Detecting and tracking populations at-risk of Alzheimer's disease: studying asymptomatic cognitive profiles and symptomatic clinical presentations

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Declaration of authorship and originality

I, Ivanna Micol Pavisic, 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 penetrant autosomal dominantly inherited condition. Due to its clinical and neurophysiological similarities with sporadic AD features, it represents an important clinical group in its own right but also offers a potential model for AD. This thesis is largely based on the longitudinal FAD study but also includes data from 'Insight 46' in an attempt to broaden the scope of these investigations to other 'at-risk' cohorts. The overarching aim of the thesis is to study the early subtle cognitive changes (with a particular focus on visual short-term memory but also subjective cognitive decline) and the symptomatic presentations (both cognitive and clinical) that accompany disease progression in AD.

The key findings were that over time, presymptomatic mutation carriers (PMCs) had a faster rate of decline in visual short-term memory (VSTM) function, specifically in the ability to remember the location *and* the target identity. This relational binding deficit was strongest in the most challenging task condition: 3-items, 4s delay (high load, longest delay), and is clinically relevant as it shows sensitivity in tracking individuals during preclinical AD stages. Consequent eye movement investigations of VSTM function, revealed a stronger cognitive effort for PMCs compared to controls during encoding, a finding which may increase the diagnostic value of relational binding tasks.

Other important findings were: the higher incidence of subjective cognitive decline symptoms in two otherwise different populations "at-risk" of AD: PMCs carriers and amyloid-positive ~70-year-old participants and the ineffective VSTM function and much smaller influence of mutation specificity on survival time variance in comparison to variance in age at onset for symptomatic FAD individuals.

Together, this work has implications for the interpretation of cognitive and clinical data, the understanding of heterogeneity in FAD and may help detect and track subtle cognitive decline of potential value to clinical practice.

IMPACT STATEMENT

As the prevalence of AD continues to rise rapidly, there is a pressing need to better understand the early preclinical stage – the stage during which future disease-modifying treatments are most likely to be effective. Similarly, greater insight into the timing and nature of cognitive and clinical changes, may increase the chances of therapeutic success and better patient quality of life.

The results presented in this thesis provide novel evidence that a specific cognitive function like relational binding is sensitive to tracking populations at-risk of AD like FAD. This effect was seen for presymptomatic carriers within an average distance of 5.8 (SD 1.8) years to expected symptom onset and was strongest in the most challenging task conditions: 3-items, 4s delay. Consequent analysis revealed these deficits related to the integrity and efficacy of encoding and maintenance processes, caused by the advancing preclinical AD state. Taken together these findings have implications on investigations of relational binding in preclinical AD as they favour a focus on high load conditions (3-items) in a continuous domain. The basis of this recommendation also accords with resource models of working memory suggesting that the *precision* of recall declines *continuously* as the number of items to be remembered increases. I propose that a continuous representation of binding accuracy instead of the previously proposed categorical representation of error, could be better suited to evaluate the *quality* or *resolution* of a memory representation. Furthermore, as greater cognitive effort in PMCs was specifically associated with accurate performance in this continuous measure of relational binding accuracy, this supports the suitability of this measure as a preclinical cognitive marker and emphasises how eye-tracking could provide an additional layer to the understanding of preclinical AD changes – not only from a methodological standpoint – but also in regards to increased sensitivity.

Equally important to understanding AD progression, is the clinical finding that the natural history of FAD appears to have changed over time with longer survival through generations and a considerable variance in disease duration affected by factors other than genetic mutations. This has direct implications to the support provided to patients and their families as well as our understanding of the effects of drug treatment on survival.

Finally, and in order to illustrate the larger scope of this thesis, the subjective cognitive decline features observed in both presymptomatic FAD carriers and ~70-year-old participants with elevated levels of β-amyloid, is a concrete example of the potential transferability of FAD findings

into AD more broadly. It also advocates for further comparisons between populations ‘at-risk’ of AD specifically *during* preclinical stages.

Dissemination of results to the research community is in progress and will continue through publication in scientific journals, presentation at international conferences and public engagement activities. I have also had the great privilege of meeting and supporting individuals and families living with, or affected by, FAD through the FAD Rare Dementia Support group over the years, discussing research ideas and presenting findings with, and to, them.

ACKNOWLEDGEMENTS

Firstly, I would like to thank FAD and Insight 46 participants for their generous contribution and enthusiasm over the years without which this study would not have been possible. To FAD participants, I have immense respect for you all. I know I will cherish many moments and reflections for the rest of my life.

I would like to thank Dr Yoni Pertzov who despite not being a PhD supervisor, has provided guidance, knowledge and support throughout the years. I am grateful to my PhD supervisors, Professor Sebastian Crutch, Professor Nick Fox, Dr Aida Suarez Gonzalez and Dr Natalie Ryan– who despite being on maternity leave for part of my PhD was supportive and available with a new born baby! It has been a privilege to learn from you all. I am also grateful to Dr Jennifer Nicholas for her statistical support.

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AAO = age at onset

AYO = proximity to actual age at symptom onset

Aβ = β-amyloid

Aβ+ = β-amyloid positive

Aβ- = β-amyloid negative

AD = Alzheimer's disease

APP = amyloid precursor protein gene

APOE = apolipoprotein gene

CDR = clinical dementia rating

CI = confidence interval

CSF = cerebrospinal fluid

DRC = Dementia Research Centre

DT = total dwell time on fractals

Eq = equality

EYO= proximity to expected age at symptom onset

FAD = familial Alzheimer's disease

IQR = interquartile range

IWG = International Working Group for New Research Criteria for the Diagnosis of AD

LTM = long-term memory

LOAD = late onset Alzheimer's disease

MCI = mild cognitive impairment

MMSE = mini mental state examination

MRI = magnetic resonance imaging

MTL = medial temporal lobe

NIA-AA = National Institute on Aging – Alzheimer's Association

NSHD = National Survey of Health and Development (the British 1946 Birth Cohort)

PACC = Preclinical Alzheimer Cognitive Composite

PET = positron emission tomography

PMC = presymptomatic mutation carrier

Pr = proportional time spent looking at the target

PSEN1 = presenilin 1 gene

PSEN2 = presenilin 2 gene

S = total number of shifts between fractals

SAD = sporadic Alzheimer's disease

SCD = subjective cognitive decline

SD = standard deviation

SEP = socio-economic position

STM = short-term memory

SUVR = standard uptake volume ratio

UCL = University College London

VES = visual exploration strategies

VSTM = visual short-term memory

WAIS-R = Wechsler Adult Intelligence Scale - Revised

WASI = Wechsler Abbreviated Scale of Intelligence

WMS-R = Wechsler Memory Scale - Revised

1. GENERAL INTRODUCTION

1.1. Alzheimer's disease

1.1.1. A brief history and size of the problem

In 1901, Aloysius 'Alois' Alzheimer described the first case of Alzheimer's disease (AD), a patient named Auguste Deter who presented with multiple cognitive deficits including reduced episodic memory, disorientation, expressive aphasia, impaired comprehension and unpredictable behaviour. After the patient had passed in 1907, Aloysius alongside his colleague Gaetano Perusini reported post-mortem findings including: extracellular plaques of dystrophic neurites, intracellular neurofibrillary tangles (NFTs), and cortical atrophy in the brain, which remain the pathological hallmarks of AD to this day. However, it was not until the 1980s that it was possible to identify the core constituent of plaques as being the protein β-amyloid (Aβ) (Masters et al., 1985). Although AD is strongly liked in the public imagination with old age, it is particularly noteworthy that Auguste Deter, the focus of Alzheimer's landmark study, was only 56 years-old and living with young onset dementia.

Over 100 years after Alzheimer's original publication, AD is now recognized as a major public health problem. There are 50 million people living with dementia globally. AD is the most common form and is estimated to contribute to 60-70% of cases. (World Health Organization, 2020). In 2012 the World Health Organisation published a report 'Dementia: a public health priority' advocating for action at international and national levels. In 2015, 46.8 million people were estimated to have dementia and this number is expected to double every 20 years reaching 75 million in 2030 and 131.5 million in 2050. In addition to the incalculable human impact, the economic cost is staggering – in the UK alone the estimated current cost is £26 billion per year, and this is projected to more than double by 2040 (Prince et al., 2014).

A number of advances in AD research have had an impact on the way we view and talk about the condition. Historically, the diagnosis of AD dementia was achieved through a process of exclusion: patients with a dementia syndrome without any of the following: 1) dementia: loss of autonomy; 2) elimination of other causes of dementia; blood exams: endocrinopathies, infectious or inflammatory disorders; 3) computerised tomography scan/ magnetic resonance imaging (CT-Scan/MRI): vascular lesions, tumour; hydrocephalus (Dubois, 2018; Jack et al., 2018). In the last two decades, the disease has gained a clearer definition based on: clinical phenotype (typically

the amnestic syndrome of the hippocampal type) supported by the presence of biomarkers (considered the biological signatures of the disease (Dubois et al., 2007, 2010, 2018)).

The most recent National Institute on Aging and the Alzheimer Association (NIA-AA) Research Framework (Dubois, 2018; Jack et al., 2018) established a shift in AD diagnosis from a clinical consequence of the disease (syndromal construct) to the molecules and biological processes underlying it (biological construct). AD is now considered a continuum of progressive pathophysiological changes confirmed by post-mortem examination or in vivo markers (grouped into those of Aβ deposition, pathologic tau, and neurodegeneration [AT (N)] (**Figure 1.1**).

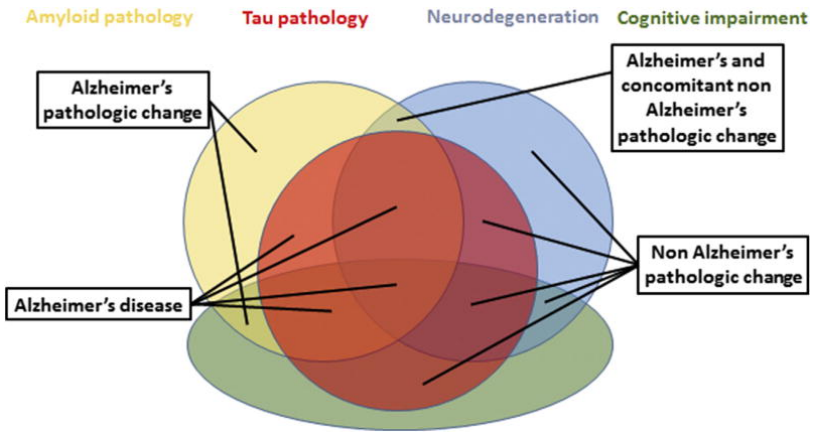


Figure 1.1 NIA-AA Research Framework.

Reprinted from (Jack et al., 2018) under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License (CC BY NC ND).

While post-mortem confirmation of pathology is still the gold standard for the definitive diagnosis of AD, new technologies (imaging and fluid biomarkers) have been developed which allow the detection of Aβ, tau pathology and neurodegeneration *in vivo*. These have made the study of pathological changes possible and revealed that these begin 20-30 years before the onset of symptoms, with Aβ pathology being the first to accumulate (Jack, 2013; Palmqvist et al., 2019). This period of pathological changes in the absence of symptoms is referred to as the preclinical stage of AD and is discussed in greater detail later (section 1.2).

1.1.2. Hallmarks of AD

From a **clinical and cognitive perspective**, AD is characterized by gradually progressive cognitive decline involving multiple cognitive domains over time. Whilst most cases of AD begin with amnestic symptoms (i.e. memory for recent events) indicating medial temporal lobe (MTL) dysfunction, there is increasing recognition of atypical AD syndromes where loss of episodic memory is not the leading feature (Dubois et al., 2014). Some atypical forms of AD include:

- Posterior cortical atrophy (PCA)- characterized by early visuo-perceptual and visuo-spatial dysfunction, indicating early prominent parieto-occipital involvement (Crutch et al., 2017).
- Logopenic aphasia (LPA)- characterized by word finding difficulties and impaired sentence repetition, indicating posterior temporal lobe dysfunction (Gorno-Tempini et al., 2008).
- Behavioural/dysexecutive variant- characterized by frontal lobe dysfunction leading to behavioural difficulties (Ossenkoppele et al., 2015).

Common features across these variants include an insidious onset, with slow progression thereafter and prominent impairment of cerebral cortical function more than subcortical regions. The reason for the same pathological entity among different syndromes, also known as the 'paradox of syndromic diversity' is not well understood (Warren et al., 2012). Nonetheless, as the disease progresses, these clinically diverse syndromes tend to converge to involve the same combination of domains, strengthening the theory that AD affects a common network of multiple vulnerable brain regions (Warren et al., 2013).

In addition to these cognitive and clinical features, neuropsychiatric symptoms are also relatively common with a diagnosis of AD, including depression anxiety and agitation (Lyketsos et al., 2011; Mega et al., 1996). Symptoms of subjective cognitive decline (SCD) in particular, can be an early presenting feature of clinical disease, sometimes preceding objective cognitive decline (Ownby et al., 2006).

Neuropathological features will not be discussed in depth in this thesis however the pathological hallmarks of AD include: the presence of extracellular plaques of dystrophic neurites (core constituents identified as being amyloid in the 1980s (Masters et al., 1985)), NFTs (main

constituent identified as hyperphosphorylated form of microtubule-associated protein tau (Wood et al., 1986)) and cortical atrophy (**Figure 1.2**).

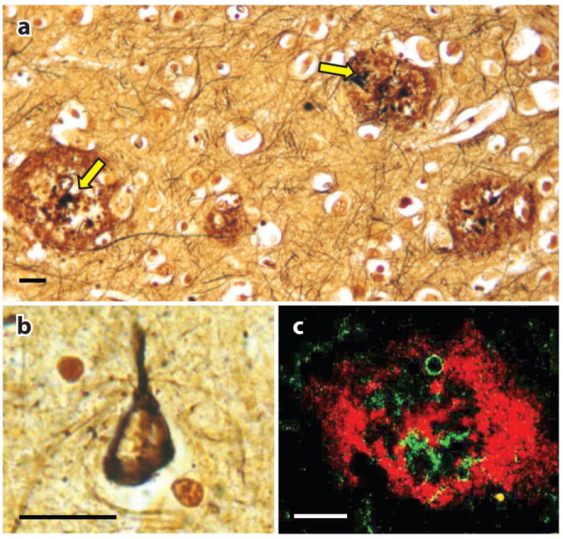


Figure 1.2 The pathological hallmarks of AD

From (O'Brien & Wong, 2011) with permission from Annual Review of Neuroscience. Brain sections from a patient with dementia are stained with silver, revealing **(A)** neuritic plaques and **(B)** an NFT. The plaques in panel **A** consist of an amorphous reddish protein (Aβ) with dystrophic neurites (yellow arrows, dark black material). In **C** an Aβ plaque stained with an anti-Aβ antibody (red) shows infiltrating microglia stained with an IBA1 antibody (green). Each line is 40 microns. Aβ=β-amyloid; NFT=neurofibrally tangle; Ionized calcium binding adaptor molecule 1.

Amyloid is a chemically heterogenous protein defined by a β-pleated sheet structure. The amyloid fibrils in AD plaques are composed of the Aβ peptide, a 39-43 amino acid residue peptide produced by cleavage from a larger amyloid precursor protein (APP). APP is a transmembrane protein expressed at high levels in the brain (Bayer et al., 1999; O'Brien & Wong, 2011). Enzymes recognized to cleave APP include α-secretase, β-secretase and γ-secretase (**Figure 1.3**). α-secretase is considered to be part of the non-amyloidogenic pathway in APP processing. It precludes Aβ formation as it cleaves within the segment of APP that would otherwise give rise to Aβ. Alternatively, APP may undergo sequential cleavage by β and γ secretase. Extracellular cleavage by β-secretase generates a soluble extracellular fragment, and is followed by the cleavage of APP within its transmembrane domain by γ-secretase. An unusual property of γ-secretase is that it cleaves APP sequentially typically generating peptides from 39–43 amino acids in length: the Aβ protein.

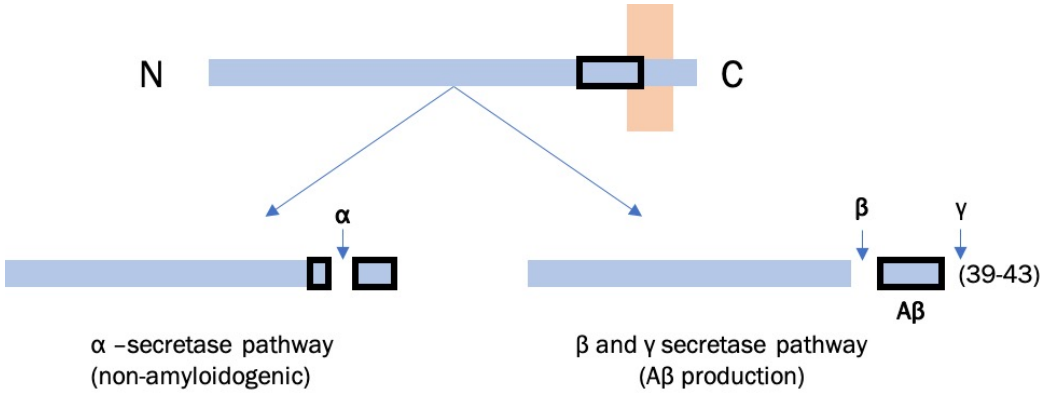


Figure 1.3 Cleavage of APP to produce Aβ.

The Aβ peptide is present in unaffected individuals and may have a normal physiological role. In AD however, an imbalance develops between Aβ production and Aβ clearance. Of the different potential Aβ peptides (i.e. $Aβ_{39}$ to $Aβ_{43}$), the major species in Aβ production are $Aβ_{40}$ and $Aβ_{42}$. Longer forms of the peptide are more prone to aggregation and are thought to be the more neurotoxic – perhaps through the formation of oligomers. $Aβ_{42}$ may have a role in seeding and is prominent in the cores of neuritic plaques of AD patients.

Tau is a protein expressed in all axons of the central nervous system and its primary role is thought to promote assembly and stability of microtubules (Cleveland et al., 1977), which provide cytoskeletal stability and facilitation of intracellular transport. All tau species present within the paired helical filaments of NFTs are hyperphosphorylated. Tau pathology demonstrates a relatively consistent anatomical pattern of progression in all AD patients (Braak & Braak, 1991). It typically appears first in the entorhinal cortex (stages I-II), before appearing in to the limbic cortex (stages III-IV) and then the neocortex (stages V-VI). It has subsequently been found that the anatomical distribution of cortical tau pathology is much more closely associated with both cortical atrophy and the severity and pattern of cognitive impairment, than amyloid is (Arriagada et al., 1992; Bierer et al., 1995; Whitwell et al., 2008) (**Figure 1.4**).

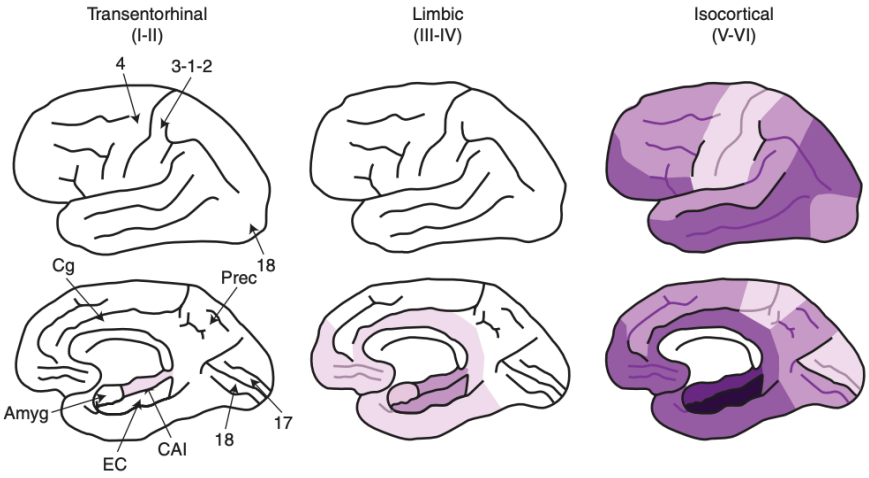


Figure 1.4 Spatiotemporal pattern of neurofibrillary neurodegeneration, following staging of tau pathology in AD.

From (Serrano-Pozo et al., 2011) under the terms of the Creative Commons Attribution License (CC BY). Shading indicates the distribution of NFTs with darker colours representing increasing densities. Amyg=Amygdala; EC=Entorhinal cortex; CA1=Cornus ammonis 1 hippocampal subfield; Cg=Cingulate cortex; Prec=Precuneus; 4= Primary motor cortex; 3-1-2=Primary sensory cortex; 17= Primary visual cortex; 18=Associative visual cortex. The first NFTs consistently appear in the transentorhinal (perirhinal) region (stage I) along with the entorhinal cortex proper, followed by the CA1 region of the hippocampus (stage II). Next, NFTs develop and accumulate in limbic structures such as the subiculum of the hippocampal formation (stage III) and the amygdala, thalamus, and claustrum (stage IV). Finally, NFTs spread to all isocortical areas (isocortical stage), with the associative areas being affected prior and more severely (stage V) than the primary sensory, motor, and visual areas (stage VI).

Although the defining **histopathological hallmarks** of AD are Aβ plaques and NFTs, it is the loss of neurons seen on histopathological examination that is thought to link most closely to clinical decline. Some cortical regions have been found to be affected more than others, with medial temporal regions, the posterior cingulate cortex and superior parietal regions particularly vulnerable.

An additional observation made on histopathological assessment has been changes in the numbers of non-neuronal glial cells, and in particular an increase in the number of microglia – the primary immune cell of the central nervous system (Brun & Englund, 1981). Whilst not the focus of this thesis, there is now great interest in better understanding the role of neuroinflammation in AD, and how it interplays with the other pathological processes discussed above.

A study by Murray and colleagues (Murray et al., 2011), proposed neuropathologically defined subtypes of AD with distinct clinical characteristics. In this study, the authors classify AD cases

into typical, hippocampal sparing, or limbic predominant. Findings relevant to this thesis are summarised in **Table 1.1.**

Table 1.1 Summary of AD-subtypes characteristics.

	Hippocampal sparing	Typical	Limbic predominant
NFTs	⇑ cortical areas, ⇓ hippocampus		⇓ cortical areas, ⇑ hippocampus
Hippocampal atrophy	⇓ than typical and limbic predominant subtypes		
Age at onset	Youngest AAO	Mean AAO in the middle of both subtypes	Oldest AAO
Disease duration	⇓ than typical	⇑ than hippocampal sparing	⇑ than hippocampal sparing, similar to typical
Atypical clinical presentation	⇑ than typical, ⇑ than limbic predominant	⇑ than limbic predominant, ⇓ than hippocampal sparing	⇓ than typical, ⇓ hippocampal sparing
Microtubule-associated protein tau (MAPT) H1H1 genotype	⇓ than limbic predominant	Similar to limbic predominant	⇑ common than hippocampal sparing, similar to typical
***APOE* genotype** [a]	⇓ effect than typical and limbic predominant	⇑ effect than hippocampal sparing	⇑ effect than hippocampal sparing

From (Murray et al., 2011). [a] APOE ε4 carriers had smaller hippocampal area, fewer neurons, and higher hippocampal neurofibrillary tangle counts than non-carriers. NFTs=neurofibrillary tangles; AAO=age at onset; *APOE*=apolipoprotein gene.

1.1.3. Genetic risk factors in sporadic AD

Most (99%) of cases of AD are considered "sporadic" – nonetheless genetics account for up to 53% of total phenotypic variance (Andrews et al., 2020; Ridge et al., 2016). Late onset AD (LOAD) is much more common than early onset (EOAD) and has a strong genetic component. The identification of novel loci (position of a gene within a chromosome) that affect LOAD is critical for the understanding of the underlying aetiology of AD (Karch & Goate, 2015). *APOE* is the major gene known to increase the risk of the disease (Corder et al., 1993; Saunders et al., 1993). APOE encodes a polymorphic glycoprotein expressed in liver, brain, macrophages, and monocytes, participates in transport of cholesterol and other lipids and is involved in neuronal growth, repair response to tissue injury, nerve regeneration, immunoregulation, and activation of lipolytic

enzymes (Van Cauwenberghe et al., 2016). The *APOE* gene contains three major allelic variants at a single gene locus (ε2, ε3, and ε4), encoding for different isoforms (APOE2, APOE3, and APOE4) that differ in two sites of the amino acid sequence (Saunders et al., 1993). The risk effect is estimated to be threefold (or more) for heterozygous carriers (*APOE* ε34) and 15-fold for ε4 homozygous carriers (APOE ε44), and has a dose-dependent effect on onset age (Corder et al., 1993; Saunders et al., 1993). APOE binds to Aβ and is thought to support clearance of soluble Aβ and Aβ aggregations. Yet, APOE ε4 is thought to be less efficient in mediating this Aβ clearance (Deane et al., 2008).

Genome-wide association studies (GWAS) have identified polymorphisms in or near several genes that are associated with AD risk. Until 2018, the largest GWAS study of AD had identified 19 risk loci (Lambert et al., 2013). In 2018 and 2019, three new GWAS in AD were published, expanding the total number of risk loci to 40 (Jansen et al., 2019; Kunkle et al., 2019; Marioni et al., 2018). The actual risk variants represented by these GWAS associations remain largely unidentified (Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2009, 2013; Naj et al., 2011; Seshadri et al., 2010). An overview of some single-locus AD-susceptibility genes identified by GWAS alongside their function and characteristics is shown below (**Table 1.2**) (Van Cauwenberghe et al., 2016).

Although the total number of AD risk genes remains elusive, there is good evidence suggesting that, in combination, they have a substantial impact on AD predisposition. These AD risk genes affect various processes, roughly falling into four pathways: Aβ metabolism, lipid metabolism, immune and complement system/inflammatory response, and cell signalling (**Figure 1.5**).

Table 1.2 Overview of the single-locus AD-susceptibility genes identified by GWAS and meta-analysis: function and characteristics.

Gene	Pathway	Function	Effect on APP pathway	Effect on tau pathway
SORL1	Endosomal vesicle cycling	Vesicle trafficking	Aβ generation and clearance	-
BIN1	Endosomal vesicle cycling	Clathrin-mediated endocytosis	-	Tau toxicity
CR1	Immune response	Regulation of complement activation	Aβ clearance	-
CLU	Cholesterol and lipid metabolism	Chaperone function; regulation of cell proliferation	Aβ aggregation and clearance	-
PICALM	Endosomal vesicle cycling	Trafficking of synaptic vesicle proteins	APP trafficking and Aβ clearance	Co-localisation in NFTs
ABCA7	Lipid metabolism and immune response	Efflux of phospholipids and phagocytosis	Aβ clearance	-
FERMT2	Cytoskeletal function and axonal transport	Actin assembly and cell shape modulation	-	Tau toxicity
CASS4	Cytoskeletal function and axonal transport	Scaffolding protein unknown function (in Drosophila ortholog binds to CD2AP ortholog)	-	-
EPHA1	Endosomal vesicle cycling and immune system	Brian development, modulating cell migration, axon guidance, and synapse development and plasticity	-	-
PTK2B	Cell migration and synaptic function	Ion signaling and induction of long-term potentiation in the hippocampal CA1 neurons	-	-
CD2AP	Endosomal vesicle cycling	Cytoskeletal reorganization and vesicle movement	Aβ clearance	Protection against tau toxicity
INPP5D	Immune response	Regulation of gene expression and posttranslational modification of proteins, microglial and myeloid function	-	-
MEF2C	Immune response, neural development, synaptic function	Synaptic plasticity	-	-
CD33	Immune system and inflammatory response	Cell-cell interactions and cell functions in the innate and adaptive immune systems	Aβ clearance	-

From (Van Cauwenberghe et al., 2016). *SORL1*= sortilin related receptor 1; *BIN*=bridging integrator 1; *CR1*=complement C3b/C4b receptor 1; *CLU*= clusterin; *PICALM*= phosphatidylinositol-binding clathrin assembly protein; *ABCA7*= TP-binding cassette transporter A); *FERMT2*=fermitin family member 2; *CASS4*= Cas scaffolding protein family member 4; *EPHA1*=EPH receptor A1; *PTK2B*=protein tyrosine kinase 2 beta; *CD2AP*=D2-associated protein; INPP5D=inositol polyphosphate-5- phosphatase; *MEF2C*=myocyte enhancer factor 2C; *CD33*= CD33 molecule.

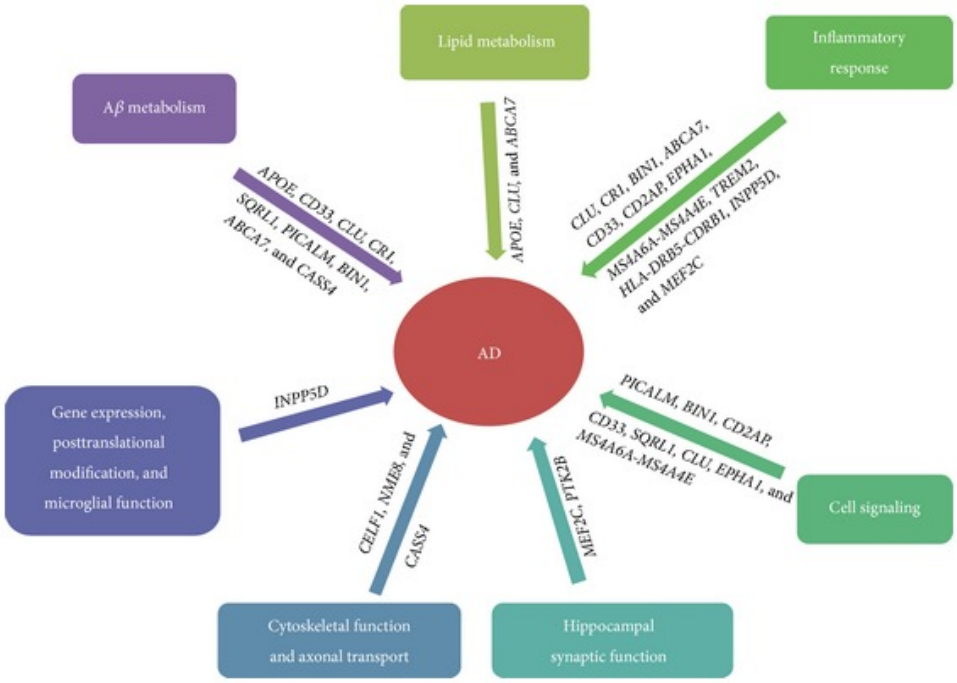


Figure 1.5 Proposed pathways of susceptibility genes involved in the pathogenesis of AD.
From (Zou et al., 2014) under the terms of the Creative Commons Attribution License (CC BY)).

Approaches in smaller datasets have shown evidence of rare coding variants in genes with moderate to large effects on LOAD risk including *PLD2* and *TREM2* (Cruchaga et al., 2014; Guerreiro et al., 2010; Jonsson et al., 2013; Kim et al., 2009). The identification of these rare variants is also valuable as it provides further insight into pathways that may be central to the disease pathogenesis.

Dominantly inherited mutations in β-amyloid precursor protein *(APP),* presenilin 1 (*PSEN1*) and presenilin 2 *(PSEN2)* cause EOAD (Guerreiro et al., 2012; Holtzman et al., 2011) (see section 1.3). Yet, increasing evidence suggests there are additional variants in *APP* and *APP*-modifying genes that alter AD risk in LOAD cases (Benitez et al., 2013; Cruchaga et al., 2012; Jin et al., 2012).

The image below portrays a summary of the rare and common variants genes that contribute to AD risk (**Figure 1.6**).

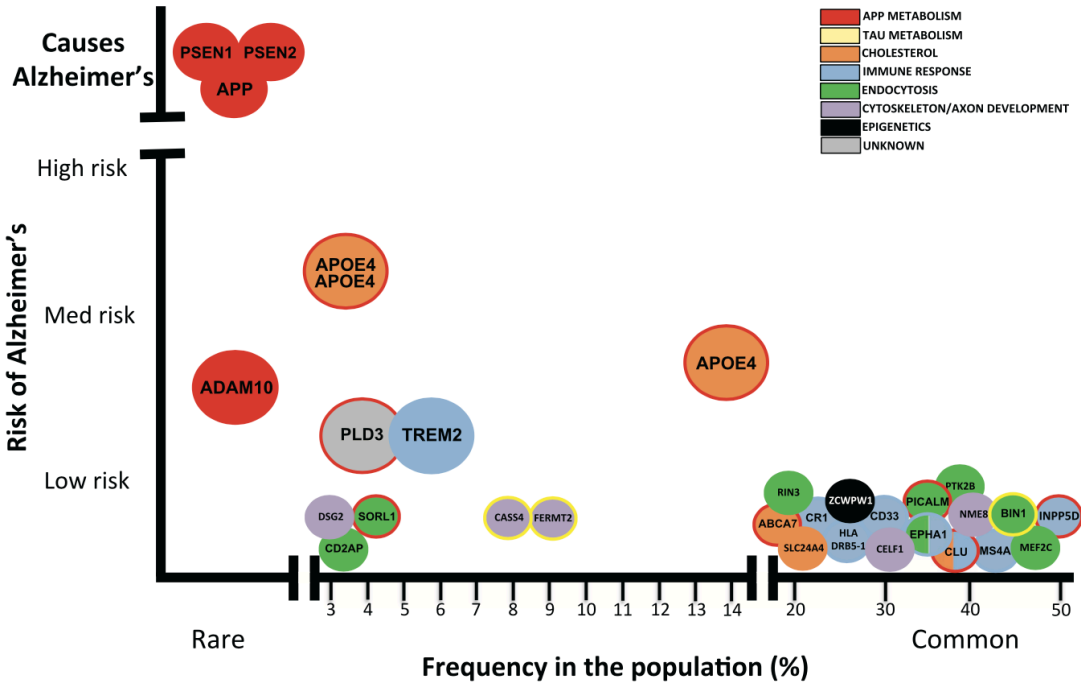


Figure 1.6 GWAS, genome-wide association studies

From (Karch & Goate, 2015) with permissions from Elsevier).

Whilst modifiable risk factors (e.g. treatable medical conditions and lifestyle choices) will not be the focus of this thesis, they also play a role in the development of AD (Edwards III et al., 2019). These include comorbidities (e.g. vascular diseases; type II diabetes; traumatic brain injury; epilepsy and depression) and lifestyle (e.g. physical activity; sleep disturbance; diet; smoking and alcohol).

Unfortunately, there are currently no disease-modifying treatments available for AD. The field has suffered from a series of disappointing failures from clinical trials of drugs that have targeted Aβ pathology. As AD has such a long preclinical window, research suggests this may be the most beneficial time to provide disease-modifying therapies. While individuals with preclinical AD are, by definition, cognitively "normal", there is increasing evidence that subtle changes can be detected and at a group level there is strong support for a period of several years of decline prior to diagnosis.

1.2. Preclinical AD

1.2.1. Theoretical models of presymptomatic AD

Efforts to create standardized biomarker-based criteria for preclinical AD have been led by two main groups: The International Working Group for New Research Criteria for the Diagnosis of AD (IWG) (Dubois et al., 2007, 2010, 2014, 2016) and the NIA-AA (Jack et al., 2012, 2016, 2018; Sperling et al., 2011). The evolution of the criteria over the years reflects the advances in the understanding of the disease, the biomarkers that become available and the debates about how the disease should be conceptualised. The biomarkers included in these preclinical AD criteria are derived from neuroimaging or cerebrospinal fluid (CSF) sampling. Blood-based biomarkers for AD, in development, are not discussed in this thesis (Zetterberg, 2019).

In 2012, Bateman and colleagues (Bateman et al., 2012) showed a range of biomarker changes in this preclinical window in familial Alzheimer's disease variant (FAD): $A\beta_{42}$ concentrations in CSF appeared to decline ~25 years before expected onset compared to non-carriers; Aβ deposition as measures by Pittsburgh compound B - positron- emission tomography (PIB-PET) was detected ~15 years before; increases in levels of tau in the CSF and in brain atrophy were detected approximately 15 years before; cerebral hypometabolism and impaired episodic memory approximately 10 years before and global cognitive impairment starting 5 years before expected onset (**Figure 1.7**). This study offered possible candidates and time-windows for clinical trials in FAD with potential generalizability to sporadic AD (SAD).

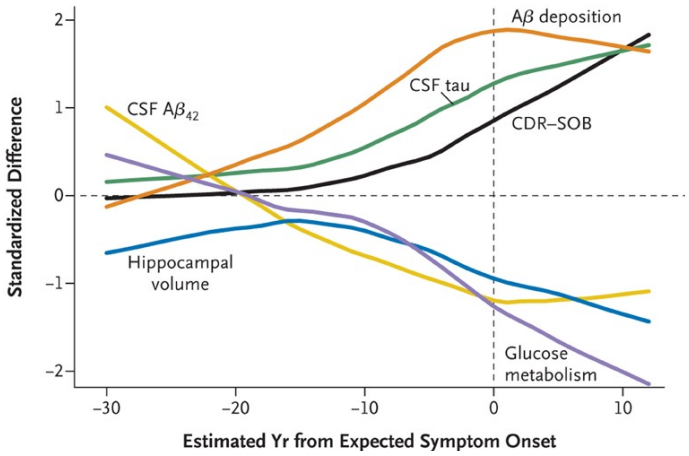


Figure 1.7 Clinical and biomarker changes in dominantly inherited Alzheimer's disease

From (Bateman et al., 2012) with permission from (Publisher of the New England Journal of Medicine), Copyright Massachusetts Medical Society.

Table 1.3 below summarizes the main changes in AD conceptualisation in its various stages. Notably, the latest criteria have been conceived as frameworks for research and are not currently recommended for use in clinical practice.

Table 1.3 Latest published criteria for preclinical AD.

	AD is:	**Preclinical AD is:**	**Biomarkers of AD pathology:**
IWG-Updated criteria for preclinical AD (Dubois et al., 2016)	A pathological entity defined by amyloid and tau pathology.	**Preclinical AD** = cognitively-normal individuals with biomarker evidence of abnormal amyloid and tau The following two classifications are no longer considered as preclinical AD but may precede it: • **Asymptomatic at-risk for AD** = cognitively-normal individuals with biomarker evidence of abnormal amyloid or tau (but not both). • **Presymptomatic AD** = cognitively-normal individuals who carry a proven autosomal dominant mutation for AD.	• abnormal Aβ-PET or abnormal Aβ in CSF • abnormal tau-PET or abnormal tau in CSF
Updated NIA-AA criteria (Jack et al., 2018)	A purely pathophysiologic entity, with no reference to clinical symptoms.	Using the ATN framework (A: amyloid; T=tau and N=neurodegeneration), individuals are placed into five categories based on their biomarker profiles. Three of these categories form the Alzheimer's continuum: • Alzheimer's pathologic change (A+T-N-) • AD (A+T+N-, A+T+N+) • Alzheimer's and concomitant suspected non-Alzheimer's pathologic change (A+T-N+) The other two categories are not part of the continuum: a) Normal AD biomarkers (A-T-N-) b) Non-Alzheimer's pathologic change (A-T+N-, A-T-N+, A-T+N+) Any of these biomarker profiles may be combined with one of three cognitive stages: cognitively unimpaired, mild cognitive impairment (MCI) or dementia. **Preclinical AD** = cognitively-unimpaired individuals with an "AD" biomarker profile. **Preclinical Alzheimer's pathologic change** = cognitively- unimpaired individuals with an "Alzheimer's pathologic change" biomarker profile.	• A is defined by either abnormal Aβ-PET or abnormal Aβ in CSF • T is defined by either abnormal tau-PET or abnormal p-tau in CSF • N is defined by either hypometabolism on FDG-PET, abnormal t-tau in CSF, or atrophy in regions characteristic of AD on MRI

Aβ=β-amyloid; AD=Alzheimer's disease; CSF=cerebrospinal fluid; FDG=International Working Group for New Research Criteria for the Diagnosis of AD; NIA-AA=National Institute on Aging – Alzheimer's Association; PET=positron emission tomography

1.2.2. Dominance of the amyloid hypothesis

The amyloid hypothesis has dominated the field for the last 25 years (Selkoe & Hardy, 2016). It argues that the accumulation of Aβ plaques between neurons is the primary cause of AD and arises from an imbalance between the production and clearance of Aβ. Pivotal evidence came from the discovery of the genetic mutations causing FAD (see section 1.3 for details). Mutations in the *APP* or the presenilin genes (*PSEN1* and *PSEN2)* are involved in generating either more Aβ peptides or a higher proportion of longer and more amyloidogenic peptides. A similar phenomenon is seen in Down's syndrome due to the duplication of chromosome 21 which contains the *APP* gene. The authors proposed that the pathological sequence is likely to be 1) Aβ deposition, followed by 2) tau phosphorylation and NFT formation and 3) neuronal damage and then death. Hence, this model postulates, tau pathology is a downstream process to the initial deposition of Aβ. The proposed order has not gone unchallenged. The poor correlation between fibrillar Aβ deposition and clinical status (Giannakopoulos et al., 2003, Arriagada et al., 1992) and the universality of tau pathology in AD, provide support to consider AD as a 'tauopathy'. Further evidence in support of the amyloid hypothesis includes 1) the observation that the accumulation of Aβ pathology begins several years before the appearance of tau pathology and neurodegeneration (Bateman et al., 2012; Jack et al., 2013; Pletnikova et al., 2018); 2) the documented neuronal toxicity of Aβ in animal studies (Selkoe & Hardy, 2016); 3) evidence that the *APOE* ε4 allele – the biggest risk factor for AD after age impairs clearance of Aβ (Selkoe & Hardy, 2016).

Criticism of the amyloid hypothesis is rooted in the failure of drug trials targeting Aβ in patients with AD or mild cognitive impairment (MCI) (e.g. 'Aducanumab' which was rejected by the US Food and Drug Administration – FDA – in November 2020 on the grounds of their being not enough evidence of its effectiveness in slowing cognitive decline) (https://www.alzforum.org/therapeutics/aducanumab). Some argue that the hypothesis is too simplistic and linear (e.g. (Edmonds et al., 2015)) and the time is overdue to embrace other models of AD. The decision to focus on AD pathology in isolation from other processes has created concerns of the validity of the criteria in real life and worries that it has diverted research efforts away from other candidates (Louie, 2019; McCleery et al., 2019).

1.2.3. Amyloid deposition and ageing: The British 1946 Birth Cohort

Aβ pathology is related to a number of factors (Corder et al., 1994; Farrer et al., 1997), perhaps the most recognized one being age (Jack et al., 2008; Rodrigue et al., 2009). The British 1946 study is part of the MRC National Survey of Health and Development (NHSD) (Lane et al., 2017; Lu et al., 2019) and focuses on Aβ pathology and its relationship to cognition, mental health, imaging and life-course variables. As the study members were aged ~70 at the time of recruitment into Insight 46, the prevalence of dementia was expected to be low – around 3% (Prince et al., 2014) – but a sizeable minority of participants were expected to be in the preclinical stages of AD, with meta-analytical data suggesting significant Aβ pathology could be expected in around 15-25% of individuals at this age (Jansen et al., 2015).

The cohort provides a unique opportunity for understanding neurodegeneration in the context of ageing, the life course, and the complex factors and interactions that influence the progression of neurodegeneration and related pathologies.

For the purpose of this thesis, discussions on the Insight 46 study will be limited to symptoms of SCD and their relationship to preclinical AD - and specifically amyloid (Reisberg et al., 2008; Rentz et al., 2013).

1.2.4. Limitations of studying presymptomatic sporadic AD

Despite the growing availability of AD biomarkers (not discussed here), issues remain when trying to recruit individuals to study presymptomatic AD. No biomarker is 100% sensitive and specific, as it is currently not possible to determine with certainty, at the point of recruitment, whether or not an individual definitely has presymptomatic AD based on biomarkers alone. It is also not feasible to estimate how far an individual meeting criterion for preclinical AD, is from symptom onset at a given time point. It is therefore challenging to study the preclinical stages of SAD given that the lack of genetic certainty and absence of clinical symptoms makes it relatively unlikely for individuals to see a neurologist that early.

One alternative to these limitations is to study individuals with presymptomatic FAD. FAD and its utility in AD research are discussed in detail in the following section.

1.3. Familial Alzheimer's disease

The vast majority of AD is sporadic, with no clear pattern of autosomal dominant inheritance within families. However, in the 1930s, some cases of AD occurring at an early age and with clustering within families were observed. In 1991, a genetic linkage study of a family from Nottingham (Family 23), in whom young onset AD was highly prevalent, led to the discovery of the first FAD pathogenic mutation in *APP* gene on chromosome 21 (Goate et al., 1991). The identification of pathogenic mutations in two other genes: *PSEN1* gene on chromosome 14 and the *PSEN2* gene on chromosome 1 (Levy-Lahad et al., 1995; Sherrington et al., 1995) followed. Over 200 autosomal dominantly inherited genetic mutations have now been described across *APP*, *PSEN1* and *PSEN2*, the vast majority of which appear to be fully penetrant (Ryan & Rossor, 2010) (an individual with the mutation will develop the condition if they live long enough), although some rare reports of reduced penetrance exist (Thordardottir et al., 2018). FAD is also referred to as autosomal dominantly inherited Alzheimer's disease (ADAD) are represents less than 1% of all AD cases (Bateman et al., 2011).

1.3.1. Relevance in the context of AD research

Despite its low prevalence, FAD has proven extremely important in our understanding of SAD (Fox et al., 1997; Rossor et al., 1996). Unlike *in vivo* biomarkers which lack diagnostic certainty, FAD does not (Ryman et al., 2014; Thordardottir et al., 2018). This provides a unique opportunity to study changes across the AD continuum from presymptomatic stages through to cognitive and clinical decline.

Crucially, identifying at-risk individuals allows the study of AD many years before symptoms onset when early brain and physio-pathological changes can open opportunities for disease modifying treatments. When clinical symptoms arise, the amount of brain damage and burden is to date, irreversible and the possibility of drug success decreases considerably. Administering interventions at an appropriate earlier time is therefore critical to increase these chances.

As presymptomatic FAD mutation carriers are usually relatively young, they also tend to have less comorbidity, allowing for investigations of direct pathological consequences of AD without having to account for other co-existing pathologies. This opens routes for identifying subtle and suitable markers for tracking the natural course of the condition as well as any change due to treatment.

1.3.2. The amyloid precursor protein gene

The V717I missense mutation in the *APP* gene (London mutation) was the first mutation identified and thought to cause a relative increase in longer Aβ moieties such as $A\beta_{42}$ relative to $A\beta_{40}$ (Goate et al., 1991). APP is a transmembrane protein playing a role in neural plasticity and regulation of synapse formation although its exact function is not fully understood. To date, 58 *APP* mutations have been described, accounting for around 20% of FAD cases (Tang et al., 2016) ("ALZFORUM").

As discussed in section 1.1.2, APP can be enzymatically cleaved along one of two parallel pathways. The majority of pathogenic APP mutations lie within or close by either the β-secretase or γ-secretase cleavage sites. In addition to point mutations, *APP* duplications can give rise to FAD (Rovelet-Lecrux et al., 2006). This finding is also consistent with the frequent occurrence of young onset AD in individuals who have Down's syndrome, where an extra copy of chromosome 21 (and the *APP* gene) is present.

1.3.3. The presenilin genes

PSEN1 mutations account for 70-80% of all FAD. The *PSEN1* gene has 13 exons (although only exons 3-12 code the PSEN1 protein comprising the eight transmembrane domains). Over 180 pathogenic *PSEN1* mutations (missense mutation and deletions) are reported, with the majority located in areas thought to lie close to PSEN1's transmembrane domains ("ALZFORUM"). The PSEN1 protein forms part of the γ-secretase complex that is responsible for the transmembrane cleavage of APP to form Aβ (De Strooper et al., 1998).

Like PSEN1, PSEN2 also forms an important part of the γ–secretase complex (Cruts & Van Broeckhoven, 1998). Pathogenic mutations in *PSEN2* are less common than both *APP* and *PSEN1* mutations accounting for no more than 5% of total FAD cases.

1.3.4. Predicting age at onset

As well as being able to predict who will (mutation carriers) and will not (non-carriers) develop the condition, it is also possible to predict with relatively high accuracy at what age an individual mutation carrier will develop symptoms (Ryman et al., 2014) based upon reported age at onset (AAO) in the family or other mutation carriers. This means that it is possible to estimate, at a given

time point, how many years from symptom onset an individual is likely to be, usually referred to as “estimated years to symptom onset”. In sporadic AD, this approximation is not possible, and so following individuals over time longitudinally to the point in which they develop symptoms, is the only way of knowing the AAO with any accuracy.

A systematic review of papers describing FAD, covering 3,275 individuals, by Ryman and colleagues (Ryman et al., 2014) reported three methods to predict an individual’s AAO-based on parental AAO (r^2=0.38); mean age of family members (r^2=0.49) and mean onset of all others with the same mutation (r^2=0.52) - all of which showed significant correlations with the actual years to onset. Following generational and regional differences in clinically diagnosing AD, it is generally more reliable to define “onset” as the development of the first symptom of progressive cognitive decline, rather than the time of formal diagnosis. Historically, parental AAO has been the most commonly used method. The retrospective way of ascertaining AAO, has been found to produce similar estimates to information obtained contemporaneously in clinical notes at the time of original assessment (Doody et al., 2004). Nonetheless, estimations of age at expected symptom onset have a degree of error. Within certain families, AAO has been found to show significant variation and both genetic and environmental factors have been proposed as possible modifying factors but the underlying reasons remain uncertain. Importantly however, Ryan and colleagues (Ryman et al., 2014) showed there was no linear relationship between AAO and disease duration.

1.3.5. Heterogeneity in FAD

The heterogeneity in FAD has become an increasingly studied and recognised concept over the years (Ryan et al., 2016; Ryman et al., 2014; Tang et al., 2016) (**Figure 1.8**). Although a number of studies have revealed subtle differences between FAD and SAD (Harvey & Rossor, 1995; Kennedy et al., 1995; Rossor et al., 1996); the clinical features, duration and cognitive presentation are comparable between the two (Rossor et al., 1996; Ryan & Rossor, 2010; Swearer et al., 1992). This overall similarity presents great opportunities given the diagnostic certainty in FAD.

Atypical presentations, characterized by presentation with cognitive dysfunction other than memory, occur in both FAD and SAD. Language and behavioural variants are seen in both (Ryan et al., 2016; Ryan & Rossor, 2010) but the visual variant PCA typically occurs in sporadic disease with only one case report of PCA due to *PSEN1* mutation (Sitek et al., 2013). Recognizing this

clinical variability is crucial, as an accurate diagnosis - often missed in patients with atypical presentations - is the starting point for patient management and key to finding treatment (Ryan et al., 2016; Van der Flier, 2016). **Table 1.4** shows a summary of three studies looking at the cognitive and non-cognitive neurological manifestations of FAD occurring at some point in the disease.

It has been proposed that a significant amount of phenotypic variability is explained by differences in underlying mutations between and within genes (in the *PSEN1* group, 72% of AAO variance was explained by the specific mutation) (Ryan et al., 2016). Moreover, whether a *PSEN1* mutation is located pre or post codon 200 influences AAO: *PSEN1* mutations pre-codon 200 are generally associated with an earlier AAO than those after codon 200 (Mann et al., 2001; Ryan et al., 2016; Shea et al., 2016) – an observation linked to a more severe angiopathy and a greater burden of white matter hyperintensities on MRI in individuals with a pre-codon 200 mutation in comparison to pre-codon 200 (Mann et al., 2001; Ryan et al., 2015). Additionally, a later AAO has been described in atypical presentations in comparison to amnestic presentations (Ryan et al., 2016; Tang et al., 2016), questioning whether disease duration might be longer in both atypical presentations and pre-codon 200 in comparison to their counterparts. This established that genotype-phenotype variations could inform our understanding of AD pathophysiology (Mann et al., 2001).

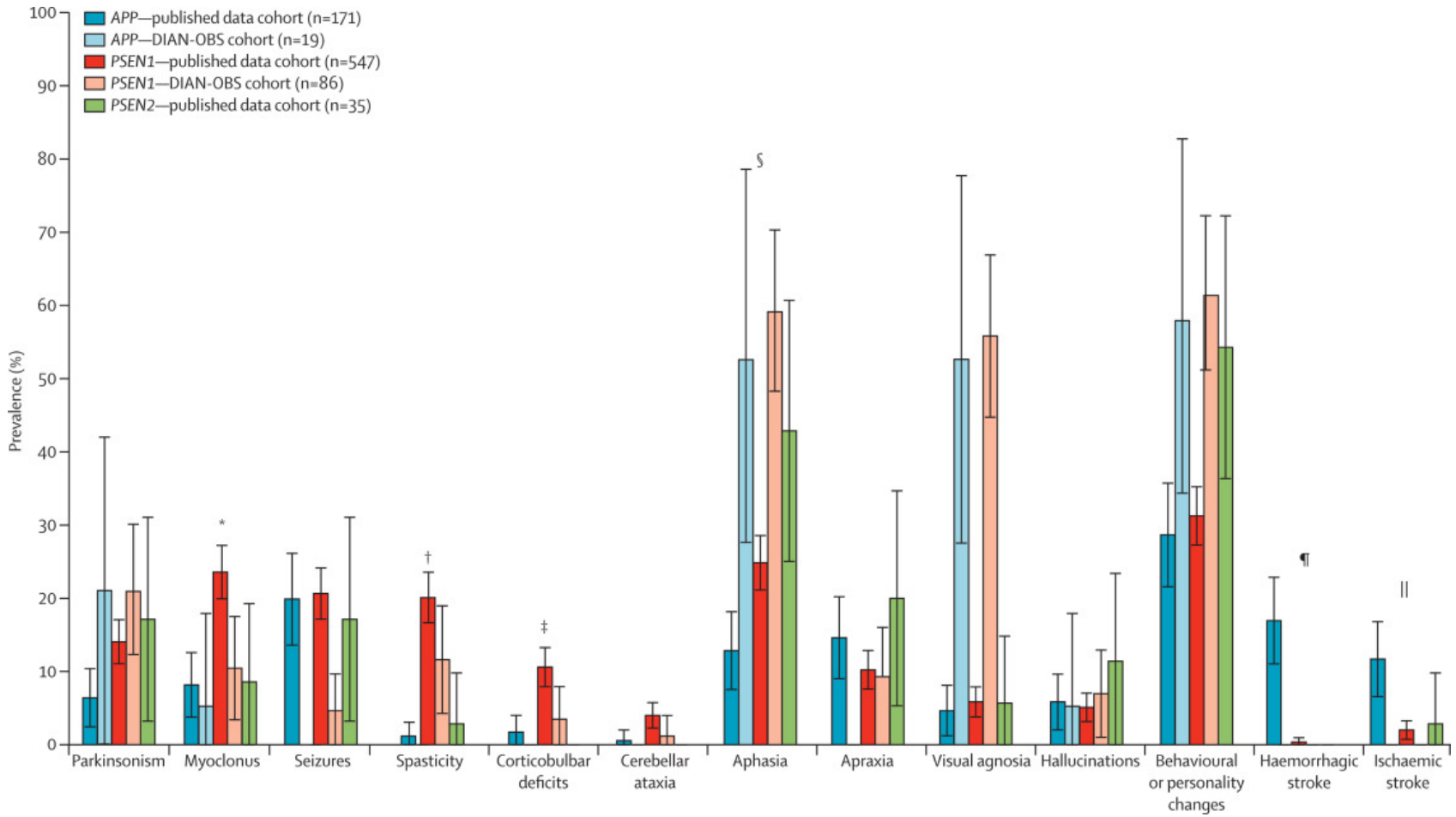


Figure 1.8 Summary of the prevalence of non-amnestic features in FAD

From (Tang et al., 2016) with permissions from Elsevier under the terms and conditions provided by Elsevier and Copyright Clearance Center. Comparison between the frequencies reported by the multicenter Dominantly Inherited Alzheimer's Disease Network (DIAN) and a literature search of the published FAD literature. Rates for *PSEN2* carriers in DIAN-OBS were not calculated as there were only two symptomatic individuals in that group. Although significant variability in symptom prevalence is observed between mutations in the three genes in the reported literature, there were few differences between *APP* and *PSEN1* in the DIAN-OBS cohort. Error bars shown are 95% confidence intervals.

Table 1.4 Three studies investigating the prevalence of clinical features in FAD cohorts over the course of the disease (as opposed to presenting features).

	Motor features (myoclonus and seizures)	**Cognitive, neuropsychiatric or behavioural features**	**Pyramidal signs**	**Extra-pyramidal signs**	**Cerebellar signs**	**Strokes**
107 DIAN-OBS cohort (Tang et al., 2016)[a]	Myoclonus= 9% Seizures= 3%	In >50% individuals. Aphasia= 57.9% Apraxia= 7.5% Visual agnosia= 55.1% Hallucinations= 6.5% Behaviour or personality changes= 61.7%	Spasticity= 9.3%	Parkinsonism= 11.2%	15.0%	Haemorrhagic stroke= 0 Ischaemic stroke= 0
Systematic review of 188 publications; N=1228 (Detailed neurological examination available for N=753) (Tang et al., 2016)[b]	Myoclonus=20% Seizures=20%	In <30% individuals. Aphasia=23.0% Apraxia= 11.7% Visual agnosia= 5.6% Hallucinations= 5.6% Behaviour or personality changes= 31.7%	Spasticity= 15.0%	Parkinsonism= 12.5%	3.1%	Haemorrhagic stroke= 4.1% Ischaemic stroke= 4.2%
213 patients with *PSEN1* and *APP* (medical history and neurological examination findings available for N=121) (Ryan et al., 2016)[c]	***APP***: Myoclonus= 33% Seizures= 25% ***PSEN1***: Myoclonus= 47% Seizures= 24%	***APP***: Amnestic presentations= 97%. Non-amnestic= 3% ***PSEN1***: Amnestic=84%. Non-amnestic presentation= 16%.	***APP:*** NA ***PSEN1***: 25%	***APP:*** not present. ***PSEN1***: 14.0% extrapyramidal signs i.e. rigidity	***APP:*** not present. ***PSEN1***: 3.0% i.e. ataxia	NA

[a] Tang and colleagues' reports from the DIAN-observational study (DIAN-OBS) on behalf of the DIAN consortium. [b] Tang and colleagues' reports on the literature and DIAN-OBS.
[c] Ryan and colleagues describe cognitive and neurological features in a series of participants over the years (1987-2015).
NA= not applicable; DIAN= Dominantly Inherited Alzheimer's Network. *APP*=amyloid precursor protein gene; *PSEN1*=presenilin 1 gene.

A number of factors could explain differences in prevalence. Selection or sampling and measurement bias (Moulder et al., 2013; Van der Flier, 2016) and the timing and number of visits certainly have an influence when looking at the prevalence of symptoms. For example, shorter follow-up periods may result in lower prevalence of certain symptoms such as seizures and myoclonus that may sometimes be higher in prevalence at later stages of the disease. Younger AAO and advanced disease stages have also been related to higher frequency of non-cognitive clinical features (Tang et al., 2016). Thus, non-cognitive clinical manifestations are possibly influenced by disease severity, environmental and genetic factors. Tang and colleagues (Tang et al., 2016) postulate that non-cognitive clinical features seem to affect a small proportion of individuals with mild to moderate FAD and case reports might overestimate their prevalence, while underestimating cognitive neurological features. Large population-based systematic protocols and longer follow-ups could address these concerns. Accurately determining the prevalence of clinical and neurological signs and symptoms is important to define the disease clinically, understand its prognosis, impact on patients and inform the conduct of research (Tang et al., 2016).

Although *APOE* ε4 is a major risk factor for SAD (Farrer et al., 1997), evidence of its effect on FAD are less clear (Pastor et al., 2003; Ryman, et al., 2014; Sorbi et al., 1995; Wijsman et al., 2005) and some research has even suggested a beneficial effect of ε4-carriership. For instance, *APOE* ε4 carriership has been observed in association with resistance to certain infections (Smith et al., 2019) and also associated with a slight advantage in certain cognition functions (Tuminello & Han, 2011; Zink et al., 2019). This effect has been explained by the antagonistic pleiotropy, whereby a gene has both beneficial and detrimental effects, with the detrimental effects often manifesting later in life when the forces of natural selection are weaker (Austad & Hoffman, 2018)). Shea and colleagues reported that *APOE* ε4 status did not affect the AAO, age of death, or duration of clinical course of the disease when considering all patients together (irrespective of the gene) and when considering the mutated gene (Shea et al., 2016). Other studies have looked at the influence of *APOE* ε4 allele and reported mixed results. In a family with an *APP* p.Val717Ile mutation (N=17 affected), having at least one copy of the *APOE* ε4 was associated with a younger symptom onset (Sorbi et al., 1995). However, *APOE* genotype was not found to influence AAO in a group of individuals with *APP* mutations at codon 692 or 693 (N=41) (Haan et al., 1994). In *PSEN1* mutations, a modifying effect on AAO by ε4 allele has not been reported (Van Broeckhoven et al., 1994). Though, it did appear associated with an earlier AAO in a large Colombian kindred with the *PSEN1* p.Glu280Ala mutation (N=52 affected) (Pastor et al., 2003). For *PSEN2*, younger ages at onset in *APOE*

ε4 carriers have been reported in a lineage of Volga German descent carrying the same *PSEN2* p.Asn141Ile mutation (N=74 affected) (Wijsman et al., 2005).

A number of studies have also suggested different patterns of regional atrophy between *APP* and *PSEN1* mutations with *APP* patients having smaller hippocampi compared to *PSEN1*, despite being similar in terms of disease severity (Scahill et al., 2013). Indeed, Voxel-based morphometry (VBM) and cortical thickness effects-maps suggest subjects with *PSEN1* mutations might have more cortical involvement and reduced whole-brain, grey and white matter volumes compared with *APP* subjects. Moreover, white matter atrophy appears relatively more localised in individuals with *APP* mutations, compared to a rather extensive white matter involvement of occipital, parietal and frontal lobes in *PSEN1* which might explain the greater occurrence of atypical clinical features including spastic paraparesis in some patients with *PSEN1* mutations.

Differences in neuropsychological profiles between FAD mutations have also been studied and (although not the focus of this thesis) include the greater memory impairment in *APP* compared to *PSEN1*. This distinction has been linked to greater involvement of the medial temporal and limbic regions in *APP* compared to *PSEN1*, which conversely show a greater impairment of the non-memory domains and imaging findings of greater cortical loss (Scahill et al., 2013).

Over the years, increasing research on FAD has led to the establishment of an international initiative: The Dominantly Inherited Alzheimer's Network (DIAN), discussed in more detail below.

1.3.6. The Dominantly Inherited Alzheimer's Network

DIAN is an international registry of individuals at-risk for developing FAD, established in 2008. One of its aims is to investigate the order of AD pathophysiological changes that occur in presymptomatic mutation carriers (individuals who carry a mutation but have not yet developed symptoms) and to some extent represent an equivalent to individuals meeting criteria for preclinical AD in sporadic cases (Moulder et al., 2013).

In 2009, a DIAN clinical trials committee was formed to evaluate potential trial designs and determine which therapeutic targets were likely to be most responsive to treatment; the trials committee was then transitioned to the DIAN Trials Unit (DIAN-TU). The study has been investigating whether two drugs called 'gantenerumab' and 'solanezumab' may have an effect in slowing down the underlying disease process in FAD. Both of these drugs are monoclonal

“antibodies” that bind to amyloid protein and may thereby help remove it from the brain. Whilst further detailed analyses are awaited, the top line results were announced in February 2020. Unfortunately, this initial analysis showed that neither drug was effective in its main aim of slowing down cognitive decline. This failure may lie either in the lack of sensitivity of the outcome and/or in the selection of individuals (i.e. symptomatic individuals being too severe affected and presymptomatic being too far away from expected onset at the start of the trial to show a meaningful reduction in cognitive decline). However, a number of analyses are ongoing and an open label extension study established for the gantenerumab arm - the impact upon biomarkers or indeed survival, is not yet known but may inform future trials.

1.4. Scope of the PhD

My research is largely based on data from the longitudinal FAD study at the DRC. This PhD has the overarching goal of deepening our understanding of populations at-risk of AD like FAD, by studying the subtle preclinical cognitive changes and cognitive and clinical features that accompany disease progression.

The following chapter introduces my specific research questions and hypotheses, the background to the unanswered questions in this field and reviews the relevant literature.

2. BACKGROUND AND RESEARCH QUESTIONS

2.1. Rationale

In the majority of AD cases, the first, most salient and clinically relevant symptom is a progressive loss of episodic memory, more marked for recent events (Dubois et al., 2007; McKhann et al., 1984). Since the discovery of AD, studies of memory deficits have experienced a considerable shift in conceptualization.

As pathophysiological changes begin many years prior to clinical manifestations, what was primarily used to describe the clinical picture of a patient, transitioned into a multifaceted and multi-layered patient-research concept: a symptom, a clinical manifestation, a cognitive marker of preclinical AD and even a subjective memory complaint-to name a few.

As a starting point, I will first address the symptomatic phase of the condition, specifically evaluating phenotype-genotype interactions in relation to disease duration and survival in order to illustrate the complexity of symptomatic manifestations-including amnestic ones and establish some grounds to investigate preclinical investigations (Chapter 4).

I will then focus on visual short-term memory (VSTM) impairments in a longitudinal cohort where the majority of participants are presymptomatic and study how such changes occur in relation to disease progression and proximity to estimated symptom onset (EYO) (Chapter 5).

In order to explore these deficits in greater detail, I next evaluate how a relatively novel technique like eye-tracking, may deepen our understanding of VSTM impairments in FAD during encoding (Chapter 6).

Lastly and in order to extend memory impairments into another dimension, I consider features of SCD in presymptomatic FAD and an older, elderly population 'at-risk of AD' due to the increased likelihood of Aβ deposition with older age (Chapter 7).

As the title of this thesis suggests, the common theme in all this work lies in addressing AD from two relevant angles: 1) through the investigation of subtle preclinical changes (with a particular focus on VSTM function and features of SCD – both of which have been suggested as sensitive markers of preclinical AD (Jessen, 2014; Parra et al., 2010a)) and 2) by evaluating the symptomatic features in relation to the progression of the disease. The rationale for this lies in the assumption that this 'dual focus' – on sensitive preclinical markers of disease *and* the symptomatic phase – provides a better understanding of AD as a whole (rather than focusing on one or the other). It is relatively common to hear the phrase "targeting the right people at the right time" when discussing therapeutic treatments in AD for instance. While efforts tend to focus on understanding the preclinical change, important value also lies in

investigating changes that occur in the symptomatic phase, not only to improve the quality of life of those already diagnosed, but also because understanding the common course of the condition itself is as crucial for treatment interventions (i.e. to measure the effect of a given drug on an affected individual, the average survival of the disease and the factors that affect it, need to be characterised first).

Overall, I will aim to address the following topics: 1. Survival in FAD (Chapter 4); 2. VSTM function over time (Chapter 5); 3. VSTM function and eye movements (Chapter 6); 4. Features of SCD in populations "at-risk" of AD (Chapter 7).

Research questions with clear links to each data chapter are discussed next, followed by a review of the relevant literature forming the basis for the hypothesis.

2.2. Research questions

The specific research questions for each data chapter are described next with short descriptions outlining how this work links together.

As a starting point, I focus on a retrospective symptomatic FAD cohort. The overall question I aim to answer is: **i) What are the survival estimates of individuals with *PSEN1* and *APP* mutations and how do genotype and phenotype differences influence these estimates?**

More specifically my research questions for Chapter 4 are:

1. Is there a difference in survival time between *PSEN1* and *APP* mutation carriers?

2. How much of the variance in survival is explained by genotype (i.e. mutation & family)?

3. Is there a difference in survival time between amnestic (typical) and non-amnestic (atypical) presentations?

4. Is there a difference in survival time between *APOE* ε4-carriers and *APOE* ε4-non carriers?

5. Is there a difference in survival time between sexes?

6. Given the broad range of data (year of birth range: 1879-1983), is there an indication of a generational effect?

7. What is the relationship between survival and AAO?

8. Within *PSEN1*, is there a difference between mutations pre- and post-codon 200?

Estimates of the duration of a disease are valuable both in a research and clinical setting. Nonetheless, as in many aspects of science there is no such thing as 'one size fits all'. It is

therefore paramount to describe this variability and attempt to investigate factors that might influence this estimate. The main scope here is thus to review the current findings and methods used to study disease duration in FAD and establish limitations and outstanding questions from the literature – see section 2.3. Furthermore, following the significant amount of variability in AAO explained by differences in underlying mutations between and within genes described in section 1.3.5. of Chapter 1, analogous investigations will be performed for survival estimates.

Following this retrospective investigation in symptomatic FAD, the focus turns to the early preclinical changes in AD. The overall question I aim to answer here is: **ii) How do VSTM impairments vary in symptomatic and presymptomatic FAD mutation carriers with disease progression?**

More specifically, my research questions for Chapter 5 are:

1. Are the cross-sectional deficits in VSTM and VSTM binding in preclinical AD, also reflected in longitudinal decline in task performance? In other words, can VSTM – precisely the "What was where?" task – track preclinical AD changes?

2. Is there a relationship between EYO and VSTM function?

3. For comparison, is longitudinal decline in presymptomatic FAD carriers and symptomatic mutation carriers seen in other more traditional neuropsychology tasks?

VSTM and VSTM binding tasks have been suggested as cognitive marker of AD including presymptomatic stages of the condition. However, there is a need for longitudinal studies that can follow individuals at-risk of AD or FAD from a presymptomatic stage through to AD. I investigated how these impairments related to the progression of the disease. The principal scope of this chapter is to evaluate the longitudinal change of VSTM in presymptomatic and symptomatic FAD mutation carriers. The scientific basis for these questions – including the brain-behaviour foundation for testing VSTM in the first place, will be reviewed in section 2.4.

Narrowing the preclinical focus further, I turn to a relatively novel and non-invasive technique – eye-tracking – and evaluate how it may provide greater understanding of the memory processes behind these behavioural task outcomes. The overall question I aim to answer here is: **iii) What is the relationship between eye movements and memory performance in a FAD cohort?**

More specifically, my research questions for Chapter 6 are:

1. Is there a difference in low-level oculomotor characteristics (e.g. saccade amplitude or velocity) between presymptomatic FAD carriers and controls and symptomatic FAD carriers and controls?

2. Is there a difference in the visual search strategies – measured by eye movement patterns (e.g. fixation duration, time spent fixating the stimuli) – between presymptomatic FAD carriers and controls and symptomatic FAD carriers and controls?

3. Can these visual search strategies predict VSTM performance?

A growing body of evidence suggests a link between eye movements and memory (Hannula et al., 2010). The aim of this chapter is to establish whether oculomotor characteristics, specifically during encoding, may deepen the understanding of VSTM impairments described in FAD. Eye movements studies in AD and the scientific basis for investigating eye movements as a proxy to cognitive processes, will be reviewed in section 2.5.

Finally, in order to extend the scope of the thesis, I investigate SCD – proposed to be an indicator of preclinical AD – as an example of the possible links that can be made between populations at-risk of AD in future investigations. The overall question I aim to answer here is: **iv) Are symptoms of SCD reported by two at-risk populations (i.e. Insight 46 and FAD) associated with β-amyloid and mutation carrier status respectively?**

More specifically, my research questions for Chapter 7 are:

1. What is the relationship between SCD – measured by the MyCog questionnaire (Rami et al., 2014) – and preclinical AD (measured by amyloid positivity in Insight 46 or mutation status in FAD)?

2. Are SCD features associated with a) family history of AD (only for the Insight 46 cohort as this was an inclusion criterion for FAD); b) objective cognition; c) affective symptoms; and d) life-course variables?

SCD has been reported as an early feature of AD and several studies have shown associations between SCD and AD biomarkers (e.g.(Jessen et al., 2014)). Nonetheless, substantial variation exists and considerations on SCD and preclinical AD while accounting for affective symptoms are lacking.

These topics are reviewed in the following sub-sections. Each sub-section ends with a summary of the implications for my research. As Chapter 7 includes the only non-FAD study, I will review the relevant literature of SCD in the appropriate data chapter instead.

2.3. What are the genotype and phenotype influences on disease duration in FAD?

2.3.1. Disease duration in FAD

Disease duration, the time an individual has symptomatic disease, is often calculated by subtracting the age at symptom onset to the age of the individual's death. The average duration in FAD varies in the literature; whilst some report a more aggressive disease course for FAD compared to SAD, others suggest this is only modestly shorter (9.7 ± 5.1 years) than the average course of 11.3 years from symptom onset to death reported in SAD (Godbolt et al., 2004; Waring et al., 2005).

Drawing direct comparisons between the two is often problematic as survival is likely to be affected by factors such as age (younger, healthier patients may survive longer than older frailer patients with additional comorbidities may) and estimates for AAO can be highly subjective (Ryan & Rossor, 2010).

There have only been a few comprehensive systematic reviews of AAO and disease course in FAD (Canevelli et al., 2014; Ryman et al., 2014; Shea et al., 2016). In a symptom onset meta-analysis of 3,275 individuals with *PSEN1* and *PSEN2* mutations, Ryman and colleagues also carried out a sub-analysis of 600 individuals with known ages at death (45% of their dataset) (Ryman et al., 2014). They did not find a linear relationship between AAO and the progression of the disease ($p>0.5$). However, on further investigation, the authors detected an inverted U-shape relationship between these two variables, whereby patients with early (younger than 35 years) or late (older than 65 years) onset each had a shorter disease course than patients with onset in midlife (35-65).

Shea and colleagues' systematic review (Shea et al., 2016), reported that patients with *PSEN1* mutations had the lowest AAO and age at death (mean 43.3 ± 8.6 years and 50.5 ± 9.7 years, respectively; $p<0.001$) and patients with *PSEN2* mutations had the oldest AAO and age at death (mean 58.1 ± 9.5 years and 71.8 ± 10.6 years, respectively; $p<0.001$) and longest disease duration (median 11 years, $p=0.03$). In this respect, it is worth noting that Canevelli and colleagues' systematic review (Canevelli et al., 2014) showed a mean disease duration of 10.8 years, ranging from 3 to 25 years for *PSEN2* mutations.

In conclusion, studies demonstrate considerable variation and little is known about the factors which influence this variability.

2.3.2. Approaches to study disease duration

Research into preclinical cognitive deficits and their relationship with disease progression is crucial. A series of approaches to study disease duration in AD have been proposed. Perhaps the first distinction lies in its design: a) prospective studies investigate an outcome during a time-period (this time period is either practised or estimated); b) retrospective studies in which the investigations in relation to an outcome are done backwards. These can either by hypothesis-driven (e.g. observational longitudinal studies) or data-driven (e.g. event-based modelling, change-point analysis).

Longitudinal studies employ continuous or repeated measures to follow particular individuals (within a predefined groups) over prolonged periods of time often years or decades (Caruana et al., 2015). This study type is particularly useful for evaluating the relationship between risk factors and the development of disease, and the outcomes of treatments over different lengths of time. They have the advantage over cross-sectional studies as may assess when the changes occur.

Data-driven progression models on the other hand, have emerged in recent years as a family of computational approaches for analysing progressive diseases (Oxtoby et al., 2018). Instead of regressing against predefined disease stages (Bateman et al., 2012; Ridha et al., 2006; Scahill et al., 2013) or learning to classify cases from a labelled training database (Klöppel et al., 2008; Young et al., 2014), generative data-driven progression models construct an explicit quantitative disease signature without the need for a priori staging. However, the greatest limitations of some of these model versions, is the assumption of a common or average disease trajectory among individuals (Archetti et al., 2019).

Change-point analyses (CPA, e.g. event-based modelling) are designed to detect subtle changes in incidence and characterize changing trends in time series (Kass-Hout et al., 2012). Some of the limitations include that various sources of variation may influence the data readings and consequently the performance of the change-point methods. While some of these sources may be identifiable, it is not the case for all.

Though each of these methods may prove useful for studying disease duration and its progression when the information of an individual's age at death is known, limitations exist when survival estimations are needed and datasets are incomplete (unknown ages at death).

Survival analysis or time-to-event analysis, is a set of methods for analysing data where the outcome variable is the time until the occurrence of an event of intertest. In some patients, the expected event (e.g. success of treatment or an individual's passing), does not occur until

after the end of the trial, or even not at all. This means that the only information available on these patients is that no event has yet occurred as of a particular point in time. This is known as censoring. The probability that a patient has survived up to a certain point in time is calculated using the Kaplan–Meier method (Goel et al., 2010), often used to graphically represent a survival time curve.

2.3.3. Implications for my research

Studies have looked at the relationship between AAO and cognitive presentation, neurological features and mutation in FAD and even investigated differences in disease duration between genes. However, studies investigating survival time between genes (*APP* and *PSEN1)*, *APOE* ε4 status, sexes, cognitive presentations, mutation positions (within *PSEN1)* and the extent to which disease duration varies by mutation and family within each gene, all while accounting for censoring, are lacking. My first data chapter, Chapter 4, will explore these questions in detail.

In the next section I will outline the relevant literature for the second data chapter: Chapter 5.

2.4. What is the evidence for VSTM impairments as markers for preclinical AD?

2.4.1. Memory impairments and AD

As mentioned above, in the majority of AD cases, the first, most salient and clinically relevant symptom is a progressive loss of episodic memory, more marked for recent events. This is referred to as an amnestic presentation or typical AD (Dubois, 2018, 2014), and from a radiological point of view is characterised by atrophy of the MTL and particularly the hippocampus. In the very early stages of the disease, the clinical syndrome selectively often affects this one cognitive domain, and has minimal impact on day-to-day functioning, a period often referred to as the MCI stage. MCI due to AD refers to the symptomatic pre-dementia phase of AD where the degree of cognitive impairment is not normal for age (Albert et al., 2011; Dubois et al., 2014).

Memory has been the focus of AD research for many years (e.g. (Dubois et al., 2007; McKhann et al., 1984)). One of the most popular models for studying memory remains Atkinson-Shiffrin's, (Atkinson & Shiffrin, 1968), also known as the modal or multi-store model. This considers memory as a sequence of three stages from sensory to short-term memory

STM to long-term memory (LTM) rather than a unitary process and classifies memory according to its duration and content (**Figure 2.1**).

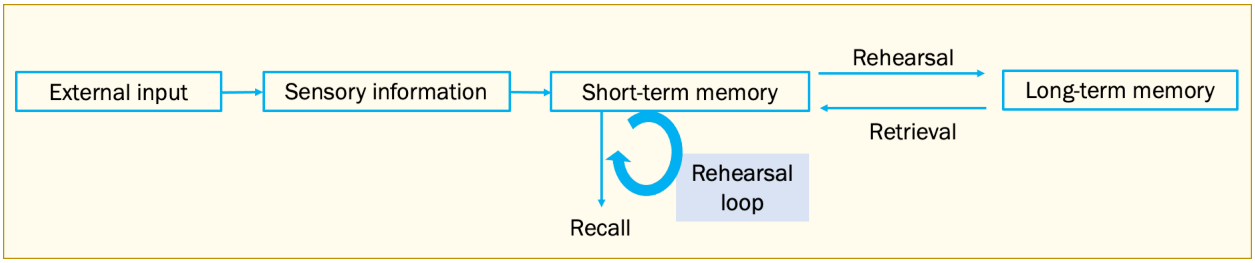


Figure 2.1 Diagram of human memory following the modal or multi-store model by Richard Atkinson and Richard Shiffrin, 1968 (Atkinson & Shiffrin, 1968).

While long-term episodic memory impairments have been widely documented (e.g. (Greene et al., 1996; Hodges, 2000)), relatively less attention has been devoted to STM deficits in AD (Liang et al., 2016).

2.4.2. Models of working memory and common conceptions

Short-term memory (STM) is the mechanism used to retain information over seconds. VSTM is the memory system that affects the operation of the visual domain. WM refers to the short-term storage and manipulation of information for a short period of time (lasting on the order of seconds) (Baddeley, 2003; Ma, Husain, & Bays, 2014). Whereas performance on WM tasks improves with brain development from childhood to early adulthood, it declines in the elderly (Ma, Husain, & Bays, 2014). Models of WM have extensively undergone revisions and refinements over the years, with the first, proposed by Baddeley and Hitch in 1974 (Baddeley & Hitch, 1974) comprising three components: a central executive, a verbal storage system called the phonological loop and a visual storage system called the visuo-spatial sketchpad (Baddeley, 2003). This was later modified to include links to LTM via the visuo-spatial sketchpad and the phonological loop. The inclusion of the episodic buffer followed and was presumably controlled by the central executive system and capable to retrieve information from the store and where necessary manipulate and modify it (Baddeley, 2000). The episodic buffer attracted considerable attention, leading to questions about the functions and structures of WM and its neurobiological underpinnings (Baddeley, 2007) and finally resulting in the latest revised model of WM (Baddeley et al., 2011) (**Figure 2.2**).

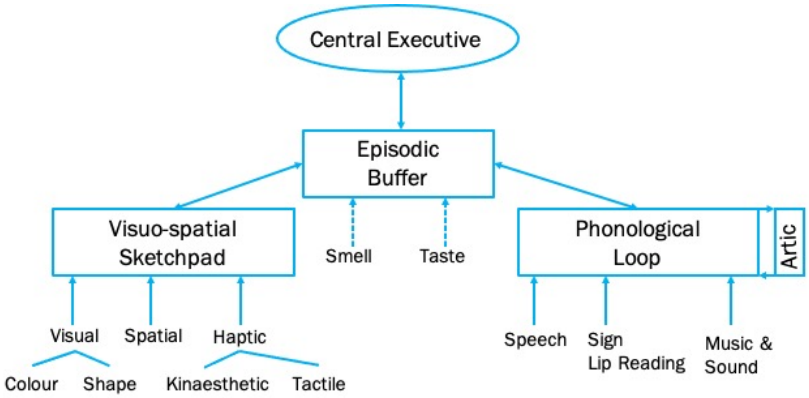


Figure 2.2 A revised model of working memory (Baddeley et al., 2011)

This latest revision of the WM model, places the episodic buffer at the heart, as a passive system, yet one that serves an important integrative role because of its capacity to bind information from a number of different dimensions into unified "chunks" (Baddeley et al., 2011; Cowan, 2001). The model carries a number of assumptions including the belief that a conscious access to the phonological loop or sketchpad takes place via the buffer. These subsystems themselves act as lower-level buffers allowing information (visual or spatial for the visuo-spatial sketchpad and language-related i.e. speech for the phonological loop) to be combined. The classical view is that WM is limited in capacity, holding a fixed, small number (K) of items. Miller's "magical number" seven (Miller, 1956) or Cowan's "magical number" four (Cowan, 2001) refers to the normal span (average number of items, or "chunks") healthy adults can store in their STM. A highly influential proposal has been that items retained in WM are held in three or four independent object "slots", one for each item stored (Luck & Vogel, 1997). The approach postulates WM is either 'all or none': an object gets into a memory slot (is remembered correctly), or it does not (is not remembered at all). However, relatively recent work has led to substantial understanding of the structure and organization of WM with reasons to reconsider the classical view mentioned above. Specifically, studies showed how the *precision* of recall declines continuously as the number of items to be remembered increases (Ma, Husain, & Bays, 2014). In addition, increasing the saliency of a stimulus causes it to be stored with enhanced precision at the cost of poorer memory for other stimuli (Ma, Husain, & Bays, 2014). Although the interpretation of these results remains an active area of debate, a 'competing slot model' where every item is stored with either high precision or not at all, cannot explain these findings (Luck & Vogel, 1997; Ma, Husain, & Bays, 2014). Conversely, a 'resource model' where WM is a limited resource *flexibly distributed between all items*, may (Alvarez & Cavanagh, 2004; Bays et al., 2009; Bays & Husain, 2008; Fougnie et al., 2012; Franconeri et al., 2013; Gorgoraptis et al., 2011; Keshvari et al., 2013; van den

Berg et al., 2012; Wilken & Ma, 2004). Importantly, 'resource models' postulate it is not the number of items remembered that makes resources limited but rather the *quality* of memory precision. They are based on two premises (Bays et al., 2009; Palmer, 1990; Wilken & Ma, 2004): 1) the internal representations of sensory stimuli are noisy (they are corrupted by random, unpredictable fluctuations) 2) the level of this noise increases with the number of stimuli in memory. Therefore the more resources allocated to an item, the less noise is present in its representation and the more precise its recall (Ma, Husain, & Bays, 2014).

This flexibility in memory allocation, represents a crucial distinction between competing slot and resource accounts of WM (Bays & Husain, 2008). A growing body of evidence suggests that, rather than limited to a fixed storage distribution, memory resources can be unevenly distributed so that prioritized items are stored with enhanced precision compared to others (Bays et al., 2011; Gorgoraptis et al., 2011; Zokaei et al., 2011). Indeed, this recall advantage appears to come with a cost to other stimuli in memory, recalled with less precision (Bays & Husain, 2008; Gorgoraptis et al., 2011). In contrast to the slot framework, resource models of WM state that the same resources are engaged whether one or multiple visual items are stored (Ma, Husain & Bays, 2014). A neural basis for resource models lies in the number of action potentials used to encode memories (van den Berg et al., 2012). This correspondence between resources and the amplitude of neural activity is supported by various lines of evidence (Ma, Husain & Bays, 2014): 1) theoretical models propose that neuronal gain is proportional to the precision of encoding of the stimulus (Seung & Sompolinsky, 1993); 2) WM resources are similar to attentional resources (Awh & Jonides, 2001; Mazyar et al., 2012) and attention modulates neural gain (McAdams & Maunsell, 1999); and 3) neuronal spiking is costly and the benefits of more spikes in encoding stimuli might be overweighted by the energy spent leading to a decreased precision of each time (van den Berg et al., 2012).

There is increasing acknowledgement in the field that understanding why information is forgotten is at least as important as understanding how information is encoded and retained (Davis & Zhong, 2017; Richards & Frankland, 2017; Sadeh et al., 2014; Sadeh & Pertzov, 2020). In recent years, a renewed focus on the 'passage of time' as a significant cause of memory loss (Davis & Zhong, 2017; Hardt et al., 2013; Migues et al., 2016), in addition to interference (as opposed to the more traditional view of interference being the sole factor (Underwood, 1957)) emerged. Some researchers sustain that given the striking similarities between time-dependent forgetting of information over short (interval of a few seconds; STM or WM paradigms) and long timescales (interval of several minutes to days, weeks, and even months), this provides evidence in support of a single, hippocampus-based, mechanism

underlying memory at both short and long timescales: "a unifying account of hippocampal forgetting" (Sadeh & Pertzov, 2020). A summary of the conceptual similarities in support of this argument is outlined in **Table 2.1.**

Table 2.1 Conceptual similarities between short and long-time scales.

	Short Timescales & Long Timescales
Hippocampus	The MTL and the hippocampus in particular, support memory over both timescales (Buffalo et al., 1998; Ezzyat & Olson, 2008; Holdstock et al., 2000; Olson et al., 2006). This is in contrast to the traditional accounts which regards STM and LTM as two distinct systems, supported by district brain regions (Alvarez et al., 1994; Atkinson & Shiffrin, 1971; Baddeley & Warrington, 1970; Cave & Squire, 1992) and more recent ones: (Davelaar et al., 2005; Talmi et al., 2005).
Relational binding (the process of encoding representations regarding relations between two (or more) entities of information (Ryan et al., 2013) (see section 2.4.3 for more details on relational binding).	The hippocampus play a crucial role in learning novel associations-namely in relational binding (see (Olsen et al., 2012) for review) and supports mnemonic functions (relying on relational binding) regardless of the duration of the study-test interval (Yonelinas, 2013)[1]
Time forgetting patterns	Manifested in the weakening of hippocampus-based relational memory (long: (Brubaker & Neveh-Benjamin, 2012; Hockley & Consoli, 1999; Sweegers & Talamini, 2014; Talamini & Gorree, 2012); short:(Pertzov et al., 2012, 2013, 2017; W. Zhang & Luck, 2009)) occur in both timescales.
Encoding duration and rate of forgetting	Some research suggests forgetting does not depend on the total time allocated to learning/encoding (Cohen-Dallal et al., 2018; Hintzman & Stern, 1984) in either time scale.
Manner in which information is forgotten: gradual *vs* all-or-none fashion	Loss of information across short and long timescales is usually reflected in gradual loss, but at times also in complete loss of accessibility (Ma et al., 2014; Sekeres et al., 2018)[2]

[1]'Why did earlier studies suggest that the hippocampus is not necessary for memories across short durations?' Sadeh and Pertzov (Sadeh & Pertzov, 2020) suggest, memory tasks employed in such studies did not rely on relational binding (i.e. they did not involve the creation of novel associations between entities.

[2] Tulving's ideas (Tulving & Pearlstone, 1966) regarding the availability *vs* accessibility of memories also appears to support the a graded memory decline: although items might not be *accessible* at a certain point in time, their memory traces still exist. Hence, the *availability* of inaccessible traces is manifested the fact that items may be retrieved at a later occasion, given cues or memory tests. MTL=medial temporal lobe; STM=short-term memory; LTM=long-term memory.

Furthermore, a cognitive construct which has driven considerable amount of revision of WM models is binding – the cognitive function known to support the integration of features necessary to maintain a coherent representation of an object in immediate memory. In fact, to account for such operations in WM, Baddeley proposed the episodic buffer in the first place, arguing that it could be the locus of binding functions (Baddeley, 2000). Further work over the years has led to the proposal of a dissociation between two binding processes within WM: conjunctive binding supported by the visuo-spatial sketchpad and relying on a cortical network

including sub-hippocampal structures (parietal-occipital temporal network; entorhinal & perirhinal cortices) and relational binding supported by the episodic buffer and relying on the frontal-parietal-MTL network and dependent on the integrity of the hippocampus (Jonin et al., 2019). Further details on VSTM binding – including the distinction between relational and conjunctive binding – is discussed in the next sub-section.

2.4.3. VSTM binding

In daily living, binding on a temporary basis in STM is essential to keep track of, for example, changing patterns of traffic while driving, or whether a white and round or the yellow and more elongated pill has just been taken (Parra et al., 2010b).

Over the past decade, STM binding tests in the visual domain (i.e. VSTM), have attracted much attention in the field of preclinical AD. VSTM is a memory system that stores visual information for a few seconds and is used for ongoing cognitive tasks (Hollingworth et al., 2009). As Baddeley and colleagues argue, the observation that visual binding is specifically impaired in AD as compared to healthy controls is intriguing, yet at the same time difficult to interpret in terms of specific neural systems (Baddeley et al., 2011).

There is strong support for the independence of different feature dimensions which later combine to form one representation (e.g.(Wang et al., 2017)). Neuropsychological evidence suggests that representing single and bound features in perception may be supported by different brain mechanisms with occipital, parietal and temporal regions important for perceptual feature binding (Cohen & Rafal, 1991; Friedman-Hill et al., 1995; Parra et al., 2009) and the prefrontal cortex activated when WM for conjunctions of features is explored (Mitchell et al., 2006; Prabhakaran et al., 2000).

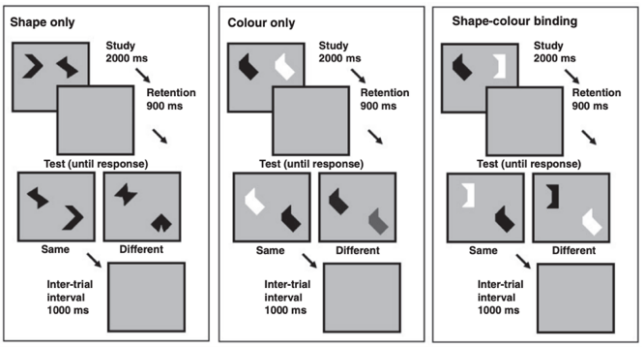
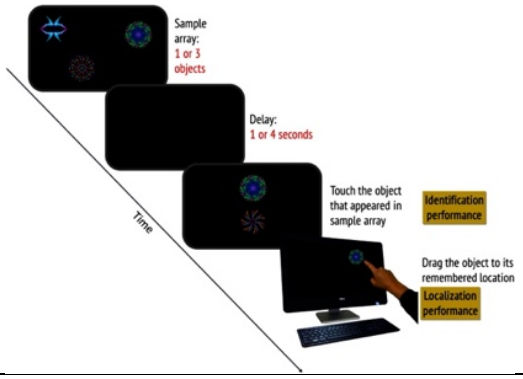
Whether binding features within integrated objects in visual WM, require dedicated resources is still controversial (e.g. (Brockmole & Henderson, 2008; Luck & Vogel, 1997; Wheeler & Treisman, 2002)), with differences in opinions likely to reflect: the difference in the type of bound information (e.g. same or different types of features); the number of objects to be remembered (Alvarez & Cavanagh, 2004; Brady et al., 2011) and whether or not object location is one of the features included in the binding (e.g. (Hollingworth & Rasmussen, 2010; Logie et al., 2011)). Indeed, neuroimaging studies show that processing bindings between visual features in visual WM that involve object location engages the hippocampus and prefrontal cortical regions not involved in processing the individual features (e.g. (Piekema et al., 2006; Prabhakaran et al., 2000)); whereas memory bindings involving conjunctions of

surface features (e.g. colour-shape) does not involve regions other than those involved in processing single features in visual WM (Parra et al., 2014; Shafritz et al., 2002; Xu & Chun, 2006).

As previously mentioned, relational binding, is thought to be reliant on the hippocampus and adjacent MTL structures. As a matter of fact, a study by Pertzov and colleagues on patients with MTL damage provided evidence of the MTL's involvement in linking together different types of information, likely to be represented in different brain areas (Pertzov et al., 2013). Furthermore, this proposal considers the hippocampus as the neural structure responsible for maintaining links relating separate aspects of memory and enabling flexible recombination of memory parts. The hippocampus receives its major cortical input from the medial entorhinal cortex (MEC) and the lateral entorhinal cortex (LEC); with the MEC providing the spatial input ("where"), and the LEC the non-spatial input ("what"). The hippocampus then combines the two streams in the dentate gyrus (DG) (Knierim et al., 2014).

Some conceptual differences of conjunctive and relational binding – including their relationship with the hippocampus – are outlined in **Table 2.2**

Table 2.2 Conjunctive *vs* relational binding

	Conjunctive binding	**Relational binding**
Definition	The integration of features within an object, ultimately forming a single representation of the item with multiple elements (Moses & Ryan, 2006).	The association of an object identity's to other 'independent' features such as its location, context or source (Hannula et al., 2015).
Experimental example	E.g. *Change-detection paradigm*: individuals are presented with two consecutive visual arrays of stimuli (separated by a short delay), which appear simultaneously on the screen and are composed of polygons, colours or combinations of polygon–colour targets. The participant is then asked to decide whether the two arrays were identical or different, as the stimuli in the second array may change. Memory performance of only the polygons and colours are contrasted with performance of the polygon–colour combinations (binding) (Parra, et al., 2009; Parra et al., 2010a). 	E.g. *Delayed-reproduction task*: individuals are presented with a sample array of 1 or 3 abstract objects (for 1 or 3 seconds respectively) followed by a delay of 1 or 4 seconds. Following this delay, participants are asked to report which one of two objects was presented (object identity) and move the selected object to its correct location (object location) on a touch screen. Using a continuous scale, binding of the object's identity to its location is measured (Liang et al., 2016; Pertzov et al., 2012). 
Brain regions	Is supported by a network involving the entorhinal and perirhinal cortex as well as occipital-parietal regions (Parra et al., 2013; Shafritz et al., 2002; Xu & Chun, 2006).#	Engages a network where the hippocampus plays an essential role (Mayes et al., 2007; Parra et al., 2015a); it also relies on regions of the default network (posterior cingulate cortex/precuneus, lateral parietal and medial prefrontal cortex) (Bastin et al., 2014; Mayes et al., 2007).#
Hippocampus role	Successful performance does not depend on the integrity of the hippocampus (Baddeley, 2010; Mayes et al., 2007; Parra et al., 2015a).	Successful performance is dependent the hippocampus (e.g. FAD study: (Liang et al., 2016) and patients with focal MTL damage (Pertzov et al., 2013)).
Effect on ageing	Appears preserved across lifespan in healthy ageing (Parra et al., 2009; Parra et al., 2019; Rhodes et al., 2016).	Seems to decline as the hippocampus degenerates with age regardless of risk for AD (Fan et al., 2017; O'Shea et al., 2016).

Conjunctive binding: figure from (Parra et al., 2010a) with permissions from. Oxford University Press under the terms and conditions provided by Oxford University Press and Copyright Clearance Center; Relational binding: figure from (Liang et al., 2016) under the terms of the Creative Commons Attribution License (CC BY).

#VSTM binding tasks have also demonstrated sensitivity for symptomatic stages of AD and proven useful for the differential diagnosis of sporadic AD (e.g. (Della Sala et al., 2012; Parra et al., 2009; Parra et al.,2010b; Zokaei & Husain, 2019)). MTL=medial temporal lobe; AD=Alzheimer's disease; FAD=familial Alzheimer's disease.

Whether one type of binding (relational or conjunctive) is best suited for investigations of AD populations is subject to debate with some suggesting that the insensitivity of conjunctive binding to ageing favours it over relational binding (Didic et al., 2011; Parra, 2017). Yet, others argue that sensitivity to ageing does not disqualify a task from being appropriate to investigating AD given that a test should have the highest predictive power when comparing patients' performance to that of *age-matched* controls (Liang et al., 2017).

Regardless of the type of binding, two broad types of VSTM spatial processing are recognized: allocentric and egocentric processing. Allocentric (object-to-object) processing, encodes information about the location of one object or its parts with respect to other objects whereby the location of one object is defined relative to the location of other objects. This is often assessed by altering the viewpoint, colours and textures between the initially presented and target images. Egocentric (self-to-object) processing on the other hand, represents the location of objects in space relative to the axes of the self (left-right, front-back, up-down) (Coughlan et al., 2018) (**Figure 2.3**). Extensive evidence suggests that the hippocampus has a key involvement in spatial memory (Chan et al., 2016), a notion that dates back to the discovery of "place cells" (O'Keefe & Dostrovsky, 1971). Following the identification of "grid cells" by May-Britt and Edvard Moser in 2005, it was proposed that the hippocampal formation also contained information regarding direction and distance allowing construction of a cognitive map of the environment (i.e. the belief that "hippocampus is the locus of the brain's internal map of the spatial environment") (Moser et al., 2015; O'Keefe & Nadel, 1978).

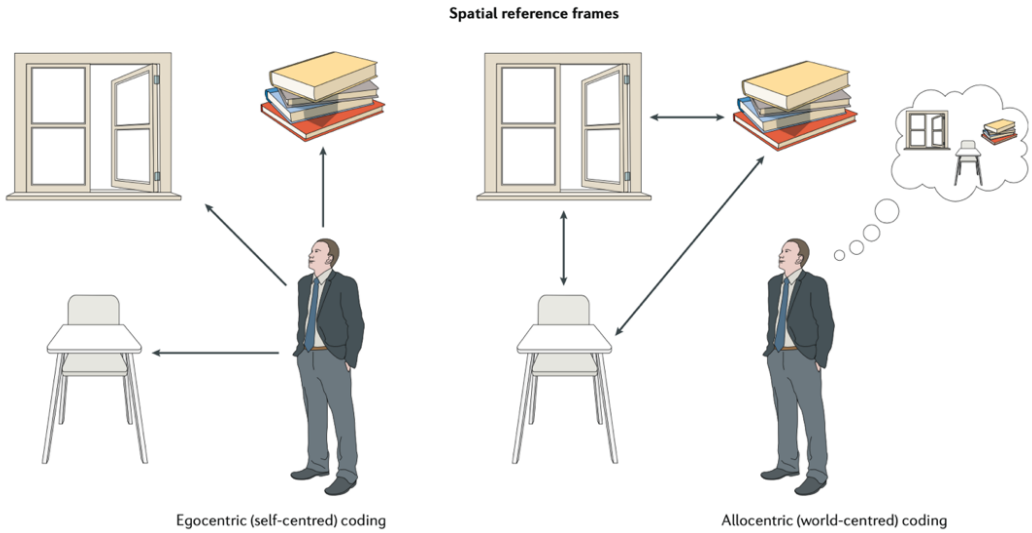


Figure 2.3 Egocentric and allocentric spatial coding.

Left: egocentric self-centred navigation from the view point of then navigator and right: allocentric strategies are based on the navigator's perception of landmark positions relative to other landmarks. From (Coughlan et al., 2018) with permissions from Springer Nature (Copyright © 2018, Springer Nature).

Contrary to verbal tasks such as recognition memory tests, which are also sensitive to the hippocampus and other MTL structures affected in AD (Delacourte et al., 1999), VSTM binding tasks appear to be less susceptible to semantic interference (i.e. the notion that recall of an object is slower in the context of a semantic category coordinate distractor word compared to an unrelated distractor word (Lupker, 1979; Rosinski, 1977)) and cognitive reserve (CR such as education and cultural background (Parra et al., 2011), whereby the onset of dementia and decline in cognitive testing, among individuals who had high occupational or educational attainment, is delayed (Rentz et al., 2013; Stern, 2012)). VSTM tasks also seem less affected by practice or learning effects as the repeated presentation of non-verbal/abstract stimuli is quickly overwritten (Colzato et al., 2006; Logie et al., 2009). Moreover, changes in VSTM and relational binding specifically, are thought to be specific to early cognitive changes in AD as relational binding processes require the linking of object features (e.g. the 'what' and the 'where') from independent memory stores – a process shown to be particularly sensitive to presymptomatic AD even when other 'context-free' more traditional tasks were not (Liang et al., 2017; Parra et al., 2010).

Various experimental designs assessing STM and binding exist, each with different characteristics. For instance, free recall paradigms (where the free-recall of two or three common objects and two or three primary colours presented as individual features - unbound or integrated into unified objects-bound is tested) are more dependent on self-initiated processing and hence tend to be more sensitive to ageing (Danckert & Craik, 2013) than change-detection paradigms. Change-detection paradigms (see

for a description of the task) and free-recall tasks are both considered less sensitive to ageing, education and cultural background (Parra et al., 2011) in comparison to relational binding for instance.

Finally, to conclude this section I briefly consider the relationship between attention and memory as one example of the complex interactions between memory and other cognitive processes. Memory retrieval is usually associated with activation of the parietal cortex, which is also implicated in the attentional system (Pereira et al., 2014). Some findings suggest MTL structures may play a perceptive role on the perirhinal cortex when discriminating between different conditions of feature ambiguity (Bussey et al., 2002). Overt shifts of attention are also thought to be associated with higher accuracy of performance in relational visuo-spatial memory tasks whereby attention facilitates memory for the relationship between objects (Olsen et al., 2014). Activation of parietal lobe regions – known to be involved in the visuo-spatial attention and in oculomotor planning – may reinforce spatial representations and

consequently produce accurate memory recognition of previously studied items (Pereira et al., 2014). Various studies report that attention and memory (visuo-spatial memory mainly) together, may influence the formation of visual representations, affecting encoding and maintenance (Hitch et al., 2020). Importantly, attentional processes play other roles besides facilitating retrieval and their interaction with memory is rather complex.

According to the feature integration theory (Treisman & Gelade, 1980) when attention is focused on an object, all its attributes (e.g. shape, colour, motion, and texture) are rapidly bound into a unified representation that is then used by higher level cognitive processes. The first stage of perception consists of *pre-attentive processing* which generates a set of feature maps of their spatial distributions. The second stage consists of *focused attention*, which binds together information from a particular location in the various feature maps and leads to the perception of a multifeatured object at the location in question. The multifeatured object file is a temporary episodic representation which might undergo interference in store (Kahneman et al., 1992). Indeed, if multiple objects are presented in the visual field, a competition between multiple objects for selection is established as a 'race' towards VSTM (Hitch et al., 2020).

Furthermore, attention may play a more important role in maintaining feature bindings in VSTM than in maintaining individual features (Che et al., 2019). The feature integration theory also proposes that features are 'retraced' in order to check whether the binding of feature is correct. This process of retracing is referred as the "re-entrant process", depends on attention and is particularly necessary for binding but not required for features (Bouvier & Treisman, 2010). Binding representations are fragile and easily disrupted by the encoding of further feature combinations (Gao et al., 2017; Shen et al., 2015). This fragility and disruption depend both on the sequential order of encoding and the similarity of features (i.e. colour) which is also dependent on attentional requirements (Allen et al., 2006). It is often quite difficult to tease apart the effects of attention and memory and further research into this relationship in AD pathology is needed.

2.4.4. Implications for my research

The research studies mentioned above confirm VSTM binding deficits are detectable at preclinical stages of AD. Nonetheless, longitudinal studies which evaluate preclinical VSTM impairments in relation to disease progression and EYO, are lacking. My second data chapter, Chapter 5, explores this in more detail.

In the next section I will outline the relevant literature for the third data chapter: Chapter 6.

2.5. Can the study of eye movements be a complementary and promising novel approach for investigations of preclinical?

2.5.1. Some approaches for the detection of preclinical AD

Cognitive behavioural assessments have long been considered the gold standard for the diagnosis and prediction of AD progression (Rentz et al., 2013). However, a number of studies have failed to find a relationship between cognitive performance and some biomarker evidence of AD in clinically asymptomatic at-risk individuals (Aizenstein et al., 2008; Jack et al., 2008; Mormino et al., 2009). Novel measures should ideally be simple, cost-effective, and capable of capturing subtle cognitive changes occurring at preclinical stages of AD, that can differentiate these individuals from healthy ageing – and function as predictive markers. Findings from Hedden and colleagues' meta-analysis of 7,140 subjects (64 studies) (Hedden et al., 2013) found that amyloid pathology (plasma assays of amyloid- $A\beta_{40}$ and $A\beta_{42}$ monomers, and PET imaging using PiB, florbetapir, and florbetaben) appeared to have a greater influence on memory-related systems in clinically normal older adults than other cognitive domains. While tests of episodic memory have a stronger association with biomarker evidence of preclinical AD, some retrospective studies indicate executive function tasks (e.g. Dual tasking (MacPherson et al., 2012)), may also be indicative of preclinical decline given associations with preclinical markers (e.g. Aβ deposition) (Grober et al., 2008; Rentz et al., 2013). See **Table 2.3** for an overview of tests showing sensitivity to preclinical AD.

Table 2.3 A selection of tests showing sensitivity to subtle cognitive changes associated with biomarker evidence of preclinical AD (either due to preclinical biomarker evidence or mutation status in presymptomatic FAD).

Test*	Cognitive function	Validation
Memory capacity/binding test (Buschke, 2013); (Rentz et al., 2010)[1]	Verbal associative binding	**1)** 34 HC, impairments in second-list learning associated with amyloid burden.
The 4 Mountains Test (Bird et al., 2010); (D. Chan et al., 2016); (R. A. Wood et al., 2016); (Ritchie et al., 2018)[2]	Allocentric spatial memory	**2)** 188 HC (aged 40-59), of whom 94 individuals had a parent with dementia. The 4MT was found to be a better predictor of risk than tests of episodic memory, verbal fluency, or executive functioning suggesting allocentric rather than egocentric processing may be a potential indicator of risk for LOAD.
Face Name Associative Memory (FNAME) (Amariglio et al., 2012)[3]; (Rentz et al., 2011)	Cross-modal associative binding	**3)** 210 HC, good test–retest and discriminate validity for name, occupation and summary scores, useful across all educational strata. **4)** 45 HC, decrease in face name *vs* face occupation associated with amyloid burden.
STM binding test- (conjunctive binding: (Parra, et al., 2010a)[5]; relational binding: (Liang et al., 2016)[6]).	Visual recognition, feature & item binding; change detection & delayed-reproduction tasks	**5)** Conjunctive binding: 30 PMCs with p.Glu280Ala *PSEN1* mutation showed impairment in VSTM binding, suggesting STM binding may be a preclinical marker for FAD. **6)** Relational binding: 12 PMCs with *PSEN1* and *APP* mutations showed greater misbinding of object identity and location than 50 HC. Hippocampal volume loss across FAD patients (asymptomatic and 8 symptomatic) was associated with object-location binding.
Behavioural pattern separation object test (Stark et al., 2013)[7]	Visual recognition, pattern separation	**7)** 98 HC aged 20-89 years. The age-impaired (based on their delayed word recall performance in the RAVLT) showed impairments in pattern separation but not in recognition performance whereas 11 MCI were impaired in both.
Spatial pattern separation (Kluger et al., 1999)[8]; (Lau et al., 2012)	Visual recognition, pattern separation, spatial discrimination	**8)** 37 HC, spatial pattern separation performance was associated with reduced bilateral hippocampal volume and with the CSF Aβ42/pTau181 ratio. In contrast, a paragraph recall test that is sensitive to the MCI stage of AD, was not sensitive to these biomarker correlates of preclinical AD.
Discrimination and transfer task (Myers et al., 2002, 2008)[9]	Spatial discrimination	**9)** 37 HC, reduced transfer performance was associated with mild-to-moderate hippocampal atrophy in HC and associated with clinical impairment 2 years later. Performance also correlated with CSF Aβ42 and the Aβ42/pTau181 ratio.
Accelerated long-term forgetting (Weston et al., 2018)[10]	Accelerated forgetting in LTM	**10)** Compared to 14 HC, 21 PMCs on average 7.2 (4.5) years to expected onset, did not show any difference for the initial learning or 30-min recall of three tasks (list, story, and figure recall). However, the proportion of material recalled at 7 days was lower in PMCs than non-carriers for list (p=0.0002), story (p=0.0048), and figure (p=0.012) recall.
Dual tasking task (MacPherson et al., 2012)[11]	Dual-task impairments	**11)** 39 PMCs on average 12.4 years to expected onset, showed dual tasking impairments despite normal performances on other standard neuropsychological tests of cognition and memory – compared to 29 HC.

Generalization tasks (Myers et al., 2003); (Petok et al., 2018)[12]	Generalization (the ability to transfer previous learning to novel but familiar recombinations)	**12)** Impairment in 32 PMCs on average 15.2 (SD 8.5) years to expected onset, and even worse impairment among those with smaller left hippocampal volume – compared to 11 HC.
Circle-tracing task (Macpherson et al., 2017)[13]	Visuomotor integration	**13)** Six 'direct' trials where participants could see their arm and their tracing path on the tablet and 6 'indirect' trials where the participant's arm was covered by a box, were compared. Accuracy and speed to trace a circle with serial-subtraction tested. Across both conditions, 19 PMCs on average 7.0 years to expected onset, made more errors than 12 HC (difference=0.297 [95% CI 0.062–0.532], p=0.013) but there was no significant difference in tracing speed.
Composite scores (Ringman et al., 2005)[14]	1. Language 2. Visuo-spatial 3. Executive function/working memory 4. Verbal memory or language composite scores	**14)** In the four composite scores in 97 PMCs (median 14.9 years to expected onset, range: 1-25) performed worse on the executive function/working memory tests (p<0.001) and the visuo-spatial tests but not on verbal memory tests (p=0.059) or language tests (p=0.930) compared to 106 HC.
DIAN battery (Storandt et al., 2014)[15]: 1. Logical memory (Story, immediate and delayed recall from the WMS-R (Wechsler, 1987)). 2. Semantic categorization accuracy performance (E. E. Smith et al., 1974) 3. Digit symbol task (from the WAIS-R; (Wechsler & De Lemos, 1981) 4. Simon task (Simon, 1969)	1. Verbal episodic memory 2. Retrieval from semantic memory under high attentional demands. 3. Processing speed and attention 4. Attention switching	**15)** Compared to HC, 89 PMCs on average 12.7 (8.1) years to expected onset, performed significantly worse at baseline for 1 & 2. In addition, presymptomatic deficits were also observed in relation to EYO for 1, 3 and 4.
Performance and Verbal IQ (Fox et al., 1998)[16]	Fluid intelligence/verbal reasoning – Verbal IQ Fluid intelligence/non-verbal reasoning – Performance IQ	**16)** During a 6-year follow up period, in a group of 53 asymptomatic at-risk FAD within 5 years of expected onset, individuals who became clinically affected-on average 2.6 (SD 1.4) years later – had significantly lower verbal memory (p=0.003) and performance (p=0.030) scores at their first assessment.

A selective review adapted from (Rentz et al., 2013). HC=healthy controls; Aβ=amyloid beta; AD=Alzheimer's disease; LOAD=late onset AD; FAD=familial Alzheimer's disease; PMCs: presymptomatic mutation carriers; aMCI=amnestic mild cognitive impairment; *APOE* ε4=apolipoprotein gene ε4; CSF=cerebrospinal fluid; RAVLT=Rey auditory verbal learning test. PMCs=presymptomatic carriers; WMS-R: Wechsler Memory Scale-Revised; WAIS-R: Wechsler Adult Intelligence Scale-Revised; EYO=proximity to expected age at symptom onset. LTM=long-term memory. The underline and number in the 'Test' column, indicates the study for which the data in the 'Validation' column was drawn from.

Cognitive composites (a single score formed by combining scores from multiple cognitive tests), have also attracted much attention over the past years, hence their inclusion in **Table 2.3**). The US FDA has recently declared openness to cognitive composite end-points (Kozauer & Katz, 2013) with the rationale that a combination of measures covering different cognitive domains may be sensitive to cognitive decline when effects are too small to be detectable on individual tests. An advantage of composite scores are their correlation with neuroimaging and CSF biomarkers and hence sensitivity to preclinical AD (e.g. (Ayutyanont et al., 2014; Mormino et al., 2017)) which is why they are often used as outcome measures in clinical trials (e.g. (Weintraub et al., 2018)). Furthermore, composite scores also decrease the probability of Type I errors (false positives) due to the reduction in the number of outcomes measures in comparison to individual tests. However, there are also disadvantages to the use of composite scores such as the difficulties in determining with certainty whether an overall increase in score reflects improvements in all domains or improvements in some and impairments in others. This is because sometimes a score may tap onto multiple cognitive domains which are not necessarily that closely related to each other. Composite scores may therefore mask important differences apparent in individual component scores and, in doing so, increase the chances of Type II errors (false negatives) (Riordan, 2017).

In this thesis, the focus of preclinical cognitive assessments will mainly lie in a specific cognitive function: relational binding – measured using the "What was where?" task. Where other traditional neuropsychology tasks are discussed they are presented for the purpose of comparison. This is because I am primarily interested in understanding whether this specific cognitive function is related to disease progression and whether it is sensitive to tracking preclinical decline. In doing so, I am conscious of the fact that Type I errors may arise, but I have made an 'a prior' consideration that, for the purpose of my investigations, the consequence of Type II errors would be worse than Type I errors. This is because while finding a signal would represent the first indication that relational binding might be sensitive to tracking presymptomatic changes in FAD, the aim of my work at this stage is restricted to a proof of principle (and would not cause harm to an individual as the effect of a false positive in a clinical trial might).

FAD is a rare condition and this limits the sample size available for testing a specific hypothesis. If additional restrictions are put in place, the probability of finding an effect reduces even further and this may not necessarily reflect a lack of signal but rather that more statistical

power is needed. For this reason, results are interpreted with caution (see section 3.7.3.3 for reflections on multiple corrections and statistical power).

Notably, the one chapter which includes a composite score is Chapter 7 (the PACC: Preclinical Alzheimer's Cognitive Composite score). This is because the focus there is to investigate SCD as another preclinical marker of AD and not to evaluate the sensitivity of objective cognitive assessments to preclinical AD. Therefore, the purpose of PACC in that scenario exclusively lies in adjusting the model for objective cognitive performance and in doing so, a stringent approach is preferred to avoid confounding effects on the variable of interest (SCD).

Taken together, a number of neuropsychology tests and techniques to study cognition have evolved over the years and a technique which has relatively recently emerged is eye-tracking. This is discussed in the next sub-section.

2.5.2. Rationale of using eye-tracking to study memory

While VSTM binding has become a popular cognitive function for the study of preclinical AD over the past decade, its function is not well understood. VSTM research has typically investigated the capacity (Alvarez & Cavanagh, 2004; Luck & Vogel, 1997) and representation formats (Gopher et al., 1996; Hollingworth et al., 2005; Jiang et al., 2000; Luck & Vogel, 1997; Phillips, 1974), with the question of VSTM function relatively neglected. A proposed role for VSTM is that it establishes correspondence whilst viewing (Hollingworth et al., 2009), specifically between objects visible on separate fixations. Viewing constitutes fixations and saccades that break up visual information. Visual information is thoroughly processed during fixations (still periods of time between eye movements which normally last between 150ms and 300ms (Rayner, 1998)), driven by rapid eye movements, saccades, which direct the fovea towards a particular element of interest (Martinez-Conde et al., 2004). The input for vision is therefore divided into a series of discrete episodes. To span the perceptual gap between fixations, a *transsaccadic memory* for the virtual properties of the scene must be maintained across each eye movement. Evidence indicates that visual memory across saccades depends on the VSTM system originally identified by Phillips (Phillips, 1974). Transsaccadic memory exhibits properties similar to those found in VSTM for example it has capacity of 3-4 objects (Irwin, 1992; Luck & Vogel, 1997; Pashler, 1988); has lower spatial precision than sensory memory (Irwin, 1991; Phillips, 1974) and maintains object-based representations with its capacity dependent on the number of objects (Gopher et al., 1996; Luck & Vogel, 1997).

“I *see* your point”, “*show* me what you mean”, “it opened my *eyes*” are all expressions used in the English language as synonyms for “understand”. Buffalo & Meister (Meister & Buffalo, 2016) suggest this is because vision is a primate’s primary sensory modality used to extract information from the surrounding world. Tracking eye movements when an individual is looking at a scene might therefore provide insight into the way information is processed. Behind eye movement patterns, are complex cognitive functions like attention, executive control and WM (Fernández et al., 2015; Grady et al., 2001; Hayhoe & Ballard, 2005; Hoffman & Subramaniam, 1995; Itoh & Fukuda, 2002; Milea et al., 2005). Indeed, recent investigations on eye movements during the performance of a cognitive task, suggest that pupil dilation – defined by “a stimulus-induced increase in pupil diameter, relative to a pre-stimulus baseline period” (Goldinger & Papesh, 2012) – increases with increasing task demands (Porter et al., 2010). Two interpretations of these findings have been proposed; the first being that pupil dilation reflects the demands or load of the task and the second that pupil dilation actually reflects the effort created in response to the task demand, in other words – the cognitive effort. The suggestion that pupil reflexes signal brain activity during cognitive events, was proposed a while back (Kahneman & Beatty, 1966). The evidence comes from the observation that in dark-adapted conditions, which inhibit the parasympathetic nervous system, pupils still dilate in response to cognitive demand (Steinhauer & Hakerem, 1992). The authors suggest this is because the pupil activity corresponds to activity in the locus coeruleus (which indicates high levels of attention (Aston-Jones & Cohen, 2005)) (Goldinger & Papesh, 2012). In addition to pupil dilation, some authors (Meister & Buffalo, 2016) have taken the hypothesis that eye movements can measure cognition further, suggesting that fixation is arguably a “currency of memory,” as the strength of recognition depends on the number and duration of fixations made during encoding (Kafkas & Montaldi, 2011; Molitor et al., 2014).

Two broad positions related to the role of eye movements exist. One suggests that memory for different aspects (e.g. items, spatial, non-spatial relationships and temporal order) guide eye movement behaviour. For instance in one study, participants viewed three objects one at a time and despite consecutive simultaneous representation, they tended to inspect the objects in the order matching the originally experienced temporal sequence (Hannula et al., 2010; Ryan & Villate, 2009). The second view is that eye movements precede and contribute to a conscious recollection of previously learned associations (Hannula & Ranganath, 2009; Moscovitch, 2008). Some argue that the rapid disproportionate viewing effects (fixation time, duration and number) developing far in advance of behavioural responses, suggests an obligatory nature of memory on eye movements and not the other way round (Parker, 1978).

Taken together, the evidence suggests that abnormal patterns of eye movements may therefore serve as an indirect surrogate to investigate cognitive functions (Fernández et al., 2018). Eye movement investigations in AD specifically, are discussed next.

2.5.3. Eye-tracking as a tool to study symptomatic and preclinical AD

Over the years, eye movement have also been used as means to further our understanding of the pathophysiology of common disorders like AD (Fernández et al., 2018). For example, in an important study, Porter and colleagues showed that pupil dimension was maximal in AD patients when processing at resource limits, but then fell once processing demands had exceeded these limits (Porter et al., 2010).

Researchers have also tried to identify oculomotor mechanisms that can highlight cognitive deficits present in the early course of AD. One of the tasks known for its high sensitivity to early memory impairments (and damage to the MTL), is the 'visual-paired comparison' task, assessing memory recognition by focusing on the tendency that subjects will explore novel items in more detail. Interestingly, a similar pattern of viewing distribution between both novel and previous seen images in observed in MCI subjects (Crutcher et al., 2009). Eye-tracking characteristics from AD and MCI studies are summarized in **Table 2.4**.

Table 2.4 Oculomotor characteristics in AD and MCI.

Population	Finding
AD	Difficulties processing colour information, contrast sensitivity, object and face recognition (Cronin-Golomb et al., 1993; Alice Cronin-Golomb et al., 2007; Gilmore, Cronin-Golomb, et al., 2005; Gilmore, Groth, et al., 2005; Kurylo et al., 1994; Pache et al., 2003; Rizzo et al., 2000).
	Less focused exploration movements, with fewer fixations inside the area of interest. Longer fixations and smaller saccade amplitudes (Mosimann et al., 2004).
	Impairments in high-order visual perceptive function like divided attention, selective attention, visual memory (Rizzo et al., 2000) and semantic interference (Loewenstein et al., 2004).
MCI	Fixation duration, saccade orientation, and pupil diameter – have been suggested to improve the classification accuracy of MCI patients (Lagun et al., 2011)).
	Altered visual search strategies and eye movement behaviours, with deficits in smooth pursuit eye movements, an increased number of saccades, as well as increased attentional deficits and eye blinks (Müller et al., 1991).
	Altered saccadic inhibition: a fMRI study showed decreased activation in the frontal eye fields when compared to healthy controls during anti-saccade performance (Alichniewicz et al., 2013).
	Impairments in an anti-saccade task distinguish between patients with the amnesic MCI (aMCI) and the non-amnesic variants of MCI (naMIC) whereby aMCI make greater errors compared to naMCI (Wilcockson et al., 2019).

AD=Alzheimer's disease; MCI=mild cognitive impairment; fMRI=functional magnetic resonance imaging.

Some eye movement investigations have been carried out in VSTM binding experiments and in fact, a version of the relational binding task (with real life objects) described in section 2.4.3 was first administered in healthy controls using eye-tracking (Pertzov et al., 2012). In these original investigations, Pertzov and colleagues found evidence that fixation order had a significant effect on task performance; specifically, performance decreased with more fixations to other objects following the last fixation on the target (post-target fixations) whereas performance enhanced with increasing the number of fixations on the target object (Pertzov et al., 2012). In this respect, other research has suggested that stronger memories were associated with image regions attracting more and longer fixations during encoding (Hannula et al., 2010; Pertzov et al., 2009). Furthermore, a study by Fernández and colleagues (Fernández et al., 2018) examined eye movements during a VSTM conjunctive binding and found that binding impairments in patients with mild AD were accompanied with a reduction in mean fixation duration during encoding. Yet, eye-tracking studies in preclinical AD are lacking and there may be useful links between eye movements and memory – like the evidence linking eye movements with cognitive effort during encoding described in symptomatic AD – which could broaden our understanding of preclinical AD as a whole and even increase the sensitivity to detecting preclinical AD.

2.5.4. Implications for my research

Having described the evidence linking eye movements and cognition, my work investigates the association between VSTM relational binding and eye movements in a preclinical AD cohort like FAD. As my hypotheses for this chapter suggest, I will evaluate whether eye movements during stimuli presentation, reveal deficits in encoding. A greater understanding of VSTM impairments in preclinical AD stages has important implications especially if the added value of eye-tracking reveals more sensitive to detecting preclinical AD than previous behavioural outcomes on their own. Although the literature around pupillometry and fixation order is interesting, my focus on visual search strategies is guided by the assumption that the different strategies participants use to perform the task (e.g. focusing on one or two fractals *vs* spending a similar amount of time fixating all three fractals) is also informative of the underlying memory process. Furthermore, this approach is consistent with the higher-order visual impairments (e.g. visuo-spatial strategies and attention difficulties) described for symptomatic sporadic AD in some reports (e.g. (Pereira et al., 2014; Tales et al., 2002)) and with the higher mean diffusivity observed in presymptomatic FAD carriers compared to

controls in areas of the brain associated with the visuo-spatial imagery like the precuneus (Weston et al., 2020). This will be further explored in Chapter 6.

Now that research questions and relevant background has been described, the final section of this chapter will define the specific hypothesis with clear links to the research questions out forward for each data chapter.

2.6. Hypothesis

The central hypothesis linking all the work presented in the thesis, is the assumption that genetic (i.e. carrying a mutation for FAD) or pathological (i.e. being amyloid-positive in Insight 46) components, provide important basis for the cognitive and clinical profiles in AD. Indeed, I hypothesise that the cognitive and clinical profile described in the literature in cross-sectional studies, will also hold true and even become more pronounced as the disease progresses.

The specific hypotheses for each chapter with direct links to the research questions are described next.

i) Survival in FAD: Chapter 4

1. No hypothesis is made with regards to survival estimates between *APP* and *PSEN1* mutation carriers given the inconsistencies in literature.

2. Comparable to AAO (Ryan et al., 2016; Tang et al., 2016), a moderate percentage of variance in survival time (above 50%), will be explained by genetic factors such as: genes, mutation and family.

3. Individuals with an atypical presentation will have a longer survival compared to those exhibiting an amnestic presentation.

4. No hypothesis is made for *APOE* ε4 carriership in relation to survival given the inconsistencies in literature.

5. As the risk of FAD is dependent on Mendelian inheritance, there will be no difference in survival time between sexes.

6. Due to improvements in quality of life and health care, individuals born in more recent generations will have longer survival compared to those born in older generations.

7. AAO and survival time will not show a linear relationship.

8. Individuals carrying a *PSEN1* mutation located in the pre-codon 200 region, will have a longer survival in comparison to those carrying a *PSEN1* mutation in the post-codon 200 region.

ii) VSTM function over time: Chapter 5

1. Over time, a faster rate of decline in VSTM relational binding function will be observed in FAD mutation carriers (symptomatic and asymptomatic) compared to controls.

2. VSTM impairments in presymptomatic individuals will become more pronounced with EYO, yet deficits in symptomatic individuals will plateau after a certain stage as performance remains poor at every visit.

3. Longitudinal decline will also be observed in recognition memory tests, but at a later stage to VSTM relational binding deficits in both asymptomatic and symptomatic individuals.

iii) Eye movements and VSTM function: Chapter 6

1. FAD mutation carriers will have similar low-level oculomotor characteristics in comparison to controls.

2. The distribution of fixation time among the stimuli will be less balanced for presymptomatic and symptomatic carriers compared to controls.

3. Longer fixation durations on the stimuli will be associated with better VSTM performance.

iv) SCD and preclinical AD: Chapter 7

1. Amyloid-positivity and FAD mutation status will respectively be associated with symptoms of SCD, above and beyond effects of anxiety and depression on SCD.

2. Having a family history of AD (over not having one – in the Insight 46 cohort) and objective cognitive deficits (measured by the PACC score), will independently be associated with higher a SCD score. No hypothesis of the effect of life-course variables on SCD is made given the inconsistencies in the literature.

3. GENERAL METHODOLOGY

The majority of the work in this thesis is drawn from a cohort of individuals with families affected by FAD (Chapters 4, 5 and 6) and one data chapter includes findings from the Insight 46 study (Chapter 7). In this chapter a general overview of FAD and Insight 46 methodologies are described following a brief description of the studies included in this thesis.

3.1. Studies included in this thesis

All FAD studies (VSTM longitudinal study, VSTM eye-tracking and survival in FAD study) were part of the ongoing FAD local study. This includes families known to be affected by FAD, with genetic mutations in either *PSEN1* or *APP*. Forty-eight participants were included in the VSTM longitudinal study between July 2012 and June 2018 (data collection had already began when I started by PhD in October 2017); 52 participants were considered in cross-sectional VSTM eye-tracking study between June 2018 and October 2019 and records from 256 individuals were collected from July 1987 up to September 2019 for the FAD retrospective survival study (**Figure 3.1**).

The last data chapter included in this thesis is part of the Insight 46 study, a neuroimaging project involving members of the MRC National Survey of Health and Development (NSHD, the British 1946 birth Cohort) all born during the same week in March 1946. These participants have been studied ever since, providing a rich dataset of measures (e.g. physical and mental health, cognition, lifestyle). Its purpose in this thesis is to represent another cohort at-risk of AD. Four-hundred and sixty participants were included in the cross-sectional SCD study and attended a baseline assessment between May 2015 and January 2018 (time period referred to as 'Phase 1') (**Fig 3.1**). For reference, **Figure 3.1** illustrates other Insight 46 projects I was involved in but did not lead throughout the course of my PhD. These projects are represented by white circles and not discussed hereafter.

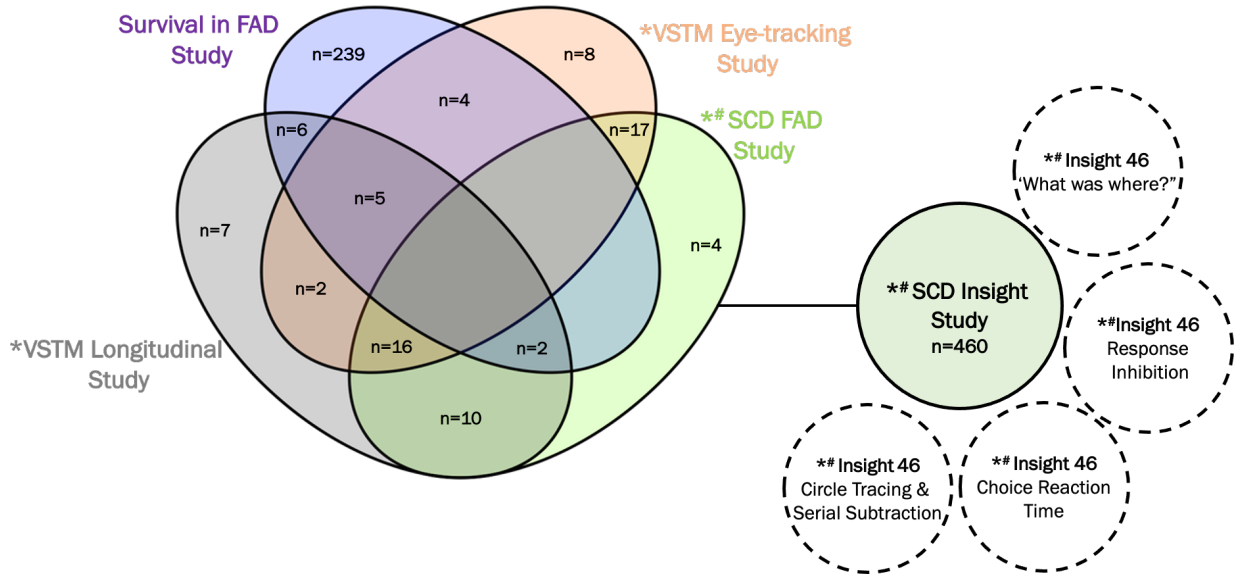


Figure 3.1 Diagram of the studies included in this thesis.

N represents the number of participants. The overlap between circles in the FAD studies indicates participants took part in more than one study. White circles represent projects from Insight 46 I contributed to but did not lead (Dr. Kirsty Lu from Insight 46 led these projects); *: study includes preclinical AD research; * #: study is exclusively based on preclinical AD research. The horizontal line represents one link between these two preclinical cohorts. AD=Alzheimer's disease; FAD: Familial Alzhiemer's disease; VSTM: visual short-term memory; SCD: subjective cognitive decline.

3.2. FAD participants

3.2.1. Participant recruitment

As mentioned above, most work in this thesis is drawn from studies of a cohort of individuals who come from families known to be affected by FAD. FAD participants came from a number of sources:

1. Families already known to the DRC through previous participation in research
2. Individuals seen in the Specialist Cognitive Disorders Clinic at the National Hospital for Neurology and Neurosurgery
3. DIAN/DIAN TU research team at the DRC
4. Individuals who contacted the DRC after hearing about research through other means (e.g. department website, media coverage or Rare Dementia support groups)

3.2.2. Inclusion criteria

Individuals were eligible if they either had i) a diagnosis of FAD; or ii) were at-risk by virtue of a parent or sibling being affected, or having been affected, by FAD. For the two VSTM studies, healthy individuals with no family history of dementia were also recruited.

In addition, each participant from the FAD study was required to meet the following criteria:

1. Able to provide informed consent (written informed if the participant had capacity and written informed consent from participant's consultees if cognitive impairment prohibited written informed consent).
2. Assessed as able to comply with the demands of the study
3. Age over 18 at time of enrolment
4. Fluent in English (to allow for reliable cognitive testing)
5. Absence of significant active central nervous system or medical disorders (to avoid confounding effects on outcome measures).

For the retrospective survival in FAD study, the dataset also included individuals who had a diagnosis of FAD but had sadly passed by the time of data analysis. This information was collected by family relatives over the years.

3.2.3. Clinical assessment and classification of groups

A semi-structured interview was conducted with each participant to ascertain information relating to both his or her general health and potential symptoms of cognitive decline. Separately, each participant nominated a close informant, who was interviewed separately in order to gain additional insight into the participant's level of functioning.

The Clinical Dementia Rating (CDR) scale was used to provide further information relating to the participant's day-to-day functioning (Morris, 1993). The CDR incorporates information obtained from both the individual being assessed (i.e. the participant) and the informant separately, and assesses a number of different areas, including i) memory, ii) orientation, iii) judgment and problem solving, iv) community affairs, v) homes and hobbies, and vi) personal care (**Appendix 1**). A global score is calculated, which relates to the participant's degree of impairment and falls into the following categories:

I. No impairment
II. Questionable impairment
III. Mild impairment
IV. Moderate impairment
V. Severe impairment

The mini-mental state examination (MMSE, (Folstein and McHugh 1975)), which gives a score between 0 and 30 (30 being the best), was also used as part of the clinical assessment to provide further information to assist in forming the clinical impression.

A detailed physical neurological examination was performed on each participant to assess for possible non-cognitive signs of FAD, or of any evidence of other central or peripheral nervous system pathology. A general examination of the cardiorespiratory and gastrointestinal systems was also performed as standard-not discussed further in this thesis.

Individuals were defined as symptomatic if consistent symptoms of cognitive decline were reported by the participant and/or their informant, and the global CDR was >0. If these criteria were not met but the participant carried a genetic mutation causing FAD, they were said to be presymptomatic.

Participants therefore broadly fell into one of the following groups:

- **Symptomatic FAD participants who were alive at their last contact with the centre (N=60):** individuals with a confirmed clinical diagnosis of FAD, and a confirmed mutation in either *PSEN1* or *APP*.
- **Symptomatic FAD participants with a known death (N=190):** individuals with a confirmed clinical diagnosis of FAD, and a confirmed mutation in either *PSEN1* or *APP* who had sadly passed by September 2019.
- **At-risk participants (N=56):** asymptomatic individuals who, by virtue of having an affected parent (with a confirmed genetic diagnosis), were at 50% risk of having inherited a mutation, and thereby at-risk of developing symptomatic FAD in the future. This group included asymptomatic carriers and asymptomatic non-carriers who were then assigned to the corresponding group (i.e. non-carriers were added to the healthy control group and a presymptomatic mutation carrier (PMC) group created*****). Six of these at-risk participants transitioned into symptomatic carriers during the course of my PhD.
- **Healthy controls (N=13):** individuals with no family history of AD.

Where possible, efforts were made to recruit controls who were age and gender matched; however in the case of at-risk participants, for whom mutation status was unknown at the point of recruitment, recruitment of matched-controls was not possible, although it was expected that approximately 50% of at-risk individuals would be non-carriers and that the ages between carriers and non-carriers should be similar.

Specific numbers for each of the studies are reference in the corresponding data chapters. *****PMCs were separated into late PMCs and early PMCs considering EYO from the data's median split. Late PMCs were those 'closest to' the expected onset and early PMCs those

'furthest away'. The median split was dependent on the data and whilst there was an overlap in the individuals included, they were tested at different time points. Hence, details on group classification are provided in the corresponding data chapter (VSTM longitudinal study: Chapter 5; VSTM eye-tracking study: Chapter 6, SCD study: Chapter 7). See section 3.2.4 next, for more details on EYO.

3.2.4. Estimating the number of years to likely onset

Expected age at symptom onset was calculated for each participant by subtracting the participant's current age from the age at which their affected parent first developed progressive symptoms of cognitive decline. The age at which their parent developed progressive symptoms was determined by detailed discussion with all available family members. This method for calculating expected onset is used in many FAD studies (though in some studies the parent's AAO is replaced by the mean AAO for the family or mutation) (Bateman et al., 2012), and has been shown to provide relatively accurate estimates of time to onset (Ryman et al., 2014). EYO allows prediction of how far from symptom onset an asymptomatic individual is at a given time point, if they are indeed a carrier of a mutation. EYO can be thought of as a proxy marker of disease stage, spanning both presymptomatic and symptomatic disease phases. Hence, it is possible to separate individuals into different disease stages sub-groups based on EYO, i.e. those who are further and closer to predicted onset at a given time point.

3.2.5. Family mutations

A total of 84 different FAD families, with 54 different mutations (46 *PSEN1*, 8 *APP)*, were involved across the three FAD studies. Family mutations form all three FAD studies are listed in **Table 3.1**

Table 3.1 Family mutations represented in the FAD cohort across all FAD studies.

Gene	Mutation	Exon	Number of families	Number of individuals
APP				
	p.Ala692Gly	17	1	4 D
	p.Val715Ala	17	1	1 D
	p.Val717Gly	17	1	11 D; 3 S
	p.Val717Ile	17	7	23 D; 5 S; 7 AR
	p.Val715Leu	17	1	2 D; 2 S; 1AR; 1 AR,S
	p.Thr719Asn	17	1	2 D
	p.Val717Phe	17	1	1 S
	p.Val717Leu	17	1	1 AR
PSEN1				
	Intron 4	4	5	22 D; 5 S; 6 AR
	p.Tyr115Cys	5	2	2 D; 1 AR
	p.Ala79Val	4	1	1 S
	p.Tyr115His	5	1	5 D; 2 S; 1 AR
	p.Thr116Asn	5	1	1 D
	p.Glu120Lys	5	2	6 D; 1 S, 1AR
	p.Ser132Ala	5	2	3 D; 2 AR
	p.Met139Val	5	3	14 D; 4 S; 2 AR
	p.Ile143Phe	5	1	1 D; 1 S
	p.Met146Ile	5	2	6 D; 4 AR; 4 AR,S
	p.Leu153Val	5	1	3 D, 1 AR
	p.Tyr154Cys	5	1	1 S
	p.Val142Ile	5	1	1 D; 1 S
	p.Leu166Arg	6	1	1 S
	p.Leu166del	6	1	1 S
	ΔE167 p.Ile168del	6	1	1 D
	p.Leu171Pro	6	2	3 D; 2 S
	p.Glu184Asp	7	3	4 D; 2 S; 2 AR, 3 AR,S
	p.Ile202Phe	7	1	2 D; 3 AR
	p.Gln222Pro	7	1	1 D
	p.Gly206Val	7	1	1 D
	p.Gly206Ala	7	2	1 S
	p.Ile229Phe	7	1	3 D
	p.Leu235Val	7	2	4 D, 1S

	p.Phe237Leu	7	1	1 S
	p.Leu250Ser	7	1	6 D; 1 S
	p.His214Tyr	7	1	3 AR
	p.Ala246Cys	7	1	3 D; 1 S; 2 AR
	p.Ala260Val	8	1	1 S
	p.Cys263Phe	8	1	1 S
	p.Pro264Leu	8	4	4 S; 2 AR, 1 AR,S
	p.Pro267Ser	8	1	2 D; 1 S
	p.Arg269His	8	4	2 D; 3 S; 2 AR
	p.Arg278Ile	8	1	7 D; 2 S; 5 AR
	p.Glu280Gly	8	3	20 D; 1 S, 4 AR; 1 AR,S
	p.Phe283Leu	8	1	9 D; 2 S
	p.Leu282Pro***	8	1	1 S
	p.Ser290Cys	9	1	4 D; 1 S
	ΔE9*	9	1	1 S
	p.Arg377Met	11	1	1 D
	p.Gly378Val	11	1	3 D; 1 S
	p.Gly394Val	11	1	1 D
	p.Pro436Ser	12	1	3 D; 3 S
	p.Thr291Ala & p.Ala434Thr**	9 &12	1	1 D
	p.Leu424Val	12	1	1 D
	p.Pro433Ser***	12	1	1 D

The number of individuals, across all three FAD studies is reported alongside the number of families with each mutation. Individuals are classified into the following categories: deceased (D), symptomatic (S), asymptomatic but at-risk (AR), asymptomatic but transitioned into S in at least **one** study (AR,S). Details relating to how many at-risk participants for each mutation were mutation carriers is not given to preserve genetic blinding and ensure it is not possible for any at-risk individual to attempt to deduce their mutation status. *The exon 9 deletion (NM_000021.3:c.869-1G>T; p.S290C;T_S319del) commonly referred to as ΔE9. **One patient had both Thr291Ala on exon 9 and Ala434Thr on exon 12 (Ryan et al. 2016). *** Novel mutations.

3.3. Insight 46 participants

3.3.1. Participant recruitment

Participants from NSHD had the following caveats: 1) only singleton babies were included (not twins or multiples); 2) the sample was stratified by social class, taking all babies whose fathers had an agricultural or non-manual occupation, and one in four babies whose fathers had a manual occupation; 3) only babies born to married mothers were included, since the

stratification by social class was based on the father's occupation (and in the 1940s it was relatively uncommon for unmarried couples to co-habit) (Wadsworth et al., 2006). A recruitment flow-chart for the Insight 46 cohort is provided in **Figure 3.2** and further details have been published here (James et al., 2018; Lane et al., 2017). Individuals were sent an invitation by post and then screened by telephone if interested. 502 participants were recruited into Phase 1.

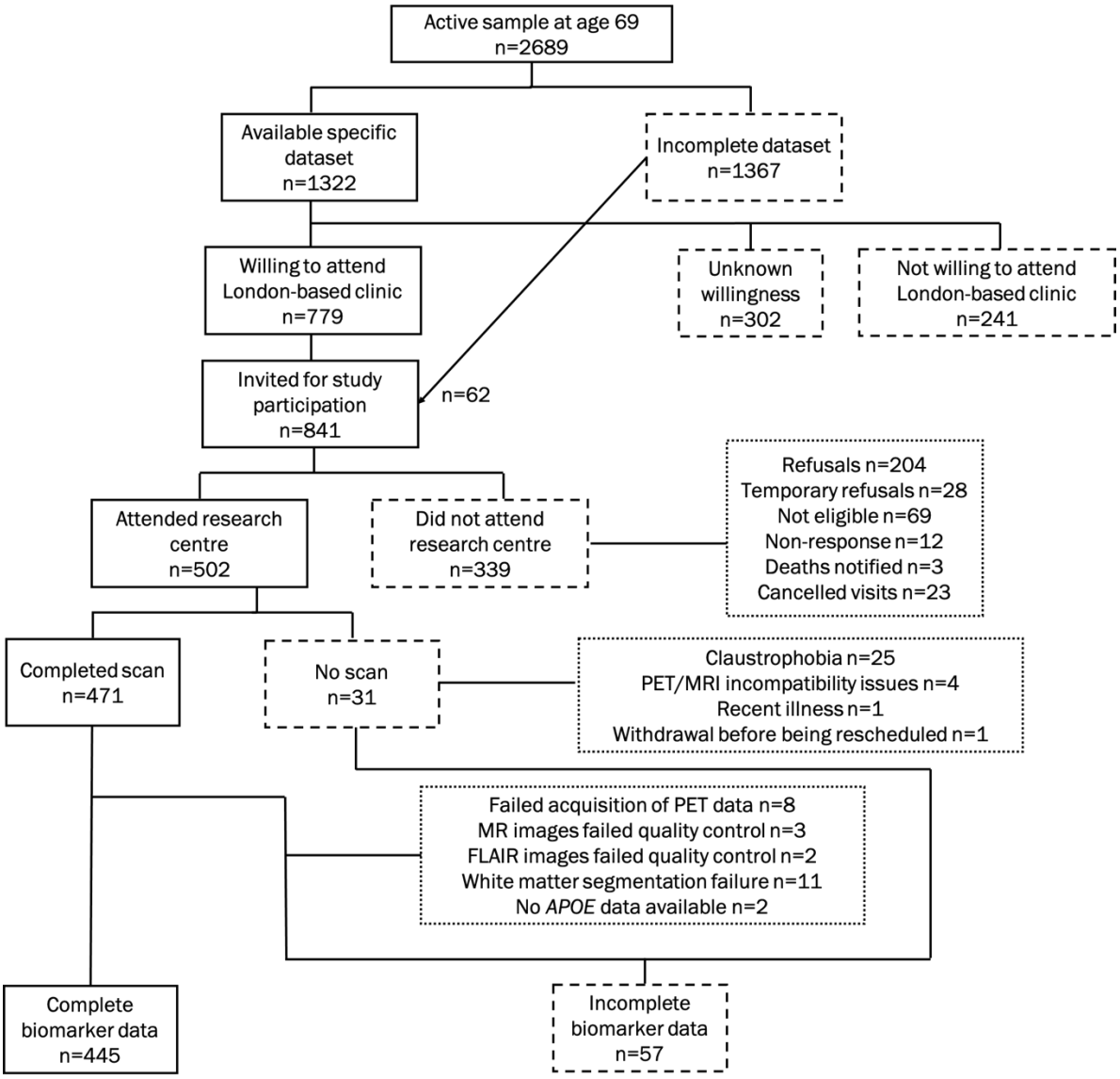


Figure 3.2 Flow chart of recruitment and data acquisition

From (Lu et al. 2019) with permissions from Wolters Kluwer Health, Inc. and Copyright Clearance Center. The specific dataset refers to a set of life-course data which formed the original criteria for Insight 46 eligibility (see section 3.3.2 for further details). To reach the larger target sample size, criteria were relaxed to remove the requirements for a previous measure of lung function, smoking or physical exercise, enabling recruitment of a further 62 individuals. FLAIR = fluid attenuated inversion recovery MRI; MR=magnetic resonance; PET= positron emission tomography.

3.3.2. Inclusion criteria

In order to avoid a priori decisions as to who might be at-risk of cognitive decline, entry criteria to Insight 46 was based only on maximising the life-course data available for analysis (Lane et al., 2017). The minimum life-course dataset included:

1. Attendance at a clinical visit age 60-64
2. Parental socio-economic position (SEP): at least one indicator of occupational social class or education.
3. Cognition: memory and processing speed from the 60-64-year collection and at least one set of measures at either age 8, 11 or 15
4. Early physical growth trajectories: birth weight and at least one measure of height and weight at ages 4-15
5. Educational attainment: highest qualification by age 26
6. Mental health: teacher rating of behaviour and temperament at ages 13 or 15, and at least one measure of affective symptoms at ages 36, 43, 53 or 60-64
7. Blood pressure, lung function, adult height and weight: at least one measure of each at ages 36, 43, 53 or 60-64
8. Health behaviour: at least one measure of smoking and physical exercise at ages 36, 43, 53 or 60-64
9. Blood: either age 53 or 60-64 samples

In addition, participants were eligible if they were able to attend a clinic-based visit at University College London (UCL) and had no contraindications to MRI or PET, such as severe claustrophobia, or metal within the body (e.g. pacemakers and intracranial clips) (Lane et al., 2017).

3.3.3. Clinical assessment and classification of groups

Full details of the clinical assessment in Insight 46 are provided in the protocol paper (Lane et al., 2017). Specifics are provided below only for those variables which I have used in subsequent analyses.

As for the FAD study, all Insight 46 participants completed the MMSE (Folstein et al., 1975), a standard personal and family history of neurological illness or cognitive impairment and a medication history.

A PET scan determined the amyloid group. β-amyloid PET and MRI data were collected simultaneously during a 60-minute scanning session on a Biograph mMR 3T PET/MRI

scanner (Siemens Healthcare, Erlangen, Germany) with IV intravenous injection of 370 MBq of the Aβ-PET ligand (Galvin et al., 2005), F^{18}-Florbetapir (Amyvid) (Lane et al., 2017). A cut-point of Aβ positivity was determined using a Gaussian mixture model and defined as 0.6104 representing the 99th percentile of the lower (Aβ negative) Gaussian and in accordance with previous studies this gave a dichotomous variable of amyloid status: Aβ+ (elevated levels of β-amyloid) or Aβ- (normal levels of β-amyloid) (Lu et al., 2019). See Chapter 7 for more details. Of the 460 participants included in this study, 40 were missing PET data. Forty-two out of the 502 met criteria for neurological or psychiatric condition and were subsequently excluded from the SCD analysis. This definition included the following:

I. Clinical evidence of dementia, Parkinson's disease and other neurodegenerative disorders (n=8)
II. Psychiatric disorder requiring anti-psychotic medication or electroconvulsive shock therapy (n=4)
III. Epilepsy requiring active treatment (n=6)
IV. Radiological evidence of traumatic brain injury or major neurosurgery (n=2)
V. Clinical diagnosis or radiological features of multiple sclerosis (n=3)
VI. Clinical diagnosis of stroke, or radiological evidence of cortical ischaemia or haemorrhage consistent with previous cortical stroke (n=18)
VII. Radiological evidence of possible brain malignancy (n=1)

This resulted in 460 participants included in the SCD study, 343 amyloid negative and 77 amyloid-positive individuals (16.7%), which is around the expected prevalence for this age (Jansen et al., 2015).

3.4. Consent and ethical considerations

Both FAD and Insight 46 studies reported in this thesis, were carried out at UCL, Queen Square Institute of Neurology in conjunction with the National Hospital for Neurology and Neurosurgery, UCL Hospitals NHS Trust. The FAD study was approved by The National Hospital for Neurology and Neurosurgery and Institute of Neurology Joint Research Ethics Committee (subsequently, National Research Ethics Service Committee, London Queen Square, REC ref 11/LO/0753). For Insight 46, ethical approval was granted by the National Research Ethics Service (NRES) Committee London (14/LO/1173). All participants gave written informed consent.

All studies were carried out in accordance with the declaration of Helsinki and followed the framework provided by the Mental Capacity Act (2005) relating to research and capacity (Bray, 2005). Capacity was assessed on an individual basis by a clinician with experience in the assessment of patients with cognitive impairment. Detailed discussions with the participant and their informant formed the foundation of this assessment. Whilst individuals lacking capacity were not recruited, a participant may lose capacity during the course of the study after previously giving informed consent. In such a case, and if the individual was still able to participate, the researcher would be responsible for identifying an appropriate consultee who knows the participant well, who would then be consulted regarding whether or not the participant should continue.

Given that most participants in the FAD studies were 'at-risk' (i.e. they had not previously undergone predictive clinical genetic testing, and so were unaware of their genetic status, and wished this to remain the case) additional ethical considerations were made. It was paramount to ensure that any genetic testing done as part of the research was done in a way that ensured the participant would never be made aware of the result. A specific standard operating procedure was put in place in relation to this point, and several steps taken to ensure this was the case, as outlined below:

- Those who performed the genetic analysis in the laboratory would only communicate the results to specific pre-determined individuals (i.e. the study statisticians), who would themselves never have direct contact with the participants.
- The clinicians, psychologists, and radiographers who carried out assessments on the participants, including the author, remained blind to the participants' genetic status, which prevented them from either accidentally indicating the genetic result to the participant, or allowing knowledge of the genetic result to bias their objective clinical assessment.
- Any publications resulting from the work would not depict results in any way that may make it be possible to attempt to deduce the genetic status of any at-risk individual.
- For studies in which there was a risk of unblinding and I performed the statistical analysis (e.g. VSTM eye-tracking study, SCD FAD study), identifiable information was removed from working spreadsheets, participant ID anonymized and genetic status information limited to the following groups: non-carriers (controls), symptomatic carriers and based on the median split of EYO in the dataset, early PMCs or late PMCs. Any original spreadsheet containing identifiable information were hereafter locked using password protection with a password unknown to me.

3.5. Materials and measures

3.5.1. Clinical and life-course data

In addition to MMSE and CDR scores, clinical data for the FAD studies included: information on mutation, family, AAO, age at death (where available) and cognitive presentation (amnestic *vs* atypical – see Chapter 4 for details on how this was defined). Most clinical data were collected during assessment with the study clinician. Information on age at symptom onset or date of passing was gathered through conversations with individuals as part of the research visit or over the phone by myself or the clinical nurse, Helen Rice (HR). For all survival analysis, the time of last contact with the centre had to be recorded for appropriate and necessary censoring of data.

Complementary to this background assessment, anxiety and depression were also evaluated since neuropsychiatric symptoms may precede cognitive decline in AD pathology (Ownby et al., 2006). For the FAD cohort this was done using the Hospital and Depression Scale (HADS) questionnaire which provides separate quantitative scores for both depressive symptoms and symptoms of generalized anxiety (Zigmond & Snaith, 1983) and for Insight 46 participants, mental health was assessed in two ways: 1) using the 28-item version of the General Health Questionnaire (GHQ-28) (Goldberg & Hillier, 1979) and 2) using the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1983).

In addition, information on life-course variables was also collected. National Reading Test scores (NART; see **Table 3.2**) instead of education levels were considered when adjusting VSTM models. This is in accordance with previous reports using the same task (e.g.(Liang et al., 2016)), and is also in line with reports suggesting NART as a better predictor of premorbid IQ in comparison to demographic variables like education due to the resistance of NART to neurological impairment and age-related cognitive decline and its high correlation with measured intelligence (Bright et al., 2002). For Insight 46 however, in line with the life-course nature of the cohort (individuals were studied throughout their lifetime), previous reports of this cohort (Lu et al., 2019) and the absence of NART scores, models were adjusted for three life-course variables instead: SEP, highest education position and childhood cognitive ability (see Chapter 7 a definition of each variable and details how they were collected).

3.5.2. *APOE* genotyping and blood sampling acquisition (where applicable)

For FAD studies, blood samples were acquired for DNA testing. Samples were collected in 10ml ethylenediaminetetraacetic acid (EDTA) coated Vacutainer™ tubes (BD, Oxford, UK).

DNA was extracted and Sanger sequencing performed to establish the presence or absence of a pathogenic FAD mutation, as has been described previously (Janssen et al., 2003). *APOE* ε4 status was determined by the Medical Research Council (MRC) Prion Unit (London, UK) using minor groove binding probe genotyping assays (TaqMan, Applied Biosystems).

For the Insight 46 study, *APOE* genotyping was conducted at LGC, Hoddesdon UK and participants classified into two categories based on the presence of the *APOE* ε4 allele: ε4-carriers and ε4 non-carriers.

3.5.3. Subjective cognitive outcomes: MyCog and AD8 questionnaires

For both FAD and Insight 46 studies, the participant's perception of cognitive decline (subjective cognitive decline) was measure using the MyCog questionnaire, a brief validated tool that is part of the Subjective Cognitive Decline-Questionnaire (SCD-Q) (Rami et al., 2014). It involves list of 24 yes/no questions assessing perceived decline over the last two years in instrumental activities of daily living that include memory, language and executive tasks. AD8 was used to capture the informant's perspective of the participant's cognition (Galvin et al., 2005). The AD8 correlates well with the CDR scale, and has high sensitivity and specificity for detecting cognitive impairment (Galvin et al., 2005, 2006). For more details on subjective cognitive outcomes see Chapter 7.

3.5.4. Objective cognitive outcomes: Neuropsychology battery

A comprehensive neuropsychology battery was administered in FAD studies by myself or another trained psychologist over the years. An overview of the cognitive tests is provided in **Table 3.2.** Some of these tasks are standardised clinical neuropsychological tests that have been widely used in studies of preclinical AD and the others are more novel. For Insight 46, the only reference to neuropsychology test in this thesis, is the Preclinical Alzheimer's Cognitive Composite (PACC) given its sensitivity to subtle cognitive decline in the preclinical phase of AD (Donohue et al., 2014). Data was collected by one of the psychologists in the team (see **STATEMENT OF ATTRIBUTIONS**).

Table 3.2 Neuropsychology measures administered as part of the studies.

Name of Test	Source	Cognitive domain	Brief description
Mini-Mental State Examination	(Folstein et al., 1975).	Global cognition	A 30-point screening tool for cognitive impairment, which covers multiple cognitive domains including orientation to time, and place, registration, recall, attention, calculation, visuo-spatial function, language, repetition, writing, reading, following a 3-stage command.
Vocabulary	Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler 1999).	Fluid intelligence/ verbal reasoning	Participants are asked to provide the definition of a series of words. It is a graded test related to education.
Block design		Fluid intelligence/non-verbal reasoning	Participants are shown a pattern on a sheet and asked to create the pattern using blocks.
Similarities		Fluid intelligence/ verbal reasoning	Participants are read two words and asked to state in which way such two words are alike.
Matrix reasoning		Fluid intelligence/non-verbal reasoning	Participants are shown a matrix of geometric shapes with a piece missing and are required to select the missing piece from five options.
Recognition memory test for words (RMT-words)	(Warrington, 1984).	Recognition memory	Individuals are presented with a series of 50 words, then presented with pairs of words (a target and a foil) and instructed to choose the word which they were previously exposed.
Recognition memory test for faces (RMT-faces)	(Warrington, 1984).	Recognition memory	Individuals are presented with a series of 50 faces, then presented with pairs of faces (a target and a foil) and instructed to choose the face which they were previously exposed.
Digit span forwards	(Wechsler, 1945).	Short-term memory	Subjects are read a sequence of numbers and asked to repeat the same sequence back to the examiner in order (forward span) or in reverse order (backward span).
Digit span backwards	(Wechsler, 1945).	Working memory	
Spatial digit span	(Wechsler, 1997)	Visuo-spatial working memory	Subjects are shown a sequence (in the form of blocks) and asked to copy the sequence both in order (forwards) or reverse order (backwards).
British Picture Vocabulary Scale (BPVS)	(Dunn & Dunn, 2009).	Vocabulary	Individuals are presented with four pictures at the same time with a word in the middle and asked to choose the picture that best described the meaning of the word.
Graded Naming Test (GNT)	(McKenna & Warrington, 1983).	Naming	Individuals are presented with one picture at a time and asked to provide the name for it.
Arithmetic	(Jackson & Warrington, 1986).	Numeracy	Individuals are asked to perform a series of calculations (additions and subtractions).
Camden Paired-Associated Learning (Camden PAL)	(Warrington, 1996).	Associative Memory	Individuals are read and shown two associated words at a time (verbal-paired associated) and then presented with one of the words and asked to recall the second associated word.
Visual Object and Space Perception Battery- Object Detection (VOSP OD)	(Warrington & James, 1991).	Visuo-spatial perception	Subjects are shown four silhouettes and asked to choose one which reassembled a real-life object (e.g. a chair).

National Reading Test (NART)	(Nelson, 1991).	Premorbid IQ	Individuals are presented with a list of 50 words in order of increasing difficulty and asked to read this out-loud. The number of errors made is recoded.
Stroop	(Stroop, 1935).	Executive function	Individuals look at colour words and are asked to name the colour of the ink the words are printed in while ignoring the actual word meaning.
Usual and Unusual views	(Warrington & Taylor, 1973).	Visuo-spatial perception	Individuals are shown 20 photographs of objects taken from conventional and 20 unusual views and asked to identify the object.
Verbal Fluency	(Newcombe, 1969)	Executive function	Individuals are asked to name as many words starting with a letter (e.g. F) as possible within 1 minute.
Category Fluency	(Benton, 1968)	Executive function	Individuals are asked to name as many words from a particular category (e.g. animals) as possible within 1 minute.
Trails Making Test A & B	(Delis et al., 2001; Reitan & Wolfson, 1995).	Executive function/ Speed and fluid cognitive abilities. Part A: motor and visual search speed/ Part B: self-shifting and inhibition	In part A, participants are asked to draw lines to connect the numbers in ascending order. In part B, the circles include numbers and letters and participants are asked alternate between number and letters (i.e. 1-A-2-B-3-C, etc.).
Digit Symbol Substitution Test (DSST)	Wechsler Adult Intelligence Scale-Revised (WASI-R) (Wechsler & De Lemos, 1981).	Processing speed and attention	Participants are given a code table of digits paired with symbols. On a worksheet with rows of digits, they are asked to fill in the corresponding symbols as quickly and accurately as possible. The score is the number of symbols completed correctly within 90 seconds.
“What was where?”	Task designed by Dr Pertzov (Pertzov et al., 2012).	Visual short-term memory	Participants are shown 1 or 3 objects on a screen and asked to remember the objects and their location. After a delay or 1 or 4 seconds, they are required to identify the learned object from a distractor and place it in its remembered location (see Chapter 5).
Preclinical Alzheimer’s Cognitive Composite (PACC) *	Original test included the Free and Cued Selective Reminding Test (FCSRT)(Donohue et al., 2014) instead of the FNAME (Lu et al., 2019).	Detect and track subtle cognitive changes in preclinical AD.	This composite score comprises the following tests: MMSE (Folstein et al., 1975), Logical Memory IIa from the Wechsler Memory Scale-Revised (Wechsler 1987), DSST (Wechsler, 1981) and the 12-item Face-Name Test (FNAME) (Papp et al., 2014).

AD=Alzheimer’s disease.

3.5.5. Eye-tracking data

All eye-tracking data was recoded for a sample of FAD participants using a desktop-mounted infrared video-based eye tracker, Eyelink 1000Plus (SR Research, Canada). The Eyelink 1000 plus is a highly flexible eye-tracker, with sampling of eye movements of up to 1000 Hz per second, and an accuracy of 0.15 degrees (deg). Participants used a chin and head rest to provide stability and maintain a constant viewing distance throughout the experiment. Fixations and saccades were defined by the Eyelink system, using standard velocity and acceleration thresholds (30 deg/s and 8000 deg/s^2). Periods during which no saccadic movement occurred were automatically identified as fixation periods. All the data were obtained from recordings with an average Cartesian prediction error of < 1 deg during the validation procedures.

3.6. Data processing

Clinical data were collected from family folders and paper records (i.e. AAO, cognitive presentation and age at death if available) and imputed in a Microsoft Excel (Microsoft Inc) sheet. Where information was missing from a FAD family, myself or my supervisor Dr. Natalie Ryan (NR) contacted either an active participant or a spouse or relative by email or phone (with their consent). In cases where establishing contact was unsuccessful, I recoded the last year of contact with the centre and used this information for censoring the data.

Traditional neuropsychology data was inputted from the central neuropsychology spreadsheet and any missing data recorded. Any output file from computer tasks (e.g. tests forming the PACC score such as the FNAME) was automatically created and the data visually inspected after collection.

All eye-tracking data was visually inspected using Data Viewer and trials and/or participants were excluded if there was the signal loss that would have interfered with the data analysis and interpretation of results (e.g. no fixations were recorded on a trial).

I visually inspected the questionnaires for any missing data for the MyCog questionnaires, mental health variables (anxiety and depression) and life-course variables (e.g. childhood cognitive ability, education, SEP).

All data were collated in Microsoft Excel (Microsoft Inc) and then imported to Stata v14 (StataCorp 2015). I wrote programs on Stata v14 to clean the data and generate the outcome variables of interest, described in the relevant Chapters (4 to 7).

3.7. Data analysis

Chapters 4 to 7 represent the data chapters of my PhD and details on analyses will be discussed there. However, my general approach is defined below in section 3.7.1 followed by an explanation of the statistical approaches.

All analyses were conducted in Stata v14 or later (StataCorp 2015, 2017). Results were considered statistically significant if the chance of false positive finding was below 0.05 (statistical significance was set at $p<0.05$) and Bonferroni corrections considered where appropriate.

3.7.1. General approach

Due to the requirements for myself and other staff seeing FAD participants for research visits, to remain blind to the genetic status of all at-risk participants, statistical analyses were done in collaboration with the department statistician, Dr Jennifer Nicholas (JN). Each analysis was prospectively planned by myself after conversations with my supervisors and outcome variables alongside corresponding covariates chosen. JN then either performed the statistical analyses herself (VSTM longitudinal project) or separated participants into mutation carriers and non-carriers blinding participant ID so that I could perform the analysis (all other FAD projects involving FAD PMC presented here).

All FAD studies reported in this thesis primarily describe comparisons between two broad groups in relation to control performance: 1) Symptomatic FAD mutation carriers and 2) presymptomatic FAD mutation carriers. Controls were a combination of any healthy controls not from FAD families and those at-risk FAD family members who test negative for a mutation (non-carriers). PMC, were further separated into early PMCs and late PMCs in an attempt to acknowledge the influence of EYO on presymptomatic change (**Figure 3.3A**).

The Insight 46 study reported in this thesis compared individuals who were amyloid-positive to those who were amyloid-negative (**Figure 3.3B**).

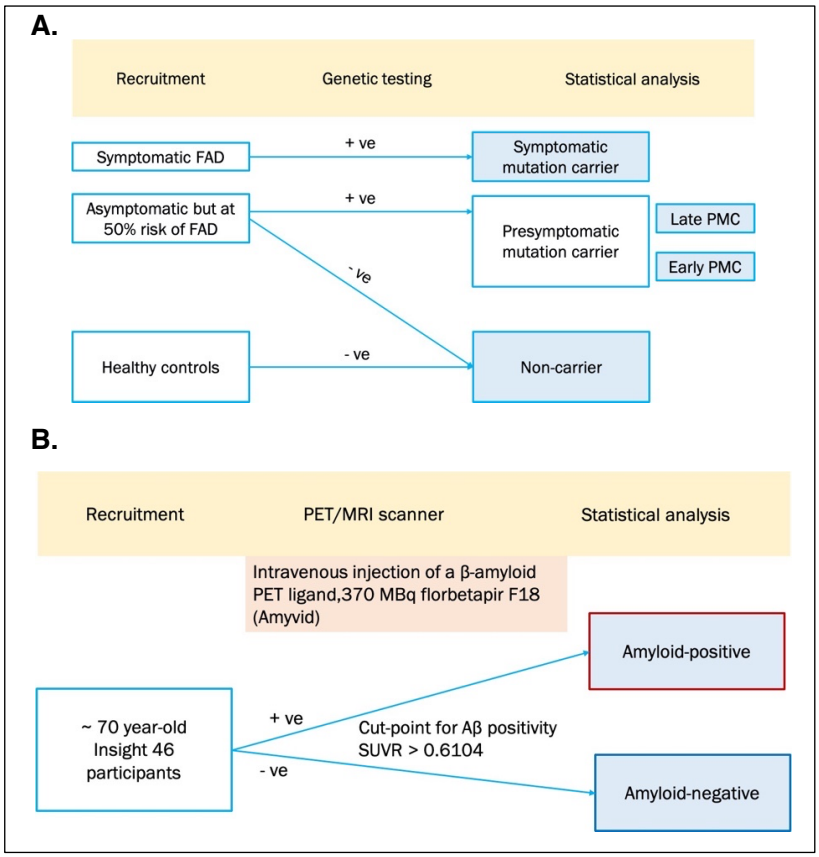


Figure 3.3 Separation of participants into the different groups for analysis.

A. FAD studies. **B.** Insight 46 study. PMC=presymptomatic mutation carrier. Aβ+: amyloid-positive; Aβ-: amyloid-negative. SUVR= Standard Uptake Volume Ratio.

The general statistical approach depended on the research questions.

For FAD studies, the comparison was based on i) mutation status and EYO: non-mutation carriers, early PMCs, late PMCs and symptomatic carriers) or ii) purely on gene membership for the symptomatic study (*PSEN1 vs APP* mutations). For the Insight 46 study, amyloid status (Aβ+ *vs* Aβ-) was the principal comparison of interest.

In the same way, variables of interest varied depending on the question to be answered and the associated outcomes (e.g. Chapter 4: survival time; Chapter 5: VSTM function such as localisation performance; Chapter 6: eye movements such as fixation duration; Chapter 7: SCD measured by the MyCog questionnaire).

Exactly how these questions were investigated varied depending on the characteristics of the measure being investigated and the number of data points available (including whether longitudinal or cross-sectional data were used).

3.7.2. Covariates

In advance of my analysis, variables that would be theoretically expected to have an effect on the values of measures of interest was identified. These were then included as covariates in order to adjust for any confounding effects. This approach was chosen because although the presence of a statistically significant difference between groups would indicate the need to adjust for this, the absence of a statistically significant difference does not rule out the possibility that the variable may still exert a confounding influence. Regression analysis allows these 'nuisance' variables to be adjusted for statistically. Age is known to be closely associated with neuronal loss, both in normal aging and neurodegenerative disease (Scahill et al., 2013). Sex differences in dementia (Podcasy & Epperson, 2016) as well as brain size (Barnes et al., 2010) and cognition (Jäncke, 2018) are still unclear but increasingly recognized as topics of interest in the literature. Hence, for all analyses age (or year of birth for survival models) and sex were considered as covariates. Depending on the specific nature of the variable being investigated, other predictors were included (see Chapters 4 to 7 for details).

3.7.3. Statistical models

I reviewed the statistical approaches of previous studies that have been used for similar papers to the data presented in this thesis. Previous papers on the VSTM task reported here, "What was where?", used repeated measures analysis of variance (ANOVA) where each participant was given a mean score for each condition and the mean scores were entered in each model (e.g.(Pertzov et al., 2012)). Yet, disadvantages of the ANOVA approach are that information is lost by the reduction of data to mean scores, particularly information about within participant variability- and it is heavily reliant on the assumption that the outcome is normally distributed. Multivariable models were therefore used for VSTM and SCD studies.

Previous studies addressing disease duration have often estimated 'disease length', including only patients who have died, by subtracting an individual's AAO from their age at death. This leads to an intrinsic bias against longer disease durations as individuals who are affected, but have not yet passed away, cannot be included (Armstrong, 2014; Kartsonaki, 2016). Survival models were therefore employed as they allow for unbiased estimations when datasets are incomplete.

3.7.3.1. Group comparisons

For Chapter 4, the primary group comparison was between *APP* and *PSEN1* mutations. Kaplan-Meier survival estimate was used for descriptive statistics and Weibull multilevel parametric survival analysis (using an accelerated failure-time model) was used to compare the survival function of different groups of patients and test the specific hypothesis.

For Chapters 5, 6 and 7 multivariable modelling was used. The exact nature of regression model depended on a number of factors, including the nature of the outcome variable (continuous or dichotomous) and whether the residuals were normally distributed.

For continuous outcomes (other than survival time), appropriate transformations were applied so that the data more closely approximated normal distribution (e.g. log-transformation for distance measures in the "What was where?" task). For dichotomous measures (e.g. correct *vs* incorrect response), logistic regression was used. For outcomes where a transformation was not appropriate but skew was still a concern, bootstrapping was used to produce bias-corrected and accelerated 95% confidence intervals from 2000 replications. For trial-by-trial responses, data was clustered by participant.

For the only longitudinal study, Chapter 5, which included follow-up data, mixed effects modelling, including both fixed and random effects, was used to examine outcomes of interest and their relationship to EYO in mutation carriers and age in the controls.

3.7.3.2. Associations with predictors of performance

After comparing between groups, associations between variables of interest and other predictors were assessed. Some examples include: Chapter 4: mutation position, generational effects and cognitive presentations all in relation to survival; Chapter 5: rates of cognitive change in association with disease severity; Chapter 6: eye movements as predictors of VSTM performance; Chapter 7: associations between MyCog score (continuous scale of subjective cognitive decline), mental health variables and life-course predictors (childhood cognitive ability, education and SEP).

3.7.3.3. Consideration of correction for multiple comparisons

Corrections for multiple comparisons has been and still is a highly debated topic within the scientific community with some arguing that performing many comparisons increases the likelihood of obtaining at least one false-positive result with each additional test, while others

state this is not always appropriate as these 'corrections' reduce the likelihood of detecting the effect of interest (O'Keefe, 2003). Furthermore, arguments against correction of multiple comparisons include: the reduction of statistical power (with the argument that falsely declaring the "insignificance" would be no less unethical than incorrectly exaggerating its effects would be (e.g., (Begg & Berlin, 1988)); the inconsistent application and 'unprincipled practice' (e.g. methods like ANOVAs are routinely subjected to familywise correction, whereas other techniques like multiple regressions are not even if the same number of significance tests is involved (O'Keefe, 2003; Smith et al., 2002)); the publication bias (i.e. the unethical tendency to present only two significant results from a group of ten knowing that the presentation of ten might lead to the suggest of a familywise adjustment (Matsunaga, 2007)) and the difficulty in establishing whether studies are portions of a unique larger study (e.g. would investigators be required to update 'old' papers with 'updated' *p* values if additional 'sub-studies' were to follow using as similar dataset) (Althouse, 2016; Matsunaga, 2007). This last scenario raises an important point that has direct relevance to one of my data chapters – Chapter 5 – where in addition to carrying out a longitudinal analysis, I included more participants and analysed cross-sectional differences between controls and patient groups with some overlap between participants. These corrections effectively penalizes an association for being found in a large study rather than in a small study (Althouse, 2016).

One popular approach for correcting for Type I errors, are family wise procedures which adjust the likelihood of making Type I errors. However, this correction is done at *each test* so that their alphas sum up to 0.05 for the overall H_0 (null hypothesis), resulting in the overall Type II error rate being inflated. While the False Discovery Rates (FDR) (Benjamini & Hochberg, 1995) approach seems to partially address this 'inflation issue', the method is appropriate for Bayesian methodologies and not frequentist like the ones presented here.

Therefore, although each model contained several predictors, after careful considerations of the literature and discussions with the department statistician (JN), corrections for multiple comparisons were not applied with the exception of the comparison of survival estimates between exons. The assumption underlying the practice of correcting for multiple comparisons is that the first explanation for non-null findings is chance, which may lead to errors of interpretation (Rothman, 1990). Correction for multiple comparisons is often appropriate in scenarios where the result will be used to justify a decision with significant impact (e.g. in a clinical trial where a drug may be licensed based on a positive effect on any one outcome measure) or when a large analysis is conducted without prior hypotheses (e.g. a genome-wide association study where it is statistically likely that numerous false positives would be

detected) (Althouse, 2016). However, when exploratory studies are performed these corrections are thought to be less critical provided that there is a clear statement that subsequent studies should be conducted to confirm the observed association.

For these reasons, where significant results are detected in this thesis, they are interpreted cautiously with reference to previous literature. Importantly, as previously raised, this was done with the assumption that the risk of Type II errors would be more detrimental than Type I errors due to the exploratory nature of these investigations (e.g. survival in FAD, longitudinal VSTM change in FAD, eye movement investigations in FAD). Notably, in accordance with the growing debate around *p* values (i.e. whether they should be used at all to established significance (Ranstam, 2012)), effect sizes and confidence intervals are also presented. Nonetheless, the risk of Type I errors remains a limitation of these approaches and it is paramount that future studies replicate these investigations. For this reason, for each data chapter, I will state what the ideal sample size would be if investigations were to be replicated, the significance set at 0.05 and the statistical power at 80%. For simplicity these values are presented with the assumption that future studies would follow the same design. Calculations are based on the relationship that exists between the *p* value and the observed sample size and power as well as the central limit theorem (i.e. the distribution of the sample is normal). For example, if the observed *p* value was *p*=0.046, and the observed sample size N=92; the sample size required to replicate this effect would be N=189. This is because a *p* value close to 0.05 means there was a 50% chance of observing an effect and a 50% chance of not observing an effect. Therefore, with a desired statistical power of 80% the sample size would need to be approximately double the original sample size or N=189 as stated above. The sample size was calculated in three steps:

1. Z statistic (or **Zstat**) for the stated *p* value	ABS I invnorm*[1] (*p* value*[2])/2 I
2. Calculation of the required **Ez** for the stated level of power	ABS Iinvnorm(1-power*[3])I + ABS Iinvnorm(1- α*[4]/2)I
3. Calculation of the required sample size	a) (**Ez** / **Zstat**)^ 2 = X b) X * (n1*[5])

Where:
ABS = absolute difference
*[1]: invnorm is a function that returns normally distributed random numbers with mean 0 and standard deviation 1 (i.e. in line with the central limit theorem).
*[2]: the observed *p* value from my findings
*[3]: power is 80% or 0.8
*[4]: α = 0.05
*[5]: n1 = the sample size which yield the observed *p* value in a particular finding

4. DISEASE DURATION IN FAD: A SURVIVAL ANALYSIS

This chapter focuses on the estimated survival time of *APP* and *PSEN1* mutation carriers and their relationship with genotype and phenotype interactions. A paper based on this chapter has been published in *Neurology, Genetics* (Pavisic et al., 2020a).

4.1. Introduction

As mentioned earlier, there are currently no disease-modifying treatments for AD. Studying factors that may influence survival time may offer leads to potentially useful interventions as well as improving prognostic information (Armstrong, 2014). FAD, may be a suitable candidate to investigate survival variability due its similarity to sporadic AD (pathologically and clinically), its genetic certainty and the somewhat predictable age at symptom onset in families (Ryman et al. 2014)

It is well documented that AAO is older for *APP* than *PSEN1* mutation carriers (Ryman et al. 2014; Tang et al. 2016; Ryan et al. 2016). However, findings from disease duration are far less consistent (Shea et al. 2016; Canevelli et al. 2014; Ryman et al. 2014). In some studies, *PSEN1* mutation carriers have shown shorter lengths of disease duration than *APP* and *PSEN2* (Shea et al., 2016), but considerable variation exists. A meta-analysis by Ryman and colleagues found a mean disease duration of 9.7 years (SD ± 5.1 years) (Ryman et al. 2014) which is comparable to some longitudinal studies of AD where mean survival from onset was 11.3 years for all patients and 12.1 years for patients with onset below 60 years of age (Waring et al., 2005). One reason for the large variance in disease duration may be that cross-sectional analysis struggles to account for an intrinsic bias against longer disease durations and any generational effects which may arise due to increased awareness within the family or improvements in clinical care. *APOE* ε4 influences on disease duration are also inconsistent. While it is well established that *APOE* ε4 status increases the likelihood of AD risk (Corder et al., 1993; Saunders et al., 1993), its effects on disease duration are less clear. Much remains unknown about the complexities of interactions between different genetic risk factors and their influence on phenotype.

The better we can account for the predictable variation between individuals – either due to genotype or phenotype variations or a combination of both – the better the quality of care for patients and the greater the likelihood of informing clinical trials towards more personalised approaches. The FAD local study is uniquely placed to address this as it has established a research relationship with participants and their families over many years.

Consistent with the approach outlined in section 3.7, this chapter addresses differences in survival time between *APP* and *PSEN1* genes, *APOE* ε4 carriers and ε4 non-carriers, sexes, cognitive presentations (typical or amnestic *vs* atypical or non-amnestic), and *PSEN1* mutation position in relation to codon 200 – all while accounting for censoring. The main hypothesis is that similar to AAO, a substantial amount of variability in survival will be explained by genetics (e.g. difference between genes, mutation and family). Other predictions include the longer survival for individuals carrying a mutation in the pre-codon *vs* post-codon 200 region (due to a greater burden of white matter hyperintensities for post-codon 200); longer survival for those born in earlier *vs* older generations (due to better quality of care over and greater alertness of symptoms); no differences between sexes and no direct associations between AAO and survival. No hypothesis is made for *APOE* ε4 status in relation to survival given the inconsistencies in literature.

4.2. Methods

4.2.1. Study design and participants

Families with histories suggestive of FAD were referred from clinical and research centres across the UK and Ireland between July 1st, 1987 and September 2nd 2019. Clinical and genetic data from these families was evaluated (**Table 4.1**). Inclusion criteria for the study were a family history suggestive of FAD and known age at symptom onset. Exclusion criteria were a neurodegenerative condition other than FAD, unknown age at symptom onset, unknown year of birth and no information on last year of contact with the centre.

Table 4.1 *PSEN1* and *APP:* characteristics of the sample included in the analysis.

N=256	*APP*	*PSEN1*	*APP* + *PSEN1*
No. Families	12	64	76
No. Mutations	7	45	52
Total No. Symptomatic individuals	**55**	**201**	**256**
Sex (males %)	**32 (58.2 %)**	**94 (46.7 %)**	**126 (49.2%)**
No. known deaths	**43 (78.2 %)**	**147 (73.1 %)**	**190 (74.2 %)**
Cognitive Presentation			
No. with available data	**37**	**101**	**138**
Amnestic	36 (97.3 %)	83 (82.2 %)	119 (86.2 %)
Atypical	1 (2.7 %)	18[a] (17.8 %)	19 (13.8 %)
***APOE* Genotype**			
No. with available data	**31**	**96**	**127**
***APOE* ε4 carrier**	7 (22.6 %)	36 (37.5 %)	43 (33.9 %)
APOE 44	1	3	4
APOE 34	6	31	37
APOE 24	0	1	1
***APOE* ε4 non-carrier**	24 (77.4 %)	60 (62.5 %)	84 (66.1 %)
APOE 23	3	5	8
APOE 33	21	54	75
APOE 22	0	1	1

[a] one additional individual was subsequently excluded as the motor presentation preceded cognitive symptoms.

One individual was excluded from the cognitive presentation analysis as they had a motor presentation that preceded cognitive symptoms. One participant with two *PSEN1* substitutions (p.Thr291Ala and p.Ala343Thr) was excluded from the exon analysis because it was unclear whether pathogeneity was due to one or both of these amino acid substitutions (Ryan et al. 2016). Twelve additional individuals were excluded from all analysis: five due to uncertainty in year of last contact (information necessary for censoring) and seven due to unknown year of birth (variable considered as a covariate in all models) (**Figure 4.1**). The intron 4 mutation was classified as involving exon 4 because it is located just outside this exon.

In total, 256 individuals were included in the analyses (201 with *PSEN1* and 55 with *APP* mutations) (**Figure 4.1**). For this study, written informed consent was obtained from the participant consultee if cognitive impairment prohibited written informed consent.

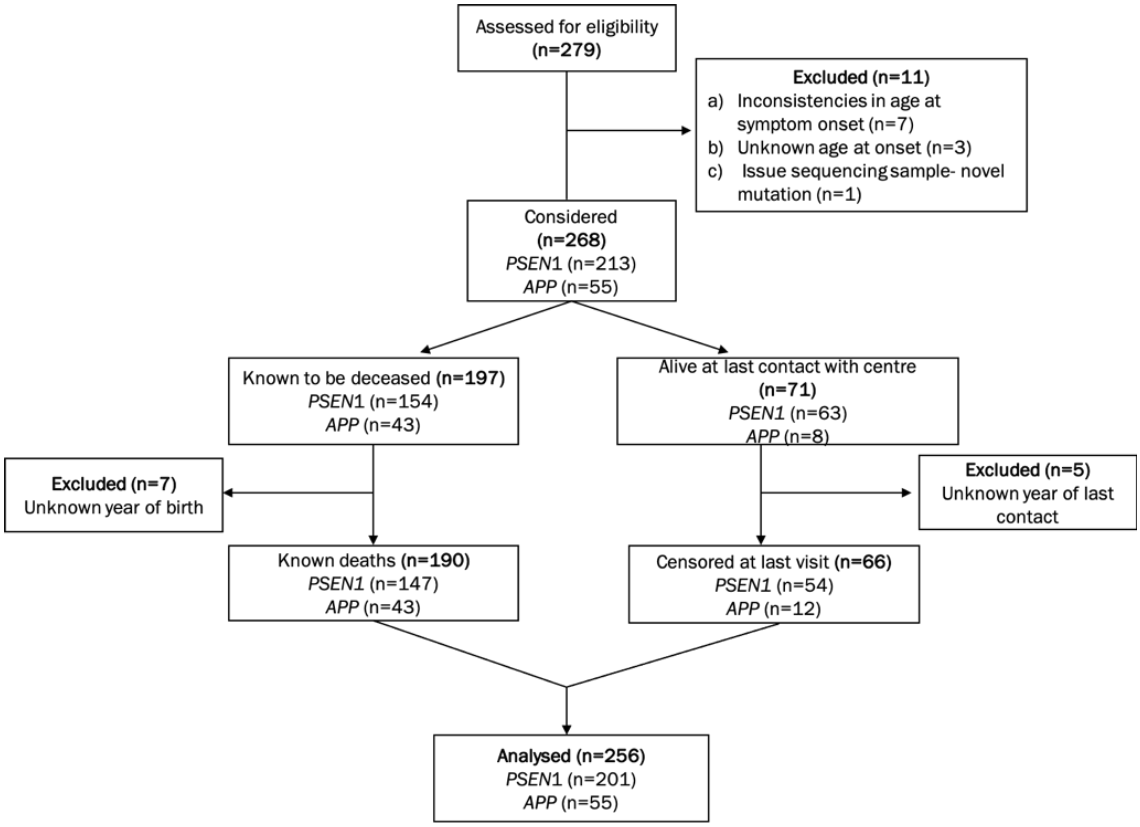


Figure 4.1 Flowchart for the analysis inclusion process

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4.2.2. Procedures and data collection

Contemporaneous records were evaluated to determine the following variables:

- **AAO (in years):** defined as the age at which progressive symptoms of cognitive, behavioural, or motor changes were first noticed by someone who knew the patient well; and the nature of the initial symptoms.
- **Cognitive presentation:** classified as either amnestic, for those with initial memory symptoms, or atypical, for those with non-amnestic initial symptoms such as behavioural change or symptoms of language or executive dysfunction or dyscalculia.
- **Age at death (in years)**: ascertained from examination of medical records, post-mortem reports and interviews with living relatives.
- **Disease duration (in years)**: calculated by subtracting the age at death from the AAO where this was available (N=197) and where this was not available the disease duration at censoring (survival) was calculated from the age of the individual at their last assessment (N=71).

- ***APOE* ε4 status:** determined by the presence or absence of at least one ε4 allele (ε4 carriers *vs* ε4 non-carriers)
- **Exon position**: dependent on the specific mutation and found here: https://www.alzforum.org/mutations.

As described in previous work, individuals with novel variants in *PSEN1* or *APP* were assessed for the presence of additional mutations in other dementia-related genes using the MRC Dementia Gene Panel (Ryan et al. 2016; Beck et al. 2014). All novel sequence variants were absent from the Genome Aggregation Database (https://gnomad.broadinstitute.org/).

4.2.3. Statistical analysis

Differences in survival between *APP* and *PSEN1* genes, *APOE* ε4 carriers and ε4 non-carriers, cognitive presentation, sex, exon number, and *PSEN1* mutation position in relation to codon 200 were investigated. The Kaplan-Meier survival estimate was used for descriptive statistics (i.e. survival plots) and Weibull multilevel parametric survival analysis (using an accelerated failure time model) was used to compare the survival function of different groups of patients and test the specific hypothesis. Following the second-order relationship between disease duration and AAO in Ryman and colleague's meta-analysis (Ryman et al. 2014), I predefined that I would investigate a quadratic term for AAO and test an interaction with gene. Sex, year of birth (range: 1879-1983) and gene were included as fixed effects and family (as a proxy to mutation) as a random effect in all survival models. The intra-class correlation coefficient (ICC) was used to quantify the proportion of variance in disease duration explained by mutation and family:

$$ICC = \frac{\sigma_2^2}{\sigma_2^2 + \sigma_1^2}$$

Where:

- σ_2^2 is family membership variance or mutation specificity variance
- σ_1^2 is individual variance

For the Weibull accelerated failure time model, the individual errors follow a Gumbel distribution with variance given by:

$$\sigma_1^2 = \frac{\pi^2}{6p^2}$$

Where:

- p= ancillary parameter of the Weibull distribution

An ICC of 0 indicates none of the variability in disease duration is explained by the random variable (e.g. family) while an ICC of 1 would indicate all of the variability is explained by the random variable.

Linear mixed effects models with random effects for mutation and family and fixed effects for sex, year of birth and gene were used to compare differences in AAO between genes and cognitive presentations (within *PSEN1* mutations).

Bonferroni correction for multiple comparisons was applied for comparison of survival estimates between exons.

4.3. Results

Age at symptom onset was available for all 256 individuals included (201 with *PSEN1*, 55 with *APP* mutations). Age at death was available for 190 of those individuals (77.0% of the dataset: 147 *PSEN1*, 43 *APP* mutations). **Table 4.2** below shows details on the specific mutations.

Table 4.2 Mutations carried by the individuals in the cohort (N=256).

	Exon	No. of families	No. affected individuals (range)	Mean AAO, years (range)	Mean age at death, years (range)	Mean disease duration, years (range)
APP			**N=55**			
p.Ala692Gly	17	1	4	46 (39-54)	59 (51-65)	12.8 (8-21)
p.Val715Ala	17	1	1	42	51	9.0
p.Val717Gly	17	1	14	51 (40-61)	64 (57-74)	13.2 (6-23)
p.Val717Ile	17	6	28 (1-10)	52 (42-63)	64 (54-75)	10.2 (4-23)
p.Val717Leu	17	1	5	49 (48-51)	62 (60-64)	12.5 (9-16)
p.Thr719Asn	17	1	2	46	56 (55-56)	9.5 (9-10)
p.Val717Phe	17	1	1	38	NA	NA
PSEN1			**N=201**			
Intron 4 (g.23024delG)	4	4	27 (2-21)	38 (34-45)	47 (41-69)	9.9 (5-27)
p.Ala79Val	4	1	1	52	NA	NA
p.Tyr115Cys	5	2	2	39 (34-44)	50 (44-55)	10.5 (10-11)
p.Tyr115His	5	1	7	34 (30-40)	42 (41-46)	8.2 (5-11)
p.Thr116Asn	5	1	1	34	43	9.0
p.Glu120Lys	5	2	7 (2-5)	35 (31-39)	44 (37-52)	7.7 (3-16)
p.Ser132Ala	5	1	3	59 (58-60)	70 (67-73)	11.0 (9-13)
p.Met139Val	5	4	18 (3-8)	40 (35-48)	50 (41-75)	10.1 (5-27)
p.Ile143Phe	5	1	2	56 (53-59)	60	7.0
p.Met146Ile	5	2	6	48 (43-50)	55 (47-60)	7.2 (3-12)
p.Leu153Val	5	1	3	35 (35-36)	44 (41-49)	8.7 (6-13)
p.Tyr154Cys	5	1	1	41	NK	NK
p.Val142Ile	5	1	2	51 (50-51)	64	14.0
p.Leu166Arg	6	1	1	40	NA	NA
p.Leu166del	6	1	1	38	NK	NK
Δ167 p.Ile168del	6	1	1	43	52	9.0
p.Leu171Pro	6	1	5	42 (40-43)	51 (47-57)	9.0 (5-15)
p.Glu184Asp	7	3	9 (1-5)	41 (36-47)	52 (48-58)	10.8 (6-14)
p.Ile202Phe	7	1	2	48 (47-48)	60 (53-67)	12.5 (5-20)
p.Gln222Pro	7	1	1	45	NK	NK
p.Gly206Val	7	1	1	30	36	6.0
p.Gly206Ala	1	1	1	55	NK	NK
p.Ile229Phe	7	1	3	33 (32-34)	35 (34-37)	2.3 (2-3)
p.Leu235Val	7	1	5	52 (44-59)	61 (53-67)	9.7 (8-12)
p.Phe237Leu	7	1	1	47	NK	NK
p.Leu250Ser	7	1	7	52 (47-56)	59	6.5 (4-11)
p.Ala246Cys	7	1	4	55 (48-60)	64 (53-73)	7.7 (5-13)
p.Ala260Val	8	1	1	40	NK	NK
p.Cys263Phe	8	1	1	59	NK	NK
p.Pro264Leu	8	3	5 (1-2)	48 (44-56)	NK	NK
p.Pro267Ser	8	1	3	39 (38-41)	49 (45-52)	9.5 (7-12)
p.Arg269His	8	3	5 (1-2)	56 (50-62)	67 (64-69)	16.0 (14-18)

p.Arg278Ile	8	1	9	49 (41-59)	64 (52-71)	14.1 (8-21)
p.Glu280Gly	8	3	22 (1-14)	41 (38-49)	53 (45-71)	11.6 (5-32)
p.Phe283Leu	8	1	11	47 (42-48)	54 (48-58.6)	7.6 (6-12)
p.Leu282Pro***	8	1	1	41	NA	NA
p.Ser290Cys	9	1	5	42 (41-44)	51 (48-54)	8.8 (6-13)
ΔE9*	9	1	1	45	NK	NK
p.Arg377Met	11	1	1	38	49	11.0
Gly378Val	11	1	4	45.5 (41-50)	50 (45-54)	4.0
p.Gly394Val	11	1	1	40	NK	NK
p.Pro436Ser	12	1	6	46 (44-50)	60 (56-69)	14.3 (12-19)
p.Thr291Ala and p.Ala434Thr**	9 & 12	1	1	42	47	5.0
p.Leu424Val	12	1	1	45	51	6.0
p.Pro433Ser***	12	1	1	37	66	29

**The exon 9 deletion (NM_000021.3:c.869–1G→T; p.Ser290Cys; Thr291_Ser319del) is commonly referred to as ΔE9.

** One patient had both Thr291Ala on exon 9 and Ala434Thr on exon 12 (Ryan et al. 2016).

*** Novel mutations

AAO= age at onset; NA= not applicable as individuals were still alive; NK= not known.

4.3.1. Estimated survival in *PSEN1* and *APP* mutation carriers

Considering only individuals with known age at death (N=190), the mean disease duration was 10.4 (SD 5.3) years, range: 2-32 years. Survival analysis (N=256) revealed a 75% probability of surviving at least seven years, 50% of surviving at least ten years, 25% of surviving at least fourteen years and an estimated mean survival of 11.6 [95% CI 10.4, 12.9] years. There was no evidence for a difference in estimated survival between *APP* and *PSEN1* mutation carriers (**Table 4.3**, p=0.474, **Figure 4.2**).

Considering the cohort as a whole, family membership explained 18% (ICC 0.18; p<0.001) of the variability in disease duration and mutation specificity explained 6% (ICC 0.06; p=0.188). In patients with a *PSEN1* mutation, 25% of the variance in disease duration was explained by family membership (ICC 0.25, p<0.001) and 10% by a specific mutation (ICC 0.10, p=0.129). Data were not analysed separately for *APP* mutations due to small numbers (7 mutations, 12 families).

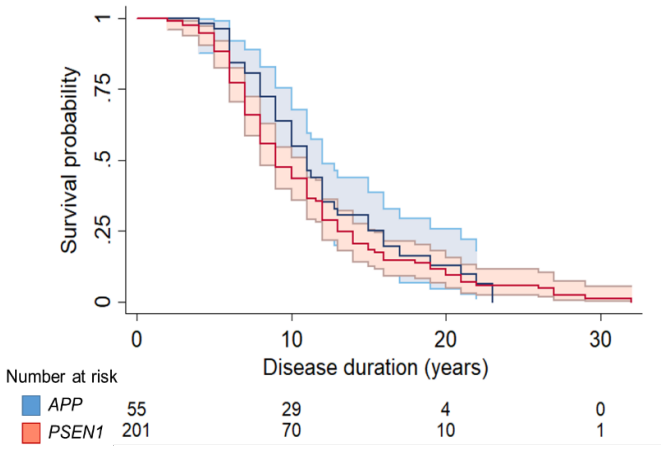


Figure 4.2 Survival probability by gene.

Unadjusted Kaplan-Meier survival plots show the estimated survival probability by disease duration for *PSEN1 vs APP*. The blue line references *APP* and the red line *PSEN1*. 95% confidence intervals and number of individuals still alive per disease duration length: by 10 years, by 20 years and by 30 years are also shown. From (Pavisic, et al., 2020a) with permissions from Wolters Kluwer Health, Inc. and Copyright Clearance Center & Neurology © Genetics).

4.3.2. Relationship between survival and age at onset

The distribution of AAO of the sample is shown in **Figure 4.3A**. In accordance with previous work (Ryan et al. 2016), AAO was significantly later for individuals with *APP* mutations (mean age 50.6 (SD 5.6), range 38-63) than those with *PSEN1* mutations (43.5 (7.2), range: 30-62; $p<0.001$) (**Figure 4.3B**). In patients with *PSEN1* mutations, 72% of the variance in AAO was explained by mutation (ICC 0.72, $p<0.001$). Mutation and family membership together explained 80% of the variance in age at symptom onset (ICC 0.80, $p<0.001$). Considering both genes together, 67% of the variance was explained by mutation and 72% by mutation and family together.

No linear relationship between an individual's AAO and the estimated length of disease course was observed (Time Ratio (TR)=1.00 [0.99, 1.01], $p=0.286$). However, there was a significant interaction with gene (TR = 1.05 [1.02, 1.08], $p=0.001$). While in *PSEN1* mutations, later AAO were associated with longer disease durations (disease duration increased by 1.8 [0.3, 3.4] % for every 1 year increase in AAO, $p=0.018$); in *APP* later ages at onset were associated with shorter disease durations (disease duration decreased by 3.0 [0.9, 4.7] % for every 1 year increase in AAO, $p=0.005$) (**Figure 4.3C**). Plotting the disease course for all affected individuals with known ages at death revealed a an 'inverted-U' shape relationship between AAO and disease course, like that reported by Ryman and colleagues (Ryman et al. 2014): patients with early (younger than 40 years) or late (older than 50 years) onset each had shorter disease duration than patients with onset in midlife (40–50 years) irrespective of the gene ($\chi 2$

= 6.12, p=0.047; considering AAO as a quadratic term). However, including the gene-interaction abolished this quadratic association (χ^2=1.33, p=0.515), indicating gene membership may have driven the 'inverted-U' shape effect.

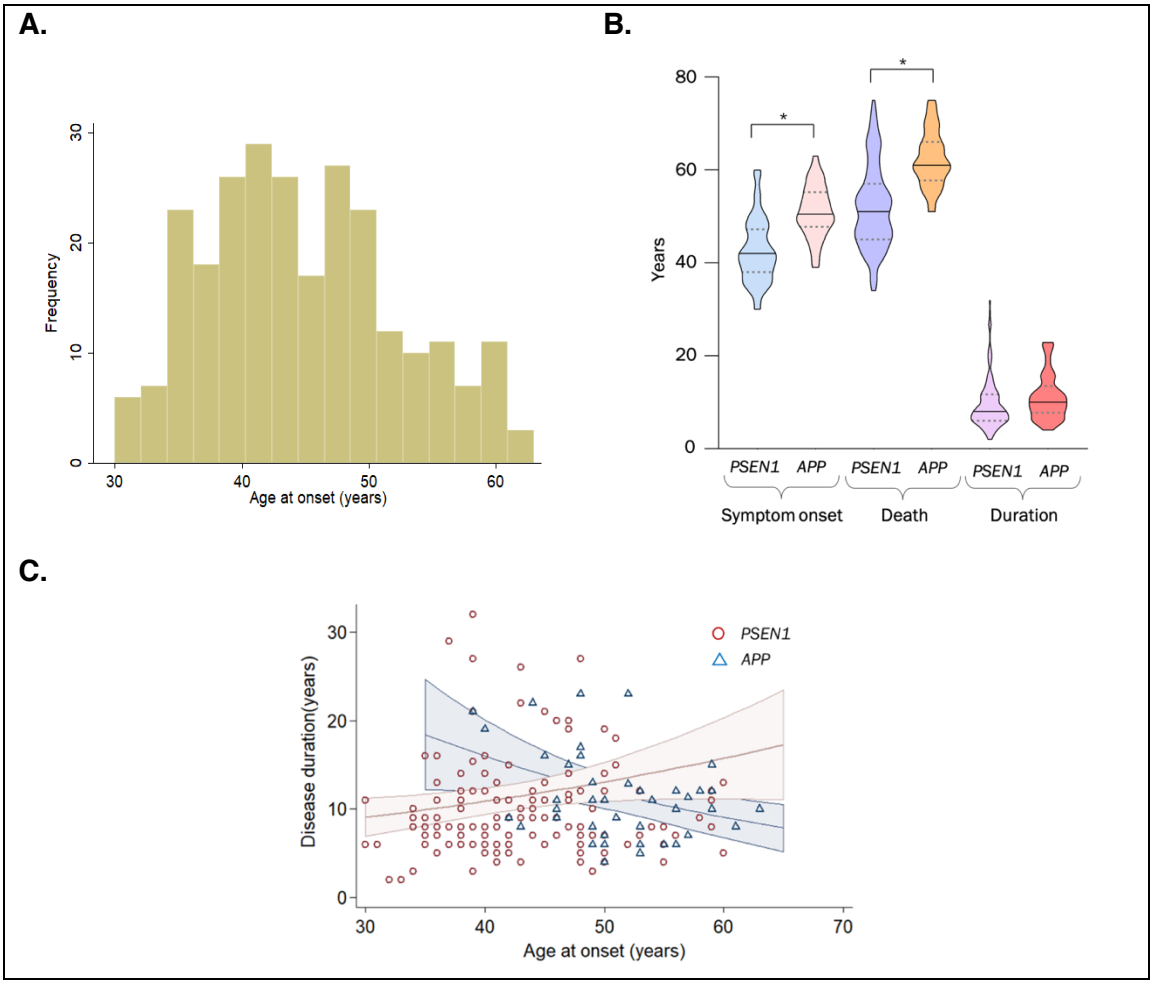


Figure 4.3 Survival and age at onset.

A. Histogram of the age at onset distribution considering the cohort as a whole. **B.** Violin plots show the distribution of age at symptom onset, at death and disease duration for *PSEN1 vs APP*. Data are median (line) with median IQR (upper and lower dotted lines). Age at onset: 42 (38-48) years *vs* 50 (48-55) years; age at death: 52 (46-58) years *vs* 61 (58-66) years and disease duration: 8 (6-12) years *vs* 10 (8-13) years. '*' indicates significant difference between groups. **C.** Scatter plot shows the association between age at symptom onset and age at death in *PSEN1 vs APP*. The solid line represents the line of best fit from the survival model, adjusted for sex, year of birth and clustered by family membership for each gene. Shaded area represents 95% confidence intervals. Markers show the unadjusted raw data: hollow blue triangles represent individuals with *APP* mutations and hollow red circle markers, individuals with *PSEN1* mutations. From (Pavisic et al., 2020a) with permissions from Wolters Kluwer Health, Inc. and Copyright Clearance Center & Neurology © Genetics).

4.3.3. Generational effects

Irrespective of the gene, an individuals' year of birth appeared to influence survival and AAO; with AAO being earlier and duration longer in more recent times. Disease duration increased

by 0.6 [0.2, 1.0] % for every increase in 1 year of birth (TR= 1.01 [1.00, 1.01], *p*=0.003). AAO decreased by 0.04 [0.01, 0.07] years for every increase in 1 year of birth (*p*=0.004). Further analysis revealed the greatest difference in survival time was between individuals born before and after 1931: estimated survival: 9.1 [7.7, 10.4] years *vs* 12.2 [10.8, 13.5] years (**Figure 4.4**). However, there was no significant difference in the effect of AAO on survival between individuals born before or after the 1930s (45.5 [43.3, 47.7] years *vs* 44.6 [42.7, 46.5] years, estimated difference: 0.9 [-2.1, 0.4] years, *p*=0.181). The effect of year of birth on survival time remained significant when adjusting for AAO (0.6 [0.2, 1.0] % increase in survival for every increase in 1 year of birth, *p*=0.002; survival estimates pre *vs* post births in the 1930s: 8.9 [7.6, 10.3] years vs 12.2 [10.9, 13.5] years, *p*<0.001).

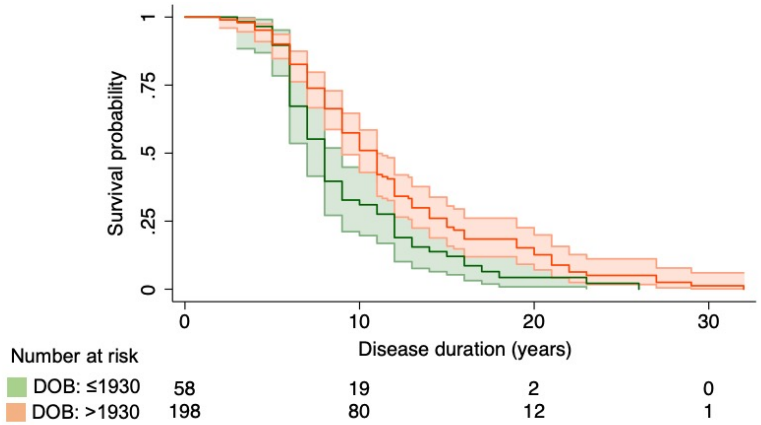


Figure 4.4 Survival probability pre- and post- births in the 1930s.

Unadjusted Kaplan-Meier survival plot showing survival by disease duration for individuals born before and after the 1930s for the cohort as a whole. The green line references individuals born by 1930 and the orange line after 1930. 95% confidence intervals and number of individuals still alive per disease duration length: by 10 years, by 20 years and by 30 years are also shown. DOB: date of birth. From (Pavisic et al., 2020a) with permissions from Wolters Kluwer Health, Inc. and Copyright Clearance Center & Neurology © Genetics).

4.3.4. Sex

Sex did not appear to affect disease duration either for the cohort as a whole (**Table 4.3**, *p*=0.895, **Figure 4.5**) or for genes separately (females *vs* males: *PSEN1*: 11.8 [9.4, 14.2] years *vs* 12.3 [10.0, 14.6], *p*=0.739; *APP:* 11.8 [10.0, 13.6] years *vs* 11.7 [9.7, 13.6], *p*=0.870).

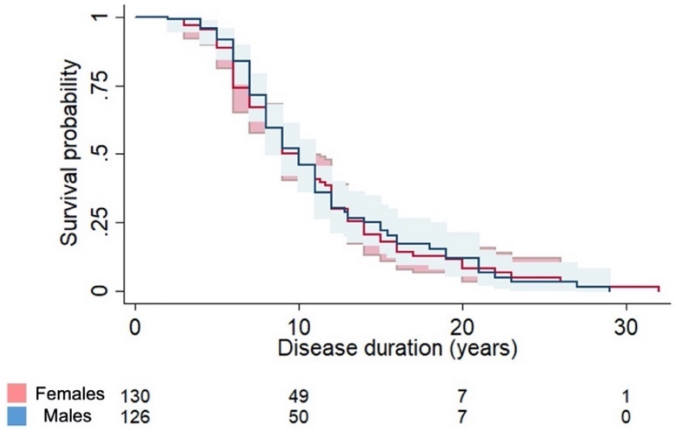


Figure 4.5 Survival probability by sex.

Unadjusted Kaplan-Meier survival plot shows the estimated survival probability of disease duration by sex for the cohort as a whole. 95% confidence intervals and number of individuals still alive per disease duration length: by 10 years, by 20 years and by 30 years are also shown. Cranberry= females; Blue=males.

4.3.5. *APOE* ε4 status

Considering the cohort as a whole, *APOE* ε4 status did not have an effect on AAO (ε4 carriers= 44.0 [41.8, 46.1] years *vs* ε4 non-carriers=44.5 [42.5, 46.5], *p*=0.495) or in genes separately (ε4 carriers *vs* ε4 non-carriers: *PSEN1*: 42.5 [40.3, 44.8] *vs* 43.4 [41.2, 45.5], *p*=0.376; *APP:* 48.3 [43.9, 52.7] *vs* 49.0 [45.9, 52.2], *p*=0.701). Survival analysis (N=127) revealed similar survival estimates between ε4 carriers and ε4 non-carriers for *APP* and *PSEN1* mutations together (**Table 4.3**, *p*=0.100, **Figure 4.6**).

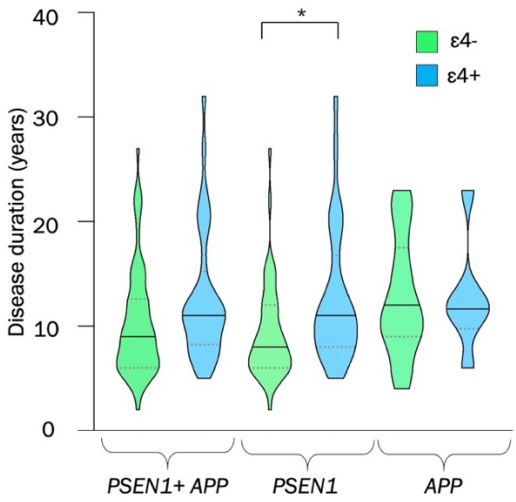


Figure 4.6 Violin plots show the distribution of disease duration by *APOE* status.

ε4 non-carrier *vs* ε4 carrier for *PSEN1* & *APP* genes together and *PSEN1* and *APP* separately. Data are median (line) with median IQR (upper and lower dotted lines). '*' indicates significant difference between groups. *PSEN1* & *APP*: 9 (6-12.4) years *vs* 11 (8.5-14.5); *PSEN1*: 9 (7-12) years *vs* 14 (11-20) and *APP*: 12 (9-16) years *vs* 11.7 (11-13). From (Pavisic, et al., 2020a) with permissions from Wolters Kluwer Health, Inc. and Copyright Clearance Center & Neurology © Genetics).

There was no significant interaction between *APOE* ε4 status and gene (TR = 1.35 [0.87, 2.08], *p*=0.180). Yet, further analysis revealed an effect seemingly restricted to *PSEN1* mutations (N=96): ε4 carriers had longer survival time compared to ε4 non-carriers (**Table 4.3**, *p*=0.046) (**Figure 4.7A**). *APOE* ε4 status did not have an effect on disease duration in the small group of individuals with *APP* mutations (N=31) (**Table 4.3**, *p*=0.738, **Figure 4.7B**). Adjusting models for AAO revealed similar results (ε4 carriers *vs* ε4 non-carriers: *PSEN1*: 13.4 [10.9, 15.9] *vs* 10.6 [9.0, 12.2], *p*=0.033; *APP:* 13.7 [9.9, 17.5] *vs* 12.5 [10.5, 14.5], *p*=0.560).

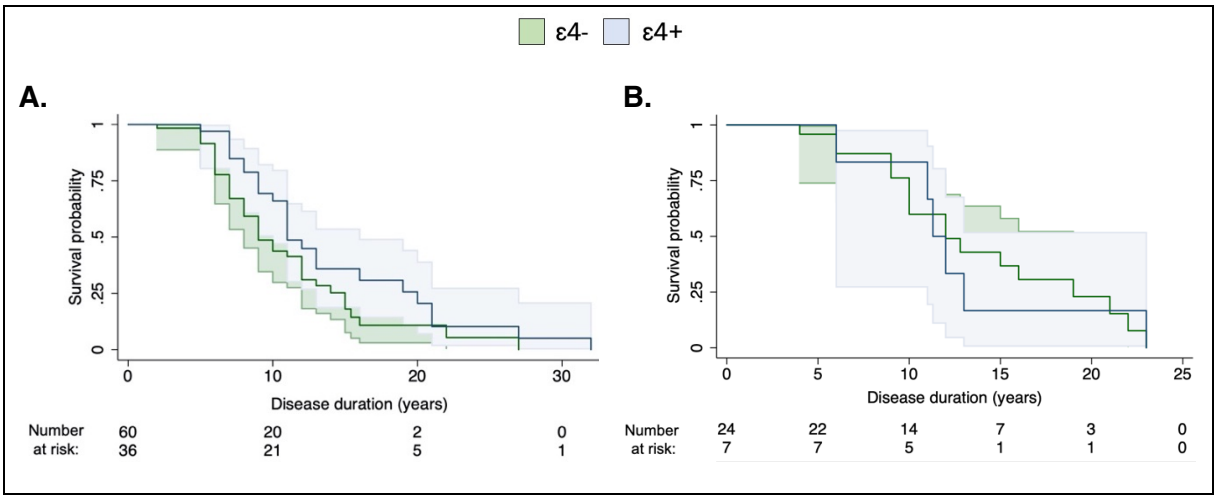


Figure 4.7 Survival probability by *APOE* ε4 status for *APP* and *PSEN1*.

Unadjusted Kaplan-Meier survival plots show the estimated survival probability by disease duration for *APOE* ε4 status for **A.** *PSEN1* mutations. **B.** *APP* mutations. 95% confidence intervals and number of individuals still alive per disease duration length: by 10 years, by 20 years and by 30 years are also shown. Blue=ε4 carriers; green=ε4 non-carrier. From (Pavisic et al., 2020a) with permissions from Wolters Kluwer Health, Inc. and Copyright Clearance Center & Neurology © Genetics).

I then examined *APOE* ε4 heterozygous and homozygous groups separately considering the dose-dependent effects ε4 on the risk of SAD. Due to statistical power limitations for homozygous carriers (N=4), I report heterozygous ε4 carrier results only (N=38). In the whole cohort, there was a trend towards carriers of one ε4 allele having a 20.5 [0.5, 45.8] % longer survival time compared to ε4 non-carriers (3.7 [11.3, 16.0] years *vs* 11.4 [10.0, 12.9] years, *p*=0.056). Within the *PSEN1* cohort, the possession of one ε4 allele was associated with a 29.4 [3.6, 61.6] % longer survival time (13.7 [11.1, 16.3] years *vs* 10.6 [9.0, 12.1] years, *p*=0.023) (**Figure 4.8A**). Comparing carriers of one ε4 allele to ε4 non-carriers in the *APP* cohort, did not reveal any differences (12.3 [7.4, 17.3] years *vs* 13.5 [10.1, 17.0] years, *p*=0.677, **Figure 4.8B**). Lastly, considering AAO in these models did not change results either (*PSEN1*: 14.0 [11.2, 16.8] years *vs* 10.6 [9.1, 12.2], *p*=0.017; *APP:* 14.0 [9.2, 18.8] years *vs* 12.5 [10.4, 14.5], *p*=0.550).

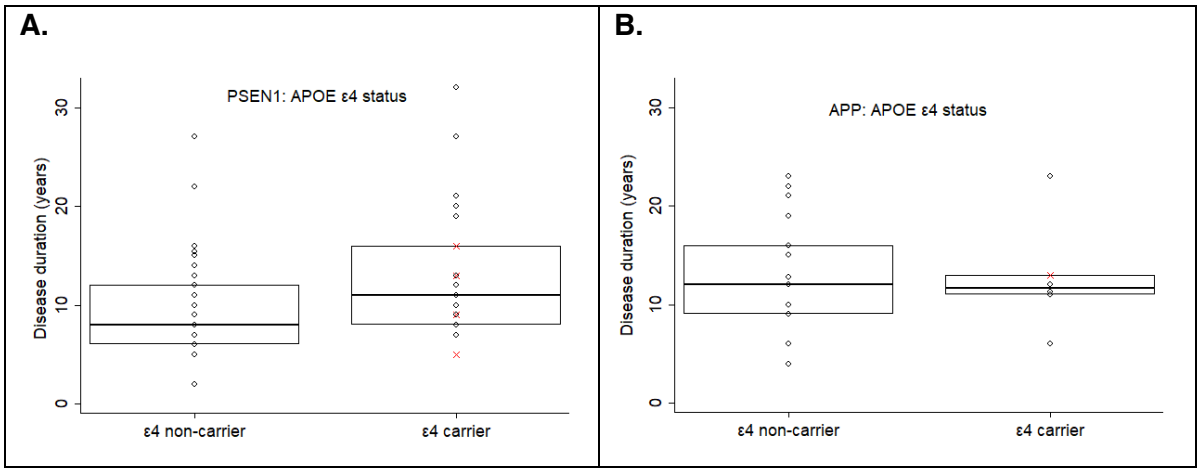


Figure 4.8 Disease duration by *APOE* ε4 genotype for *APP* and *PSEN1*.

Individual data is shown: boxplot shows median values for each group and lower and upper percentiles (25-75). **A.** *PSEN1* mutations: disease durations by *APOE* ε4 status: ε4 non-carrier=8 years (6-12); ε4 carrier=11 years (8-16). **B.** *APP* mutations: disease durations by *APOE* ε4 status: ε4 non-carrier =12 years (9-16); ε4 carrier=11.6 years (11-39). ε4 homozygous carriers are indicated by red crosses in both groups. From (Pavisic et al., 2020a) with permissions from Wolters Kluwer Health, Inc. and Copyright Clearance Center & Neurology © Genetics).

4.3.6. Cognitive presentation

Within the *PSEN1* group, individuals with atypical presentations had a 49.2% longer survival time compared to those with amnestic presentations (**Table 4.3**, p=0.009, **Figure 4.9**). Only 8% of the variance in survival time between individuals with the same cognitive presentations in *PSEN1* mutations was explained by family membership (ICC 0.08, p=0.157). The difference in estimated survival time between cognitive presentations was replicated combining *APP* and *PSEN1* groups (**Table 4.3,** p=0.013).

There was no significant interaction between the cognitive presentation and *APOE* ε4 status (p=0.401) or AAO (p=0.574). Nonetheless, following some literature of later ages at onset for atypical compared to amnestic presentations (Ryan et al. 2016) (not observed here in *PSEN1* mutations amnestic: 42.4 (SD 7.3), range: 30–62 years *vs* atypical: 45.4 (5.7), 38–58 years, p=0.592), survival models were re-run after adjustment for AAO and similar results emerged (atypical *vs* amnestic: *PSEN1*: 17.2 [12.1, 22.3] *vs* 11.6 [10.0, 13.2], p=0.011; *PSEN1* and *APP:* 17.3 [12.4, 22.2] *vs* 12.0 [10.6, 13.3], p=0.015). There was no significant interaction between the cognitive presentation and *PSEN1* codon 200 position either (p=0.887). The independent effect of *PSEN1* mutation position on survival estimates is discussed in the next section.

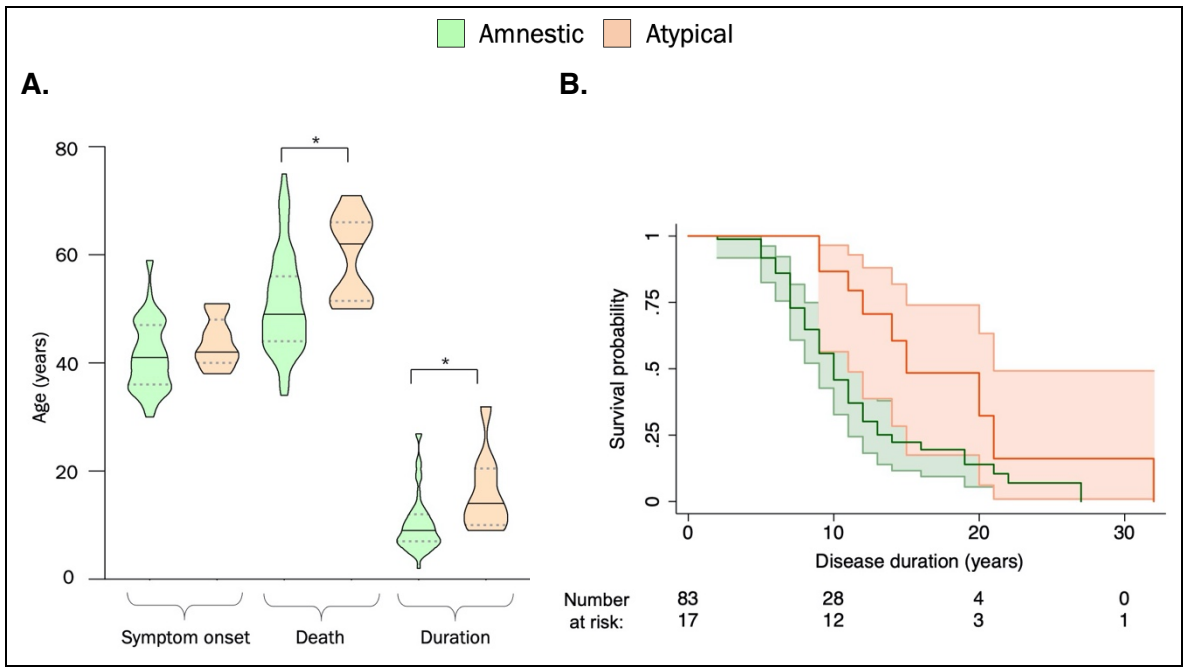


Figure 4.9 Symptom onset, age at death, disease duration and survival probability by cognitive presentation.

A. Violin plots show the distribution of age at symptom onset, at death and disease duration by cognitive presentation: amnestic *vs* atypical. Data are median (line) with median IQR (upper and lower dotted lines). '*' indicates significant difference between groups. Age at onset: 41 [36-47] years vs 44 [41-50] years; age at death: 49 [44-56] years vs 62 [52-66] years and disease duration: 9 [7-12] years vs 14 [11-20] years. **B.** Unadjusted Kaplan-Meier survival plot shows the estimated survival probability by disease duration for cognitive presentations. 95% confidence intervals and number of individuals still alive per disease duration length: by 10 years, by 20 years and by 30 years are also shown. Green= amnestic presentations; Orange=atypical presentations. From (Pavisic et al., 2020a) with permissions from Wolters Kluwer Health, Inc. and Copyright Clearance Center & Neurology © Genetics).

4.3.7. *PSEN1* mutation location

Survival time did not differ between individuals with *PSEN1* mutations located pre- or post-codon 200 (**Table 4.3**, p=0.746) and considering AAO in these models did not change results (11.9 [9.7, 14.2] *vs* 11.4 [9.4, 13.3], p=0.713).

Some individuals with *PSEN1* mutations in exon 8 (N=40) appeared to reach long disease durations (mean duration (in those with known age at death) exon 8: 11.3 (SD 5.9), range: 5–32 years; **Figure 4.10**). After adjusting for multiple comparisons (28 comparisons: Bonferroni correction), mutations located in exon 8 (N=58) had longer survival estimates than in those in exon 11 (N=6) (14.0 [10.8, 17.2] years *vs* 6.2 [3.4, 9.0] years, p=0.034). Fifteen percent of the variability in survival time among those with a mutation on the same exon was explained by family membership (ICC 0.15, p=0.004).

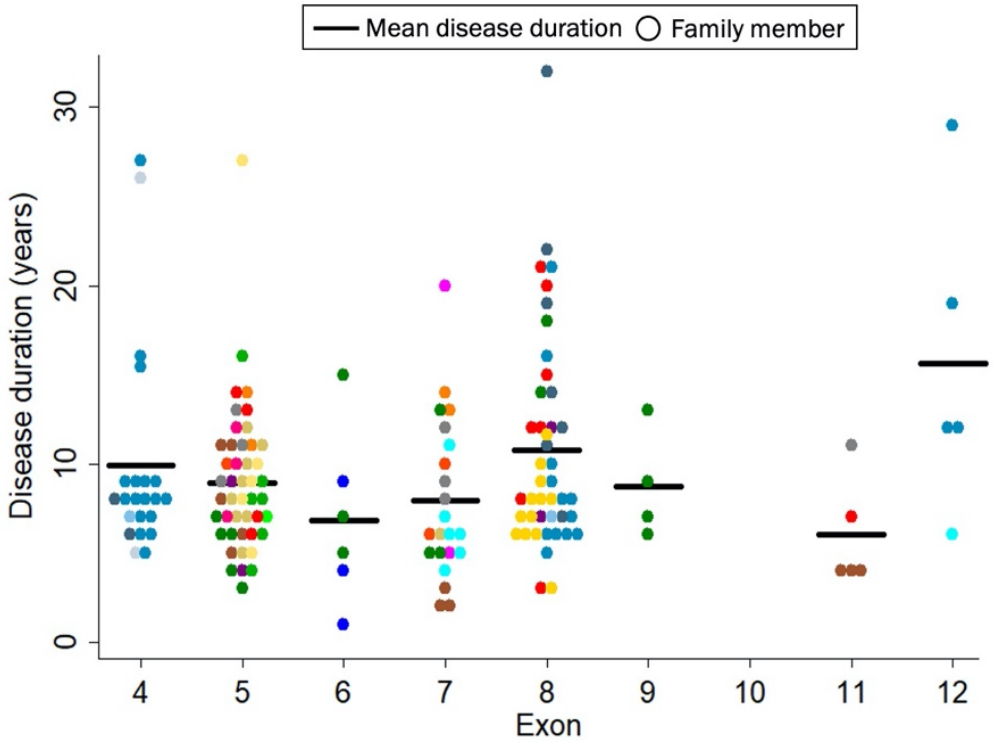


Figure 4.10 *PSEN1* mutation carriers: disease duration by exon position.

Each dot represents one individual's disease duration. Within each exon, different colours represent separate families; multiple families with the same mutation are indicated by different shades of the same colour (blue, green, purple, or pink). Bars indicate mean disease duration (in years) for mutations involving each exon. From (Pavisic et al., 2020a) with permissions from Wolters Kluwer Health, Inc. and Copyright Clearance Center & Neurology © Genetics).

Table 4.3 Disease duration, estimated mean survival time, and effects from survival model comparison.

	Disease duration: Mean (SD) (years)	Disease duration: Range (years)[a]	Estimated survival [95% CI] (years)[b]	TR [95 %CI]
Genes			**N=256**	
APP	11.2 (4.9)	4–23	12.5 [9.7, 15.3]	Reference
PSEN1	9.7 (5.3)	2–32	11.4 [10.1, 12.7]	0.91 [0.72,1.17]
Sex	**N= 190**		**N=256**	
Females	10.0 (5.4)	2–32	11.7 [10.3, 13.1]	Reference
Males	10.1 (5.2)	2–29	11.6 [10.1, 13.1]	0.99 [0.86, 1.13]
***APOE* status**				
PSEN1* & *APP	**N=92**		**N=127**	
ε4 non-carriers	10.4 (5.2)	2–27	11.3 [9.9, 12.7]	Reference
ε4 carriers	12.8 (6.4)	5–32	13.2 [11.1, 15.3]	1.16 [0.97, 1.39]
PSEN1	**N= 69**		**N=96**	
ε4 non-carriers	9.4 (4.7)	2–27	10.6 [9.0, 12.1]	Reference
ε4 carriers	12.9 (6.7)	5–32	13.2 [10.8, 15.5]	**1.24 [1.00, 1.54] ***
APP	**N= 23**		**N=31**	
ε4 non-carriers	12.7 (5.8)	4–23	13.4 [10.3, 16.6]	Reference
ε4 carriers	12.7 (5.6)	6–23	12.6 [8.4, 16.9]	0.94 [0.65, 1.35]
***APOE* genotype**				
PSEN1* & *APP				
ε4 non-carriers	10.4 (5.2)	2–27	11.4 [10.0, 12.8]	Reference
ε4 heterozygous carriers	13.2 (6.6)	6–32	13.7 [11.3, 16.0]	1.20 [1.00, 1.46]
PSEN1				
ε4 non-carriers	9.5 (4.7)	2–27	10.6 [9.0, 12.1]	Reference
ε4 heterozygous carriers	13.4 (6.8)	7–32	13.7 [11.1, 16.3]	**1.30 [1.04, 1.62] ***
APP				
ε4 non-carriers	12.8 (5.8)	4–23	13.5 [10.1, 17.0]	Reference
ε4 heterozygous carriers	12.7 (6.3)	6–23	12.3 [7.4, 17.3]	0.91 [0.59, 1.40]
Cognitive presentation				
PSEN1* & *APP	**N= 87**		**N=139**	
Amnestic	10.9 (5.5)	2–27	11.9 [10.6, 13.1]	Reference
Atypical	15.5 (7.1)	9–32	17.1[12.4, 21.9]	**1.44 [1.08, 1.93] ***
PSEN1	**N= 60**		**N=102**	
Amnestic	10.2 (5.4)	2–27	11.2 [9.8, 12.6]	Reference
Atypical	15.9 (7.4)	9–32	16.7 [12.0, 21.5]	**1.49 [1.11, 2.01] ****
***PSEN1*: Codon 200**	**N=147**		**N=201**	
Post	9.9 (5.9)	2–32	11.9 [9.9, 14.0]	Reference
Pre	9.3 (4.7)	3–27	11.5 [9.3, 13.7]	0.96 [0.76, 1.22]

[a] Disease duration was calculated from individuals with known ages at death only. [b] Estimated mean survival additionally included any censored data. Times Ratio (TR), 95% CI (confidence intervals) and *p* value encompass the effects of the survival model. Bold=significant; *: significant at $p < 0.05$; **: significant at $p<0.01$.

4.4. Discussion

4.4.1. Summary

In this retrospective cohort study, I investigated various predictors of survival including genes, AAO, year of birth, sex, *APOE* ε4 status, cognitive presentation and *PSEN1* mutation position. I also evaluated the extent to which survival variance was explained by family membership and mutation specificity. The main finding was that survival was influenced by mutation and family specificity to a much lesser extent than AAO. Furthermore, no differences in duration were observed between *PSEN1* and *APP* genes, pre- *vs* post- codon positions (within *PSEN1)* or between sexes. Yet, survival increased over time and was longer for atypical presentations compared to amnestic ones. Lastly, within *PSEN1* mutation carriers, there was some indication that ε4 carriership yield a longer survival compared to individuals who were non-ε4 carriers. These themes will be discussed in greater detail in the following sub-sections.

4.4.2. Survival estimates for *PSEN1* and *APP* genes

Individuals with *APP* mutations had, on average, similar estimated survival time to individuals with *PSEN1* mutations, despite the later AAO observed for *APP* compared to *PSEN1* mutations. Nevertheless, there was great variability in survival estimates for both the *PSEN1* (2–32 years) and *APP* (4–23 years) groups and unlike AAO, mutation type and family membership explained relatively little of this variance. In this respect, it may be relevant that family membership accounted for a slightly larger proportion of variance in survival than mutation type, although shared environmental factors could also contribute to this finding.

4.4.3. Relationship between survival and age at onset

In accordance with Ryman and colleagues meta-analysis (Ryman et al. 2014), there was a trend for longer disease duration in individuals with an AAO of 40–50 years (compared with <40 years or >50 years). The reasons for this are unclear. Ryman and colleagues argue this is consistent with a model in which highly pathogenic mutations may cause early-onset disease with a rapidly progressive course, while mutations with more gradual pathogenesis may cause later onset of disease, but could also show decreased survival times from a decrease in amyloid clearance with advancing age (Ryman et al. 2014).

Examination of *PSEN1* and *APP* mutation carriers separately suggested that whilst in *PSEN1* mutations, later ages at onset were associated with longer disease durations, in *APP* later

ages at onset were associated with shorter disease durations. Although it is unclear why these differences between *APP* and *PSEN1* exist, I argue that different paths of disease course between genes may underly the 'inverted-U' shape relationship between AAO and survival observed also in other studies (Ryman et al. 2014). More specifically, *APP* mutation carriers often show a more localised pattern of atrophy (in the medial temporal and limbic regions) compared to the rather extensive white matter involvement of occipital, parietal and frontal lobes in *PSEN1* mutation carriers. However, not much is known about how these patterns of atrophy progress with time and whether certain brain regions affected have more direct implications on functions of everyday life and ultimately quality of life than others. Hypothetically, the impairment of MTL regions early on may led to a reduced degree of independence from an earlier stage and subsequent faster progression. In this regard, it is relevant to note that recent work advocates binding as an important cognitive function for everyday life activities (Calia et al., 2020) – and this function is thought to be supported by MTL regions.

4.4.4. Effect of year of birth and sex

Results indicate that individuals born after 1930 had longer survival time compared to those born in previous generations and that AAO was earlier with more recent years of birth. These suggest that gradually (with no step change), onset or recognition of onset, has come earlier. This may partly be due to greater awareness within families: with onset coming about two years earlier over the course of two generations (~50 years). Yet, survival increased over and above this as the difference in AAO between births pre- and post-1930s was smaller than the difference in survival time (0.9 years *vs* 3.1 years). Hence, the increase in survival cannot exclusively be explained by earlier awareness of symptoms and may also relate with the fact that care, as well as life expectancy, has improved over the years. Notably, antibiotics would have become widely available by the time individuals born after the 1930s were clinically affected (Aminov, 2010).

Findings do not provide evidence for sex differences in survival in FAD. Females appear to be at a greater risk of sporadic AD (Podcasy & Epperson, 2016). Although this may be explained by a number of factors including: a) greater longevity (Podcasy & Epperson, 2016); b) differences in risk factors and dementia including *APOE* ε4 (e.g. female ε4 carriers were twice as likely as ε4 non-carriers to have dementia compared to males (Altmann et al., 2014)); c) life-style factors (e.g. sex differences in brain development set the stage on which lifestyle and health conditions exert an influence (Podcasy & Epperson, 2016)) and d) childhood

intelligence (dementia risk is higher for lower scoring categories and this association is stronger for females (Russ et al., 2017)), the relevance of sex differences in FAD remains unclear.

4.4.5. *APOE* ε4 status

Findings suggest that carrying an *APOE* ε4 allele may be associated with increased disease duration in individuals with *PSEN1* mutations, but not in *APP* mutation carriers. Nevertheless, this result would need confirmation as I was not able to demonstrate a significant difference between the two genetic groups in the effect of *APOE* ε4 (gene x *APOE* ε4 status interaction). Interestingly, the rare *APOE* ε3 Christchurch p.Arg136Ser mutation has recently been reported to delay onset of cognitive symptoms by three decades in a carrier of the Colombian *PSEN1* p.Glu280Ala mutation (Acosta-Baena et al., 2011; Arboleda-Velasquez et al., 2019). Furthermore, while the *APOE* ε4 hypothesis of antagonistic pleiotropy is controversial, it is interesting to note that a gene associated with greater risk of sporadic AD may be associated with longer survival in inherited conditions like FAD (at least specifically for individuals with *PSEN1* mutations). These findings indicate that the advantage of *APOE* ε4 may to some extent, explain for the survival of this gene is humans and highlights the possibility of beneficial effects on specific aspects also in later life. It also emphasises how much remains unknown about the complexities of interactions between different genetic risk factors and their influence on disease onset and survival. Larger studies that consider the full range of *APOE* genotypes and follow individuals over time are needed to untangle the multi-faceted effects of the *APOE* genotype. Considering a 0.05 level of significance and 80% power, the sample size required to replicate *APOE* ε4 findings in *PSEN1* mutation carriers is N=189 and for the cohort as a whole (*APP* & *PSEN1* mutation carriers) it is N=368.

4.4.6. Cognitive presentations and *PSEN1* mutation location

Despite phenotypic and pathological differences reported between *PSEN1* mutations located before and beyond codon 200 (Ryan et al. 2016; Ringman et al. 2016; Mann et al. 2001), similar to other reports (Ringman et al., 2016; Ryan et al., 2015), survival estimates did not differ between these mutation groups. Atypical presentations have been reported to be more common with *PSEN1* mutations beyond codon 200 and the prevalence of atypical symptoms also differs markedly between exons, with non-amnestic cognitive presentations and pyramidal signs particularly common with mutations located in exon 8 (Ryan et al. 2016).

Findings from the current study suggest that individuals with exon 8 mutations may also have particularly long disease durations. An intronic polymorphism in PSEN1 between exon 8 and exon 9 has been reported to show a significant association with late onset disease (Hutton & Hardy, 1997; Wragg et al., 1996). There may be differences in the disease process induced by variants located in this region of *PSEN1*, which drive later ages at symptom onset, longer disease durations and atypical presentations. Considering a 0.05 level of significance and 80% power, the sample size required to replicate the difference in survival between amnestic and atypical cognitive presentations in *PSEN1* mutation carriers (where most variability in cognitive presentation is observed in comparison to *APP* mutation carriers) is N=117.

4.4.7. Study limitations

The study has a number of limitations. First, individuals born over a range of 100 years were included. Whilst this brought the strength of allowing to study generational effects, it may somewhat limit how much findings on disease duration may be generalized to newly diagnosed patients. While the analysis was adjusted for year of birth, replication in larger cohorts of more recently diagnosed individuals is needed. Second, cognitive presentations were classified as 'atypical' on the basis that the initial symptoms did not involve memory but instead comprised behavioural change, language impairment, dyscalculia or executive impairment. Atypical symptoms are often more difficult to recognize as signs of FAD and even sporadic AD, leading to a possible underrepresentation of this group. Nonetheless, the atypical group had a longer disease duration, supporting the notion that there may be biological differences in those with atypical presentations, which underpin both the atypical presentation and the longer disease durations. Third, it was not possible to consider the effects of lifestyle (Rosenberg et al., 2020) (e.g. exercise) or life-course (e.g. socio-economic position or education) factors on survival. This is particularly important as some epidemiological studies indicate that higher education attainment results in longer survival in the healthy population although reasons for this remain unknown (e.g. does education directly causes this difference in outcome by affecting behaviours such as smoking, or is this difference due to other factors like socio-economic or genomic differences (Davies et al., 2018)). Future investigations should study the effect of these variables on survival rates, particularly in light of the finding that genetic factors contribute relatively little to the variance in disease duration in the cohort presented here. Lastly, although the study includes a relatively large number of cases, considering the rarity of FAD, the sample size could be considered a limitation and

further investigation of survival in larger FAD cohorts will be an important direction for future research.

4.4.8. Conclusions

A number of factors may contribute to phenotypic variability in FAD. This represents an important characterisation of variability in survival in FAD while accounting for censoring and is one of the first steps towards allowing patients and their families to plan for the future. The fact that a relatively small variance in survival was explained by mutation specificity was surprising. Furthermore, the seemingly longer survival for individuals with atypical presentations compared to amnestic presentations has direct implications to individuals living with the condition and may also inform the interpretation of disease-modifying trials. More specifically, it remains paramount to try to understand the reasons behind these findings and while estimating survival with a 'number' is a crucial starting point, more detailed investigations of the stages in this progression are needed. The next sub-section will describe this in more detail.

4.5. FAD stages

As an extension to this project, I next considered a 'staging' framework of disease progression in FAD with my supervisor NR.

This description of FAD is based on a seven-stage framework describing the progression of typical Alzheimer's disease (see: https://www.alz.org/alzheimers-dementia/stages), developed by Dr. Barry Reisberg, clinical director of the New York University School of Medicine's Silberstein Aging and Dementia Research Center. These original descriptions of the stages of typical AD are reproduced in the **Appendix 2.** For some individuals, FAD may closely resemble typical SAD. For others, the changes experienced may remain partially distinct until the latest stages.

When given a diagnosis of FAD, people naturally ask what will happen next, how long will they be able to progress with a particular activity, or when and what care will they need. Such questions are often met with the response "we don't know" or "it's different for each person". FAD can affect people in different ways. For example, there can be variability in the symptoms that people experience, the age at which they begin and how quickly they progress. Whilst true, these responses are not terribly helpful. Regardless of this between-person variability, there is a somewhat similar pattern and timing of the "stages" that people with FAD go through.

There are inevitably exceptions, but for most people with FAD having a framework that describes these 'stages' may be helpful in understanding where one is in terms of the disease course. This will hopefully be valuable for discussions regarding treatment, support and planning both for the individual and their families and for professionals, especially as many professionals may never have met someone with FAD.

The latest NIA-AA Research Framework (Jack et al., 2018), included a syndromal staging of cognitive continuum (applicable to all members of a research cohort independent of biomarker profile, see **Appendix 3**) and a numeric clinical staging (only applicable to individuals in the AD continuum). The numeric clinical staging is outlined below.

Stage 1: Performance within expected range on objective cognitive tests

Cognitive test performance may be compared to normative data.

Does not report recent decline in cognition or new onset of neurobehavioral symptoms of concern.

No evidence of recent cognitive decline or new neurobehavioral symptoms by report of an observer (e.g., study partner) or by longitudinal cognitive testing if available.

Stage 2: Normal performance within expected range on objective cognitive tests

Transitional cognitive decline: decline in previous level of cognitive function, which may involve any cognitive domain(s) (i.e., not exclusively memory).

May be documented through subjective report of cognitive decline that is of concern to the participant.

Represents a change from individual baseline within past 1–3 years, and persistent for at least 6 months.

May be corroborated by informant but not required.

Or may be documented by evidence of subtle decline on longitudinal cognitive testing but not required.

Or may be documented by both subjective report of decline and objective evidence on longitudinal testing.

Although cognition is the core feature, mild neurobehavioral changes (e.g. changes in mood, anxiety, or motivation) may coexist. Neurobehavioral symptoms should have a clearly defined recent onset, which persists and cannot be explained by life events.

No functional impact on daily life activities.

Stage 3: Performance in the impaired/abnormal range on objective cognitive tests

Evidence of decline from baseline, documented by the individual's report or by observer (e.g., study partner) report or by change on longitudinal cognitive testing or neurobehavioral behavioural assessments.

May be characterized by cognitive presentations that are not primarily amnestic.

Performs daily life activities independently, but cognitive difficulty may result in detectable but mild functional impact on the more complex activities of daily life.

Stage 4: Mild dementia

Substantial progressive cognitive impairment affecting several domains, and/or neurobehavioral disturbance. Documented by the individual's report or by observer report or by change on longitudinal cognitive testing.

Clearly evident functional impact on daily life, affecting mainly instrumental activities. No longer fully independent/requires occasional assistance with daily life activities.

Stage 5: Moderate dementia

Progressive cognitive impairment or neurobehavioral changes. Extensive functional impact on daily life with impairment in basic activities. No longer independent and requires frequent assistance with daily life activities

Stage 6: Severe dementia

Progressive cognitive impairment or neurobehavioral changes. Clinical interview may not be possible.

Complete dependency due to severe functional impact on daily life with impairment in basic activities, including basic self-care.

From (Jack et al., 2018). **For stages 1–6**: Cognitive test performance may be compared to normative data of the investigator's choice, with or without adjustment (choice of the investigators) for age, sex, education, etc. **For stages 2–6**: Although cognition is the core feature, neurobehavioral changes—for example, changes in mood, anxiety, or motivation—may coexist. For **stages 3–6**: Cognitive impairment may be characterized by presentations that are not primarily amnestic.

Though useful, this is only applicable for individuals meeting the ATN framework in the Alzheimer's continuum. I felt there was a lack of focus on various aspects; namely 1) atypical presentations (i.e. non-amnestic); 2) the consideration of at-risk individuals who may not meet ATN criteria or carry a genetic mutation but still have subjective cognitive complaints and or affective symptoms; 3) the distinction between symptoms and cognitive decline; and 4) a need to incorporate milestones (e.g. functional) for both the individual and the support system around them.

The purpose of the framework presented here is to describe how symptoms and abilities in individuals affected in any way by FAD may change with time and to acknowledge that despite a common underlying condition, people may have different symptoms and experiences. Unlike Dr. Reisberg's framework, the stages defined here are addressed from three points of view: the individuals, the support system of family and friends around them and professionals (in this case clinicians).

To highlight the fact that many individuals will not know their genetic status and therefore be "at-risk" of FAD we outline two possible routes (**Figure 4.11**). One reflects the initial stages of the condition and the other a series of possible "states" someone without a genetic mutation might experience when knowing FAD runs in the family. For example, it is understandably quite common for individuals at-risk of FAD to develop concerns about their memory as they

approach the age at which their parent developed symptoms. Factors such as stress, anxiety and depression can exacerbate problems further, sometimes causing them to progress to a stage at which they score poorly on objective cognitive tests. Recognition that this situation can occur in both gene positive and negative individuals, is clearly important as factors like depression or anxiety may mimic the onset of disease (causing unnecessary additional worries) and most importantly because anxiety or depression are treatable.

- **Stage/State 1:** No symptoms (but known to have an increased risk of FAD from family history or genetic test)
- **Stage/State 2:** Very mild symptoms
- **Stage/State 3:** Mild symptoms with cognitive decline
- **Stage 4:** Moderate symptoms with cognitive decline
- **Stage 5:** Moderately severe symptoms with cognitive decline
- **Stage 6:** Severe symptoms with cognitive decline
- **Stage 7:** Very severe symptoms with cognitive decline

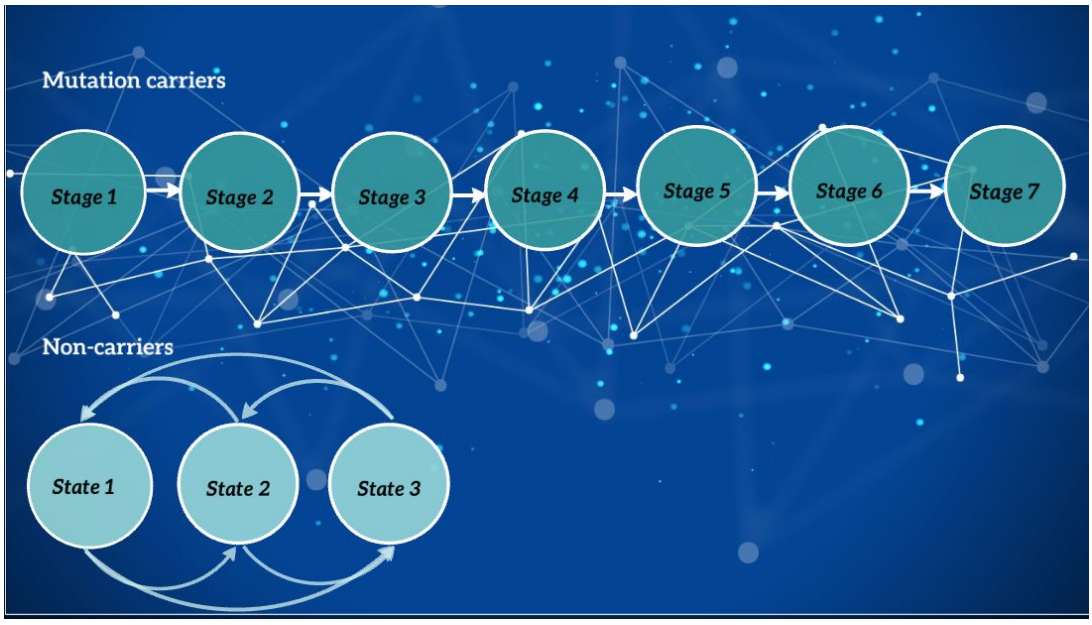


Figure 4.11 Schematic representation of the dynamic phases of FAD.

Additions to the seven-stage framework of typical AD are referenced in **blue**.

Stage/State 1: No symptoms but known to have an increased risk of AD (from family history or genetic test).

Entry criteria: No symptoms but known to have an increased risk of AD (from family history or genetic tests).

The person does not experience any memory, behavioural or other cognitive problems. They have no difficulty with limb movements. No symptoms are noticed by their family or friends or detected during a medical assessment. The individual is able to carry out all usual duties and activities.

Exit criteria for Stage/Stage 1 and entry criteria for Stage/State 2: memory or other cognitive problems may occur in both gene positive and gene negative individuals (although the causes for these are different).

Stage/State 2: Very mild symptoms

The person may feel as if he or she is having memory or other cognitive difficulties (see below). Sometimes, the individual's family, friends and co-workers may also notice symptoms. Occasionally, symptoms may be noticed by the people who know the individual well but not by the individual. At this level, the individual's function in work, social and home environments is not significantly impacted.

In all cases, a medical assessment does not detect any evidence of cognitive impairment.

This level may reflect the earliest signs of AD in individuals that are gene positive but it may also reflect effects of stress, anxiety or depression in those who are gene negative. It is important to exclude treatable causes such as anxiety and depression as this can allow mutation negative individuals to then move back to state 1.

There are three different versions of this stage/state depending on who reports symptoms, and individuals may move between these sub-groups at different times and in different orders.

2.1 Symptoms reported by the individual alone

2.2 Symptoms reported by the individual and their family, friends or co-workers

2.3 Symptoms reported by the individual's family, friends or co-workers but not by the individual

The most common symptoms noticed at this stage involve memory (termed 'amnestic' symptoms by medical professionals). However, for some people the initial symptoms do not

involve memory. The individual may instead have a change in behaviour, for example becoming more withdrawn and less interested in things, or becoming more impulsive, agitated or irritable. Rarely at this stage, they may develop abnormal beliefs (delusions) or hallucinations. Some people may have initial symptoms involving cognitive abilities other than memory, such as calculation skills. Others may develop 'dysexecutive' problems, which means difficulties with planning, organising and carrying out complex tasks. Sometimes the first symptoms involve expressive language, with difficulty finding words or articulating them.

This stage can therefore be further classified by whether the initial symptoms are: **amnestic (A), behavioural (B) calculation (C), dysexecutive (D) or expressive language (E) problems.**

Rarely, the first symptoms may not be cognitive at all but for example may involve **motor function (M).** For example, limb stiffness or jerking movements (myoclonus).

Exit criteria for Stage/State 2 and entry criteria for Stage/State 3: a) in gene positive individuals, difficulties with memory or other aspects occur more often and a detailed medical assessment may start to reveal difficulties; b) in gene negative individuals, if anxiety and depression are treated subjects may revert back to state 1. However, if this is not the case a transition into state 3 is possible.

Stage/State 3: Mild symptoms with cognitive decline

In gene positive individuals, early-stage AD can be diagnosed in some, but not all cases. However, as with the previous level, difficulties may also reflect effects of stress, anxiety or depression in those who are gene negative. It is important to exclude treatable causes such as anxiety and depression as this can allow mutation negative individual to then move back to state 2 or even state 1. In this level difficulties are mild and individuals may now be unable to carry out activities at the same level (e.g. taking longer or completing them less effectively) but are still able to look after their own affairs without assistance.

Friends, family or co-workers notice difficulties. During a detailed medical interview, doctors are able to detect a problem. Sometimes, the individual may have reduced insight into their problems. This stage may therefore be classified as either:

3.1 Symptoms reported by the individual and their family, friends or co-workers

3.2 Symptoms reported by the individual's family, friends or co-workers but not by the individual

Common stage 3 difficulties include: forgetting information that one has just read or been told; losing or misplacing items; increasing trouble with planning or organizing; having noticeably greater difficulty performing tasks in social or work settings; noticeable problems coming up with the right word or name, trouble remembering names when introduced to new people; finding personal belongings; trouble keeping track of appointments including with doctors and meetings with other people; change of habits.

Exit criteria for Stage/State 3 and entry criteria for Stage 4: progressive symptoms often detected during medical assessment. Difficulties with more complex activities progress to the extent of requiring some help at work or at home, for example, with managing finances, preparing meals (i.e. instrumental activities of daily living, IADL). Only individuals who are gene positive will progress to the next stages.

Stage 4: Moderate symptoms with cognitive decline

At this point, a careful medical assessment is able to detect clear-cut and progressive symptoms and objective deficits on cognitive testing. At this level, difficulties may occur with more complex activities at work or at home such as managing finances or preparing meals (i.e. IADL) and the individual may need some help with these.

Common symptoms include: forgetfulness for recent events, impaired ability to perform challenging mental arithmetic, greater difficulty performing complex tasks, such as planning dinner for guests, paying bills or managing finances. Individuals may become withdrawn, especially in socially or mentally challenging situations.

In some patients, there may be additional motor problems such as small involuntary jerking movements, particularly of the fingers (myoclonic jerks). More rarely, there may be limb stiffness, difficulty walking, and problems with coordination or tremor. Visuo-spatial/perceptual abilities may also become affected at this stage (e.g. difficulties recognizing where an object ends and the next starts perceiving objects at night, judging distances). Some people develop seizures, although this occurs more commonly in the later stages of the illness.

Exit criteria for Stage 4 and entry criteria for Stage 5: individuals require help with some basic day-to-day activities (i.e. ADL) such as dressing but are still able to attend to their own bodily needs without assistance (e.g. personal hygiene and continence management).

Stage 5: Moderately severe symptoms with cognitive decline

Gaps in memory and thinking are noticeable, and individuals begin to need help with day-today activities. At this stage, those with AD may: be unable to recall their own address or telephone number or the high school or college from which they graduated; become confused about where they are or what day it is; need help choosing proper clothing for the season or the occasion; still remember significant details about themselves and their family; still require no assistance with eating or using the toilet; develop changes in food preferences (e.g. sweet tooth or preferences for cuisine that relates to a particular time of life).

Exit criteria for Stage 5 and entry criteria for Stage 6: individuals may need assistance with daily activities that attend to their own bodily needs including eating, bathing and grooming. Bowel and bladder problems may also start to emerge.

Stage 6: Severe symptoms with cognitive decline

Memory continues to worsen; personality changes may take place and individuals need extensive help with daily activities including attending own bodily needs.

At this stage, individuals may lose awareness of recent experiences as well as of their surroundings; tend to wander or become lost; remember their own name but have difficulty with their personal history; distinguish familiar and unfamiliar faces but have trouble remembering the name of a spouse or caregiver; need help dressing properly and may, without supervision, make mistakes such as putting pyjamas over daytime clothes or shoes on the wrong feet; experience major changes in sleep patterns (e.g. sleeping during the day and becoming restless at night); need help handling details of toileting; have increasingly frequent trouble controlling their bladder or bowels; experience personality and behavioural changes, including suspiciousness and delusions (such as believing that their caregiver is an impostor) or compulsive, repetitive behaviour. The person may also repetitively articulate certain words or sounds.

Exit criteria for Stage 6 and entry criteria for Stage 7: individuals may lose the ability to respond to their environment and eventually to control movement to the point of requiring constant (nursing) care and attention. Swallowing often becomes impaired.

Stage 7: Very severe symptoms with cognitive decline

In the final stage of this disease, individuals are completely dependent on others for their personal care. They lose the ability to respond to their environment, to carry on a conversation and, eventually, to control movement. They may still say words or phrases but often lose the ability to smile, to sit without support and to hold their heads up. Reflexes become abnormal. Muscles grow rigid. Swallowing is impaired. Maintaining adequate nutrition, hydration and skin integrity can be an issue at this stage.

Exit criteria for Stage 7: end of life. Although AD and other degenerative diseases are life-shortening illnesses, another condition or illness (such as pneumonia) may actually be the cause of the person's death. Pneumonia is listed as the cause of death in up to two thirds of people with dementia. The person's ability to cope with infections and other physical problems will be impaired due to the progression of the disease. In some people, no specific cause of death is found, other than AD.

Interview with a carer

I was able to carry out one interview with a carer of an individual carrying a *PSEN1* p.Pro264Leu mutation located in exon 8. The cognitive presentation was predominantly behavioural (atypical). Notably, it was important to also document some of the caveats of the staging framework. In the PCA staging framework, my colleagues have showed that no one framework will describe every individual equally well as everybody is different. Yet, rather than denying this difference, sharing the framework with individuals with or affected by FAD to see how well these stages describe their experiences or indeed ways in which it diverges is crucial- especially if this is to be a useful document for them. Other caveats previously documented by my colleagues working on PCA include: some people may be diagnosed earlier or later in the overall course of their FAD and may thus not necessarily 'begin' their journey in stage/state 1; perspectives change and hence the use of language 'mild', 'moderate', 'severe' in the early days after a diagnosis might seem very different 10 years later in the illness.

For this interview, the FAD stages framework was shared in advance and on the day of the interview each stage was discussed with the carer. **Table 4.4.** highlights the complexity and variance in the symptoms experienced. Any additions (purple) or discrepancy (red) was noted and discussed. Once this valuable conversation side of the interview was complete, the final step was a visualization and involved the use of post-it notes. I had prepared three sets of post-it notes referencing: a) the stage (yellow); b) symptoms mentioned in the framework

(blue); and c) cognitive functions (green). The carer was then asked to order these in the way which felt most representative to their loved one's journey (**Figure 4.12**). This sorting of items/variables according to subjective experience references a 'Q-sort methodology' in qualitative research.

There are a number of plans to use this document as a guide for those in FAD families and the possibility of future web surveys to capture a clearer picture of the middle and late stages of FAD, the speed at which people progress through these individual stages and further information about heterogeneity across different FAD mutations.

Table 4.4 Findings from the interview carried out.

Stage/State 1: No symptoms		
Entry criteria: *No symptoms but known to have an increased risk of AD (from family history or genetic tests).*	The person does not experience any memory, behavioural or other cognitive problems. They have no difficulty with limb movements. No symptoms are noticed by their family or friends or detected during a medical assessment.	***Exit criteria:*** *Memory **or other cognitive functions** may occur in both gene positive and gene negative individuals.*
Stage/State 2: Very mild symptoms		
Entry criteria: *Memory **or other cognitive functions** occur but medical assessment does not detect difficulties.* **2.1** Symptoms reported by the individual alone **2.2** Symptoms reported by the individual and their family, friends or co-workers **2.3** Symptoms reported by the individual's family, friends or co-workers but not by the individual	Stage 2 subtypes: Amnestic (A), behavioural (B) calculation (C), dysexecutive (D), expressive language (E), motor (M) problems. **Symptoms:** -Aggressive behaviour towards one self or other people -Planning/executive function remained preserved -Visuo-spatial memory may start to become impaired i.e. reflective surfaces may be a problem -Insight might be preserved -Mobility: preserved -Obsessive behaviours i.e. food, money, personal possessions. -Sense of humour may be preserved. -May develop abnormal beliefs (delusions) or hallucinations (L)	***Exit criteria:*** *a) In **gene positive** individuals, difficulties with memory or other cognitive functions may start to occur more often and a detailed medical assessment may start to reveal difficulties.* *b) In **gene negative** individuals, if anxiety and depression are treated subjects may revert back to state 1. However, if this is not the case a transition into state 3 is possible.*
Stage/State 3: Mild symptoms with cognitive decline		
Entry criteria: *Medical assessment detects difficulties in one or more of these aspects: memory, orientation and judgement, problem solving, community affairs, home & hobbies, personal care.* **3.1** Symptoms reported by the individual and their family, friends or co-workers **3.2** Symptoms reported by the individual's family, friends or co-workers but not by the individual	**Symptoms:** -Forgetting information that one has just read or been told -Losing or misplacing items -Increasing trouble with planning or organizing. -Having noticeably greater difficulty performing tasks in social or work settings -Noticeable problems coming up with the right word or name -Trouble remembering names when introduced to new people -Finding personal belongings	***Exit criteria:*** *Progressive symptoms detected during medical assessment.* *Difficulties may be detected preparing meals, snacks at home, or managing finances.* *Individuals who are gene negative will also exit this level and regress to previous states.* *Only individuals who are gene positive will progress to the next stages.*

	-Keeping of appointments including doctors and meetings with other people -Increase habit of writing things down -Mobility might still be preserved. -Only in hindsight partners might notice some problems with gait. -Sense of humour might sometimes still be preserved. -Hallucinations about people and animals -Tantrum-like behaviours; burst of anger	
Stage 4: Moderate symptoms with cognitive decline		
Entry criteria: *Progressive symptoms detected during medical assessment.* At this point, a careful medical assessment is able to detect clear-cut and progressive symptoms and objective deficits on cognitive testing.	**Symptoms:** -Forgetfulness for recent events -Impaired ability to perform challenging mental arithmetic -Greater difficulty performing complex tasks, such as planning dinner for guests, paying bills or managing finances. -Withdrawn-social or mentally challenging situations. -In some patients, additional motor problems such as small involuntary jerking movements, particularly of the fingers (myoclonic jerks). -More rarely, there may be limb stiffness, difficulty walking(E), and problems with coordination or tremor. -Visuo-spatial/perceptual abilities may also become affected at this stage (e.g. difficulties recognizing where an object ends and the next starts perceiving objects at night, judging distances). -Some people develop seizures, although this occurs more commonly in the later stages of the illness (like stage 6 or 7).	***Exit criteria:*** *Individuals need help with more basic day-to-day activities such as dressing and sometimes more general difficulties with mobility (E).*
Stage 5: Moderately severe symptoms with cognitive decline		
Entry criteria: *Individuals need help with more basic day-to-day activities such as dressing and sometimes more general difficulties with mobility(E).*	**Symptoms:** - Gaps in memory and thinking are noticeable, and individuals begin to need help with day-today activities. -Be unable to recall their own address or telephone number or the high school or college from which they graduated -Become confused about where they are or what day it is -Need help choosing proper clothing for the season or the occasion and sometimes even putting them on -Still remember significant details about themselves and their family	***Exit criteria:*** *Individuals may need more extensive help with daily activities including eating, bathing and grooming.* *Bowel and bladder problems may also start to emerge.*

	-Still require no assistance with eating or using the toilet -Develop changes in food preferences (e.g. sweet tooth or preferences for cuisine that relates to a particular time of life) (E). -Some individuals may no longer be able to feed themselves. -Loss of speech -Walking may start to become a more prominent problem.	
Stage 6: Severe symptoms with cognitive decline		
Entry criteria: *Individuals may need more extensive help with daily activities including eating, bathing and grooming.* *Bowel and bladder problems may also start to emerge.*	**Symptoms:** - Memory continues to worsen, personality changes (E) may take place and individuals need extensive help with daily activities. -Lose awareness of recent experiences as well as of their surroundings. -Tend to wander or become lost -Remember their own name but have difficulty with their personal history -Distinguish familiar and unfamiliar faces but have trouble remembering the name of a spouse or caregiver -Need help dressing properly (E) -Experience major changes in sleep patterns -Need help handling details of toileting (E) -Have increasingly frequent trouble controlling their bladder or bowels (E) -Experience personality and behavioural changes, including suspiciousness and delusions (such as believing that their caregiver is an impostor) or compulsive, repetitive behaviour. The person may also repetitively articulate certain words or sounds (E). -May still be responsive to sounds and surroundings. -May lose the ability to sit without support.	***Exit criteria:*** *Individuals may lose the ability to respond to their environment and eventually to control movement.* *Swallowing often becomes impaired.*
Stage 7: Very severe symptoms with cognitive decline.		
In the final stage of this disease, individuals lose the ability to respond to their environment, to carry on a conversation and, eventually, to control movement. They may still say words or phrases (E). At this stage, they also lose the ability to smile, to sit without support and to hold their heads up. Reflexes become abnormal. Muscles grow rigid and stiff (E). Swallowing is impaired. Maintaining adequate nutrition, hydration and skin integrity can be an issue at this stage.		

Any additions to each stage are referenced in purple and any discrepancy mainly in the order of symptoms described is referenced in red; E=earlier (i.e. symptom appeared earlier than mentioned in the stage); L= later (i.e. symptom appeared later than mentioned in the stage); AD=Alzheimer's disease.

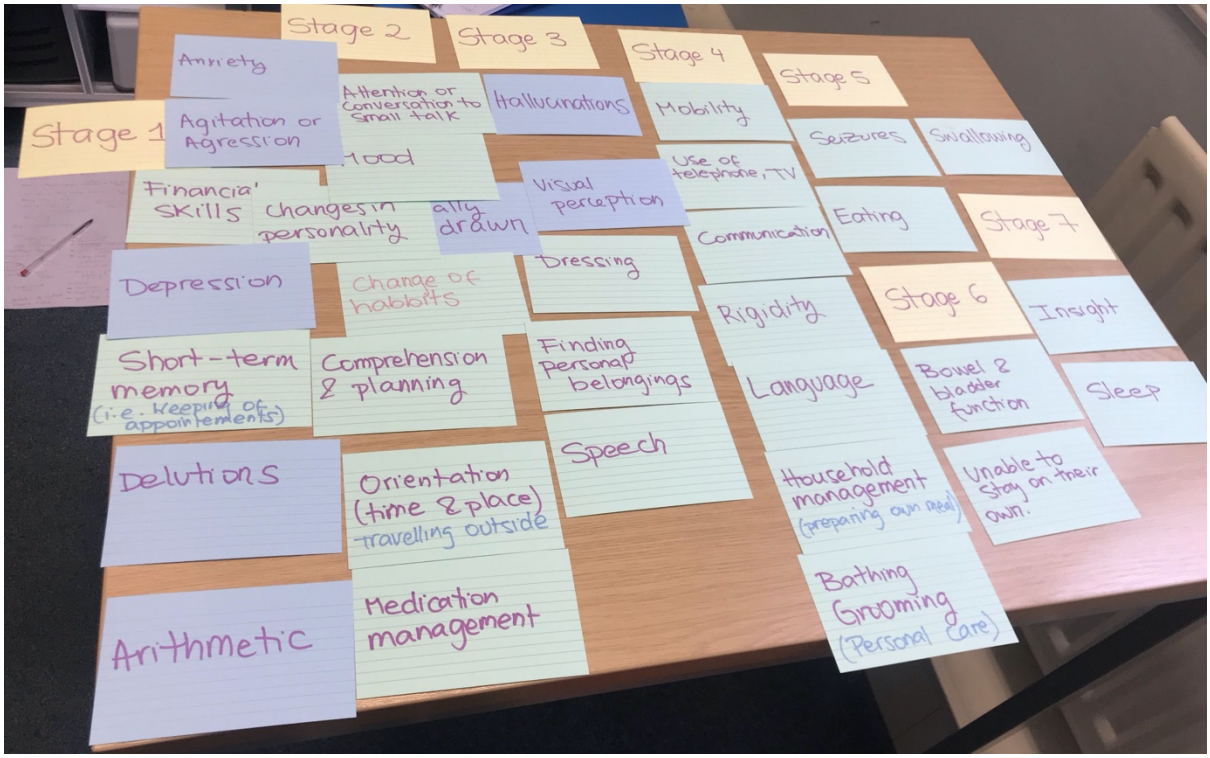


Figure 4.12 Picture shows the order in which symptoms where mentioned.
During the interview, the carer placed each card in proximity to the corresponding stage.

This framework was also discussed at a FAD Rare Dementia Support group meeting in May 2019. Reflections arising from this meeting include the need to establish the degree of severity of each symptom as well as a differentiation of when specific symptoms started and when the function was completely lost (i.e. problems with speech may arise in stage 3 and speech might be completely lost by stage 5). In addition, where possible, it might be helpful to establish a relationship between the loss of function and activities of daily living. For instance, dressing apraxia may result from impairments relating to gait, visuo-spatial abilities, attention or a mixture of all. In this regard, it is relevant to mention once again the work by Calia and colleagues, as an example of the important associations between activities of daily living and cognition and more specifically how memory binding may account for impairment of abilities that support instrumental functions (Calia et al., 2020). VSTM binding will be discussed in the next data chapter.

5. VSTM DEFICITS IN FAD: A LONGITUDINAL OBSERVATIONAL STUDY

This chapter focuses on the VSTM deficits in FAD and their relationship to disease progression and EYO.

5.1. Introduction

It is well established that episodic memory impairment is a central, defining feature of AD (Dubois et al., 2007; McKhann et al., 1984). By contrast, less research has focused on STM, the ability to temporarily *maintain* information over seconds (Atkinson & Shiffrin, 1968, 1971). Classically, the STM has been tested using 'span' measures where participants are asked to remember a string of stimuli (Groeger et al., 1999). Although such quantal measures have been fundamental to developing our understanding of memory function, they are not as sensitive to detecting changes in memory *resolution* due to their binary nature (correct *vs* incorrect recall). In 2014, Ma and colleagues (Ma, Husain & Bays, 2014) proposed a new approach to study the resolution with which items are retained, arguing that just because an individual fails to recall an item correctly does not imply they had no memory of it at all. Delayed-reproduction tasks (e.g. Peich, Husain, and Bays 2013; Pertzov et al. 2012, 2013) rely on remembering a feature and reproducing the exact stored features after a retention period using a *continuous analogue* response space (Bays et al., 2009; Gorgoraptis et al., 2011; Wilken & Ma, 2004). In recent studies, delayed-reproduction tasks have been reported to be more sensitive than conventional span measures of STM, especially to investigate clinical populations (Zokaei et al., 2015).

A number of studies have found deficits in presymptomatic or preclinical stages of AD (e.g. (Liang et al., 2016; Parra et al., 2010a)) including that by Liang and colleagues in which presymptomatic FAD carriers showed deficits for object-location binding and localisation of the target position, in the high load condition of the "What was where?" task (Liang et al., 2016). "What was where?" is a relational binding delayed-reproduction task which measures memory for object identification, localisation and object-location binding under different conditions of memory load and delay (thought to increase difficulty of performance (Pertzov et al., 2012)).

While promising results emerged from these studies, longitudinal investigations that follow individuals at-risk of AD or FAD from a presymptomatic stage through MCI and symptomatic AD are lacking (Liang et al., 2017).

In light of these findings, a series of questions remained unanswered:

1. Are the cross-sectional deficits in VSTM binding in preclinical AD also reflected in longitudinal decline in task performance?

2. Given that an individual's expected age at symptom onset may be estimated from their parental onset, what is the relationship between an individual's VSTM performance and EYO at the time of testing?

3. For comparison, is longitudinal decline of cognition in presymptomatic and symptomatic mutation carriers seen in other more traditional neuropsychology tasks?

The aim of this study is to first evaluate cross-sectional VSTM performance in a larger sample to that of Liang and colleagues (Liang et al., 2016). Secondly, to investigate how VSTM in both presymptomatic and symptomatic FAD mutation carriers, changes with EYO. Finally, for comparison I evaluate longitudinal decline in traditional neuropsychology tasks. The main hypothesis is that, analogous to previous cross-sectional investigations, a faster rate of object-location binding (measured by the proportion of swap errors) will be detected in presymptomatic and symptomatic FAD carriers in comparison to controls. Furthermore, VSTM impairments will become more pronounced with EYO up until a plateau is reached and cognitive demands exceed available resources resulting in poor performance at every visit. Lastly, a faster rate of decline in presymptomatic carriers compared to controls will also be observed in traditional neuropsychology memory tasks (e.g. recognition memory) but at a later time than changes in relational binding due to the greater sensitivity of relational binding to preclinical AD.

5.2. Methods

5.2.1. Study design and participants

As previously mentioned in the **GENERAL METHODOLOGY,** individuals at-risk of FAD were recruited into the study if there was an autosomal dominant family history of AD and known pathological mutation in *PSEN1* or *APP* genes in at least one affected family member. Additionally, for this study, healthy individuals (without a family history of AD) were recruited from our research database. Inclusion criteria required participants to have normal or corrected-to-normal visual acuity and colour vision and ≥ 70% average accuracy in identification performance at baseline visit (see (Liang et al., 2016)).

Genetic results available for all at-risk individuals, on either clinical or research basis but research genetic results were only shared with the statistician involved in the study and were not disclosed to the participants or to other researchers.

The study included symptomatic carriers, PMCs and controls. Symptomatic individuals were mutation carriers who had cognitive symptoms consistent with AD. PMC individuals were mutation carriers who had not developed symptoms and who scored zero on the CDR scale (Morris, 1993). Control participants consisted of both non-carriers (at-risk individuals who tested negative for pathological mutations) and healthy individuals (from our research database). As per Liang and colleagues, EYO was used as an approximation of how far individuals (presymptomatic and symptomatic) were from symptom onset (Liang et al., 2016). This was based on an individuals' age at the time of assessment subtracted from the age at which their affected parent developed symptoms (Bateman et al., 2012; Ryman et al., 2014). I also considered how performance varied continuously with actual years to/from symptom onset (AYO) for symptomatic carriers and PMC who converted into symptomatic carriers throughout the study (n=3). Actual age at onset or age at onset (AAO) was defined as the age at which progressive symptoms of FAD, were first noticed by the individual or someone who knew the patient well.

As well as investigating how performance varied continuously with EYO (grouping symptomatic and presymptomatic carriers together), I grouped individuals by their symptom status and proximity to symptom onset at the baseline assessment into: symptomatic carriers; 'early' PMCs (more than 8.5 years from expected onset), 'late' PMCs (within 8.5 years from expected onset) and controls. The cut-off of 8.5 years corresponded to the median split of EYO of PMCs in this dataset.

The cross-sectional analysis included 99 individuals: 67 non-carriers and 32 mutation carriers, 9 of whom were symptomatic. Differences between this cross-sectional study and Liang and colleagues (Liang et al., 2016) were: the addition of n=17 at-risk (mutation carriers and non-carriers) individuals; n=2 symptomatic carrier and the exclusions of n=1 at-risk and n=1 symptomatic individuals (see **Figure 5.1** for reasons). Note that mutation status of these at-risk individuals is not disclosed to prevent unblinding of genetic status. Baseline characteristics of the cross-sectional N=99 study is presented in **Table 5.1.**

The longitudinal analysis included 48 participants who attended between 2 and 5 visits (median 3), at intervals ranging from 0.5 to 3.9 years (median 1.3) (Mean follow-up time: controls= 2.8 [SD 1.7] years, range=1-6; early PMC= 3.7 [1.7] years, range=1-6 years; late PMC=3.4 [1.7] years, range=1-6; symptomatic=2.6 [0.7] years, range=2-4)). Baseline performance for the longitudinal cohort is presented in **Table 5.2.** Importantly, when follow-up lengths were compared between groups it was noted that data was 'missing at random'. In other words, there was no indication that the differences in follow-up length were due to a

specific reason or that reasons for this differed between groups. The assumption that was therefore made was that if individuals were observed for a longer period, they would follow a similar trajectory.

Figure 5.1 below provides further details on the analyses and participants included.

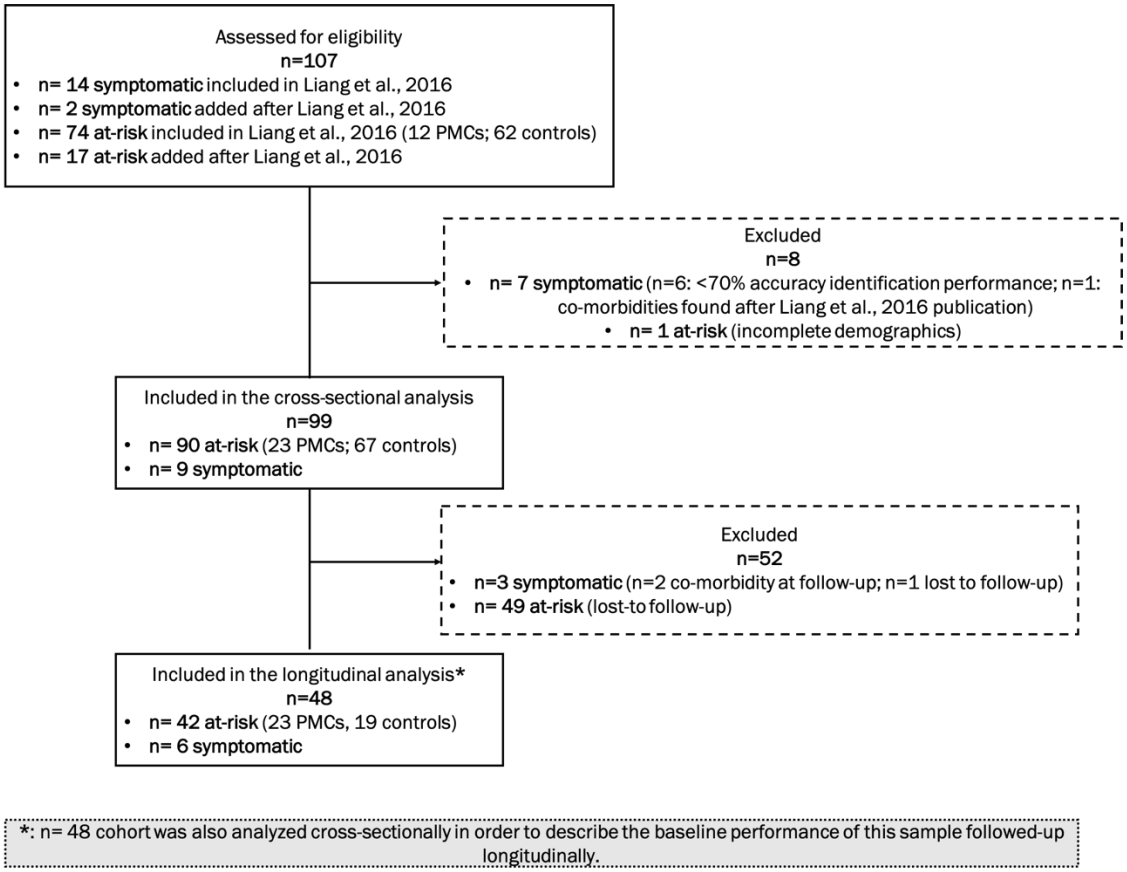


Figure 5.1 Participants included in the analyses.

PMC=presymptomatic mutation carrier. Note that mutation status is not explicitly stated for n=17 at-risk to preserve blinding of genetic status.

5.2.2. Procedures and data collection

The study protocol included a clinical and neuropsychological assessment and the "What was where?" VSTM task.

Detailed interviews were conducted with individuals at-risk of FAD and their close informants by a neurologist to assess for the presence of cognitive or behavioural symptoms attributable to AD. AD was diagnosed in accordance with the Dubois criteria (Dubois et al., 2007, 2010). The MMSE (Folstein, and McHugh 1975), the CDR (Morris, 1993) and HADS questionnaire (Zigmond & Snaith, 1983) were administered.

The neuropsychological test battery, traditionally conducted in the department, was performed by a psychometrician and included measures of various cognitive domains: episodic memory (RMT for words and faces; (Warrington, 1996)); working memory (digit span (Wechsler 1987)); intellectual function (WASI) (Wechsler 1999)); executive function (Stroop (Stroop, 1935)); confrontational naming (GNT (McKenna & Warrington, 1983); vocabulary (BPVS (Dunn & Dunn 2009)); arithmetic (Graded Difficulty Arithmetic Test (Jackson & Warrington, 1986)), visual perception (VOSP OD (Warrington & James, 1991)); processing speed (digit symbol test (Wechsler, 1981)) and estimated premorbid intelligence (the NART (Law and O'Carroll 1998; Nelson 1982). Of note, completion rate for all neuropsychology tests was not 100% due to changes in the battery over the years (see **Table 5.2**).

The stimuli and procedure of the 'What was where?' task have been described in detail in previous papers (Liang et al., 2016; Pertzov et al., 2012) (**Figure 5.2**). Participants sat approximately 42 cm in front of an interactive touch-sensitive screen (Dell Inspiron One 2320) with a 1920 × 1080-pixel matrix corresponding to approximately 62 × 35 deg of visual angle. In each trial, 1 or 3 fractals were displayed on the screen in random locations, presented on a black background. The locations of the fractals were generated in a pseudo-randomised manner by a MATLAB script (MathWorks, Inc). The script imposed the following restrictions: fractals were always at least 9 deg away from each other to avoid crowding and to ensure that there was a clear zone of 4.5 deg around each fractal which is necessary for the calculation of swap errors (see experiment outcomes), and fractals were at least 6.5 deg from the centre of the screen and 3.9 deg from the edges. Participants were asked to look at the fractals and to try to remember their identities and locations. 1-fractal trials are referred to as 'low load' and 3-fractal trials are referred to as 'high load'. The low load trials were displayed for 1 second (s) whereas the high load trials were displayed for 3 s. This was followed by a blank screen for either a short or long delay (1s or 4s), and then a test array appeared in which 2 fractals were displayed along the vertical meridian. One of these fractals had appeared in the memory array on the previous screen (the target) and the other was a foil or distractor. The foil was not an unfamiliar object, but was part of the general pool of 60 fractal presented across the experiment in another trial (all fractals were generated using: http://sprott.physics.wisc.edu/fractals.htm; see **Appendix 4** for further details on fractals used). Participants were instructed to touch the fractal that they remembered seeing and drag it to the location where they think it was originally presented. This provided a continuous measure of localisation error. There was no time-limit for reporting the location – the tester pressed the space bar to initiate the next trial when the participant was ready. Each participant performed a practice block of 10 trials followed by two test blocks. Each test block consisted

of 10 trials with 1 fractal and 40 trials with 3 fractals and a balanced number of trials with 1s or 4s delay between memory and test arrays. The 100 trials were the same for all participants (i.e. the same fractals were presented in the same locations) but the trials were presented in a random order for each participant. The reason for this was to avoid the results being confounded by practice effects on the one hand (familiarity with the procedure could cause performance to improve throughout the task) and by interference effects on the other hand (as fractals appear more than once during the task, the foil in the test array could be recognised from a previous trial, which could increase the likelihood of errors in object identification throughout the task).

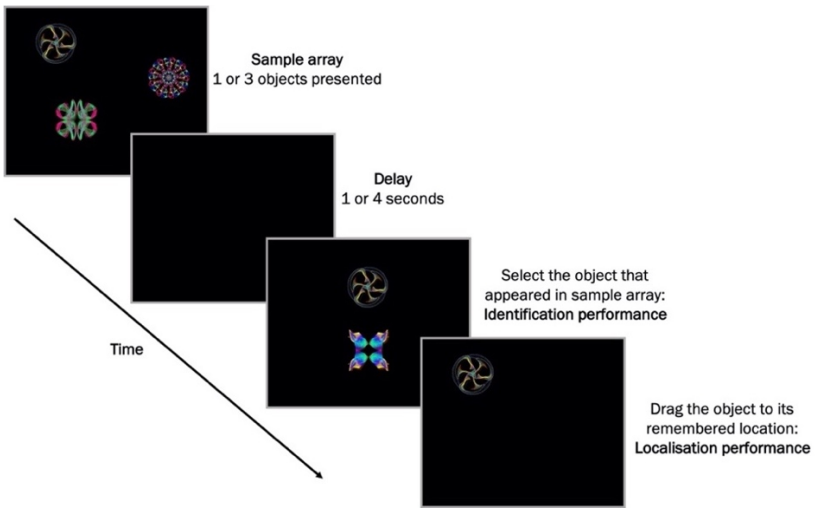


Figure 5.2 Schematic of "What was there?" (adapted from (Liang et al., 2016) under the terms of the Creative Commons Attribution License (CC BY)).

As for the previous cross-sectional study (Liang et al., 2016), the experiment outcomes were:

- **Identification performance:** proportion of trials where the correct object was chosen.
- **Localisation error:** the distance (in deg of visual angle) between the centre of the target object once placed in its remembered location and its true (original) location in the memory array (only correctly identified objects).
- **Swap errors:** the percentage of correctly identified objects placed within 4.5 deg eccentricity of other fractals in the original array so that an object could not be swapped with more than one object (3-items condition only).
- **Nearest item control (NIC):** the distance (in deg of visual angle) between the location reported by the participant and the closest of the three original locations from the memory array. It provides an index of localisation precision regardless of object identity (Liang et al., 2016; Pertzov et al., 2013) (3-items condition only).

5.2.3. Statistical analysis

In all analysis, group (e.g. late PMCs *vs* controls) was the main predictor of interest. Baseline demographics and neuropsychology scores were compared between symptomatic carriers, early PMCs, late PMCs and controls using ANOVA, or Kruskal-Wallis test where the distribution of the variable was skewed. Fishers' exact test was used to compare the sex distribution between the groups.

All models included a random slope and intercept to allow clustering by participant (with separate random intercept terms for controls and mutation carriers where these additional terms improved model fit). The models for localisation and NIC additionally included a random effect of visit, nested within the participant. All analysis of VSTM was adjusted for delay (1 *vs* 4s), block (1 *vs* 2), number of items (1 *vs* 3, where relevant), sex, age at baseline, and NART at baseline.

Due to a skewed distribution localisation error and NIC were log transformed and the proportion of swap errors was square root transformed before analysis. Interaction tests were used to examine whether group differences in cross-sectional performance, changes in performance over time, or the relationship with EYO varied by delay, block and number of items.

5.2.3.1. Cross-sectional analysis

VSTM performance at the baseline visit was compared between controls and each of symptomatic carriers, early PMCs and late PMCs using logistic regression models for object identity and linear regression model for all other measures. Robust standard errors were used to account for repeated measures.

5.2.3.2. Longitudinal analyses

In order to address the first research question (i.e. are the cross-sectional deficits in VSTM binding in preclinical AD also reflected in longitudinal decline in task performance?); rates of change in VSTM function (for each metric) were investigated between controls and each of symptomatic carriers, early PMCs and late PMCs. In order to address the second research question (i.e. what is the relationship between an individual's VSTM performance and EYO at the time of testing?), I investigated the association between VSTM performance and EYO as a continuous measure (in presymptomatic and symptomatic mutation carriers). This

continuous approach was performed recognizing that although separating PMCs by the median split, is widely used (e.g. (Weston et al., 2020)) especially in genetic conditions where the chances of exhibiting symptoms increases with proximity to onset – it also reduces the variance in the predictor. In addition, for symptomatic carriers at baseline (n=6) and late PMCs who converted into symptomatic throughout the study (converters' n=3), I examined the association between VTSM performance and AYO as a continuous measure. The rationale for this was that, where available, actual age at onset would provide a more precise estimation than expected age at onset, of how VSTM function varied with proximity to onset.

Longitudinal change in object identity was analysed using a mixed effects logistic regression model and analysis of the other VSTM outcomes used a linear mixed effects model. The linear mixed effects models also included separate residual error terms for symptomatic mutation carriers, PMCs, and controls to allow for heteroscedasticity.

The models examining proximity to onset (EYO and AYO, as a continuous measure) in mutation carriers included age at visit as a predictor to account for any effects of healthy ageing. Estimation of the effect of age included data from both controls and mutation carriers in order to have an estimation of the predicted mean difference between controls and mutation carriers by EYO and AYO. This predicted mean performance was calculated for controls and by EYO and AYO in the carriers, setting age and NART at the average of the sample and for an equal balance of sexes and task conditions (block, delay and load).

Finally, in order to address the third research question (i.e. is longitudinal decline of cognition in presymptomatic and symptomatic mutation carriers seen in other more traditional neuropsychology tasks?), analysis was conducted to compare longitudinal change in neuropsychology performance between controls and each of symptomatic carriers, early PMCs and late PMCs. For each outcome, rates of change were compared between group by including group at the baseline assessment and an interaction between group at the baseline assessment and follow-up length as predictors in each model.

Mixed effects linear regression was used for analysis of verbal IQ, performance IQ, arithmetic, GNT and NART. A mixed effects logistic regression model was used for RMT words, RMT faces and VOSP. Mixed effects ordinal logistic regression model was used for digit span forwards and digit span backwards. All models adjusted for sex, age at baseline, and NART at baseline. Controls were included in all models to allow for changes with increasing age.

The random effects included in the linear regression models were: a random intercept for GNT and NART and a random slope and intercept for carriers and a separate random intercept for controls for performance IQ, verbal IQ and arithmetic. All the mixed effects linear regression

models had separate residual error terms for symptomatic mutation carriers, PMCs, and controls to allow for heteroscedasticity. The regression models for RMT words, RMT faces, VOSP, digit span forwards and digit span backwards included separate random intercepts for carriers and controls.

5.3. Results

5.3.1. Cross-sectional cohort N=99

5.3.1.1. Demographics and traditional neuropsychology

Sixty-seven controls and 32 carriers with cross-sectional data were available for the VSTM binding task. Early PMCs were on average 12.9 years away from expected onset and compared to controls were on average younger (p=0.062) and had lower scores in: verbal IQ (p=0.013), BPVS (p=0.004) and NART measures (p=0.006). Late PMCs were on average 5.8 years from expected onset and compared to controls, had significantly lower education (p=0.023) and baseline anxiety (p=0.035) and depression scores (p=0.020) and had significantly lower scores for verbal IQ (p=0.007) but similar scores on remaining measures. Symptomatic carriers were on average 3.0 years after expected onset, and as expected were older (p=0.026), had lower MMSE (p<0.001), higher global CDR (p<0.001) and significantly worse scores on neuropsychology tasks including arithmetic (p=0.007), RMT for words (p<0.001), digit span (p=0.014) and Stroop (p=0.001) (**Table 5.1**).

5.3.1.2. VSTM performance

Consistent with previous reports, across the sample as a whole, VSTM performance was significantly worse with higher load (3-items *vs* 1-item) (p<0.001 for all metrics). Longer delay (1 *vs* 4s) was also associated with worse localisation, NIC performance (both p<0.001), and identification performance (p=0.008) but did not affect swaps proportion (p=0.255).

Symptomatic carriers had 44.0 [95% CI 25.4, 56.7] % lower odds of correctly identifying the target (difference in OR=0.57, p<0.001), 46.0 [20.1, 77.5] % greater localisation error (p<0.001), 17.5 [1.4, 36.2] % greater NIC error (p=0.032) and made a greater proportion of swap errors (p<0.001) in comparison to controls (**Table 5.1,** **Figure 5.3**). While there was a trend for higher NIC error in the late PMC group (10.5 [-1.4, 23.9] % greater error p=0.085), no significant differences were observed at a presymptomatic level (**Table 5.1**). For comparison between localisation and NIC error, in the 3-items condition, symptomatic carriers

had significantly greater localisation error compared to controls (67.2 [41.4, 97.5] % greater error, $p<0.001$). There was little evidence that either PMC group had greater localisation error than controls (adjusted difference: late PMC 5.1 [-10.4, 23.4] % difference, $p=0.534$; early PMC -0.9 [-21.0, 24.3] % difference $p=0.938$). The finding of much smaller difference in localisation between symptomatic carriers and controls after NIC suggests that some of the greater localisation error in this group at baseline may be accounted for by a tendency to mislocalise the fractal to the location of the nearest fractal (regardless of whether it was the target).

There was no significant interaction between group and delay, block or number of items in identification, localisation or NIC performance metrics. However, there was a significant interaction between delay and the proportion of swap errors ($p=0.039$), whereby symptomatic carriers showing larger differences compared to controls in the long delay than the short delay. Although there was no significant interaction with block ($p=0.110$), I investigated performance in the first block by delay following Liang and colleagues finding of a significantly higher proportion of swap errors in the PMC group compared to controls, in the first block long delay condition (Liang et al., 2016). Symptomatic carriers made a greater proportion of swap errors in both blocks (both blocks $p<0.001$), but there was evidence for higher swaps in the 4s, block 1 condition, only. No further significant differences emerged in the 4s, block 1 condition, (early PMCs: $p=0.057$, late PMCs: $p=0.996$) (**Table 5.1**).

Table 5.1 Baseline demographics, neuropsychology and VSTM performance by group for N=99.

	Controls (N=67)	Early PMCs (N=12)	Late PMCs (N=11)	Symptomatic carriers (N=9)
Demographics				
Sex: N (%) Male	34 (50.7)	3 (25.0)	7 (63.6)	6 (66.7)
Age (yrs)	39.4 (8.1)	34.8 (6.4)	37.0 (5.0)	**48.1 (9.8)****
EYO (yrs)	NA	-12.9 (4.7)	-5.8 (1.8)	3.0 (4.1)
AYO (yrs)	NA	NA	NA	3.1 (4.0)
Education (yrs)	15.4 (2.7)	14.3 (2.5)	**13.3 (2.5)***	13.9 (2.9)
MMSE	29.5 (0.8)	29.3 (0.9)	29.5 (0.8)	**25.1 (3.7)****
CDR global	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	**0.6 (0.2)****
Anxiety	6.1 (3.8)	7.9 (4.6)	**3.9 (3.9)***	7.0 (4.5)
Depression	3.2 (2.8)	2.9 (4.0)	**1.3 (1.6)***	2.4 (2.1)
Neuropsychology tests				
Performance IQ	110.5 (16.3)	106.0 (15.7)	101.4 (10.1)	100.4 (12.1)
Verbal IQ	109.9 (14.9)	**96.1 (15.1)***	**95.4 (13.5)****	99.4 (18.8)
Arithmetic total/24	16.7 (6.8)	13.9 (5.0)	14.3 (4.6)	**10.3 (5.8)****
RMT faces/50	41.1 (7.2)	41.0 (4.2)	43.8 (4.5)	40.3 (3.7)
RMT words/50	47.0 (5.0)	48.7 (2.2)	46.5 (2.8)	**35.3 (10.0)****
Digit span forwards/8	7.1 (1.2)	6.8 (1.0)	7.4 (1.1)	**6.0 (1.5)***
Digit span backwards/7	5.2 (1.2)	5.7 (1.3)	5.4 (1.1)	4.3 (1.6)
BPVS/150	142.5 (8.8)	**135.0 (14.4)****	139.8 (10.1)	135.9 (11.8)
GNT/30	20.9 (4.6)	17.8 (5.8)	19.2 (5.4)	18.8 (7.2)
NART/50	31.8 (8.9)	**24.1 (8.6)****	27.7 (10.7)	25.4 (13.2)
VOSP OD/20	18.0 (2.8)	17.8 (1.8)	18.3 (1.3)	17.6 (1.5)
Stroop (s)	50.3 (14.0)	45.8 (12.2)	52.6 (14.1)	**78.2 (22.4)****
VSTM performance				
Identification (% correct)				
Overall	91.6 (4.8)	90.2 (6.3)	92.0 (3.9)	**81.9 (5.0)****
Localisation error (deg)				
Overall	4.4 (1.3)	4.5 (1.3)	4.6 (1.1)	**7.8 (1.8)****
1-item # all delays	2.4 (0.9)	2.2 (0.3)	2.5 (0.8)	3.8 (1.8)
3-items # all delays	6.5 (2.0)	6.8 (2.4)	6.6 (1.7)	**11.7 (2.4)****
NIC error (deg)				
Overall: all delays	3.7 (0.9)	3.8 (0.9)	4.1 (0.6)	**4.9 (1.3)***
Swap error (%)				
Overall	10.6 (5.3)	11.7 (4.7)	10.2 (5.9)	**22.6 (8.1)****
Block 1, 1s	12.0 (8.4)	12.4 (9.2)	9.9 (5.0)	21.2 (12.6)
Block 1, 4s	13.2 (8.7)	18.7 (9.2)	15.0 (10.8)	**23.2 (18.0)***

Unadjusted mean values are given with SD unless otherwise stated. SD = standard deviation; NA= not applicable; PMC= presymptomatic mutation carrier; EYO=estimated years to/from symptom onset (a negative value indicates a younger age than their estimated age at symptom onset); AYO=actual years to/from onset (positive values indicate years post onset); Anxiety and depression scores from HADS= hospital anxiety and depression scale; NIC=nearest item control; IQ=intelligence quotient; MMSE=mini mental state examination; CDR=clinical dementia rating scale; RMT=recognition memory test; GNT=graded naming test; VOSP OD=object decision from the visual object and space perception battery. Digit spans forwards and backwards are taken from the WMS-R= Wechsler Memory Scale. Neuropsychology data were available at baseline for: 64 participants for performance IQ, verbal IQ; 98 for arithmetic total, GNT, NART, VOSP; 99 for RMT faces, RMT words, digit span forwards, digit span backwards; 71 for BPVS; and 78 for Stroop (s). #localisation measures are presented by item-number to allow for comparison with NIC findings. Bold=significant; *: the difference between the patient group and controls for that variable was significant at $p<0.05$. **: the difference between the patient group and controls for that variable was significant at $p<0.01$.

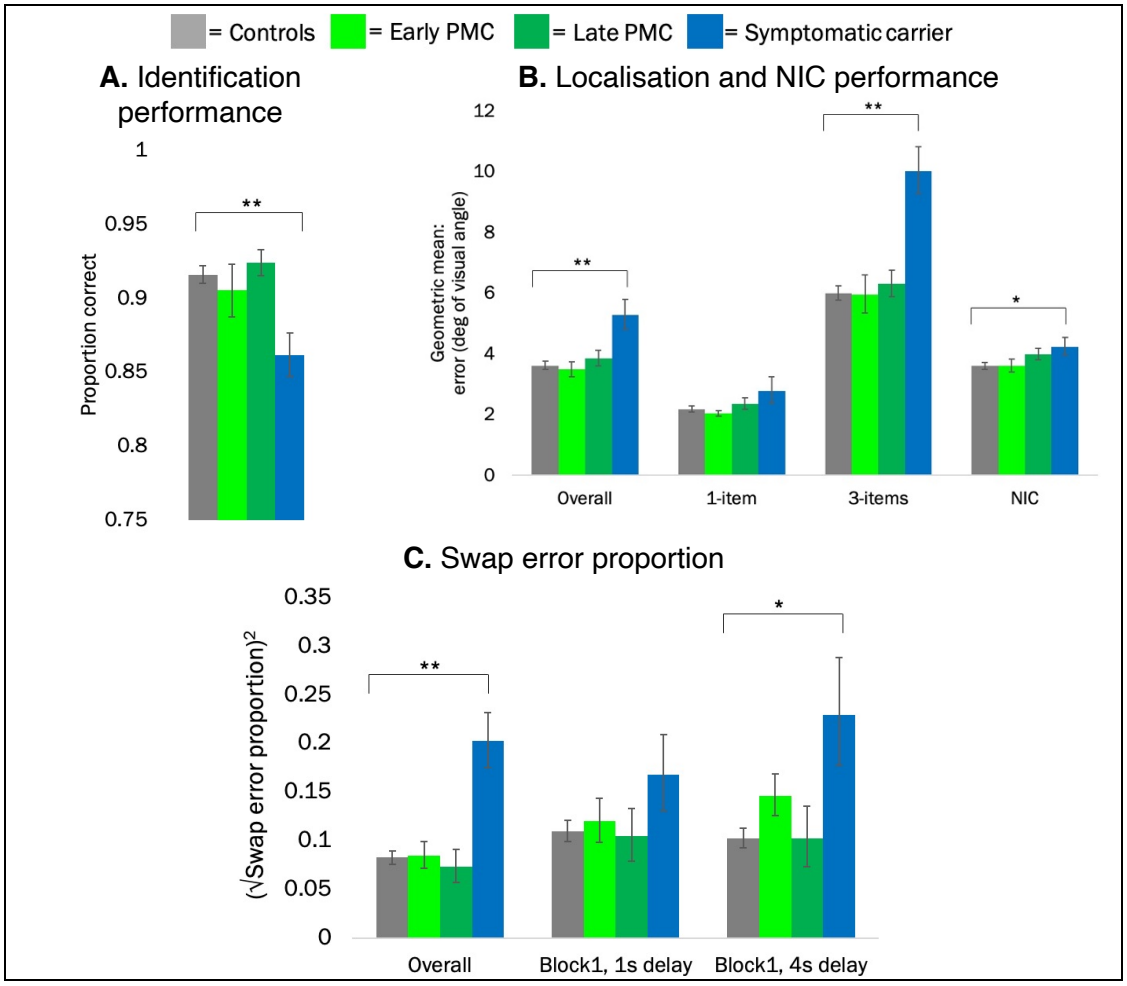


Figure 5.3 Cross-sectional mean performance by group (from model adjusted for age, sex and NART) for N=99.

A. Identification performance; **B.** Localisation and NIC error; **C.** Swap error proportion overall and by delay in block 1. Error bars show +/- standard error of the mean. PMC=presymptomatic mutation carrier. NIC=nearest item control. *: significant at $p<0.05$.; **: significant at $p<0.01$.

A more direct comparison between this N=99 cohort and the cohort published in Liang and colleagues report (Liang et al., 2016), is shown in **Figure 5.4**. This is restricted to the two metrics showing difference at a presymptomatic level in Liang and colleagues original reports: localisation error and swap error proportion. In order to avoid unblinding participant genetic status, groups are divided into: controls, PMCs (early and late together) and symptomatic carriers.

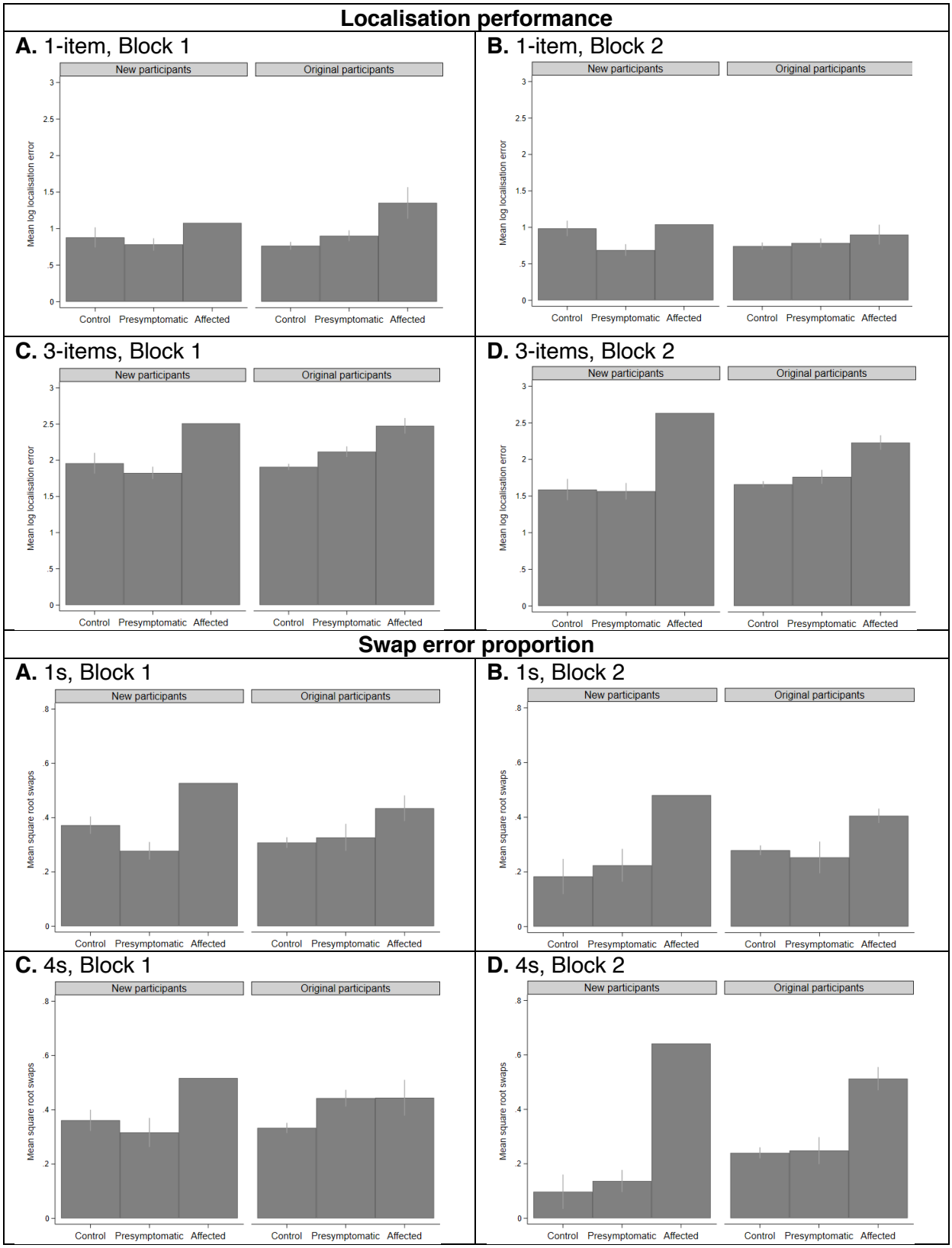


Figure 5.4 Mean performance (from model adjusted for age, sex and NART) by group for the 'new participants' added since Liang and colleague's publication compared to the 'original participants' included in that study.

Group is categorised into: control, presymptomatic and symptomatic or affected to avoid unblinding. Reason for missing error bar are: 1) the score was for one person; 2) there was no variability in score (e.g. 100% correct identification).

As **Figure 5.4** shows, the cross-sectional findings presented do not entirely replicate to those of Liang and colleagues (Liang et al., 2016). Specifically, while symptomatic participants showed a poorer performance in all metrics in both studies, PMCs (early or late) did not show evidence of a greater swap error proportion in in the highest-load, longest-delay condition as reported previously by Liang and colleagues' (Liang et al., 2016) (see Discussion for possible reasons for this).

Baseline performance of the longitudinal sample (N=48) is described next.

5.3.2. Longitudinal cohort N=48

5.3.2.1. Demographics and traditional neuropsychology at baseline

Forty-eight individuals completing at least 2 annual visits were included in the longitudinal analysis: 19 controls; 20 individuals who remained presymptomatic throughout the duration of the study (12 early PMCs, 8 late PMCs); 3 converters (participants who were late PMCs at baseline but had symptoms at their last follow-up visit) and 6 symptomatic carriers. Similar to the N=99 cohort, early PMCs were on average younger than the control group (p=0.041) and 12.6 years before expected onset. Late PMCs were on average 5.8 years before expected onset and had slightly lower baseline anxiety (p=0.023) and depression (p=0.049) scores. As expected, symptomatic carriers were older (p=0.029), had lower MMSE (p=0.002), higher global CDR (p<0.001) and were on average 2.7 years after expected onset at the baseline visit (**Table 5.2**).

There was evidence that compared to controls, early PMCs had higher scores for backwards digit span (p=0.049); late PMCs had significantly lower values for performance IQ (p=0.005) and symptomatic individuals had significantly worse scores on arithmetic (p=0.018), RMT for words (p<0.001), Stroop (p=0.019) and tended to have lower performance IQ scores (p=0.076). At the first visit in which Camden PAL was introduced (on average 1.3 years after baseline of other tests), the symptomatic carriers had significantly lower score than controls (p=0.007), no differences were observed for PMCs (early PMCs: p=0.747; late PMCs: p=0.352).

5.3.2.2. VSTM performance at baseline

Symptomatic carriers had 41.0 [16.5, 38.3] % lower odds of correctly identifying the target (OR=0.59, p=0.003), 53.0 [18.1, 98.3] % greater localisation errors (p=0.002) and made a greater proportion of swap errors (p<0.001) in comparison to controls. There was also a trend

for symptomatic participants to have greater NIC error (15.8 [1.4, 35.9] %, p=0.080). Late PMCs were significantly worse than controls in the NIC error at baseline (16.9 [3.6, 31.9] %, p=0.015) but no significant differences were seen from controls for the other measures (**Table 5.2**). Early PMCs had similar performance to controls on all measures (**Table 5.2,** **Figure 5.5**).

A significant interaction of group with block was observed for the identification measure (p=0.020) with symptomatic carriers showing a much larger difference from controls in block 2 than in block 1. A trend towards an interaction of group with increasing load (p=0.067) was seen for the localisation measure (p=0.067), whereby symptomatic carriers showed greater differences from controls in the 3-items *vs* 1-item condition. There were no significant interactions with the proportion of swap errors (delay: p=0.117; block: p=0.273). However, following previous reports (Liang et al., 2016), I investigated performance in the first block by delay. Significant differences from controls were only seen for the symptomatic carriers (**Table 5.2**, 1s: p=0.021; 4s: p=0.008).

Table 5.2 Baseline demographics, neuropsychology and VSTM performance by group for N=48 in the longitudinal analyses.

	Controls (N=19)	Early PMCs (N=12)	Late PMCs (N=11)	Symptomatic carriers (N=6)
Demographics				
Sex: N (%) Male	9 (47.4)	3 (25.0)	7 (63.6)	4 (66.7)
Age (yrs)	41.2 (9.4)	**34.8 (6.4)***	37.0 (5.0)	**50.0 (11.8)***
EYO (yrs)	NA	-12.6 (4.7)	-5.8 (1.8)	2.7 (4.3)
AYO (yrs)	NA	NA	NA	-2.0 (2.4)
Education (yrs)	14.2 (2.7)	14.3 (2.5)	13.3 (2.5)	15.0 (3.0)
MMSE	29.6 (0.7)	29.3 (0.9)	29.5 (0.8)	**25.3 (3.7)****
CDR global	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	**0.6 (0.2)****
Anxiety	6.9 (3.9)	7.8 (4.8)	**3.9 (3.9)***	6.0 (4.7)
Depression	3.5 (3.4)	3.1 (4.2)	**1.3 (1.6)***	1.7 (1.8)
Neuropsychology				
Performance IQ	113.5 (7.5)	106.5 (15.0)	**101.4 (10.1)****	104.3 (12.1)
Verbal IQ	106.7 (15.9)	96.9 (14.6)	95.4 (13.5)	102.7 (20.3)
Arithmetic total/24	17.2 (5.4)	13.9 (5.0)	14.3 (4.6)	**11.2 (6.3)***
RMT faces/50	42.5 (4.2)	41.1 (4.2)	43.8 (4.5)	41.2 (4.3)
RMT words/50	47.6 (2.8)	48.7 (2.2)	46.5 (2.8)	**38.0 (4.0)****
Digit span forwards/8	6.8 (1.2)	6.8 (1.0)	7.4 (1.1)	6.3 (1.5)
Digit span backwards/7	4.8 (1.1)	**5.7 (1.3)***	5.4 (1.1)	4.8 (1.6)
BPVS/150	140.9 (9.5)	135.0 (14.4)	139.8 (10.1)	140.5 (11.1)
GNT/30	20.3 (4.3)	17.8 (5.8)	19.2 (5.4)	22.0 (4.6)
NART/50	28.6 (9.0)	24.1 (8.6)	27.7 (10.7)	29.7 (12.1)
VOSP OD/20	17.9 (2.1)	17.8 (1.8)	18.3 (1.3)	18.2 (1.2)
Stroop (s)	49.8 (12.8)	47.6 (8.7)	52.6 (14.1)	**73.3 (25.8)***
Camden PAL/24	19.4 (3.7)	18.9 (2.9)	17.0 (6.0)	**9.2 (6.7)****
VSTM performance				
Identification (% correct)				
Overall	91.3 (4.6)	89.9 (6.3)	92.0 (3.9)	**83.7 (5.3)****
Block 1	88.9 (6.5)	89.8 (5.9	91.3 (4.3)	85.0 (5.5)
Block 2	93.6 (5.4)	90.2 (8.8)	92.7 (5.3)	**82.3 (6.1)****
Localisation error (deg)				
Overall	5.1 (1.1)	5.8 (2.0)	5.7 (1.5)	**9.6 (1.6)****
1-item, (all delays) #	2.3 (0.7)	2.3 (0.3)	2.5 (0.8)	3.1 (1.7)
3-items, (all delays) #	5.9 (1.4)	6.8 (2.5)	6.6 (1.8)	**11.6 (1.8)****
NIC (deg)				
Overall: all delays	3.3 (0.6)	3.5 (0.8)	**3.7 (0.6)***	4.0 (0.8)
Swap error proportion				
Overall	9.4 (3.1)	12.2 (4.6)	10.2 (5.9)	**25.8 (7.7)****
Block 1, 1s	12.1 (6.3)	12.9 (9.0)	9.9 (5.0)	**24.2 (14.9)***
Block 1, 4s	13.9 (5.9)	18.3 (9.4)	15.0 (10.8)	**30.1 (18.5)****

Unadjusted mean values are given with SD unless otherwise stated. SD = standard deviation; NA= not applicable; PMC= presymptomatic mutation carrier; EYO=years to/from predicted symptom onset (a negative value indicates a younger age than their estimated age at symptom onset); AYO=actual years to/from onset (negative values indicate years post onset; Anxiety and depression measures scores were taken from the HADS= hospital anxiety and depression scale; IQ=intelligence quotient; Digit spans forwards and backwards were taken from the WMS-R= Wechsler Memory Scale; RMT=recognition memory test; GNT=graded naming test. #localisation measures are separated by item-number to allow for comparison with NIC findings. Neuropsychology data were available at baseline for: 47 participants for performance IQ, verbal IQ and Stroop; 39 participants for Camden PAL (introduced into battery around 2014); for all remaining test 48 participants were included. Bold=significant; *: the difference between the patient group and controls for that variable was significant at $p<0.05$; **: the difference between the patient group and controls for that variable was significant at $p<0.01$.

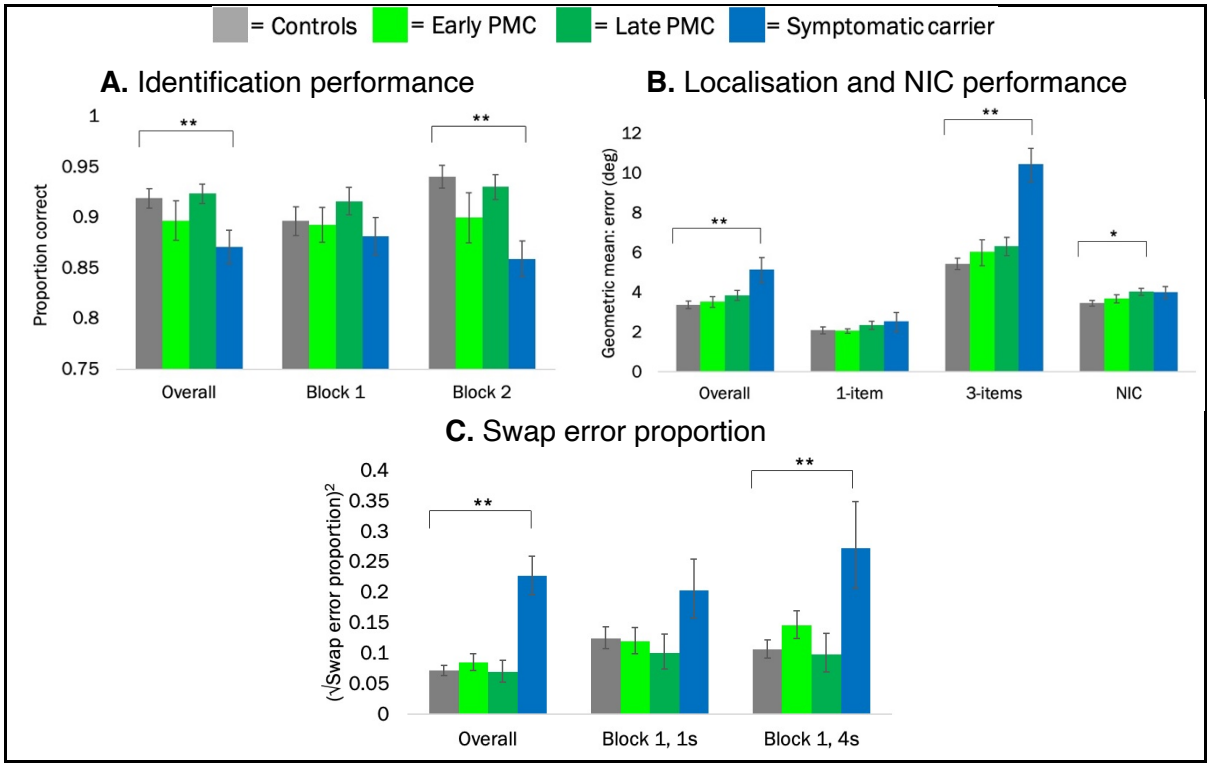


Figure 5.5 Baseline mean performance by group (from model adjusted for age, sex and NART) for N=48.

A. Identification performance; **B.** Localisation by memory-load and NIC error; **C.** Swap error proportion overall and by delay in block 1. Error bars show +/- standard error of the mean. PMC=presymptomatic mutation carrier; NIC=nearest item control. *: significant at $p<0.05$.; **: significant at $p<0.01$.

5.3.2.3. Longitudinal VSTM performance

Considering all groups and visits together, delay (all p<0.001, but swap proportion $p=0.048$) and load (all $p<0.001$) had a significant effect on all measures (where relevant). Block number also had a significant effect on localisation, NIC and swap proportion (all $p<0.001$) with a trend in the same direction for identification performance ($p=0.055$) (**Table 5.3**).

There was weak evidence that older age at baseline tended to be associated with identification ($p=0.070$); localisation ($p=0.110$) and NIC ($p=0.055$) but no significant on swap error rate ($p=0.341$). Neither sex nor NART showed a significant association any of the VSTM measures (**Table 5.3**).

Table 5.3 Effect of VSTM variables and demographics on longitudinal VSTM performance.

Change per year	VSTM performance [95% CI]			
	Identification	**Localisation error**	**NIC error**	**Swap proportion**
Delay (1s as reference)	**25 [16, 33] % lower odds****	**24 [20, 28] % (greater) ****	**14 [11, 17] % greater ****	**coefficient=0.024 [0.000, 0.047] greater ***
Load (1-item as reference)	**76 [70, 81] % lower odds****	**2.3 [1.9, 2.7] times (greater) ****	NA	NA
Block (block 1 as reference)	12 [0, 26] % higher odds	**-12 [-10, -15]% (smaller) ****	**-6 [-4, -9] % smaller****	**coefficient = -0.068 [-0.091, -0.044] ****
Age (per 1-year increase)	2 [0, 4] % higher odds	0.8 [0.2, 1.9] % (greater)	0.7 [0.0, 1.5] % (greater)	coefficient = 0.001 [-0.001, 0.004]
Sex (females as reference)	19 [-12, 42] % higher odds	3.9 [-18.1, 12.8] % (greater)	-4.9 [-15.2, 6.8] % (smaller)	coefficient = 0.026 [-0.021, 0.073]
NART (per point increase)	1 [0, 3] % increase	-0.4 [-1.3, 0.4] % (decrease)	-0.2 [-0.8, 0.4]% (decrease)	coefficient = -0.002 [-0.004, 0.001]

NA= not applicable; CI = confidence intervals; VSTM=visual short-term memory; NIC= nearest item control; NART=National Adult Reading Test (estimating premorbid intelligence). Bold=significant; *: significant at $p<0.05$; ** significant at $p<0.01$

5.3.2.3.1. Rates of change

Within-group VTSM performance

Throughout the course of the study, VSTM performance: remained similar for controls (identification: $p=0.913$; localisation: $p=0.737$; NIC: $p=0.607$; swap errors: $p=0.937$) and early PMCs (identification: $p=0.850$; localisation: $p=0.826$, swap errors: $p=0.231$) with a weak trend for decreasing NIC error with time ($p=0.057$). In late PMCs and symptomatic carriers, performance decreased for localisation (late PMC: $p=0.011$, symptomatic: $p=0.033$) and NIC errors (late PMC: $p=0.045$, symptomatic: $p=0.004$). In addition, there was evidence for decreasing identification performance for symptomatic carriers ($p=0.011$) but not in late PMCs ($p=0.217$) or for swap error performance in either group (late PMC: $p=0.943$, symptomatic: $p=0.237$) (see the first row of each variable in **Table 5.4** for the effect size corresponding to changes within group per year).

Group differences in VSTM performance

Identification performance

There was no significant difference in the rate of change of identification performance between either of the PMC groups and controls (early: $p=0.830$; late: $p=0.395$, see second row for each

variable in **Table 5.4** for effect sizes). Symptomatic carriers however, showed a faster reduction in identification performance over time (p=0.036), with 42.8 [2.50, 66.4] % lower odds of correct identification than controls at baseline decreasing to 64.7 [37.1, 80.2] % lower by year 3 (**Figure 5.6A**). There was no significant interaction between group and load (p=0.451), delay (p=0.557) or block (p=0.408) in rates of change differences.

Localisation performance

Late PMCs and symptomatic carriers showed a trend towards a faster rate of decline in localisation performance compared to controls (late PMCs: p=0.082; symptomatic carriers: p=0.066). No differences in rates of change were observed between early PMCs and controls (p=0.946) (see **Table 5.4** for effect sizes).

There was a significant interaction between delay and group in the rate of change (p=0.036), and both load (p<0.001) and delay (p=0.002) had a significant effect on differences in performance between groups. There was a significant interaction of the effect of delay on rate of change in the late PMC group (p=0.013) such that in the 4s, but not 1s delay conditions, late PMCs showed significantly greater increase in localisation error over time than was seen in the controls (1-item, 4s: p=0.043; 3-items, 4s: p=0.008 *vs* 1-item, 1s: p=0.825; 3-items, 1s: p=0.800, **Table 5.4**). The late PMC group had significantly higher localisation error than controls from 2 years after baseline, with the greatest difference in the 3-items, 4s condition (difference 11.0 [-10.0, 36.8] % at baseline, increasing to 35.4 [5.4, 73.8] % at 3 years) (**Figure 5.6B**).

Symptomatic carriers generally had a faster increase in localisation error than controls, but this only reached statistical significance in the 3-items, 1s condition (p=0.043, see **Table 5.4** for effect sizes). No further significant interaction effects on the rate of change were observed. There were no significant differences between early PMCs and controls in any condition (overall: p=0.946, see **Table 5.4** for effect sizes).

NIC performance

PMCs did not show a significant difference in the rate of NIC error per year compared to controls (early: p=0.281; late: p=0.215, **Table 5.4**). Symptomatic carriers on the other hand, had faster increase in NIC error compared to controls (p=0.015, **Figure 5.6C**) (see **Table 5.4** for effect sizes).

While no significant interactions of group rate of change emerged for delay (p=0.364) or block (p=0.986), delay conditions were evaluated separately given the findings for localisation error. Symptomatic carriers showed a significantly faster increase in NIC error compared to controls after 4s (p=0.013) and a trend in the same direction after 1s (p=0.114, **Table 5.4**). In the late PMC group, there was a suggestion for a faster increase in NIC error compared to controls specific to the long delay (p=0.064) as no difference was seen after 1s (p=0.778, **Table 5.4**). As the late PMC group had a smaller difference to controls in NIC error than in the localisation error (both 4s conditions), this suggests that some, but not all, of the difference from controls in localisation error could be accounted by a tendency to mislocalise the fractal to the location of the nearest fractal (regardless of whether it was the target). However, some of the difference from controls remained with NIC, indicating that part of the increase in localisation error was specific to the target distance rather than exclusively an effect of mislocalising the fractal.

Swap performance

There was no significant difference in rate of change in swap error performance over time between either PMC groups and controls (early: p=0.389; late: p=0.917). Although symptomatic carriers made a greater proportion of swap errors compared to controls (p<0.001), there was no significant difference in the rate of change (p=0.309, **Figure 5.6D**) (see **Table 5.4** for effect sizes).

Though there was only weak evidence towards an interaction of block (p=0.086) and delay (p=0.089) for their effects on the differences in rate of change between groups, I specifically examined the 4s block 1 condition following Liang and colleagues finding of higher swap errors in PMCs in this condition (Liang et al., 2016). While there was a trend for higher swap error proportion in late PMCs compared to controls (p=0.099), this effect did not reach statistical significance. No differences were observed for early PMCs *vs* controls (p=0.830) and despite a higher proportion of swaps overall (p<0.001), symptomatic carriers showed no difference in rate of change compared to controls in this condition either (p=0.946, **Table 5.4**).

Table 5.4 Rates of change in VSTM metrics. The first row indicates the change over time within each group (change/year). The second row compares the rate of change for each patient group to that of controls (difference in change/year).

Change per year	Adjusted mean [95% CI] Group difference [95% CI] (control as reference)			
	Controls	**Early PMCs**	**Late PMCs**	**Symptomatic carriers**
Identification performance: Odds ratio for correct response				
Overall	1.00 [0.92, 1.08]	1.01 [0.93, 1.09]	0.94 [0.86, 1.03]	**0.85 [0.75, 0.96]***
	NA	1.01 [0.91, 1.13]	0.95 [0.84, 1.07]	**0.85 [0.73, 0.99]***
Localisation error: % error				
Overall	0.4 [-2.1, 3.1]	0.3 [-2.4, 3.1]	**4.1 [0.9, 7.4]***	**7.0 [0.6, 13.8]***
	NA	-0.1 [-3.8, 3.7]	3.6 [-0.4, 7.9]	6.5 [-0.4, 13.9]
3-items	0.4 [-2.2, 3.1]	1.4 [-1.5, 4.3]	**4.0 [0.7, 7.5]***	**7.3 [0.5, 14.6]***
	NA	0.9 [-3.0, 5.0]	3.6 [-0.7, 8.1]	6.9 [-0.5, 14.7]
3-items, 1s	0.8 [-2.2, 3.9]	0.0 [-3.3, 3.4]	1.4 [-2.3, 5.3]	**9.9 [1.7, 18.8]***
	NA	-0.8 [-5.2, 3.8]	0.6 [-4.1, 5.6]	**9.0 [0.3, 18.5]***
3-items, 4s	0.0 [-3.0, 3.1]	2.7 [-0.7, 6.2]	**6.9 [2.9, 11.0]****	4.8 [-3.4, 13.6]
	NA	2.7 [-1.9, 7.4]	**6.9 [1.8, 12.2]****	4.7 [-3.9, 14.2]
1-item	0.6 [-3.2, 4.4]	-3.3 [-7.3, 0.8]	4.4 [-0.5, 9.5]	5.5 [-4.7, 16.9]
	NA	-3.9 [-9.1, 1.7]	3.8 [-2.3, 10.3]	5.0 [-5.9, 17.0]
1-item, 1s	0.9 [-3.1, 5.1]	**-4.6 [-8.8, -0.2]***	1.7 [-3.4, 7.0]	8.1 [-3.3, 20.8]
	NA	-5.5 [-11.0, 0.4]	0.7 [-5.6, 7.5]	7.1 [-4.9, 20.5]
1-item, 4s	0.2 [-3.8, 4.3]	-2.0 [-6.3, 2.4]	**7.2 [1.8, 12.8]****	3.0 [-7.7, 15.0]
	NA	-2.2 [-7.9, 3.9]	**7.0 [0.2, 14.2]***	2.8 [-8.6, 15.7]
NIC error: % error				
Overall	0.6 [-1.6, 2.7]	2.3 [-0.1, 4.8]	**2.7 [0.1, 5.5]***	**7.6 [2.3, 13.1]****
	NA	1.8 [-1.4, 5.1]	2.2 [-1.2, 5.7]	**7.0 [1.3, 12.9]****
1s #	0.9 [-1.5, 3.4]	1.4 [-1.4, 4.3]	1.5 [-1.6, 4.8]	6.0 [-0.3, 12.8]
	NA	0.5 [-3.2, 4.3]	0.6 [-3.4, 4.7]	5.1 [-1.7, 12.3]
4s #	0.2 [-2.3, 2.7]	**3.3 [0.4, 6.2]***	**4.1 [0.8, 7.5]***	**9.3 [2.5, 16.6]****
	NA	3.1 [-0.7, 7.0]	3.9 [-0.2, 8.2]	**9.1 [1.8, 17.0]****
Swap error: √proportion				
Overall	-0.001 [-0.014, 0.013]	-0.010 [-0.026, 0.006]	0.001 [-0.018, 0.019]	-0.016 [-0.043, 0.011]
	NA	-0.009 [-0.030, 0.012]	0.001 [-0.022, 0.024]	-0.015 [-0.045, 0.014]
Block 1, 4s	-0.014 [-0.035, 0.005]	-0.011 [-0.036, 0.013]	0.014 [-0.014, 0.041]	-0.017 [-0.059, 0.026]
	NA	0.004 [-0.028, 0.036]	0.029 [-0.005, 0.063]	-0.002 [-0.049, 0.045]

Adjusted mean difference in rate of change per year in the carrier group, compared to rate of change in controls. CI= Confidence intervals; NIC=nearest item control; NA=not applicable; PMC=presymptomatic mutation carrier. #The NIC measure is shown by delay for comparison with the localisation error measure. Bold=significant; *: significant at $p<0.05$. **: significant at $p<0.01$

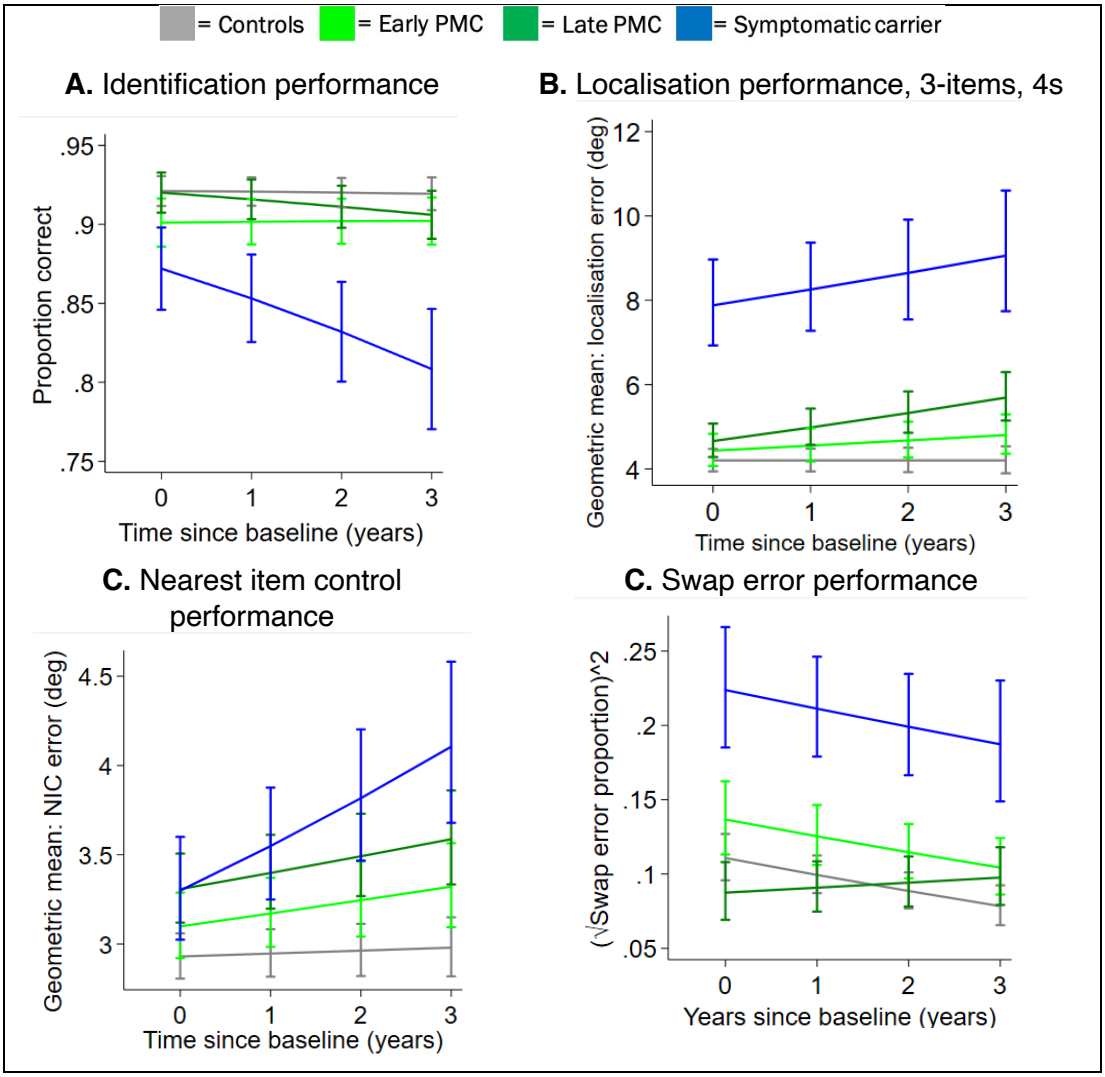


Figure 5.6 Longitudinal estimated mean performance by group (from model adjusted for age at baseline, sex and NART).

A. Identification performance. **B.** Localisation error performance for the 3-items, 4s condition. **C.** Nearest item control performance-overall score. **D.** Swap error performance. PMC=presymptomatic mutation carrier; NIC=nearest item control. Error bars indicate +/- standard error by time from baseline visit. Number of participants seen per time point: visit 1 (baseline) = 48 (100% of all participants); visit 2 = 48 (100% of all participants); visit 3 = 34 (58% of controls; 83% of early PMCs; 82% of late PMCs; 67% of symptomatic carriers); visit 4 = 11(21% of controls; 25% of early PMCs; 36% of late PMCs; 0% of symptomatic carriers).

Interim summary

In response to the first research question, the analysis carried out so far indicates that over time, late PMCs had a faster rate of decline in localisation performance (greater error) compared to controls. This effect was strongest in the most challenging task conditions (high load, long delay) with the late PMCs showing a significantly worse performance to that of controls two years after the baseline visit. Furthermore, symptomatic carriers had a faster rate of decline in most metrics but the swap error proportion in comparison to controls. The next

section will investigate how performance varied continuously when individuals approached their expected and actual ages at onset.

5.3.2.3.2. Relationship with proximity to symptom onset

Identification performance

Considering all FAD carriers (presymptomatic and symptomatic), there was no significant association between identification performance and EYO (p=0.120, **Figure 5.7A**), nor were there any significant interactions between task-conditions (load: p=0.298; delay: p=0.705 and block: p=0.573). Nonetheless, identification performance significantly decreased with AYO (p<0.001) (**Figure 5.7B**).

Localisation performance

For all FAD carriers, a significant association between EYO and worse localisation error (p=0.024) was observed. There was a significant interaction with load (p<0.001) and delay (p=0.002), such that the localisation deficit associated with closer proximity to onset was greater in the 3-items and 4s condition (i.e. when the memory demands were greatest), but there was no interaction with block (p=0.137).

Results were therefore examined by load and delay. Both 3-items conditions, showed a significant increase in localisation error with EYO (or more years post onset) (1s: p=0.036; 4s: p=0.002). The association was strongest in long delay (difference from controls at -5 years: 1s =19.1 [1.5, 39.8] %, p=0.032 *vs* 4s =23.9 [5.5, 45.4] %, p=0.009). In the 3-items, 4s model (**Figure 5.7D**), a statistically significant difference in mean localisation error between FAD carriers (presymptomatic and symptomatic) and controls was observed from 6 years before expected onset (difference=20.1 [5.5, 41.0] %; p=0.024).

Localisation error significantly increased with AYO within symptomatic carriers and converters (p<0.001) (**Figure 5.7E**).

NIC performance

Considering all FAD carriers, there was a trend towards greater NIC error with EYO (p=0.068). Although there was no significant interaction with delay (p=0.082), delay conditions were examined separately for comparison to localisation error performance. In the 4s condition,

there was a significant association with EYO (p=0.036, **Figure 5.7G**), with a difference in NIC error between FAD carriers and controls observed 6 years prior to EYO (difference=13.0 [-6.8, 27.0] %, p=0.023). There was also a significant association between worsening NIC performance and AYO overall (p=0.002) and in the 4s condition (p=0.005, **Figure 5.7H**).

Swap performance

There was no significant association between the proportion of swap errors and EYO in all mutation carriers (p=0.123, **Figure 5.7J**) nor between swap errors and actual years to onset in the symptomatic group with converters (p=0.863) (**Figure 5.7K**).

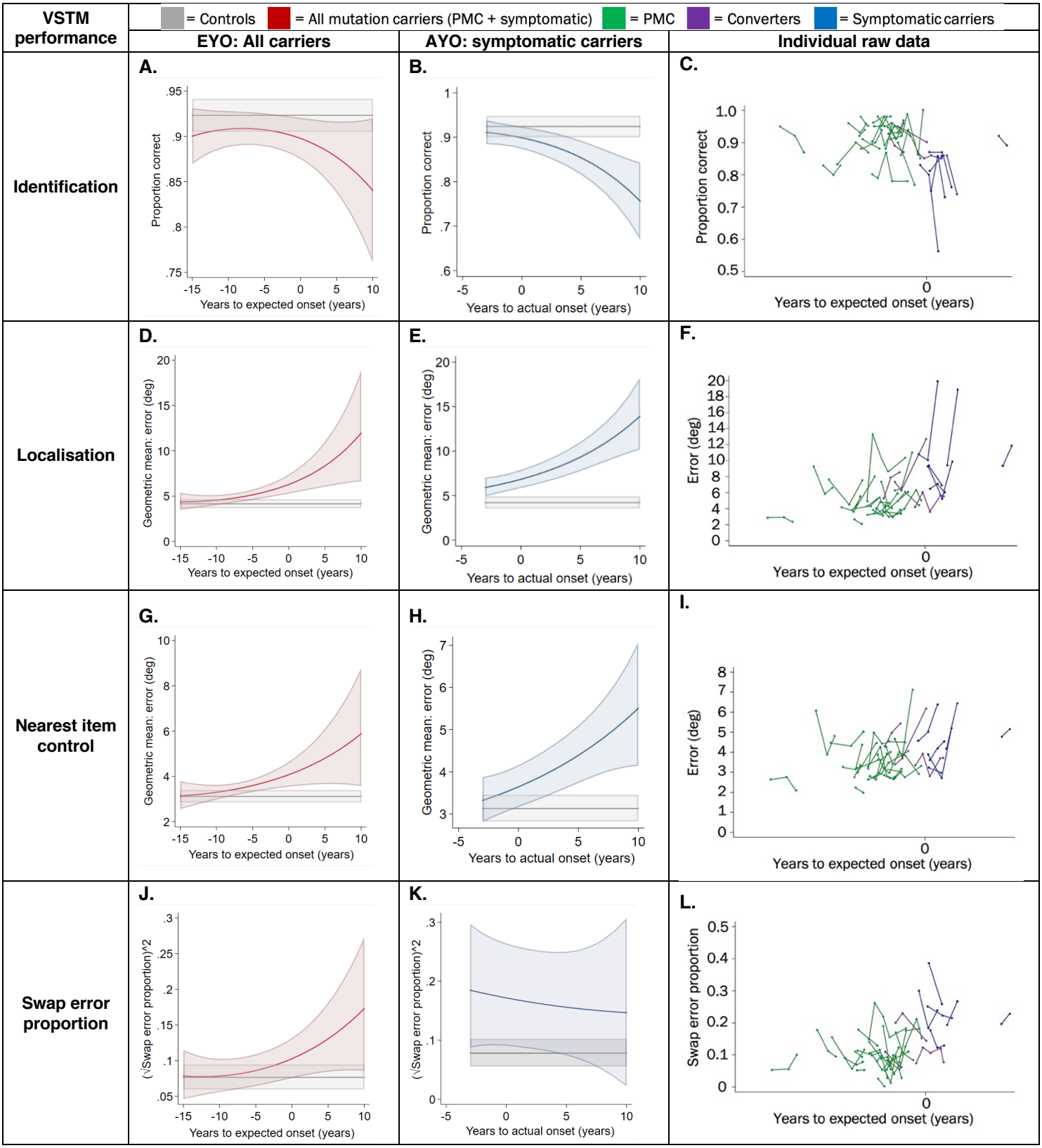


Figure 5.7 Relationship between VSTM performance and proximity to symptom onset.
Column 1: predicted mean (from model adjusted for age, sex and NART) performance against EYO. Column 2: predicted mean (from model adjusted for age, sex and NART) performance against AYO. Column 3: individual unadjusted data for each VSTM metric where visits are marked as dots and connected for each participant. Row 1: Identification performance (across all task conditions); Row 2: Localisation performance: 3-items, 4s condition; Row 3: NIC performance 4s condition; Row 4: Swap error proportion (across all task conditions).
For columns 1 and 2 shaded area indicates 95% confidence intervals.

Interim summary

In response to the second research question, the analysis on expected and actual age at onset and VSTM showed that localisation performance decreased with EYO and AYO. Once again, this effect was seen most strongly in the most challenging tasks conditions (high load and long delay). In addition, identification performance decreased AYO but not EYO.

The next section will investigate how performance varied for each patient group in comparison to controls in more traditional neuropsychology tasks.

5.3.2.4. Longitudinal change in traditional neuropsychology

Following the finding of a faster rate of decline in localisation performance for the late PMC group, I next investigated whether there was any evidence of such a difference in the more traditional neuropsychology tasks.

The following cognitive functions were evaluated: fluid intelligence/verbal and non-verbal reasoning (verbal and performance IQ from the WASI), recognition memory for words and faces (RMT), working memory (digit span backwards), STM (digit span forwards), visual perception (VOSP OD) and paired associated learning (Camden PAL). The focus on these tasks was based on a) traditional literature (e.g. (Fox et al., 1998)) and b) tests focusing on memory – within the available longitudinal battery. In addition, I also evaluated visual perception taking into account the visual component of the "What was where?" task (see discussion for the limitations associated with the lack of comparison of "What was where?" to more modern and sensitive tasks to preclinical AD (e.g. FCSRT) and the lack of a perceptual test using the same stimuli).

A significant difference between late PMCs and controls on the RMT words was observed approximately 1 year later than the presymptomatic changes observed in localisation performance (i.e. from 3 years after baseline), with 35 [45.6, 22.2] % greater rate of decline per year (**Table 5.5**, $p<0.001$, **Figure 5.8**). A difference between controls and the early PMC group was seen for RMT for faces although in the opposite direction to that expected with a better performance than controls (**Table 5.5**, $p=0.034$). No further significant group differences emerged at a presymptomatic level. A greater rate of decline than controls was detected for symptomatic carriers only in performance IQ ($p<0.001$) and digit span backwards (**Table 5.5**, $p=0.031$). This is most likely due to poor performance of this group at every visit (e.g. RMT for words and Camden PAL) or a decline in performance in some but not all visits in (e.g. VIQ).

Finally, while a direct comparison between Camden PAL and the VSTM is slightly inaccurate as the two tasks do not share the same baseline, it is interesting to note that no difference in the rate of change compared to controls was observed in any of the groups (early PMCs: p=0.646; late PMCs: p=0.151, symptomatic carriers: p=0.798, **Table 5.5**).

Table 5.5 Rates of change in traditional neuropsychology tasks by group. The first row indicates the change over time within each group (change/year). The second row compares the rate of change for each patient group to that of controls (difference in change/year).

Change per year	Adjusted mean [95% CI] Group difference [95% CI] (control as reference)			
	Controls	**Early PMCs**	**Late PMCs**	**Symptomatic carriers**
PIQ (points)	**1.16 [0.38, 1.94]****	1.23 [-0.14, 2.60]	0.33 [-1.27, 1.94]	**-2.72 [-4.78, -0.65]****
	NA	0.07 [-1.51, 1.65]	-0.83 [-2.61, 0.96]	**-3.88 [-6.08, -1.67]****
VIQ (points)	0.30 [-0.72, 1.33]	1.17 [-0.56, 2.91]	0.30 [-1.73, 2.34]	1.54 [-2.77, 5.86]
	NA	0.87 [-1.15, 2.89]	-0.0007 [-2.28, 2.28]	1.24 [-3.20, 5.68]
RMT words (%)	-14.10 [-31.96, 1.34]	0.11 [-14.48, 12.85]	**25.82 [17.67, 33.17]****	4.12 [-9.97, 16.41]
	NA	12.46 [-0.07, 28.27]	**34.99 [22.23, 45.65]****	25.82 [17.67, 33.17]
RMT faces (%)	-5.34 [-14.51, 3.10]	**-19.77 [-30.36, -10.05]****	-8.30 [-19.44, 1.81]	1.97 [-19.61, 12.07]
	NA	**-13.70 [-28.05, -0.96]***	-2.80 [-16.95, 9.63]	3.20 [-15.93, 19.17]
Digit span forwards (%)	-2.89 [-47.38, 28.16]	-24.54 [-82.49, 15.02]	22.59 [-30.78, 54.18]	19.62 [-74.61, 63.00]
	NA	-21.03 [-104.56, 28.39]	24.77 [-42.15, 60.19]	21.88 [-83.72, 66.78]
Digit span backwards (%)	-22.98 [-74.49, 13.33]	-1.47 [-49.00, 30.91]	30.96 [-10.09, 56.70]	58.57 [-3.88, 83.48]
	NA	17.49 [-38.64, 50.90]	43.86 [-1.00, 68.79]	**66.31 [9.39, 87.47]***
VOSP OD (%)	-14.66 [-33.97, 1.86]	-11.37 [-29.49, 4.21]	-19.60 [-49.19, 4.12]	-14.48 [-60.97, 18.59]
	NA	2.87 [-20.55, 21.74]	-4.30 [-36.70, 20.41]	0.16 [-45.37, 31.43]
Camden PAL (points)	-0.09 [-0.31, 0.13]	-0.02 [-0.17, 0.12]	**-0.30 [-0.51, -0.10]****	-0.17 [-0.77, 0.43]
	NA	0.06 [-0.20, 0.32]	-0.22 [-0.52, 0.08]	-0.08 [-0.73, 0.56]

CI=confidence intervals; PIQ= performance IQ; VIQ= verbal IQ; RMT=recognition memory test; GNT= graded naming test; VOSP OD= visual object and space perception battery object decision; PAL=paired associate learning. Bold=significant; *: significant at p<0.05. **: significant at p<0.01

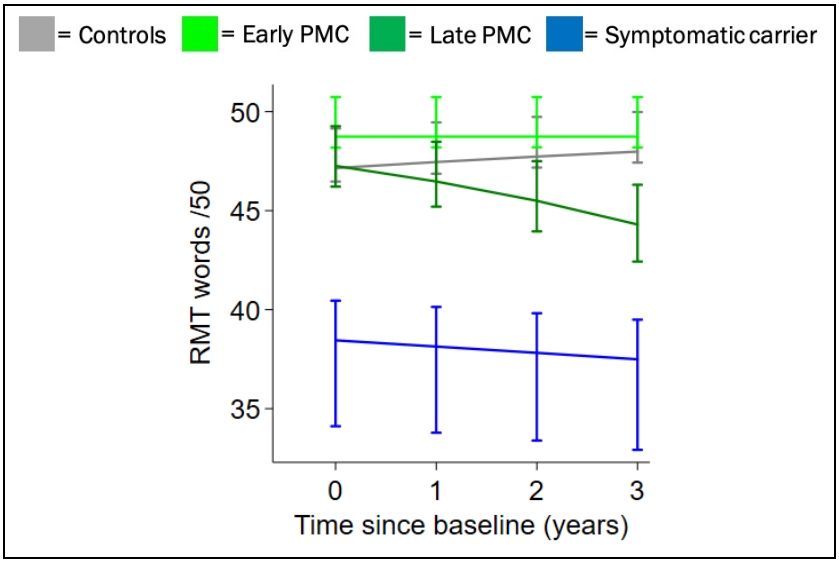


Figure 5.8 Longitudinal estimated mean performance for RMT for words by group (from model adjusted for age at baseline, sex and NART).

PMC=presymptomatic mutation carrier. RMT=recognition memory test. Error bars indicate +/- standard error by time from baseline visit. Number of participants seen per time point: visit 1 (baseline) = 48 (100% of all participants); visit 2 = 43 (74% of controls and 100% patient groups); visit 3 = 33 (58% of controls; 83% of early PMCs; 73% of late PMCs; 67% of symptomatic carriers); visit 4 = 11 (21% of controls; 25% of early PMCs; 36% of late PMCs; 0% of symptomatic carriers).

Interim summary

In response to the third research question, the analysis on more traditional neuropsychology tasks, showed a significant rate of decline in recognition memory – specifically RMT for words for the late PMCs compared to controls. Importantly, this effect was significant three years after the baseline visit– approximately 1 year later to the changes observed in the "What was where?" task.

In light of the longitudinal impairments observed in the localisation performance metric for late PMCs, the next section will investigate motor function over time (see below for further details).

5.3.2.5. Longitudinal change in 'motor function'

Finally, I considered the possibility that the greater error at localising the target for late PMCs could be due to a motor difficulty rather can a deficit in recall (i.e. participants were less precise at selecting the fractal in the first place before dragging it to its remembered location). In order to evaluate this, I carried out a post-hoc analysis where I calculated the absolute distance from the selected location within the fractal to its centre, every time a correct fractal was selected. This was quantified as the 'deviation from the centre' (see Figure 5.9).

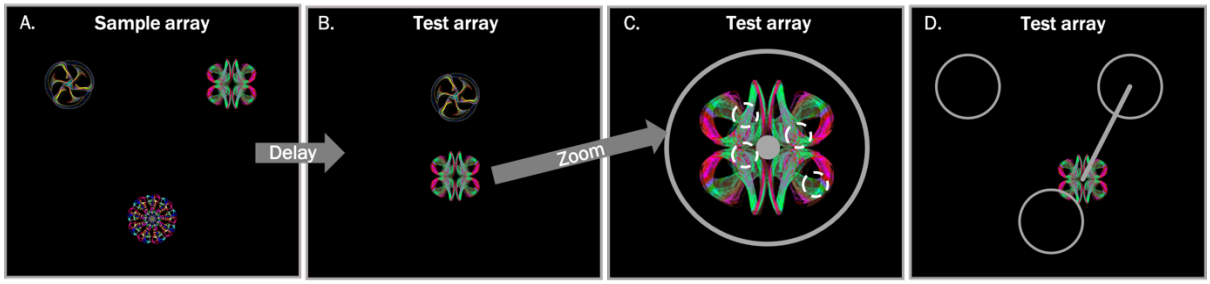


Figure 5.9 Illustration of the motor function estimation.

A. Sample array where either 1 or 3 objects are presented (3 in this case). **B.** The participant is asked to make a choice between two fractals where one is the target and the other a distractor or foil. **C**. The deviation from the centre is calculated by measuring the distance within the fractal between the centre of the actual fractal (grey circle) and the position of the participant's finger the first time they select the correct fractal (different positions within the fractal are illustrated as white dotted circles). **D.** The localisation error measure where the distance from the centre of the fractal to the participants' chosen location within the array is measured.

There was no difference in the rate of change of the mean deviation from the centre between early PMCs and controls (difference in change per year *vs* controls = -2.6 [-8.8, 4.0] %, p=0.429). Compared to controls, late PMCs showed a significantly slower increase in the deviation (i.e. they were more precise than controls; difference in change per year *vs* controls = -7.7 [-13.9, 1.0] %, p=0.024) and a trend towards a slower increase was also observed for symptomatic carriers (i.e. they were more precise than controls, difference in change per year *vs* controls = -9.1 [-17.5, 0.2] %, p=0.054).

Delay and load also had significant effects on the deviation, whereby longer delay and higher load were associated with smaller deviation from the centre (both p<0.001). While participants were not explicitly asked to select the centre of the stimuli when making a choice, the significant effect of delay and load as well as the smaller deviation for late PMCs compared to controls, suggests that the faster decline in VSTM observed in the localisation metric for late PMCs cannot be explained by a motor impairment, especially as late PMCs were more precise over time when selecting the fractal in comparison to controls.

5.4. Discussion

5.4.1. Summary

In this chapter I investigated VSTM function over time using the "What was where?" task, with a particular interest in studying relational binding longitudinally. More specifically, I evaluated a) differences in the rate of change between symptomatic and presymptomatic carriers compared to controls; b) how VSTM varied continuously with proximity to onset and c) whether

longitudinal decline was also be seen in more traditional measures of neuropsychology. The main finding was that 'late' PMCs (within 8.5 years of expected onset) had a faster decline in the rate of localisation performance in long delay conditions, compared to controls, with a significant difference apparent approximately 2 years after the baseline visit. Importantly, this effect preceded changes in traditional measures of recognition memory, which were observed approximately 3 years after the baseline visit. Interestingly, localisation performance was also the only VSTM metric to show a significant association with EYO with strongest effects observed in long delay conditions up to 6 years prior to estimated symptom onset. Other important findings include: the faster rate of decline in identification and localisation performance (though localisation effects were only found significant in one condition – 3-items, 1s – most likely due to small numbers in this group) and the decreasing identification performance with increasing AYO for symptomatic carriers (as opposed to EYO).

Taken together, these findings indicate a preferential effect of localisation deficits in FAD carriers, especially in those who were presymptomatic. These findings will be discussed in greater detail in the following sub-sections.

5.4.2. Preferential effect on localisation performance – what is this metric really measuring?

Relational binding in the "What was where?" task is conventionally measured using the 'swap error proportion' metric. In this approach, if the fractal is placed within 4.5 deg of another fractal in the memory array, it is considered to be 'swapped'. While this pre-defined threshold measures this misbinding as a proportion of error (in comparison to change-detection paradigms which compare accuracy between bound and unbound conditions but fail to quantify the error itself), the results presented in this thesis argue that this metric (swap error proportion) may not be as sensitive to preclinical AD as previously thought. Instead, I will next argue why localisation performance may be better suited to account for relational binding and possible reasons why a higher proportion of swap error was not observed in the patient groups in comparison to controls despite previous evidence from the literature (Liang et al., 2016).

Localisation performance measures the distance (in degrees of visual angle) between the centre of the target object once placed in its remembered location and its true (original) location in the memory array *after* the correct fractal has been identified. Therefore, by definition, this metric indicates the resolution or quality of recall of the following memory representation: the object's identity *bound* to its exact location and the greater the error. I argue that in order to count as a 'swap', the fractal must be localised 'close enough' to another

fractal location. However, if localisation performance is poor, then this proportion might be underrepresented. Indeed, the finding of a greater localisation error of late PMCs over time, may explain why the swap error proportion metric did not evidence any deficits at a presymptomatic level. Whilst it is possible that with a later longitudinal sample, swap error in PMCs would have been observed, the lack of significant association with EYO, suggests this metric may not be that well suited to preclinical AD as previously thought and most importantly to this chapter, it lacks sensitivity to preclinical *decline*. Instead, the new proposed metric of relational binding – i.e. localisation error – perhaps more appropriately labelled as 'relational binding accuracy', may. This proposal is also in line with more recent views of working memory models, specifically resource models, which describe how the allocation of memory resources is limited by the flexibility with which resources are distributed between objects. In this regard it is relevant to note that precision decreased (the degree of error increased) when three objects were presented in comparison to one.

Similar to change-detection paradigms, this new approach does not allow to quantify misbinding as a proportion. Nevertheless, the novelty lies in the quantification of precision in a continuous spectrum whereby more error indicate less precision of the memory representation. As these effects were predominantly present in long delay conditions, the impairment observed over time in late PMCs may be related to a difficulty in maintenance processes rather than memory encoding or retrieval. Importantly, localisation performance deficits were not observed in the early PMC group, raising important considerations as to when this task (or cognitive function as a whole) may be sensitive to tracking preclinical decline in AD. In other words, are relational binding deficits dependent on PMCs being relatively close to expected onset? If so, how close do they have to be? Notably, the EYO analysis on localisation performance showed that significant differences between carriers and controls were seen from 6 years to expected onset and early PMCs were on average 12.6 (SD 4.7) years to expected onset in comparison to late PMCs who were 5.8 (1.8) years. Thus, it is possible that the combination of a relatively far distance to expected onset and the small sample size of early PMCs, did not result in higher VSTM impairments nor more specifically in relational binding deficits (as both the swap error proportion and the more novel conception of binding measured by the localisation performance metric, were similar to controls in this group).

Now that the new proposal of evaluating relational binding has been described, I will next compare the findings presented here with previous literature and discuss possible reasons for the inconsistency in swap error findings.

5.4.3. Integrating and comparing VSTM results with previous literature

The cross-sectional investigations described here revealed VSTM impairments for object identity, localisation and swaps in symptomatic carriers but unlike previous reports from our centre, there was no evidence of a greater swap error proportion in our sample of PMC (Liang et al., 2016) (in either early or late PMCs) in comparison to controls.

This lack of replicability in findings raises important questions of the task's validity and reliability for preclinical AD. Looking at Liang and colleagues' cross-sectional finding of a higher proportion of swap errors in the 4s delay condition (Liang et al., 2016), it appears that a sample size of N=71 (for a replication study that included PMCs and controls) would be required to find a significant difference (set at 0.05) with 80% statistical power. Yet, we did not observe a significant effect with a total sample of N=99 (including 19 controls and 23 PMCs). In this regard, it is worth noting that in both cross-sectional analyses presented in this thesis (N=99 and N=48), the NIC error for the late PMC group was higher than controls (this was seen as a trend for N=48: p=0.085 and a significant difference in N=48: p=0.015). While this metric is difficult to intercept on its own, when the task was first designed, this metric was described as "a measure of localisation error subtracting out the effects of swaps" (Pertzov et al., 2013). In other words, the NIC measures the distance (in deg of visual angle) between the location reported by the participant and the *closest of the three original locations* from the memory array (3-item conditions only). Thus, if the participant places the fractal 'close enough' to the target location, then localisation and NIC errors will be the same. However, if the participant places the fractal 'close enough' to the position of another fractal in the memory array, the localisation error will be greater than the NIC error and this may be an indication that the participant correctly remembered the target but *swapped* its location with that of another fractal. Consequently, it remains plausible that late PMCs were showing an indication of misbinding at baseline but that the binary nature of the 'swap error' proportion metric was not sensitive enough to detect this.

A number of additional reasons may explain the lack of replication of findings between Liang and colleagues (Liang et al., 2016) and the cross-sectional cohort presented here. Firstly, differences in the characteristics of the PMCs sample in comparison to Liang and colleagues' report (Liang et al., 2016) may have influenced results. The inclusion of more PMC participants (23 in our study *vs* 12 in Liang and colleagues' and 6 additional mutations – 5 *PSEN1* and 1 *APP)* meant that they were on average further from expected onset and had a broader range of EYO in comparison to Liang and colleagues' report (mean EYO=9.5 (SD 5.0) *vs* 8.5 (3.8)). This may have resulted in performance differences given that disease progression varies

between genes (with *PSEN1* mutation carriers more frequently presenting with non-amnestic cognitive symptoms than *APP* mutation carriers (Scahill et al., 2013) and even between mutations within the same gene (Pavisic et al., 2020a; Ryan et al., 2016). Therefore, the combination of gene differences and a broader EYO distribution may have increased the variability in performance resulting in greater 'noise' which would have been captured as variance in continuous metrics (localisation and NIC measures) but resulted in greater inconsistencies when classifying a response that relies on a specific threshold such as swap errors. Furthermore, the late PMC group presented here had lower anxiety scores compared to controls (in both N=99 and N=48 samples) and this was not the case for Liang and colleagues' PMC group (in which patients and controls had similar anxiety scores comparable to my control group (Liang et al., 2016). While high anxiety levels have shown to negatively impact cognition (Okon-Singer et al., 2015) and visual working memory specifically (e.g. (Spalding et al., 2020)), implications of low anxiety scores on cognition are complex and it is difficult to establish whether or not this could have carried some advantage for late PMCs performance especially in light of the reduced insight that may be observed sometimes in presymptomatic stages of FAD. Secondly, the separation between of PMCs into 'early' and 'late' was a different approach taken to the original report. Although this may have caused differences in results, **Figure 5.4** clearly shows that the proportion of swaps was not higher than controls in the 'new' participants as it had been for the 'old' participants described by Liang and colleagues (Liang et al., 2016). Lastly, as a relatively accurate localisation is required for a response to count as a swap, swap errors may have been underrepresented in the sample (in both symptomatic and presymptomatic carriers) especially in light of the localisation error finding. The non-significant interaction between the rate of swap error proportion and delay in our longitudinal analysis was also surprising, yet the worsening localisation particularly for longer delays may have veiled this interaction too.

Taken together, whether or not the preclinical differences in swap error proportion described by Liang and colleagues were due to chance remains unknown. However, a novel and important preclinical finding from my investigations is the faster decline of in VSTM performance, specifically in the localisation performance metric. This deficit was interpreted as a specific relational binding problem and is based on a novel proposal in which relational binding is measured as accuracy or precision in a continuous analogue scale.

More broadly, these longitudinal findings may be explained by a 'unified account of hippocampal forgetting across short and long timescales', proposed recently (Sadeh & Pertzov, 2020). Accelerated forgetting refers to a long-term memory process, whereby new

material appears to be encoded and retained normally over periods of up to 30 min but is then forgotten at an abnormally rapid rate over the following hours to weeks (Weston et al., 2018). As the precision of localisation performance gradually declined with time (i.e. instead of demonstrating a complete loss of access), I propose that a process similar to accelerated forgetting may be behind the deficits observed in 'late' PMC, whereby forgetting over just a few seconds is associated with decreases in precision at an abnormally rapid rate. As this effect was observed for the localisation error measure, this suggests the association of the object's identity to *its location* may have been predominantly forgotten at a faster rate.

5.4.4. Neuropsychology considerations

In addition to investigating VSTM function longitudinally, performance for presymptomatic and symptomatic carriers was compared in more traditional neuropsychology tasks. Interestingly, while verbal and performance IQ measures showed lower values for PMCs at baseline, there was no evidence for a faster rate of decline compared to controls. This is in accordance with one of the first studies of FAD in PMCs (Fox et al., 1998), which showed individuals who became clinically affected had significantly lower performance IQ scores at their first assessment.

Another task which has shown promise as a sensitive cognitive marker of preclinical AD is associative learning like the Camden PAL (Bastin et al., 2014; Pereira et al., 2014). While a direct comparison is not possible due to the differences in baseline visits, this study does not provide evidence that associative learning is sensitive to subtle preclinical decline. Importantly, a faster rate of decline was observed in RMT for words in late PMCs compared to controls. Decline in recognition memory tests have traditionally been associated with AD (e.g.(Diesfeldt, 1990)) and while most sensitivity has been described in symptomatic AD, some reports suggests recognition discriminability for amnestic MCI patients with biomarker evidence of prodromal AD (Goldstein et al., 2019). I propose that the recognition memory findings may be explained by the multicomponent nature of tasks and the fact that accurate performance requires the integrity of a variety of processes. For example, certain brain areas which are active during the episodic retrieval of these tests (e.g. the right anterior prefrontal cortex (Rugg et al., 1998) or the entorhinal cortex (Weston et al., 2016)), might also overlap with the neural correlates of binding (frontal-parietal-MTL network for conjunctive binding and parietal-occipital-temporal networks for relational binding) (Jonin et al., 2019). These findings are also consistent with a recent event-based modelling study by O'Connor and colleagues, showing that RMT declined ~ 5 years to EYO, around a half a decade after long-term memory tests

like ‘long-term accelerated forgetting’ (O’Connor et al., 2020). Hence it is possible that tests measuring specific cognitive functions share some commonalities with regards to which brain areas are activated. Longitudinal imaging studies including fMRI are therefore needed to further determine which regions of the brain show significant deficits in preclinical AD in comparison to controls and in which order.

Taken together, these results raise a relevant point about possible inherent psychometric properties which are best fit for detection or screening *vs* those best fit for tracking cognitive decline in preclinical AD. This will be discussed as a broader theme in the **GENERAL DISCUSSION**.

5.4.5. Study limitations

The current study has several limitations. First, despite the increased sample size in comparison to the previous cross-sectional study (23 presymptomatic carriers in this study *vs* 12 presymptomatic carriers in (Liang et al., 2016)), this remains relatively small due to the low prevalence of FAD. Considering a level of significance of 0.05 and a statistical power of 80%, a sample size of N=54 (for a replication study that included all 4 groups of which N=33 would be late PMCs and controls), would be required to investigate whether findings in the 3-item, 4s condition for late PMCs are replicated. Moreover, increasing the data points available at each time point (especially for later visit where there were more individuals lost to follow-up) would also increase the statistical power associated with these findings. While individuals from all groups were lost to follow-up at random, the current findings may be biased towards an underrepresentation of what the symptomatic trajectory due to the small numbers of symptomatic carriers at later visits. Second, age and educational differences between groups may confound comparisons. Nonetheless, all models included an adjustment for age and NART (as an estimation of premorbid IQ) and most importantly, age was similar between controls and late PMCs where the novel and significant differences were found. Third, it is possible that by considering all FAD carriers together, the heterogeneity in the progression of the disease between genes and mutations may have affected our results. However, creating mutation-based subgroups would not have been possible due to issues around validity of modelling such small groups. In addition, the late PMC group was heterogenous in that individuals EYO spanned within 8.5 years before expected onset; mean=-5.8 (SD 1.8) years and these estimations are inevitably imprecise given the within-family variation in AAO (Ryman et al., 2014). In relation to this, a DIAN observational study (Bateman et al., 2017), looking at disease progression in FAD carriers showed that decline of cognition (measured by

cognitive composite scores) was not linear. Indeed, it was in light of the complexity in disease progression that PMCs were divided with respect to EYO (i.e. PMC closest to onset might perform differently to those furthest way). However, it is important to acknowledge that this approach also carries limitations as participant close to each other on the distribution might be classified into different groups, reducing the variability of the predictor. For these reasons, subsequent analysis on the associations between VSTM performance and EYO as a continuous measure presented here may serve as a complementary approach. Fourth, the lack of inclusion of more modern and sensitive tasks to preclinical AD (e.g. FCSRT) limits the comparison of "What was where?" to other tasks and cognitive functions carrying evidence of sensitivity to preclinical AD. This comparison is important for future work to inform clinical recommendations further. Moreover, findings in the "What was where?" task may also be explained by the attention and frontal/executive demands of this task (with the localisation measure being particularly sensitive due to its continuous nature), rather than the visuo-spatial or memory aspects *per se*. A comparison with other neuropsychology tasks measuring these cognitive tasks (e.g. Stroop) would have increased the confidence that results may be signalling a somewhat specific VSTM deficit (relational binding). In this regard, it is relevant to note that post-hoc comparison of motor function between controls and each patient group did not suggest motor deficits were behind localisation performance differences between PMCs and controls, as precision increased with time in all patient groups compared to controls. Furthermore, the lack of a perceptual test (using the same stimuli) represents an important limitation as it remains uncertain whether some of the deficits observed may be due to a failure to perceive the fractal in the first place.

Lastly, the qualitative observation of VSTM performance in 'converters' showed that for all VSTM metrics, performance did not follow a unique pattern. For some participants, scores worsened while for others they remained stable. Reporting this substantial variability – possibly resulting from the 100 trials completed by participants at every visit in addition to the limitations previously mentioned – is important as it raises novel considerations of the use of such tasks at an individual level although more data points are needed to evaluate this further.

5.4.6. Conclusions

Taken together these findings highlight that evaluating the *degree* of error on a continuous scale may be a sensitive measure of longitudinal decline in the preclinical stages of FAD. More specifically, the proposal of a continuous analogue scale to measure relational binding accuracy – using 'localisation performance' is novel. In combination, the significant association

of this metric with EYO and the significantly faster decline of performance for PMCs seen on average 6 years to expected onset in comparison to controls, has important implications for the direction of future work. The fact that the strongest effects were seen in the most challenging task conditions (3-items, 4s delay) indicates this may be the direction to take in larger studies. Indeed, it remains paramount to combine conjunctive and relational binding approaches if we are to advance our understanding of the sensitivity of this cognition function (binding) in preclinical AD populations.

While a relevant follow-up investigation would have been to focus on longitudinal imagining and evaluate how structural or functional correlates may better determine the use of this tasks as a tool to screen or monitor conditions like FAD – I wished to focus on eye-tracking instead. The rationale for this was to exclusively investigate non-expensive and non-invasive approaches for the study of preclinical changes in this thesis. Hence, the next data chapter will evaluate whether measuring the cognitive effort required to complete this task may a) increase the sensitivity to preclinical AD; b) provide further insight into some of the inconsistencies found in this work in comparison to previous literature.

6. A CLOSER LOOK AT VSTM DEFICITS IN FAD

This next chapter investigates how viewing behaviour, measured by tracking eye movements, may provide further insight into the VSTM impairments in symptomatic and presymptomatic FAD. A paper based on this chapter has been published in *Scientific Reports* (Pavisic et al., 2021a).

6.1. Introduction

There is currently increasing acknowledgement in the field that understanding why information is forgotten is at least as important as understanding how it is encoded and retained (Davis & Zhong, 2017; Richards & Frankland, 2017; Sadeh et al., 2014; Sadeh & Pertzov, 2020).

While measuring the *precision* of recall has been reported to be more sensitive than conventional span measures which only index the *number* of items held in memory (Zokaei et al., 2015), explicit verbal responses require conscious recollection. Yet, eye movements may reveal information for elements of previous experience without such reports (Hannula et al., 2010), making it as a suitable candidate to study memory processes. The nature of human visual processing is such that one region of the visual scene is sampled at a time, by directing the high-acuity foveal portion of the retina to successively fixated regions (Hannula et al., 2010). Such patterns of exploration, captured by gaze position across time, appear to be particularly influenced by two types of factors: the physical properties of the elements ('bottom-up') and the contextual information available ('top-down') (Duc et al., 2008). A prevalent view of such sequential sampling is that at every fixation, the oculomotor system faces competition between exploring different aspects of an object or scene *vs* maintaining fixation to allow for in-depth cortical processing (the 'exploration–exploitation dilemma') (Kietzmann & König, 2015). The 'linear approach to threshold explaining space and time' (LATEST) model of gaze deployment, claims that each decision to move the eyes is *"an evaluation of the relative benefit expected from moving the eyes to a new location compared with that expected by continuing to fixate the current target"* (Tatler et al., 2017). Theoretically, the eyes move when the evidence that favours shifting to a new location outweighs that favouring remaining at the present location (Tatler et al., 2017).

Several studies have now reported that viewing behaviour is strongly related to hippocampal activity (e.g. (Meister & Buffalo, 2016)) and the results of various investigations have suggested that viewing behaviour is an integral part of the *memory formation* process supported by the hippocampus (Chan et al., 2011; Kafkas & Montaldi, 2011; Loftus, 1972; Molitor et al., 2014). Further, one functional magnetic resonance imaging (MRI) study showed

that activity in the hippocampus predicted expressions of relational memory in subsequent eye fixation patterns, even when explicit, conscious retrieval failed (Hannula & Ranganath, 2009). More recently, some researchers have also proposed that eye movements may serve as indirect surrogates to investigate VSTM (Fernández et al., 2018; Pertzov et al., 2012) and perhaps most relevant for the current study is the finding that better recall or 'stronger memories' are associated with image regions that attract more fixations during encoding (Hannula et al., 2010; Pertzov et al., 2009).

To understand the potential role and validity of VSTM delayed-reproduction tasks as cognitive preclinical AD markers, it is necessary to determine whether VSTM deficits may arise from alterations in: i) correctly *maintaining* the features of an item; ii) variability in the ability to access the memory *(retrieval)* or iii) *encoding* stimuli in the first place. Studying eye movements may help unveil the source of impairment. For instance, if individuals carrying a genetic mutation for FAD, have a different eye movement pattern than controls during the initial presentation of the stimuli, could this be an indication of an encoding impairment? Moreover, how do different visual search strategies relate to the accuracy of task performance?

Although VSTM impairments were not detected cross-sectionally for PMCs in the previous chapter, evidence of preclinical AD deficits in VSTM and VSTM binding specifically, have been previously reported in the literature (e.g. (Liang et al., 2016; Parra et al., 2010a)) and the reasons for these inconsistencies are unknown. Importantly, the lack of cross-sectional differences between PMCs and controls in the previous chapter may have been due to a) lack of statistical power b) lack of sensitivity of the task to subtle changes or c) no real presymptomatic deficits (i.e. previous results were due to chance). In this regard, it is relevant to investigate memory processes using techniques such as eye-tracking which may provide another level of detail by measuring cognitive effort and possibly shed light into some of these inconsistencies.

The main hypothesis in this chapter is that, encoding – indexed indirectly by overall time spent fixating a stimulus – will be particularly affected in symptomatic and presymptomatic FAD individuals. Following the indication from the previous chapter that relational binding accuracy may be more accurately measured by localisation performance than swap error proportion, I anticipate that the greatest differences will be found in this metric. Lastly, I hypothesize that low-level oculomotor deficits will not be observed in neither presymptomatic nor symptomatic carriers in comparison to controls.

6.2. Methods

6.2.1. Study design and participants

In this cross-sectional observational study, 52 participants were recruited: 26 carriers of mutations in *PSEN1* or *APP* and 26 healthy controls. Of the mutation carriers 9 had progressive cognitive symptoms and 17 were PMCs.

All participants underwent clinical assessment, including a semi-structured interview, neurological examination and the CDR (Morris, 1993), depression and anxiety questionnaires (HADS) (Zigmond & Snaith, 1983) and a standard neuropsychology battery (**Table 6.1**). EYO was calculated as described in Chapter 5 and in this case the median split corresponded to 6 years. Based on results from genetic tests and clinical assessments, individuals were classified as symptomatic carriers, 'early' PMCs (more than 6 years to expected onset), 'late' PMC (at least 6 years from expected onset) and controls. Symptomatic individuals were those who had a positive genetic test and cognitive symptoms consistent with AD and scored higher than zero on the CDR scale (Morris, 1993). PMCs were at-risk individuals who had a positive genetic test but did not have symptoms and who scored zero on the CDR scale (Morris, 1993). Control participants consisted of both non-carriers (at-risk individuals who tested negative for pathological mutations) and healthy individuals (from the research database) recruited for the study.

6.2.2. Procedures and data collection

The VSTM task "What was where?" has previously been described in Chapter 5. The experiment was run on a Dell 2120 desktop computer with a 23-inch screen at a viewing distance of 42 cm and the same design was implemented: 10 trials with 1 fractal and 40 trials with 3 fractals and a balanced number of trials with 1 or 3 fractals (displayed for 1 or 3s respectively) and 1 or 4s delay between memory and test arrays. Following Liang and colleagues' finding (Liang et al., 2016) that testing confined to only 50 trials was sufficient to distinguish FAD cases from controls; the eye-tracking experiment consisted of 50 trails.

As for Chapter 5, the behavioural metrics of task performance were:

- **Identification performance**: proportion of trials where the correct object was chosen.
- **Localisation error**: the distance (deg) between the centre of the target object once placed in its remembered location and its true (original) location in the memory array (only for correctly identified objects).

- **Swap errors**: the percentage of correctly identified objects placed within 4.5 deg eccentricity of other fractals in the original array (3-items condition only).
- **NIC error**: the distance (deg) between the centre of the target object once placed in its remembered location and the location of the nearest fractal from the memory array (3-items condition only).

Eye movements were recorded at 1000 Hz using a desktop-mounted infrared video-based eye tracker (Eyelink 1000Plus; SR Research). Participants sat in front of the computer screen resting their head on a chin rest in order to provide stability and maintain a fixed viewing distance. The eye-tracking camera did not obstruct the computer screen but was placed below it. Before the experiment began, I ensured the participant was able to reach the computer touch-screen comfortably without obstructing the eye-tracking camera in the process. A drift correction procedure was used before each individual trial. For each fractal, the diameter of the region of interest (ROI) was 8.4 deg. The fractal diameter was 5.7 deg wide which resulted in a 1.35 deg ROI border around each fractal.

All eye-tracking recordings were visually inspected using Data Viewer to check for any signal loss that would interfere with data analysis and interpretation of results. One participant (late PMC) was excluded from all analysis due to intermittent signal loss throughout the experiment. Blinks were identified and removed using Eyelink's automated blink detection. Vision was binocular in case there was a problem with one eye (e.g. poor eyesight, watery, or dry eye) but ultimately only eye movements from the right eye were recorded for all participants as no issues were detected.

In order to test the hypothesis that encoding – indexed indirectly by the overall time spent fixating a stimulus – might be particularly affected in presymptomatic FAD individuals, I examined four metrics related to perception of the stimuli.

The predictions associated with each visual exploration strategy (VES) metrics (measured for each trial) during the 3s viewing period were:

a) Prediction 1. A greater amount of time spent fixating the stimuli (all 3-fractals), would result in a more accurate VSTM performance.

Metric: Total dwell time on fractals (DT): sum of the total fixation time on all fractals.

b) Prediction 2. A greater amount of time spent fixating the *target* (unknown to the participant at the time of viewing) *in proportion* to the overall time spent fixating all fractals, would result in a more accurate VSTM performance.

Metric: Proportional time spent looking at the target (Pr): time spent fixating the target (the item that was later probed) divided by the total time spent fixating all fractals.

c) Prediction 3. A more even or equal distribution of the fixation time on all three fractals (e.g. dividing viewing time equally between all three fractals *vs* spending 80% of the time viewing one fractal and 20% of the time viewing the other two), would result in a more accurate VSTM performance.

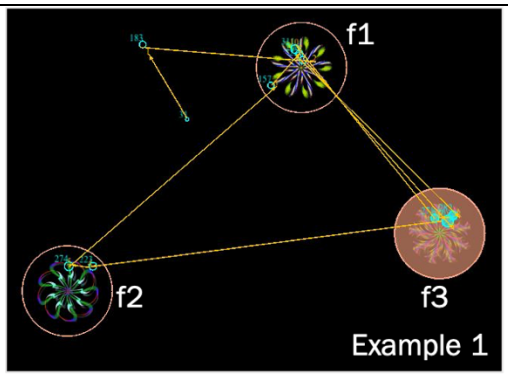
Metric: Equality (Eq): homogeneity in the distribution of the time spent fixating on fractals. I generated a metric between 1 and 0, where 1 represents a completely equal distribution of fixation time between the 3 fractals (f) i.e. f1=1000ms; f2=1000ms; f3=1000ms and anything lower than 1 represents a less homogenous or less equal distribution of fixation time e.g. f1=3000ms; f2=0ms; f3=0ms where Eq=0 or f1=1800ms; f2=1200; f3=0ms where Eq=0.4. Values between 0 and 1 indicate fixation time was split between fractals, with smaller values designating less equal fixation times. For example, a value of 0.5 indicates that the maximum proportional difference in dwell time was 0.5, which could represent f1=1500ms; f2=0ms; f3=1500ms or f1=2000ms; f2=500ms; f3=500ms. Importantly, this metrics represents a novel estimation of dwell time as it gives weighting to the *way* in which viewing time is proportionally distributed among stimuli.

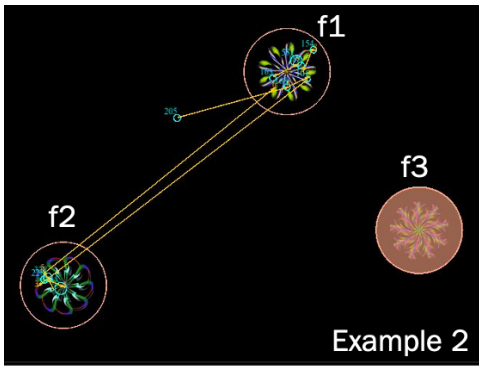
d) Prediction 4. Shifting the gaze between one or more fractals, would result in better VSTM performance in comparison to focusing all viewing time on less fractals (i.e. making less shifts).

Metric: Total number of shifts between fractals (S): total number of eye movements between fractals.

Notably, VES metrics were not considered in the same regression models. While DT and Eq are measuring different things and are not correlated (spearman's ρ=0.54, p<0.001), they are somewhat associated. For example, an unequal distribution of viewing time – such as focusing on one or two fractals instead of three – may be more likely when the overall fixation time on all three fractals is shorter. Equally a longer total dwell time may result in a more even distribution of fixation time across all three fractals – though this was not always the case as shown by the specific predictions for each metric and the merely moderate correlation among them.

For the formulas used to generate each VES metric see **Figure 6.1**.





Measures	Equation	Examples
DT = Total dwell time on fractals	DT=∑ (f1+f2+f3)	**Example 1:** DT_{f1}=497ms; DT_{f2}=489ms; DT_{f3}=789ms → DT=1755ms **Example 2:** DT_{f1}= 748ms; DT_{f2}=886ms; DT_{f3}=0ms → DT=1634ms
Pr = Proportion of time spent on target	$Pr=\frac{f3}{DT}$	**Example 1:** Pr = 0.45 **Example 2:** Pr = 0
Eq = Equality in scanning strategy	$\mathrm{InEq}=\frac{\lvert f1DT-f2DT\rvert+\lvert f1DT-f3DT\rvert+\lvert f2DT-f3DT\rvert}{\mathrm{DT}}$ $Eq=1-\frac{InEq}{2}$	**Example 1:** Eq = 1 – 0.17= 0.83 **Example 2:** Eq = 1- 0.54 = 0.46
S = Total shifts between fractals	S= ∑ (number of times gaze was shifted to a new fractal)	**Example 1:** S_{f1}=3; S_{f2}=1; S_{f3}=4 → S=8 **Example 2:** S_{f1}=1; S_{f2}=1; S_{f3}=0 → S=2

Figure 6.1 Visual exploration strategy measures with examples from sample array.

Figure adapted from (Pavisic et al., 2021a) under the terms of the Creative Commons Attribution License (CC, BY). Highlighted fractal represents the target (f3), the identity of which was not known to the participant at the time of viewing. InEq=Inequality. f1, f2 and f3 are fractal 1, 2, and 3 respectively. DT_{f1}, DT_{f2} and DT_{f3} are the dwell times on fractal 1, 2, and 3 respectively. S_{f1}, S_{f2} and S_{f3} are the total shifts on fractal 1, 2 and 3 respectively.

In order to account for any low-level oculomotor differences, I also evaluated basic oculomotor metrics (defined for each trial) between groups using Eyelink's automated detection algorithm:

- **Saccade amplitude** (deg): average amplitude of each saccade.
- **Saccade velocity** (deg/ms): average velocity of each saccade.
- **Peak saccade velocity** (deg/ms): the highest velocity reached during the saccade.
- **Saccade duration** (ms): average time between the start of a saccade and its end.

- **Number of saccades per second** (saccades/s): The number of saccades that were made after the target appeared, excluding blinks (disappearance of the pupil) and excluding saccades smaller than 2 deg (Shakespeare et al., 2015).

6.2.3. Statistical analysis

Baseline demographics and neuropsychology scores were compared between controls and each of symptomatic carriers, late PMCs and early PMCs using ANOVA or Kruskal-Wallis test where the distribution of the variable was skewed. Fishers' exact test was used to compare the sex distribution between the groups.

Behavioural performance on the VSTM task was compared between groups. As the focus of this paper was on exploration strategies, VES metrics and any associations between measures of task performance with eye-tracking was restricted to the 3-items condition only. As in Chapter 5, localisation error and NIC error were both log-transformed and swap error proportion was square root transformed before analysis due to skewed distributions. Analysis of object identity used a logistic regression model and analysis of the other VSTM outcomes used a linear mixed effects model. Models used robust standard errors to account for clustering by participant.

VES metrics and basic oculomotor characteristics, on each trial, were compared between groups using multivariable linear regression models. Examination of residuals was performed to check model fits. For outcomes with skewed distributions (saccade amplitude, saccade duration, average saccade velocity, peak velocity, DT and Eq) bootstrapping, clustered on individual to account for repeated measures, was used to produce bias-corrected and accelerated (BCA) 95% confidence intervals (CIs) from 2,000 replications.

To investigate the relationship between VSTM and VES, I used multivariable linear regression models, where the outcome was either the log of localisation error, log of NIC error or sqrt swap error proportion and logistic regression models where the outcome was identification performance. The predictors for these models were group, sex, age at assessment, NART scores and the VES metrics (DT, Pr, Eq or S) for a total of four analysis (for each VSTM metric) – see below.

- Model 1= NART, sex, age, DT
- Model 2 = NART, sex, age, Eq
- Model 3 = NART, sex, age, S
- Model 4 = NART, sex, age, Pr

Interactions were examined between each VES metric and group and between each VES metric, group and delay where relevant.

All models were adjusted for sex, age and NART and delay (1 *vs* 4s). As saccade amplitude, velocity and duration are closely linked to one another, they were each included as covariates in corresponding models.

For each variable, participants were excluded if their overall performance deviated by 2.5 standard deviations (SD) from either side of the mean of each group. This was done given the varied nature of eye-tracking data and in order to keep consistency in the way in which variables were treated throughout analysis.

6.3. Results

6.3.1. Demographics and traditional neuropsychology

As expected, symptomatic carriers were older than controls (p=0.006), had lower MMSE scores (p<0.001) and reported lower symptoms on anxiety (p=0.016). PMCs and controls were well-matched for age (early: p=0.935, late: p=0.479); early PMCs reported higher depression scores (p=0.034) and late PMCs had slightly lower education levels (p=0.025). No further significant differences were observed for demographics characteristics between groups (**Table 6.1**). Neuropsychology differences were only seen between symptomatic carriers and controls in cognitive functions affected in AD such as fluid intelligence/non-verbal reasoning (p<0.001), recognition memory (faces: p=0.002, words: p<0.001) and STM (digit span forwards: p=0.016). Differences were also seen in category fluency, executive function (Stroop and Trails), paired associate learning (Camden PAL), processing speed (digit symbol substitution) and visuo-spatial working memory (spatial digit span) (all p<0.001). There was also some evidence for lower visuo-spatial detection in symptomatic carriers (VOSP OD: p=0.036). No significant differences were observed between PMCs and controls for neuropsychology measures (**Table 6.1**).

Table 6.1 Participant demographics and neuropsychology.

	Controls (N=26)	Early PMCs (N=7)	Late PMCs (N=9)	Symptomatic carriers (N=9)
Demographics				
Gender (male: female)	11: 15	2: 5	3: 6	7: 2
Age (years)	38.5 (11.8)	38.1 (4.7)	41.3 (7.6)	**50.0 (10.4)****
EYO (years)	NA	-13.9 (7.0)	-3.2 (2.8)	3.9 (5.7)
MMSE	29.9 (0.3)	29.4 (0.5)	29.8 (0.4)	**25.0 (2.6)****
NART [a]	29.7 (8.0)	26.9 (11.1)	31.4 (3.5)	30.9 (10.2)
Education (years)	16.2 (2.1)	16.3 (2.4)	**14.4 (2.7)***	15.4 (2.0)
CDR (global) [b]	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	**1.7 (2.1)****
Anxiety [c]	6.9 (4.0)	9.0 (3.5)	7.0 (4.5)	**4.1 (2.0)***
Depression [c]	2.0 (3.0)	**3.9 (3.8)***	2.0 (2.8)	2.3 (2.8)
Neuropsychology tests				
Verbal IQ [b]	101.6 (8.5)	102.0 (12.2)	105.9 (14.1)	97.2 (14.6)
Performance IQ [b]	115.9 (13.7)	112.1 (15.2)	114.8 (12.0)	**92.0 (14.1)****
Arithmetic total/24 [d]	11.9 (7.9)	9.7 (4.9)	12.9 (4.7)	7.3 (6.8)
RMT faces/50 [b]	45.4 (3.6)	44.3 (4.2)	45.1 (2.8)	**37.7 (7.3)****
RMT words/50 [b]	48.9 (1.6)	50. 0 (0.0)	47.0 (3.2)	**34.4 (5.8)****
Digit span forwards/8 [b]	7.2 (0.7)	6.9 (0.9)	7.3 (0.7)	**6.2 (1.3)***
Digit span backwards/7 [b]	4.9 (1.2)	4.9 (1.2)	5.2 (0.8)	4.3 (1.6)
BPVS/150 [f]	140. 8 (8.0)	136.9 (14.2)	143.4 (3.9)	140.7 (9.7)
Verbal Fluency [b]	15.3 (5.1)	16.0 (2.0)	16.3 (4.2)	13.3 (6.1)
Category Fluency [b]	24.5 (6.2)	22.3 (3.4)	24.1 (4.9)	**15.9 (5.9)****
GNT/30 [f]	19.2 (4.7)	18.3 (5.8)	22.9 (1.6)	18.7 (5.9)
VOSP OD /20 [f]	18.6 (1.1)	17.7 (2.4)	19.1 (1.0)	**17.1 (2.3)***
Stroop ink (s) [f]	48.4 (11.3)	51.4 (11.4)	48.3 (10.0)	**99.3 (43.0)****
Camden PAL/24 [a]	19.8 (4.4)	18.6 (3.2)	19.9 (5.0)	**6.7 (4.5)****
Digit symbol/93 [b]	65.9 (11.8)	65.6 (4.6)	66.7 (11.7)	**31.1 (12.5)****
Spatial forwards/9 [f]	6.4 (0.8)	5.4 (1.3)	5.9 (0.9)	**4.1 (1.5)****
Spatial backwards/9 [f]	5.8 (1.0)	4.9 (1.6)	5.1 (0.9)	**3.4 (1.5)****
Trails A (s) [b]	24.9 (7.2)	24.6 (9.9)	21.0 (4.9)	**53.3 (37.2)****
Trails B (s) [g]	54.2 (16.5)	58.1 (22.6)	46.3 (5.6)	**153.6 (90.3)****

Unadjusted mean values are given with SD unless otherwise stated. SD= standard deviation; NA=not applicable; PMC=presymptomatic mutation carrier; EYO=estimated years to/from symptom onset (a negative value indicates a younger age than their estimated age at symptom onset); Anxiety and depression from the HADS=hospital anxiety and depression scale; MMSE=mini-mental state examination CDR=clinical dementia rating scale; IQ=intelligent quotient; RMT=recognition memory test; BPVS=British Picture Vocabulary Scale; GNT=graded naming test; NART=National Adult Reading Test; PAL=paired associated learning; Digit spans forwards and backwards are taken from the WMS-R=Wechsler Memory Scale. [a] n= 48; [b] n= 38; [c] n= 39; [d] n= 37; [e] n=44; [f] n=43; [g] n=42. Bold=significant; *: the difference between the patient group and controls for that variable was significant at p <0.05; **: the difference between the patient group and controls for that variable was significant at p<0.01.

6.3.2. Behavioural metrics of task performance

Consistent with previous studies and Chapter 5 (Liang et al., 2016; Pertzov et al., 2012, 2015), performance was significantly influenced by load (1-item *vs* 3-items: p<0.001). Delay also had a significant effect in all (1s *vs* 4s: p<0.001) but the identification performance metric (p=0.140 unlike previous reports which showed an effect of delay on identification performance too);

such that irrespective of the group, participant performance was worse with higher load and longer delays.

Overall symptomatic carriers had on average 65.4 [41.5, 79.5] % lower odds of correct identification ($p<0.001$) and significantly higher localisation error ($p<0.001$) in comparison to controls (**Table 6.3,** **Figure 6.2**). No significant differences were observed for PMCs (identification: early: $p=0.618$; late: $p=0.635$ and localisation error: early: $p=0.702$; late: $p=0.853$).

In the 3-items condition, compared to controls symptomatic carriers had on average 63.1 [38.3, 78.0] % lower odds of correct identification ($p<0.001$), higher localisation and NIC error (both $p<0001$) and showed a trend towards a higher proportion of swaps (difference in square root of swap error: coefficient=0.066 [-0.004, 0.135], $p=0.063$). PMCs showed similar performances to controls in NIC error (early $p=0.929$; late: $p=0.862$) and in the proportion of swaps (early $p=0.473$; late: $p=0.467$) (**Figure 6.2**, see **Table 6.3** for effect size).

There was a weak interaction between group and delay in the proportion of swaps, whereby early PMCs had a higher proportion of swaps after a 4s *vs* 1s delay ($p=0.059$) compared to controls. However, this effect did not reach statistical significance and may have been driven by the trend for a lower proportion of swaps after a 1s delay in this group compared to controls ($p=0.075$) (**Figure 6.2**).

Table 6.2 VSTM performance by group. The first row indicates the adjusted mean and the second row indicates the adjusted group difference with control as the reference group.

	Adjusted mean [95% CI] Group difference [95% CI] (control as reference) Controls	Early PMCs	Late PMCs	Symptomatic carriers
Identification performance: % correct & Odds ratio for correct response				
Overall	90.8 [88.8, 92.8] NA	92.0 [88.2, 95.8] 1.16 [0.64, 2.10]	89.8 [85.7, 93.8] 0.88 [0.53, 1.48]	78.1 [70.4, 85.9] **0.35 [20.5, 58.5]****
3-items	88.9 [86.4, 91.4] NA	90.0 [85.4, 94.7] 1.12 [0.62, 2.03]	87.6 [82.5, 92.6] 0.88 [0.51, 1.50]	75.0 [66.5, 83.5] **0.37 [0.22, 0.62]****
Localisation error: Geometric mean (deg, back-transformed from log-transformation) & % error difference				
Overall	4.43 [4.00, 4.91] NA	4.24 [3.47, 5.17] -4.33 [-23.61, 19.82]	4.52 [3.79, 5.38] 1.93 [-16.71,24.75]	7.05 [5.90, 8.41] **59.03 [29.67, 95.05]****
3-items (all delays) #	5.11 [4.53, 5.76] NA	4.83 [3.95, 5.91] -5.40 [-25.35, 19.87]	5.32 [4.30, 6.59] 4.21 [-18.20, 32.76]	9.14 [7.45, 11.22] **78.94 [41.47, 126.33]****
3-items, 1s	4.58 [4.07, 5.16] NA	4.06 [3.11, 5.30] -11.41 [-33.59, 18.18]	4.79 [3.74, 6.12] 4.43 [-20.38, 36.97]	8.58 [6.95, 10.59] **87.19 [45.44, 140.92]****
3-items, 4s	4.92 [4.33, 5.58] NA	4.91 [4.13, 5.84] -0.14 [-19,70, 24.20]	5.04 [4.27, 5.94] 2.46 [-16.39, 25.57]	7.79 [6.17, 9.83] **58.32 [22.15, 105.21]****
NIC error: Geometric mean (deg, back-transformed from log-transformation) & % error difference				
All delays #	3.50 [3.26, 3.76] NA	3.53 [2.92, 4.28] 0.92 [-17.46, 23.39]	3.54 [3.15, 3.99] 1.23 [-11.77, 16.15]	5.24 [4.37, 6.28] **49.70 [22.63, 82.76]****
1s	3.33 [3.06, 3.62] NA	3.31 [2.84, 3.87] -0.55 [-16.37, 18.26]	3.32 [2.93, 3.76] -0.26 [-14.16, 15.90]	4.98 [4.21, 5.89] **49.46 [23.55, 80.81]****
4s	3.69 [3.40, 4.00] NA	3.77 [2.96, 4.80] 2.30 [-20.45, 31.55]	3.80 [3.37, 4.28] 2.98 [-10.59, 18.61]	5.53 [4.42, 6.91] **49.89 [17.25, 91.62]****
Swap error √proportion				
All delays	0.119 [0.094, 0.144] NA	0.102 [0.061, 0.144] -0.017 [-0.064, 0.030]	0.142 [0.084, 0.200] 0.231 [-0.040, 0.087]	0.185 [0.121, 0.249] 0.066 [-0.004, 0.135]
1s	0.112 [0.082, 0.142] NA	0.056 [0.003, 0.109] -0.056 [-0.117, 0.006]	0.1448 [0.083, 0.207] 0.330 [-0.037, 0.103]	0.158 [0.066, 0.250] 0.046 [-0.051, 0.143]
4s	0.126 [0.087, 0.166] NA	0.147 [0.096, 0.198] 0.021 [-0.039, 0.081]	0.139 [0.071, 0.206] 0.012 [-0.066, 0.091]	0.213 [0.146, 0.279] **0.086 [0.004, 0.169]***

VSTM=visual short-term memory; PMC=presymptomatic mutation carrier; #: localisation error in the 3-item condition across delays is presented for an overall comparison with NIC error. Note that as delay length did not have a significant effect on identification performance, results are not shown by delay. Bold=significant; *: significant at $p<0.05$; **: significant at $p<0.01$.

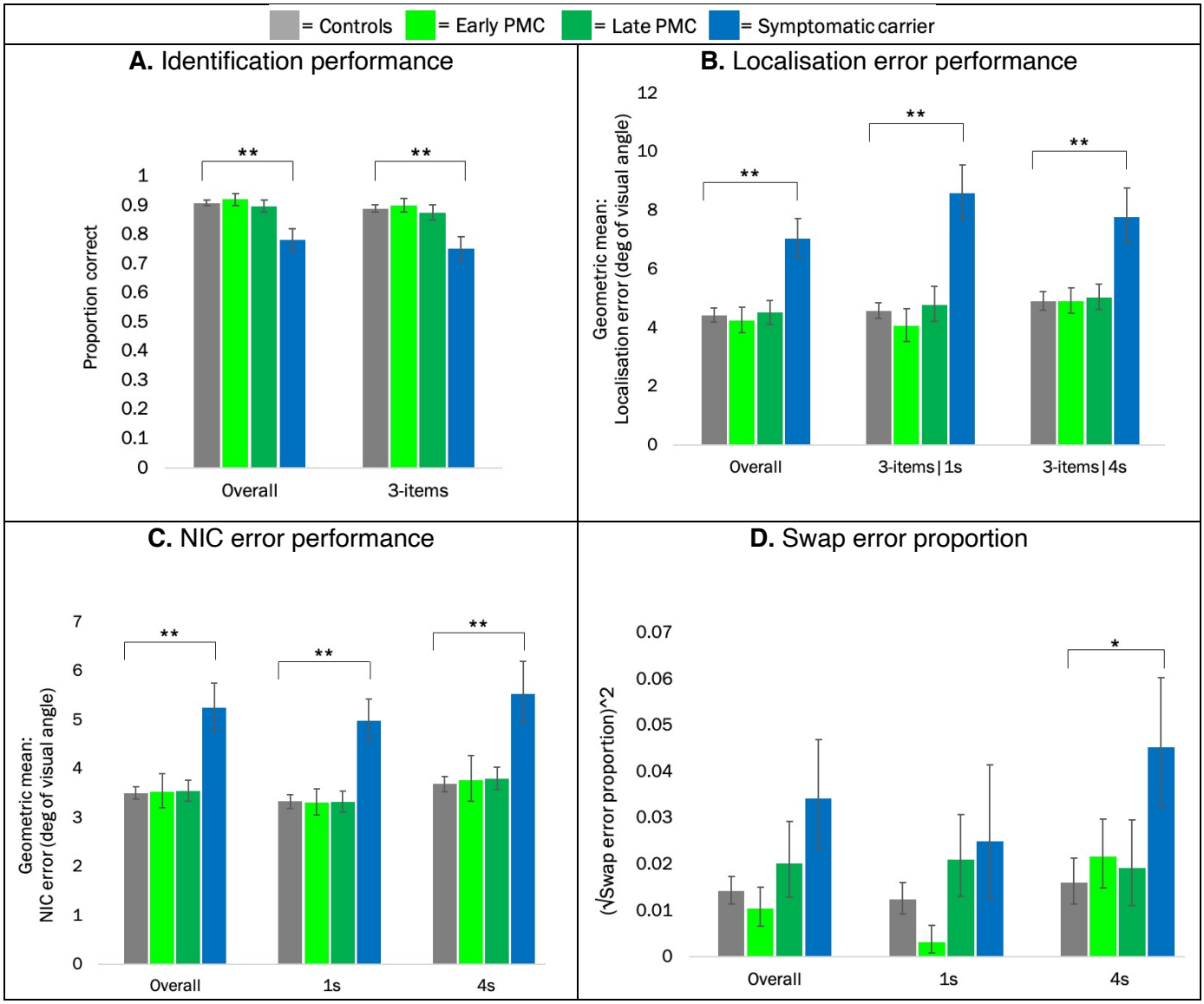


Figure 6.2 Behavioural VSTM mean performance by group (adjusted for age, sex and NART).

Figure adapted from (Pavisic et al., 2021a) under the terms of the Creative Commons Attribution License (CC, BY). **A.** Identification accuracy overall and for the high load. **B.** Localisation error overall and by delay for the high load. **C.** NIC error overall and by delay. **D.** Swap error overall and by delay. Error bars show +/- standard error of the mean. PMC=presymptomatic mutation carrier. NIC=nearest item control. *: significant at $p<0.05$.; **: significant at $p<0.01$.

6.3.3. Visual exploration strategies and basic oculomotor characteristics

Compared to controls, the symptomatic group spent on average 276ms less time fixating the stimuli (total dwell time on fractals, **Table 6.3,** Figure 6.3A) and showed a less homogenous distribution of fixation time among fractals (with a lower equality score: 0.12 points lower in equality score, Figure 6.3B and a trend for fewer shifts between fractals, $p=0.181$ **Figure 6.3C, Table 6.3**). There was no difference between either of the PMC groups and controls in any of these three VES metrics (**Table 6.3**).

As the target (the fractal that would be probed) was unknown to the participant at the time of viewing, there was no difference between each patient group and controls in the proportion of time spent looking at the target (**Table 6.3,** Figure 6.3D).

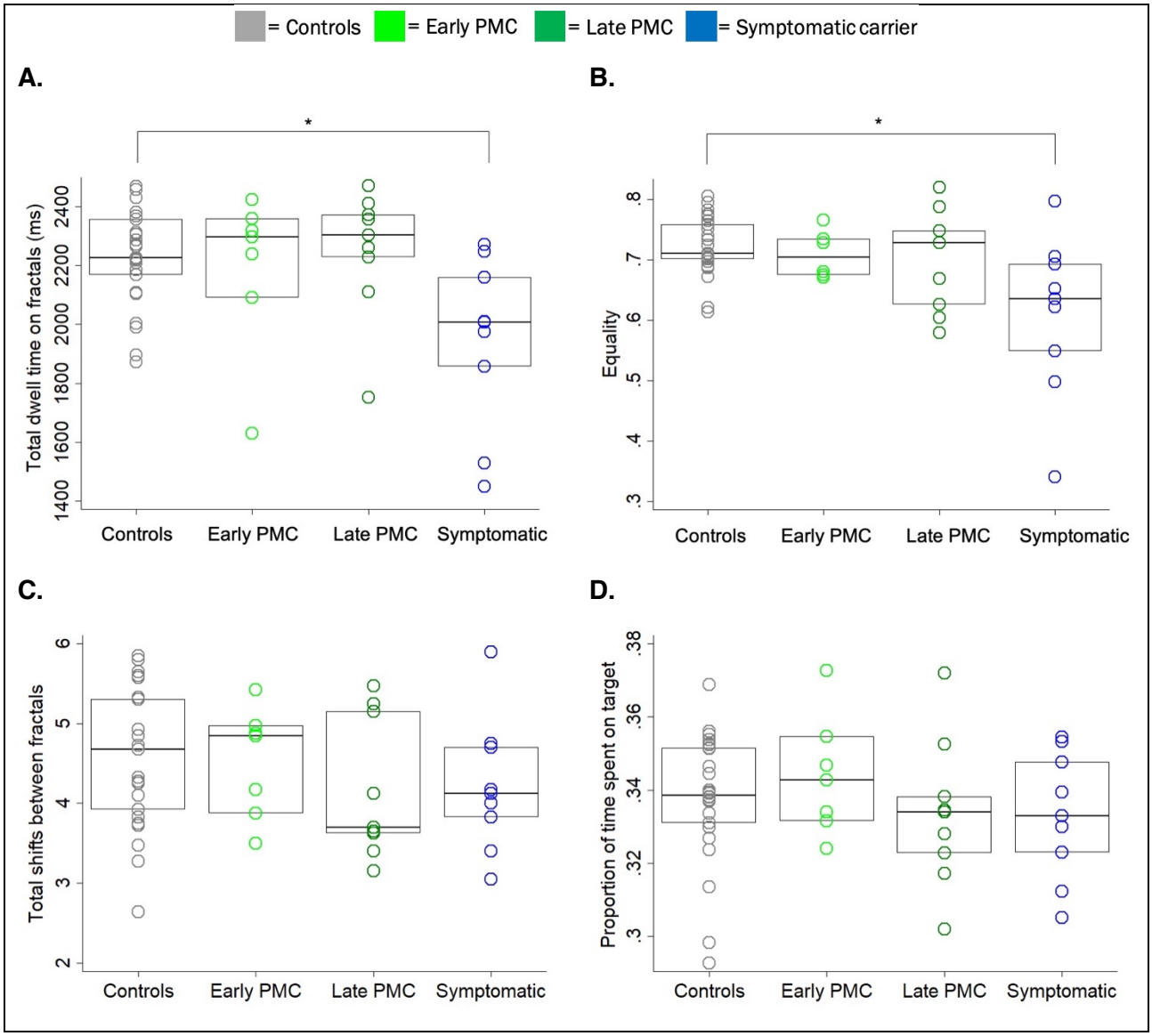


Figure 6.3 Unadjusted visual exploration strategy metrics by group.

Figure reprinted from (Pavisic et al., 2021a) under the terms of the Creative Commons Attribution License (CC, BY). Each data point represents one participant**. A.** Total dwell time on fractals. **B.** Equality index. Note that the x-axis does not start with zero. **C.** Total shifts between fractals. **D.** Proportion of time spent on the target fractal (unknown to the participant). *: significant at p <0.05 from bias-corrected and accelerated (BCA) approach. Box represent median and interquartile (IQR) range.

Compared to controls, basic oculomotor characteristics revealed no significant differences in any of the patient groups (**Table 6.3**) and while blinks were removed from the analysis, a separate investigation revealed a similar number between groups (**Table 6.3**).

Table 6.3 Eye-tracking metrics by group. The first row indicates the adjusted mean and the second row indicates the adjusted group difference with control as the reference group.

	Adjusted mean [95% CI] Group difference [95% CI] (reference controls)			
	Controls	**Early PMCs**	**Late PMCs**	**Symptomatic carriers**
Visual exploration strategies				
Total dwell time on fractals- 'DT' (ms) #	2224.2 [2151.0, 2297.5] NA	2197.4 [1998.5, 2396.2] -26.9 [-236.3, 182.5]	2258.7 [2117.3, 2400.1] 34.5 [-126.1, 195.0]	1947.7 [1756.7, 2138.7] **-276.5 [-483.7, -69.4] ***
Equality- 'Eq' #	0.72 [0.70, 0.75] NA	0.71 [0.68, 0.75] -0.01 [-0.05, 0.03]	0.70 [0.65, 0.76] -0.02 [-0.08, 0.04]	0.60 [0.52, 0.68] **-0.12 [-0.20, -0.04] ***
Total shifts between fractals- 'S'	4.5 [4.2, 4.9] NA	4.6 [4.0, 5.1] 0.02 [-0.6, 0.7]	4.3 [3.7, 4.8] -0.3 [-0.9, 0.4]	4.1 [3.5 to 4.6] -0.5 [-1.1, 0.2]
Proportion of time spent on target- 'Pr'	0.34 [0.33, 0.34] NA	0.34 [0.33, 0.35] 0.004 [-0.01, 0.02]	0.33 [0.32, 0.35] -0.004 [-0.02, 0.01]	0.34 [0.32, 0.35] -0.003 [-0.02, 0.01]
Basic oculomotor tasks				
Saccade amplitude (deg) #	4.41 [4.39, 4.44] NA	4.42 [4.36, 4.47] 0.008 [-0.05, 0.07]	4.43 [4.39, 4.48] 0.02 [-0.03, 0.07]	4.37 [4.31, 4.44] -0.04 [-0.10, 0.03]
Saccade duration (ms) #	39.00 [38.83, 39.16] NA	38.83 [38.51, 39.14] -0.17 [-0.54, 0.20]	38.98 [38.72, 39.21] -0.03 [-0.30, 0.25]	38.72 [38.38, 39.06] -0.27 [-0.63, 0.09]
Saccade velocity (deg/ms) #	94.84 [94.35, 95.34] NA	95.56 [94.73, 96.40] 0.72 [-0.22, 1.65]	95.67 [94.83, 96.51] 0.83 [-0.15, 1.80]	93.81 [92.87, 94.75] -1.03 [2.13, 0.07]
Peak velocity (deg/ms) #	157.80 [152.64, 162.96] NA	156.80 [150.33, 163.27] -1.00 [-8.78, 6.78]	155.12 [150.49, 159.75] -2.69 [-9.51, 4.14]	164.20 [155.81, 172.60] 6.40 [-3.78, 16.58]
Number of saccades per second (sacc/s) #	5.0 [4.9, 5.2] NA	5.1 [4.8, 5.4] 0.03 [-0.3, 0.4]	5.0 [4.7, 5.3] -0.08 [-0.4, 0.3]	5.0 [4.8, 5.2] -0.06 [-0.3, 0.2]
Blinks per trial	5.9 [5.4, 6.4] NA	5.4 [3.7, 7.1] -0.5 [-2.3, 1.2]	5.6 [5.0, 6.1] -0.4 [-1.1, 0.4]	5.9 [5.2, 6.6] -0.003 [-0.9, 0.9]

PMC=presymptomatic mutation carrier. NA=not applicable. Bold=significant; *: significant at $p < 0.05$; # from bias-corrected and accelerated (BCA) approach.

6.3.4. Visual exploration strategies as predictors of VSTM performance

While the only deficits detected on individual behavioural VSTM and low-level oculomotor functions were amongst symptomatic individuals, I next explored VES as predictors of VSTM function. The rationale for this was that if encoding deficits were present in FAD carriers in comparison to controls, these would be detected when evaluating eye movements in association with task performance.

Identification performance

Across the whole sample, increasing DT and Eq were respectively associated with greater odds of correct identification. For every 100ms in DT in a trial, the odds of correct identification increased by 4.1 [0.8, 7.3] % (p=0.015). Similarly, higher Eq score resulted in greater odds of correct identification (p=0.006). To put this into context, with Eq=0.5 *vs* Eq=1: % correct identification: 83.2 [80.2, 86.3] % *vs* 89.50 [86.6, 92.5] %.

Identification performance was not significantly associated with the number of eye movement shifts (saccades) between fractals (p=0.291). However, increasing Pr resulted in greater odds of correct identification/decreasing error (OR=2.82, p=0.037). To put this into context, Pr= 0.33 (33% of fixation time on the target) = 86.0 [83.7, 88.3] % correct identification *vs* Pr=1 (100% of fixations on the target): 92.3 [87.2, 97.3] %.

All significant association between VES metrics and identification performance persisted in the same direction when excluding symptomatic carriers (DT: p=0.025, Eq: p<0.001 and Pr: p=0.038).

As delay did not have a significant effect on identification performance, interaction tests were restricted to group and VES metrics. There was trend for an interaction between DT and early PMCs, whereby for every 100ms in DT in a trial, the odds of correct identification decreased by 11.8 [-1.0, 24.5] %, however this did not reach statistical significance (p=0.070). No significant interactions with DT and late PMCs (p=0.906) or symptomatic carriers (p=0.162) emerged nor were there significant interactions between group and Eq (early PMCs: p=0.982; late PMCs: p=0.801; symptomatic carriers: p=0.262) or between group and Pr (early PMCs: p=0.291; late PMCs: p=0.172; symptomatic carrier: p=0.159).

Localisation performance

Across the sample as a whole, increasing DT and Eq were both associated with decreasing localisation error. For every 100ms in DT in a trial, localisation performance decreased by 2.1 [0.6, 3.5] % (p=0.006). Similarly, higher Eq score resulted in a reduction of localisation error (p=0.021). To put this into context, with Eq=0.5 *vs* Eq=1 the geometric localisation error (back-transformed from log transformation) was 6.0 [5.5, 6.6] deg *vs* 5.2 [4.5, 5.8] deg. Both of these associations persisted in the same direction when excluding symptomatic carriers (DT: p=0.035, and Eq: p=0.021).

There was no signification interaction between group and DT (early PMCs: p=0.339; late PMCs: p=0.427; symptomatic: p=0.475); group and Eq (early PMCs: p=0.331; late PMCs:

p=0.961; symptomatic: *p*=0.838) or group delay and Eq (early PMCs: *p*=0.628; late PMCs: *p*=0.388; symptomatic: *p*=0.355). However, a significant interaction between group, delay and DT emerged, whereby for every 100ms DT increase in a trial, late PMCs showed a smaller localisation error in the 1s *vs* 4s delay conditions compared to controls (group x delay x DT interaction coefficient= -5.7 [-10.5, -1.0] %, *p*=0.019). This suggested a stronger association between DT and localisation error in the 1s compared to the 4s delay condition for late PMCs compared to controls which was not observed in other groups (interaction coefficients: early PMCs= -0.2 [-5.5, 5.2] %, *p*=0.954; symptomatic= 0.3 [-5.1, 5.7] %, *p*=0.907). Due to the significant interaction between group x delay and DT, delay conditions were investigated further for late PMCs.

Specifically, this meant that in the 1s delay condition for every 100ms in DT, localisation error decreased by 4.6 [1.0, 8.3] % more in the late PMC than control group (*p*=0.014). As a result, with shorter DT, performance for late PMCs was worse (greater error) than controls whereas with longer DT, a similar localisation performance was observed (**Figure 6.4A**). No significant interactions emerged with DT and other groups in the 1s delay condition (early PMC: *p*=0.498, symptomatic: *p*=0.672) nor with any of the groups and DT in the 4s delay condition (early PMC: *p*=0.326, late PMC: *p*=0.675 and symptomatic carriers: *p*=0.508, **Figure 6.4B**).

Localisation error was not significantly associated with the number of eye movement shifts (saccades) between fractals (*p*=0.266) or the proportion of time spent on the target (*p*=0.128).

NIC performance

Across the sample as a whole, increasing DT and Eq were respectively associated with decreasing NIC error. For every 100ms in DT in a trial, NIC performance decreased by 1.2 [-0.02, 2.4] % error (*p*=0.054). Similarly, a higher Eq score resulted in a reduction of NIC error (*p*=0.008) in that trial. To put this into context, with Eq=0.5 *vs* Eq=1, the geometric NIC error (back-transformed from log transformation) resulted in 4.0 [3.7, 4.3] deg *vs* 3.5 [3.2, 3.8] deg, respectively. Increasing 'Pr', was also significantly associated with a reduction in NIC error (*p*=0.001) with Pr= 0.33 *vs* Pr=1, resulted in geometric NIC error=3.8 [3.5, 4.0] deg *vs* 3.0 [2.6, 3.5] deg, respectively. When excluding symptomatic carriers, associations with NIC performance remained significant for Pr only and as a trend for Eq (DT: *p*=0.293 and Eq: *p*=0.072; Pr: *p*<0.001).

Interaction tests between group and VES metrics for NIC error, revealed a trend between DT and late PMCs and symptomatic carriers, whereby for both groups increasing DT resulted in

smaller error than controls: for every 100ms NIC error decreased by 2.3 [-0.2, 5.0] %, p=0.070 for late PMCs and 2.1 [-0.2, 4.4] %, p=0.075 for symptomatic carriers (this was not the case for early PMCs: -1.9 [-4.4, 0.5] %, p=0.122). There was also a trend between Pr and symptomatic carriers, whereby increasing Pr resulted in *greater* error: geometric NIC error =1.5 [1.0, 2.3] deg, p=0.055 than controls (this was not the case for early PMCs: p=0.756 or late PMCs: p=0.937). Yet, none of these interactions reached statistical significance and no interaction effects were observed with delay and DT (early PMCs: p=0.393; late PMCs: p=0.103, symptomatic: p=0.442) or delay and Pr (early PMCs: p=0.272; late PMCs: p=0.632, symptomatic: p=0.193).

However, there was a significant interaction between group and Eq across delays for symptomatic carriers and a trend for late PMCs, whereby a greater Eq score resulting in lower NIC error (early PMCs: p=0.536; late PMCs: p=0.084; symptomatic: p=0.028). No significant interaction between group, Eq and delay emerged (early: p=0.723; late: p=0.767 symptomatic: p=0.063) but given the trend in symptomatic carriers, delay conditions were evaluated separately for this group. The association between a greater equality score and lower NIC error appeared specific to the 4s delay condition (1s: p=0.205, **Figure 6.4C** *vs* 4s: p=0.027, **Figure 6.4D**).

NIC error was not significantly associated with the number of eye movement shifts (saccades) between fractals (p=0.705).

Swap performance

Across the sample as a whole, swap error proportion was not associated with DT (p=0.926), Eq (p=0.903) or Pr (p=0.334). However, there was a trend for a greater proportion of swaps with increasing eye movement shifts between fractals (√swap error proportion: 0.016 [0.00004, 0.031], p=0.051). Interestingly, this effect strengthened when excluding symptomatic carriers (√swap error proportion: 0.020 [0.004, 0.036], p=0.018).

There was no significant interaction between group and S although there was a trend for a lower proportion of swap error with increasing shifts for early PMCs (√swap error proportion difference: -0.033 [-0.072, 0.005], p=0.086; late PMCs: p=0.863; symptomatic: p=0.111).

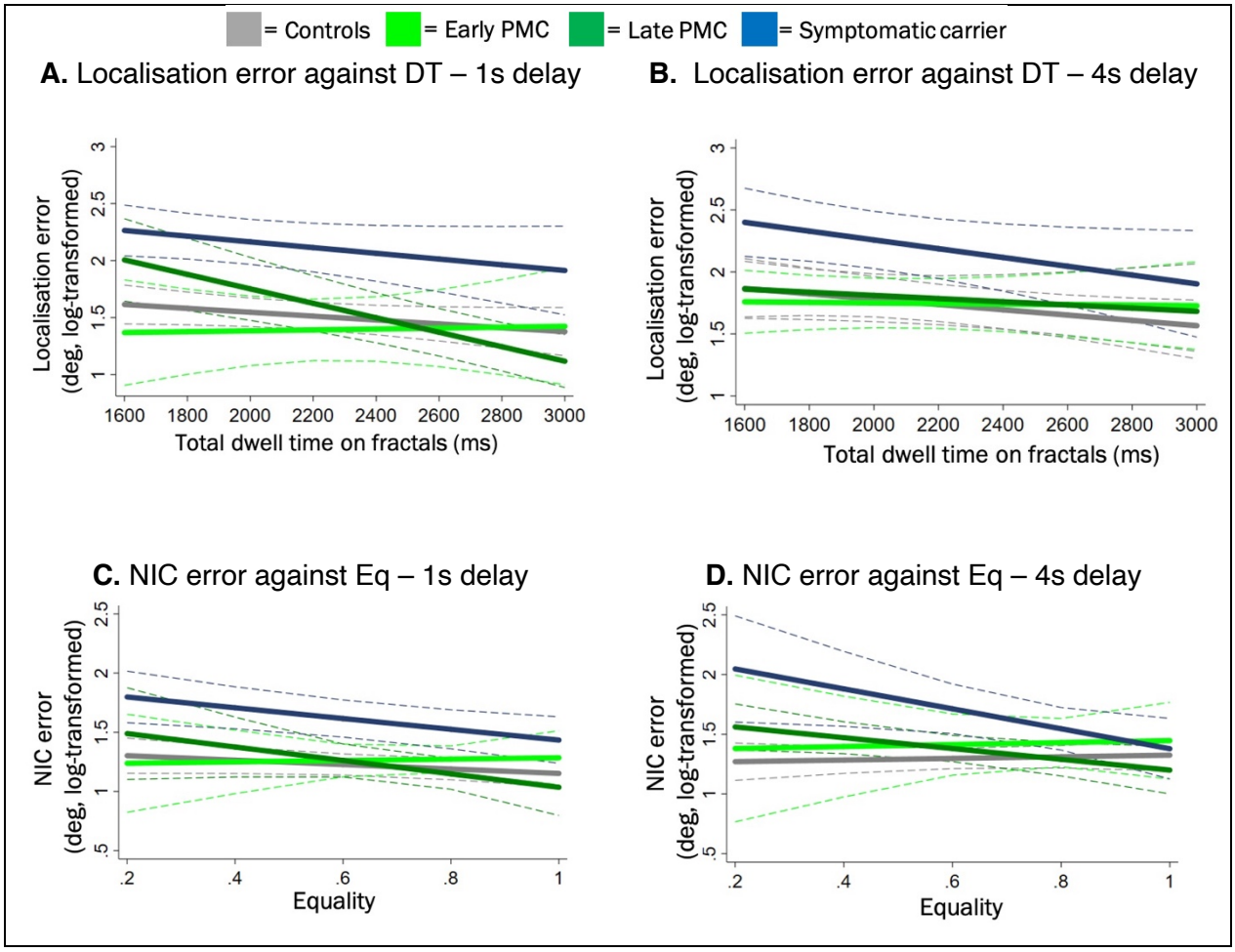


Figure 6.4 VES metrics against VSTM performance.

Figure adapted from (Pavisic et al., 2021a) under the terms of the Creative Commons Attribution License (CC, BY). **A.** 1-second delay condition by the total dwell time on fractals (DT). **B.** 4-seconds delay condition by the total dwell time on fractals (DT). **C.** 1-second delay condition by Equality score (Eq). **D.** 4-seconds delay condition by Equality score (Eq). Complete lines represent best fit lines of the interaction between group and each predictor from the multivariable regression model with localisation or NIC error as the outcome and NART, sex and delay and DT as predictors for panels A. & B. and Eq as predictor for panel C. & D. VES=visual exploration strategy; DT=total dwell time on fractals; Eq=equality. Incomplete lines represent 95% confidence intervals for each group.

Finally, I investigated whether VSTM performance between groups changed when considering VES as additional predictors in regression models (**Table 6.4**). In order to allow for a comparison between models, **Table 6.4, Table 6.5** and **Table 6.6** exclude participants with VES>2.5 SD.

Table 6.4 VES as predictors of VSTM performance: across delays.

	Adjusted mean [95% CI] Group difference [95% CI] (control as reference)				
	Adjusted by NART, sex, delay# and:	**Controls**	**Early PMCs**	**Late PMCs**	**Symptomatic carriers**
Identification (% correct) Odds Ratio for correct response		88.8 [86.1, 91.4] NA	88.8 [84.0, 93.7] 1.01 [0.57, 1.77]	87.6 [82.5, 92.7] 0.89 [0.52, 1.54]	75.1 [66.7, 83.8] **0.38 [0.22, 0.64]****
	DT	88.5 [86.0, 91.1] NA	88.1 [83.3, 92.9] 0.96 [0.56, 1.62]	87.1 [82.2, 92.1] 0.88 [0.53, 1.47]	77.4 [69.7, 85.0] **0.43 [0.26, 0.73]****
	Eq	88.3 [85.7, 91.0] NA	88.6 [83.9, 93.3] 1.03 [0.60, 1.76]	87.4 [82.7, 92.1] 0.92 [0.55, 1.52]	77.2 [69.1, 85.3] **0.44 [0.25, 0.76]****
	S	88.6 [85.9, 91.3] NA	88.6 [83.6, 93.6] 1.00 [0.56, 1.77]	87.8 [83.0, 92.5] 0.92 [0.55, 1.57]	76.0 [67.3, 84.8] **0.40 [0.23, 0.70]****
	Pr	88.7 [86.1, 91.4] NA	88.9 [84.0, 93.6] 1.01 [0.57, 1.77]	87.6 [82.5, 92.7] 0.90 [0.52, 1.55]	75.3 [66.7, 83.8] **0.38 [0.22, 0.65]****
Localisation: Geometric mean error (deg) & % error difference		5.04 [4.45, 5.70] NA	5.16 [4.35, 6.11] 2.37 [-17.13, 26.45]	5.30 [4.27, 6.58] 5.26 [-17.60, 34.48]	9.28 [7.59, 11.34] **84.11 [45.66, 132.70]****
	DT	5.08 [4.52, 5.70] NA	5.28 [4.47, 6.22] -4.00 [-15.10, 27.40]	5.38 [4.36, 6.64] 6.00 [-16.23, 34.10]	8.70 [7.24, 10.45] **71.24 [37.56, 113.19]****
	Eq	5.08 [4.50, 5.73] NA	5.17 [4.34, 6.17] 1.92 [-17.87, 26.47]	5.32 [4.30, 6.58] 4.69 [17.84, 33.41]	9.00 [7.39, 10.96] **77.32 [40.69, 123.50]****
	S	5.00 [4.43, 5.65] NA	5.11 [4.40, 5.93] -2.11 [-15.97, 24.09]	5.34 [4.30, 6.62] 6.70 [-16.37, 36.15]	9.48 [7.78, 11.55] **89.43 [50.05, 139.14]****
	Pr	5.04 [4.46, 5.70] NA	5.16 [4.35, 6.11] -2.35 [-17.11, 26.39]	5.29 [4.26, 6.58] 5.01 [-17.86, 34.24]	9.28 [7.60, 11.33] **84.02 [45.78, 132.29]****
NIC: Geometric mean error (deg) & % error difference		3.50 [3.26, 3.77] NA	3.84 [3.35, 4.40] 10.00 [-5.40, 27.92]	3.34 [3.13, 3.98] 0.96 [-11.96, 15.78]	5.25 [4.39, 6.29] **50.27 [23.15, 83.37]****
	DT	3.52 [3.28, 3.78] NA	3.90 [3.40, 4.47] 10.82 [-4.61, 28.75]	3.57 [3.19, 4.00] 1.43 [-11.17, 15.82]	5.03 [4.27, 5.94] **42.99 [19.00, 71.82]****
	Eq	3.53 [3.28, 3.79] NA	3.85 [3.36, 4.42] 9.22 [-6.22, 27.19]	3.54 [3.17, 3.97] 0.46 [-11.99, 14.66]	5.11 [4.34, 6.01] **44.87 [20.99, 73.47]****
	S	3.50 [3.25, 3.76] NA	3.84 [3.35, 4.39] 9.64 [-5.70, 27.49]	3.54 [3.14, 3.98] 1.03 [-11.96, 15.94]	5.26 [4.42, 6.26] **50.24 [24.18, 81.78]****
	Pr	3.50 [3.26, 3.77] NA	3.84 [3.35, 4.40] 9.64 [5.80, 27.62]	3.53 [3.13, 3.98] 0.73 [12.28, 15.68]	5.25 [4.39, 6.29] **49.92 [22.98, 82.77]****
Swap error √proportion		0.125 [0.101, 0.149] NA	0.098 [0.052, 0.144] -0.027 [-0.078, 0.024]	0.142 [0.085, 0.199] 0.017 [-0.045, 0.079]	0.180 [0.112, 0.248] 0.055 [-0.018, 0.128]
	DT	0.125 [0.100, 0.149] NA	0.097 [0.051, 0.143] -0.027 [-0.079, 0.024]	0.142 [0.085, 0.198] 0.017 [-0.45, 0.079]	0.183 [0.111, 0.255] 0.058 [-0.019, 0.135]
	Eq	0.125 [0.101, 0.149] NA	0.098 [0.052, 0.144] 0.017 [-0.045, 0.080]	0.142 [0.085, 0.199] 0.054 [-0.021, 0.129]	0.179 [0.110, 0.249] 0.054 [-0.021, 0.129]
	S	0.122 [0.100, 0.144] NA	0.095 [0.05, 0.139] -0.027 [-0.076, 0.022]	0.146 [0.091, 0.202] 0.024 [-0.036, 0.084]	0.188 [0.122, 0.253] 0.066 [-0.004, 0.135]
	Pr	0.125 [0.100, 0.149] NA	0.098 [0.052, 0.144] -0.027 [-0.078, 0.024]	0.143 [0.086, 0.200] 0.018 [-0.044, 0.080]	0.180 [0.113, 0.247] 0.055 [-0.017, 0.128]

PMC=presymptomatic mutation carrier; NIC=nearest item control; CI=confidence intervals; DT=total dwell time on fractals; Pr=proportion of time spent looking at the target; S=total number of shifts between fractals; Eq=Equality; VES= visual exploration strategies; NA=not applicable. Bold=significant; *: significant at $p<0.05$; **: significant at $p<0.01$.

Table 6.5 VES as predictors of VSTM performance: 1s delay.

	Adjusted mean [95% CI] Group difference [95% CI] (control as reference)				
	Adjusted by NART, sex[#] and:	**Controls**	**Early PMCs**	**Late PMCs**	**Symptomatic carriers**
Localisation: Geometric mean error deg & % error difference		4.51 [3.98, 5.11] NA	4.43 [3.52, 5.58] -1.67 [-23.94, 27.11]	4.76 [3.69, 6.16] 5.72 [-20.36, 40.33]	8.73 [7.06, 10.79] **93.62 [49.43, 150.88]****
	DT	4.54 [4.06, 5.10] NA	4.52 [3.60, 5.68] -0.38 [-22.67, 28.33]	4.86 [3.80, 6.22] 7.06 [-18.28, 40.27]	8.19 [6.71, 9.99] **80.47 [41.92, 129.48]****
	Eq	4.54 [4.01, 5.14] NA	4.44 [3.50, 5.64] -2.16 [-24.86, 27.39]	4.77 [3.71, 6.13] 5.12 [-20.60, 39.16]	8.51 [6.85, 10.58] **87.58 [44.10, 144.17]****
	S	4.49 [3.96, 5.10] NA	4.41 [3.56, 5.47] -1.73 [-23.27, 25.85]	4.78 [3.70, 6.19] 6.55 [-19.93, 41.77]	8.82 [7.17, 10.85] **96.45 [51.75, 154.31]****
	Pr	4.51 [3.97, 5.11] NA	4.44 [3.53, 5.59] -1.44 [-23.70, 27.32]	4.76 [3.68, 6.16] 5.72 [-20.41, 40.45]	8.72 [7.05, 10.79] **93.61 [49.39, 150.91]****
NIC: Geometric mean error deg & % error difference		3.33 [3.05, 3.63] NA	3.55 [3.22, 3.92] 6.74 [-6.11, 21.33]	3.31 [2.91, 3.77] -0.47 [-14.71, 16.15]	4.98 [4.19, 5.93] **49.71 [23.05, 82.14]****
	DT	3.34 [3.07, 3.64] NA	3.60 [3.25, 3.98] 7.58 [-5.39, 22.34]	3.35 [2.97, 3.79] 0.30 [-13.54, 16.36]	4.80 [4.10, 5.60] **43.46 [20.13, 71.33]****
	Eq	3.35 [3.07, 3.66] NA	3.56 [3.21, 3.95] 6.19 [-6.96, 21.19]	3.32 [2.94, 3.74] -1.05 [-14.55, 14.58]	4.86 [4.14, 5.70] **44.85 [20.67, 73.87]****
	S	3.32 [3.05, 3.63] NA	3.55 [3.22, 3.90] 6.71 [-5.90, 21.00]	3.32 [2.92, 3.78] -0.12 [-14.51, 16.68]	5.01 [4.23, 5.92] **50.66 [24.50, 82.32]****
	Pr	3.33 [3.05, 3.63] NA	3.57 [3.23, 3.94] 7.20 [-5.74, 21.93]	3.31 [2.91, 3.77] -0.45 [-14.73, 16.22]	4.98 [4.20, 5.91] **49.69 [23.29, 81.74]****
Swap error √proportion		0.116 [0.084, 0.147] NA	0.066 [0.007, 0.124] -0.050 [-0.116, 0.016]	0.145 [0.083, 0.207] 0.029 [-0.041, 0.099]	0.156 [0.061, 0.251] 0.040 [-0.061, 0.142]
	DT	0.116 [0.084, 0.147] NA	0.066 [0.007, 0.124] -0.050 [-0.116, 0.016]	0.144 [0.083, 0.207] 0.029 [-0.041, 0.099]	0.126 [0.062, 0.250] 0.040 [-0.060, 0.141]
	Eq	0.115 [0.084, 0.147] NA	0.066 [0.007, 0.124] -0.050 [-0.116, 0.016]	0.145 [0.083, 0.207] 0.039 [-0.041, 0.099]	0.157 [0.061, 0.253] 0.041 [-0.062, 0.144]
	S	0.114 [0.082, 0.145] NA	0.064 [0.008, 0.121] -0.050 [-0.114, 0.014]	0.147 [0.086, 0.208] 0.033 [-0.035, 0.102]	0.160 [0.065, 0.254] 0.046 [-0.056,0.147]
	Pr	0.116 [0.084, 0.148] NA	0.064 [0.005, 0.123] -0.052 [-0.119, 0.015]	0.145 [0.084, 0.206] 0.029 [-0.041, 0.099]	0.156 [0.061, 0.251] 0.040 [-0.062, 0.142]

PMC=presymptomatic mutation carrier; NIC=nearest item control; CI=confidence intervals; DT=total dwell time on fractals; Pr=proportion of time spent looking at the target; S=total number of shifts between fractals; Eq=Equality; VES= visual exploration strategies; NA=not applicable. Bold=significant; *: significant at $p<0.05$; **: significant at $p<0.01$.

Table 6.6 VES as predictors of VSTM performance: 4s delay.

	Adjusted mean [95% CI] Group difference [95% CI] (control as reference)				
	Adjusted by NART, sex[#] and:	**Controls**	**Early PMCs**	**Late PMCs**	**Symptomatic carriers**
Localisation: Geometric mean error (deg) & % error difference		5.65 [4.80, 6.66] NA	6.00 [4.97, 7.26] 6.21 [-17.49, 36.73]	5.93 [4.90, 7.17] 4.83 [-17.76, 33.63]	9.88 [7.69, 12.69] **74.67 [29.59, 135.44]****
	DT	5.71 [4.87, 6.69] NA	6.17 [5.13, 7.43] 8.14 [-15.19, 37.90]	6.00 [4.98, 7.22] 5.03 [-16.88, 32.74]	9.24 [7.29, 11.72] **61.91 [20.86, 116.90]****
	Eq	5.71 [4.86, 6.71] NA	6.04 [4.96, 7.35] 5.80 [-18.14, 36.73]	5.95 [4.92, 7.20] 4.29 [-17.99, 32.62]	9.52 [7.48, 12.13] **66.92 [24.50, 123.80]****
	S	5.60 [4.78, 6.55] NA	5.91 [4.96, 7.06] 5.65 [-16.79, 34.13]	5.98 [4.95, 7.22] 6.82 [-15.73, 35.41]	10.22 [7.91, 13.20] **82.64 [35.56, 146.06]****
	Pr	5.67 [4.82, 6.67] NA	5.99 [4.93, 7.27] 5.63 [-18.30, 36.56]	5.90 [4.88, 7.14] 4.11 [-18.36, 32.77]	9.89 [7.70, 12.70] **74.45 [29.63, 134.77]****
NIC: Geometric mean error (deg) & % error difference		3.69 [3.40, 4.01] NA	4.15 [3.40, 5.07] 12.45 [-9.13, 39.15]	3.79 [3.35, 4.28] 2.62 [-11.06, 18.41]	5.55 [4.44, 6.93] **50.27 [17.54, 92.12]****
	DT	3.72 [3.43, 4.03] NA	4.23 [3.47, 5.17] 13.95 [-7.79, 40.81]	3.82 [3.39, 4.30] 2.79 [-10.66, 18.27]	5.28 [4.27, 6.53] **42.18 [12.13, 80.28]****
	Eq	3.72 [3.43, 4.03] NA	4.17 [3.40, 5.11] 12.08 [-9.62, 38.98]	3.80 [3.38, 4.28] 2.18 [-11.20, 17.58]	5.37 [4.39, 6.57] **44.52 [15.66, 80.59]****
	S	3.69 [3.40, 4.02] NA	4.16 [3.39, 5.09] 12.51 [-9.29, 39.57]	3.78 [3.35, 4.27] 2.41 [-11.19, 18.10]	5.53 [4.47, 6.83] **49.55 [18.56, 88.65]****
	Pr	3.70 [3.40, 4.02] NA	4.14 [3.39, 5.07] 12.10 [-9.58, 38.98]	3.78 [3.35, 4.26] 2.24 [-11.31, 17.86]	5.55 [4.44, 6.94] **50.13 [17.37, 92.04]****
Swap error √proportion		0.134 [0.009, 0.175] NA	0.130 [0.091, 0.169] -0.004 [-0.056, 0.047]	0.139 [0.073, 0.205] 0.005 [-0.073, 0.082]	0.204 [0.134, 0.274] 0.070 [-0.017, 0.157]
	DT	0.134 [0.092, 0.175] NA	0.128 [0.088, 0.167] -0.006 [-0.058, 0.046]	0.139 [0.072, 0.204] 0.004 [-0.074, 0.083]	0.211 [0.130, 0.292] 0.077 [-0.019, 0.174]
	Eq	0.135 [0.094, 0.175] NA	0.130 [0.091, 0.170] -0.004 [-0.056, 0.047]	0.139 [0.073, 0.205] 0.004 [-0.074, 0.082]	0.203 [0.131, 0.275] 0.070 [-0.020, 0.156]
	S	0.130 [0.092, 0.168] NA	0.124 [0.082, 0.166] -0.006 [-0.06, 0.047]	0.144 [0.080, 0.208] 0.014 [-0.060, 0.088]	0.217 [0.144, 0.209] **0.087 [0.001, 0.173]***
	Pr	0.134 [0.093, 0.175] NA	0.130 [0.091, 0.169] -0.004 [-0.056, 0.047]	0.139 [0.073, 0.205] 0.005 [-0.073, 0.083]	0.204 [0.134, 0.274] 0.070 [-0.016, 0.157]

PMC=presymptomatic mutation carrier; NIC=nearest item control; CI=confidence intervals; DT=total dwell time on fractals; Pr=proportion of time spent looking at the target; S=total number of shifts between fractals; Eq=Equality; VES= visual exploration strategies; NA=not applicable. Bold: significant; *: significant at $p<0.05$; **: significant at $p<0.01$.

Including VES metrics as additional predictors in regression models resulted in comparable differences between groups in identification performance; slightly *greater* differences between

late PMCs and controls and slightly *reduced* differences between symptomatic carriers and controls for the localisation error measure. For instance, in the 1s condition where the significant interaction between late PMC and DT was observed, the percentage localisation error increased in late PMCs, when considering DT as a predictor in comparison to the reference model (adjusted by NART and sex only). However, these effects did not yield statistically significant differences between groups and effect sizes remained small.

6.4. Discussion

6.4.1. Summary

In this chapter, I investigated how eye-tracking data could deepen our understanding of VSTM changes in a preclinical AD population. I assessed memory performance using a delayed-reproduction paradigm (Pertzov et al., 2012, 2013) with a continuous analogue scale measuring the *precision* of memory recall and evaluated whether eye movements could predict VSTM performance. My main hypothesis was that encoding – indexed indirectly by overall time spent fixating a stimulus – might be particularly affected in FAD individuals. Overall, greater time spent viewing the stimuli increased VSTM performance accuracy across all groups. The key finding was that the relationship between eye movements during encoding and VSTM performance, differed between FAD mutation carriers and controls even at presymptomatic stages. More specifically, following a 1s delay, late PMCs (within 6 years to expected symptom onset), showed a stronger reliance on the dwell time on fractals (DT) than controls to achieve an accurate localisation performance. These results suggest that a greater cognitive effort was required in late PMCs to achieve a level of localisation performance comparable to that of controls. Other important findings include: the overall shorter dwell time on fractals and the more unequal distribution of viewing time among fractals in the symptomatic carrier group in comparison to controls. Lastly, no differences in low-level oculomotor performance were observed for symptomatic or presymptomatic carriers in comparison to controls. These findings will be discussed in greater detail in the following sub-sections.

6.4.2. Viewing behaviour and VSTM performance

Across the sample as a whole, several measures of VES predicted behavioural task performance. The time spent fixating the stimuli (total dwell time) and a more equal distribution of this time among fractals were both associated with better recall of object identity and location (of the target fractal: localisation and the nearest fractal: NIC). As expected, the proportion of time spent fixating the target fractal (the item that was later probed) was also associated with better performance (with significant association on object identity and NIC and a trend in the same direction for localisation error). The total number of saccadic shifts and swap error performance only showed an association with each other with some indication that more shifts resulted in a lower proportion of swaps for early PMCs, however this trend did not reach statistical significance and may have been driven by the somewhat overall lower proportion of swaps in this group compared to controls. Taken together, findings are in accordance with the literature that viewing behaviour is an integral part of the *memory formation* process given that VSTM performance was significantly associated with most VES metrics (Chan et al., 2011; Kafkas & Montaldi, 2011; Loftus, 1972; Molitor et al., 2014).

Compared to controls, symptomatic carriers showed a shorter dwell time on fractals and a less homogenous distribution of this fixation time among the three fractals (with a lower equality score and a trend for fewer shifts). While the shorter dwell time on fractals may result from a slower exploration strategy, there was some evidence of impaired visual perception and executive function suggesting this may have also contributed to this finding. Yet, there was no evidence of basic oculomotor impairment in this group suggesting that a reduced engagement with the stimuli and more fixations on other parts of the screen, may also explain this finding. Since investigations in healthy individuals suggest that memory for object identity and location improves with the number and duration of fixations in a cumulative manner (Pertzov et al., 2009) and with increasing exploration of different aspects of an object or a scene (the ‘exploration-exploitation dilemma’); eye movements in symptomatic carriers may be at the root of some of the VSTM impairments reported here and in the literature (Liang et al., 2016). In line with this, the group difference between symptomatic carriers and controls was smaller when adjusting for those VES metrics (e.g. NIC error decreased when Eq was considered). Notably, this effect was smaller for identification performance suggesting either that continuous metrics like localisation or NIC a) may be more sensitive at picking up subtle differences between groups or; b) spatial components may be more sensitive to viewing behaviour all together.

Symptomatic carriers also showed some evidence that a greater proportion of time spent looking at the target fractal resulted in greater NIC error. As the target was unknown to the participant at the time of viewing, this result may reflect the less homogenous distribution of fixation time among fractals and possibly, an ineffective encoding given that fixation time on the target, resulted in poorer NIC performance.

Compared to controls, there was no indication that either of the PMC groups had significantly different eye movement characteristics or worse VSTM performance respectively. However, there was evidence that the predictive effect of eye movements on VSTM performance (and hence the relationship between eye movements and VSTM function) differed between groups. More details are discussed in the hypothesis proposed below.

6.4.3. The 'weakening encoding' hypothesis in presymptomatic FAD

Compared to controls, late PMCs showed a significantly stronger reliance on the total stimuli fixation time for accurate localisation performance in the 1s condition. If accurate performance relies on fixation time (as a proxy to encoding time (Hannula et al., 2010)), why might the relationship between dwell time and localisation error be stronger in late PMC individuals compared to controls? I propose that the integrity and efficiency of encoding processes might be weakened in late PMCs owing to the advancing preclinical AD state, with more time gradually required to effectively encode the stimuli. Additionally, this narrowing window between the time required to encode, and the time available to encode during this fixed presentation time, may have led to a reduction in the variability of dwell times associated with subsequent accurate localisation performance (i.e. stronger association for late PMCs than controls). This overreliance may thus be interpreted as a greater susceptibility to poorer performance. The hypothesis presented here is comparable to that suggested by Bondi and colleagues in episodic memory, whereby another group of individuals at-risk of AD (by virtue of the *APOE* ε4 allele) appeared to require additional cognitive effort to achieve comparable performance levels on tests of episodic memory encoding (Bondi et al., 2005). Accounting for VES metrics yield somewhat higher localisation error for late PMCs compared to controls (i.e. with the same DT, late PMCs had worse localisation error) however this did not result in significant group differences. Notably, the weakening encoding effect may be too subtle to be reflected in task performance especially given that late PMCs required a longer DT to perform at control level, but most DT were within quite a

narrow range. Hence, although rather speculative, these findings suggest that with shorter DT, larger localisation error differences between late PMCs and controls would be observed. Nonetheless, this hypothesis requires further investigation.

Localisation error is a measure of the distance from the exact location of the *target* to the position selected by the participant (for correctly identified objects). Consequently, from a theoretical point of view, it may to some extent represent a measure of '*correct binding'* in a *continuous scale* (of the object's identity to its correct location). So, why was the stronger association between dwell time and localisation error in the late PMC group only seen in the 1s delay condition? As reported previously (Liang et al., 2016; Pertzov et al., 2012), longer 4s delays lead to poorer performance across all subjects. Pertzov and colleagues argue this may relate to the erosion of the representation in memory due to the limitations of the episodic memory buffer (the time over which the object's representations are maintained in memory) (Pertzov et al., 2009, 2012). As memory of the object's identity and location are thought to be held in different brain regions (Darling et al., 2006; Kessels et al., 1999; Pertzov et al., 2012; Postma et al., 2008) and hence not tightly bound in the episodic buffer, they need to be actively linked *over time* for the correct recall of which object was where. Such effects may therefore mask the more subtle relationship between dwell time and localisation error-which is more reflective of processes at encoding than processes during maintenance and retrieval.

The findings in the NIC metric, are in support of the hypothesis proposed above. The main difference between localisation error and the NIC measures is that the latter considers the distance to the closest fractal as opposed to the correct fractal. Therefore, if localisation and NIC measures are considered in a continuum, just as localisation performance may account for 'correct binding' and NIC may represent a measure of 'incorrect binding'. In line with this, it is feasible that the stronger reliance of DT was specific to the localisation error measure i.e. to the *binding* between the object's identity and location.

6.4.4. Final considerations on results and study limitations

Lastly contrary to some literature (Liang et al., 2016; Parra et al., 2010, 2015b), but in accordance with the previous chapter in this thesis, no difference between PMCs and controls were observed in the binary measure of swap errors (misbinding). Interestingly, unlike other behavioural outcomes, swap error proportion was not associated with the majority of eye-tracking measures.

This suggests that other mechanisms not accounted for here, might explain swap error performance. Furthermore, as mentioned in Chapter 5, localisation deficits (evidenced here by greater possible susceptibility to error) may have underrepresented the swap error proportion.

Similar to other studies using this task (Liang et al., 2016; Pertzov et al., 2012, 2015), it is important to acknowledge that the various significant effects in task conditions may have led to false positives and that this study was mostly exploratory. As mentioned in Chapter 5, given the continuous nature of the localisation and NIC error, and the measures sensitivity to quantitative change (as opposed to binary outcomes like identification performance or swap error proportion), subtle differences were detected.

The current study has a number of limitations. In addition to the limitations mentioned in Chapter 5 which are specific to FAD (e.g. the small sample size due to the low prevalence of the condition); from an eye-tracking perspective, the extent to which age-related differences in viewing patterns (Chan et al., 2011; Shih et al., 2012) contribute to memory and hippocampal activity in older adults in unknown (Voss et al., 2017). Crucially, the most significant finding was in the late PMC group, well-matched for age. Moreover, similar to previous reports (Liang et al., 2016), late PMC had lower education levels than controls. While studies suggest VSTM tasks like the one presented here, are impervious to education and intercultural background (Parra et al., 2011; Yassuda et al., 2019), this requires further exploration. Considering a 0.05 level of significance and 80% power, the total sample size required to replicate the late PMC finding of a greater reliance on the total dwell time in order to achieve an accurate localisation performance is N=66 (of which N=45 would be late PMCs and controls).

6.4.5. Conclusions

To the best of my knowledge, I present the first characterization of viewing behaviour in FAD mutation carriers performing a VSTM task.

In summary, findings show how visual search strategies predict VSTM function in a preclinical cohort like FAD. This observation is novel and may explain some of the variance and inconsistencies previously described. For example, if participants spend a ‘sufficient’ and ‘optimal’ amount of time viewing the stimuli their performance will be better than if fixation time is shorter or the distribution of time among fractals is less equal – and without eye-tracking this could not been quantified. More specifically, the finding of a weakening encoding in late PMCs (within 6

years to expected symptom onset) evidenced by the stronger reliance of dwell time on fractals in order to achieve a localisation performance comparable to that of controls, shows that the inclusion of eye movements as markers of subtle cognitive deficits may increase the sensitivity of VSTM tasks to preclinical AD in comparison to behavioural summary metrics of task performance on their own. Importantly, this effect was specific to the localisation performance metric, proposed as a novel measure of relational binding accuracy in the previous chapter.

Lastly, one important limitation of this work is the assumption that VSTM deficits observed in this patient group are entirely explained by the underlying FAD mutation. In this regard it is relevant to note the work of Koppara and colleagues describing how individuals with SCD had binding deficits (specifically under the 3-item conditions) (Koppara et al., 2015). While the specific contributions of SCD symptoms to VSTM function were not evaluated here – primarily due to differences in testing time points – SCD symptoms have been associated with an increased risk of AD and even described as 'preclinical AD' indicators (Jessen et al., 2014) or clinical indicators of early AD (stage 2, according to the NIA-AA Research Framework (Jack et al., 2018)). Interestingly, the observation that binding deficits were specific to the 3-items condition in individuals with SCD, creates a parallel with the work presented in this thesis, whereby the stronger impairments in presymptomatic carriers were observed in the most challenging task conditions (3-items). Hence, given that relational binding deficits and SCD have both been associated to preclinical AD in the literature, the study of SCD in populations at-risk of AD represented an interesting addition to the thesis. The next chapter will therefore evaluate SCD in the Insight 46 cohort (where individuals are at-risk of AD due to ageing and amyloid deposition) as well as FAD (where individuals are at-risk of AD due to a genetic mutation).

7. SUBJECTIVE COGNITIVE DECLINE IN POPULATIONS AT-RISK OF AD

This chapter focuses on the SCD in populations at-risk of AD and their relationship with mental health and a) amyloid status or b) FAD mutation carriership. A paper based on this chapter looking at Insight 46 data has been published in *Journal of Neurology, Neurosurgery and Psychiatry* (Pavisic et al., 2021b).

As the concept of SCD has not been discussed in the **GENERAL INTRODUCTION** of this thesis, I will first provide an overview of the literature on SCD. This will be followed by a sub-chapter on SCD in Insight 46 and then a sub-chapter on SCD in presymptomatic FAD. Considering the differences in demographics (e.g. age) and possible lifestyle factors (most individuals in the FAD cohort had a preconceived notion of having a 50% chance of inheriting AD), direct comparisons between the Insight 46 and FAD will be restricted to speculations in the discussion only.

7.1. Overview

SCD is defined as the self-reported worsening of cognitive abilities, in subjects who are unimpaired on objective cognitive tests (Colijn & Grossberg, 2015; Tandetnik et al., 2015). In 2014, a conceptual framework on SCD in preclinical AD associated SCD with an increased risk of future objective cognitive decline (Jessen et al., 2014; Mitchell et al., 2014; Slot et al., 2018); risk of MCI and dementia (Buckley et al., 2016; Gifford et al., 2014; Jessen et al., 2010; Slavin et al., 2015). As SCD manifests prior to the onset of clinical impairment (Sperling et al., 2011), there is potential to target populations for early prevention trials (Molinuevo et al., 2017).

The SCD-initiative (SCD-I) (Jessen et al., 2014), proposes a framework for SCD research whereby this terminology relates to: “a self-experienced persistent decline in cognitive capacity (compared to previously normal cognitive status and unrelated to an acute event) and normal performance on cognition” (Jessen et al., 2014). The SCD-I working group outlined the following key points, definitions and considerations.

Key points

1. SCD occurs at the preclinical stage of AD and may serve as a ‘symptomatic indicator’ of preclinical AD since a) longitudinal data supports SCD as a risk factor for future cognitive

decline, MCI and AD dementia (e.g. (Dufouil et al., 2005; Glodzik-Sobanska et al., 2007; Jessen et al., 2010; Reisberg et al., 2010; van Oijen et al., 2007)); b) there is cross-sectional biomarker evidence for an increased prevalence of preclinical AD in those with SCD (e.g. (Perrotin et al., 2012; Wang et al., 2013)); and c) individuals with SCD and biomarker evidence for AD are at increased risk of future cognitive decline and progression to MCI and AD dementia (Peter et al., 2014; van Harten et al., 2018).

2. Current knowledge is insufficient to comprehensively define the specific features of SCD in preclinical AD which may be variable and expressed heterogeneously.
3. SCD by itself may never be sufficient to diagnose preclinical AD as it is neither required for the diagnosis of preclinical AD nor is it necessarily present in all cases of preclinical AD.
4. Numerous causes of SCD other than preclinical AD exist (e.g. normal aging, psychiatric and neurologic disorders other than AD, or related to effects of medication and substance use).

It addition, any definition of SCD is a trade-off between being overinclusive (high sensitivity and high false positive rates) and being too restrictive (high specificity and high false negative and high screening failure rates) (Jessen et al., 2014). In the 2014 framework, Jessen and colleagues (Jessen et al., 2014) argue that while these definitions and considerations may be thought as overinclusive, this is a preferred approach given that the specific features of SCD in preclinical AD are not yet well known.

Definitions

1. *Subjective* refers to the self-perception of cognitive performance and is conceptually independent of performance on a cognitive test (objective cognition).

2. *Cognitive* refers to any cognitive domain and is not restricted to memory as individuals may often report memory decline when they are actually experiencing decline in another cognitive domain and vice-versa.

3. *Decline* refers to a subjectively experienced worsening of cognitive capacities. It reflects the progressive nature of cognitive deterioration in AD. The authors suggest some characteristics of this decline increase the likelihood of an association with preclinical AD such as: 1) the association

of decline with a particular concern (worries) (Jessen et al., 2010); and 2) the belief that one's own cognitive capacity is inferior compared with others of the same age group (Amariglio et al., 2012; Perrotin et al., 2012).

Considerations

1. Recording the time frame and age at onset of SCD. There is evidence that the onset of SCD within a few years may be more predictive of cognitive decline and AD than the presence of SCD for several years (Chary et al., 2013; Dufouil et al., 2005; Treves et al., 2005). A reported age of onset at 60 years or older is proposed as there is increasing prevalence of AD-related neuropathological alterations starting at midlife, which may trigger SCD after neuronal dysfunction affects cognitive abilities. At younger age cutoffs, the likelihood of SCD due to causes other than AD increases (Jessen et al., 2014).

2. Recording whether impairment is detected by the informant or not. There is evidence suggesting that informant report may be a better predictor of objective performance than self-report and may facilitate identification of very early decline related to AD (e.g., (Reisberg et al., 2008; Slavin et al., 2010)). It is also worth noting that other studies suggest the earliest changes in cognition are best perceived by the individual rather than by an observer (Caselli et al., 2014).

3. The presence of major psychiatric disorders should be an exclusion criterion. Psychiatric disorders can be associated with SCD and in the context of research on preclinical AD, SCD should not be confounded by other conditions that affect the subjective experience of cognitive capacity. Nonetheless, the relationship between SCD and affective symptoms is complex and symptoms of depression and anxiety may also be manifestations of preclinical AD. Hence, accounting for affective symptoms in statistical models (possibly excluding individuals fulfilling criteria for major psychiatric disorders) should be considered.

4. If available, information on *APOE* ε4 should be recorded given the genetic risk factor of AD.

5. Record the setting in which information is collected (i.e. population-based studies; volunteer samples and medical-help seeking samples). In a research or medical environment, the terms complaint is frequently used. However, in some countries, these terms and their equivalents have negative connotations and this will impact how and if complaints are recorded.

In combination, the features outlined thus far comprise the SCD-plus criteria:

SCD-plus criteria

- Subjective decline in memory*, rather than domains of cognition
- Onset of SCD within the last 5 years
- Age at onset ≥ 60 years
- Concerns (worries) associated with SCD
- Feeling of worse performance than others of the same age group

**: Although the cognitive profile can be varied, memory is often the first domain affected in preclinical AD.*

If available, record:

- Confirmation of cognitive decline by an informant
- Presence of *APOE* ε4 genotype
- Biomarker evidence of AD (defines preclinical AD)

In 2017, Molinuevo and colleagues published a series of reflections on the Jessen and colleagues' proposed framework (Molinuevo et al., 2017). As an example of some unanswered questions in the field, a few points are mentioned below:

- Discussions on thresholds or cut-offs for subjective cognitive measures to determine when an individual has "significant" cognitive concerns are lacking. This makes it difficult to compare findings across studies.
- While most studies appear to take a categorical approach to SCD, there may be a potential value of continuous approaches that capture features such as frequency and severity of the subjective cognitive profile.
- There may be an added value to SCD if assessed longitudinally.
- Factors such as demographics, knowledge of genetic risk, and medical issues may impact SCD.

7.2. Insight 46

7.2.1. Introduction

As a 'symptomatic indicator of preclinical AD', SCD has been suggested one of the first symptoms of AD (Buckley et al., 2016; Gifford et al., 2014; Jessen et al., 2010; Rönnlund et al., 2015; Slavin et al., 2015). Research showing an association between SCD and AD biomarkers includes: similarities in grey matter atrophy between SCD and AD individuals (Peter et al., 2014); low cerebrospinal fluid (CSF) $A\beta_{1-42}$, increased amyloid deposition on PET imaging (Perrotin et al., 2012), and structural and functional changes on MRI in brain areas typically affected in AD (Jessen, 2014; Reisberg et al., 2008; Studart & Nitrini, 2016). Taking this further, some have evaluated the associations of SCD within specific cognitive domains (e.g. memory, language, executive function) with AD biomarkers (e.g. grey matter volume) (Valech et al., 2019). Nonetheless, considerable variation exists. For instance previous studies found that within individuals with subjective complaints who seek medical help, those considered to be in the preclinical stage of the AD continuum (amyloid marker-positive and tau pathology-negative, or amyloid marker-positive and tau pathology positive) ranged from 7 to 40% (Jessen et al., 2018; van Harten et al., 2013; Wolfsgruber et al., 2017).

Two important studies investigating SCD in relation to AD are worth mentioning here; the first is the DZNE-Longitudinal Cognitive Impairment and Dementia (DELCODE) study and the second is the Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset (adni.loni.usc.edu) – specifically a report by Zhang and colleagues (Zhang et al., 2018). The DELCODE study is an observational longitudinal memory clinic-based multicentre study in Germany (where individuals are ≥60 years of age) with the aim to improve characterization of the early, preclinical stage of AD with a focus on SCD patients (defined by the 'presence of subjectively reported decline in cognitive functioning with concerns as expressed to the physician of the memory centre' and objective performance within a normal range (Jessen et al., 2018)). A recent report from this group showed that several SCD-plus features or SCD domains were relevant predictors of AD pathology (Miebach et al., 2019). More specifically, memory and language complaints were most frequent while complaints in the planning domain were relatively rare with only 10% of participants reporting them. Additionally, onset of subjective decline within 5 years, confirmation of cognitive decline by an informant and decline-related worries, were all associated with lower $A\beta_{42}$ levels (Jessen et al., 2018). The ADNI database has also provided important information on the risk factors that predict amyloid positivity in patients with SCD. The primary aim of this widely available

database is to investigate clinical, neuropsychological, neuroimaging and other biomarkers in relation to the progression of MCI and early AD. Recently, investigations in SCD patients aged ~70 (where subjective memory complaints were defined by a score of >16 in the first 12 items of the Cognitive Change Index or CCI (Bower et al., 2014), a CDR score of 0, a MMSE score ≥ 24, normal performance on objective performance, and no informant-reported memory complaints) showed that: being female, *APOE* ε4 carrier and having a history of cigarette smoking, were all significant risk factors of amyloid positivity with SCD participants (Zhang et al., 2018).

While this evidence of associations between SCD and biomarkers support its validity as a concept, SCD often coexists with affective symptoms (low mood and anxiety) (Molinuevo et al., 2017) and this is something that previous studies (including DELCODE and ADNI) have not comprehensively addressed. Individuals with SCD may sometimes be more introspective and sensitive to perceived changes in their own mental status (Jessen, 2014). Affective symptoms such as depression and anxiety are themselves associated with increased risk of dementia (Buckley et al., 2013; Edmonds et al., 2014; Montejo et al., 2014; Potvin et al., 2011; Silva et al., 2013; Stogmann et al., 2016) and self-reported memory problems (Richards et al., 2014). The relationship is very complex and it is still unclear to what extent depression and anxiety are risk factors for neurodegeneration and/or prodromes of cognitive decline and/or to what extent both may stem from common causes earlier in life (Cipriani et al., 2015; Muliyala & Varghese, 2010; Pietrzak et al., 2015). As Richards and colleagues argue, there are a number of possible scenarios underlying this relationship: anxiety and depression a) directly cause cognitive impairment, or at least lower the threshold for its manifestation; b) are emotional responses to emerging cognitive impairment; c) are risk indicators, being a manifestation of a shared neuropathological substrate that underlies both cognitive decline and mood disturbance (Richards et al., 2014). Crucially, to disentangle the specific associations between SCD and progression to cognitive impairment it is important to account for the influence of affective symptoms and in doing so evaluate the extent to which anxiety, depression and amyloid status all contribute to SCD.

In addition, there has been relatively little research into the influence of 'life-course' factors such as childhood cognitive ability, education (Derouesné et al., 1993; Jorm et al., 1997; van Oijen et al., 2007) and SEP, which have been shown to influence cognition throughout adulthood (Richards et al., 2019). Controlling for these factors may increase our ability to detect subtle associations between SCD and AD pathology.

Taken together, questions remain about SCD and AD biomarkers while accounting for affective symptoms and other factors that influence SCD and its association with AD biomarkers including life-course variables and family history of AD.

In this chapter, I first aim to investigate associations between SCD symptoms and amyloid status in a population-based sample of ~70-year-olds when rates of dementia are low at ~3% (Prince et al., 2014) but the prevalence of amyloid pathology is already significant at ~15-25% (Jansen et al., 2015). Secondly, I investigate whether symptoms of SCD are associated with family history of AD (Haussmann et al., 2018) and lower objective neuropsychology scores all while accounting for amyloid status, affective symptoms and life-course variables in Insight 46. Finally, I assess whether any relationships between SCD and amyloid are driven by a specific cognitive domain (memory, language or executive function).

The main hypothesis is that amyloid positivity will be associated with greater SCD symptoms, after accounting for affective symptoms. In addition, symptoms of SCD will be associated with lower objective neuropsychology scores and a family history of AD.

7.2.2. Methods

7.2.2.1. Study design and participants

Recruitment procedures have previously been described. For the Insight 46 neuroscience sub-study, 502 NSHD participants were recruited and assessed at UCL between May 2015 and January 2018.

In line with Molinuevo and colleagues' (Molinuevo et al., 2017) recommendations of SCD research studies, participants with cognitive impairment (defined as MMSE (Folstein et al., 1975) <26; exhibiting signs of objective impairment) and major neurological and psychiatric conditions (which might result in subjective complaints due to an acute event) were excluded from analyses. This resulted in 460 participants included in the SCD study, 343 amyloid negative and 77 amyloid-positive individuals (see **GENERAL METHODOLOGY** for details).

7.2.2.2. Procedures and data collection

Subjective cognitive decline outcomes

Symptoms of SCD were measured using the MyCog questionnaire, a brief validated tool that is part of the SCD-Q (Rami et al., 2014) (see **Appendix 5**). As no single gold standard instrument or sufficiently validated cut-off on any scale can currently differentiate individuals with SCD from those without SCD in a clinical setting (Jessen, 2014; Jessen et al., 2020; Molinuevo et al., 2017) and in accordance with other approaches (Valech et al., 2019), I considered MyCog scores in a continuous spectrum (instead of dichotomizing the sample into SCD and non-SCD groups). A higher total score (max. 24) indicated greater perceived cognitive decline.

As well as an overall score, studies have generated sub-scores of this questionnaire referencing specific cognitive domains (Valech et al., 2019):

- MyCog memory concern (items 1-11) [max. 11]
- MyCog language concern (items 12-17) [max. 6]
- MyCog executive function concern (items 18-24) [max. 7]

SCD investigations were informed by the SCD-plus criteria (Jessen et al., 2014): participants were over the age of 60; most had an onset of SCD within the past 5 years; *APOE* ε4 genotype was considered and the informant's perception of the participant's cognition investigated.

As in the original SCD-Q, a series of questions about general perception of cognitive function preceded the MyCog questionnaire: a) "Do you perceive memory or cognitive difficulties?"; b) "In the last two years has your cognition or memory declined?". Additionally, if the participant replied 'yes' to question a) and/or question b): the following questions would be asked: c) "Do you perceive memory or cognitive difficulties more than other people the same age?"; d) "At what age did these start?" and e) "Would you ask a doctor about these difficulties?". These questions were not designed for quantitative purposes but administered to provide an overview of concerns and in order to establish whether participants wished for their GP to be contacted based on these concerns.

To evaluate the informant's perception of the participant's cognitive decline the AD8 tool was used (Galvin et al., 2005). As previously mentioned, the AD8 consists of eight questions and has been shown sensitive to detecting early cognitive changes associated with dementia and to correlate with CDR scores (Galvin et al., 2007; Morris, 1993).

Biomarker measures

As mentioned in the **GENERAL METHODOLOGY**, β-amyloid PET and multimodal MRI data were collected on a single Biograph mMR 3T PET/MRI scanner (Siemens Healthcare, Erlangen, Germany), with IV injection of 370 MBq of the Aβ-PET ligand (Galvin et al., 2005), F^{18}-Florbetapir (Amyvid). Cortical fibrillar Aβ deposition was quantified using a SUVR, calculated from a composite cortical region of interest with a reference region of eroded subcortical white matter, using 10 minutes of static steady-state florbetapir data ~50 minutes post injection. A cut-point for Aβ positivity was determined at SUVR >0.6104. Aβ-PET attenuation correction was performed using a pseudo-CT method (Lane et al., 2017), but for 26 participants, this reconstruction was not available due to technical issues and one based on the ultrashort echo time (UTE) MRI sequence was used instead (Lu et al., 2019). Of the 460 participants included in this study, 40 were missing PET data.

APOE genotype, determined from DNA analysis of blood samples, was classified into two categories based on the presence of the ε4 allele: ε4 non-carrier (no ε4 allele: 69.9%) or ε4 carrier (heterozygous: 27.5% and homozygous: 2.6%).

Neuropsychological testing

The results of cognitive testing (previously described (Lu et al., 2019)), were used to derive the PACC (Donohue, et al., 2014), a measure developed to detect and track subtle cognitive changes in preclinical AD phase (Baker et al., 2016). A higher PACC score indicated better performance. As described previously (Lu et al., 2019), the FCSRT was replaced with the 12-item Face-Name test (FNAME-12) (Papp et al., 2014). As PACC performance is significantly associated with life-course variables (Lu et al., 2019), childhood cognitive ability, education and SEP were also considered in the analysis (see below).

Life-course and clinical variables

Childhood cognitive ability was measured at age 8 using 4 tests of verbal and nonverbal ability devised by the National Foundation for Education Research (Pigeon & Douglas, 1964). The sum of scores from these 4 tests was standardised into *z* scores representing overall cognitive ability. If these data were missing, the equivalent score from the tests at age 11 was used (or if this was

missing, the score from age 15 was used) (Lu et al., 2019). These standardized scores were based on the full NSHD cohort.

Educational attainment was represented as the highest educational or training qualification achieved by age 26, grouped into 5 categories: no qualification, below O-levels (vocational), O-levels and equivalents, A-levels and equivalents, higher education (degree and equivalents) (Lu et al., 2019).

SEP was derived from participants' own occupation at age 53, or earlier if this was missing. Occupations were coded according to the UK Registrar General's Standard Occupational Classification, then classified into 6 categories: unskilled, partly skilled, skilled manual, skilled nonmanual, intermediate, professional (Lu et al., 2019).

As mentioned in the **GENERAL METHODOLOGY**, two mental health measures were available: 1) the 28-item version of the GHQ-28 (Goldberg & Hillier, 1979) and 2) the STAI (Spielberger et al., 1983). The GHQ-28 previously assessed during the NSHD data collection at ages 68-69 (James et al., 2018) measures depression and general health with a validated threshold (>=5) indicating severity consistent with a 'mental health disorder' or caseness. The STAI contains 20 items assessing trait anxiety (how the individual feels generally) and 20 examining state anxiety (anxiety at the present moment; measured on the same day as the MyCog questionnaire). Each item is rated on a 4-point scale from "almost never" to "almost always". Higher scores indicate greater anxiety.

Lastly, information on relevant family history was collected. I categorised participants as having a family history of AD if they reported that one or more of their parents or siblings had a diagnosis of AD or "Dementia-not otherwise specified" (NOS), recognising that in previous decades non-specific diagnoses of dementia were common and the majority of them were likely to be cases of AD (similar to (Panza et al., 2010)). Diagnoses of other specific types of dementia (e.g. vascular, frontotemporal, dementia with Lewy bodies), or other neurodegenerative or psychiatric conditions were not included in the family history of dementia category. Participants were asked to report the age at onset of their relative's symptoms.

7.2.2.3. Statistical analysis

Participant characteristics were initially compared between amyloid-positive and amyloid-negative groups using t-tests, or Wilcoxon rank-sum test where the distribution of the variable was skewed. Chi-squared test was used to compare group distribution for any binary variable.

A multivariable regression model with MyCog as the outcome, AD8 score as the predictor and age and sex as covariates was used to evaluate if the informant's perspective (AD8 score) was consistent with the participant's perspective. In this analysis, all cognitively unimpaired participants (N=460) were included. To assess whether symptoms of SCD (defined in a continuous basis through MyCog scores) were associated with amyloid status a multivariable regression model with MyCog as the outcome and amyloid status and sex and age as predictors (Model 1) was used. I then added the measures of affective symptoms in a stepwise manner to evaluate their associations with MyCog scores and to see whether their inclusion in the model affected the adjusted mean difference in MyCog scores between amyloid groups (Models 2-4). In the same way, I added the PACC measure of objective cognitive performance (Model 5) and three life-course variables that have consistently been shown to predict objective cognition (Lu et al., 2019; Richards et al., 2019): childhood cognitive ability, education and SEP (Model 6). Finally, family history of AD was considered as a predictor (Model 7). These analyses was based on the 420 cognitively-unimpaired participants with available PET data (see (Lu et al. 2019)).

The multivariable linear regression models contained the following predictors:

- **Model 1** = Amyloid status, age, sex
- **Model 2** = Model 1 + trait anxiety
- **Model 3 =** Model 2 + state anxiety
- **Model 4 =** Model 3 + GHQ-28 (mental health disorder yes/no)
- **Model 5 =** Model 4 + PACC
- **Model 6=** Model 5 + childhood cognitive ability, education and SEP
- **Model 7** = Model 6 + family history of AD (yes/no)

Although findings on the relationship between sex and symptoms of SCD are equivocal (Buckley et al., 2013), evidence of sex differences in mental health (anxiety and affective symptoms) (Riecher-Rössler, 2017) and AD prevalence (Podcasy & Epperson, 2016) exist. Therefore, I

investigated sex differences by testing for interactions between sex and predictors of interest in all regression models. In the sample of all cognitively-unimpaired participants (N=460), I also tested for sex differences in anxiety scores, general mental health (GHQ-28) and informant perspective after dichotomizing AD8 score based on a validated cut-off for informant concern (≥2 points) (Galvin et al., 2005)-using Wilcoxon rank-sum tests or chi-squared test respectively.

Examination of residuals was performed to check model fits. For outcomes with skewed distributions, bootstrapping was used to produce bias-corrected and accelerated 95% CIs from 2,000 replications.

7.2.3. Results

7.2.3.1. Symptoms of SCD in this sample

Participant characteristics are provided in **Table 7.1**. Of the 460 individuals included in this analysis, 44.8% reported that they perceived memory or cognitive difficulties and 45.4% that they perceived a decline in cognition over the past two years (mean age at onset: 63.2 [SD 10.3], range: 20-70). Of those who perceived difficulties or decline in the last two years, 8.8% reported this was worse than peers of the same age and 2.3% would seek medical advice (**Table 7.1**).

AD8 scores were significantly associated with MyCog scores, showing agreement between participant and informant perspectives: MyCog scores increased by 0.94 points ([95% CI 0.25, 1.63], p=0.007) for every 1-point increase in AD8 scores. Informants of male participants were more likely to report concerns ($\chi 2$=6.35, p=0.012): of the 19 participants with AD8 ≥2, 15 were male.

Table 7.1 Participant characteristics.

	All participants	N=420	
		β-amyloid-positive	β-amyloid-negative
N	460	77	343
Sex, % female	49	44	51
Age at assessment, mean (SD) (range)	70.7 (0.70) (69.2–71.8)	70.6 (0.7) (69.4–71.8)	70.6 (0.7) (69.2–71.8)
MMSE, mean (SD) (range) *	29.3 (0.89) (26–30)	29.1 (1.0) (26–30)	29.3 (0.9) (26–30)
PACC, z-score, mean (SD) (range) *	-0.05 (0.68) (-2.43–1.72)	-0.10 (0.73) (-2.10–1.31)	0.08 (0.66) (-2.43–1.72)
Highest education qualification, %			
None	16.2	16.9	16.1
Below O-levels (vocational)	5.0	6.5	4.3
O-levels or equivalent	24.8	24.7	25.5
A-levels or equivalent	36.3	33.8	36.5
Degree or equivalent	17.8	18.2	17.9
Childhood cognitive ability mean (SD) (range) [a]	0.39 (0.74) (-1.60–2.50)	0.41 (0.74) (-1.59–2.47)	0.44 (0.74) (-1.37–2.50)
Adult socio-economic position, %			
Unskilled	1.0	1.3	0.6
Partially unskilled	4.8	3.9	5.4
Skilled manual	9.6	9.1	9.3
Skilled nonmanual	21.5	16.9	22.0
Intermediate	52.0	53.3	51.9
Professional	11.3	15.6	10.5
APOE* status, % ε4-carrier *[b]**	30.1	59.7	23.2
SCD questions			
Perceive memory or cognitive difficulties (% yes)	44.8	49.4	45.2
Perceive decline in cognition or memory over past 2 years (% yes)	45.4	50.6	45.5
Difficulties or decline worse than peers (% yes)	8.8 [c]	12.8 [d]	8.2 [e]
Would seek medical advice (% yes)	2.3 [c]	6.4 [d]	1.5 [e]
SCD age onset, mean (SD) (range)	63.2 (10.3) (20–70) [c]	63.6 (10.2) (20–70) [d]	62.9 (10.7) (20–70) [e]
SCD≥ 60 years, %	93.0	90.9	93.3
Total MyCog score (out of 24): mean (SD) (range) *	4.4 (3.9) (0–23)	5.2 (3.6) (0–15)	4.3 (3.9) (0–23)
AD8, mean (SD) (range) [f]	0.2 (0.6) (0–5)	0.4 (1.0) (0–5)	0.1 (0.4) (0–3)
AD8, % AD8 ≥2 *	4.1	10.4	2.6
Trait anxiety, mean (SD) (range)	31.8 (7.9) (20–65)	31.1 (8.0) (20–65)	31.9 (7.8) (20–64)
State anxiety, mean (SD) (range)	29.6 (7.9) (20–61)	29.3 (7.1) (20–52)	29.8 (8.1) (20–61)
Mental health disorder prevalence at age 69, % yes [g]	7.0	10.4	6.2
Family history of 'AD', % yes * [h]	23.9	33.8	22.5

[a] Z-scores for childhood cognitive ability were based on the full NSHD cohort of n = 5362, so the mean for Insight 46 participants indicates that they had higher childhood cognitive ability on average than their peers not recruited to this substudy. [b] n = 458 (the participants that had *APOE* status information available). [c] n = 260 (the participants who answered 'yes' either to perceiving memory or cognitive difficulties or decline in cognition or memory in the last two years). [d] n = 47 (the participants who answered 'yes' either to perceiving memory or cognitive difficulties or decline in cognition or memory in the last two years). [e] n = 195 (the participants who answered 'yes' either to perceiving memory or cognitive difficulties or decline in memory or cognition in the last two years). [f] n = 459 (one informant questionnaire was not completed). [g] n=452 (the participants that completed the GHQ-28 questionnaire). [h] Of these participants with a family history, their affected relatives were: mother=70.0%, mean age at onset (SD): 82.2 (9.1) years; father=28.2%, 78.5 (8.8) years; siblings=8.2%, 75.5 (10.0) years (do not add up to 100% as some people had multiple relatives with a family history of dementia). *The difference between the amyloid groups for this variable was significant at $p < 0.05$; ** The difference between the amyloid groups for this variable was significant at $p < 0.01$.

7.2.3.2. Associations with amyloid

Aβ+ individuals reported greater concerns on most items of the MyCog questionnaire (**Figure 7.1**). Multivariable regression showed that Aβ+ individuals tended to have higher MyCog scores than Aβ- (adjusted mean=5.2 [4.3, 6.0] *vs* 4.3 [3.9, 4.7], *p*=0.080, Model 1) (**Table 7.2**, see also unadjusted means in **Table 7.1**).

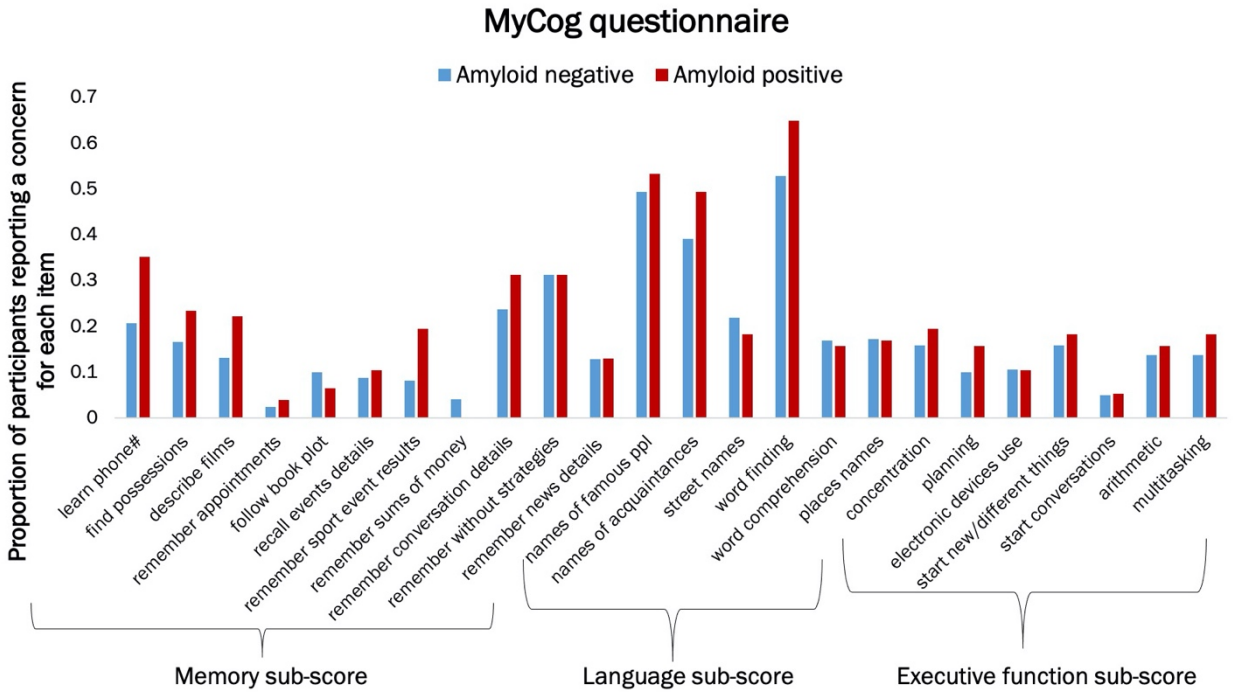


Figure 7.1 MyCog scores by β-amyloid status.

Figure adapted from (Pavisic et al., 2021b) under the terms of the Creative Commons Attribution License (CC, BY). Bar graphs shows the proportion of participants responding a concern for each item by β-amyloid status (N=460). 'Memory', 'language' and 'executive function' concern scores are referenced are will be further discussed in section 7.2.3.6.

The difference in MyCog scores between the amyloid groups was slightly greater in males than females, but this interaction was not statistically significant (interaction coefficient: -1.22 [-2.85, 0.41], *p*=0.143). Neither sex nor the age at assessment had a significant effect on MyCog scores (**Table 7.2**).

7.2.3.3. Impact of affective symptoms on SCD and amyloid associations

There was no difference in affective symptoms scores between amyloid groups (**Table 7.1**). As expected, trait anxiety, state anxiety and the mental health disorder variable (GHQ-28) all showed significant positive associations with MyCog scores when examined separately (regression coefficient=0.14 [0.08, 0.19] increase in MyCog scores per trait anxiety point $p<0.001$; 0.07 [0.02, 0.12] per state anxiety point, $p=0.004$; mental health disorder: 1.91[-0.18, 4.00] MyCog points higher compared to those who did not meet criteria for mental health disorder, $p=0.074$). However, trait anxiety was the only variable showing a significant association with SCD when considering all affective variables together (**Table 7.2**, Model 4). This suggests the relationship between SCD symptoms and affective symptoms was mostly explained by this factor (**Figure 7.2A**).

After adjustment for state anxiety and trait anxiety (Model 2 and Model 3), Aβ+ individuals had higher MyCog scores compared to Aβ- (5.3 [4.4, 6.1] *vs* 4.3 [3.9, 4.7], $p=0.044$) (**Table 7.2**). This indicated that Aβ+ participants had greater concerns about their cognition above and beyond any differences in general tendencies towards anxiety.

Accounting for GHQ-28 scores, slightly attenuated associations with amyloid (Aβ+ = 5.2 [4.4, 6.0], Aβ- = 4.4 [3.9, 4.7], $p=0.053$, Model 4) (**Table 7.2**).

Consistent with the literature, females reported greater anxiety than males (mean trait anxiety score: males = 30.6 [SD 7.5], range: 20-65, females = 33.0 [8.2], 20-64, $p=0.0007$; mean state anxiety score: males = 28.6 [7.5], 20-52, females = 30.7 [8.1], 20-61, $p=0.004$) and higher prevalence of 'case-level emotional symptoms/mental health disorder' (males: 4.8% *vs* females: 10.9%, $\chi 2=-5.88$, $p=0.015$). Interactions between sex and anxiety variables in Model 4 revealed steeper associations between anxiety and MyCog scores in females compared to males (interaction coefficient for state anxiety = 0.09 [-0.01, 0.19], $p=0.078$, **Figure 7.2B**). Similar effects were observed for trait anxiety, but this did not reach statistical significance (0.09 [-0.03, 0.20], $p=0.134$). No such interaction was observed for GHQ-28 scores (-0.43 [-5.50, 4.63], $p=0.868$).

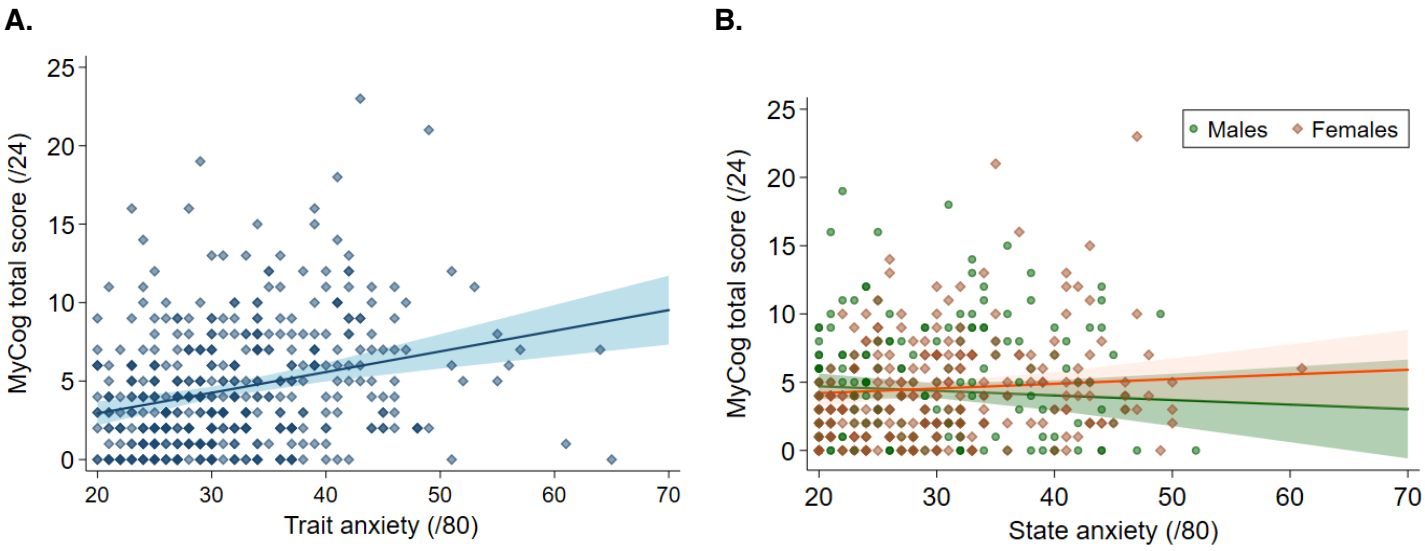


Figure 7.2 Total MyCog score against anxiety.

Figure adapted from (Pavisic et al., 2021b) under the terms of the Creative Commons Attribution License (CC, BY). Scatter plot shows the raw data (N=460) of MyCog against anxiety measures. **A.** Trait anxiety: the blue line is the line of best fit from the multivariable regression model (adjusted for sex age at assessment and affective symptoms, Model 4). **B.** State anxiety: The green and orange lines are the lines of best fit from the multivariable regression for males and females respectively (adjusted for sex, age at assessment and affective symptoms, Model 4). The shaded areas represent 95% confidence intervals. Note that the minimum possible trait anxiety score is 20.

7.2.3.4. Objective cognitive assessments: PACC score

As previously reported (Lu et al., 2019), individuals who were Aβ+ had lower scores on the PACC compared to Aβ- (regression coefficient=-0.17 [-0.31, -0.03], *p*=0.019). However, PACC scores were not independently associated with MyCog scores, although the coefficient was in the predicted direction (Model 5, *p*=0.194, **Table 7.2**). There was no significant interaction between sex and PACC (*p*=0.834). Childhood cognitive ability (0.25 [-0.30, 0.81], *p*=0.371), SEP (-0.11 [-0.52, 0.29], *p*=0.588) and education (0.14 [-0.21, 0.48], *p*=0.433) were not independently associated with MyCog scores (once adjusted for all predictors from Model 5). Considering these factors together further attenuated the association between amyloid positivity and higher MyCog scores (Model 6; *p*=0.139, **Table 7.2**). Of note, the regression coefficient between amyloid positivity and MyCog considering life-course variables without PACC (adjusted for all predictors from Model 4) was 0.78 higher MyCog points for Aβ+ [-0.13, 1.69] *vs* Aβ- (*p*=0.093), suggesting PACC and life-course predictors together attenuated associations with amyloid. There were no significant interactions between life-course factors and sex.

7.2.3.5. Family history of AD/Dementia-NOS

Individuals with a family history of AD/Dementia-NOS had a nominally higher prevalence of *APOE* ε4 (ε4 carriers: 37.3%) compared to those without a family history of AD/Dementia-NOS (ε4 carrier: 27.9%; $\chi 2=3.51$, $p=0.061$). The affected relatives (mostly parents, but some siblings, **Table 7.1**) had an average age of onset of 80.6 (SD 9.3) years.

There was no evidence of a significant association between total MyCog score and family history of AD/Dementia-NOS ($p=0.764$) (Model 6, **Table 7.2**). No significant interaction between sex and family history was observed ($p=0.429$).

Adopting a purer definition of family history of AD, including only individuals who reported a relative affected by a specific diagnosis of AD, also did not reveal a significant association with the total the MyCog score ($p=0.276$). No significant interaction with sex emerged either ($p=0.540$).

Figure 7.3 below shows a visual summary of the regression models considered.

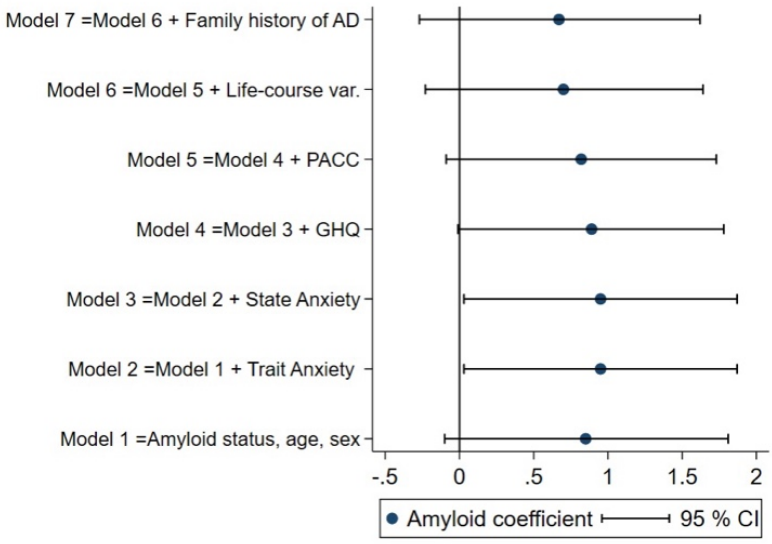


Figure 7.3 Amyloid coefficient as a predictor for each regression model.

Figure reprinted from (Pavisic et al., 2021b) under the terms of the Creative Commons Attribution License (CC, BY). Amyloid coefficients (adjusted difference in mean MyCog score for amyloid-positive and amyloid-negative groups) from each regression model with 95% confidence intervals (N=420). Note that a positive coefficient indicates higher MyCog scores in the amyloid-positive group.

Table 7.2 Predictors of MyCog in N=420.

	Coefficient [95% confidence interval for each model]						
	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 5**	**Model 6**	**Model 7**
Amyloid status (negative as reference)	0.85 [-0.10, 1.81]	**0.95 [0.03, 1.87]***	**0.95 [0.03, 1.87]***	0.89 [-0.01, 1.78]	0.82 [-0.09, 1.73]	0.70 [-0.23, 1.64]	0.67 [-0.27, 1.62]
Age	0.07 [-0.46, 0.61]	0.05 [-0.48, 0.57]	0.05 [-0.48, 0.58]	0.05 [-0.49, 0.60]	0.04 [-0.49, 0.58]	0.19 [-0.37, 0.75]	0.18 [-0.38, 0.74]
Sex (male as reference)	0.19 [-0.54, 0.92]	-0.10 [-0.80, 0.60]	-0.10 [-0.80, 0.60]	-0.14 [-0.85, 0.56]	0.0005 [-0.71, 0.71]	-0.03 [-0.84, 0.77]	-0.03 [-0.83, 0.78]
Trait anxiety		**0.14 [0.08, 0.19]****	**0.14 [0.08, 0.20]****	**0.13 [0.07, 0.19]****	**0.13 [0.07, 0.19]****	**0.14 [0.08, 0.21]****	**0.14 [0.08, 0.21]****
State anxiety			-0.002 [-0.05, 0.05]	-0.003 [-0.06, 0.05]	-0.007 [-0.06, 0.05]	-0.02 [-0.07, 0.04]	-0.02 [-0.07, 0.04]
Mental health disorder (no as reference)				1.10 [-1.00, 3.20]	1.05 [-1.02, 3.11]	1.21 [-0.87, 3.29]	1.20 [-0.90, 3.29]
PACC *z* score					-0.40 [-1.00, 0.20]	-0.47 [-1.28, 0.33]	-0.48 [-1.29, 0.33]
Childhood cognitive ability						0.15 [-0.42, 0.71]	0.14 [-0.43, 0.71]
Education (per category)						0.12 [-0.25, 0.48]	0.11 [-0.26, 0.48]
SEP (per category)						0.02 [-0.41, 0.45]	0.02 [0.41, 0.45]
Family history of AD/Dementia-NOS (no as reference)							0.25 [-0.67, 1.17]
R^2	0.008	0.084	0.084	0.088	0.093	0.102	0.103

Multivariable regression models were used so each association is independent of all others. R^2 gives the proportion of variance in each cognitive outcome that is explained by the combined predictors. AD=Alzheimer's disease; NOS=not otherwise specified; SEP = socio-economic position; PACC=Preclinical Alzheimer's composite score. R^2 gives the proportion of variance in the outcome that is explained by the combined predictors. Bold=significant; * Significant at $p < 0.05$; **Significant at $p < 0.01$

Finally, I performed an analysis for each cognitive domain in attempt to unveil whether the associations between MyCog and amyloid status and trait anxiety were driven by one particular domain. The same regression models were considered with each cognitive domain as the outcome: 'memory', 'language' or 'executive function' concerns.

7.2.3.6. MyCog domains

Associations with amyloid

Model 1 revealed a trend for more 'memory' concerns in Aβ+ compared to Aβ- individuals (regression coefficient=1.95 [95% CI 1.53, 2.38] *vs* 1.51 [1.31, 1.71], p=0.070). No differences were observed in the concerns classified under 'language' (amyloid status difference coefficient = 0.22 [-0.16, 0.61], p=0.258) or 'executive function' (amyloid status difference coefficient = 0.19 [-0.10, 0.48], p=0.193).

While there was no evidence of a difference in overall MyCog scores between sexes in any of the domains (memory: p=0.564; language: p=0.140 and executive function: p=0.356), the memory concerns score was the only one to showed an interaction between amyloid status and sex (interaction coefficient = -0.85 [1.70, 0.006], p=0.048), whereby the difference in memory concerns between amyloid groups was smaller in females than males (**Figure 7.4**). Further analysis by sex revealed an effect seemingly restricted to males: Aβ+ had 0.83 [0.09, 1.57] significantly higher scores than Aβ- (p=0.028), whereas no evidence for a difference in MyCog was observed between amyloid groups in females (p=0.927). A similar interaction was observed for the executive function concern score but this trend did not reach statistical significance (-0.50 [-1.00, 0.08], p=0.096). This effect was not observed for the language concern score (-0.02 [-0.78, 0.75], p=0.967).

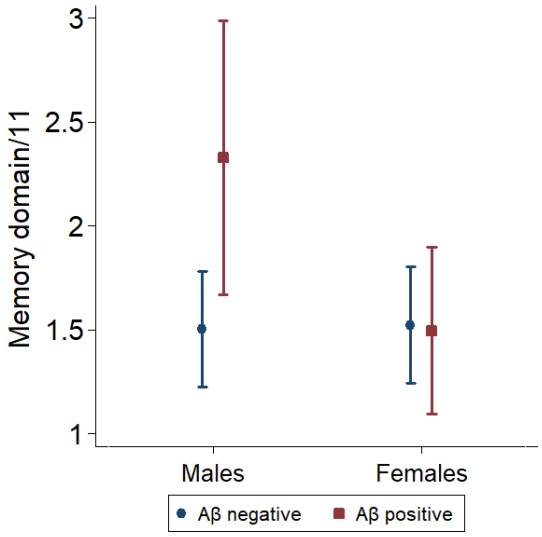


Figure 7.4 Memory concern score by amyloid status for males and females.

Memory concern score (adjusted means predicted from the multivariable regression, Model 1) by sex and amyloid status with 95% confidence intervals. Note that a higher score indicates higher memory concerns.

Effect of affective symptoms

For all cognitive domains higher trait anxiety scores were associated with higher concern scores (all $p<0.001$, Model 2, **Table 7.3**). However, only the executive function concern score showed an interaction between trait anxiety and sex, whereby the relationship between SCD symptoms relating to 'executive function' and anxiety scores was stronger in females compared to males (**Figure 7.5**) (interaction coefficients: executive function = 0.04 [0.001, 0.07], $p=0.041$; memory= 0.03 [-0.02, 0.08], $p=0.266$; language= 0.02 [-0.02, 0.06], $p=0.333$). Considering state anxiety in the regression, did not materially change results (Model 3, **Table 7.3**). Similar to trait anxiety, there was a trend for a stronger relationship between state anxiety and executive function concerns for females compared to males (interaction coefficient=0.03 [0.00004, 0.07], $p=0.050$). No significant interactions were observed for the other domains (memory: 0.03 [-0.02, 0.07], $p=0.301$; language: 0.03 [-0.007, 0.07], $p=0.107$).

GHQ-28 scores were not associated with any of the cognitive domains in MyCog (memory: $p=0.351$; language: $p=0.315$; executive function: $p=0.460$, **Table 7.3**) and no significant interactions between GHQ-28 scores and sex emerged either (memory: -0.74 [-3.31, 1.82], $p=0.571$; language: 0.10 [-1.66, 1.87], $p=0.909$; executive function: 0.21 [-1.01, 1.44], $p=0.739$).

The interaction between amyloid status and sex for the memory concern score remained as a trend after adjustment for affective symptoms (Model 4, amyloid status difference coefficient = -0.84 [-1.67, 0.03], *p*=0.051).

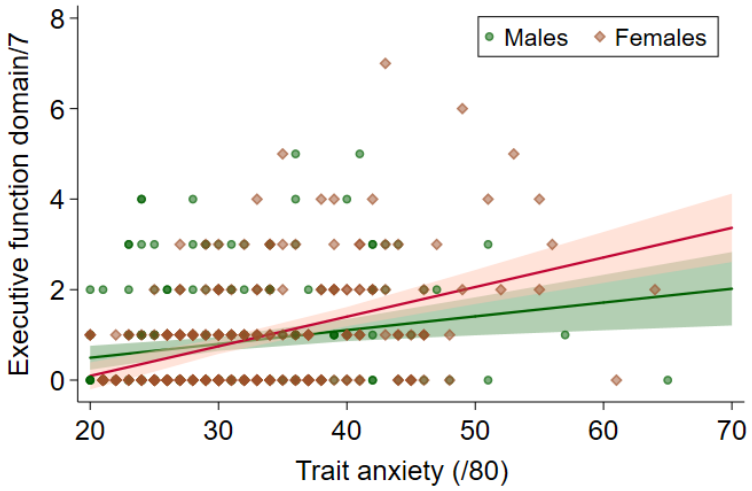


Figure 7.5 Executive function concerns against trait anxiety.

Scatter plot shows raw data of the 'executive function' concern score against trait anxiety. The lines are the line of best fit from the multivariable regression for males and females. Shaded areas represent the corresponding 95% confidence intervals from the model (adjusted for age at assessment, sex and trait anxiety, Model 2).

Effect of objective performance and life-course predictors

Higher SCD symptoms relating to the language domain were associated with lower PACC scores, however this trend did not reach statistical significance (**Table 7.3**, *p*=0.053). Such associations were not observed for the memory and executive function concern scores (**Table 7.3**, *p*=0.423 and *p*=0.571, respectively). No significant interactions between sex and PACC emerged (memory: -0.003 [-0.62, 0.61], *p*=0.992; language: -0.07 [-0.55, 0.41], *p*=0.772; executive function: -0.06 [-0.42, 0.30], *p*=0.738).

Life-course variables were not associated with any of the MyCog cognitive domains when variables where considered separately: childhood cognitive ability (memory: 0.14 [-0.12, 0.40], *p*=0.283; language: 0.20 [-0.04, 0.43], *p*=0.100; executive function: -0.09 [-0.25, 0.08], *p*=0.324); SEP (memory: -0.05 [-0.25, 0.15], *p*=0.615; language: -0.06 [-0.23, 0.11], *p*=0.468; executive function: 0.0006 [-0.11, 0.11], *p*=0.992); education (memory: 0.02 [-0.15, 0.20], *p*=0.816;

language: 0.08 [0.06, 0.21], *p*=0.256; executive function: 0.04 [0.06, 0.14], *p*=0.421) or together (Model 6, **Table 7.3**).

The interaction between amyloid status and sex for the memory concern score remained significant after adjustment for objective performance (-0.84 [-1.68, 0.003], *p*=0.049) and as a trend after adjustment for objective performance and life-course variables (-0.82 [-1.69, 0.05], *p*=0.064).

Family history of AD/Dementia-NOS

Similar to total MyCog scores, there was no significant association between family history and any of the domain scores (**Table 7.3**; memory: *p*=0.837; language: *p*=0.425; executive function: *p*=0.354); nor were there any significant interactions between family history and sex (memory: -0.72 [-1.57, 0.14], *p*=0.101; language: 0.19 [-0.58, 0.97], *p*= 0.625; executive function: -0.22 [-0.79, 0.35], *p*=0.445). The interaction between amyloid status and sex for the memory concern score remained as a trend after adjustment for family history (-0.83 [-1.70, 0.04], *p*=0.062).

Adopting a more restrictive definition of family history of AD did not reveal any significant associations either (memory: 0.17 [-0.57, 0.90], *p*=0.655; language: 0.50 [-0.06, 1.05], *p*=0.079; executive function: 0.10 [-0.36, 0.55], *p*=0.670), nor were there any significant interactions with sex (memory: -0.67 [-2.12, 0.79], *p*=0.368; language: 0.14 [-0.98, 1.25], *p*=0.815; executive function: -0.31 [-1.19, 0.57], *p*=0.485).

Table 7.3 Predictors of MyCog by domain in N=420.

Memory Language Executive	**Coefficient [95% confidence interval for each model]**						
	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 5**	**Model 6**	**Model 7**
Amyloid status (negative as reference)	0.44 [-0.04, 0.91] 0.22 [-0.16, 0.61] 0.19 [-0.10, 0.48]	**0.47 [0.003, 0.94]*** 0.25 [-0.12, 0.63] 0.22 [-0.05,0.50]	**0.47 [0.003, 0.94]*** 0.25 [-0.12, 0.63] 0.22 [0.05, 0.50]	0.43 [-0.02, 0.89] 0.23 [-0.14, 0.61] 0.22 [-0.05, 0.50]	0.41 [-0.05, 0.88] 0.19 [-0.19, 0.57] 0.21 [-0.07, 0.50]	0.36 [-0.12, 0.83] 0.14 [-0.24, 0.52] 0.21 [-0.08, 0.50]	0.36 [-0.11, 0.84] 0.12 [-0.27, 0.51] 0.19 [-0.11, 0.49]
Age	0.04 [-0.21, 0.28] 0.05 [-0.17, 0.27] -0.01 [-0.18, 0.16]	0.03 [-0.22, 0.27] 0.04 [-0.18, 0.26] -0.02 [-0.19, 0.15]	0.03 [-0.21, 0.28] 0.04 [-0.18, 0.26] -0.02 [-0.19, 0.22]	0.05 [-0.21, 0.30] 0.05 [-0.18, 0.27] 0.04 [-0.21, 0.13]	0.04 [-0.21, 0.30] 0.04 [-0.18, 0.26] -0.04 [-0.21, 0.13]	0.11 [-0.15, 0.37] 0.09 [-0.15, 0.32] 0.004 [-0.18, 0.17]	0.11 [-0.16, 0.37] 0.08 [-0.15, 0.31] 0.009 [-0.18, 0.16]
Sex (male as reference)	-0.14 [-0.48, 0.22] 0.21 [-0.10, 0.51] 0.12 [0.10, 0.34]	-0.24 [-0.57, 0.10] 0.12 [-0.18, 0.42] 0.02 [-0.19, 0.23]	-0.23 [-0.57, 0.10] 0.12 [-0.18, 0.43] 0.01 [-0.20, 0.22]	-0.26 [-0.60, 0.09] 0.13 [-0.18, 0.44] 0.02 [-0.23, 0.20]	-0.21 [-0.56, 0.14] 0.21 [-0.10, 0.53] 0.00005 [-0.22, 0.22]	-0.25 [-0.65, 0.14] 0.24 [-0.10, 0.58] -0.02 [0.26, 0.23]	-0.25 [-0.65, 0.14] 0.24 [-0.10, 0.58] -0.01 [-0.26, 0.24]
Trait anxiety		**0.05 [0.02, 0.07]**** **0.04 [0.02, 0.06]**** **0.05 [0.04, 0.07]****	**0.05 [0.02, 0.08]**** **0.04 [0.02, 0.07]**** **0.05 [0.03,0.07]****	**0.05 [0.02, 0.08]**** **0.04 [0.02, 0.07]**** **0.04 [0.02, 0.06]****	**0.05 [0.02, 0.08]**** **0.04 [-0.02, 0.07]**** **0.04 [0.02, 0.06]****	**0.05 [0.02, 0.08]**** **0.05 [0.02, 0.07]**** **0.05 [0.03, 0.07]****	**0.05 [0.02, 0.08]**** **0.05 [0.02, 0.07]**** **0.05 [0.03, 0.07]****
State anxiety			-0.006 [-0.03, 0.02] -0.003 [-0.03, 0.02] 0.007 [-0.01, 0.03]	-0.005 [-0.03, 0.02] 0.005 [-0.03, 0.02] 0.007 [-0.01, 0.03]	-0.006 [-0.03, 0.02] -0.007 [-0.04, 0.02] 0.007 [-0.01, 0.03]	-0.009 [-0.04, 0.02] -0.01 [-0.03, 0.01] 0.002 [-0.02, 0.02]	-0.009 [-0.04, 0.02] -0.01 [-0.03, 0.01] 0.003 [-0.02, 0.02]
Mental health disorder (no as reference)				0.51 [-0.57, 1.59] 0.36 [-0.35, 1.08] 0.22 [-0.37, 0.81]	0.50 [-0.57, 1.57] 0.34 [-0.36, 1.03] 0.22 [-0.37, 0.80]	0.54 [-0.55, 1.63] 0.38 [-0.33, 1.08] 0.29 [-0.29, 0.87]	0.54 [-0.55, 1.63] 0.37 [-0.34, 1.08] 0.29 [-0.31, 0.88]
PACC *z* score					-0.12 [-0.42, 0.17] -0.23 [-0.46, 0.003] -0.05 [-0.23, 0.13]	-0.14 [-0.52, 0.25] **-0.32 [-0.61, -0.03]*** -0.02 [-0.26, 0.22]	-0.14 [-0.52, 0.25] **-0.32 [-0.61, -0.04]*** -0.02 [-0.26, 0.22]

Childhood cognitive ability						0.12 [-0.15, 0.40] 0.15 [-0.10, 0.40] -0.13 [0.31, 0.05]	0.13 [0.15, 0.40] 0.15 [-0.10, 0.39] 0.14 [-0.32, 0.05]
Education (per category)						-0.01 [-0.20, 0.18] 0.05 [-0.10, 0.19] 0.08 [-0.03, 0.18]	0.009 [-0.20, 0.18] 0.05 [-0.10, 0.19] 0.07 [-0.03, 0.18]
SEP (per category)						-0.02 [-0.23, 0.20] 0.01 [-0.17, 0.19] 0.03 [-0.09, 0.15]	0.02 [-0.23, 0.20] 0.006 [-0.18, 0.19] 0.03 [-0.09, 0.15]
Family history of AD/Dementia-NOS (no as reference)							-0.05 [-0.48, 0.39] 0.16 [-0.23, 0.56] 0.13 [-0.15, 0.42]
R^2	0.010 0.007 0.006	0.050 0.046 0.106	0.051 0.046 0.107	0.055 0.049 0.110	0.057 0.058 0.110	0.064 0.070 0.118	0.064 0.072 0.120

Multivariable regression models were used so each association is independent of all others. R^2 gives the proportion of variance in each cognitive outcome that is explained by the combined predictors. AD=Alzheimer's disease; NOS=not otherwise specified; SEP = socio-economic position; PACC=Preclinical Alzheimer's composite score. R^2 gives the proportion of variance in each outcome that is explained by the combined predictors. Bold=significant; * Significant at $p < 0.05$; **Significant at $p < 0.01$.

7.2.4. Discussion

7.2.4.1. Summary

In this large population-based sample of older adults of approximately the same age, I investigated symptoms of SCD in relation to amyloid, affective symptoms, objective performance and family history of AD/Dementia-NOS.

The key finding was that SCD symptoms, measured using MyCog, were significantly associated with Aβ positivity even after accounting for symptoms of anxiety. Other important findings include: the fact that symptoms of SCD were not significantly associated with family history of AD, objective performance or life-course variables. Furthermore, items classified under the 'memory' and 'executive function' domains within MyCog, showed significantly higher scores in Aβ+ compared to Aβ- individuals. Conversely, no significant differences between Aβ+ and Aβ- individuals were observed among the items classified in the 'language' domain. These themes will be discussed in greater detail in the following sub-sections.

7.2.4.2. Amyloid and SCD symptoms

Amyloid-positive individuals showed higher SCD symptoms compared to those who were amyloid-negative. This is in accordance with previous evidence that SCD symptoms are an early sign of AD, measurable at preclinical stages and correlating with accumulating pathology (Jessen et al., 2020) and with previous investigations in participants of similar age (e.g. DELCODE: (Jessen et al., 2018) and ADNI: (Zhang et al., 2018)). The results suggest that subtle manifestations of the effects of AD pathology in terms of SCD are detectable at age 70, when participants are still likely to be quite a few years away from the onset of dementia. However, the increase of less than one point in total MyCog scores from Aβ- to Aβ+ is small, reflecting the generally low MyCog scores in this cohort. In this context, it is perhaps relevant to note that around half of this cohort reported subjective cognitive difficulties (lower percentage compared to cohorts of similar age (Jessen et al., 2010; van Harten et al., 2018)) but, of these individuals, only 9% said that this was worse than their peers and 2% that they would report their concerns to a doctor. Long-term prospective studies (e.g. (Verlinden et al., 2016)), in individuals who eventually developed dementia, suggest that SCD occurs on average around 10 years before dementia diagnosis. As the mean onset of SCD symptoms in this sample was 63.2 (10.3) years, longitudinal follow-ups might further unveil the specificity and sensitivity of SCD symptoms as markers for

preclinical AD. In this regard it is also relevant to note that informant concern was relatively uncommon in our dataset in comparison to previous reports of participants of the same age (e.g. (Jessen et al., 2018)) even among those reporting concerns with memory or cognition in general. Importantly, this may be due to the properties of the AD8 questionnaire itself which is restricted to only eight questions and is often used as a screening tool for dementia.

The analysis of concerns by cognitive domain revealed that those items classified under the 'memory' and 'executive function' domains may have possibly led the difference observed between amyloid-positive and amyloid-negative individuals. This is in accordance with the literature suggesting subtle changes in preclinical AD in both of these cognitive domains (Pereira et al., 2014) and with other reports indicating that individuals who were amyloid-positive were particularly affected in 'memory related items' with SCD scores (Jessen et al., 2018). Some researchers even suggests objective executive performance impairments may precede memory deficits matching with the deposits of extracellular amyloid that appears in the basal neocortex first, and only later involves the hippocampus (Harrington et al., 2013). While the relationship and interactions between different brain regions remains complex, the general consensus is that both functions may be impaired at preclinical stages of AD.

7.2.4.3. Associations with affective symptoms

While all mental health measures were associated with MyCog scores, trait anxiety was the only measure with an independent effect on MyCog total scores and each of its domain, with higher anxiety scores resulting in higher MyCog scores. This association is consistent with a similar association in this cohort at age 53 (Richards et al., 2014) and has a number of interpretations: subjective symptoms may be capturing a tendency for negative thoughts rather than a preclinical indicator of AD; experiencing cognitive decline (whether subjective or objectively measurable) may increase the likelihood for individuals to develop heightened general anxiety traits; and finally as anxiety is associated with increased risk of dementia, individuals with higher anxiety may be more likely to be on the dementia pathway (and thus to be experiencing cognitive decline). However, the findings presented here show that anxiety levels did not differ between the amyloid groups, consistent with a recent study that found no association between anxiety and amyloid or tau pathology, although they did observe an association between repetitive negative thinking and greater pathological burden (Marchant et al., 2020). This highlights the complexities of interpreting

SCD symptoms in a clinical context, as individuals seeking medical help for their cognitive concerns may have diverse profiles of affective symptoms, and current evidence does not support predictions about risk of progression from SCD to dementia on an individual basis (Howard, 2020).

In accordance with the literature (Green et al., 2019), females reported higher trait anxiety scores compared to males. The significant interaction between sex and trait anxiety for the 'executive function' concern score suggests that women with higher anxiety might be more likely to have concerns that they are under-performing on daily tasks that require planning, judgement and problem-solving. As women are at a greater risk of developing AD and mental health problems are associated with a greater risk of dementia, it is possible that anxiety differences may play a role in the disparity in dementia risk between sexes (Podcasy & Epperson, 2016). In this context, it is worth noting that I found female participants to have better cognition from the perspective of their informants (lower AD8 scores), which is consistent with a previous finding in this cohort that females performed 0.39 SD higher than males on the PACC (see (Lu et al., 2019)). However, no overall sex differences in participant-reported SCD symptoms were observed.

The significant interaction between sex and amyloid in the 'memory' concern score, may also result from the disparity in dementia risk, the differential impact of affective symptoms on SCD, or the fact that females tend to be more introspective and observant than males. Nonetheless, future research should investigate this further.

7.2.4.4. Associations with objective performance

While amyloid positivity was associated with symptoms of SCD and has previously been related to lower objective cognitive performance in this cohort (Lu et al., 2019), there was no statistically significant relationship between MyCog score and objective cognitive performance once accounting for amyloid status. As argued by Jessen and colleagues, SCD symptoms may be independent of objective cognitive performance because the latter represents a cross-sectional measure whereas the former describes change over a time period (often years) (Jessen, 2014). Notably, some criteria of SCD exclude objective impairment and rather consider it as an indication of MCI instead of SCD (Bondi et al., 2014; Jessen et al., 2020). In this context, it is worth noting that the objective cognitive measure, PACC, was adjusted for childhood cognitive ability, education and SEP – three variables that accounted for a significant proportion of the variance in

PACC scores (Lu et al., 2019) – so these adjusted scores did to some extent reflect whether participants' objective cognitive performance was better or worse than expected. However, this is still conceptually different to a subjective experience of change over the last two years. Longitudinal follow-up of Insight 46 participants will address the question of whether self-perceived decline is related to change in performance on objective cognitive tests. Analysis of concerns by domain revealed some evidence for an association between PACC scores and SCD symptoms relating to language. While this finding was slightly surprising, limitations on this domain analysis are discussed further on.

7.2.4.5. Family history of AD/Dementia-NOS

Some studies (Haussmann et al., 2018; Heun et al., 2003; Tsai et al., 2006) have evaluated SCD in individuals with a family history of AD and showed higher SCD in those with first-degree family history. This may be explained by the increased risk of AD due to inheritance of genetic risk factors, but could also reflect the fact that individuals who have witnessed cognitive impairment in close relatives may have heightened vigilance to cognitive changes or heightened concerns about their own cognition (Heun et al., 2003). In an effort to account for diagnostic uncertainty at the time, I considered individuals with a family history of AD/Dementia-NOS in the same group if any first-degree relative had AD or dementia-NOS, and found a prevalence (~25%) similar to other studies of participants of a similar age (Haussmann et al., 2018). Family history was not significantly associated with SCD symptoms in this cohort. While a trend for *APOE* ε4 carriership in those individuals with a family history was observed, it is possible that a stronger association with family history might emerge in the cohort in the coming years, as the affected relatives were mostly parents with an age of onset several years older than the current age of participants.

7.2.4.6. Reflections on the SCD plus criteria and study limitations

While the aim of this study was not to explicitly test the SCD-plus criteria, a number of aspects from these criteria were none the less considered. SCD symptoms were evaluated in relation to cognitive domains, and memory (as well as executive function) appeared to drive differences in SCD symptoms between amyloid statuses; the age at SCD symptom onset was, for the most part, after the age of 60 years; SCD symptoms in relation to peers of the same age was measured

and reported; *APOE* ε4 status was taken into consideration in relation to family history and the informant's perspective, taken into account.

This study has a number of limitations. Firstly, performance on MyCog may be influenced by factors not account for here, such as medication use and personality traits (e.g. neuroticism and anxiety sensitivity *vs* openness and conscientiousness) (Kliegel et al., 2005; Molinuevo et al., 2017; Pearman & Storandt, 2004; Slavin et al., 2010), limiting its specificity (Jessen et al., 2014). Secondly, NSHD participants are all white (Lu et al., 2019), limiting the generalisability of findings to more diverse populations. Thirdly, the classification of MyCog items into 'domains' (Rami et al., 2014) is likely to be imprecise, as many daily tasks are inherently multi-domain and some of the items classified as 'language' arguably have a strong memory component (e.g. those relating to remembering the names of famous people, acquaintances and streets/cities). Thus, it is important to consider the domain findings with caution. Lastly, participants with missing neuroimaging data were more likely to have mental health problems (James et al., 2018) and individuals with major neurological and psychiatric conditions were excluded. These individuals are hence likely to be underrepresented in analyses. Strengths of this study include the large sample size, the population-based nature of the cohort (meaning that levels of subjective cognitive symptomatology may be more representative of the general population than many other studies) and the very small age-range (meaning that findings are robust to the confounding effects of age-related changes in SCD). For consistency with other data chapters, considering a 0.05 level of significance and 80% power, the total sample size required to replicate the findings of a higher MyCog total score in Aβ+ compared to Aβ- after accounting for self-reports of anxiety (Model 3) is N=851.

7.2.4.7. Conclusions

In summary, findings show independent effects of anxiety and β-amyloid status on symptoms of SCD in a cognitively-normal cohort at age ~70. As amyloid-positive individuals perceived greater cognitive decline at an age where dementia prevalence is low, this suggests that the presence of SCD symptoms may have some utility in identifying people at-risk of developing AD dementia in older age, provided that the influence of affective symptoms is carefully considered.

7.3. FAD

7.3.1. Introduction

Similar to Insight 46, I wished to evaluate SCD symptoms in a group of FAD participants (N=49): 25 controls (in this case all non-carriers with a family history of FAD), 13 early PMCs and 11 late PMCs. As this thesis is largely focused on evaluating populations at-risk of AD and the data were available, this comparison seemed appropriate. There have been some studies looking at SCD symptoms in FAD, the largest one being as part of the DIAN study (107 PMCs *vs* 109 non-carriers) by Laske and colleagues showing a low prevalence of SCD symptoms (investigated though a *single* item), in PMCs (12.1 % *vs* 9.2% in non-carriers, Chi-square test p=0.478) (Laske et al., 2015). Nonetheless, similar to SCD investigations in older adults (Buckley et al., 2017), considerable variation exists with some studies reporting significant differences (e.g. Memory Complaint Scale (Acosta-Baena et al., 2011) in the Colombian kindred: 25 PMCs *vs* 26 non-carriers, p=0.02) (Norton et al., 2017).

Similar to Insight 46, in this subsection I first investigate associations between SCD symptoms and mutation status and second, I consider the effect of affective symptoms and objective cognition on this association. Family history is not considered here as inclusion criteria for the FAD study was to have a family history of AD. Finally, I assess whether the informant's perspective matched that of the participants and if SCD symptoms are driven by a specific cognitive domain ('memory', 'language' or 'executive function').

The main hypothesis is that mutation status will be associated with greater symptoms of SCD, after accounting for affective symptoms.

7.3.2. Methods

7.3.2.1. Study design and participants

As for the other FAD studies presented in this thesis, participants were divided into: controls; symptomatic carriers; early PMCs (more than 5 years from expected onset) and late PMCs (within 5 years to expected onset). The median split for PMCs here was 5 years. Similar to Insight 46 study, symptomatic participants were excluded from the analysis as the focus was on preclinical or in this case – presymptomatic stages of AD.

7.3.2.2. Procedures and data collection

The same MyCog questionnaire (as above) was used to measure the participants' SCD symptoms and AD8, the informant's perspective.

Affective symptoms in this cohort were evaluated using the HADS questionnaire (Zigmond & Snaith, 1983).

Objective performance was measured by verbal and performance IQ (Fox et al., 1998) and not VSTM since the "What was where?" task was not administered in the same time point as MyCog. In addition, similar to the PACC score for Insight 46, the rationale was to *adjust* models for objective cognition using a comprehensive cognitive measure (of fluid intelligence and verbal or non-verbal reasoning respectively) rather than a specific cognitive function like VSTM or relational binding. This is also in accordance with one of the first studies (Fox et al., 1998) in presymptomatic FAD showing that individuals who later became clinically affected had lower performance IQ scores (p=0.030) at their first assessment, when they were seemingly unaffected.

7.3.2.3. Statistical analysis

Baseline demographics and neuropsychology scores were compared between controls and early PMCs and between controls and late PMCs using ANOVA, or Kruskal-Wallis test where the distribution of the variable was skewed. Fishers' exact test (instead of Chi-squared test) was used to compare the sex distribution between the groups as this is more appropriate for smaller sample sizes.

A multivariable regression model with MyCog as the outcome, AD8 score as the predictor and age and sex as covariates were used to assess the informant's perspective in relation to MyCog. To assess whether symptoms of SCD (defined in a continuous basis through MyCog scores) were associated with mutation status I used a multivariable regression model with MyCog as the outcome and mutation status and sex and age as predictors (Model 1). I then added the measures of affective symptoms in a stepwise manner to evaluate their associations with MyCog scores and to see whether their inclusion in the model affected the adjusted mean difference in MyCog scores between PMCs and controls (Models 2-3). In the same way, I added two measures of objective performance (verbal and performance IQ, Models 4-5), and education level (instead of

NART in order to keep the comparison between FAD and Insight 46 as similar as possible, Model 6).

The multivariable regression models were as follows:

- **Model 1** = Mutation status (early or late PMCs *vs* controls), age, sex
- **Model 2** = Model 1 + Anxiety score from HADS
- **Model 3** = Model 2 + Depression score from HADS
- **Model 4** = Model 3 + Verbal IQ
- **Model 5** = Model 4 + Performance IQ
- **Model 6** = Model 5 + Education level (in years)

Once again, interactions with sex and each predictor were tested. Examination of residuals was performed to check model fits. For outcomes with skewed distributions, bootstrapping was used to produce bias-corrected and accelerated 95% CIs from 2,000 replications.

7.3.3. Results

Participant characteristics are reported in **Table 7.4**. There was no difference in sex distribution between groups ($\chi 2=3.31$, $p=0.206$). Although late PMCs tended to be slightly older and have lower MMSE scores than controls, this effect did not reach statistical significance (MMSE: $p=0.090$; age: $p=0.073$). No significant differences were observed between controls and early PMCs for age or MMSE scores. All groups reported similar levels of affective symptoms (anxiety: $p=0.495$; depression: $p=0.553$); similar verbal IQ ($p=0.727$) and performance IQ ($p=0.400$) scores as well as education levels ($p=0.287$).

Table 7.4 Participant characteristics of the FAD cohort.

	All participants	Controls	Early PMCs	Late PMCs
N	49	25	13	11
Sex, % female	51.0	56.0	61.5	27.3
Age, mean (SD) (range)	39.0 (8.0) (23–62)	39.1 (9.5) (23–62)	37.5 (6.9) (29–53)	40.5 (5.0) (32–49)
MMSE, mean (SD) (range)	29.6 (1.0) (24–30)	29.8 (0.7) (28–30)	29.8 (0.4) (29–30)	28.9 (1.8) (24–30)
CDR global, mean (SD) (range)	0.0 (0.0) (0–0)	0.0 (0.0) (0–0)	0.0 (0.0) (0–0)	0.0 (0.0) (0–0)
HADS-Anxiety, mean (SD) (range) [a]	6.9 (4.2) (0–16)	7.0 (4.0) (0–16)	6.8 (5.2) (0–16)	6.4 (3.8) (1–14)
HADS-Depression, mean (SD) (range) [a]	2.5 (2.8) (0–11)	2.8 (2.7) (0–9)	2.4 (3.4) (0–11)	2.2 (2.7) (0–8)
Verbal IQ [a]	103.5 (11.4) (76–120)	105.1 (9.4) (88–119)	102.0 (11.5) (85–120)	101.5 (15.6) (76–119)
Performance IQ [a]	114.3 (14.7) (63–137)	116.9 (12.6) (87–137)	115.0 (11.9) (97–134)	107.5 (20.0) (63–133)
Education (years) [a]	15.0 (2.4) (9–18)	15.3 (2.1) (12–18)	15.3 (2.1) (12–18)	13.8 (3.2) (9–18)
Total MyCog score (out of 24): mean (SD) (range)	2.9 (4.4) (0–21)	1.6 (2.2) (0–7)	3.8 (5.5) (0–21)	4.6 (6.0) (0–19)
AD8, mean (SD) (range), % [b]	0.1 (0.4) (0–2)	0.0 (0.0) (0–0)	0.2 (0.6) (0–2)	0.3 (0.5) (0–1)
AD8, % AD8 ≥2	2.3	0	7.7	0

[a] n=48; [b]n=44; PMC = presymptomatic mutation carrier; MMSE: mini-mental state examination; HADS= Hospital Anxiety and Depression Scale.

7.3.3.1. Symptoms of SCD in this sample

Unlike the Insight 46 cohort, the participant's report of SCD symptoms was not in accordance with the informant's (1.57 [-2.95, 6.10], p=0.496) and results did not change when considering mutation status (0.74 [-3.92, 5.39], p=0.757). However, it is worth noting that 87.8% of informants of PMCs scored 0 on the AD8 questionnaire. There was no difference in the distribution of informant perception of male and female participants either ($\chi 2$=0.003, p=0.957).

7.3.3.2. Associations with mutations status

Overall, early and late PMCs reported greater concerns on most items of the MyCog questionnaire (**Figure 7.6**) compared to controls yet, Model 1 did not show any evidence for differences in MyCog between PMCs and controls (early PMCs: p=0.143; late PMCs: p=0.122, **Table 7.5**, see also unadjusted means in **Table 7.4**).

There was no significant interaction between mutation status and sex (early PMCs: p=0.496; late PMCs: p=0.252).

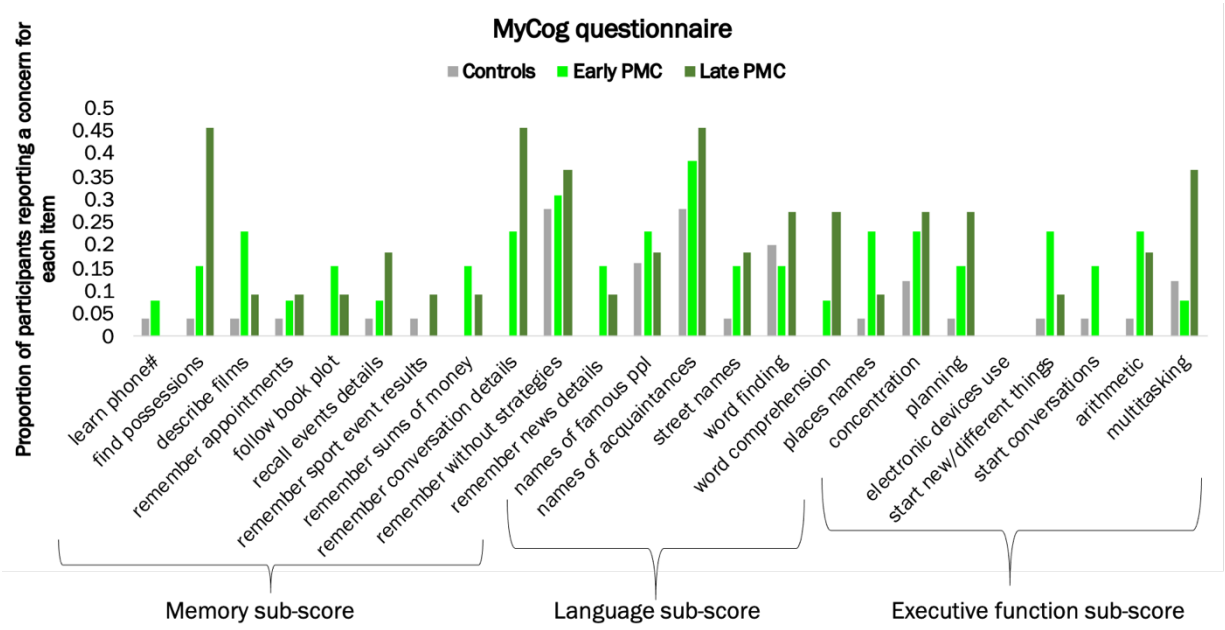


Figure 7.6 MyCog scores by mutation status.

Bar graphs shows the proportion of participants responding a concern for each item by mutation status (N=49). 'Memory', 'language' and 'executive function' concern scores are referenced are will be further discussed in section 7.3.3.6.

7.3.3.3. Impact of affective symptoms on SCD and mutation associations

Similar to the Insight 46 study, reports of higher anxiety and depression were independently associated with higher MyCog scores when considered separately. For every one-point increase of the HADS-A, MyCog increased by 0.61 points (**Table 7.5**, Model 2, p=0.001, **Figure 7.7**) and for every one-point increase in HADS-D, MyCog increased by 0.81 points (p=0.003). Nonetheless, when affective symptoms were considered together, reports of higher anxiety

tended to be associated with higher MyCog scores (p=0.068) whereas no effect was observed for HADS-D (p=0.395, **Table 7.5**, Model 3).

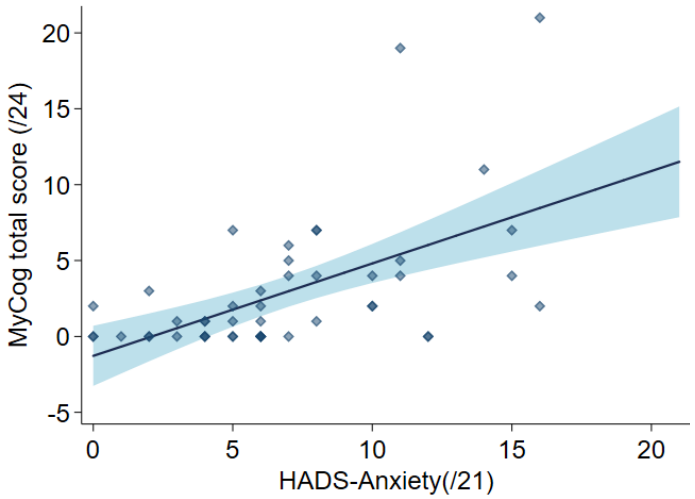


Figure 7.7 Total MyCog against HADS-Anxiety.

Scatter plot shows the raw data (N=48) of MyCog against trait anxiety. The blue line is the line of best fit from the multivariable regression model (adjusted for sex age and HADS-Anxiety, Model 2). The shaded areas represent 95% confidence intervals. HADS=Hospital Anxiety and Depression Scale.

There was no evidence for a significant interaction between affective symptoms and sex (HADS-A: -0.13 [-0.84, 0.57], p=0.709; HADS-D: 0.70 [-0.29, 1.69], p=0.164). The addition of the affective symptoms as predictors revealed a significant association between mutation status and MyCog scores, whereby both PMCs groups had higher MyCog scores than controls (Model 3: early PMCs: p=0.027; late PMCs: p=0.042, **Table 7.5**).

7.3.3.4. Effect of objective performance and education

Similar to the Insight 46 findings, neither verbal nor performance IQ were significantly associated with MyCog scores (**Table 7.5**; Model 4-verbal IQ: p=0.233; Model 5- performance IQ: p=0.162). However, considering objective performance in the model, revealed that only late PMCs had statistically higher MyCog scores (**Table 7.5**; early PMCs: p=0.057; late PMCs: p=0.032) and including education levels did not materially change the results (**Table 7.5**). There was no significant interaction between objective performance and sex (verbal IQ: -0.11 [-0.38, 0.15], p=0.412; performance IQ: -0.04 [-0.21, 0.13], p=0.663) nor between years of education and sex

(-0.19 [-1.18, 0.80], *p*=0.711). Although education levels are commonly considered in combination with objective performance, education level was also included as an additional predictor to Model 3 and no significant associations with MyCog observed (-0.18 [-0.46, 0.10], *p*=0.202).

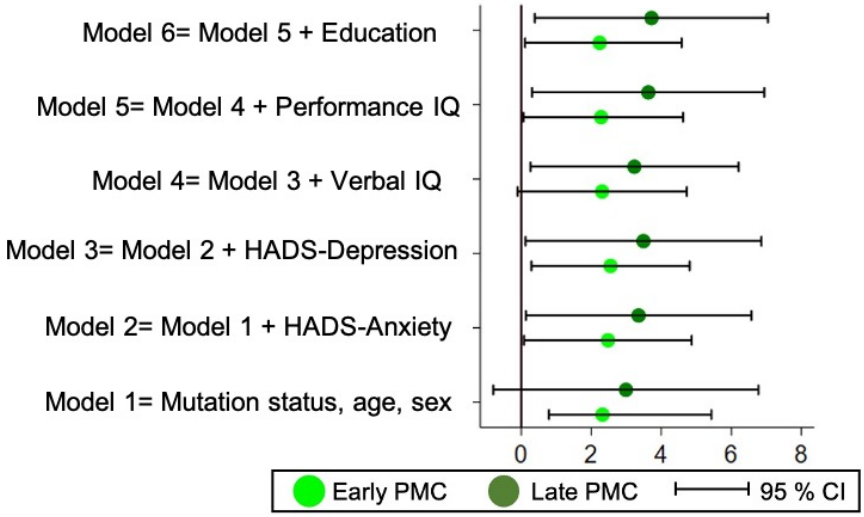


Figure 7.8 Mutation status coefficient as a predictor for each regression model.

Mutation status coefficients of each model with 95% confidence intervals (N=49). Note that a coefficient of 0 indicates no difference in the association with MyCog between mutation status. PMC= presymptomatic mutation carrier.

7.3.3.5. Age and sex

Overall, sex did not have a significant association with MyCog scores in this cohort, suggesting males and females reported similar symptoms on SCD. Age appeared to have effects in some regression models, whereby greater age was associated with higher MyCog scores, although the effect size remained relatively small (**Table 7.5**, Model 3: *p*=0.040; Model 5: *p*=0.028; Model 6: *p*=0.040).

Table 7.5 Predictors of MyCog in the FAD cohort (N=49).

	Coefficient [95% confidence interval for each model]					
	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 5**	**Model 6**
Mutation status (control as reference)	Early: 2.32 [0.79, 5.43] Late: 2.99 [-0.80, 6.77]	**Early: 2.48 [0.08, 4.87]* Late: 3.35 [0.13, 6.57]***	**Early: 2.55 [0.29, 4.81]* Late: 3.49 [0.12, 6.86]***	Early: 2.31 [-0.10, 4.73] **Late: 3.23 [0.26, 6.21]***	Early: 2.28 [0.06, 4.62] **Late: 3.63 [0.31, 6.94]***	Early: 2.24 [0.11, 4.58] **Late: 3.72 [0.39, 7.04]***
Age	0.09 [-0.06, 0.24]	**0.10 [-0.02, 0.21]***	0.09 [-0.02, 0.19]	**0.12 [0.006, 0.23]***	**0.13 [0.01, 0.25]***	**0.14 [0.007, 0.28]***
Sex (male as reference)	0.43 [-1.91, 2.77]	-0.10 [-2.07, 1.87]	0.05 [-2.06, 2.15]	0.28 [-1.64, 2.21]	0.26 [-1.62, 2.14]	0.30 [-1.65, 2.26]
Anxiety-HADS		**0.61 [0.25, 0.97]****	0.45 [-0.04, 0.94]	0.46 [-0.01, 0.98]	**0.50 [-0.007, 1.00]***	0.50 [0.0003, 1.00]
Depression-HADS			0.33 [-0.43, 1.09]	0.22 [-0.57, 1.00]	0.23 [-0.54, 1.00]	0.23 [-0.57, 1.02]
Verbal IQ				-0.09 [-0.24, 0.06]	-0.15 [-0.35, 0.05]	-0.16 [-0.38, 0.05]
Performance IQ					0.07 [-0.03, 0.17]	0.07 [-0.03, 0.17]
Education (years)						0.11 [0.47, 0.70]
R^2	0.117	0.445	0.466	0.508	0.536	0.538

Multivariable regression models were used so each association is independent of all others. R^2 gives the proportion of variance in each cognitive outcome that is explained by the combined predictors. PMC = presymptomatic mutation carrier. R^2 gives the proportion of variance in the outcome that is explained by the combined predictors. Bold=significant; * Significant at $p < 0.05$; **Significant at $p < 0.01$

7.3.3.6. MyCog domains

Similar to Insight 46, SCD relating to 'memory' concerns were higher in mutation carriers compared to controls once affective symptoms were considered (**Table 7.6**, early PMCs: p=0.065; late PMCs: p=0.035). In addition, the executive function concern score also showed a similar effect for both PMCs groups compared to controls (**Table 7.6**, early PMC: p=0.023; late PMC: p=0.016). No significant associations were observed for the language concern score (**Table 7.6**, early PMC: p=0.116; late PMC: p=0.165).

There was no significant interaction between mutation status and sex for any of the domains (memory: early PMCs: 1.04 [-1.41, 3.49], p=0.403; late PMCs: -1.43 [-4.41, 1.54], p=0.345; language: early PMCs: 0.35 [-1.46, 2.15], p=0.708; late PMCs: -1.06 [-3.59, 1.46], p=0.409); executive function: early PMCs: 0.39 [-1.22, 2.00], p=0.635; late PMCs: -1.34 [-2.72, 0.04], p=0.058).

HADS-A had a significant effect on all MyCog domains (**Table 7.6**, memory: p=0.004; language: p=0.007; executive function: p<0.001) and there was no significant interaction with sex for any of the domains (memory: -0.06 [-0.39, 0.28], p=0.740; language: -0.08 [-0.34, 0.19], p=0.576; executive function: -0.0005 [-0.14, 0.14], p=0.994). HADS-D did not show a significant association with any of the domain (**Table 7.6**; memory: p=0.695; language: p=0.472; executive function: p=0.065) nor was there a significant interaction with sex (memory: 0.34 [-0.17, 0.86], p=0.190; language: 0.26 [-0.11, 0.63], p=0.167; executive function: -0.10 [-0.12, 0.32], p=0.386).

Similar to Insight 46, objective performance did not show a significant association with MyCog scores for any of the domains (verbal IQ-memory: p=0.119; language: p=0.414; executive function: p=0.437; performance IQ-memory: p=0.110; language: p=0.670; executive function: p=0.050) and there was no significant interaction with sex either. Considering education levels in regression models did not materially change results (**Table 7.6,** and a post-hoc analysis using NART instead performed as a sanity check, yield similar results) and there were no significant interactions between education and sex for any of the domains (memory: p=0.929; language: p=0.719; executive function: p=0.414). Overall, sex did not have a significant association with MyCog scores in this cohort. Age appeared to have effects in some models but effect sizes remained relatively small.

Table 7.6 Predictors of MyCog for each domain in the FAD cohort (N=49).

Memory Language Executive	**Coefficient [95% confidence interval for each model]**					
	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 5**	**Model 6**
Mutation status (control as reference)	Early: 1.15 [-0.38, 2.68] Late: 1.47 [0.31, 3.20] Early: 0.55 [-0.42, 1.51] Late: 0.78 [-0.54, 2.09] Early: 0.62 [-0.25, 1.49] Late: 0.78 [-0.11, 1.64]	Early: 1.22 [-0.07, 2.51] **Late:** **1.63 [0.11, 3.15]*** Early: 0.59 [-0.25, 1.43] Late: 0.86 [-0.35, 2.08] **Early:** **0.67 [0.09, 1.24]*** **Late:** **0.85 [0.16, 1.55]***	Early: 1.24 [-0.07, 2.54] **Late:** **1.67 [0.11, 3.22]*** Early: 0.61 [-0.18, 1.40] Late: 0.91 [-0.37, 2.19] **Early:** **0.70 [0.22, 1.17]*** **Late:** **0.92 [0.21, 1.62]***	Early: 1.08 [-0.25, 2.41] **Late:** **1.51 [0.18, 2.84]*** Early: 0.57 [-0.31, 1.44] Late: 0.84 [-0.37, 2.05] **Early:** **0.67 [0.15,1.19]*** **Late:** **0.88 [0.25, 1.51]***	Early: 1.06 [-0.23, 2.35] **Late:** **1.72 [0.22, 3.23]*** Early: 0.56 [-0.32, 1.45] Late: 0.90 [-0.44, 2.24] **Early:** **0.66 [0.17, 0.15]*** **Late:** **1.00 [0.32, 1.70]***	Early: 1.04 [-0.25, 2.34] **Late:** **1.76 [0.26, 3.26]*** Early: 0.56 [-0.30, 1.43] Late: 0.89 [-0.51, 2.29] **Early:** **0.63 [0.14, 1.12]*** **Late:** **1.06 [0.41,1.71]***
Age	0.04 [-0.03, 0.11] 0.04 [-0.01, 0.05] 0.01 [-0.03, 0.06]	0.04 [0.02, 0.10] 0.04 [0.00001, 0.08] 0.02 [-0.01, 0.05]	0.04 [-0.01, 0.09] 0.03 [0.002, 0.07] 0.01 [-0.01, 0.04]	**0.06 [0.002, 0.12]*** **0.04 [0.007, 0.08]*** 0.02 [-0.01, 0.04]	**0.07 [0.007, 0.13]*** 0.04 [0.00002, 0.09] 0.02 [-0.007, 0.05]	**0.07 [0.004, 0.14]*** 0.04 [-0.009, 0.10] 0.03 [-0.002, 0.05]
Sex (male as reference)	0.08 [-1.00, 1.67] 0.33 [0.50, 1.15] 0.02 [-0.63, 0.68]	-0.16 [-1.10, 0.78] 0.19 [-0.62, 0.99] -0.13 [-0.59, 0.34]	0.04 [-0.01, 0.91] 0.23 [-0.60, 1.07] -0.06 [-0.51, 0.39]	0.09 [-0.94, 0.96] 0.30 [-0.49, 1.09] 0.03 [-0.45, 0.40]	-0.003 [-0.92, 0.92] 0.30 [-0.50, 1.10] -0.03 [-0.44, 0.37]	0.01 [-0.95, 0.98] 0.30 [-0.52, 1.11] -0.008 [-0.43, 0.41]
Anxiety-HADS		**0.27 [0.08, 0.45]**** **0.16 [0.04, 0.28]**** **0.18 [0.10, 0.26]****	0.23 [-0.006, 0.46] 0.11 [-0.07, 0.30] **0.11 [-0.08, 0.21]***	**0.25 [0.02, 0.48]*** 0.12 [-0.08, 0.32] **0.11 [0.006, 0.22]***	**0.26 [0.03, 0.49]*** 0.12 [-0.08, 0.33] **0.12 [0.01,0.22]***	**0.26 [0.03, 0.49]*** 0.12 [-0.08, 0.33] **0.12 [0.01, 0.22]***
Depression-HADS			0.08 [-0.31, 0.47] 0.10 [-0.18, 0.38] 0.15 [-0.009, 0.31]	0.01 [-0.37, 0.39] 0.07 [-0.24, 0.39] 0.14 [-0.03, 0.30]	0.02 [-0.35, 0.39] 0.07 [-0.24, 0.39] 0.14 [-0.02, 0.30]	0.01 [-0.36, 0.39] 0.08 [0.25, 0.40] 0.14 [-0.02, 0.30]

Verbal IQ				-0.05 [-0.12, 0.01] -0.02 [-0.08, 0.03] -0.01 [-0.05, 0.02]	-0.09 [-0.18, 0.004] -0.03 [-0.11, 0.05] -0.03 [-0.08, 0.01]	-0.09 [-0.19, 0.004] -0.03 [-0.12, 0.06] -0.04 [-0.09, 0.004]
Performance IQ					0.04 [-0.009, 0.09] 0.01 [-0.04, 0.06] 0.02 [-0.00002, 0.05]	0.04 [-0.01, 0.09] 0.01 [-0.04, 0.06] 0.02 [-0.003, 0.05]
Education (years)						0.05 [-0.22, 0.32] -0.006 [-0.26, 0.25] 0.07 [-0.06, 0.21]
R^2	0.122 0.087 0.106	0.405 0.285 0.537	0.410 0.302 0.605	0.481 0.323 0.616	0.517 0.328 0.659	0.519 0.328 0.671

Multivariable regression models were used so each association is independent of all others. R^2 gives the proportion of variance in each cognitive outcome that is explained by the combined predictors. PMC = presymptomatic mutation carrier. R^2 gives the proportion of variance in each outcome that is explained by the combined predictors. Bold=significant; * Significant at $p < 0.05$; **Significant at $p < 0.01$

7.3.4. Discussion

7.3.4.1. Summary

Interestingly, despite very different ages and experiences of seeing AD within one's family the FAD cohort showed a similar pattern of performance to Insight 46, arguable a distinct cohort at-risk of AD.

The findings presented in this data chapter add to the emerging evidence that symptoms of SCD are detectable at presymptomatic stages of FAD – in this case, within an expected onset range of -16.5 to 3.3 years. In summary, both early PMCs (more than 5 years from expected onset) and late PMC (within 5 years to expected onset) showed significantly higher MyCog scores compared to controls once affective symptoms were considered in the models. Furthermore, items classified under the 'memory' and 'executive function' domains within MyCog, showed higher scores in late PMCs and early PMCs compared to controls (with a trend observed for the memory domain score in early PMCs – possibly due to the lower consistency of SCD in individuals who are further way from expected symptom onset in comparison to late PMCs). Conversely, no significant differences between PMCs and controls were observed among the items classified under the 'language' domain.

7.3.4.2. Reflections on FAD findings and comparisons with Insight 46

Consistent with the notion that subjective complaints increase with age (Garcia-Ptacek et al., 2016), but perhaps surprising given the family history of the FAD subjects, MyCog scores were overall lower in the FAD cohort compared to Insight 46. Moreover, a greater proportion of variance in scores was explained by the predictors included in FAD models compared to Insight 46. Yet, it is relevant to note there was variability in the measures included (i.e. anxiety scores from the HADS *vs* trait and state anxiety scores from the STAI questionnaires). A recent study comparing anxiety measures between different questionnaires, suggested that state anxiety measured by the STAI, showed a higher prevalence rate of anxiety compared to HADS-A (39% *vs* 23%) (Emons et al., 2019). While cut-off scores were not used here, also for these reasons, it is important to acknowledge direct comparisons between this cohorts and questionnaires remains speculative. As previously mentioned, affective symptoms are well recognized as risk factors of dementia (Richards et al., 2014). A study by Ringman and colleagues (Ringman et al., 2015), showed that while some psychiatric symptoms were prominent during the presymptomatic phase

of FAD, depressive symptoms were not. The lower scores of HADS-D (and smaller variance) in comparison with HADS-A may explain why anxiety was more strongly associated with MyCog than depression scores.

As for the Insight 46 cohort, objective performance was not associated with symptoms of SCD and adjustment for education levels did not materially change results. Further reflections on comparisons between subjective and objective cognition will be discussed in Chapter 8; section 8.2.3.1.

Additional limitations of this study in the FAD cohort include the lack of information on lifestyle factors, some of which (e.g. sleep problems and stress) have been associated with both subjective and objective cognitive problems (Miley-Akerstedt et al., 2018). In addition, the sample size was relatively small and included individuals from different pedigrees making the data more subject to within group heterogeneity and this was not accounted for in the analysis. Considering a 0.05 level of significance and 80% power, the total sample size required to replicate the higher MyCog total score in late PMCs compared to controls after accounting for all variables in Model 6, is N=80 (of which N=59 would be late PMCs and controls).

In summary, symptoms of SCD appear detectable in FAD PMCs, above and beyond effects of affective symptoms, education level and objective cognition, from five years to expected symptom onset.

While direct comparisons between these two at-risk cohorts are novel and complex; findings from this data chapter accord with the notion that symptoms of SCD may be sensitive markers of preclinical AD.

8. GENERAL DISCUSSION

8.1. Summary

In this thesis I investigated clinical features, neuropsychological changes and symptoms of SCD in populations at-risk of AD with results largely based on data from the FAD study. My key findings with reference to the overarching hypotheses stated in section 2.2.2 are summarized as follows:

i) There was no difference in survival time between *PSEN1* and *APP* mutations. Unlike AAO, survival estimates were influenced to a much lesser extent by genotype (measured by family membership as a proxy to mutation specificity). In addition, atypical presentations (non-amnestic) had longer survival compared to typical (amnestic) presentations; longer survival times were observed for earlier compared to later generations and there was some indication that *APOE* ε4 carriership conferred an advantage in terms of survival for *PSEN1* carriers.

ii) There was evidence for a faster rate of decline in VSTM function for PMC individuals (within 8.5 years to expected symptom onset) compared to controls. Unlike previous reports describing preclinical effects in the swap error proportion metric, this effect was specific to the localisation error measure which also showed a significant association with EYO and appeared earlier than changes in more traditional measures of recognition memory. Compared to controls, symptomatic individuals showed faster rates of decline in all VSTM metrics but swap error performance which remained poor at every visit. In addition, identification performance was significantly associated with proximity to actual symptom onset.

iii) Symptomatic carriers showed less effective visual exploration strategies compared to controls. While superficially PMCs (within 6 years to expected symptom onset) appeared to have a similar viewing behaviour compared to controls, associations with VSTM performance revealed evidence supportive of a weakening encoding process specific to the localisation error measure (proposed as a novel measure of relational binding accuracy) in this group.

iv) As expected, SCD was more pronounced in the ~70-year-old amyloid-positive individuals and ~40-year-old PMCs (within 5 years to expected symptom onset) respectively, compared to amyloid-negative and FAD non-carriers, of similar demographics. These differences were observed above and beyond effects of mental health on SCD.

Making reference to my overarching hypothesis in section 2.2.2, genetic (carrying a mutation for FAD) and pathological (being classified as ‘amyloid-positive’ in Insight 46) components did

provide an important basis for clinical and cognitive outcomes: FAD carriers and amyloid-positive individuals showed a degree of impairment compared to their respective control groups. Yet, the patterns of impairment were not entirely consistent with what had been observed in the literature at a given time point as the disease progressed. In combination, this thesis presents important and novel evidence of VSTM function (e.g. relational binding) and clinical features (e.g. survival), specifically in relation to FAD progression, as well as a relevant parallel between two distinct cohorts at-risk of AD. Conceptually, I present a new proposal for measuring relational binding and probe sub-processes which may be captured using eye-tracking.

These results are discussed in more detail below, followed by a discussion of common themes emerging from this thesis, the strengths and limitations, and directions for future work.

8.2. Key results and interpretations

Before symptomatic and presymptomatic findings are discussed it is important to note that a number of additional variables not accounted for here, may have influenced results. Some examples include environmental aspects, epigenetic contributions (**Table 8.1**), as well as the timing of (for cross-sectional studies) and intervals between (for longitudinal studies) observations. The design and variables considered play a key role in understanding and interpreting the empirical findings.

Table 8.1. below provides an overview of the concepts or variables investigated in this thesis.

Table 8.1 Representation of the concepts studied in this thesis by AD 'stage'.

	Presymptomatic	Symptomatic
'Traditional neuropsychology' (Chapters 5, 6)	*	*
VSTM function (Chapters 5, 6)	*	*
VSTM 'relational binding' (Chapters 5, 6)	*	*
Viewing behaviour (Chapter 5)	*	*
Cognitive presentations (Chapters 5, 6)		*
APOE status (Chapter 4)		*
Gene (Chapter 4)		*
Family membership & mutation specificity (Chapter 4)		*
Mutation position-*PSEN1* gene (Chapter 4)		*
Expected age at symptom onset (Chapters 4, 5, 6)	*	*
Actual age at symptom onset (Chapters 4, 5)	NA	*
Age at death (Chapter 4)	NA	*
Environmental factors		
Epigenetic factors		

VSTM=visual short-term memory; *APOE*= apolipoprotein gene; *PSEN1=presenilin 1;* NA=not applicable.

While the main focus of this thesis was on the preclinical aspects of AD, I will first discuss how impairments observed in symptomatic carriers provided the necessary context and starting point for subsequent preclinical discussions.

8.2.1. Symptomatic findings

The analysis of survival time revealed that only a small proportion of variance could be explained by family membership across the whole cohort of 256 individuals and this finding was replicated when restricted to *PSEN1* mutations. While unexpected, these results are consistent with the growing body of evidence, mainly in sporadic AD, that epigenetic factors (other than gene mutation, (Millan, 2014)) and lifestyle factors (or 'modifiable risk factors', (Edwards III et al., 2019)) have a significant impact on quality of life and survival. Also possibly related to lifestyle factors and improvements in health care over the years, were the "generational effects" observed with longer survival for earlier generations.

The significantly longer disease duration for atypical compared to amnestic presentations was surprising. Yet, atypical presentations tend to be more common with *PSEN1* mutations beyond codon 200 and with mutations located in exon 8 (Ryan et al., 2016). Interestingly, survival analysis showed individuals with exon 8 mutations had particularly long disease durations. Thus, as suggested in Chapter 4, there may be differences in the disease process induced by variants

located in this region of PSEN1, which drive later ages at symptom onset (Hutton & Hardy, 1997; Wragg et al., 1996), longer disease durations and atypical presentations.

Antagonistic pleiotropy is a leading evolutionary explanation for aging and non-communicable disease (Austad & Hoffman, 2018; Byars & Voskarides, 2020), however, its putative benefits remain controversial (Tuminello & Han, 2011). As previously mentioned, the longer survival for ε4 carriers in comparison to ε4 non-carriers observed within *PSEN1* mutation carriers may be explained by this antagonist pleiotropy hypothesis whereby a gene has both beneficial and detrimental effects, with the detrimental effects often manifesting later in life when the forces of natural selection are weaker. In this regard, it is relevant to note that some studies observed superior performance for ε4 carriers in the "What was where?" task: specifically, ε4 carriers had a more accurate localisation than ε4 non-carriers after delays of a few seconds (Zokaei et al., 2017, 2019, 2020). However, no biomarker data were available in these investigations so the contribution of preclinical AD pathology could not be evaluated. In future, a more direct comparison between different at-risk populations (such as Insight 46 and FAD) performing the same task with biomarker data available would provide further insight into the complex interactions of amyloid, *APOE* and genetic mutation. It remains intriguing to understand at what age these gene effects become beneficial and whether the presence of other genetic factors favours or limits these effects. In addition, it is possible that some of the variance in VSTM function (across all groups) could be explained by *APOE* status.

Other notable symptomatic findings included a faster rate of decline in VSTM function compared to controls, the significant association between identification performance and AYO and the ineffective visual exploration strategies during stimuli presentation. Taken together, results from Chapter 5 and Chapter 6, suggest a gradual decline in the ability to effectively encode abstract and complex scenes which may be at the root of at least some of the memory impairments observed in this cohort. Notably, attention and executive control (e.g. impairments disengaging with the stimuli after viewing) may have also contributed to such deficits particularly as memory retrieval is usually associated with activation of the parietal cortex, which is also implicated in the attentional system (Pereira et al., 2014). Furthermore, there is evidence that attention facilitates memory for the relationship between objects in relational visuo-spatial memory tasks (Olsen et al., 2014) and that attention and visuo-spatial memory together influence the formation of visual representations, possibly affected here during encoding and maintenance in symptomatic carriers (Hitch et al., 2020)

Hypothetically, cognitive symptomatic findings may also be in line with the pattern of atrophy described in FAD, whereby significant white matter differences were observed between non-carriers and symptomatic carriers in the: cingulum and fornix which form input and output connections to the MTL; cingulate and precuneus; and significant grey matter differences in the: thalamus and putamen, temporal lobe, precuneus, and cingulate gyrus (Cash et al., 2013), regions believed to be involved in object perception, planning, attention and encoding just to name a few – though it is difficult to comment given the lack of MRI data.

Taken together, the heterogeneity revealed amongst affected members of mutation carriers in FAD families, appears consistent with the growing (clinical, cognitive, neuroimaging and neuropathological) documentation of phenotypic heterogeneity amongst individuals in sporadic AD (Lam et al., 2013). To date most of the current clinical trials consider AD as a single phenomenon, often neglecting the benefits in treatments that might arise from discriminating between disease subtypes for instance. The findings from this thesis are in accordance with the appreciation that this heterogeneity must be reflected in disease monitoring, management, and especially in trial designs aimed at measuring 'effective' responses to treatments. Consequently, if much variation exists how does this impact how we measure 'effective' responses to treatments (i.e. given that heterogeneity of disease and its progression is inherent to the condition, what is considered an effective response to treatment?)

Another relevant concept arising from symptomatic findings, is the urgent necessity to describe and pre-define stages. Critical work arising from the specific studies on kindreds, like the Colombian kindred *PSEN1* p.Glu280Ala mutation (Acosta-Baena et al., 2011) and the most recent staging system from the NIA-AA Research Framework (Jack et al., 2018) for SAD. The new FAD stages presented in this thesis attempt to incorporate heterogeneity within our "stages" description of progression in order to provide individuals and families with a more tangible concept. Further research into how the so called 'biological changes' affect individuals and their families is indispensable and integrative approaches incorporating both qualitative and quantitative methods may be best placed to address this.

Now that symptomatic findings have been discussed, I turn to the question of: how did impairments start? The next sub-section will discuss key results and interpretations for preclinical findings arising from this thesis.

8.2.2. Preclinical objective findings

In summary, the longitudinal analysis revealed late PMCs, within 8.5 years to expected onset, had a faster rate of decline of localisation performance with significant effects seen from 2 years after the baseline visit (before changes in more traditional neuropsychology tasks including those measuring episodic memory). This suggested that spatial (i.e. location) and perceptual (i.e. identity) information together, were less effectively remembered (e.g. encoded, maintained or retrieved) as the disease progressed. Localisation performance was also significantly associated with EYO suggesting a progressive decline as a function of expected age at symptom onset. Perhaps non-coincidentally, the cross-sectional eye-tracking experiment revealed late PMCs, within 6 years to expected onset, showed a stronger reliance than controls on the time spent fixating the stimuli in order to achieve localisation accuracy.

8.2.2.1. Which processes are affected?

I next speculate on which process within STM may be impaired if results are to be integrated. **Figure 8.1** shows a visual representation of this following the presymptomatic FAD findings presented in this thesis.

If forgetting and remembering are considered at opposite sides of the same spectrum, longitudinal VSTM findings suggested that forgetting happened at an "accelerated rate" in late PMCs (within 8.5 years to expected onset) and that this effect was specific to the localisation performance metric (**Figure 8.1**). Furthermore, the overreliance of late PMCs (within 6 years to expected onset) on the stimuli fixation time for this same metric may indicate a 'compensatory mechanism' whereby PMCs compensate an encoding deficit by requiring more time to effectively encode (**Figure 8.1**). As previously argued in Chapters 5 and 6, localisation performance may conceptually be viewed as 'correct binding' of the object's identity to its correct location. Impairments were observed in this measure and not in the nearest item control measure (a purer measure of localisation to any fractal) or identification performance (identity of the object) as it was the identity *and* location of the object together which, requiring greater cognitive load, were more vulnerable to forgetting. From an encoding point of view, the "re-entry hypothesis described in section 2.4.3 (whereby features are 'retraced' to check whether binding of features is correct) has been suggested as a necessary process for 'binding' and is particularly fragile to disruption during encoding (Bouvier & Treisman, 2010; Gao et al., 2017; Shen et al., 2015). In line with this,

if localisation performance is considered a measure of 'relational binding', there may be processes specific to AD pathology in presymptomatic FAD which make these processes even more vulnerable.

A number of explanations underlying forgetting have been proposed with one being that 'the passage of time' is a significant cause of memory loss (Davis & Zhong, 2017; Migues et al., 2016; Sadeh et al., 2014; Sadeh & Pertzov, 2020) (in addition to the more traditional view that interference is the sole factor (Underwood, 1957)). The conceptual similarities between short- and long-timescales, described in section 2.4.2, allow for the speculation that similar to LTM, in STM memories depending on recollection are more vulnerable to 'decay over time' than interference, whereas memories depending on familiarity are more vulnerable to interference (Sadeh et al., 2014). Recollection involves reinstatement of an event from memory whereas familiarity does not involve reinstatement but is accompanied by the feeling that an item had been previously encountered (Sadeh et al., 2014). To a certain extent it could be argued that "What was where?" relies more on recollection than familiarity given that fractals were presented more than once throughout the experiment (i.e. the target in one trial could act as a distractor in another trial). As this 'passage of time' had a stronger detrimental effect on the performance of PMCs compared to controls, one potential interpretation is that this mechanism of forgetting is not only more pronounced in presymptomatic FAD following the effect observed between visits but may also have a short-term effect in maintenance given the specificity to deficits after a 4s delay (**Figure 8.1**).

Lastly, another hypothesis proposed in LTM, is that retrieval success is associated with 'rapid replay of content' (Liu et al., 2019; Wimmer et al., 2020). Observations from animal studies have identified offline reactivation of sequences of hippocampal place cells that reflect past and future trajectories, which are thought to support memory consolidation, retrieval and planning (Jadhav et al., 2012; Ólafsdóttir et al., 2018; Pfeiffer & Foster, 2013; Wimmer et al., 2020). A similar process to this reactivation may be impaired in presymptomatic FAD in VSTM during encoding or retrieval (**Figure 8.1**).

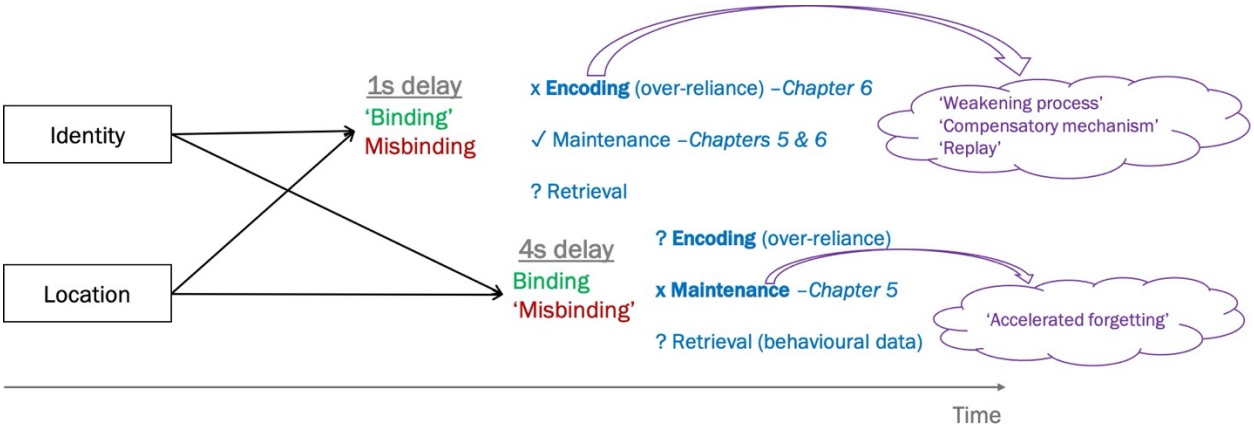


Figure 8.1 Schematic of memory components hypothesized to be impaired in FAD PMCs.

Black text outlines the different components of an object (i.e. identity and location) which are either correctly or incorrectly bound after a 1s and 4s delays. Note that 'binding' and 'misbinding' specifically refer to the continuous measures of localisation performance (binding/correct binding=green) and nearest item control (misbinding/incorrect binding=red). Blue text references the stage of the memory processes and whether evidence from the chapters presented in this thesis suggest any deficits: tick=preserved; cross=impaired. Chapter 5 mainly represents behavioural longitudinal findings and Chapter 6 eye-tracking cross-sectional. Of note, encoding deficits are referenced with '?' after a 4s delay as findings from Chapter 5 raise the possibility that encoding may also be affected but perhaps 'masked' by the more prominent maintenance deficits. Purple text indicates the hypothetical processes discussed in text. Note that the grey arrow indicates the passage of time.

8.2.2.2. Implications of my findings to recent views of working memory

The work presented in this thesis also has conceptual implications for the more recent views of working memory. More specifically, the proposal that precision indexes binding functions (a property previously allocated to 'swap error proportions'), provides in my opinion, a better candidate to measure the accuracy with which that bound representation is held in memory.

The "What was where?" task was originally designed following the idea that just because an individual fails to recall an item correctly does not necessarily mean that it was completely abolished from memory (all or none scenario) (Liang et al., 2016). While the 'swap error proportion' metric enables the quantification of this misbinding, the evidence provided in this thesis suggests its binary nature may not be that well suited to quantify this imprecision.

Resource models (Bays & Husain, 2008; Palmer, 1990; Wilken & Ma, 2004) indicate that the resources available limit the *quality* of the memory representation in such a way that resources can be unevenly and flexibly distributed (Bays & Husain, 2008) in order to enhance the precision of one object at the cost of other stimuli held in memory, as memory resources are shared

between items (Ma et al., 2014). Furthermore, recall variability has been shown to gradually and *continuously* increase with increasing set size (rather than exhibiting the step decline that would be expected on reaching capacity of a fixed number of items (Luck & Vogel, 1997)). Considering these empirical observations, I argue that the metric representing precision should also share these properties and that 'localisation performance', being continuous and sensitive to this gradual decrease is best suited to account for relational binding accuracy.

Importantly, as previously argued this novel estimation of relational binding accuracy does not allow for the quantification of misbinding as a proportion (i.e. the decrease in relational binding accuracy is measurable but misbinding errors themselves are not) and this remains a limitation of the proposal. Yet, the comparison between localisation and NIC error, to a certain extent, allows some quantification of this misbinding: a similar value in localisation and NIC error would imply the item location was not swapped with that of another fractal whereas a considerably lower NIC error in comparison to localisation error would. Indeed, this example shows the value of analysing data using regression models (clustering data points by participant), rather than summary scores in ANOVAs – especially when recently proposed resource model propose that precision is itself variable across items and trials, even when set size is kept fixed (Fougnie et al., 2012; van den Berg et al., 2012).

Exactly how working memory handles features and bindings is unknown and various theories exist. The feature map theory proposes that features belonging to different feature dimensions can be processed in parallel, for example, in the form of feature maps over space. In this way, storage and retrieval of feature conjunctions occur by extracting the features at a single location from different maps (Schneegans & Bays, 2019). Indeed, important for the reflections of my findings to broader views of working memory is the notion that space may play an important role in combining features even when location is not a feature to encode (binding between colour and shape is thought to be at least in part mediated by their shared location (Schneegans & Bays, 2019; Treisman & Zhang, 2006)). This is yet another reason why a metric measuring location is appropriate to measure binding sensitively.

Interestingly, some authors argue it is not the absolute object location that is relevant for an accurate binding of features but rather the *relative* location within a spatial configuration of objects (Hollingworth, 2007; Hollingworth & Rasmussen, 2010). The mechanisms underlying VSTM impairments are complex and various lines of research exist. One viewpoint is that VSTM retains information using a retinotopic frame of reference: a coordinate system with respect to the retina

that retains view-dependent information. Alternatively, information might be retained using an allocentric frame of reference: a coordinate system with respect to the scene that retains view-invariant information. While the basis of VSTM memory remains under debate and these processes were not explicitly investigated in this thesis, the hippocampus is necessary to construct and store allocentric representations and is also proposed as one of the earlier regions affected by AD pathology (Chan et al., 2016; Fox et al., 1998; Liang et al., 2017) suggesting these processes might have been affected in PMCs performing this task. Importantly, other studies have shown that atrophy in the entorhinal cortex precedes atrophy in the hippocampus (Braak & Braak, 1991; Parra, 2017). Longitudinal imaging studies in preclinical populations like presymptomatic FAD are required to investigate this further.

More broadly, the evidence provided in Chapters 5 (evaluating longitudinal VSTM function) and Chapter 6 (VSTM function and eye-tracking), accord with the recommendation that a higher load (i.e. 3-items) is best suited for detecting subtle changes in function in presymptomatic changes (whereas lower load e.g. 1-item is best suited for dysfunction in symptomatic stages of AD (Koppara et al., 2015)). While this represents preliminary work and future investigations should evaluate relational binding and conjunctive binding together in preclinical populations which are culturally diverse, taken together, the novel proposal of a continuous measure of binding in addition to the high load consideration suggests these two factors may also be important when conceptualizing working memory models. Interestingly, a recent report by Jonin and colleagues (Jonin et al., 2019), argued that the visuo-spatial sketchpad could support conjunctive binding rather than the previously proposed episodic buffer (Baddeley et al., 2011). Although it is not possible to make neuroanatomical inferences here, the frontal-parietal MTL network has been suggested as a neural correlate of the episodic buffer (Prabhakaran et al., 2000) and future fMRI investigations should assess which areas of the brain are activated during the encoding, maintenance and retrieval of individual and bound features in relational binding tasks like “What was where?”.

In summary, in my opinion, the main value of this work is not to argue for or against a particular theoretical position but rather to encourage theoretically-informed revisions to the cognitive and psychometric properties of WM tests as applied to the detection of earliest cognitive change in neurodegenerative disease populations. Indeed, the continuous scale, high load, long delay and the role of space may be important when investigating relational binding in preclinical AD. Lastly, in light of the preliminary eye-tracking findings, it may also be relevant to consider the ‘duration of

viewing' or 'efficacy and integrity of encoding' within memory models, as a more direct determinant of the accuracy and precision with which memory representations are recalled.

The next section will discuss broader considerations when interpreting cognitive change in preclinical AD.

8.2.2.3. Emerging issues and cautions in interpreting objective preclinical cognitive change.

The literature on presymptomatic cognitive deficits in FAD is somewhat mixed and there are number of possible reasons for this. Firstly, the nature of cognitive tasks administered varies; for example, the semantic categorization accuracy task mentioned in the previous section, was developed to measure attention control in semantic memory retrieval (Jackson et al., 2012) and there is a strong contribution of attention to episodic memory performance in the logical memory task (Balota et al., 2002). Similarly, it is likely that the "What was where?" task engages other cognitive functions such as executive function and attention indicating that performance between individuals may vary depending on both strategic grounds and slightly different combinations of cognitive impairments. Secondly, findings reported are likely to depend largely on the sample (i.e. the stage of presymptomatic impairment measured by EYO). As previously mentioned, EYO was variable between studies and while it is a useful estimate it remains an approximation (see section 8.6.1.2 for more limitations on EYO as a measure). Third, sensitivity to cross-sectional *vs* longitudinal change is conceptually different and this may also explain why the previously reported swap errors in FAD were not replicated longitudinally. For example, a number of tests mentioned in the previous section were sensitive to preclinical AD but only some to longitudinal change or EYO (e.g. MMSE and Logical Memory ((Bateman et al., 2012)) and the reasons for this remain somewhat uncertain. Finally, subtle cognitive impairment does not always translate into poor performance in a behavioural task and this is largely due to the psychometric properties and statistical power needed to detect change (see section 8.3 and section 8.4 for more discussion on sensitivity of metrics). This was seen from the eye-tracking findings in Chapter 5 and while not explored here, cognitive reserve is also likely to play a key role.

8.2.3. Preclinical subjective findings

In summary, symptoms of SCD were higher in FAD PMCs within 5 years to expected onset and amyloid-positive individuals compared to FAD non-carriers and amyloid-negative individuals respectively. These findings are consistent with the literature associating SCD with greater risk of dementia, FAD mutation status (Norton et al., 2017) and highlight continuous scales might be more sensitive than single-item approaches (Laske et al., 2015). With regards to the FAD cohort, it is important to emphasize EYO plays a crucial role in subjective experiences of cognition and mental health and it is not unusual for individuals at-risk of FAD to experience considerable anxiety about the possibility of developing memory problems, especially as they grow close to the age at which their parent developed symptoms (Ryan & Rossor, 2011).

While mental health problems have been linked to dementia, the extent to which affective symptoms and SCD overlap or reflect independent neurobiological mechanisms remains uncertain (Liew, 2019; Richards et al., 2014). The significant association between anxiety scales and symptoms of SCD in both Insight 46 and the presymptomatic FAD, is in accordance with the literature yet does not allow for causality assumptions (i.e. mental health problems → lower the threshold for symptoms of SCD → increasing the risk of AD which consequently result in objective impairment).

8.2.3.1. Comparing objective and subjective cognition

Symptoms of SCD play a role in an individual's perception of cognitive abilities. While no independent association between objective and subjective performance was observed from the findings presented here, research suggests subjective experiences have an overall effect on individual performance (Spalding et al., 2020) and this may closely related to a) how close individuals are to expected symptom onset (for the FAD cohort) and b) ageing (for the Insight 46 cohort). Analogously, analysis of SCD by cognitive domain revealed that concerns within MyCog classified under the 'memory' and 'executive function' domains were significantly affected in FAD PMCs and amyloid-positive individuals. This is in line with the literature suggesting that memory and executive function are affected early in AD pathology (Alichniewicz et al., 2013; Fox et al., 1998).

Comparing objective and subjective cognition is inherently complex. Objective tests describe the cognitive capacity at a single cross-sectional time point, while subjective decline refers to a

longitudinal change (Jessen, 2014). For example, an individual whose cognition has declined from a high baseline level may perform equally to another whose cognition has remained stable at a lower level (Jessen, 2014). Furthermore, one issue intrinsic to subjective cognition is anosognosia, the loss of insight with regards to one's own impairment. While it is uncertain at what stage of AD anosognosia may occur, there is evidence that some patients with mild AD often neglect their cognitive impairment and overestimate their cognitive abilities (Jessen, 2014; Kalbe et al., 2005). As anosognosia reduces the predictive power of SCD and this was not accounted for here, results should be interpreted with caution.

8.2.3.2. Emerging issues and cautions with subjective cognition

A recent commentary titled "*Subjective cognitive decline: what is it good for?"* by Howard raised important points on the utility of SCD data (Howard, 2020). Around three quarters of people aged 70 years and older who perform normally on standard cognitive tests, report SCD (van Harten et al., 2018) yet only 14% of individuals with normal cognitive function and SCD develop dementia after a four year follow-up (Mitchell et al., 2014). Highlighting this considerable range, the author questioned SCD as a concept by claiming that *"if uncertainty surrounds when, or even if, these individuals will develop objective impairment, are measures of SCD actually helpful?"* and *"Should SCD be considered a feature of healthy cognitive ageing or a pathological diagnosis?"* (Howard, 2020). Opposing this position were Jessen and colleagues, claiming that a diagnosis of SCD provides explanation and reassurance to those who seek medical help for their concerns (Jessen et al., 2020). In this regard, it is perhaps worth noting that a stronger association between anxiety and SCD was observed in females compared to males and that some research suggests the risk factor of *APOE* ε4 is stronger in females than males, with female ε4-carriers more likely converting to MCI/AD compared to males (N=2588) (Altmann et al., 2014). This emphasises that associations between mental health, SCD and AD are complex and require further investigations. Crucially, including biomarkers, which can accurately identify the earliest clinical manifestations of AD, in such investigations is critical. Notably, subtle cognitive changes (including those indicated by symptoms of SCD) are mentioned in stage 2 of the NIA-AA criteria (Jack et al., 2018), yet there is insufficient data to establish whether biomarkers can predict clinical progression of cognitive decline at an individual level. Investigations should thus determine whether SCD is a useful marker for stage 2 and crucially whether individuals with SCD will progress to MCI or dementia (Jessen et al., 2020).

Now that symptomatic and preclinical findings have been discussed in greater detail, the next sections will explore broader and perhaps more philosophical themes which have emerged throughout the thesis.

8.3. Which signal should we be looking for and does it matter?

A number of approaches may be taken when studying cognitive functions and impairments arising from disease. Theories that focus on a particular deficit can be particularly difficult to generalise to other situations. While not the focus of this thesis, such 'pure approaches' may include neuronal networks (Chhatwal et al., 2018) and are extremely valuable to understand the pattern of network degradation associated with the spreading of AD pathology and possibly find specific targets for clinical trials. Conversely, rather than looking for a particular signal, research can focus on a 'broad spectrum' of potential targets using composite scores (previously discussed in section 2.5.1). Collectively, these somewhat opposing approaches raise the following question: Should scientific investigations be looking for *any* or a *particular* 'signal'?

For instance, would a highly specific marker be useful as an outcome for clinical trials even if it does not have a 'meaningful' impact on the patient's symptoms or quality of life? An example is the recent DIAN-TU results showing that one of the anti-amyloid drugs (gantenerumab) had significant biomarker effects, but no clinical efficacy (Tolar et al., 2020).

Determining whether tests are able to identify the likely presence or absence of a condition of interest is paramount for appropriate decision-making especially for screening tests. As Strassle and colleagues argue, there is a trade-off between statistical rigor and the 'practical realities of sample collection' (Strassle et al., 2012). In order to achieve narrow confidence intervals around estimates of sensitivity and specificity, the sample size must be large enough but this is particularly difficult when the prevalence of a condition is inherently low as is the case for FAD (Leeflang et al., 2013).

Perhaps key to answering the question 'Should scientific investigations be looking for *any* or a *particular* signal?' is to think of the purpose. Randomised controlled trials conduct research on highly selective populations and are managed in tightly controlled settings. However, in most cases samples are too selective given the clear set of inclusion or exclusion criteria (Kim et al., 2018). Somewhat opposing randomised control trials, is real-world research, which offers additional information by evaluating real-world settings and heterogenous study groups. The

survival analysis presented here pulled data from many years and various variables could have affected results making the data quite 'noisy'. However, it outlined important historical and generational effects and hopefully contributed to our understanding of FAD as a whole. The VSTM results were mostly specific to one metric, localisation performance. On one hand, this could be viewed as advantageous: if and when such tasks are used for screening purposes, this could be a non-invasive and inexpensive candidate. On the other hand, it might fail to generalized to other scenarios or have no 'beneficial impact' on the patient. Crucial to clinical practice is the transition from 1) the initial confirmation of association with the outcome of interest (e.g., VSTM impairment is associated with a diagnosis of AD) to 2) acquiring sensitivity to a treatment or an intervention (e.g., VSTM deficits decline in response to a therapy) and 3) showing a "meaningful" change in patient behaviour (e.g., change in VSTM score results in a different treatment strategy) (Pavisic et al., 2020b; Perlis, 2011). For a commentary on the translational potential of VSTM tasks (including VSTM binding) to clinical practice see (Pavisic et al., 2020b).

While it is not possible to provide a definite answer to the question 'Should scientific investigations be looking for *any* or a *particular* signal?', the reflections above suggest the answer may depend on the intended use of the findings arising from the investigations which will inevitably be bound to limitations regardless of the approach.

8.4. Continuous *vs* discrete: Which is best?

Another recurrent theme throughout the thesis was the importance of measuring the *quality* of deficit. From the experimental design of the delay-reproduction task to the inherent psychometric properties of some measures (localisation *vs* identification performance) and the way in which variables were recorded (MyCog measuring symptoms of SCD in a continuous scale as opposed to a single-item approach), discrete and continuous outcomes merit further discussion.

If clinical and cognitive heterogeneity is a feature in FAD and to some extent in AD pathology, should investigations make use of these sources of variability and be considered in a continuum where possible? While every biomarker exists on a continuum, dichotomizing values is necessary in situations which, for example, require a positive or negative result to determine eligibility (Hampel et al., 2015; Jack et al., 2017; Sperling et al., 2012). Moreover, the current research criteria for AD across the cognitive spectrum labels individuals as biomarker positive or negative (Albert et al., 2011; Dubois et al., 2014; Jack et al., 2011; McKhann et al., 2011; Sperling et al.,

2011). Nonetheless, the simplicity gained in the categorization of continuous variables may come at some cost in clinical research (Altman & Royston, 2006). Some pros and cons are listed below:

Pros

- Grouping may help in data presentation.
- Greatly simplifies the statistical analysis.
- Allows for simple interpretation of results.
- Eliminates the need for the linearity assumption.
- May improve performance of prognostic models when it creates groups with similar biological features.
- Offers a simple risk classification into high *vs* low.
- May facilitate treatment recommendations.
- Helps diagnostic criteria and decision-making.

Cons

- Information is lost (failing to account for variance) and the statistical power to detect a relationship between a variable and a patient outcome is consequently reduced.
- Does not make use of the within-category information: everyone above or beyond the cut-point is treated as equal.
- Increases the risk of a false positive result (Austin & Brunner, 2004).
- There is an underestimation of the extent of variation in outcome between groups as individuals close to but at opposite sides of the cut-point are characterised as being very different rather than very similar.
- Using two group masks any non-linearity in the relationship between the variables and outcome.
- If a dichotomisation is used cut-point may vary.

Perhaps central to these considerations is to think of the research question and properties of the deficit itself. For instance, a continuous variable is likely to have more sensitivity than a discrete outcome given that a continuous scale enables the perception of subtle change. At the same time, categorising certain variables might be beneficial especially for novel studies in which a certain

level of classification is needed. The median split of PMCs into 'late' and 'early' was an approach taken in this thesis. While the primary purpose was for the data to be blinded so that I could perform the analysis, it is important to recognize that expected age at onset is not an exact number and consequently, some individuals may be closer to onset than expected, whilst others will be further from it. This is problematic when splitting the groups into 'early' *vs* 'late' as some individuals may be in the incorrect category. Chapter 5, presented a possible solution to this problem whereby individuals were monitored over time and some 'converters' identified. Nonetheless, while more precise, expected onset remains an approximation and it is possible that a compromise is to be made.

Assessment of cognitive functions has traditionally used summary measures from standardised batteries, overlooking rich sources of variability (e.g., effort levels, response strategies). Innovative and integrative research using techniques such as eye-tracking, may offer novel ways of interpreting 'impairment' by quantifying this heterogeneity in 'cognitive effort' at an individual level. The next section discusses this theme in more detail.

8.5. Innovation within neuropsychology

Innovation within the field of dementia is paramount especially with regards to diagnosis and effective treatment. Research increasingly shows that integrative and interdisciplinary approaches are needed to capture experiences of those living with or at-risk of dementia (Kivipelto et al., 2018). Equally important, is the need for paradigms to focus on early disease detection given that the two main contributions of failures to interventional trials in AD are a) application of interventions too late in the disease process and b) lack of translatable outcomes from animal models to clinical trials in patient populations (Howett et al., 2019; Mehta et al., 2017). One example of a technique with ecological validity is immersive virtual reality (iVR) where participants navigate by real-world walking within simulated environments (e.g. (Howett et al., 2019)). The navigation system in the brain overlaps substantially with the regions affected by AD pathology in both animal models and humans (Coughlan et al., 2018). A recent virtual reality navigation task entorhinal cortex-based, differentiated patients with MCI from healthy controls, with a classification accuracy superior to reference cognitive tests considered to be highly sensitive to early AD (Howett et al., 2019).

At the same time, ecologically valid measures should be meaningful and translatable to a clinical and research setting. An example of a somewhat innovative technique in this thesis was eye-tracking mentioned in Chapter 6. Unlike traditional neuropsychology tasks which are susceptible to inter-rater and intra-rater reliability, eye-tracking allows for this reliability to be improved. It provide simplicity in use, enables large data collection (with high temporal and spatial resolution) and can be acquired without explicit reports or other overt responses (Pereira et al., 2014) (for a review on the promises and challenges for future eye-tracking application see: (Brunyé et al., 2019)). Nonetheless, eye movements can be variable and a balance is to be made between their study in naturalistic scenes which can yield very complex outcomes (Mengoudi et al., 2020) often ill-suited to predictions and their study in lower saliency experiments in which the first fixations are highly informative and predictable (Pertzov et al., 2009, 2012). One possibility around this is to create paradigms which are highly constrained semantically and perceptually (Shakespeare et al., 2013). “What was where?”, employed abstract and thus semantically meaningless stimuli, deviating from an ecologically valid and naturalistic approach, towards a controlled and constrained scenario. There are advantages to constrained paradigms which encourage ‘controlled learning’ as opposed to ‘uncontrolled learning’. For example uncontrolled learning results in variability in attentional resources and learning styles which may impact on memory performance and the ability of a test to capture underlying cognitive deficits (Loewenstein et al., 2018; Salmon & Bondi, 2009). These characteristics may have contributed to detecting subtle differences in viewing behaviour between groups in the “What was where?” task in Chapter 6. However, as previously mentioned there are strengths and limitations to every approach and this will be further discussed in the next section.

8.6. Strengths and limitations

The studies presented in this thesis had a number of strengths including: the generational effects on survival observed in a historical sample, the longitudinal follow-up data available (for the VSTM task), the use of a novel approach like eye-tracking in a rare condition like FAD; and the age-homogenous large Insight 46 sample with available demographic data from birth.

I will first discuss limitations inherent to the design, technique and data available which restricted the generalisability of findings and secondly, I will consider limitations to the interpretations of findings arising from conceptual outstanding questions in the field.

8.6.1. Representativeness of findings ('empirical limitations')

8.6.1.1. Insight 46

The very small age-range allowed for investigations into preclinical AD while disentangling disease from healthy-ageing effects (i.e. increasing brain pathologies and neurodegeneration (Jack et al., 2014; Parnetti et al., 2019) and decline in most cognitive abilities (Glisky, 2007). However, the generalisability of findings relies on the extent to which the sample is representative of the population.

A comparison with census data when study members were aged 43 concluded that the cohort remained broadly representative of the UK population of British-born adults of the same age (Wadsworth et al., 1992). A similar comparison at ages 60-64 concluded that the cohort was representative in terms of socio-economic position and rates of unemployment, although they were more likely to own a home and less likely to have limiting illness (Stafford et al., 2013). Notably, there may be a bias for healthier study members to still be alive and participating in the cohort. Yet, previous studies have recorded that only 15% of the NSHD participants had no clinical disorders at age 60-64 (based on a list of 15 disorders e.g. cancer, hypertension, diabetes) with an average of 2 disorders each (Pierce et al., 2012). As a native-born cohort reflecting the general British post-war population, all Insight 46 participants were white, and had been reported to have higher childhood cognitive ability and higher education attainment, than those no longer active (Kuh et al., 2016; Richards et al., 2019). Taken together these aspects limit generalisability to more contemporary ethnic and culturally diverse populations.

One of the main limitations for the Insight 46 SCD study, was the lack of imaging data such as tau-PET. As standard criteria for preclinical AD are based on the presence of both Aβ and tau pathology (see section 1.2.1), it was not possible to identify participants who met criteria for preclinical AD, nor to investigate how Aβ and tau pathology may interact to affect symptoms of SCD. The evidence of SCD and tau pathology is mixed. Some studies do not provide evidence that plasma tau is increased in individuals with symptoms of SCD (Müller et al., 2017) while others suggest some aspects of SCD relate to amyloid (e.g. everyday memory, everyday language, everyday organisation) and others to tau (e.g. everyday visuo-spatial, everyday planning) together with age and/or sex (Shokouhi et al., 2019). Based on previous reports, around 30% of Aβ+ 70-year-olds would be expected to have tau pathology (Jack et al., 2017a; Kern et al., 2018), and tau pathology will be present in some Aβ- individuals as well (around 15-20% (Jack et al., 2017b)).

Hence associations between Aβ and symptoms of SCD reported in this thesis may be partially explained by tau pathology.

8.6.1.2. FAD

Whilst limitations specific to the various FAD studies presented in this thesis are discussed in the relevant chapters, some overarching limitations relating to the representativeness of findings are discussed next.

With a relatively small sample size, due to the low prevalence of the condition and the limited window available to recruit, and the cognitive and clinical heterogeneity described, studies across multiple centres are required to address these investigations further. Still, the work presented in this thesis represents an important starting point and the novel techniques and statistical methods may merit additional exploration. Whilst parental AAO has been shown to correlate closely with actual AAO, and to closely relate to other methods of estimating disease, this remains a proxy measure only and is a key limitation to studying disease progression in FAD (Ryman et al., 2014). Yet, estimating actual AAO may also be subjective and challenging especially for atypical presentations (Pavisic et al., 2020a; Ryan et al., 2016) and a compromise may need to be found between statistical rigor and approximation, especially in a race for treatment.

Similar to Insight 46, the cohort presented here lacks variability in cultural background and ethnicity and race have been suggested to affect survival in SAD (Helzner et al., 2008). There is an urgent need to address this in AD research alongside effects of education and access to health care. Although anecdotal, PMCs have often shown lower education levels compared to non-carriers in our cohort. This might suggest that either symptoms of SCD, affective symptoms, subtle objective cognitive changes or a combination of all, may be causing early drop outs in education.

There are also limitations intrinsic to the study design. For example, only one study presented longitudinal data. The main reason this was done for the VSTM study, other than the increased time it would have required to collect data for the other studies too, is that there was prior evidence of presymptomatic sensitivity in FAD for this task. The retrospective survival study in Chapter 4 involved individuals born over a range of 100 years and there are inherent limitations of retrospective studies. These include: the lack of a comparison control group; the 'historical threat' (e.g. introduction of antibiotics in the 1930s and its effect on survival); what is known as 'maturation threat' (e.g. individuals living until older ages had higher chances of comorbidities

which may have influenced the outcome) and the 'social interaction threat' (i.e. the inability to account for effects of 'social interactions' or life-style on the outcome) (Tofthagen, 2012; Trochim, 2005). Nevertheless, a retrospective study design is helpful in increasing sample size even when the prevalence of a condition is relatively low, and in guiding the development of future prospective studies. Some research even suggests a benefit in including historical data in the analysis of clinical trials (van Rosmalen et al., 2018).

The lack of imaging outcomes also represented a limitation for FAD investigations. VSTM impairments have previously been associated with hippocampal volume (Liang et al., 2016) and a longitudinal analysis would have been interesting. In addition, a recent study by Norton and colleagues (Norton et al., 2020) looking at VSTM performance of PMCs (on average 11.5 years to expected onset) in relation to tau and amyloid (measured by PET) showed that VSTM performance strongly correlated with tau in entorhinal cortex and inferior temporal lobe, and with amyloid. Interestingly the non-binding "shape only" condition showed a stronger relationship to tau than the 'binding' condition emphasising the need for investigations of VSTM function with additional biomarkers. Nonetheless, clinical and cognitive aspects of neurodegenerative disease like FAD are already complex and variable and having a more direct focus on them seemed appropriate for this PhD. Furthermore, imaging is often invasive and expensive and exploring other avenues without these limitations was also of interest.

8.6.2. Conceptual reflections ('conceptual limitations')

8.6.2.1. FAD and SAD

Broadly speaking, the temporal order and progression of pathophysiological, cognitive and clinical changes are thought to be shared in FAD and SAD (Ryman et al., 2014). However, comparative studies between SAD (early and late onset together) and FAD have showed a number of differences including: earlier ages at onset and a more aggressive course for FAD, comorbidity prevalence (with cerebrovascular disease, argyrophilic grain disease, hippocampal sclerosis) and TAR DNA-binding protein-43 (TDP-43) proteinopathy present in SAD and absent in FAD as well as an Aβ load and tau pathology more severe in FAD (Cairns et al., 2015). Without a precise understanding of the biochemical distinctions between the different FAD mutations and SAD, it may be difficult to accurately interpret, compare, or broadly extrapolate outcomes of clinical trials. Some authors suggest that a comprehensive classification is required and have suggested to split

1) SAD, 2) AβPP dementias and 3) presenilin dementias in to distinct entities (Roher et al., 2016). They argue that the separation of these three 'neurodegenerative entities' will open opportunities for a better understanding of their pathophysiology and better-designed therapeutic intervention (e.g. personalised treatment) (Roher et al., 2016). Regardless of whether this should be a 'tactic' to follow, investigations in FAD with larger sample sizes are still lacking and an approach taken by many research groups, which increases sample size, is to investigate structures in the brain which are, commonly affected by AD pathology at some stage in all 'neurodegenerative entities'. A popular candidate is the hippocampus.

8.6.2.2. The role of the hippocampus

While the hippocampus is one of the most studied neuronal systems in the brain (Andersen et al., 2007), its role is still a widely debated topic. A number of theories around its function have been proposed: the declarative theory (Squire, 1986; Squire et al., 2004); multiple trace theory (Hardt et al., 2013); dual-process theory (Aggleton & Brown, 1999; Eichenbaum et al., 2007) and two others which are perhaps most relevant to this thesis: relational theory (Cohen et al., 1997; Eichenbaum & Cohen, 2001), and cognitive map theory (John O'Keefe & Nadel, 1978). In brief, the relational theory (Cohen et al., 1997; Eichenbaum & Cohen, 2001), states that the hippocampus is required to associate perceptually and conceptually distinct items that could not otherwise communicate (Bird & Burgess, 2008). This enables the relations between elements of a scene or event to be retrieved or used in novel situations, in addition to retrieval of the elements themselves. The cognitive map theory (John O'Keefe & Nadel, 1978), states that the role of the hippocampus is to construct and store allocentric representations of locations in the environment to aid flexible navigation (i.e. from a new starting position).

To date, it is still debated whether tasks involving object-location associations (e.g.(Olson et al., 2006)) engage the active maintenance of information in STM, or whether the information must be stored and subsequently recollected after a brief delay (Bird & Burgess, 2008). AD progression has also been extensively debated and studied with some authors arguing AD seems to progress in two stages, a sub-hippocampal and a hippocampal stage (Didic et al., 2011; Parra, 2017) based on the Braak staging of neurofibrillary changes for typical AD (Braak & Braak, 1991); and others claim this may not necessarily be the case (Liang et al., 2016, 2017). Structural and/or functional measurements of the hippocampus and sub-hippocampal structures made serially along with

cognitive tests including both recognition and associative memory tasks are needed to investigate this further. A number of factors are also likely to play a role in these conclusions including the stimuli and the experimental contexts. This will be further discussed in the next section with a primary focus on how eye-tracking may help to unveil this.

8.6.2.3. The hippocampus and viewing behaviour

Viewing behaviour varies across categories of stimuli and across different experimental contexts. fMRI studies show the activation of the hippocampus is *"dependent upon the type and complexity of the information presented in the stimuli being encoded"* (Stern et al., 1996). Memory effects in the hippocampus are greater when complex graphical information is used (e.g. pairs of objects and scenes) compared to more simplistic graphical information (e.g. single objects) (Kim, 2011). It is believed this pattern reflects the greater associative memory demands required of stimuli of higher complexity and greater amounts of exploratory viewing associated (Voss et al., 2017). An important outstanding question in the field is whether the hippocampus has a direct role in driving viewing behaviour or its association with viewing is a mere 'by-product' of its role in memory. In this regard, it is relevant to highlight that viewing behaviour has been linked to memory formation processes with exploratory viewing enhancing subsequent memory (Bridge et al., 2017; Henderson et al., 2005) and visual exploration impairments to hippocampal damage (Olsen et al., 2016). Research establishing whether cognitive variables per se or their effects on viewing behaviour are influencing hippocampal activity and memory is still needed (Voss et al., 2017).

I next provide a summary of potential challenges that viewing behaviour poses for the interpretation of memory experiments. These arguments are based on Voss and colleagues' opinion article (Voss et al., 2017) with additional points relevant to this thesis.

1. Intentional remembering and forgetting: Intentional remembering increases memory, hippocampal activity *and* viewing behaviour (Shih et al., 2012). Hence, influences of intentional remembering on memory may not be separated from the effects of intentionality on viewing behaviour. Conversely, intentional forgetting decreases memory and reduces hippocampal activity (Hulbert et al., 2016) but investigations on whether intentional forgetting changes viewing behaviour are lacking. This thesis provides novel evidence that forgetting caused by AD pathology is associated with change in viewing behaviour for presymptomatic and symptomatic FAD

carriers. However, effects of intentional *vs* incidental instructions on performance were not tested here.

2. Attention: Although attention shares functional neuroanatomy with oculomotor control (Corbetta et al., 1998), the extent to which attention effects on memory and hippocampal activity are due to viewing behaviour is unclear. This is particularly relevant as attention prioritization may differ from viewing behaviour (i.e. covert attention) (Voss et al., 2017). For instance while overt visual attention should be captured by tracking eye movements (Holmqvist et al., 2011), covert attention allows the eyes to fixate on a feature while covertly attending to another (Treisman & Gelade, 1980). Although eye-tracking provides valuable insight into the distribution of visual attention over a scene, this is restricted to monitoring foveal vision (Brunyé et al., 2019). This point is particularly important when considering the impact of visual exploration strategies on VSTM performance for symptomatic FAD carriers in the eye-tracking experiment.

3. Scene and/or spatial cognition: The stimulus type and complexity (e.g. scenes and spatial environments *vs* non-scenes and non-spatial stimulus) may influence both the viewing behaviour and relational demands (Bender et al., 2017; Eichenbaum, 2017). For instance, stimuli with greater visual exploration are likely to be better remembered, and this is especially true for stimuli of relatively higher complexity. While all participants viewed the same stimuli in the same order, this consideration limits the generalisability of findings.

4. Ageing: Age-related differences in viewing patterns may predict memory performance (Chan et al., 2011; Shih et al., 2012). The extent to which these viewing changes contribute to hippocampal activity is unknown. While there was no significant difference in PMCs and controls, symptomatic carriers were older and this difference in age could have affected results. Nonetheless, all models were adjusted for age.

8.7. Future directions

The work presented in this thesis can take a number of future directions. The 'FAD stages' presented towards the end of Chapter 4 may represent a starting point to investigate the functional and cognitive changes of individuals at-risk of or affected by FAD. This work will be part of the Rare Dementia Support Impact Project (Brotherhood et al., 2020) and will entail in person interviews over the coming 5 years.

With regards to the presymptomatic investigations, models of cognitive trajectories incorporating some of the novel cognitive tasks presented here as well as the other traditional neuropsychology assessments will allow examination of the relationship between timing of the cognitive deficit and disease progression. One example has recently been published by my colleague Dr Antoinette O'Connor (see **PUBLICATIONS**). Importantly, longitudinal investigations combining fMRI with VSTM binding experiments (with both relational and conjunctive paradigms) and eye-tracking are paramount if we are to better understand which areas of the brain are activated and at what time from preclinical to symptomatic disease.

Longitudinal assessments of Insight 46 participants are already on the way and will allow to investigate whether Aβ pathology and symptoms of SCD at baseline are predictive of relatively poorer cognition at follow-up and subsequent risk of dementia. Interestingly, "amyloid accumulators" – individuals whose levels of Aβ are rising from an initially normal level – may be a particularly important group for identifying the earliest changes in cognition and a suitable target group for future clinical trials (McMillan & Chételat, 2018).

Investigation of a broader range of biomarkers is also planned for both Insight 46 (e.g. *APOE* ε4, and measures of Aβ and tau pathology in CSF) and FAD studies (e.g. $A\beta_{38,}$ $A\beta_{40}$ and $A\beta_{42}$).

Looking further ahead, approximately one third of Insight 46, and one third of FAD participants have agreed to post-mortem brain donation. This will allow for investigations of pathologies and their relationship with cognition during life.

8.8. Closing summary

The work presented in this thesis add to the growing body of evidence that subtle cognitive decline is associated with preclinical AD pathology. Specifically, it provides novel evidence that viewing behaviour may explain some of the memory differences observed at symptomatic and presymptomatic stages of FAD and in doing so shows how probing memory processes via eye-tracking may increase the sensitivity of relational binding tasks to preclinical AD. The conceptualization of relational binding in a continuous spectrum of accuracy is also novel and accords with more recent models of working memory – specifically resource models – in which the *quality* or *resolution* of a memory representation is measured.

Taken together these results have implications for: survival estimations in FAD, the interpretation of memory impairments in presymptomatic FAD (at a screening level but also in relation to cognitive decline) and for the understanding of subjective cognition in preclinical and presymptomatic FAD. This work was carried out with the overarching aim of providing a better understanding of the disease to individuals affected and their families, to help inform choices of cognitive outcomes for clinical trials, and in doing so perhaps provide new avenues to target this devastating disease.

STATEMENT OF ATTRIBUTIONS

Chapter 4

I conceived and designed this study with advice from Dr Natalie Ryan and Professor Nick Fox. Clinical data were collected by Helen Rice and Drs Natalie Ryan and Antoinette O'Connor. Information on clinical features, symptom onset and date of passing where collected by Dr Natalie Ryan, Helen Rice and me from clinical notes or conversation with participants. Statistical analysis was performed by me with advice from Dr Jennifer Nicholas and I interpretated results.

Chapter 5

The "What was where?" task was designed by Dr Yoni Pertzov and Professor Masud Husain. The study was conceived and designed by Dr Yuying Liang, with advice from Professors Sebastian Crutch and Nick Fox. Dr Jennifer Nicholas performed the statistical analysis. Neuropsychology data was collected by Jessica Collins and me. Clinical data was collected by Drs Yuying Liang, Antoinette O'Connor, Philip Weston, Natalie Ryan and Professor Nick Fox. I interpretated results with advice from Drs Yoni Pertzov and Jennifer Nicholas and Professor Sebastian Crutch.

Chapter 6

I conceived and designed the eye-tracking study with advice from Dr Yoni Pertzov and Professor Sebastian Crutch. "What was where?" was adapted into an eye-tracking experiment format by me with advice from Drs Yoni Pertzov and Kurt Debono (a research support specialist from SR Research, EyeLink). Neuropsychology data was collected by Jessica Collins and me, clinical data by Dr Antoinette O'Connor and Helen Rice and eye-racking data by me. Dr Jennifer Nicholas anonymized the data for analysis separating groups by clinical status and median split, I performed the analysis and interpretation of results with advice from Dr Jennifer Nicholas.

Chapter 7

Insight 46 was conceived and planned by Professors Jonathan Schott, Nick Fox, Marcus Richards and Diana Kuh. Dr Christopher Lane, Dr Thomas Parker, Dr David Cash, Elizabeth Donnachie, Heidi Murray-Smith, Suzie Barker and Dr Michelle Byford were instrumental in designing the study

protocol and preparing the ethics application. The cognitive battery was designed by Professors Sebastian Crutch and Marcus Richards. The imaging protocol and processing pipelines were developed by Drs David Cash, Ian Malone, Marc Modat, Carole Sudre, David Thomas, Gary Zhang, Anna Barnes, John Dickson, and Professor Sebastien Ourselin. Recruitment and clinical assessments were performed by Drs Christopher Lane, Thomas Parker, Ashvini Keshavan, Sarah Buchanan and Sarah Keuss. Co-ordination and booking of participants' travel and accommodation was performed by Heidi Murray-Smith, Claudia Cramer, Molly Cooper, Elizabeth Burgnon, Jessica Collins and Dr Kirsty Lu. Neuropsychology assessments were performed by Dr Kirsty Lu, Jessica Collins, Dr Sarah James, Elizabeth Donnachie, Hannah Carr, Rebecca Street and me. Jana Klimova and Will Coath performed QC of volumetric T1, T2 and FLAIR images. Drs Ian Malone and Elizabeth Gordon managed the volumetric pipeline that generated whole brain volume and hippocampal volumes and were responsible for manual editing. The BaMoS pipeline was run by Dr Carole Sudre and BaMoS QC and manual editing was performed by Dr Christopher Lane as required. β-amyloid PET processing, imputation work and determination of the cut-point for positivity was performed by Dr David Cash. Andrew Wong and Heidi Murray-Smith coordinated the processing of *APOE* genotyping performed by LGC Hoddesdon on blood samples collected by Drs Christopher Lane, Thomas Parker, Ashvini Keshavan, Sarah Buchanan and Sarah Keuss. Processing of computerised cognitive tests was performed by Dr Kirsty Lu. Extraction of outcome variables, design of statistical models, statistical analysis and interpretation of results was performed by me with statistical advice from Dr. Kirsty Lu.

The sub-study of SCD on the FAD was conceived and design by me, with advice from Professors Sebastian Crutch and Nick Fox. Data was collected by Dr Antoinette O'Connor and Helen Rice. Dr Jennifer Nicholas anonymized the data for analysis separating groups by clinical status and median split. I performed the analysis and interpretation of results.

PUBLICATIONS

Publications that have arisen to date as a result of the work in this thesis:

Chapter 4

Pavisic, I.M., Nicholas, J.M., O'Connor, A., Rice, H., Lu, K., Fox, N.C., & Ryan, N.S. (2020). Disease duration in autosomal dominant familial Alzheimer disease. *Neurology Genetics*, 6 (5), e507. doi:10.1212/nxg.0000000000000507

Chapter 5

O'Connor A., Weston P.S.J., **Pavisic I.M.**, Ryan N.S., Collins J.D., Lu K., Crutch S.J., Alexander D.C., Fox N.C., Oxtoby N.P. (2020). Quantitative detection and staging of presymptomatic cognitive decline in familial Alzheimer's disease: a retrospective cohort analysis. *Alzheimer's Research and Therapy*, 12 (1):126. doi:10.1186/s13195-020-00695-2

Chapter 6

Pavisic I.M., Pertzov Y., Nicholas J.M., O'Connor A., Lu K., Yong K.X.X., Husain M., Fox N.C., Crutch S.J. (2021). Eye-tracking indices of impaired encoding of visual short-term memory in familial Alzheimer's disease. *Scientific Reports*. doi: 10.1038/s41598-021-88001-4

Chapter 7

Pavisic I.M., Lu K., Keuss S.E., James S-N., Lane C.A., Parker T.D., Keshavan A., Buchanan S.M., Murray-Smith H., Cash D.M., Coath W., Wong A., Fox N.C., Crutch S.J., Richards M., Schott J.M. (2021). Subjective cognitive complaints at age 70: Associations with amyloid and mental health. *Journal of Neurology, Neurosurgery and, Psychiatry*. Epub [ahead of print: 26/05/2021]. doi:10.1136/jnnp-2020-325620.

Chapter 8

Pavisic, I.M., Suarez-Gonzalez, A., & Pertzov, Y. (2020). Translating Visual Short-Term Memory Binding Tasks to Clinical Practice: From Theory to Practice. *Frontiers in Neurology*, 11:458. doi:10.3389/fneur.2020.00458

APPENDICES

Appendix 1 The Clinical Dementia Rating Scale scoring structure.

Categories	None: 0	Questionable: 0.5	Mild: 1	Moderate: 2	Severe: 3
Memory	No memory loss or slight inconsistent forgetfulness	Consistent slight forgetfulness, partial recollection of events, "benign" forgetfulness	Moderate memory loss; more marked for recent events; defect interferes with everyday activities	Severe memory loss; only highly learned material retained; new material rapidly lost	Severe memory less; only fragments remain
Orientation	Fully oriented	Fully oriented except for slight difficulty with time relationships	Moderate difficulty with time relationships; oriented for place at examination; may have geographic disorientation elsewhere.	Severe difficulty with time relationships; usually disoriented to time, often to place	Oriented to person only
Judgment and problem solving	Solves everyday problems and handles business and financial affairs, well; judgment good in relation to past performance	Slight impairment in solving problems, similarities, and differences	Moderate difficulties in handling problems, similarities, and differences; social judgment usually maintained	Severely impaired in handling problems, similarities, and differences; social judgment usually impaired	Unable to make judgements or solve problems
Community affairs	Independent function at usual level in job, shopping, and volunteer and social groups	Slight impairment in these activities	Unable to function independently at these activities although may still be engaged in some; appears normal to casual inspection	No pretense of independent function outside home. Appears well enough to be taken to function outside a family home	No pretense of independent function outside home. Appears too ill to be taken to function outside a family home
Home and hobbies	Life at home, hobbies, and intellectual interests are well maintained	Life at home, hobbies, and intellectual interests slightly impaired	Mild but definite impairment in function at home, more difficult chores abandoned, more complicated hobbies and interests abandoned	Only simple chores preserved; very restricted interests, poorly maintained	No significant function at home
Personal care	Fully capable of self-care	Fully capable of self-care	Needs prompting	Requires assistance in dressing, hygiene, keeping of personal effects	Requires much help with personal care; frequent incontinence

Appendix 2 The original 'Seven stages of Alzheimer's disease' as described for individuals with the typical sporadic presentation of AD

Stage 1: No impairment (normal function)

The person does not experience any memory problems. An interview with a medical professional does not show any evidence of symptoms of dementia.

Stage 2: Very mild cognitive decline

The person may feel as if he or she is having memory lapses — forgetting familiar words or the location of everyday objects. But no symptoms of dementia can be detected during a medical examination or by friends, family or co-workers. This stage may reflect normal age-related changes or the earliest signs of Alzheimer's disease.

Stage 3: Mild cognitive decline (Early-stage Alzheimer's can be diagnosed in some, but not all, individuals with these symptoms)

Friends, family or co-workers begin to notice difficulties. During a detailed medical interview, doctors may be able to detect problems in memory or concentration. Common stage 3 difficulties include:

-Noticeable problems coming up with the right word or name;
-Trouble remembering names when introduced to new people
-Having noticeably greater difficulty performing tasks in social or work settings
-Forgetting material that one has just read
-Losing or misplacing a valuable object
-Increasing trouble with planning or organizing

Stage 4: Moderate cognitive decline (Mild or early-stage Alzheimer's disease)

At this point, a careful medical interview should be able to detect clear-cut symptoms in several areas such as:

-Forgetfulness of recent events
-Impaired ability to perform challenging mental arithmetic for example, counting backward from 100 by 7s
-Greater difficulty performing complex tasks, such as planning dinner for guests, paying bills or managing finances
-Forgetfulness about one's own personal history
-Becoming moody or withdrawn, especially in socially or mentally challenging situations.

Stage 5: Moderately severe cognitive decline (Moderate or mid-stage Alzheimer's disease)

Gaps in memory and thinking are noticeable, and individuals begin to need help with day-today activities. At this stage, those with Alzheimer's disease may:

-Be unable to recall their own address or telephone number or the high school or college from which they graduated;

-Become confused about where they are or what day it is;

-Have trouble with less challenging mental arithmetic; such as counting backward from 40 by subtracting 4s or from 20 by 2s

-Need help choosing proper clothing for the season or the occasion

-Still remember significant details about themselves and their family

-Still require no assistance with eating or using the toilet.

Stage 6: Severe cognitive decline (Moderately severe or mid-stage Alzheimer's disease)

Memory continues to worsen, personality changes may take place and individuals need extensive help with daily activities. At this stage, individuals may:

-Lose awareness of recent experiences as well as of their surroundings

-Remember their own name but have difficulty with their personal history

-Distinguish familiar and unfamiliar faces but have trouble remembering the name of a spouse or caregiver

-Need help dressing properly and may, without supervision, make mistakes such as putting pyjamas over daytime clothes or shoes on the wrong feet

-Experience major changes in sleep patterns

-Need help handling details of toileting (for example, flushing the toilet, wiping or disposing of tissue properly)

-Have increasingly frequent trouble controlling their bladder or bowels

-Experience major personality and behavioural changes, including suspiciousness and delusions (such as believing that their caregiver is an impostor) or compulsive, repetitive behaviour like hand-wringing or tissue shredding. The person may also repetitively articulate certain words or sounds

-Tend to wander or become lost.

Stage 7: Very severe cognitive decline (Severe or late-stage Alzheimer's disease)

In the final stage of this disease, individuals lose the ability to respond to their environment, to carry on a conversation and, eventually, to control movement. They may still say words or phrases. At this stage, individuals need help with much of their daily personal care, including eating or using the toilet. They may also lose the ability to smile, to sit without support and to

hold their heads up; reflexes become abnormal; muscles grow rigid; swallowing is impaired; maintaining adequate nutrition, hydration and skin integrity can be an issue at this stage.

End of life

Although Alzheimer's disease and other degenerative diseases are life shortening illnesses, another condition or illness [such as pneumonia] may actually cause the person's death. Pneumonia is listed as the cause of death in up to two thirds of people with dementia. The person's ability to cope with infections and other physical problems will be impaired due to the progression of the disease. In some people no specific cause of death is found, other than Alzheimer's disease. Depending on the circumstances, 'Alzheimer's disease' or similar may be entered on the death certificate as the sole or main cause of death, or as a contributing factor.

Appendix 3 Syndromal staging of cognitive continuum: Applicable to all members of a research cohort independent from biomarker profiles

Cognitively unimpaired

Cognitive performance within expected range for that individual based on all available information. This may be based on clinical judgment and/or on cognitive test performance (which may or may not be based on comparison to normative data, with or without adjustments for age, education, occupation, sex, etc.).

Cognitive performance may be in the impaired/abnormal range based on population norms, but performance is within the range expected for that individual.

A subset of cognitively unimpaired individuals may report subjective cognitive decline and/or demonstrate subtle decline on serial cognitive testing.

Mild cognitive impairment

Cognitive performance below expected range for that individual based on all available information. This may be based on clinical judgment and/ or on cognitive test performance (which may or may not be based on comparison to normative data with or without adjustments for age, education, occupation, sex, etc.).

Cognitive performance is usually in the impaired/abnormal range based on population norms, but this is not required as long as the performance is below the range expected for that individual.

In addition to evidence of cognitive impairment, evidence of decline in cognitive performance from baseline must also be present. This may be reported by the individual or by an observer (e.g., study partner) or observed by change on longitudinal cognitive testing/behavioural assessments or by a combination of these.

May be characterized by cognitive presentations that are not primarily amnestic-*
Although cognitive impairment is the core clinical criteria, neurobehavioral disturbance may be a prominent feature of the clinical presentation**

Performs daily life activities independently, but cognitive difficulty may result in detectable but mild functional impact on the more complex activities of daily life, either self-reported or corroborated by a study partner.

Dementia

Substantial progressive cognitive impairment that affects several domains and/or neurobehavioral symptoms. May be reported by the individual or by an observer (e.g., study partner) or observed by change on longitudinal cognitive testing.

Cognitive impairment and/or neurobehavioral symptoms result in clearly evident functional impact on daily life. No longer fully independent/requires assistance with daily life activities. This is the primary feature differentiating dementia from MCI.

May be subdivided into mild, moderate, and severe

MCI, mild cognitive impairment. *For MCI and dementia: Cognitive impairment may be characterized by presentations that are not primarily amnestic. **Tor MCI and dementia: Although cognition is the core feature, neurobehavioral changes—for example, changes in mood, anxiety, or motivation—commonly coexist and may be a prominent part of the presentation.

Appendix 4 Fractals used in the “What was where” task

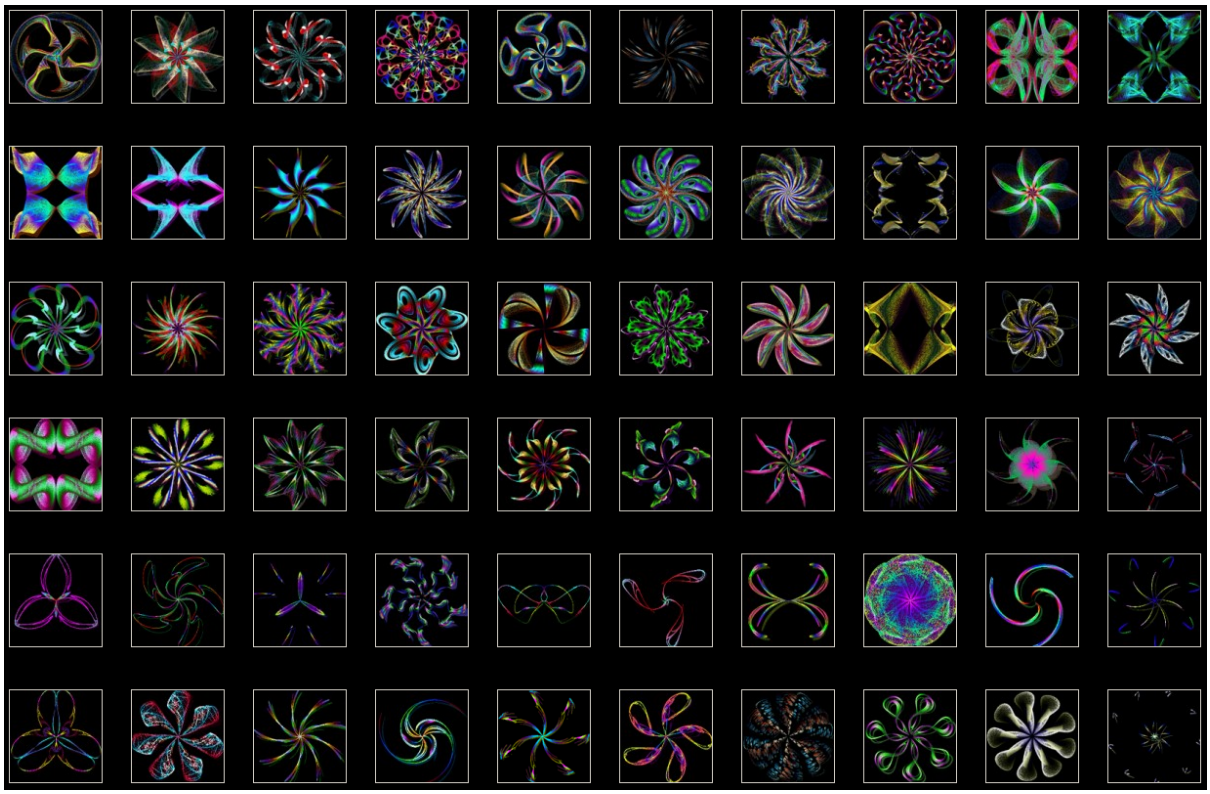


Figure reprinted from (Liang et al. 2016) under the terms of the Creative Commons Attribution License (CC BY).

Appendix 5 MyCog questionnaire

The **Subjective Cognitive Decline** questions are below, with variable names shown in red. All are Yes/No questions, apart from the onset question, which requires the age to be entered as an integer between 20 and 100, or entered as “unknown”.

Do you perceive memory or cognitive difficulties? (difficulties)

In the last two years, has your cognition or memory declined? (twoyears)

If yes, do you perceive memory or cognitive difficulties more than other people the same age? (peers)

At what age did these start? (onset)

Would you ask a doctor about these difficulties? (doctor)

Would you like the letter to your GP to report these difficulties that you have mentioned? (report)

The **MyCog** questions are below. All are Yes/No questions, apart from the total score, which is out of 24. Participants are instructed to answer YES if they believe they perform these activities WORSE than roughly two years ago (Rami et al., 2014).

I find it harder to learn new telephone numbers. (phone)

I find it harder to find personal possessions (keys, telephone, utensils, etc.) (possessions)

I find it harder to describe the plots of films. (film)

I find it harder to remember doctor's appointments. (appointments)

I find it harder to follow the plot of a book. (book)

I'm worse at recalling the details of a recent family event. (family)

I find it harder to remember the result of a recent sporting event. (sport)

I find it harder to remember sums of money (payments or debts. (money)

I find it harder to remember the details of a conversation. (conversation)

I find it harder to remember things without using strategies (lists, diary, etc.. (strategies)

I find it harder to remember the details of recent news. (news)

I find it harder to remember famous people's names. (famous)

I find it harder to remember the names of people I've met recently. (acquaintance)

I find it harder to remember street and city names. (street)

I'm worse at finding the word I want to use in a conversation. (word)

I find it harder to understand things the first time someone says them. (time)

I find it harder to remember the names of places I've visited recently. (place)

I find it harder to concentrate on what I am doing. (concentration)

I'm worse at planning things that aren't part of my daily routine (travel, excursions, etc.. (planning)

I find it harder to use electronic devices. (devices)

I find it harder to start new or different things (different)

I find it harder to start conversations. (conversations)

I find it harder to do mental arithmetic. (arithmetic)

I find it harder to do more than one thing at once without getting agitated (multitasking)

Total Score (mycog_tot)

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