alone is unlikely to account for our findings.

We observed marked ^{11}C-PiB retention in the thalamus, a pattern peculiar to the *PSEN1* group. The thalamus is not a structure traditionally associated with a heavy amyloid burden, although thalamic Aß deposition has previously been described in a post-

mortem study of *PSEN1* mutation carriers (Lippa *et al.* 1996). Specific mutations, such as M146I, have since been associated with unusually high levels of Aß pathology in the thalamus and mesencephalon (Gustafson *et al.* 1998) raising the possibility that such a pattern is codon- or mutation-specific. Post-mortem histopathological analyses have previously demonstrated immunopositive, diffuse Aß plaques in the thalamus of a *PSEN1* M139V carrier (Larner and du Plessis 2003) and small increases in thalamic ^{11}C-PiB have been noted (Kemppainen *et al.* 2006).

The cerebellum has long been used as a reference region for non-specific tracer binding in ^{11}C-PiB PET imaging as, in the SAD brain, it does not classically accumulate amyloid or retain ^{11}C-PiB (Svedberg *et al.* 2009). In accord with this we found that the sporadic AD subjects did not have raised PiB retention – with almost identical cerebellum/pons ratios when compared with the controls. However ^{11}C –PiB retention does occur in the cerebellum in FAD although there is considerable variability between subjects; this suggests, in line with previous studies (Klunk *et al.* 2007), that the cerebellum may not be an appropriate reference region in FAD and that the pons (as used here) is a suitable alternative. In order to allow comparison with other studies in AD we subsequently also calculated ratios with the cerebellum as the reference region. The respective pontine and cerebellar ratios highlight slightly different regions, though with considerable overlap. Full interpretation of these differences would require pathological correlation beyond the current scope of this study. Our results also suggest that the distribution of tissue pathology (at least in terms of plaque amyloid) associated with *PSEN1* mutations may be more varied than previously thought. This may have implications for the usefulness of PiB imaging in evaluating new therapies (see 1. 8. 2.). Finally these findings raise intriguing questions about why these different patterns of amyloid binding occur and

what their clinical and pathological implications may be – directions perhaps for future research.

4.

CHARACTERIZING FAD

I: Epistasis & single case studies

Know your enemy…and you will not

be imperilled in a hundred battles.

- Sun Tzu, *The Art of War.*

Contemporary treatment strategies are increasingly reliant upon genetic models and robust biomarkers for their development. Studies using transgenic mouse models and human FAD pedigrees continue to inform our approach to AD although, for the FAD paradigm to remain credible, clarification of the FAD/SAD relationship through an understanding of FAD's phenotypic spectrum is vital. Much progress has already been made in this regard (see 1. 4.), helping to highlight both the promise and limitations of this approach. Chapters 2 and 3, address this issue, examining the phenotypes associated with several genetic defects as well as a potential epistatic influence on the clinical phenotypes of known *APP* and *PSEN* mutations.

4. 1. Methods

An ongoing, longitudinal FAD cohort study of more than 20 years duration, has been subject to various amendments, existing in its current form since 2002. Clinical,

neuroimaging and neuropsychology data from this ongoing study have been a vital resource for a number of other studies, including those in this thesis.

This study currently involves 4 key cohorts, recruited primarily from a tertiary/quarternary dementia service and stratified according to clinical/genetic status:

- Individuals at risk of AD by virtue of an autosomal dominant family history.
- Presymptomatic, FAD mutation carriers (PMC) in whom predictive genetic testing has demonstrated a mutation (or duplication) in *APP*, *PSEN1* or *PSEN2*.
- Symptomatic, confirmed FAD mutation carriers.
- Controls (spouses or siblings of individuals from all groups in whom predictive genetic testing has shown no mutation).

Each participant is assessed annually. Subjects in the 'at risk' category were recruited when they were 5 years below the 95% confidence interval for mean AAO in their family. AAO was established through clinical assessment i.e. it corresponds to reported age at onset of symptoms as recorded on Dementia Research Centre database. With predictive genetic testing more widely available, and a greater number of pathogenic mutations discovered, an amendment was made to the study in 2002 such that it was possible to recruit presymptomatic individuals who had positive genetic tests from the time of referral (by which time they had already have received their positive test result from a geneticist). This was often at a much younger age than that stated in the original study protocol and, where they remained unaffected, they participate until they are five years older than the oldest known AAO in their family. Affected individuals participate until it is no longer possible for them to tolerate the assessments, usually by virtue of the severity of their illness. Each participant is given an information sheet and adequate time

to read and discuss it before signing a consent form. Both documents have been approved by the National Hospital for Neurology and Neurosurgery and UCL Institute of Neurology Joint Ethics Committee. Annual assessments comprise:

- Volumetric brain MRI: Imaging between 1991 and 1998 was performed on a 1.5T GE Signa scanner (General Electric Medical Systems, Waukesha, WI, USA). Volumetric T1-weighted coronal MRI scans were obtained with a spoiled gradient echo technique (256×128 matrix, field of view 24×24 cm, TR/TE/NEX/FA=35 ms/5 ms/1/35°) yielding 124 contiguous 1·5 mm thick slices. Between 2000 and 2006 imaging was undertaken on a different 1.5T GE Signa scanner running software version 5.8 (General Electric Medical Systems, Waukesha, WI, USA). Volumetric T1-weighted coronal MRI scans were obtained using an inversion recovery prepared fast spoiled gradient-echo technique (256×256 matrix, field of view 24×18 cm, TR/TE/TI/NEX/FA=14 ms/5·4 ms/650 ms/1/15°) yielding 124 contiguous 1·5 mm thick slices. An MRI Safety questionnaire was completed prior to each scan.
- Magnetic resonance spectroscopy (PMCs and controls only). 1.5 T Signa 5X system PROBE/SV, PRESS (TR=2000 msec, TE=30 msec, 192 avg).
- A T1-weighted spin echo sagittal scout is followed by six 5mm fast IR images obtained in the axial plane. A 15mm thick VOI is placed within 3 adjacent slices maximizing grey matter cingulate content, and minimizing partial volume effects
- Neuropsychological assessment.
- Clinical assessment (history, examination, MMSE).

4. 2. FAD and the ApoE4 polymorphism

Previous studies have demonstrated a modifying effect of the ApoE4 polymorphism on age at symptom onset in *APP*-associated FAD (Hardy *et al* 1993; Sorbi *et al* 1995), though

not *PSEN1*-associated FAD (Van Broeckhoven *et al* 1994). The collation of data for our FAD cohort allowed analysis of AAO, where known, in relation to ApoE genotype. The aim was to establish whether these data supported existing evidence of the effect of ApoE on AAO. Table 9 characterises available data on individuals with a known FAD mutation and ApoE genotype. Table 10 shows ApoE genotype and allele frequencies for these subjects and the corresponding data when an additional 64 subjects, in whom FAD is clinically suspected but no mutation has been demonstrated, are added. Population data from the literature are presented for comparison.

4. 2. 1. Methods

Subjects for this analysis (n=60) were selected purely on the basis that their ApoE genotype, AAO and mean family AAO (AAOf) were known. f-tests were applied and variance in the datasets found to be unequal throughout. Subsequent two-tailed, unpaired heteroscedastic t-tests were used to test two hypotheses: That ApoE4 positive FAD mutation carriers (5 *APP*, 17 *PSEN1*) had a different mean AAO to ApoE4 negative FAD mutation carriers (11 *APP*, 27 *PSEN1*), and that the differences between mean AAO and mean family AAO were greater in the ApoE4 positive group i.e. that ApoE4 carrier status modified AAO, hastening symptom onset.

Table 9: Autosomal dominant AD cases, mutation known (n=73)

Family No	*Gene*	Mutation	AAO	AAOf	ApoE Genotype	Pathology (where available)
19	*APP*	V717G	46	51.5	33	
19	*APP*	V717G	48	51.5	33	AD with CAA
19	*APP*	V717G	47	51.5	33	
19	*APP*	V717G	50	51.5	33	
19	*APP*	V717G	47	51.5	34	
19	*APP*	V717G	45	51.5	34	
23	*APP*	V717I		53.7	33	
23	*APP*	V717I	51	53.7	33	AD with diffuse Lewy body disease
23	*APP*	V717I	52	53.7	33	
23	*APP*	V717I	58	53.7	33	Diffuse Lewy body disease, AD, CAA
171	*APP*	V717L	51	45.8	33	AD
171	*APP*	V717L	48	45.8	33	
172	*APP*	V717I	56	52.5	33	
172	*APP*	V717I	47	52.5	33	AD with CAA
172	*APP*	V717I	50	52.5	34	
172	*APP*	V717I	56	52.5	34	
172	*APP*	V717I	49	52.5	34	AD with vascular malformation of the thalamus
408	*APP*	A692G			33	
175	*PSEN1*	delta4	35	37.7	33	AD
175	*PSEN1*	delta4		37.7	33	
177*	*PSEN1*	L153V	36	35	34	AD
183	*PSEN1*	E280G	42	43.5	33	
184	*PSEN1*	L250S	56	53.3	34	
184	*PSEN1*	L250S	47	53.25	34	
196	*PSEN1*	P267S	37	35.75	34	
196	*PSEN1*	P267S	38	35.75	34	
206‡	*PSEN1*	M139V	35	39	33	

206‡	*PSEN1*	M139V	39	39	33	
206‡	*PSEN1*	M139V	38	39	33	
206‡	*PSEN1*	M139V	37	39	34	
226*	*PSEN1*	R269H	51	52.3	44	AD
267*	*PSEN1*	L235V	44		33	AD
278	*PSEN1*	F283L	47	48	33	
278	*PSEN1*	F283L	46	48	33	
278	*PSEN1*	F283L	48	48	34	
283*	*PSEN1*	R377M	38	38	33	AD and cerebrovascular disease
342*	*PSEN1*	delta4			33	
346	*PSEN1*	R278I			23	
353	*PSEN1*	A260V			33	
364	*PSEN1*	E184D	41	40.5	33	
364	*PSEN1*	E184D	40	40.5	33	
37*	*PSEN1*	F237L	47		34	
53*	*PSEN1*	delta167	43	52	44	AD
74	*PSEN1*	S290Cd9	39	42.8	23	AD and CAA
74	*PSEN1*	S290Cd9	44	42.8	33	AD and CAA
74	*PSEN1*	S290Cd9	42	42.8	33	
102	*PSEN1*	E280G	40	41.8	33	
102	*PSEN1*	E280G	40	41.8	33	
102	*PSEN1*	E280G	41	41.8	34	
102	*PSEN1*	E280G	41	41.8	34	
105	*PSEN1*	delta4	36	37.4	33	AD
105	*PSEN1*	delta4	39	37.4	33	AD and CAA
105	*PSEN1*	delta4	39	37.4	33	AD and CAA
105	*PSEN1*	delta4	38	37.4	34	AD
105	*PSEN1*	delta4	33	37.4	44	AD and severe CAA
121	*PSEN1*	E120K	35	36.3	33	AD and CAA
134*	*PSEN1*	G378V	43	44.71	33	
134*	*PSEN1*	G378V	47	44.71	33	AD

148‡	*PSEN1*	M139V	36	42.1	33	
148‡	*PSEN1*	M139V	43	42.1	33	
148‡	*PSEN1*	M139V	43	42.1	34	
148‡	*PSEN1*	M139V	45	42.1	34	
156	*PSEN1*	I143F	53	55.5	33	AD
156	*PSEN1*	I143F	59	55.5	33	
156	*PSEN1*	I143F	55	55.5	33	
168	*PSEN1*	E280G	43	42.2	33	
168	*PSEN1*	E280G	47	42.2	34	AD, Lewy body pathology, pontine infarct
168	*PSEN1*	E280G	39	42.2	34	
427	*PSEN1*	delta166			34	
382	*PSEN1*	PS1			33	AD and CAA
428	*PSEN1*	Q222P			34	
267*	*PSEN1*	L235V			33	
438	*PSEN1*	M139V			33	

*also see Janssen *et al*, 2003 ‡also see Fox *et al*. 1997

Table 10: ApoE genotype and allele frequencies

		Mutation Known		All FAD Subjects		Expected SAD % (Poirier *et al* 1993; Ashford and Mortimer 2002)	Expected population % (Ashford and Mortimer 2002; Carmo Martins *et al* 2008)
		n	%	n	%		
Genotype	**22**	0	0	1	0.7	0.1	1
	23	2	2.7	4	2.9	4	12
	24	0	0.0	3	2.2	3	2
	33	46	63.0	61	44.5	35	61
	34	22	30.1	59	43.1	42	22
	44	3	4.1	9	6.6	16	2
	Total	**73**	**100**	**137**	**100**	**100**	**100**
Allele Frequencies	**2**	2	1.4	9	3.3	3	6.4
	3	116	79.5	185	67.5	59	83.6
	4	28	19.2	80	29.2	38	10
	Total	**146**	**100**	**274**	**100**	**100**	**100**

4. 2. 2. Results

Table 11: t-test results for ApoE4 hypotheses

	Mean AAO (SD)	Mean family AAO (SD)	Mean (AAO)-(AAOf) (SD)
ApoE4+ve (n=22)	**43.95** (6.34)	**45.08** (6.82)	**-1.13** (3.58)
ApoE4-ve (n=38)	**44.42** (6.67)	**45.26** (2.71)	**-0.83** (2.71)
p	0.79	0.92	0.74

4. 2. 3. Discussion

There were no significant differences between the ApoE4 positive and negative groups in terms of their mean AAO or AAOf. E4 positive subjects, on average, developed symptoms 1.13 years earlier than their family mean AAO compared to 0.83 years earlier for ApoE4 negative subjects, although this difference was not significant. The same analyses were performed after stratifying by FAD mutation and revealed no significant differences.

These data do not, therefore, match previous findings of an epistatic effect of ApoE on AAO in *APP*-associated FAD. This may have been due to the genuine absence of an effect in this cohort. However, the groups have several characteristics that would hinder

detection of such an effect if present, making a type 2 error more likely. Although previous reports have described ApoE's influence on the clinical phenotype of *APP* mutations (Hardy *et al* 1993; Sorbi *et al* 1995), no such effect has been shown in *PSEN1* mutations. The large contribution of *PSEN1* carriers to the groups may, therefore, have masked any differences, especially as the ApoE4 positive group contained a smaller proportion of *APP* carriers. The exclusion of *PSEN1* cases or further stratification by mutation may have rendered significant differences undetectable due to insufficient power related to small group size. A further limitation of the analysis is related to an inconsistency inherent in the source longitudinal study, namely that ascertainment of AAO could not have been performed by the same individual across such an extended period. Further study of the genetic basis for AD is a key requirement, not least on the subject of potential epistatic influence. Our ever-expanding ability to analyse large quantities of genetic data, using for example genome-wide association studies, will be invaluable in this regard.

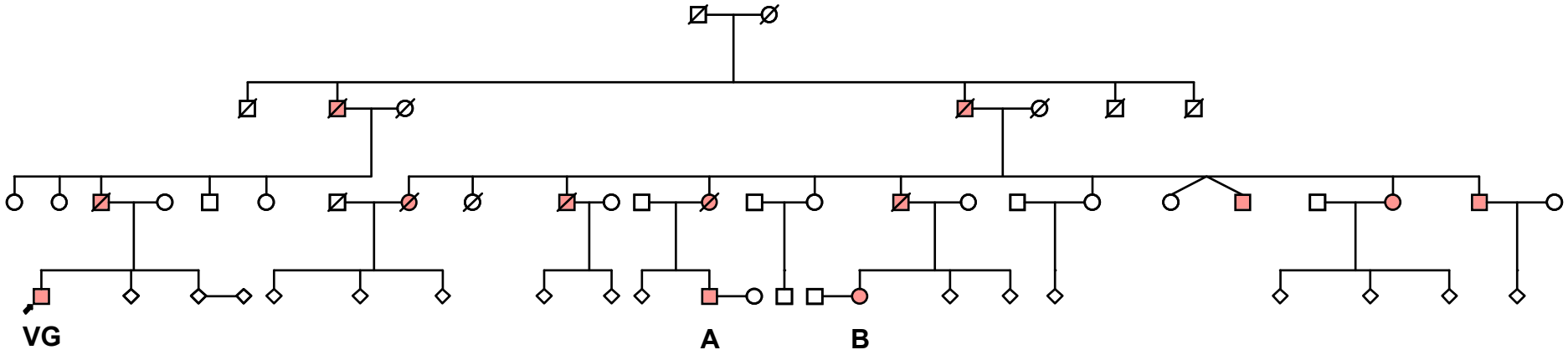
4. 3. Pure progressive amnesia and the *APP*V717G mutation.

An isolated, progressive amnestic clinical phenotype has previously been associated with the *APPV717I* (London) mutation (Gankam Kengne *et al* 2007). The following describes a similar pure progressive amnesia in association with a mutation at the same locus. Additional longitudinal, volumetric MRI and neuropsychological data are presented.

4. 3. 1. Case description

Our patient (Designated VG in Figure 13) belongs to a family with an average AAO of 51.5 years (range 40-67) with pathologically proven AD in one affected member and an *APP*V717G mutation demonstrated in the proband. The extended family tree (Figure 13) is consistent with autosomal dominant inheritance:

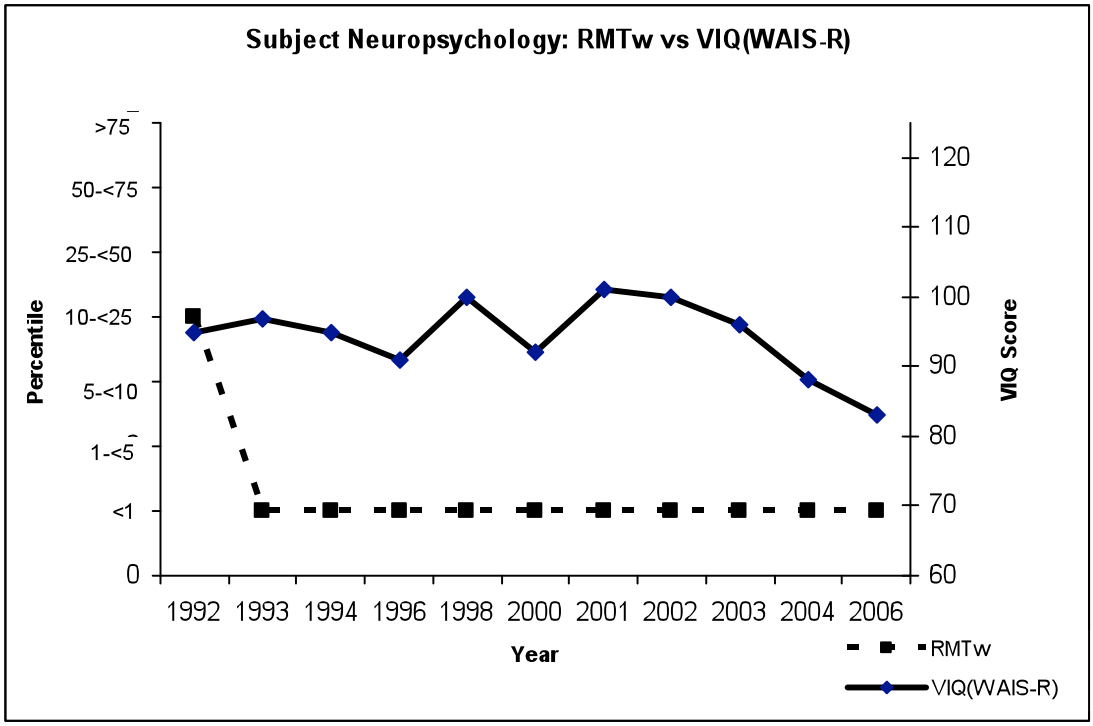
Figure 13: Pedigree



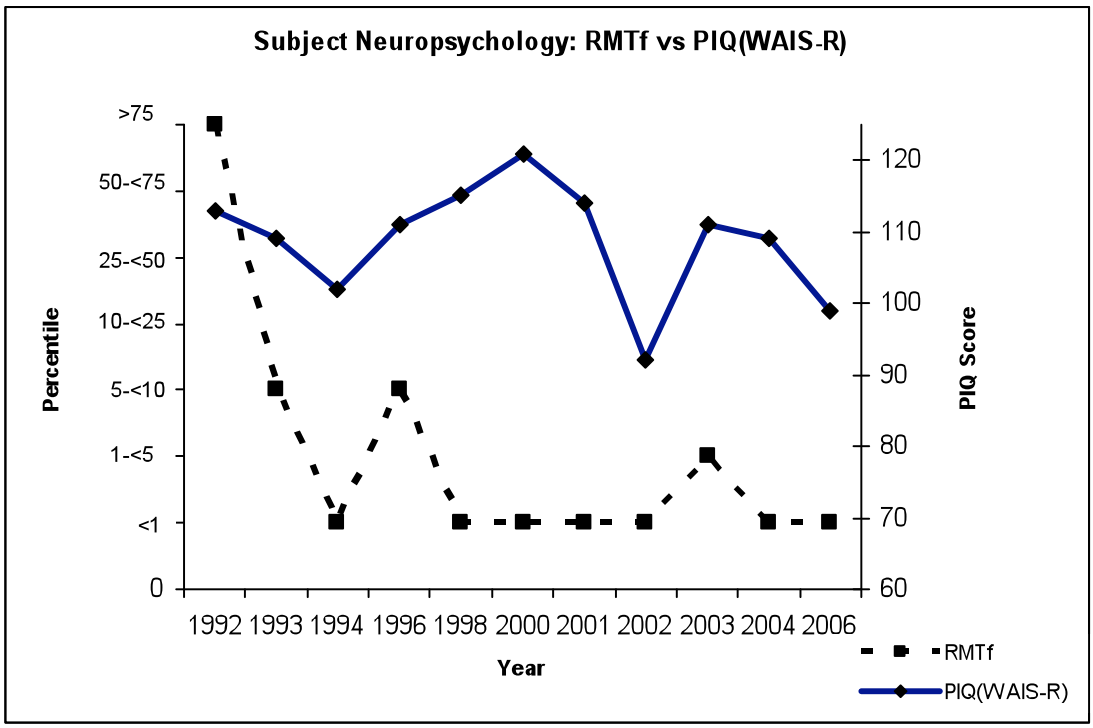
The patient first developed memory problems at 44 years-of-age, with repetitious speech and reliance upon written reminders. Over the ensuing 15 years a progressive, global impairment of memory function occurred with striking preservation of his intellectual capacity. This profile was demonstrated via longitudinal neuropsychological testing (Figures 14a and 14b): He was tested approximately annually on the verbal and performance subscales of the WAIS-R (Wechsler 1981) together with the Recognition Memory Tests for words and faces (Warrington 1984; Warrington 1996). By 46 years, his memory was globally impaired whereas his abilities on both the verbal and performance subscales of the WAIS-R remained fairly static and close to the level achieved at his first assessment.

Figure 14: Neuropsychological data

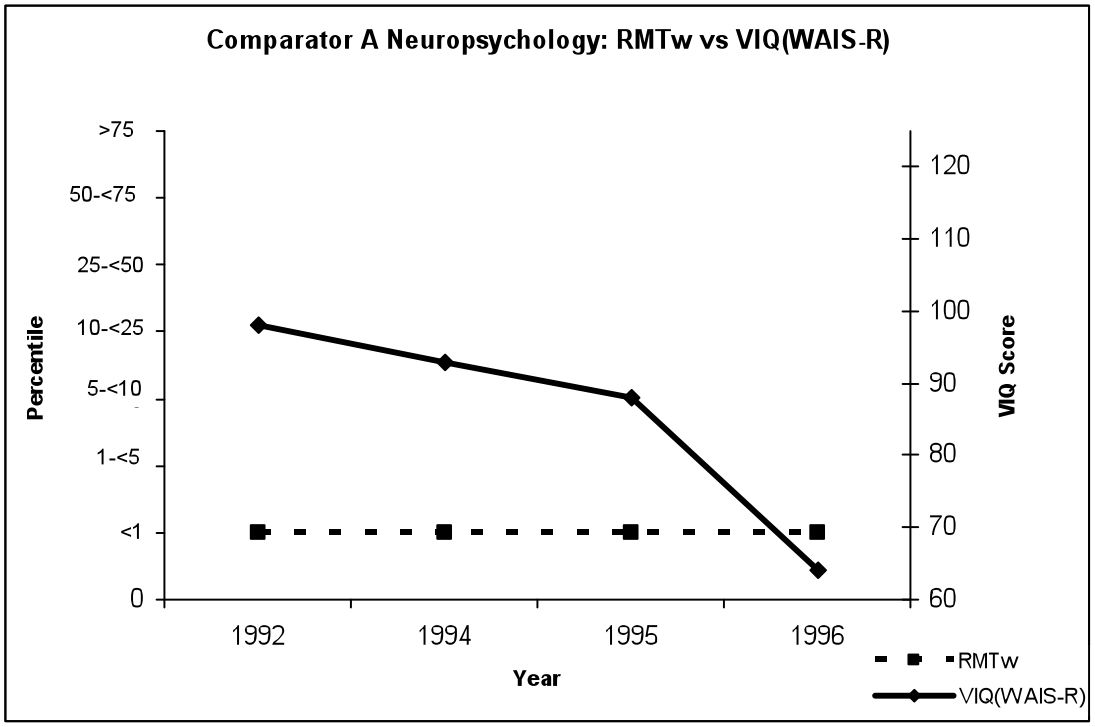
14a



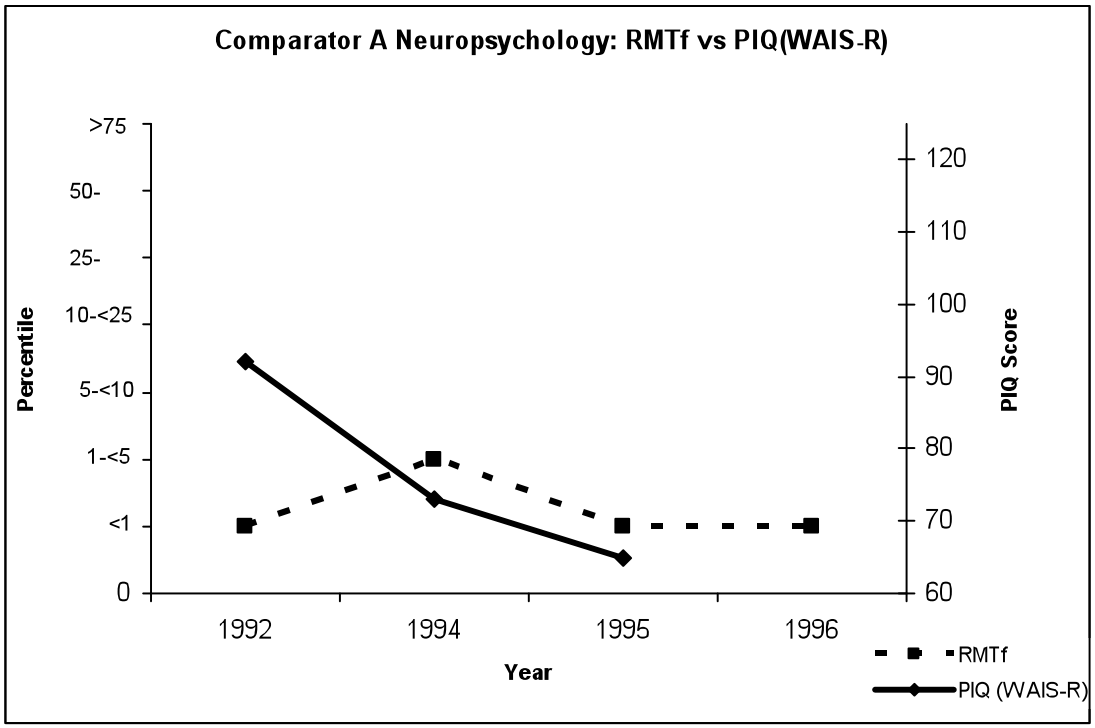
14b



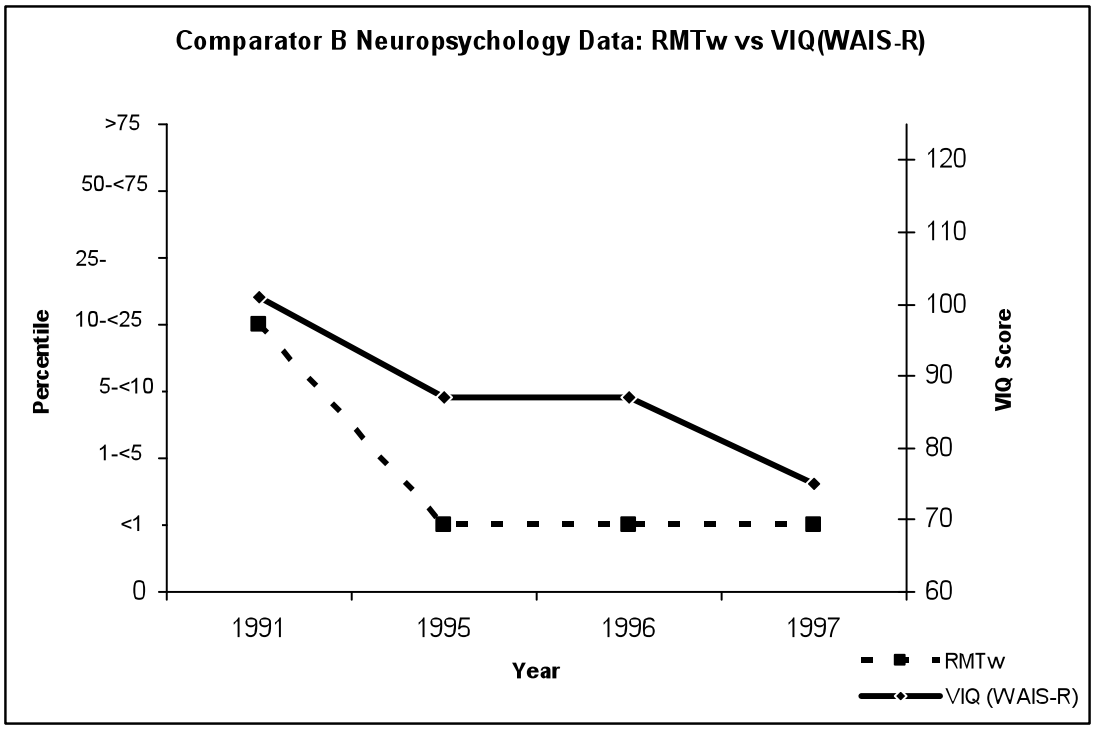
14c


Comparator A Neuropsychology: RMTw vs VIQ(WAIS-R)
Percentile
>75
50-<75
25-<50
10-<25
5-<10
1-<5
<1
0
VIQ Score
120
110
100
90
80
70
60
1992
1994
1995
1996
Year
RMTw
VIQ(WAIS-R)

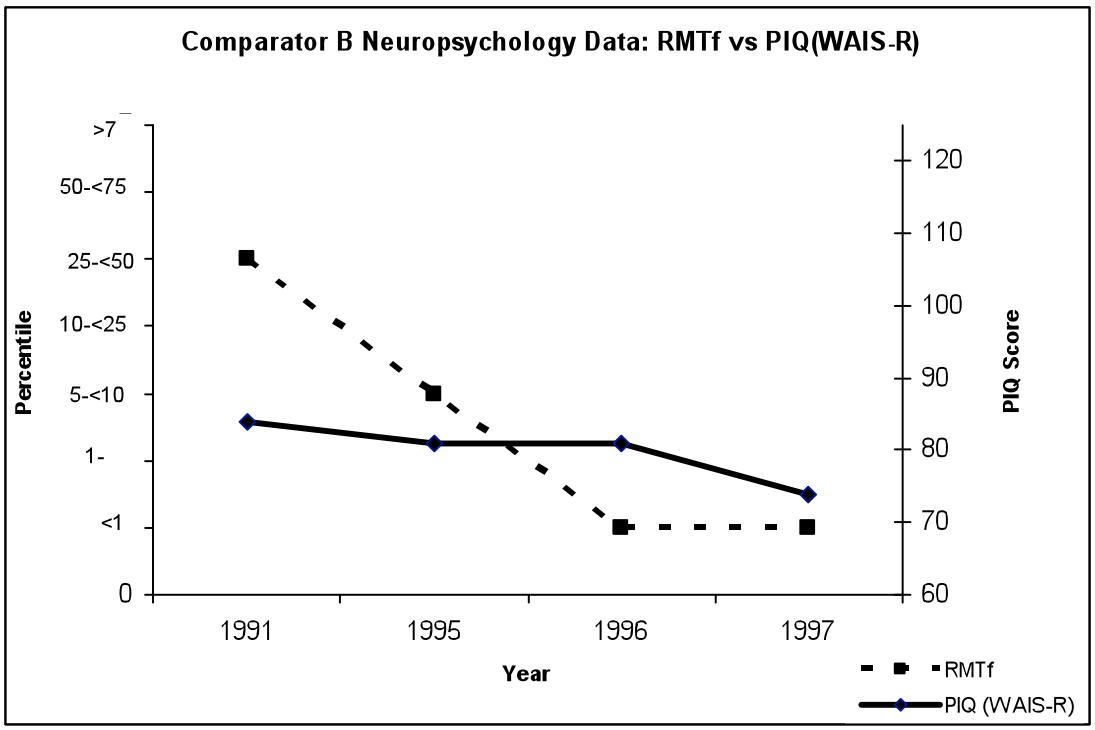
14d


Comparator A Neuropsychology: RMTf vs PIQ(WAIS-R)
Percentile
>75
50-
25-
10-<25
5-<10
1-<5
<1
0
PIQ Score
120
110
100
90
80
70
60
1992
1994
1995
1996
Year
RMTf
PIQ (WAIS-R)

14e

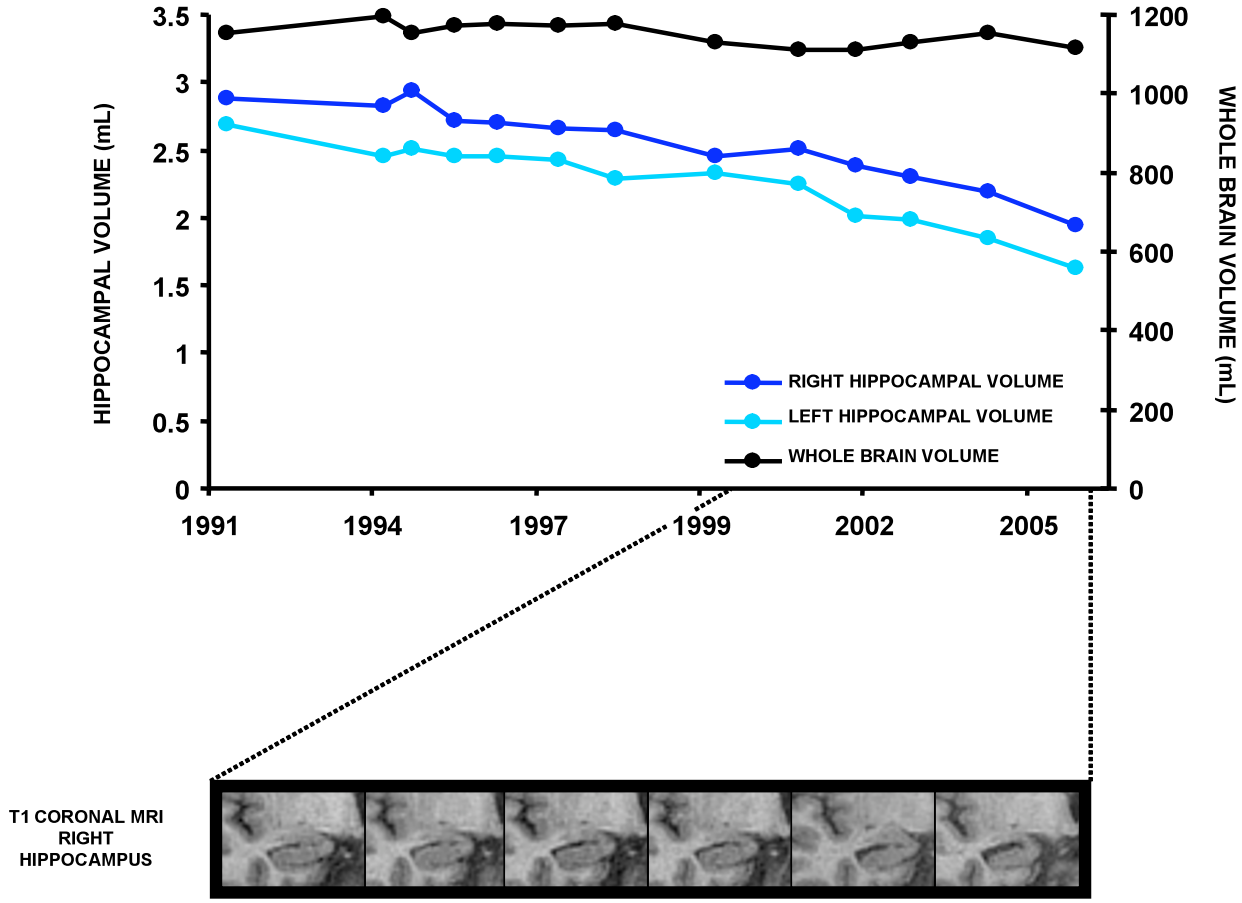

Comparator B Neuropsychology Data: RMTw vs VIQ(WAIS-R)
Percentile
>75
50-<75
25-
10-<25
5-<10
1-<5
<1
0
VIQ Score
120
110
100
90
80
70
60
1991
1995
1996
1997
Year
RMTw
VIQ (WAIS-R)

14f


Comparator B Neuropsychology Data: RMTf vs PIQ(WAIS-R)
Percentile
>7
50-<75
25-<50
10-<25
5-<10
1-
<1
0
PIQ Score
120
110
100
90
80
70
60
1991
1995
1996
1997
Year
RMTf
PIQ (WAIS-R)

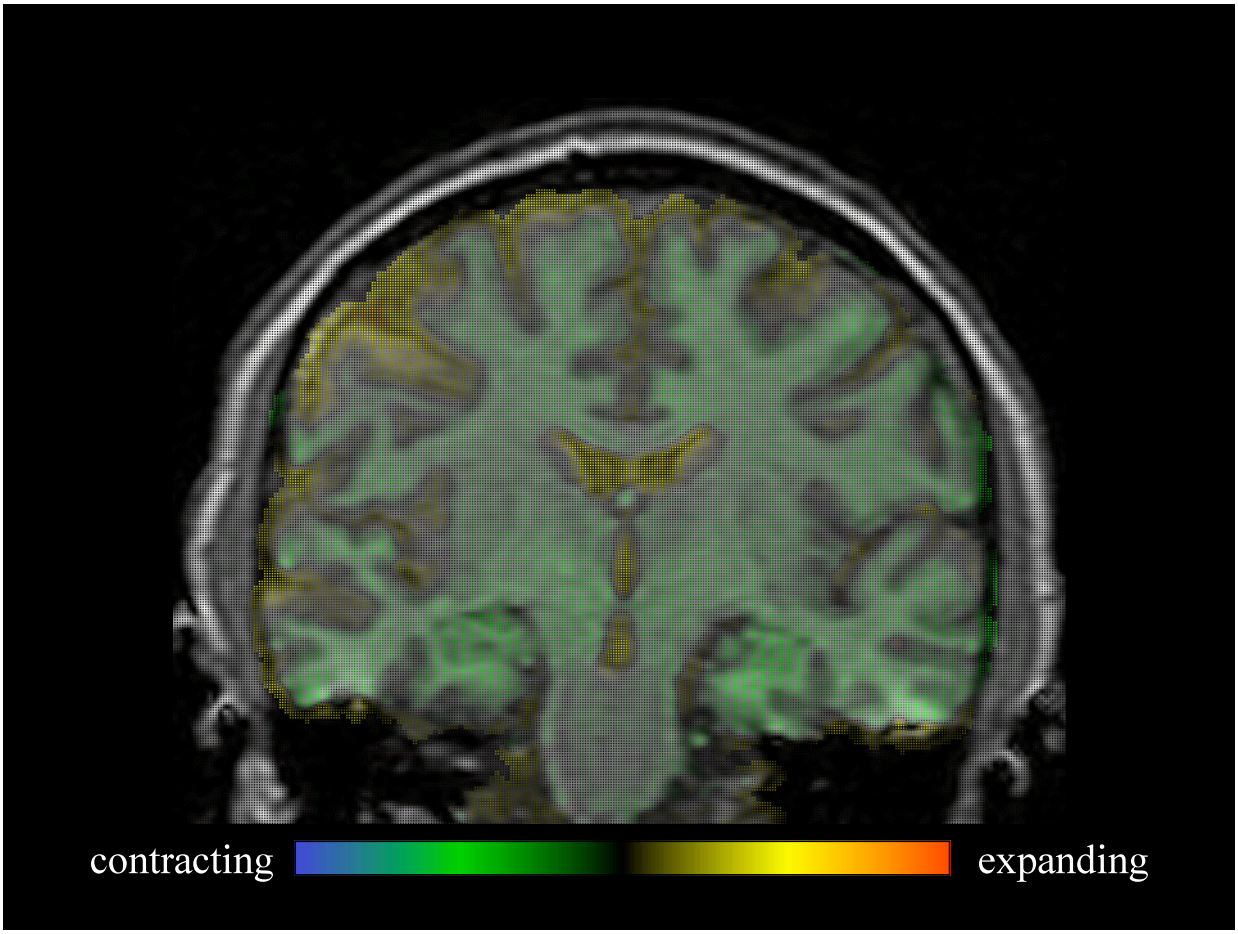
ApoE genotype was E3/3. Annual volumetric brain MRI (Figure 9) between 1991 and 1998 was performed using a 1.5T GE Signa scanner (General Electric Medical Systems, Waukesha, WI, USA). Volumetric T1-weighted coronal MRI scans were obtained with a spoiled gradient echo technique (256×128 matrix, field of view 24×24 cm, TR/TE/NEX/FA=35 ms/5 ms/1/35°) yielding 124 contiguous 1·5 mm thick slices. Between 2000 and 2006 imaging was undertaken on a different 1.5T GE Signa scanner running software version 5.8 (General Electric Medical Systems, Waukesha, WI, USA). Volumetric T1-weighted coronal MRI scans were obtained using an inversion recovery prepared fast spoiled gradient-echo technique (256×256 matrix, field of view 24×18 cm, TR/TE/TI/NEX/FA=14 ms/5·4 ms/650 ms/1/15°) yielding 124 contiguous 1·5 mm thick slices. The software package MIDAS was used for all manual hippocampal and whole brain segmentations (Freeborough *et al.* 1997). Fluid registration (Scahill *et al.* 2002) of volumetric images between 1992 and 1997 showed expansion of CSF spaces with generalised cerebral volume loss, but clear predominance of hippocampal atrophy (Figure 16).

Figure 15: Longitudinal hippocampal & whole brain volumes



Date	Left Hippocampal Volume(mm^3)	Right Hippocampal Volume(mm^3)	Total Hippocampal Volume(mm^3)	Whole Brain Volume(mm^3)
15 Jan 1992	2687	2886	5573	1154842
19 Aug 1994	2448	2821	5269	1193400
15 Feb 1995	2511	2931	5442	1154288
01 Nov 1995	2450	2709	5159	1171387
19 Jul 1996	2459	2705	5164	1174278
25 Jul 1997	2428	2661	5089	1172376
11Jul 1998	2286	2642	4928	1174848
08 Mar 2000	2326	2459	4785	1128225
03 Aug 2001	2241	2505	4746	1108732
17 Jul 2002	2016	2378	4394	1107934
25 Jun 2003	1982	2304	4286	1129148
06 Oct 2004	1848	2194	4042	1150745
21 Mar 2006	1628	1937	3565	1116479

Figure 16: Fluid registration 1992-1997.



VG's father had been affected from the age of 59 years, and also had a relatively lengthy, predominantly amnestic illness, dying at 74 years. Unfortunately, no more detailed information on the nature of his illness is available. Longitudinal neuropsychological data are available for two affected members of the same generation (designated A and B in Figure 13). These both show a contrasting pattern characterized by deterioration in both memory and non-memory domains (Figure 14).

Comparator A developed memory impairment at the age of 47 years. The initial manifestation was difficulty in learning new tasks at work and he stopped work within a

year of symptom onset. He quickly developed global cognitive decline (Figures 14c and 14d) and died aged 56 years. ApoE genotype was E3/4.

Comparator B also first experienced an insidious onset of memory problems aged 47 years. She progressed to global cognitive decline and was unable to attend for clinical or research assessments beyond the age of 52 years (Figures 14e and 14f). At our last contact she was 54 years old and dependent upon her husband for most activities of daily living. ApoE genotype was E3/4.

The mother of comparator A, whose age at symptom onset was 53 years and age at death 61 years, had pathological confirmation of AD with diffuse deposits of amyloid throughout the cortex. Neuritic plaques and NFT were abundant in all cortical regions including the hippocampus and amygdala. No other inclusion bodies were seen, but severe congophilic angiopathy was noted.

4. 3. 2. Discussion

The majority of FAD cases are associated with a predominantly amnestic clinical phenotype similar to that seen in most SAD cases. However, phenotypic heterogeneity does exist: Prominent examples include the spastic paraparesis (Kwok *et al* 1997; Crook *et al* 1998; Houlden *et al* 2000; Smith *et al* 2001; Brooks *et al* 2003) or frontal presentations (Styczynska *et al* 2004; Duarte *et al* 2004) associated with some *PSEN1* mutations. The spectrum of clinical presentations associated with the *APP* mutations is expanding. It includes CAA with ICH (Dutch mutation) (Levy *et al* 1990) and the 'intermediate' phenotypes of the Flemish mutation (Hendriks *et al* 1992b) and *APP*d (Rovelet-Lecrux *et al* 2006b) which produce CAA in association with memory-led cognitive decline.

The term pure progressive amnesia, as used by Barbeau *et al.* (2006), describes a rare amnestic syndrome of insidious onset, with memory performance that can remain stable or decline slowly over many years. Crucially, other cognitive domains and functional independence are typically preserved throughout this period. It is generally held to be a rare presentation of AD which results from MTL damage (Butters *et al.* 1996) and has been reported in association with EOAD (Stokholm *et al.* 2005), SAD (Caselli *et al.* 1998), the *APP*V717I (London) mutation (Gankam Kengne *et al* 2007) and in the context of preserved semantic processing (Fossard *et al.* 2006). To date, all cases followed up have progressed to dementia and several have had neuropathological AD confirmed (Caselli *et al* 1998).

Although EOAD and autosomal dominant early onset FAD are not equivalent, FAD forms part of a broader group of early onset disease, and it is interesting to note previous descriptions of the clinical profile of EOAD which contrast with that of our patient. In particular, previous studies have reported a higher prevalence of language impairment or other neocortical functions (Jacobs *et al* 1994) as well as a relatively more rapid progression (Rogaeva 2002) in EOAD as compared with LOAD. This contrasts starkly with VG, in whom the isolated amnesia was stable over an extended period of at least 13 years. VG also differs from two comparators in the same generation of the same family, both of whom show a decline in both memory and non-memory domains. This suggests that the unusual phenotype seen in VG is not a function of the *APP*V717G mutation *per se*.

ApoE genotype differs between VG (E3/3) and the comparators (both E3/4). ApoE status has been reported to modify AAO in *APP*V717I-associated FAD via an epistatic effect between the *APP* and ApoE genes (St George-Hyslop *et al.* 1994; Sorbi *et al* 1995).

This would suggest that the presence of the E4 allele predisposes to an earlier AAO, although this is not what we observed (subject AAO 47 years, comparator's AAO 47 and 45 years, mean AAO for family 51.5 years).

Neuroimaging in this case is consistent with the clinical features in that slow, progressive cortical atrophy was demonstrated, though with disproportionate involvement of each hippocampus. Medial temporal and posterior cingulate hypometabolism on SPECT and focal hippocampal and ERC atrophy on MRI have been previously demonstrated in pure progressive amnesia (Barbeau *et al.* 2006). However, little is known about the specific radiological profile of FAD-associated pure progressive amnesia, although MRI has previously been reported as normal (Gankam Kengne *et al* 2007). The rate and focality of brain atrophy in VG differs from previous studies of early-onset, though non-autosomal dominant, cases. For example, Frisoni *et al.* (2007) demonstrated more severe grey matter atrophy in EOAD than LOAD with neocortical areas more profoundly affected in EOAD and the hippocampus more profoundly affected in LOAD. Patients were classified according to age only (<65 years), with no information on the presence of FAD gene mutations or autosomal dominant family histories. The clinical and radiological phenotypes are congruous in this case: the symptomatic decline, while pathological, is not as rapid as one would expect in canonical AD. Similarly, the mean rate of hippocampal volume loss over a period of more than 14 years is intermediate (2.54%/year) between the values reported in cognitively normal subjects and those with AD. Previous studies have found rates of 0.80% (Du *et al.* 2004) and 0.41% (Fox *et al.* 2000) for normal controls and 5-6% (Du *et al* 2004; Hashimoto *et al.* 2005) and 2.37% (Fox *et al* 2000) for AD patients.

Our case provides further evidence for the existence of a protracted, purely amnestic phenotype related to *APP717* mutations, this time over at least 13 years. It also provides

new information regarding the nature of the regional brain volume changes associated with this clinical syndrome.

4. 4. A novel presenilin 1 deletion (p.L166del)

4. 4. 1. Case description

The proband (II-4 Figure 17) is a member of a pedigree in which there is a history consistent with autosomal dominantly inherited dementia. Her mother (I-2 Figure 17) had died at the age of 46 years after suffering from progressive memory impairment leading to dementia. The proband is a 40 year-old woman who presented with a two-year history of insidious, progressive impairment of episodic memory first noticed by her family. This would manifest chiefly as repeated questioning, forgetting names and the mislaying of objects. Other early features included topographical memory impairment and dyscalculia. Latterly, further problems had emerged such as emotional lability and an inability to plan or order tasks.

Neurological examination was normal with no myoclonus, extrapyramidal or upper motor neurone signs and a MMSE score of 16/30. At presentation she had clear, widespread cognitive impairment, most notably of verbal and visual recall, working memory and visuospatial abilities. Formal neuropsychometric testing was undertaken showing variable performance across the subtests of the WAIS but low-average and extremely low scores on verbal and performance IQ respectively. In addition she performed very poorly on tests of information processing speed, visual reasoning abilities, the Wechsler Memory Scale and Hopkins Verbal Learning Task. She achieved average performances on tests of general knowledge and abstract verbal reasoning but had substantial difficulty with mental arithmetic tasks.

MRI (Figure 18) revealed generalized, symmetrical cerebral atrophy most marked in the MTL with a few foci of high signal within the peripheral white matter. EEG revealed diffuse, poorly responsive theta activity at 4-6 Hz and frequent, intermittent runs of 3Hz, raised amplitude, notched delta activity which was generalised but with a clear bilateral, anterior emphasis. Small sharp waves were noted on occasion in the anterior regions. CSF was normal.

4. 4. 2. Genetic analysis

Analysis of *PSEN1* was conducted as described previously (Palmer *et al.* 1999). A novel 3bp CTT deletion at codon 166 was detected in the proband in both forward and reverse sequencing of exon 6. This was not detected in any of 100 normal controls.

Figure 17: Pedigree

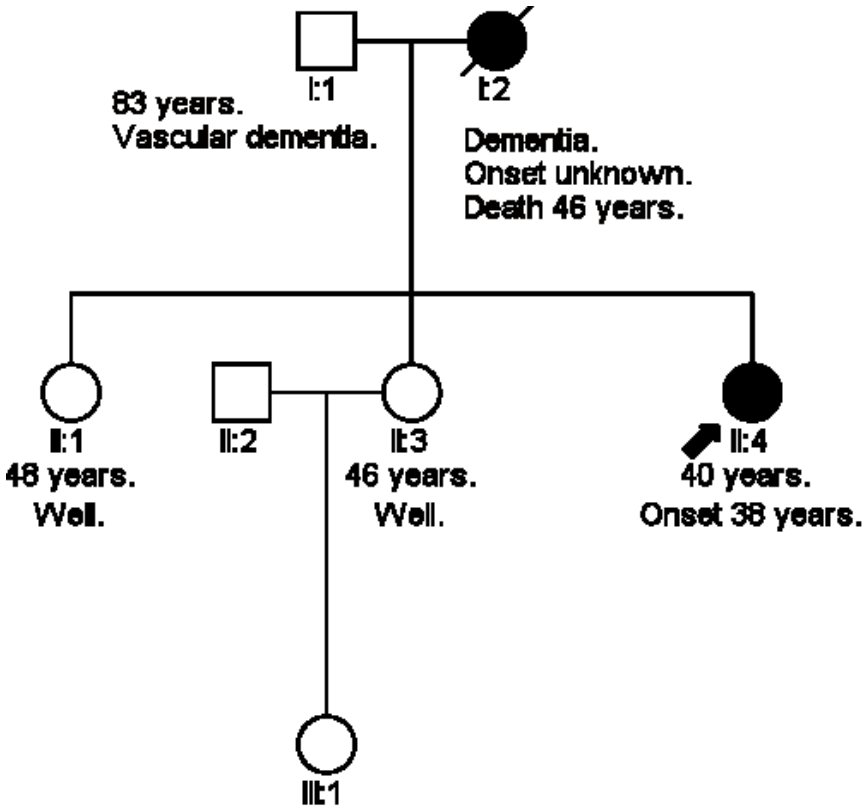


Figure 18: MRI brain, coronal T1 & axial T2

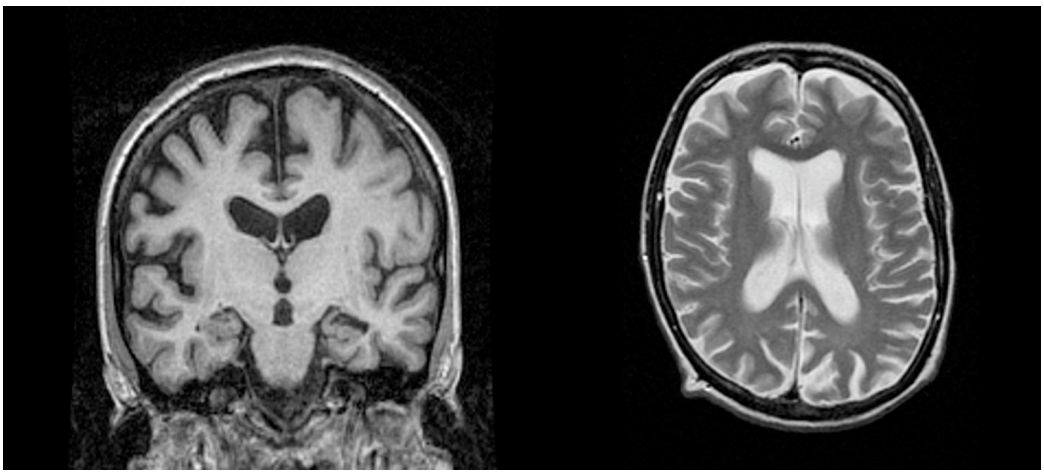
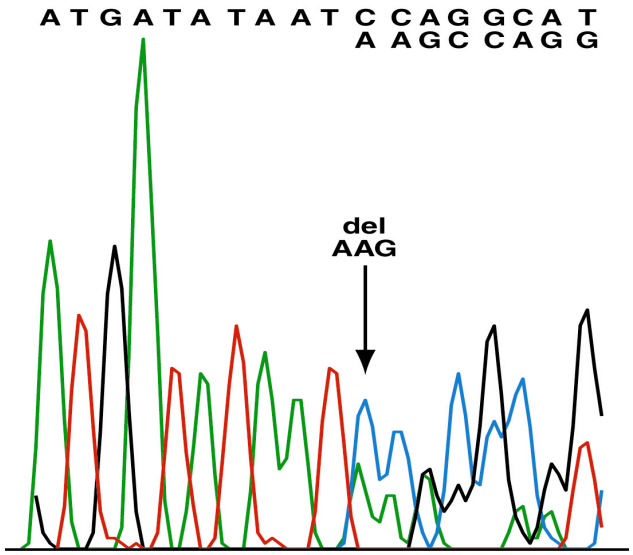


Figure 19: Sequencing electropherogram for part of the reverse compliment of *PSEN1* exon 6 showing a deletion of AAG (CTT in forward compliment, codon 166).



4. 4. 3. Discussion

Located in the third transmembrane domain of exon 6, amino acid position 166 of *PSEN1* shows significant phylogenetic conservation and in homologous proteins such as PS2 and Sell2, suggesting that the position is of functional significance. Further, this novel deletion was not detected in 100 healthy controls – suggesting that it is unlikely to represent a common, non-pathogenic polymorphism. Mutations at this site co-segregating with clinical disease have previously been reported (Ezquerra *et al.* 2000; Moehlmann *et al* 2002; Pantieri *et al.* 2005) as has a deletion at codon 167 (Janssen *et al* 2003). In two of the former cases, a clinical phenotype similar to our proband is described (Ezquerra *et al* 2000; Pantieri *et al* 2005). In the third (L166P), an aggressive onset in adolescence occurs with later spastic paraparesis (Moehlmann *et al* 2002). Other

mutations, namely *PSEN1* delta 290-319 and R278T, have been linked with spastic paraparesis in some of the affected family members (Kwok *et al* 1997).

In contrast to deletions reported at other sites on *PSEN1*, our case was associated with the 'typical' presentation of EOAD seen in most exon 6 mutations, and in most *PSEN1* mutations *per se*. The presence or absence of cotton wool plaques that accompany some other deletions remains unknown and, despite the evidence presented above, it remains difficult to comment definitively upon the pathogenicity of this novel deletion. To do so will require extending our knowledge of the family and revisiting the case in the light of any future neuropathological or genetic evidence.

4. 5. A novel presenilin 1 substitution (S132A)

4. 5. 1. Case description

The proband was seen clinically at the National Hospital for Neurology and Neurosurgery, London, presenting at the age of 64 years with a five-year history of neuropsychiatric and cognitive symptoms. Visuoperceptual and agnostic problems, reduplicative (Capgras) phenomena with persecutory delusions of imprisonment and complex visual hallucinations of animals, workmates and deceased relatives were all evident at this stage. Additional features included urinary incontinence, speech production and comprehension problems. He was noted to have myoclonic jerks of his arms and shoulders, bradykinesia, limb hypertonia and bilateral grasp and palmomental reflexes.

His family history was consistent with an autosomal dominant pattern of inheritance (Figure 20). A condition similar to that observed in the proband was noted in his mother (who died age 61 years after an ICH), a maternal aunt, a maternal uncle (who died at 65

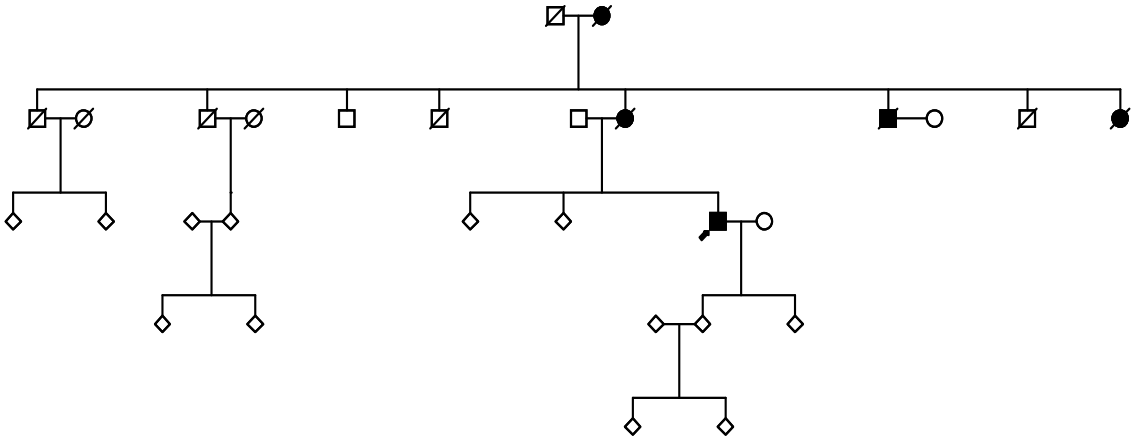
years) and his maternal grandmother who died at 76 years in an institution after a 10-year illness associated with 'cortical atrophy of (the) brain'.

EEG showed dominant slow wave activity and only occasional, poorly reactive α rhythm. MRI was too poorly tolerated to be informative. Brain CT performed at age 64 years showed mild to moderate cerebral and cerebellar atrophy.

4. 5. 2. Genetic analysis

Analysis of *PSEN1* was conducted as previously described (Palmer *et al* 1999). A novel substitution at codon 132 was detected in the proband in both forward and reverse sequencing of exon 5. The complete open reading frame of the prion protein gene was also sequenced and no mutations were detected. The codon 129 genotype was methionine valine heterozygous. It was not possible to confirm cosegregation through testing other members of the family, although the mutation was not identified in 100 normal controls.

Figure 20: Pedigree



4. 5. 3. Discussion

Codon 132 lies in exon 5 of *PSEN1* and has not previously been associated with AD-related mutation. Nearby mutations are, however, well documented and cluster in the adjacent second transmembrane domain (TMII) where 21 examples are so far recognised (see http://www.molgen.ua.ac.be/ADMutations). Examples closest to 132 include N135D (Crook *et al.* 1997), N135S (Rudzinski *et al* 2008), A136G (Fang and Jia 2007) and several substitutions at codon 139 (Boteva *et al.* 1996; Fox *et al.* 1997; Dumanchin *et al.* 1998; Campion *et al* 1999). There is considerable clinical, neuropsychological and neuropathological heterogeneity amongst TMII mutations, just as there is in the *PSEN1* mutation group as a whole, and such variety may even be seen within single mutations such as M139V. Nonetheless, mutations at codon 135 seem to have a particularly early age of onset in common, with symptoms typically developing in the fourth decade of life. Spastic dysarthria, spastic paraparesis, memory-led multi-domain cognitive decline and a generalized seizure disorder have been observed (Rudzinski *et al* 2008), as has a more typical AD-like decline with a neuropsychiatric prodrome (Crook *et al* 1997).

There are very few similarities, therefore, between the reported features of nearby mutations and the clinical phenotype observed in our proband. Although limb hypertonia was observed, he displayed a relatively late AAO with prominent behavioural and neuropsychiatric features, including visual hallucinosis. The psychiatric prodrome described by Crook *et al.* was observed in only one member of the pedigree and was not characterized by psychotic features, but rather by mood disturbance alone. The history of ICH in the mother does raise the possibility of an associated CAA, although there was no way to confirm this in the proband or mother and histopathology did not reveal significant CAA in either of the codon 135 mutations discussed above. The poorly-defined cerebellar atrophy apparent on the CT scan is consistent with previous reports of cerebellar amyloid plaques in *PSEN1* mutations (Verkkoniemi *et al.* 2001).

The possibility remains that the novel substitution observed here represents a non-pathogenic polymorphism and, in the absence of confirmed cosegregation, this is difficult to address. The strongly suggestive family history and failure to identify such a change in a sample of normal controls does, however, lend some weight to the case for its pathogenicity.

5.

CHARACTERIZING FAD

II: *APP* locus duplication

5. 1. Duplications of *APP* are a significant cause of early onset dementia in a large UK referral series.

Patients presenting before old age with AD-like features and evidence of a familial disease should be screened for FAD mutations. However, conventional sequencing will not detect gene deletions or duplications. Over recent years it has become recognised that copy number variation (CNV), caused by duplication or deletion of parts of chromosomes, are relatively common and occur with a non-random distribution across the genome (McCarroll and Altshuler 2007). The altered regions may include genes that encode for proteins known to be implicated in disease.

The association between AD and Down's syndrome has long been known, providing evidence that a higher gene dosage of *APP* is sufficient to produce an AD phenotype. Recently a number of small chromosomal duplications which include the *APP* locus have been reported (see 1. 4. 2.) with an estimated frequency of 8% (Rovelet-Lecrux *et al* 2006b), about half the contribution of missense *APP* mutations to early onset, autosomal dominant AD (Raux *et al.* 2005). Whilst these studies uncovered an important mutational mechanism at *APP*, questions remain about the frequency of these mutations in larger, less selected *APP* patient cohorts in different countries and the associated phenotypic spectrum. The following study investigated the presence and frequency of CNV at *APP* loci in a large referral cohort of UK patients, and presents an optimized methodology for cost-effective high-throughput screening of patients for such structural variation. It also examined the phenotype associated with *APP*d in this group.

5. 1. 1. Methods

5. 1. 2. Subjects

The AD cohort comprised two subgroups. One group was formed from a referral series of 381 patients for a diagnostic test of AD gene mutation, average age was 53 +/- SD 10, 47% were male. This series were screened for mutations in *APP*, *PSEN1,* and *PSEN2* although not all patients were screened for all genes as, for example, some patients were referred prior to the identification of the presenilins as causal genes. This cohort largely comprises probands with familial dementia followed-up at the Dementia Research Centre, UCL Institute of Neurology. A further 492 patients comprise a collection of patients thought to have AD but with insufficient clinical evidence to justify screening of causal genes. All patients gave informed consent for genetic analysis. The University College London Hospitals NHS Trust Local Ethics Committee gave ethical approval for the study.

5. 1. 3. Samples

Genomic DNA (gDNA) was extracted from peripheral whole blood or post mortem brain material using standard techniques. gDNA quality was assessed using agarose gel electrophoresis. Only those gDNA samples of average molecular weight >10Kb were included in the study. Concentration was assessed using a NanoDrop spectrophotometer (ThermoScientific, NanoDrop Products, Wilmington, DE) and samples were diluted to 20ng/µl in 1X tris-EDTA buffer prior to use.

5. 1. 4. Real-Time Quantitative PCR (exon-qPCR)

APP alleles were quantified on an ABI 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using the 5' nuclease assay and duplexed minor groove binding (MGB) probes designed to detect *APP* (exon 5) and *GRN* (Chr 17,

exon1). Relative quantification of *APP* using *GRN* as the internal reference was determined using the ΔCt method. Twenty nanograms of gDNA were amplified in a 25μl reaction volume containing 1X Expression Mix (Applied Biosystems), and the respective primer (900nM) and probe (200nM) sets for both the gene of interest and the internal reference using the universal protocol. Each sample was quantified in triplicate with each replicate having a different plate reference to minimise the affect of inter-well variation. One sample with trisomy 21 was included as a positive control on all plates. All samples with a dosage quotient (expressed as a ΔCT) two standard deviations from the mean were considered as potential CNVs.

5. 1. 5. Fluorescent Microsatellite Quantitative PCR (fm-qPCR)

APP alleles were quantified by the genotyping of two microsatellites located within intron 1 of *APP* (196999; Chr21: 26,460,990 – 26,461,175) and 330kb centromeric from *APP* (188463; Chr21: 25,841,358 – 25,841,530). Twenty nanograms of gDNA were amplified by denaturation at 95˚C for 1 minute followed by 24 cycles of 95˚C for 30 seconds, 53˚C for 30 seconds and 72˚C for 1 minute. Amplicons were analysed using an ABI 3130xl automated sequencer and GeneMapper software v.4.0 (ABI). In all individuals heterozygous for a given marker the allelic ratio was assessed by relative peak area. Cases were deemed CNV positive by fm-qPCR if allelic ratios were two standard deviations from the mean and if i) both of a microsatellite pair had altered allelic ratio or ii) one of a microsatellite pair had an altered peak ratio with the other genotyped as homozygous or iii) only one of a microsatellite pair had an altered peak ratio but it was the one nearest to the gene of interest.

5. 1. 6. Illumina 610 Bead Array

Samples identified as "potential CNV" by exon-qPCR and/or fm-qPCR were assayed using the Illumina bead array system according to the manufacturers protocol. All samples were assayed using the Illumina Human 610-Quad BeadChips (Illumina Inc, San Diego, CA, USA) as per manufacturer's instructions, using 200 ng of genomic DNA. These BeadChips assay more than 610,000 tag single nucleotide polymorphisms (SNP) and markers, including 60,000 CNV markers, based on the International Human HapMap project release 23 (www.hapmap.org). All the samples genotyped had a genotype success rate of more than 99%.

Individual chromosomes were examined using a method previously demonstrated to be reliable for detecting genomic copy number mutations (van de Leemput *et al.* 2007; Simon-Sanchez *et al.* 2008; Knight *et al.* 2008). We looked at the whole genome in each sample, with particular interest in the region containing *APP*, in order to be able to confirm the results previously obtained.

5. 1. 7. Results

5. 1. 7. 1. Laboratory findings

Using Exon-qPCR comparison was made between the amplification of *APP* and an internal reference marker (*APP-GRN*). 21 samples from the AD cohort were considered as potential CNVs based on the selection criteria of samples with a ΔC_t value greater than 2 standard deviations from the mean (see Table 12).

Table 12: *APP* CNV Screen

	Total Sample Number	Double homozygotes	Number Screened	Positives	False Positive Rate
Exon-qPCR	873	N/A	873	21	16
fm-qPCR	873	54	819	42	37
Both	873	N/A	873	5	0
Illumina Array	5	N/A	5	5	N/A

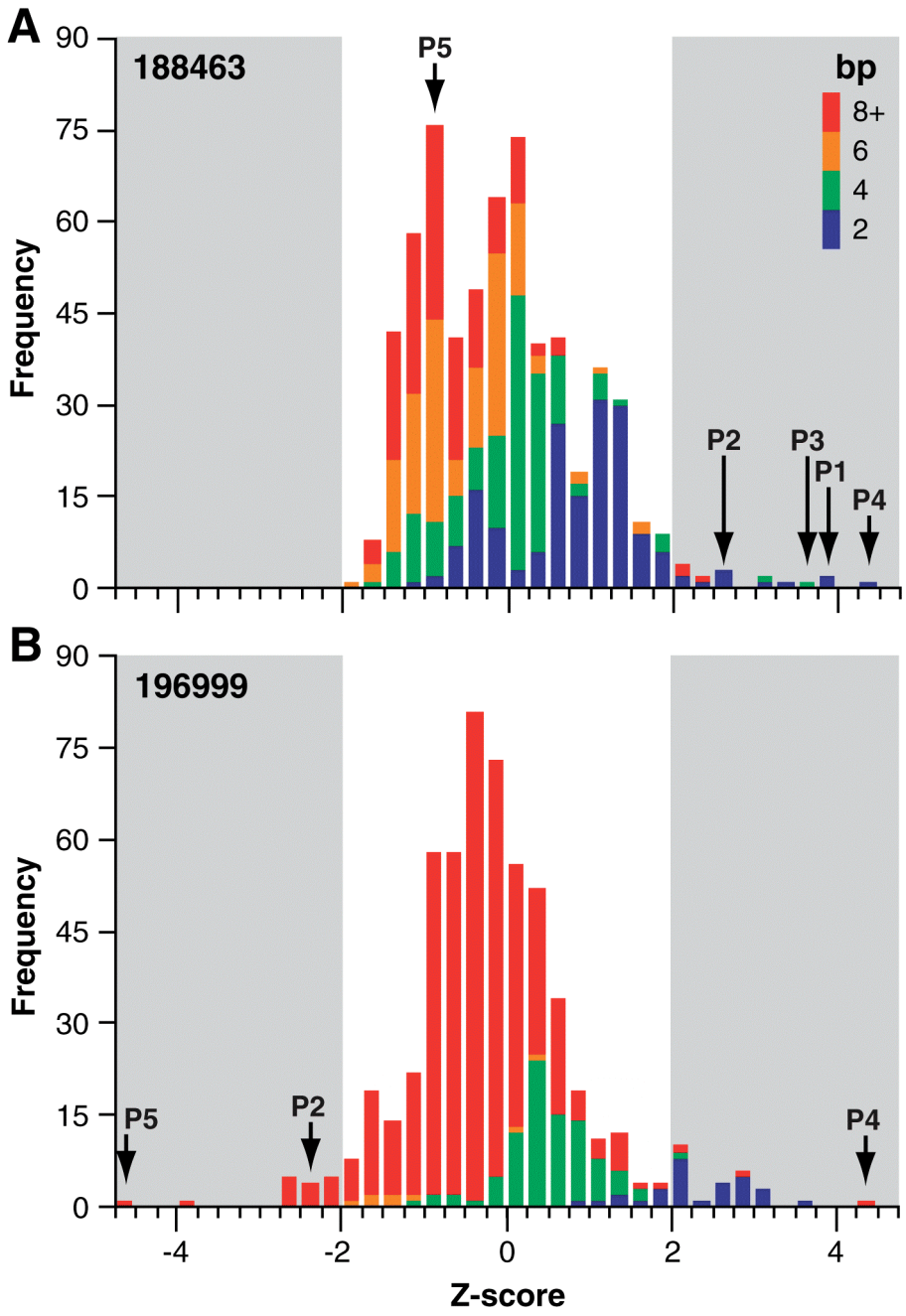
Using fm-qPCR two polymorphic microsatellites within intron 1 and 330kB centromeric to *APP* were amplified using a standard PCR protocol for analysis using fm-qPCR. A comparison of peak area was made in all heterozygous individuals for either one or both of the microsatellite pair. The samples screened were assessed for CNVs in this way with only 6.1% of individuals being double homozygotes (see Table 12). 42 samples were considered as potential CNVs. Three allelic peaks appeared on the electropherogram of Proband 3 (microsatellite 196999). This was consistent with an unbalanced translocation that does not appear to include Down's syndrome critical regions.

Five samples from the *APP* cohort were deemed positive by both exon-qPCR and fm-qPCR. All these samples were confirmed as heterozygote duplicates by the Illumina array. The complementary use of these two techniques therefore represents a highly accurate screening process for heterozygous duplications, using the Illumina array as the gold standard assay.

The Illumina 610 Bead array was used for the verification of duplication in samples that were identified as potential CNVs from exon-qPCR and fm-qPCR. These data showed

duplicated regions of Chr21q, which included the *APP* gene locus in all 5 probands identified as CNVs by both exon- and fm-qPCR. There was considerable heterogeneity in duplication size (2.77, 6.35, 4.96, 6.49Mb, see Figure 23), showing that these patients represented separate duplication events. Although Proband 3 had been identified by fm-qPCR as having three microsatellite alleles, the array revealed this patient to have an extended discontinuous duplication of Chr21q. There are no available SNP for the p arm of Chr21 so this could not be assessed. The large duplicated region is interrupted by a region of unduplicated SNP (see Figure 16). In conjunction with the microsatellite haplotyping, these data are consistent with an unbalanced translocation of Chr 21. Of the five positive individuals of the AD cohort, 4 (1%), (Probands 1, 2, 3 and 5) originated from the referral series of 381 patients for a diagnostic test of AD gene mutation and screened for *APP*, *PSEN1, PSEN2* and 1 (0.2%), (Proband 4) originated from 492 patients comprising a collection of patients thought to have AD but with insufficient clinical evidence to justify screening of causal genes.

Figure 21: *APP*d detection using alteration in the ratio of area under the curve of microsatellite alleles on electropherogram traces.



The histograms in Figure 21 illustrate the distribution of allelic peak area ratios. Variation occurring among the ratios of microsatellite allelic areas appeared to be polymodal. This observation was caused by 'stutter' peaks immediately preceding each microsatellite allele. Microsatellite stutter peaks accentuate the peak of the shorter allele to a degree that is

based upon the size differential of the two alleles. Thus, the degree of difference in base pairs between two alleles accounts for part of the variation in allelic ratios. When the two microsatellite alleles are widely separated in length, the ratio is not affected by stutter peaks, whereas when alleles are adjacent the ratio is affected most. This phenomenon accounts for most of the false positives using this method alone.

Differences in base number between allelic pairs are illustrated by different colour. Shaded areas highlight samples 2 standard deviations from the mean.

A) Microsatellite marker 188463: Proband 5 had a normal allelic peak ratio. Array analysis confirmed that this marker lay outside the duplication boundary for this individual.

B) Microsatellite marker 196999: Proband 3 had three microsatellite peaks. And Proband 1 was uninformative due to homozygosity.

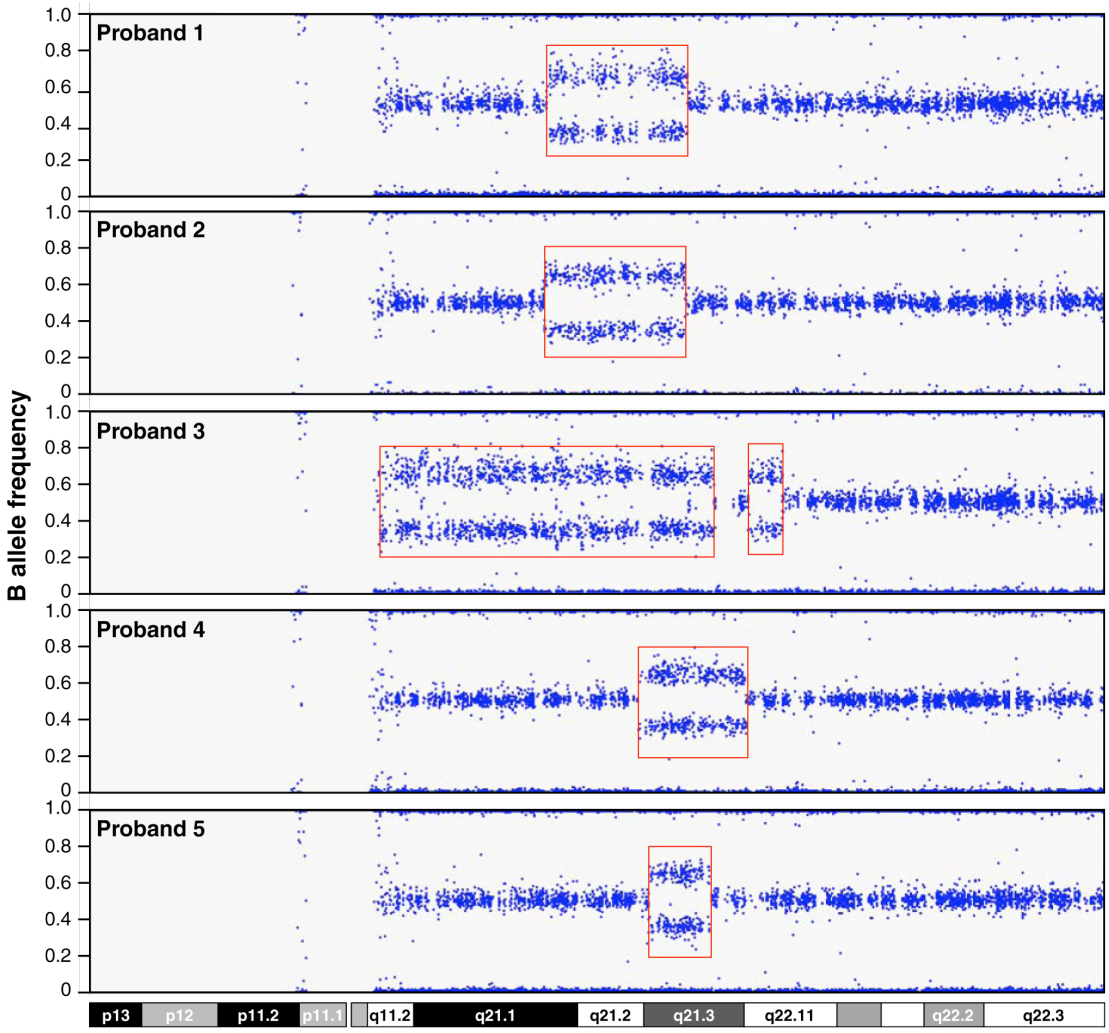


Figure 22: Confirmation of *APP*d using Illumina array technology

Heterozygous duplications in 5 probands. Regions of duplication are highlighted in red boxes. In order to examine individual chromosomes for structural mutations the visualization tool Genome Viewer version 3.2.9 within Beadstudio version 3.1.3.0 (Illumina Inc, San Diego, CA, USA) was used. An ideogram of Chr 21 and the B allele frequency is illustrated. 1 pixel=56 KB.

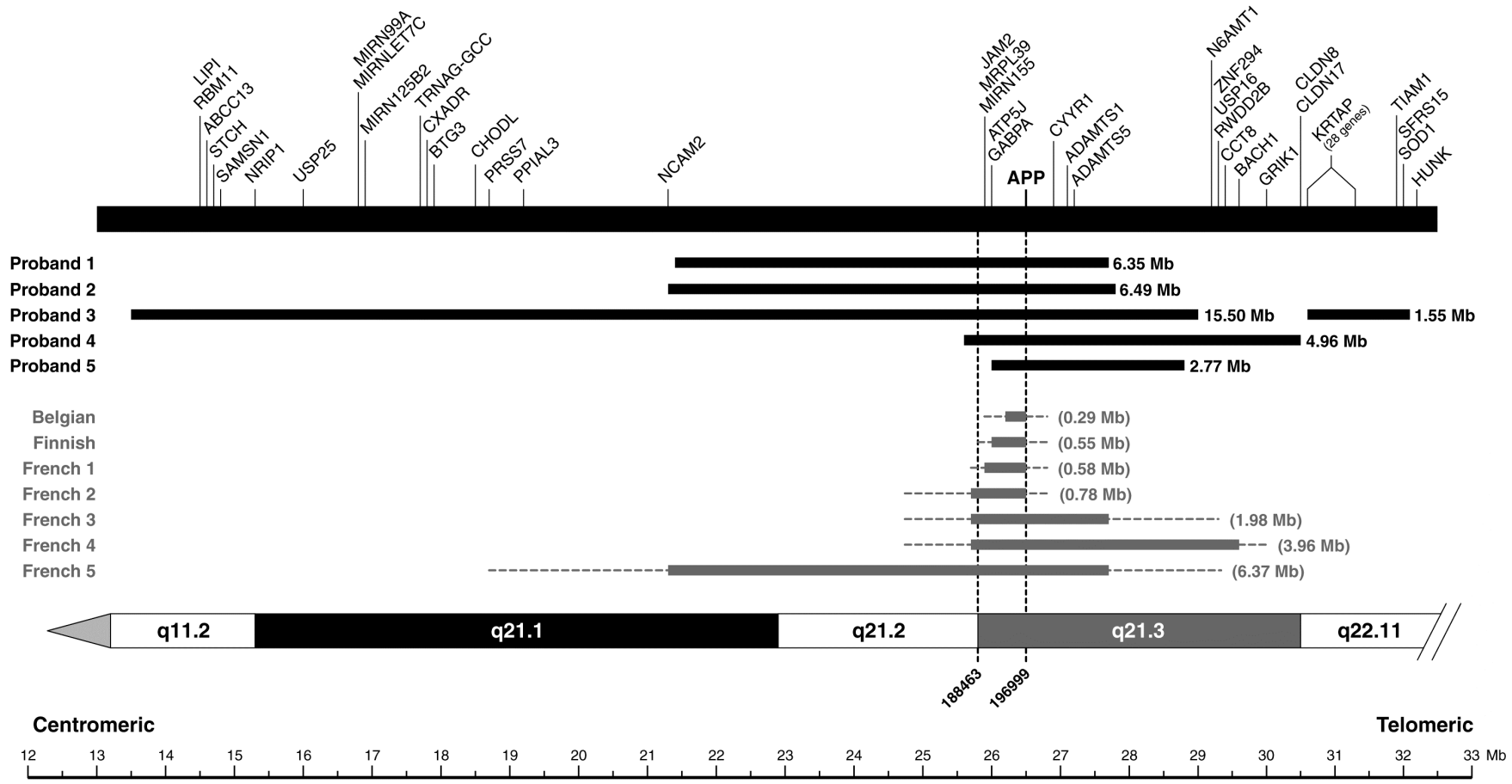


Figure 23: Chr 21 diagram showing *APP*d regions with respect to genes

*APP*d in 5 probands. Black horizontal bars indicate the extent of heterozygous duplications. Minimal sizes (in parentheses) of previously reported duplications are indicated by grey horizontal bars and the intervals of the duplication boundaries by dotted lines. Start of gene is indicated by a vertical bar. Note: Proband 1 has a partial duplication of NCAM2 and Proband 2 has a full duplication.

5. 1. 7. 2. Clinical and investigation findings

5. 1. 7. 2. 1. Proband 1

This 54y-old man had a progressive impairment of episodic memory, beginning insidiously at the age of 48y. Impairment of verbal memory was clearly impaired at presentation, although general intellectual functions were preserved. He went on to develop an apperceptive agnosia, limb apraxia and dysexecutive features. He was a former smoker. At 52y he had a generalized tonic-clonic seizure and elected not to commence anti-convulsant treatment, although donepezil 10mg was prescribed around this time. There was an autosomal dominant family history of EOAD: his father died at 62y after onset of disease in his late 40s, a paternal uncle died at 63y and a paternal aunt died at 49y, both after an undefined dementing illness (Figure 26a).

Initial MRI brain scanning showed widespread T2 FLAIR white matter hyperintensities consistent with subcortical vascular disease; multiple small, deep cerebral and cerebellar haemorrhages on T2* imaging and an old left-sided amygdala haemorrhage (see Figure 24). EEG was unremarkable. There was no history of sustained hypertension and cholesterol was 3.5mmmol/L. Tests for Notch 3 (CADASIL), *ITM2B* (Familial British Dementia), *APP, PSEN1*, *PSEN2*, *PRNP* and *MAPT* mutations were all negative. CSF analysis was unremarkable except for $_{t}$tau of 555pg/mL and $A\beta_{1\text{-}42}$ of 128pg/mL.

Table 13: Proband 1: Longitudinal Neuropsychometry

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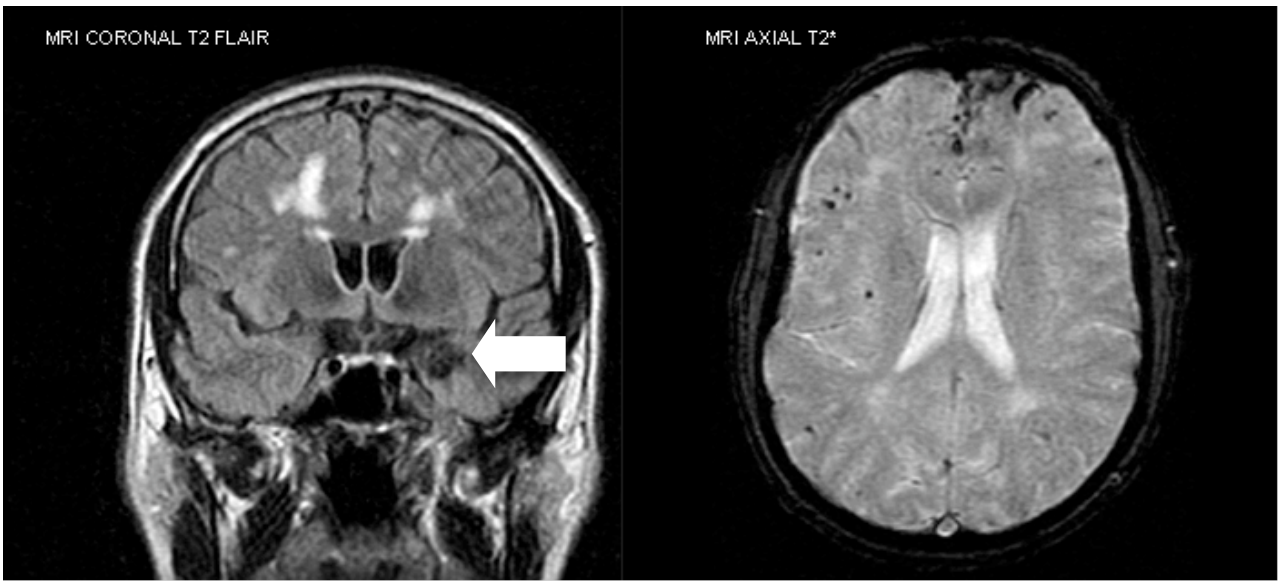
DATE(AGE) / TEST	2006(52)	2007	Jan 2008	Nov2008
VIQ	**90**	**87**	**88**	Unknown
PIQ	**76**	**80**	**78**	Unknown
RMTwords (%ile)	CHANCE	**<5**	**<5**	<5
GNT (%ile)	**25**	**5-10**	**10-25**	10
MMSE	25	23	Unknown	26

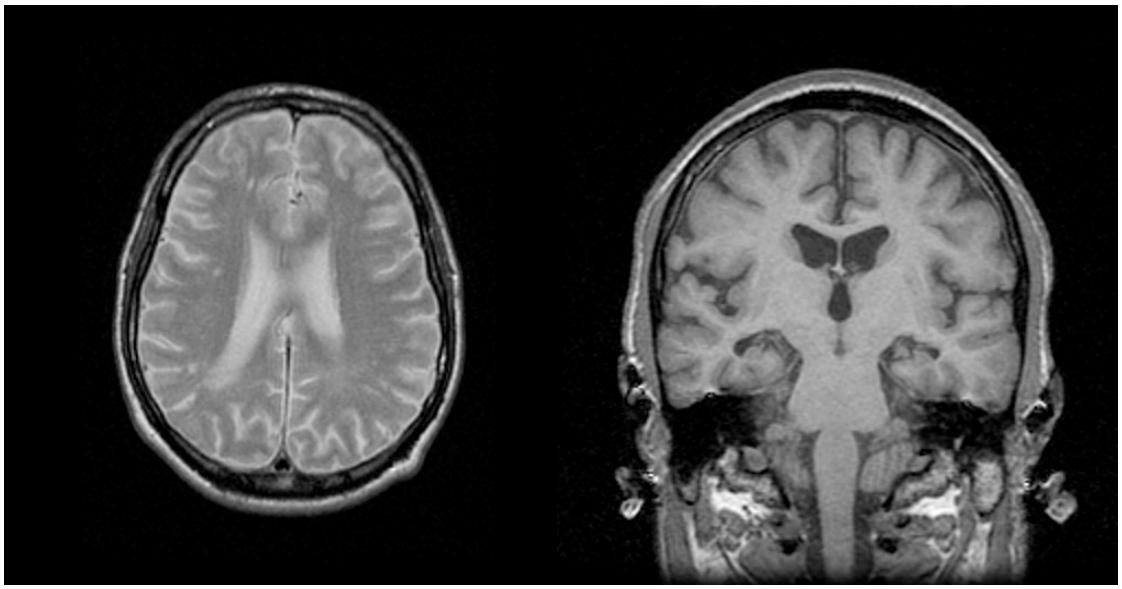
Figure 24: MRI scans from proband 1 showing extensive white matter abnormalities and an amygdala haemorrhage (arrow).



5. 1. 7. 2. 2. Proband and family 2

This man belongs to a family with a strong history of both early onset seizures and mid-life cognitive impairment (Figure 26b). He developed a variety of seizure types from the age of 11y with brief absences occurring up to five times per day and generalized tonic-clonic seizures on average once per year. A flurry of seizures at the age of 40y heralded increasing social withdrawal, apathy and an inability to follow simple commands. His cognition progressively deteriorated, including problems with memory, mood and wandering, as well as prominent myoclonus. From the age of 49y he had word-finding difficulties, which progressed to the extent that he would barely speak. He became increasingly immobile and incontinent of urine. He had no other past medical history, though he had previously been a heavy smoker. On examination at the age of 50y he had an MMSE score of 10/30, a paucity of speech, perseveration, poor concentration, moderate limb dyspraxia and visuoperceptual difficulties.

Figure 25: MRI scans from proband 2 (aged 47y) showing white matter abnormalities and hippocampal atrophy.



General neurological examination revealed a supranuclear gaze palsy affecting vertical movements, a brisk jaw jerk and brisk limb reflexes. His final MRI brain scan at age 50y revealed severe, generalized cerebral volume loss and bilateral hippocampal atrophy. There was extensive periventricular white matter signal change, more so on the right than the left. EEG showed an excess of slow wave activity with absent α-rhythm, while telemetry revealed bifrontal spike activity. CSF was acellular with normal total protein, negative oligoclonal bands and negative Whipple's PCR. Genetic screening for *APP*, *PSEN1*, *PSEN2*, *ITM2B, DRPLA, HD* and common mitochondrial mutations were negative. White cell enzymes were normal. He also underwent skeletal muscle and skin biopsies. The former showed no evidence of mitochondrial myopathy and the latter showed no Lafora bodies. Longitudinal neuropsychological testing was undertaken (Table 14) which demonstrated a relentless, slow, global cognitive decline. His sister also developed both generalized and absence seizures at the age of 11y. From the age of 50y she had a gradual, progressive impairment of episodic memory, with mild word-finding difficulties, low mood and emotional lability. Her MRI brain scan showed scattered, bilateral, peri-ventricular areas of high signal in the deep white matter, basal ganglia and pons. There was also a high signal area in the right frontal lobe consistent with an old infarct. CSF examination was unremarkable. Early onset seizures also affected his mother, a maternal aunt, one of his sisters, the daughter and granddaughter of one of his unaffected sisters as well as the grandson of another of his unaffected sisters although samples are not available for testing of segregation in these individuals. His mother died aged 64y with a long history of psychiatric problems. Lifelong generalized epilepsy was complicated by post-ictal paranoid psychosis and, although little information is available on her early life, problems with activities of daily living, self-care and an inability to remember her medication were present at least from the age of 62y.

Table 14: Proband 2: Longitudinal Neuropsychometry with corresponding EEG and imaging profile.

YEAR(AGE) / TEST	1998(44)	2000	2001	2002	2003	2004(50)
VIQ	71	78	76	75	62	63
PIQ	73	67	63	64	62	57
RMTwords	29/50	34/50	27/50	16/25*	CHANCE	CHANCE
RMTfaces	28/50	27/50	25/50	14/25*	CHANCE	CHANCE
GNT (%ile)	10-25	10-25	10-25	10	5-10	5
MMSE		22		15	10	10
MRI	FRONTAL WHITE MATTER LESIONS. NO REGIONAL ATROPHY	WHITE MATTER LESIONS BOTH HEMISPHERES NO REGIONAL ATROPHY	WHITE MATTER LESIONS BOTH HEMISPHERES BILATERAL HIPPOCAMPAL ATROPHY	→	WHITE MATTER LESIONS BOTH HEMISPHERES. GENERALIZED ATROPHIC CHANGE, GREATER ANTERIORLY. BILATERAL HIPPOCAMPAL ATROPHY RIGHT SMALLER THAN LEFT	GENERALIZED, SEVERE CEREBRAL VOLUME LOSS. EXTENSIVE WHITE MATTER CHANGE MORE PROMINENT IN RIGHT HEMISPHERE. BILATERAL HIPPOCAMPAL ATROPHY
EEG	FRONTOTEMPORAL SLOWING	TRACES OF α RHYTHM, LARGE AMOUNTS OF THETA IN BOTH HEMISPHERES	→		WIDESPREAD SLOWING. NO NORMAL RHYTHMS. NO POSTERIOR α RHYTHM	

VIQ/PIQ = VERBAL AND PERFORMANCE SUBSCALES OF WAIS-R (WECHSLER 1981)
RMTw/RMTf = RECOGNITION MEMORY TEST FOR WORDS AND FACES (*SHORT VERSION) (WARRINGTON EK 1984 & 1996)
GNT = GRADED NAMING TEST (MCKENNA & WARRINGTON 1980)
MMSE = MINI-MENTAL STATE EXAMINATION (FOLSTEIN 1975)

Information about histological post-mortem brain examination was limited, and hence inconclusive. A cavity consistent with previous haemorrhage or infarct was noted in the white matter, with abundant haemosiderin and surrounding gliosis. Several of the cortical and leptomeningeal vessels had evidence of hyaline degeneration and scanty diffuse amyloid plaques were present in the striatum.

5. 1. 7. 2. 3. Proband 3

This Greek man presented at 42 years old with a three-year history of mislaying items and forgetting events. Later problems with route finding were noted with increasing social withdrawal. Episodes of uncontrollable weeping occurred with associated malaise, rolling of the eyes and post-ictal confusion. These episodes were responsive to anti-convulsant therapy. He was an only child of two healthy parents and had no known extended family history of dementia (Figure 26e). MRI brain scans revealed generalized atrophy and EEG revealed an absence of α rhythm with disorganised activity and delta discharges. CSF analysis was unremarkable. Screening of *APP*, *PSEN1* and *PRNP* was negative. Neuropsychometry revealed a relatively preserved social façade, marked difficulty with non-verbal reasoning, dysexecutive features and impaired episodic memory. Comprehension was intact, word-retrieval problems and speech production errors (in the form of substitutions, deletions and transpositions) were evident as well as apperceptive agnosia. Reading, writing, calculation, visuoperceptual and visuospatial skills were impaired and limb apraxia was present.

5. 1. 7. 2. 4. Proband and family 4

Little clinical information about this proband is available, although it is known that she suffered from a progressive temporoparietal dementia. Later she developed extrapyramidal features, although the sporadic use of dopamine antagonists may have contributed. AAO is unknown but by 57y she was resident in a nursing home and had a severe dementia with dysphasia, dyspraxia, motor perseveration, utilization behaviour and wandering. We lost contact at this point, after obtaining the blood sample used for this analysis and determining her ApoE genotype as E3E3. Nothing is known about her only sibling but her mother is known to have died aged 49y from pathologically confirmed 'cerebral haemorrhage' and 'cystic degeneration of the brain' after a presenile dementing illness (Figure 26c shows pedigree).

5. 1. 7. 2. 5. Proband and family 5

This 55 year-old woman had progressive memory problems since the age of 48y when she fell out of bed having had a focal seizure. Seizures consisted of an olfactory aura followed by a focal seizure affecting her right arm. Complex partial and atonic seizures progressed and were refractory to therapy. She developed progressive cognitive symptoms. Aged 53y her MMSE was 16/30 and ACE 50/100. Aged 54y MMSE was 12/30 and a year later, MMSE was 4/30. Her cognitive profile was typical of AD. Apart from generally brisk reflexes, neurological examination was normal.

MRI imaging showed atrophy of the left temporal lobe compared to the right. HMPAO SPECT scan which showed some irregular diminution of perfusion of the left parieto-occipital region compared to the right. These appearances were felt to represent ischaemic change or AD.

The patient's mother died aged 66y of cancer but had progressive cognitive problems for 5 years including word finding difficulties and neologisms (see Figure 26d for pedigree).

Figure 26: Pedigrees

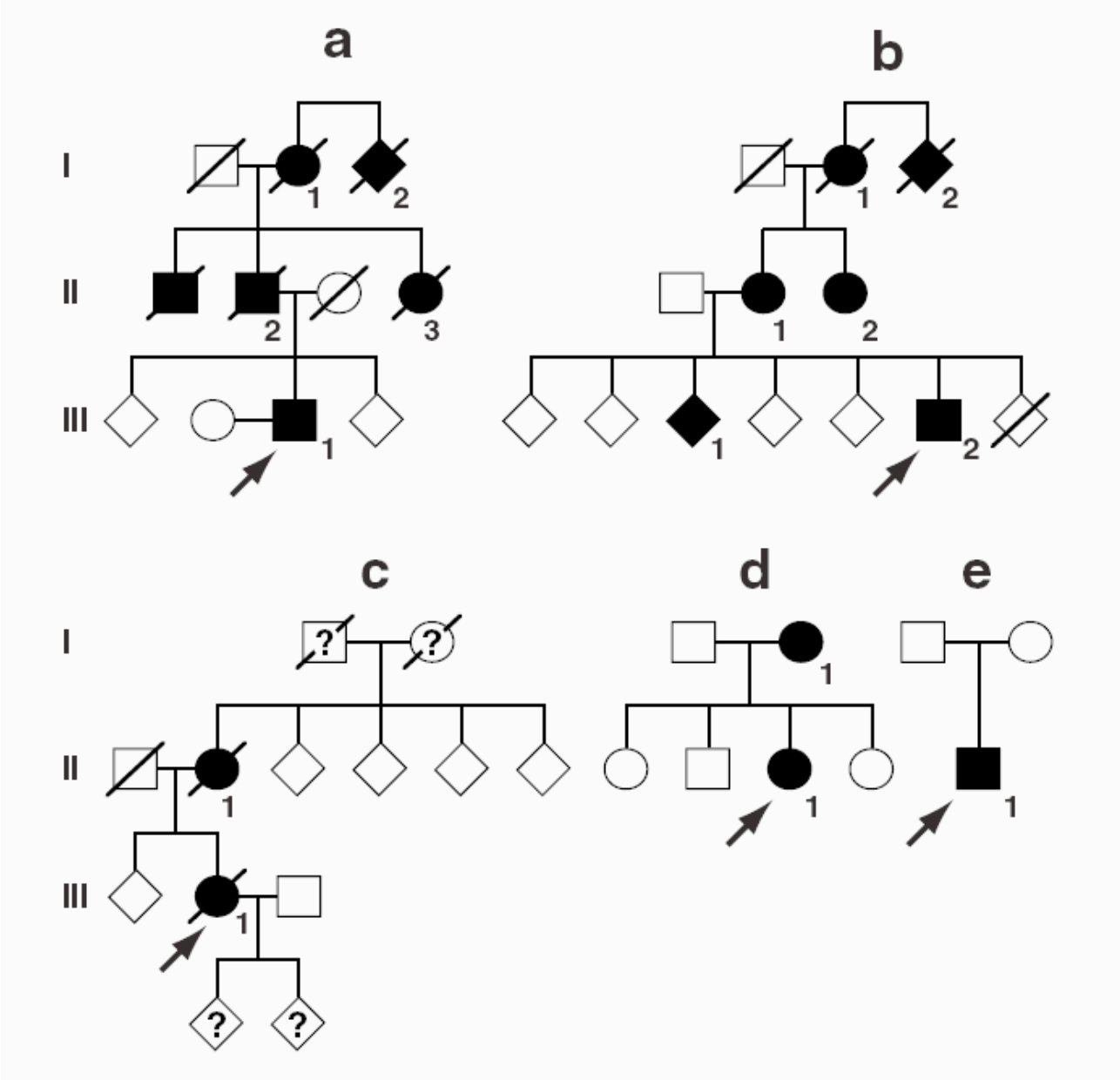


Table 15: Clinical features of 16 individuals with definite or probable *APP*d.

Pedigree	Proband	*APP*d	AAO	Age of Death	Clinical features	Seizures	ICH
AIII.1	1 (14660)	Yes	48	-	Memory loss, apraxia, dysexecutive	Late onset tonic-clonic	Yes
AI.1		-	-	-	-	-	-
AI.2		-	-	-	-	-	-
AII.1		-	-	63	Dementia	No	-
AII.2		-	Late 40s	62	Dementia	No	-
AII.3		-	-	49	Dementia	No	-
BIII.2	2 (8183)	Yes	40	-	Memory loss, myoclonus	Early onset tonic-clonic from age 11	No
BI.1		-		-	No	Early onset	-
BI.2		-		-	No	Early onset	-
BII.1		-		-	No	Early onset	-
BII.2		-		-	No	Early onset	-
BIII.1		-	50	-	Memory loss, word finding difficulty, altered mood	Generalized and complex partial seizures	No
CII.1	3 (7015)	Yes	39	-	Memory loss, social withdrawal	Probably complex partial seizures	No
DII.1	4 (19505)	Yes	-	-	-	-	No
DII.2		-	-	49	Dementia	-	Yes
EII.1	5 (17320)	Yes	48	-	Memory loss	Late onset generalized and complex partial	No
EI.1		-	61	66	Memory loss, word finding difficulty	No	No

5. 1. 8. Discussion

We describe the screening of a large and heterogeneous referral series to identify CNVs causing early onset dementia. Five probands with *APP*d were identified in the screen, four with evidence of familial dementia and one with a sporadic disease. The overall frequency of mutation in our series was 0.57% (95% CI 0.19-1.33). Although we confirm *APP*d to be relatively rare, the frequency in this series was comparable to that of missense mutations (5 different APP missense mutations in the series). The previously reported mutation frequency in FAD was 8% (95% CI 2.6-17.1) in a French series (Rovelet-Lecrux *et al.* 2006a), 2.7% (95% CI 0.32-9.3) in a Dutch series of FAD (Sleegers *et al.* 2006) and 0% in a Swedish and Finnish cohort of EOAD (95% CI 0-2.58) (Blom *et al.* 2008). Our data are not directly comparable with more selected studies as two from the five probands were not identified as having a familial disease until after mutations were identified and this aspect of the history was reconsidered. Only one from the five probands was considered likely to have CAA because of a scan finding of a small ICH.

In one proband (3) we detected a complex discontinuous *APP*d mutation associated with sporadic EOAD. This individual had three microsatellite alleles at one locus and a double allele dose at the second locus tested. The *APP*d patchily involved almost the entire Chr21q, but critically did not include the Down's Syndrome Critical Region around 21q22. The largest continuous region of duplication was 15.5Mb. These data are consistent with a different mutational mechanism from other *APP*d, an unbalanced translocation. In four probands, continuous duplications of 1.6-6.5 Mb including APP were detected; one mutation was slightly larger than any previously reported.

Although the clinical phenotype of *APP*d may vary within families, it does not appear to be influenced by the size of the duplication itself (Rovelet-Lecrux *et al.* 2007), a finding

supported by our results. Documented ages at onset range from 40-59y which is broadly in keeping with our data (range 39-61y) (Remes *et al.* 2004b; Cabrejo *et al.* 2006a). No clinical features of Down's syndrome have yet been observed although dementia and CAA seem universal, with a quarter also suffering ICH. There was also a high incidence of seizures (57%) in published series. Our data corroborate these reports with a high proportion of patients having seizures in the clinical course. One family (B) has an autosomal dominant family history of partial seizures, with partial seizures from adolescence in both patients subsequently affected by a cognitive disorder. **Whether these early seizures are coincidental, are caused by a linked gene abnormality, or a very early manifestation of CAA is unknown.** Clinically, *APP*d-related AD is intermediate between the canonical AD phenotype observed in most *APP* mutations and the frequent, CAA-associated ICH seen in the Dutch mutation. This intermediate phenotype has already prompted comparison with the Flemish *APP*A692G mutation (Hendriks *et al.* 1992a), which has similar characteristics. It seems likely that CAA does indeed underlie ICH and white matter lesions in these patients given the clear evidence of CAA-associated ICH in other *APP* mutations mentioned above. The anticipated association with microhaemorrhage is confirmed only in Proband 1 through the use of gradient echo/T2* MRI sequences (Figure 24). **These sequences had not been performed in other cases or were not available.**

The methods used, successfully identified 5 patients where duplication had occurred. 5 further samples that showed borderline abnormalities on exon-qPCR were all normal using the Illumina array. Each detection method has its own merits and neither can be used exclusively for the detection of CNV. Although not fully accurate, exon-qPCR cannot be confounded by genomic admixture due to accidental contamination. In contrast the accuracy of fm-qPCR supercedes that of exon-qPCR but is susceptible to

admixture as well as requiring heterozygosity of the marker, although this is largely resolved by typing two markers rather than one. In tandem the techniques are highly complementary and extremely effective at detecting duplications. Screening large numbers of samples as described here is a relatively powerful way of detecting and characterizing rare genetic phenomena. However, it presents ethical as well as practical challenges because the findings may have real clinical implications for the families concerned. Many samples had been held for a number of years and contact had long since been lost with individuals, meaning that the potential for communicating a positive finding was minimal. Despite this, every effort was made to give the relevant individuals the opportunity to discuss positive findings and to offer them the appropriate clinical support. The issue of sample age is a key limitation of this study as the most modern supportive investigations (such as the latest MRI sequences and CSF biomarkers) could not be incorporated into the characterizations. This factor may also have influenced diagnostic accuracy and therefore frequency estimation. The potential influence of CNVs at other loci and in other genes/diseases is an intriguing prospect and a possible focus for future research.

6.

CONCLUSIONS

Chapter 2 confirmed that structural measurement of the cerebral cortex is now both feasible, and practical, with the potential to be a useful biomarker of onset, progression and intervention. In this respect it could be considered to have achieved its aims. It demonstrated that CTh analysis may detect the pathological onset of AD some years before symptoms are apparent – the very period in which any new disease-modifying therapy would ideally be introduced. It therefore constitutes further evidence of a preclinical 'window of opportunity'. Further, by showing that diagnosis accompanies an acceleration of grey matter loss, it also highlights the consequences of failing to recognise this window and intervene appropriately. Accelerated cortical thinning might be the radiological correlate of accelerated clinical decline observed after a preclinical plateau (see 1. 6. 1.). This plateau may represent the temporary success of compensatory mechanisms and is possibly related to cognitive reserve. Particular regions in the MTL and heteromodal association cortices are identified as significant in early disease, helping to focus subsequent clinical and research imaging.

The precise nature of the relationship between cerebral Aß, macroscopic disease and clinical state remains controversial and interpretation of the ^{11}C-PiB PET data presented herein (see chapter 3) is correspondingly challenging. However, several important issues are raised by this study. It offers support for the notion of heterogeneity amongst *PSEN1* mutations both in terms of their molecular imaging profiles and, by inference, their regional pathological profiles. Some individuals displayed widespread ^{11}C-PiB retention, some showed striatal prominence, while others had more retention in the cerebellum than other areas of the brain. It is possible that, in future, ^{11}C-PiB PET could

help to sub-classify FAD more precisely and that clinico-pathological studies may be able to identify the clinical correlates of the various imaging profiles identified. Chapter 3 also demonstrates that, just as presymptomatic disease may be detected with structural and functional imaging techniques, so abnormal levels of fibrillar Aß can be detected in clinically unaffected individuals destined to develop manifest AD. ^{11}C-PiB PET is, therefore, another technique with the potential to facilitate earlier diagnosis and monitor the success of disease-modifying therapies. FAD and SAD subjects both demonstrated elevated levels of brain Aß which distinguished them from controls. However, differences were highlighted in the imaging profiles of these groups indicating that the relationship of one to another is not simple and raising issues about the limitations of the familial paradigm. As amyloid imaging becomes more widespread, confirmation that the cerebellum may not be a universally appropriate reference region is an important additional finding. So far such labour intensive, expensive structural and molecular markers have provided only incremental advances in diagnostic sensitivity and specificity. They are some way from being suitable or practical for use in the routine care of typical AD, but this situation is likely to change in light of future research and improved availability.

A viable familial paradigm demands as full a characterization of FAD as possible, and variations in clinical phenotype within FAD families provide an ideal opportunity to explore environmental and genetic factors which may underlie disease expression. Chapters 4 and 5 contribute to this. Their component studies have identified two novel pathogenic *PSEN1* mutations (p. L166del and S132A), explored their clinical, neuroimaging and neuropsychological features, and added to existing evidence of heterogeneity in this group. Similarly, through the novel association of pure progressive amnesia with the V717G substitution, they have expanded our understanding of the

varied *APP* mutation-associated syndromes. Single case studies of this kind, while useful in generating hypotheses and alerting us to unusual phenomena, have important limitations: In particular, they are descriptive rather than explanatory by nature and do not allow conclusions to be drawn about populations. Such qualitative studies have no *a priori* hypothesis and merely provide a modest, incremental contribution to an existing body of knowledge on the FAD group. They also highlight the sometimes unreliable phenotypic links between FAD and SAD and could be considered superficially damaging to the FAD paradigm in this regard. However, the continuing discovery of mutations in genes implicated in APP processing, which produce AD pathology and, in the vast majority of cases, a typical AD clinical phenotype is in itself supportive of an intimate link between FAD and SAD. In this respect these case descriptions strengthen the FAD/SAD bond. It is important that such work continues into the future, with heterogeneity a crucial area for study if we are to more precisely define said relationship. At a group level, non-canonical FAD phenotypes emphatically remain the exception and, while this situation persists, the paradigm at the heart of this thesis survives.

In chapter 5, *APP*d, to date a less well-defined phenomenon, was shown to be an important, if rare cause of familial dementia in one of the largest groups so far examined, and was found to have characteristic clinical and neuroimaging features. These include dementia, CAA, ICH and generalized seizures. The descriptive portion of the study contributes to the concept of a familial 'model' in the manner discussed above. Beyond this, my initial hypotheses were two-fold: first that large-scale screening of samples for these rare mutations was feasible and second that the prevalence of *APP*d was higher than was widely appreciated, making such screening a desirable facility. The first was proven while the second was, in a strict sense, inconclusive. However, if the unselected nature of my cohort is taken into account, the mutation frequency is relatively high i.e. it

is not dissimilar to figures found in highly selected cohorts. That *APP*d may be more common than first thought has real implications for the clinical care of families with autosomal dominant dementia, especially in cases where no missense mutation or deletion has yet been found. This study helps to highlight the ethical issues inherent in translational research where patient autonomy and informed consent must be reconciled responsibly with new discoveries, especially where they relate to long-held biological samples. The prospect that CNVs may be important in other degenerative diseases is also raised - a potential avenue for further research.

The known similarities and differences between FAD and SAD are summarized in section 1.4. although, as discussed above, the former remain more numerous and hence more compelling. The pros and cons of using this model are clear: there are persuasive, enduring similarities between FAD and SAD, which currently provide the only realistic opportunity to predict and make diagnoses with certainty. However, the apparently strong relationship between the two is inexact and incompletely defined. Proof of the validity of the FAD paradigm was not amongst the aims of this thesis and, therefore, no component study was designed to specifically address this. Rather, reference to the familial paradigm in the title was intended to reflect a methodological imperative – the need to utilise FAD to explore the potential of imaging biomarkers in identifying, monitoring and modifying AD *per se*. The validity of FAD as a model for SAD is not refuted by any findings herein although the aims did include a better understanding of relevant limitations of the paradigm through examination of comparatively rare phenotypic variations attributable to equally rare specific mutations. These more qualitative objectives were largely met through the descriptive studies presented. I can make no more assertive a statement than to say that there are, in my opinion, more

reasons to suppose features of FAD are broadly generalizable to SAD than there are to suppose they are not.

I believe this work offers grounds for optimism where the successful application of these imaging biomarkers is concerned. It also supports the view that a small number of families may hold the key to helping the many people living with AD, both now and in the future. Each story is unique, most are humbling, but those who so kindly participated in this research were united in their hope of sparing future generations their own burden. To hear their stories and to join them in this aim has been a privilege.

Ich hab mich verloren (I have lost myself)

- Auguste Deter 1850-1906

7.

APPENDICES

7. 1. NINCDS-ADRDA criteria for the diagnosis of Alzheimer's disease (McKhann *et al* 1984).

Criteria for the clinical diagnosis of PROBABLE Alzheimer's disease include:

Dementia established by clinical examination and documented by the MMSE or some similar examination and confirmed by neuropsychological tests;

- deficits in at least two areas of cognition
- progressive deterioration of memory and other cognitive functions
- no disturbance of consciousness
- onset between ages 40 and 90
- absence of systemic disorders that in and of themselves could account for the progressive deficits.

Diagnosis of PROBABLE Alzheimer's disease supported by:

Progressive deterioration of specific cognitive functions such as language (aphasia), motor skills (apraxia) and perception (agnosia)

- impaired activities of daily living
- family history of similar disorders, particularly if confirmed neuropathologically
- normal CSF and EEG
- evidence of cerebral atrophy on CT with progression documented by serial observation

Features consistent with the diagnosis:

- plateaus in the course of the disease
- associated psychiatric symptoms

- neurological abnormalities with more advanced disease including increased tone, myoclonus or gait disorder
- seizures in advanced disease
- CT normal for age

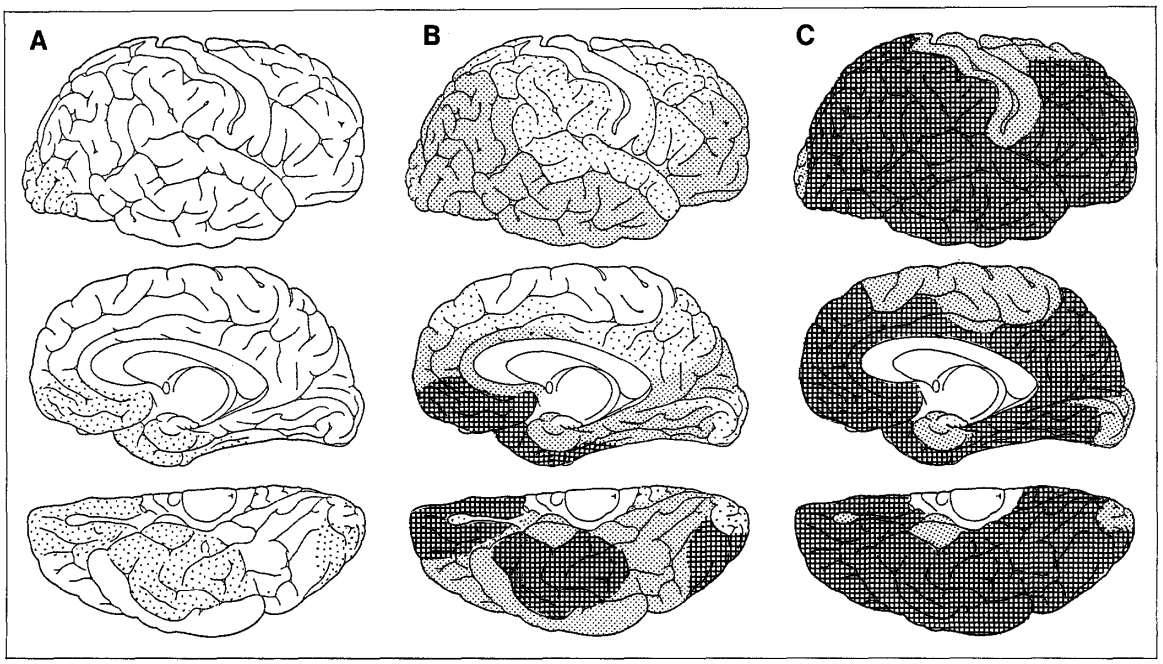
Diagnosis of PROBABLE Alzheimer's disease unlikely if:

- sudden onset
- focal neurological signs
- seizures or gait disturbance early in the disease

DEFINITE Alzheimer's disease if:

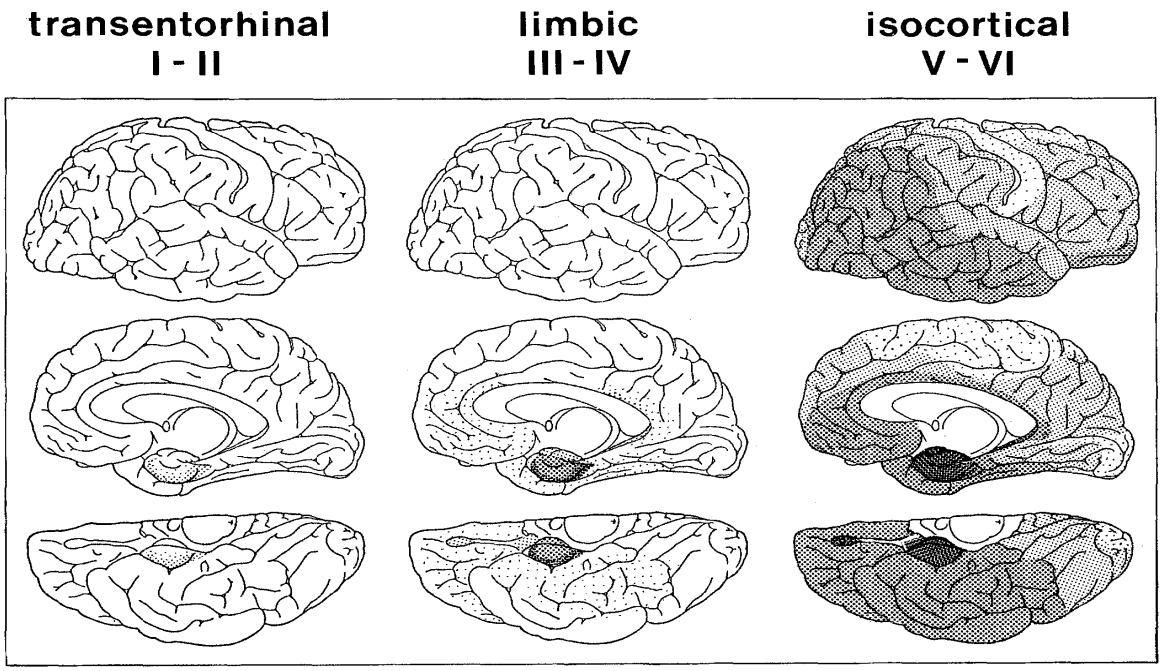
- clinical criteria for probable Alzheimer's disease
- histopathological evidence obtained from biopsy or autopsy

7. 2. Neuropathological stageing of AD-related changes (Braak and Braak 1991)



Amyloid

Fig. 1. Distribution pattern of amyloid deposits. **Stage A** Initial deposits can be found in basal portions of the isocortex. **Stage B** The next stage shows amyloid in virtually all isocortical association areas. The hippocampal formation is only mildly involved. **Stage C** In the end-stage deposits can be seen in all areas of the isocortex including sensory and motor core fields. Increasing density of shading indicates increasing numbers of amyloid deposits



Neurofibrillary changes

Fig. 4. Distribution pattern of neurofibrillary (NF) changes [neurofibrillary tangles (NFT) and neuropil threads (NT)]. Six stages (I–VI) can be distinguished. Stages I–II show alterations which are virtually confined to a single layer of the transentorhinal region (transentorhinal I–II). The key characteristic of stages III-IV is the severe involvement of the entorhinal and transentorhinal layer Pre-α (limbic III–IV). Stages V–VI are marked by isocortical destruction (isocortical V–VI). Increasing density of shading indicates increasing severity of NF changes

7. 3. Publications related to this thesis

Duplications of *APP* – but not *PRNP* – are a significant cause of early onset dementia in a large UK referral series *McNaughton D., ***Knight W. D**. *et al*

In press *Brain* August 2010 (Joint first authorship)

Knight W. D., Kim L.G., Douiri A., Frost C., Rossor M. N., Fox N. C

Acceleration of cortical thinning in familial Alzheimer's disease

Neurobiology of Aging 2009: In press (doi: 10.1016/j.neurobiolaging.2009.11.013)

W. D. Knight MRCP(UK), R. Laila. Ahsan MSc, Jessica Jackson BSc, Lisa Cipolotti PhD, Elizabeth K. Warrington DSc, Nick C. Fox FRCP, Martin N. Rossor FRCP.

Pure Progressive Amnesia & the *APPV717G* Mutation.

Alzheimer Disease and Associated Disorders 2009; 23: 410–414.

W. D. Knight, N. C. Fox, M. N. Rossor and J. D. Warren.

The Cultural Context of Visual Hallucinations.

Postgraduate Medical Journal 2008; 84: 103-105.

Rohrer JD, **Knight WD**, Warren JE, Fox NC, Rossor MN, Warren JD

Word-finding difficulty: a clinical analysis of the progressive aphasias.

Brain 2008; 131(Pt 1): 8-38.

W. D. Knight, J. Kennedy, S. Mead, M. N. Rossor, J. Beck, J. Collinge, C. Mummery.

A Novel Presenilin 1 Deletion (p.L166del) Associated with Early Onset Familial Alzheimer's Disease. *European Journal of Neurology* 2007; 14: 829-831.

7. 4. Acknowledgements and statement of personal contribution

The work presented in this thesis was carried out at the Dementia Research Centre, UCL Institute of Neurology, Queen Square, London under the directorship of Professor M. N. Rossor, MA MD FRCP FMedSci. The work was carried out between 2005 and 2009 when I held the posts of clinical research fellow at the Dementia Research Centre, UCL Institute of Neurology and honorary specialist registrar at the National Hospital for Neurology and Neurosurgery, Queen Square and St. Mary's Hospital, Paddington, London. Throughout this period I was also an investigator in a commercial phase IIa clinical trial of passive immunotherapy in AD. At the time of writing a phase III trial of the IMP (bapineuzumab) is about to commence.

The majority of this work was devised and performed personally although a substantial minority was performed with the assistance of collaborators as detailed below.

An initially MRC-funded longitudinal study had been initiated by Professor Martin Rossor which consisted of prospective clinical, radiological and neuropsychological assessment of members of FAD families (see 4. 1.). This study was the major source of data and participants for the studies described herein. Neuropsychological assessment was performed in the Department of Clinical Neuropsychology, National Hospital for Neurology and Neurosurgery under the supervision of Professor L. Cipolotti, and at the Dementia Research Centre under the supervision of Professor E. Warrington and Dr. S. Crutch.

MRI scanning was performed at UCL Institute of Neurology. I would like to acknowledge the technical assistance of Dr. J. Rohrer and Dr. A. Douiri in the

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The *APP*d study involved collaborators at several sites: neuropathological input was received from Professor T. Revesz of UCL Institute of Neurology and Professor J. Lowe of the University of Nottingham; PCR analysis of genomic DNA was performed at MRC Prion Unit, Department of Neurodegenerative Disease, UCL Institute of Neurology by D. McNaughton under the supervision of Dr. S. Mead and Professor J. Collinge.

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