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BIOMARKERS IN PRECLINICAL FAMILIAL ALZHEIMER DISEASE

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Biomarkers in preclinical familial Alzheimer disease

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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Til fjölskyldunnar minnar

(To my family)

“For small creatures such as we the vastness is bearable only through love.”

Carl Sagan

American astronomer (1934-1996)

ABSTRACT

Background: Alzheimer disease (AD) is a neurodegenerative disorder, characterized by the accumulation of β-amyloid (Aβ) plaques and tangles consisting of hyperphosphorylated tau-protein in the brain. It accounts for 60-70% of dementia cases, making it the most common cause of dementia. In rare cases the disease is inherited in autosomal dominant early onset form caused by mutations in *APP*, *PSEN1* or *PSEN2*. These familial forms of AD (FAD) allow for studies of the long preclinical stage of the disease and may thereby address unanswered questions about the natural history of AD which can be used to develop optimal tools for early diagnosis and for monitoring treatment response, as well as finding new possible treatment targets. To this end we conducted a prospective study, involving repeated clinical evaluations and collection of biomarkers from asymptomatic carriers of mutations leading to FAD with non-carriers (NC) from the same families as controls. The asymptomatic mutation carriers (MC) are good representatives of the preclinical stage of AD as they will develop symptoms of the disease in the future at an age which can be estimated based on the age at symptom onset in their family members who have already become symptomatic.

Aims: To map biomarker changes in preclinical AD, as well as their temporal trajectories and sequence, through repeated collection and analysis of biomarkers in asymptomatic FAD MC and NC.

Results: There were significant differences in the levels of the cerebrospinal fluid (CSF) biomarkers Aβ42, total-tau protein (t-tau) and phosphorylated tau-protein (p-tau), as well as in the Aβ42/p-tau ratio when comparing MC to NC, more than 7 years before the expected onset of symptoms in the MC. Aβ42 and the Aβ42/p-tau ratio were lower in MC than NC, while t-tau and p-tau were higher in MC than NC. There was a trend of Aβ42 and the Aβ42/p-tau ratio decreasing as the onset of symptoms approached in MC, while t-tau and p-tau showed a trend of increasing with approaching symptom onset. On structural magnetic resonance imaging (MRI) of the brain, the MC had reduced volume of the left precuneus, left superior temporal gyrus and left fusiform gyrus, 9 years before the expected symptom onset. However, there was no observable decline in grey matter thickness or volume as the onset of symptoms approached, making the temporality of these changes difficult to assess. In the same group of subjects there was no significant difference on neuropsychological assessments between MC and NC, but a trend of poorer results was observed in the MC regarding immediate memory, episodic memory and attention/executive function. The CSF biomarkers YKL-40, reflecting glial activation, and neurogranin, a synaptic marker, were compared between asymptomatic MC and NC and found not to differ between the groups. A longitudinal study of changes in YKL-40 and neurogranin with approaching symptom onset was also conducted, revealing an increase in YKL-40 in both MC and NC as the age of symptom onset drew nearer, with a steeper increase in MC than NC. No such correlation to years to symptom onset was found for neurogranin. The APP processing products sAPPα, sAPPβ, Aβ42, Aβ40 and Aβ38 were compared both between the MC group as a whole and the NC and between subgroups of MC carrying specific mutations and the NC. The whole MC group had lower levels of Aβ42, Aβ40 and Aβ38, as well as a lower

Aβ42/Aβ40 ratio than NC. No significant correlation was observed between any of the aforementioned APP processing products and years to symptom onset in MC. When comparing different MC subgroups to each other, the whole MC group and the NC group, some mutation specific differences in the levels of the APP processing products and their temporality emerged. During the biomarker studies presented above the presence of a statistical outlier came to our attention, an MC carrying the *PSEN1* H163Y mutation who had passed the age at symptom onset in his family but displayed no cognitive decline and no abnormalities in CSF biomarkers. This individual had been followed-up within the FAD study for 22 years and had opted for a presymptomatic genetic test, making his mutation status known to him and to the researchers involved in the study. His clinical case was characterized in paper III, with his brother serving as a control. The brother was only one year older than the outlier but had already passed away from AD at the end of the follow-up time, having displayed typical signs and symptoms of the disease in the preceding years.

Conclusions: The study revealed early preclinical changes in CSF biomarkers, reflecting Aβ aggregation, glial activation, tau phosphorylation and neurodegeneration, as well as loss of volume in specific areas in the left hemisphere of the brain on structural MRI in asymptomatic carriers of FAD mutations. When assessing the temporality of specific biomarkers in the CSF, Aβ42 and the Aβ42/p-tau ratio seemed to decrease with approaching symptom onset, while t-tau and p-tau increased as symptom onset drew nearer. These results are based on cross-sectional data, but only longitudinal studies can properly assess temporal changes, as we did for CSF neurogranin and YKL-40 (with YKL-40 increasing at a faster rate in MC than in NC). However, the overall results give an important indication of the true nature of these preclinical temporal changes. We also observed mutation specific differences in APP processing products in the CSF and characterized a case of reduced penetrance of the *PSEN1* H163Y mutation. In conclusion, the study sheds light on preclinical biomarker changes in FAD and the possible sequence of these changes. It also emphasizes the differences in phenotype between specific FAD mutations and the presence of reduced penetrance which affects the estimation of symptom onset in these families and has an impact on genetic counseling and possibly on the design of clinical trials in this population.

LIST OF SCIENTIFIC PAPERS

I. **Thordardottir S**, Kinhult-Stahlbom A, Ferreira D, Almkvist O, Westman E, Zetterberg H, Eriksdotter M, Blennow K, Graff C. Preclinical Cerebrospinal Fluid and Volumetric Magnetic Resonance Imaging Biomarkers in Swedish Familial Alzheimer's Disease. *J Alzheimers Dis*. 2015; 43(4): 1393-1402.

II. **Thordardottir S**, Kinhult-Stahlbom A, Almkvist O, Thonberg H, Eriksdotter M, Zetterberg H, Blennow K, Graff C. The effects of different familial Alzheimer´s disease mutations on APP processing in vivo. *Alzheimers Res Ther*. 2017; 9: DOI: 10.1186/s13195-017-0234-1.

III. **Thordardottir S**, Rodriguez-Vieitez E, Almkvist O, Ferreira D, Saint-Aubert L, Kinhult-Stahlbom A, Thonberg H, Scholl M, Westman E, Wall A, Eriksdotter M, Zetterberg H, Blennow K, Nordberg A, Graff C. Reduced penetrance of the PSEN1 H163Y mutation: 22-years longitudinal follow-up of a symptom free mutation carrier. *Submitted*.

IV. **Thordardottir S**, Almkvist O, Zetterberg H, Blennow K, Graff C. CSF YKL-40 and neurogranin in preclinical familial Alzheimer disease. *In manuscript*.

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LIST OF ABBREVIATIONS

AD	Alzheimer disease
ADRDA	The Alzheimer´s Disease and Related Disorders Association
AICD	APP intracellular domain
API	Alzheimer's prevention inititative
APOE	Apolipoprotein E
APP	Amyloid precursor protein
BACE	β-site APP-cleaving enzyme
CAA	Cerebral amyloid angiopathy
CDR	Clinical Dementia Rating Scale
CERAD	The Consortium to Establish a Registry for Alzheimer Disease
CheI	Cholinesterase inhibitor
CI	Confidence interval
CNS	Central nervous system
CSF	Cerebrospinal fluid
CTF	C-terminal fragment
DED	[^{11}C]-deuterium-L-deprenyl
DIAN	Dominantly Inherited Alzheimer Network
DNA	Deoxyribonucleic acid
EEG	Electroencephalography
ELISA	Enzyme-linked immunosorbent assay
EOAD	Early onset Alzheimer disease
FAD	Familial Alzheimer disease
FDG	[^{18}F]fluorodeoxyglucose
FDR	False discovery rate
GWAS	Genome-wide association studies
IGAP	The International Genomics of Alzheimer's Project
LOAD	Late onset Alzheimer disease
LP	Lumbar puncture
MAO-B	Monoamine oxidase B
MC	Mutation carrier(s)

MCI	Mild cognitive impairment
MMSE	Mini-Mental State Examination
MRI	Magnetic resonance imaging
NC	Non-carrier(s)
NFT	Neurofibrillary tangle
NIA-AA	National Institute of Aging-Alzheimer´s Association
NIA-RI	The National Institute of Aging and the Reagan Institute
NINCDS	The National Institute of Neurological and Communicative Disorders and Stroke
NMDA	N-Methyl-D-Aspartate
NPA	Neuropsychological assessment
P-TAU	Phosphorylated tau
PCR	Polymerase chain reaction
PET	Positron Emission Tomography
PiB	[^{11}C]-Pittsburgh compound B
PSEN	Presenilin
RNA	Ribonucleic acid
ROI(s)	Region(s) of interest
SAD	Sporadic Alzheimer disease
SCI	Subjective cognitive impairment
SNP	Single nucleotide polymorphism
SUVr	Standardized uptake value ratio
T-TAU	Total tau protein

1 INTRODUCTION

1.1 ALZHEIMER DISEASE – AN OVERVIEW

1.1.1 A brief history

Alzheimer disease (AD) is a neurodegenerative disorder and the most common cause of dementia, accounting for 60-70% of dementia cases (1). The first clinical description of AD was made by Alois Alzheimer, a German psychiatrist and neuropathologist, working with Emil Kraepelin in Munich at the turn of the 20th century (2). In 1901 Alzheimer encountered a 51-year old patient, Auguste Deter, suffering from pre-senile dementia with progressive memory loss, confusion and paranoid delusions. He followed Mrs. Deter's clinical course until her death 5 years later, registering widespread cognitive decline with increasing apathy and aphasia. After Mrs. Deter's death, Alzheimer received her brain for histopathological examination and described the "plaques and tangles" that are now known to be the hallmarks of AD (3). In 1997, the tissue sections from Mrs. Deter were located at the Royal Psychiatric Clinic in Munich and re-examined. The tissue sections from the cerebral cortex, originally prepared according to the Bielschowsky method, revealed an abundance of neurofibrillary tangles and amyloid plaques in the upper cortical layers, exactly as previously described by Alzheimer (4).

1.1.2 Neuropathology

1.1.2.1 Amyloid plaques and neurofibrillary tangles

For decades, AD has been neuropathologically characterized by neurodegeneration with a loss of neurons and synapses leading to progressive brain atrophy. Microscopically, it is defined by the presence of amyloid plaques and neurofibrillary tangles (NFTs) in the brain tissue (5, 6). Amyloid plaques are primarily made of extracellular aggregates of Aβ42, an amyloid β peptide composed of 42 amino acids, and fall into two categories, diffuse plaques and dense-core/neuritic plaques (7-9). Neuritic plaques stain positively with Congo-red or Thioflavin-S and are associated with dystrophic neurites, glial activation and loss of synapses and neurons. They are implicated in the pathogenesis of AD, while diffuse plaques are commonly believed to be the product of normal ageing (10). NFTs are made of hyperphosphorylated tau-protein, a protein which under physiological circumstances stabilizes microtubules within the axons of nerve cells. Tau hyperphosphorylation leads to the formation of intracellular straight and paired helical tau filaments (NFTs), which interfere with the structure and function of the affected neurons (11-14). Apart from neuritic plaques and NFTs the majority of AD cases involve multiple other pathological changes such as the deposition of TDP-43 (an RNA binding protein also associated with amyotrophic lateral sclerosis and frontotemporal lobar degeneration) (15) and α synuclein (a presynaptic protein also associated with Parkinson disease) (16).

1.1.2.2 Spatiotemporal distribution of Alzheimer pathology based on post mortem studies

Cerebral amyloidosis is an early event in AD and is now believed to arise years or decades before the onset of clinical symptoms of the disease (17-19). Typically, the earliest amyloid

plaques detected at autopsy are localized in the association neocortex, more specifically in the basal areas of the frontal, temporal and occipital lobes. The plaque pathology is then detected in other neocortical areas, apart from the primary sensory, motor and visual areas which are usually spared until the advanced stages of the disease. With disease progression, plaques also accumulate in the allocortex, including the hippocampal and entorhinal cortices (20-22). The correlation between clinical symptoms and the accumulation and distribution of amyloid plaques is not strong (23-26). NFTs have a much closer concordance with symptoms of AD as they first appear in the transentorhinal region and then the CA1 area of the hippocampus, followed by other limbic structures, the association cortex and finally the rest of the neocortex. Braak and Braak proposed a staging of NFT pathology, with stage I-II involving the transentorhinal region, stage III-IV other limbic areas and stage V-VI the neocortex (20). Loss of synapses and neurons matches the distribution of NFTs, both in space and time, and has a strong correlation to clinical symptoms. The best correlate of cognitive decline is synaptic density with synaptic loss preceding the death of neurons (24, 26-28).

1.1.2.3 Neuropathological diagnostic criteria for Alzheimer disease

In 1991, the Consortium to Establish a Registry for Alzheimer Disease (CERAD), suggested diagnostic criteria for AD based on the burden of neuritic amyloid plaques. The CERAD criteria involve scoring the density of neuritic plaques in the most severely affected region of the frontal, temporal or parietal neocortex, adjusted for the age of the subject. After incorporating clinical information on the absence or presence of dementia, subjects can be divided into three categories; possible Alzheimer disease, probable Alzheimer disease or definite Alzheimer disease (29, 30). These criteria proved to have high sensitivity but low specificity, while the opposite was true of the Braak and Braak staging described above (31).

The National Institute of Aging and the Reagan Institute (NIA-RI) combined the CERAD and Braak and Braak criteria into consensus criteria in 1997 (32), excluding age as a factor in the neuropathological diagnosis (see table 1).

Neuropathological assessment of the likelihood that Alzheimer disease accounts for a dementia should be judged as follows:	
1. High likelihood	CERAD definite, Braak and Braak V/VI
2. Intermediate likelihood	CERAD probable, Braak and Braak III/IV
3. Low likelihood	CERAD possible, Braak and Braak I/II

Table 1. The NIA-RI criteria for the neuropathological diagnosis of AD (32).

A requirement of the NIA-RI criteria is that the subject has a dementia diagnosis. As mentioned earlier, it has become apparent in recent years that AD has a long preclinical phase and the neuropathology of the disease precedes the symptoms by years or decades (18). Therefore, new

neuropathological criteria were proposed by the National Institute of Aging-Alzheimer´s Association (NIA-AA) workgroups in 2012, partly to incorporate this preclinical phase into the neuropathological diagnosis (19). Here, the requirement for the presence of dementia was abandoned and an increased emphasis was put on recording the presence of brain lesions related to conditions commonly comorbid to AD. Neuropathological results according to the NIA-AA criteria are reported in an "ABC" system, where A represents amyloidosis, B represents Braak stage and C represents the CERAD plaque score (see table 2) (19).

A. Aβ plaque score (modified from Thal et al. (22)): A0: no Aβ or amyloid plaques A1: Thal phase 1 or 2 A2: Thal phase 3 A3: Thal phase 4 or 5
B. NFT stage: B0: no NFTs B1: Braak stage I or II B2: Braak stage III or IV B3: Braak stage V or VI
C. Neuritic plaque score: C0: no neuritic plaques C1: CERAD score sparse C2: CERAD score moderate C3: CERAD score frequent

Table 2. The NIA-AA criteria for the neuropathological diagnosis of AD, Hyman et al., 2012 (19)

After staging Alzheimer pathology according to the "ABC" system above, each case receives a score of AD pathologic change according to severity; "Not", "Low", "Intermediate" or "High". The presence of "Intermediate" or "High" AD pathology is considered sufficient to explain symptoms of dementia. As mentioned earlier, the NIA-AA criteria also require that signs of comorbidities such as Lewy body disease, vascular brain injury and hippocampal sclerosis are noted.

1.1.3 Stages of Alzheimer disease

1.1.3.1 Preclinical Alzheimer disease

AD is believed to have a long preclinical phase, during which the affected individual has no or very subtle decline in cognition but manifests AD biomarker positivity (17, 18, 33, 34). The characterization of the preclinical stage of AD first became possible following the emergence of *in vivo* biomarkers, such as neuroimaging with magnetic resonance imaging (MRI) and positron emission tomography (PET) and biomarkers in the cerebrospinal fluid (CSF), reflecting cerebral amyloidosis, tau phosphorylation and neurodegeneration. The time span of preclinical AD has of yet not been fully elucidated as it seems to manifest some individual variability and could be affected by factors such as cognitive reserve, genetic profile and lifestyle (34). However, mounting evidence suggests that the preclinical stage is generally long, stretching over years or even decades (35).

In 2010, Jack et al. proposed a hypothetical model of biomarker change in AD, spanning the whole continuum of the disease, from the preclinical stage through to the stages of mild cognitive impairment (MCI) and dementia (17). The model was amended in 2013 postulating that biomarkers reflecting amyloid β deposition in the brain such as CSF Aβ42 and amyloid PET become abnormal first, followed by biomarkers reflecting tau pathology, such as CSF total tau protein (t-tau) and phosphorylated tau (p-tau). Finally, close to the onset of clinical symptoms, changes can be seen on structural MRI and on [^{18}F]fluorodeoxyglucose (FDG)-PET, signaling the onset of cerebral atrophy and neuronal death. Substantial evidence already supports this hypothetical model (33, 36-45), but prospective longitudinal studies, spanning decades, will be needed to fully understand the trajectories of different biomarkers in preclinical AD.

1.1.3.2 Mild cognitive impairment

Individuals with underlying AD pathology eventually progress from preclinical AD to mild cognitive impairment (MCI) (46), as they move along the continuum of AD. To fulfill the diagnostic criteria of MCI the patient, an informant, or a clinician responsible for the care of the patient needs to have observed a decline from the patient's previous level of performance on objective cognitive tasks. The impairment must be greater than expected based on the age and education level of the patient. It can be in one or more cognitive domains, e.g. memory, executive function, visuospatial ability, attention and/or language. Impairment in episodic memory, often referred to as amnestic MCI (47), is the form of MCI that most commonly progresses to AD dementia with an annual conversion rate of around 10% (48). This typical AD amnestic MCI phenotype, along with a positive AD biomarker(s), is often referred to as prodromal AD (49). A series of cognitive assessments showing a steeper cognitive decline over time than observed in age and education matched peers is feasible, but not necessary, as a basis for a diagnosis of MCI. An individual presenting with subjective cognitive symptoms that cannot be verified by objective cognitive testing is categorized as having subjective cognitive

impairment (SCI), a condition that has been shown to be related to increased risk of harboring underlying AD pathology (50).

In order to receive an MCI diagnosis, the patient needs to fulfill the MCI criteria above but have preserved functional abilities in daily life. That is, the patient cannot be demented. In determining that MCI is due to underlying AD pathology it is important to exclude other conditions that might be responsible for the cognitive impairment, such as vascular, traumatic, psychiatric and metabolic causes (47, 51).

1.1.3.3 Dementia

In AD, the transition from MCI to dementia is usually gradual, heralded by a loss of functional abilities in daily life. Both MCI and dementia are clinically defined entities open to subjective interpretation by the patient, knowledgeable informants and clinicians. Diagnostic guidelines for the whole of the AD continuum, the preclinical stage, MCI and dementia stages were proposed by the NIA-AA in 2011 and have already been detailed for the first two stages in the sections above.

According to these current criteria for dementia diagnosis the first step is to determine if dementia is present and thereafter if it is probably or possibly due to AD. The NIA-AA criteria for all-cause and AD dementia are detailed in table 3 (52).

The NIA-AA criteria replace the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer´s Disease and Related Disorders Association (ADRDA) criteria from 1984 (53). A patient fulfilling the NINCDS-ADRDA criteria for probable AD would also meet the new NIA-AA criteria, while a patient fulfilling the NINCDS-ADRDA criteria for possible AD would have to be reevaluated.

The NIA-AA criteria include new categories not included in previous diagnostic criteria, namely probable or possible AD dementia with evidence of the AD pathophysiological process. Here, biomarkers of AD pathophysiology reflecting amyloid β deposition and neurodegeneration have been incorporated into the criteria. Due to several issues concerning the use of AD biomarkers, including lack of standardization and of quantitative analytical techniques, the use of biomarkers is still optional in the clinical setting but can be of value for research purposes.

Criteria for all-cause dementia:

Dementia is diagnosed when there are cognitive or behavioral symptoms that:

1. Interfere with the ability to function at work or at usual daily activities.
2. Represent a decline from previous functional level.
3. Are not explained by delirium or a major psychiatric disorder.
4. Cognitive impairment is diagnosed through a combination of (1) history taking from the patient *and* a knowledgeable informant and (2) an objective cognitive assessment.
5. The cognitive or behavioral impairment involves *a minimum of two* of the following domains:
    a. Impaired ability to acquire and remember new information.
    b. Impaired reasoning and handling of complex tasks.
    c. Impaired visuospatial abilities.
    d. Impaired language functions.
    e. Changes in personality, behavior or comportment

Probable AD dementia:

Meets criteria for all-cause dementia and has the following characteristics:

A. Insidious onset.

B. Clear-cut history of worsening.

C. The initial and most prominent cognitive deficits are evident in one of these two categories; (a) Amnestic presentation or (b) Non-amnestic presentation (this includes language presentation, visuospatial presentation and executive presentation).

D. The diagnosis of probable AD dementia *should not be made* when there is evidence of substantial concomitant cerebrovascular disease, core features of dementia with Lewy bodies, prominent features of behavioral variant frontotemporal dementia, prominent features of semantic variant primary progressive aphasia or non-fluent variant primary progressive aphasia or evidence for another active neurological disease or a non-neurological medical comorbidity or use of medication that could have a substantial effect on cognition.

Possible AD dementia:

A diagnosis of possible AD dementia should be made in the two following circumstances:

A. The course is atypical, e.g. the onset is sudden or the documentation of progressive cognitive decline is insufficient.

B. The presentation is etiologically mixed, meeting all core clinical criteria for AD dementia, but has evidence of concomitant cerebrovascular disease, features of dementia with Lewy bodies or evidence of another neurological disease or a non-neurological medical comorbidity or medication use that could have a substantial effect on cognition.

Table 3. NIA-AA diagnostic guidelines for dementia due to AD, based on McKhann et al. 2011 (52).

1.1.3.4 Stages of dementia

For research and therapeutic purposes, it is important to define stages of dementia due to AD, as it progresses over a period of several years with continuing cognitive deterioration of the affected individual. The Washington University Clinical Dementia Rating Scale (CDR) (54) was developed to assess the severity of dementing illnesses. It requires information both from the patient and from a knowledgeable informant on the level of impairment in six domains; memory, orientation, judgment and problem solving, community affairs, home and hobbies and personal care. The impairment in each category is rated as absent, questionable, mild, moderate or severe, after which an algorithm is used to generate an overall category of severity. This final overall CDR score rates the patient´s symptoms as no dementia (CDR = 0), questionable dementia (CDR = 0.5), mild dementia (CDR = 1), moderate dementia (CDR = 2) or severe dementia (CDR = 3) (55).

Typical symptoms of mild AD dementia include impairment in episodic memory and executive function and changes in personality. When the patient progresses to moderate dementia these symptoms become more pronounced with added confusion, increased dependence on others for assistance with activities of daily living and behavioral and psychiatric symptoms often develop at this stage. In the final, severe, stage of AD dementia the patient becomes dependent on others for all activities of daily living, including personal care, and loses the ability to communicate. Towards the end of this stage the patient´s motoric functions deteriorate leaving the patient unable to walk, swallow or control bladder and bowel functions (56).

The CDR is a valid and reliable instrument for scoring the severity of dementia but it can be impractical as it is time consuming to administer and requires information from more than one individual. Therefore, the Mini-Mental State Examination (MMSE) was mapped onto the CDR categories in the hope that the MMSE could serve as a surrogate for the CDR (57). The MMSE is a short cognitive screening tool that can be administered in 10 – 15 minutes and only requires the patient to take part (58). The results of an MMSE are on the scale from 0 to 30, where 0 is the lowest outcome (severe impairment) and 30 the highest (no impairment). According to the results from the mapping of the CDR onto the MMSE, the MMSE is a good surrogate for the CDR with an MMSE score of 26-29 corresponding to a CDR score of 0.5 (questionable), 21 – 25 corresponding to CDR 1 (mild), 11 – 20 corresponding to CDR 2 (moderate) and 0 – 10 corresponding to CDR 3 (severe).

1.1.4 Epidemiology and economic impact

It is estimated that 46.8 million people worldwide were living with dementia in 2015 and that 60 – 70% of these patients had AD as an underlying cause (1). By 2050 this number will have risen to 131.5 million according to the World Alzheimer Report, due to the ageing of the global population (59). This increase has already become apparent in recent years with the number of people who are disabled or die from dementing illnesses having more than doubled in the

period from 1990 to 2015 (60). In 2015 the total global societal cost of dementia, including direct medical costs, direct social costs and indirect social costs, was estimated to be US$ 818 billion, equal to 1.1% of global gross domestic product (1). These costs are predicted to rise substantially in the future, in the context of increasing prevalence of dementing illnesses.

The global prevalence of all-cause dementia in people over the age of 60 is generally reported to be in the range of 5 – 8% (1). The estimated annual period prevalence of AD in this age group in a community setting is significantly higher in North America (103.6 per 1000 individuals) than in Asia (11.7 per 1000 individuals) and Europe (31.3 per 1000 individuals). Trends of regional differences have also been observed in other settings but have not been statistically significant. Explanations for these differences between regions could include differences in diagnostic criteria, variation in disease reporting, different thresholds for diagnosis, differences in age-distribution between populations, overall life expectancy and other competing risks (61). During the period from 1990 to 2015 a slight increase in the prevalence of dementia was observed in high-income North America, high-income Asia, the Caribbean and Southern sub-Saharan Africa with a modest decrease in prevalence being observed elsewhere (60).

The age-standardized prevalence of dementia is 22% higher for women than for men. The reasons for this are not fully understood but the gender difference might be attributed, at least partly, to the fact that most epidemiological studies have defined their oldest age category as 80 years and older. As women live longer they are overrepresented in this category, especially at the older end of its spectrum, and it would be feasible to break it up into smaller subcategories to see if these differences hold true in the oldest old (60, 61).

When looking specifically at Sweden, The Swedish National Board on Health and Welfare reported that 158.000 people were living with dementia in Sweden in 2012. The total cost of dementia care in Sweden amounted to 63 billion Swedish crowns in 2012, or approximately 7.4 billion US$ (62).

1.1.5 Treatment

Currently, only symptomatic treatment is available for dementia due to AD. It is not disease modifying as it does not slow down or halt the pathophysiological processes of the disease. Two categories of medication have been approved for symptomatic relief of AD dementia, acetylcholinesterase inhibitors (CheI) and N-Methyl-D-Aspartic (NMDA) receptor antagonists (63, 64).

Donepezil is a CheI that binds reversibly to and inhibits acetylcholinesterase, an enzyme that degrades acetylcholine as it is released from the presynapse (65). As AD causes an early degeneration of cholinergic synapses (66, 67), there is a deficit of acetylcholine in AD that can be ameliorated in part through the administration of donepezil. Donepezil is approved for use in mild and moderate dementia due to AD in more than 90 countries around the world and even

for severe dementia in the United States, Canada, Japan and a few other countries. Most trials on the efficacy of donepezil show modest benefits on measures of cognition, activities of daily living and behavior, but not on overall quality of life (68). These trials were usually conducted over a period of 6 months or less, but there are a few studies that support the long-term efficacy of donepezil (69, 70).

Two other CheI´s have been approved for use in mild to moderate AD, galantamine and rivastigmine. Galantamine is an acetylcholinesterase inhibitor and an allosteric potentiator of nicotinic and muscarinic acetylcholine receptors (71), while rivastigmine is a reversible inhibitor of both acetylcholinesterase and butyrylcholinesterase (72). Generally, placebo controlled trials have shown similar sustained but modest benefits of galantamine and rivastigmine as of donepezil (73-79). However, only a few studies have compared different CheI´s head-to-head. When comparing donepezil and galantamine, galantamine was found to have a slightly larger benefit regarding cognition and caregiver relief (80). Rivastigmine, which is the only CheI available as a skin patch, had less gastrointestinal side effects than the other CheI´s which are administered orally (81). Also, the rivastigmine skin patch performed slightly better on activities of daily living and global function than donepezil (82). To date, CheI´s are only approved for different stages of AD dementia, but not for MCI. According to a systematic review on all three types of CheI´s their use in patients with MCI was not associated with a delay in the onset of dementia (83).

Memantine is a noncompetitive, low- to medium-affinity antagonist of cerebral NMDA glutamate receptors and the only drug approved for treatment of AD that is not a CheI. Neuronal excitotoxicity, due to prolonged influx of Ca^{2+} ions through NMDA receptors, is believed to be involved in the pathophysiology of AD. Memantine inhibits this influx of Ca^{2+}, but as its binding is noncompetitive, the NMDA receptor can still serve its physiological purpose through activation by glutamate (84-86). In light of results from randomized clinical trials on memantine it has been approved for treatment of moderate to severe AD. In this setting, memantine treatment is effective in preventing and treating behavioral and psychiatric symptoms such as aggression, agitation, hallucinations and delusions (87, 88). A 28-week randomized controlled trial showed a modest improvement in independence, global well-being, daily function and attention in those taking memantine compared to a placebo group (89). Memantine has also been studied in combination with donepezil in patients with moderate to severe dementia due to AD. In a trial of 404 patients, lasting for 6 months, memantine augmented the positive effects of donepezil on cognition, activities of daily living, global outcome and behavior (90).

1.2 GENETICS OF ALZHEIMER DISEASE AND THE AMYLOID HYPOTHESIS

Alzheimer disease is often categorized into early onset AD (EOAD), with the debut of clinical symptoms occurring at the age of 65 or younger, and late onset AD (LOAD), when the disease becomes symptomatic after the age of 65. EOAD, which represent 2-10% of AD cases, is considered to be a genetic disorder (91, 92). Autosomal dominant mutations in three genes, *APP*, *PSEN1* and *PSEN2* are known to cause early onset autosomal dominant AD (from here on referred to as familial AD or FAD) but these known mutations account for only 5-10% of EOAD cases (91). Genetic factors also have a large influence on the occurrence of LOAD, which has an estimated heritability of 60 – 80% (93). Currently, there are 40 known genes and susceptibility loci that have either a confirmed or suspected association with increased risk of LOAD, including the most well known risk gene *APOE* (91, 94). In 2013, Lambert et al. published the results of the largest multi-center international collaborative effort in AD genetics to date, The International Genomics of Alzheimer's Project (IGAP), where 19 susceptibility loci, in addition to *APOE*, reached genome wide significance for AD (95). The study included 74.046 individuals of European ancestry in which a two-stage meta-analysis of genome-wide association studies (GWAS) was performed. Of the 19 loci reported in this study, 11 were newly identified, illustrating the statistical power of this multi-center effort. The IGAP study from 2013 included 797 Swedish AD patients and 1506 controls, making the results relevant for the Swedish population. Since 2013, IGAP and other study groups have, as previously stated, yielded a total of 40 susceptibility loci for AD (94, 96, 97).

1.2.1 Familial Alzheimer disease

Familial Alzheimer disease (FAD) is an early onset form of AD where a known pathogenic mutation in one of three genes, *APP*, *PSEN1* or *PSEN2*, is present. FAD is a very rare disease, estimated to account for <1% of AD cases (91). Mutations leading to FAD are considered to be close to 100% penetrant (98), with a predictable age at onset of symptoms, derived from the mean age at symptom onset of the affected members in each FAD family (99). However, there are a few FAD families that seem to be an exception to this rule, with wide ranges of symptom onset and suspected cases of reduced mutation penetrance (100-103).

Numerous studies on mutation carriers from FAD families have contributed valuable information on the natural history and possible pathological mechanism of both FAD and the much more common sporadic form of AD (SAD). FAD mutation carriers are believed to be able to serve as models for SAD with the only difference between FAD and SAD being the heritability and generally young age at symptom onset in FAD. Several studies have addressed the possible phenotypic differences between FAD and SAD and found no significant differences in the duration of illness, rate of cognitive decline or occurrence of non-cognitive symptoms when comparing FAD and SAD cases (104, 105). No significant differences in cognitive phenotype were found when comparing the neuropsychiatric profiles of AD patients, with and without familial AD aggregation (106). In this case however, the study did not involve cases with known FAD mutations which could mean that the results are not generalizable to FAD. Finally, the neuropathology of FAD and SAD appears to be similar, with no observed

differences between severity scores or distribution of neuritic plaques and NFTs (107, 108). However, there are numerous known mutations causing FAD (see below) and a few of these have a distinct phenotype that differs from the typical phenotype of SAD (109), even though these differences disappear when pooling together individuals from many families carrying different FAD mutations. That such differences exist is important to keep in mind when using mutation carriers from a single FAD family as models for SAD.

A great deal of what is known about the preclinical stage of AD comes from studies on cognitively asymptomatic individuals carrying FAD mutations (18). The fact that FAD mutations have a high degree of penetrance and a reliable age at symptom onset enables the mapping of the temporal trajectories of preclinical AD biomarker changes as the time point of symptom onset in each individual can be anticipated ahead of time. As FAD becomes symptomatic before the age of 65 in most cases, these subjects usually lack comorbidities such as cerebrovascular disease, which are common in older individuals with SAD. Therefore, FAD subjects allow for the study of AD in its purest form.

1.2.1.1 APP processing

Mutations leading to FAD all have an effect on the processing of the amyloid precursor protein (APP) or on the conformational structure of the β amyloid peptide. APP is a single-pass transmembrane protein with a large extracellular domain which includes the β amyloid sequence (110). Its cleavage can follow one of two pathways, an amyloidogenic pathway generating β amyloid (Aβ) and a non-amyloidogenic pathway. In the amyloidogenic pathway, APP is first cleaved by a membrane-bound protease named β-secretase (BACE1) and then by another membrane-bound protease, γ-secretase (see figure 1). Following cleavage by β-secretase a soluble APPβ (sAPPβ) ectodomain is released, with a β C-terminal membrane bound intracellular fragment (βCTF) remaining. The βCTF is then cleaved by γ-secretase, releasing an Aβ peptide, as well as an APP intracellular domain (AICD). In the non-amyloidogenic pathway, APP is cleaved by α-secretase, within the Aβ sequence, precluding the formation of Aβ. Following this cleavage, a soluble APPα (sAPPα) ectodomain is released which includes a part of the N-terminus of Aβ, leaving an α C-terminal fragment (αCTF) behind in the cell membrane. This αCTF is then cleaved by γ-secretase, releasing a 3 kDa peptide (p3) and an AICD.

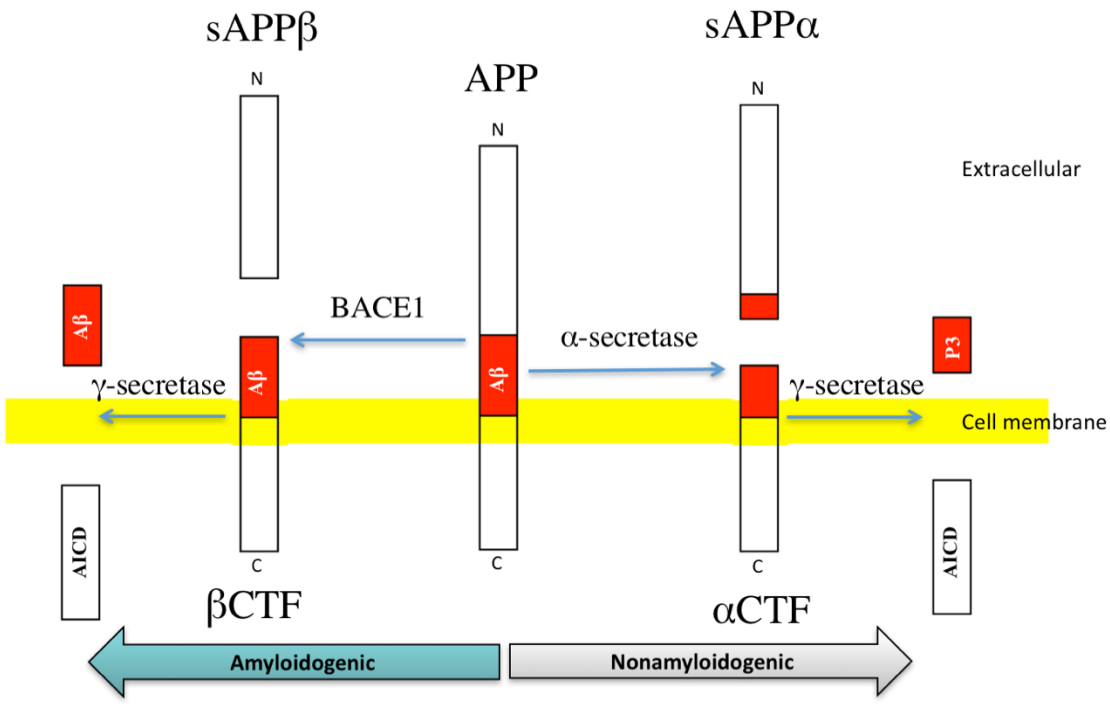

Figure 1. The amyloidogenic and non-amyloidogenic pathways of APP processing. Modified from Wang et al. 2012 (111).

The APP processing pathways described above give a somewhat simplified view of APP cleavage, as the number of proteases that cleave APP is considerably larger. These proteases include the zinc metalloproteases ADAM17, ADAM9, ADAM10 and MDC-9, all cleaving APP at the α-secretase site, as well as the aspartyl protease β-site APP-cleaving enzyme 2 (BACE2) which can cleave APP at the β-secretase site (112). BACE2 is closely related to the β-secretase mentioned above, BACE1, with BACE1 being the most abundant β-secretase in neurons. BACE1 usually cleaves APP at the β-secretase site, enabling the consequent release of Aβ, but can also cleave APP within the Aβ domain, albeit not at the same site as α-secretase (113). The recent discoveries of alternative APP fragments, e.g. N-terminally extended Aβ, has hinted at the existence of even more proteases targeting APP (114). One of these secretases, η-secretase, is a matrix metalloprotease which cleaves APP 92 amino acids upstream of the cleavage site of BACE1. This step of APP processing results in ηCTFs which in turn are cleaved by α- or β-secretase yielding Aη-α and Aη-β peptides (115). These alternative APP processing products become more abundant following the administration of β-secretase inhibitors, but their physiological and possible pathological significance remains to be elucidated (115). The cleavage of the βCTF by γ-secretase also yields different lengths of Aβ species, discussed further in chapter 1.2.1.3.

1.2.1.2 APP mutations

The *APP* gene was discovered in 1987 and localized to chromosome 21 (116-118). This discovery did not come entirely as a surprise due to the common occurrence of AD in people with trisomy 21, also known as Down syndrome (119). People with Down syndrome develop dementia approximately 30 years ahead of the general population, with a mean symptom onset of 56 years (120, 121). The first *APP* mutations leading to FAD were discovered in the early nineties (122-125), strongly underpinning the central role of Aβ in the pathogenesis of AD. The knowledge of the existence of *APP* mutations enabled the development of *APP* transgenic mouse models which have since then been central to research on AD molecular pathogenesis (126).

Currently, 58 *APP* mutations have been identified, many of which are associated with FAD and a few which are related to cerebral amyloid angiopathy (CAA) (127). The known APP mutations are generally autosomal dominant, except for the E693 delta mutation (128) and the A673V mutation (129), which are autosomal recessive. *APP* mutations have been found near the cleavage sites of γ-secretase, α-secretase and β-secretase as well as at other locations along the Aβ sequence (see figure 2). The majority of *APP* mutations with a known biological effect lead to an increase in the overall production of Aβ and/or an increase in the Aβ42/Aβ40 ratio (127), but Aβ40 is a shorter, less fibrillogenic and more common species of Aβ than Aβ42 (130). The KM670/671NL (Swedish) (123) and A692G (Flemish) (131) mutations do not change the Aβ42/Aβ40 ratio, but increase overall Aβ production. Most of the mutations located around the γ-secretase cleaving site, such as V717I (London) (122), V717G (124) and V717F (Indiana) (125), do not affect the amount of total Aβ but increase the Aβ42/Aβ40 ratio. The H677R (English) (132) and D678N (Tottori) (133) mutations increase Aβ oligomerization and cytotoxicity, but do not affect the levels of Aβ or the Aβ42/Aβ40 ratio. The same applies to the E693G (Arctic) (134, 135) and E693del (Osaka) (128) mutations which both enhance the propensity of Aβ to oligomerize and form fibrils.

One *APP* mutation stands out in having a protective effect against AD, as opposed to all the other known causal *APP* mutations. The A673T (Icelandic) mutation is located at the β-secretase cleaving site and decreases the total amount of Aβ by about 40% with the generated Aβ being less prone to aggregation than wild type Aβ (136, 137).

More of the *APP* mutations with a known mechanism are illustrated in figure 2, however this is not a complete overview of all *APP* mutations with a known effect.

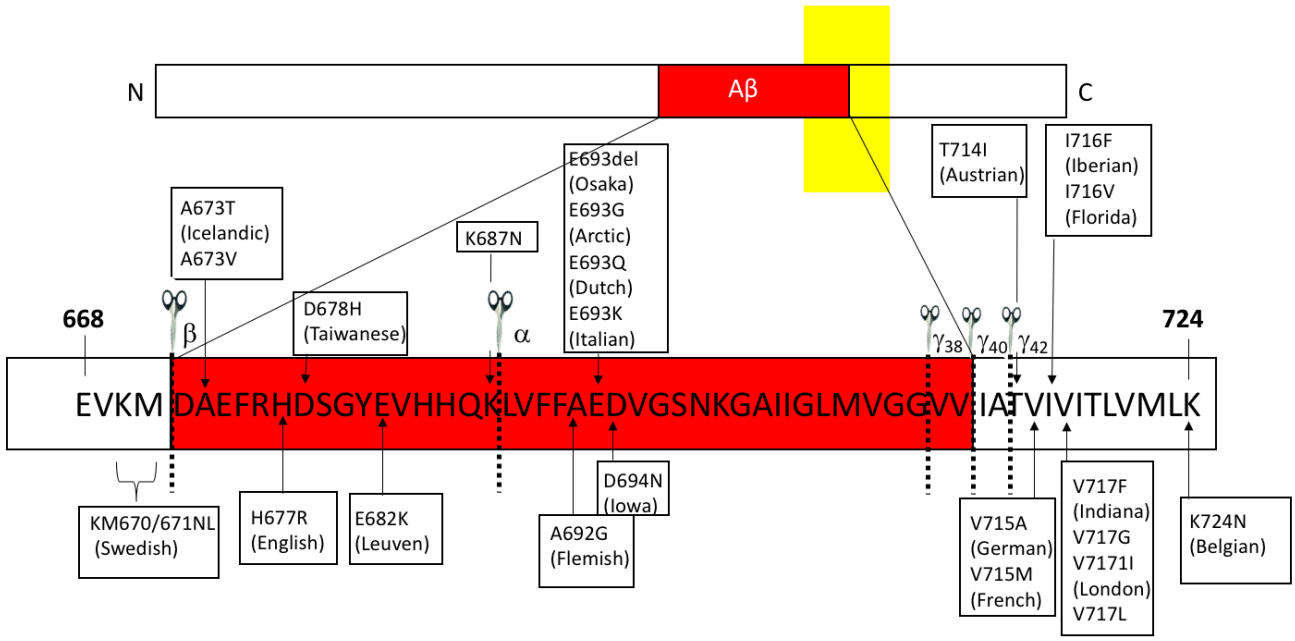

Figure 2. Locations of mutations in *APP* along the Aβ peptide sequence. The Aβ peptide sequence (in red) has been enlarged and its single amino acid code indicated in letters. The cleavage sites of the γ-secretase, α-secretase and β-secretase are illustrated with dotted lines. Modified from Bateman et al. 2011 (138).

1.2.1.3 Mutations in PSEN1 and PSEN2

The most common mutations causing FAD are located in the *PSEN1* gene on chromosome 14, with the first *PSEN1* mutation being described in 1995 (139). *PSEN2* mutations on chromosome 1, also first discovered in 1995 (140, 141), are the rarest types of FAD mutations (142, 143). To date, 241 *PSEN1* mutations and 45 *PSEN2* mutations have been reported (127).

Presenilin is one of the four proteins that constitute the γ-secretase, the others being nicastrin, anterior pharynx-defective 1 (APH-1) and presenilin enhancer 2 (PEN-2) (144). Presenilin, an aspartyl protease with nine transmembrane domains (145-148), is the catalytic subunit of γ-secretase (149). The *PSEN1* and *PSEN2* genes encode homologous presenilin proteins, presenilin-1 and presenilin-2, both being able to serve as the catalytic subunit of the γ-secretase complex (145-147). The γ-secretase cleaves APP several times, removing 3 to 4 C-terminal amino acids each time (see figure 3) (150, 151). The initial cleavage, the ε-cleavage, occurs near the transmembrane/cytoplasmic interface of APP, either between Aβ48 and Aβ49 or between Aβ49 and Aβ50. The ε-cleavage yields Aβ48 and Aβ49 peptides, depending on the starting point, which are then cleaved again, usually 2 – 3 times (152-154). The most common end product of this sequential APP cleavage is Aβ40 (constituting 80 – 90% of the Aβ peptides which are released), followed by Aβ42 (5 – 10% of released Aβ peptides) (130). Longer Aβ

peptides such as Aβ42 and Aβ43 are more hydrophobic and neurotoxic than Aβ40 and have a greater propensity to aggregate (155-157). Aβ42 is the most abundant Aβ peptide in neuritic plaques (155), with Aβ40 being the most common Aβ peptide to accumulate in blood vessels (157, 158). Aβ43 is a relatively rare and highly neurotoxic peptide (159) which is more frequent in plaque cores than Aβ40 both in the brains of patients with SAD and FAD (160).

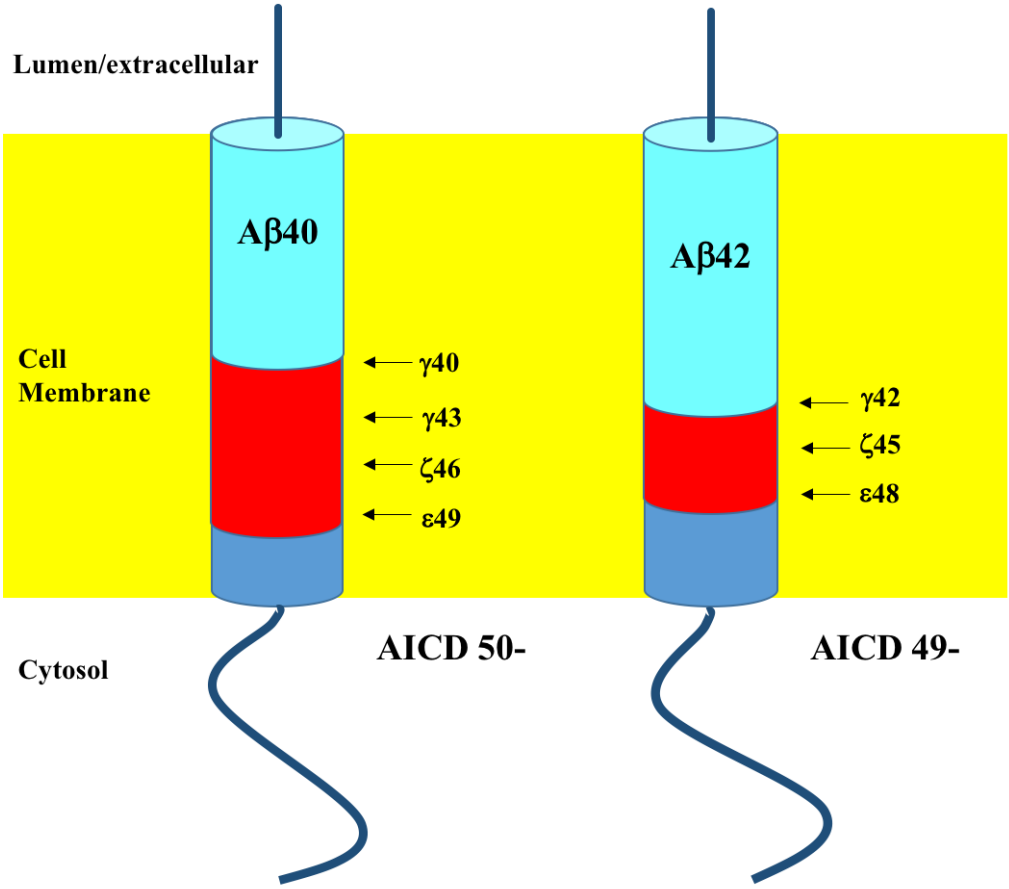

Figure 3. The sequential cleavage of APP by the γ-secretase, releasing either an Aβ40- or Aβ42-peptide. Modified from Selkoe & Hardy 2016 (161).

The γ-secretase is not only responsible for APP processing but also the cleavage of several other substrates including another type-1 transmembrane protein, the cell surface receptor Notch (162). The Notch signaling pathway is important for the differentiation of a wide variety of cell types, both during development and in adulthood (163). The fact that γ-secretase is not restricted to the APP processing pathway has proven to be a challenge in the development of AD treatment strategies based on γ-secretase inhibition.

PSEN1 mutations with a known biological effect have an influence on the γ-secretase and often seem to cause a loss-of-function of presenilin. This leads to an early halt in the sequential

cleavage of APP, resulting in a relatively increased release of longer Aβ peptides (161). Many known *PSEN1* mutations, such as the M139V, H163Y, H163R and L286V mutations, cause an increase in the Aβ42/ Aβ40 ratio but not in overall Aβ production, which is potentially due to this loss-of-function (139, 164, 165). Some *PSEN1* mutations increase the Aβ42/ Aβ40 ratio and the absolute levels of Aβ42 (164-167) and sometimes also the absolute levels of Aβ40. Others increase the Aβ42/ Aβ40 ratio but decrease the absolute levels of either Aβ40 or of both Aβ42 and Aβ40. Finally, the R278I mutation has been shown to specifically increase the amount of Aβ43. This is not an exhaustive list of all *PSEN1* mutations and their effects, but an increase in the relative or absolute amounts of longer Aβ peptides seems to be common to the majority of them.

Most of the known *PSEN2* mutations increase both the Aβ42/ Aβ40 ratio and the absolute levels of Aβ42 (140, 141, 168). However, at least one pathogenic *PSEN2* mutation (V148I) neither affects the Aβ42/ Aβ40 ratio nor the levels of Aβ42 (169). The age at symptom onset for *PSEN2* mutations is 39 – 75 years, which is a wider range and later onset than in *PSEN1* and *APP* mutations (170). The reasons for this have not been fully elucidated but there are speculations that presenilin-1 might compensate for defects in the function of presenilin-2. This has been supported by a study on knockout mice, where *PSEN2* knockouts were viable as opposed to mice with both *PSEN1* and *PSEN2* knocked out (171).

1.2.1.4 Penetrance of FAD mutations

The FAD mutations presented in the chapter above are generally considered to be close to 100% penetrant, i.e. cause clinical symptoms of AD in all mutation carriers, and have a relatively invariant and predictable age at which the onset of clinical symptoms occurs (172). However, a few cases of suspected reduced penetrance of these mutations have been described in the literature. The median age at onset for the *PSEN1* I143F mutation is 55 years but a carrier of this mutation was symptom free at the age of 68 (100). In another report the *PSEN1* A79V mutation (with a mean onset age of 64 years) was not yet penetrant in a 76- year old mutation carrier (101). In both of these cases the symptom free mutation carriers had the *APOE* ε3/ε3 genotype. In a family carrying the *PSEN1* M139V mutation there were 34 years between the individual with the youngest onset (35 years of age) and the individual with the oldest onset (69 years of age), and they both had the same *APOE* genotype (ε3/ε4). Finally, three cases of AD due to the *PSEN1* K239N mutation have been described, with a range of symptom onset from 42-71 years (based on clinical history). All of the three K239N mutation carriers had the *APOE* ε3/ε3 genotype (103). Reduced penetrance of the *APP* A713T mutation has also been reported (173), emphasizing that reduced penetrance of FAD mutations is not exclusive to *PSEN1* mutations. These variations in onset and rare cases of reduced penetrance of FAD mutations are intriguing and suggest that genetic, epigenetic or environmental factors modify the disease process.

1.2.2 Genetic risk factors for late onset Alzheimer disease

1.2.2.1 APOE

APOE is an apoliprotein, or fat binding protein, produced by several cell types including astrocytes (174). It is the main carrier of cholesterol in the brain and forms chylomicrons and intermediate density lipoprotein particles along with triglycerides, phospholipids and cholesterol (175, 176). APOE has been shown to be involved in the trafficking of APP, in APP processing and in the clearance of Aβ (177). The *APOE* gene on chromosome 19 has three alleles which code for APOE, ε2, ε3 and ε4, resulting in six possible *APOE* genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4 and ε4/ε4) (178). In 1993, the *APOE* ε4 allele was recognized as a risk allele for LOAD, increasing the risk of developing AD 3– to 4-fold for *APOE* ε4 heterozygotes (179-181). Around 25% of the general population carry at least one ε4 allele, 94% at least one ε3 allele and 15% at least one ε2 allele, making the ε2 allele the least common of the three (178). The ε2 allele has been shown to reduce the likelihood of developing AD, while the ε3 allele is considered to be neutral in this aspect (179). The mechanism by which the *APOE* ε4 allele increases AD risk has been proposed to involve its failure to efficiently mediate the clearance of Aβ (177).

1.2.2.2 Other AD risk genes

Several new loci associated with increased risk of developing AD have been discovered in recent years, but none as common and potent as *APOE* (161). ATP-binding cassette transporter 7 (ABCA7) is a lipid transporter which, like APOE, is involved in cholesterol metabolism as well as in Aβ homeostasis (182). Mutations in the *ABCA7* gene, which cause a loss-of-function of this lipid transporter, increase the risk of developing AD around threefold (183, 184). Genes related to other mechanism besides cholesterol/sterol metabolism, including inflammation/innate immune responses and endosomal vesicle recycling, have also been linked to increased AD risk (161). Complement receptor 1 (*CR1*), *CD33* and *TREM2* are an example of the former (181, 185-187). All three encode proteins expressed by microglia that have been implicated in the phagocytosis of Aβ deposits and are upregulated in response to increasing Aβ plaque load. *SORL1* is an example of the latter, encoding sortilin-related receptor 1, an endocytic trafficking factor involved in the processing of APP (188).

1.2.3 The Amyloid Hypothesis

The amyloid hypothesis was first proposed in 1991 (189-191) and is to date the most widely accepted hypothesis on the pathological processes behind AD, albeit not undisputed. According to this hypothesis it is the accumulation of β amyloid that is the driver of AD pathogenesis. This amyloid accumulation initiates other downstream processes, such as NFT formation, gliosis, loss of synapses and neuronal death, with the pathogenesis becoming increasingly complex and multifaceted as the disease progresses. There have also been

speculations around the possibility that amyloid accumulation and tau pathology might have a common upstream initiator that is currently unknown (192).

The origins of the amyloid hypothesis hail from the discoveries of the pathogenic autosomal dominant mutations in *APP*, *PSEN1* and *PSEN2* (122, 124, 125). As a vast majority of these mutations have an effect on the relative or absolute levels of Aβ42, or its propensity to aggregate, it seems logical to presume a causal relationship between Aβ42 accumulation and the early development of AD pathology observed in carriers of these mutations. The discovery that many of the gene polymorphisms which increase the risk of AD, such as *APOE* ε4, are involved in the processing of APP and/or the trafficking and clearance of Aβ have further established the central role of Aβ in AD pathogenesis. However, there are some who believe that the causality of AD is far more complicated and that Aβ aggregation is neither necessary nor sufficient to start the pathological cascade of the disease. The observation that healthy adults can have substantial amounts of Aβ plaques without any cognitive symptoms has been used as an argument in support of these views (193). Also, most clinical trials in AD patients involving monoclonal antibodies targeting Aβ have up until now been unsuccessful in halting cognitive decline (194, 195). The fact that most of the subjects in these trials have been in the clinical stage of dementia has been suggested as an explanation for this lack of treatment effect, as the disease might be irreversible in the dementia stage. Currently, ongoing clinical trials on this treatment modality are mainly aimed at subjects with MCI or at subjects who are free of cognitive symptoms but have an increased genetic risk of developing AD and/or have biomarker signs of cerebral amyloidosis. A recent study on immunotherapy with aducanumab, a human monoclonal Aβ antibody, involving patients with MCI and mild dementia due to AD, both showed a clearance of Aβ plaques from the brain and a slowing of cognitive decline (196). Treatments intended to reduce the production of Aβ42 through the inhibition of β-secretase also hold promise but are not as far along in development as immunotherapies. The amyloid hypothesis is therefore far from disproven and the clinical trials that are now underway will almost certainly shed a clearer light on its validity in the coming years.

1.3 BIOMARKERS IN ALZHEIMER DISEASE

Biomarkers that can be used *in vivo* are essential in mapping the chain of events in AD, from the preclinical stage to the clinical stage. The characterization of preclinical AD would not be possible without the use of biomarkers as no clinical symptoms have yet emerged. Biomarkers are also useful in selecting patients for clinical trials and for monitoring treatment effect. In the NIA-AA guidelines for AD diagnosis the clinical use of biomarkers is optional, but has still become widespread in the clinical setting, mainly as a supportive rather than a decisive tool. This includes the CSF biomarkers Aβ42, total tau-protein (t-tau) and phosphorylated tau-protein (p-tau), as well as FDG-PET, amyloid PET and structural MRI. These biomarkers reflect two of the hallmarks of AD, cerebral amyloidosis (CSF Aβ42 and amyloid PET) and neurodegeneration (CSF t-tau and p-tau, FDG-PET and structural MRI). Experimental

biomarkers reflecting other pathological processes are under development, both as possible diagnostic aids and for further characterization of the natural history of AD. These include novel CSF biomarkers and PET ligands as well as blood based biomarkers (197) and electroencephalographic algorithms (198, 199).

1.3.1 Biomarkers in the cerebrospinal fluid

Three core CSF biomarkers, Aβ42, p-tau and t-tau, have been extensively evaluated for use in AD diagnosis and research (200). CSF Aβ42 reflects Aβ aggregation and plaque formation, and decreases in AD to about 50% of control levels (200-202). When Aβ42 was measured in both pre- and postmortem CSF from AD patients and patients with other neurological conditions, it correlated inversely with Aβ plaque load on autopsy (203, 204). The same inverse correlation has been seen between CSF Aβ42 levels and degrees of retention of the PET amyloid ligand Pittsburgh Compound B (PiB) (205-208) which signals brain amyloidosis. A decrease in CSF Aβ42 has been observed in a few other conditions besides AD, such as dementia with Lewy bodies, a disorder also characterized by amyloid plaques, (209) and transiently in bacterial meningitis (210), probably due to degradation by proteases during the acute phase (211). This makes a decrease in CSF Aβ42 not entirely specific to AD. Studies have shown that CSF Aβ42 decreases early in the pathological cascade of AD and remains stable and low thereafter (212-215), making it an unsuitable marker of disease severity and rate of progression. CSF Aβ42 has been shown to already be reduced in prodromal AD and even in the preclinical asymptomatic stage of AD (37, 38, 44, 216-218). The exact time point at which the CSF Aβ42 reduction occurs, in relation to development of symptoms and changes in other biomarkers, still remains to be fully elucidated. However, CSF Aβ42 reduction is considered to be upstream of most, if not all, other biomarker changes related to AD pathology (34, 202).

The second AD CSF biomarker is p-tau which reflects tau phosphorylation and increases around 200% from control levels in AD, most likely before the onset of the prodromal stage, and remains steady and high thereafter (201, 202). Assays are available both for tau phosphorylated at threonine 181 (p-tau$_{181}$) and at threonine 231 (p-tau$_{231}$). These are considered to be equivalent in diagnostic accuracy (219), although there is a possibility that p-tau$_{231}$ has a somewhat greater specificity for AD than p-tau$_{181}$ (220). The increase in CSF p-tau has as of yet not been observed with certainty in other pathological conditions, making elevated p-tau seemingly exclusive to AD.

The third, and final, core AD biomarker is t-tau which represents axonal degeneration and increases around 300% early in the course AD, probably around the same time as p-tau (201, 202). T-tau is the least specific for AD of the three core CSF biomarkers, with increases in t-tau being observed in other neurological conditions such as stroke, trauma, encephalitis and Creutzfeld-Jakob disease (221-224).

Combining all three core CSF biomarkers yields higher sensitivity and specificity for AD than can be achieved using only one or two of them. The combination of reduced concentration of Aβ42 and high concentrations of p-tau and t-tau comprises the so called "AD signature" in CSF which has been shown to be highly predictive of progression to AD dementia in MCI patients (201, 225-229). An example of this is a study showing 95% sensitivity and 87% specificity of the "AD signature" in CSF in distinguishing between prodromal AD and stable MCI (229).

However, there still are several obstacles that need to be overcome before incorporating CSF biomarkers into clinical diagnostic guidelines for AD. These include interassay, intralaboratory and interlaboratory variations, which currently are considered to be unacceptably high (202). International projects, e.g. Biomarkers for Alzheimer´s disease and Parkinsons´s disease (BIOMARKAPD) (230) and the International Federation of Clinical Chemistry and Laboratory Medicine Working Group for CSF proteins (IFCC WG-CSF) (231), are underway to address these issues and to facilitate the development of standard operating procedures regarding CSF collection and analysis as well as the development of fully automated instruments for CSF assays (232).

There is great interest in developing novel CSF biomarkers reflecting other pathological processes than the core AD biomarkers, or other aspect of the same processes. The possible advantages of such development include further characterization of the natural history of AD, monitoring disease severity and possibly even treatment response, uncovering new treatment targets and increasing the sensitivity and specificity of AD diagnosis using CSF biomarkers. The Alzbiomarker database encompasses meta-analyses on original research on AD biomarkers (233), both in the CSF and in blood. Novel CSF biomarkers that have been the subject of studies over the past years and have shown some level of promise include truncated Aβ species, sAPPα and sAPPβ, Aβ oligomers, neurofilament proteins, neurogranin, VLP-1, YKL-40, BACE1 concentration and activity and sTREM2 (233).

1.3.2 Neuroimaging biomarkers

1.3.2.1 Magnetic resonance imaging

Structural neuroimaging is a required part of the clinical assessment of individuals with cognitive impairment. The purpose being to rule out causes for cognitive symptoms other than neurodegenerative diseases, e.g. cerebral infarction and neoplasms, and to assess the degree and localization of cerebral atrophy (234). Although a computerized tomography (CT) scan can suffice in some cases, magnetic resonance imaging (MRI) is the imaging method of choice in the diagnostic assessment of possible AD (234, 235). Evaluation of the hippocampus through high resolution T-1 weighted MRI is the best established and validated structural imaging marker in AD (236-240) with the medial temporal atrophy (MTA) score being widely used in the clinical setting to assess atrophy of the hippocampus (239, 241-243). Amyloid markers,

such as CSF Aβ42 and amyloid PET, are considered to be more sensitive than structural MRI in the preclinical stage of AD (34), with structural MRI markers correlating more closely to disease progression in MCI and the earlier stages of AD dementia (244, 245). An example of this is the close correlation between atrophy rates of the whole brain (246-249), entorhinal cortex (250), hippocampus (251-253) and medial temporal lobe (254) on MRI and the progression of cognitive decline in AD. Functional MRI (fMRI) complements structural MRI by providing information on the functional integrity of brain networks, making it of great interest as a potential AD biomarker.

1.3.2.2 Positron emission tomography

There are two types of PET tracers which are most widely used in AD; FDG which indicates synaptic activity and amyloid tracers which reflect the accumulation of fibrillar amyloid plaque deposition (255). FDG is a glucose analog which gives a good indication of brain metabolism as glucose is the primary energy source of the brain (256). Decreased uptake of FDG is considered to be a marker of neurodegeneration and has a characteristic pattern in dementia due to AD, involving the precuneus, posterior cingulate gyri, inferior parietal lobule, posterolateral portions of the temporal lobe, hippocampus and medial temporal cortices (257-260). Hypometabolism on FDG-PET has also been observed in patients with MCI (261, 262) as well as in cognitively healthy individuals with genetic risk for AD (38, 258, 263, 264). Despite these early pathological changes on FDG-PET, reduced FDG uptake is generally considered to occur downstream of increased brain amyloidosis detected by amyloid PET (34).

Fibrillar amyloid; neuritic plaques and CAA in particular, can be detected through PET imaging with amyloid ligands including PiB, florbetapir, florbetaben and flutemetamol (235, 265, 266). These ligands have all been shown to correlate closely with neuritic plaque load at autopsy and on cortical brain tissue biopsy (267-270) and their retention has good predictive value for the conversion from MCI to dementia due to AD (17, 207, 271, 272). An increase in amyloid load as detected on PET is an early event in AD and has been reported in asymptomatic carriers of FAD mutations as early has 15 – 20 years before the onset of the first clinically relevant cognitive symptoms (264, 273). An increase in brain amyloid burden has also been reported in cognitively healthy older adults who later progress to symptomatic AD (35, 274). Longitudinal studies have shown that the amyloid burden on PET increases in an almost linear fashion from the preclinical stage of AD through to the dementia stage, where it finally reaches a plateau (35, 245, 275) and does not correlate with disease severity in the later stages of the disease (276). Studies have shown a high level of concordance between increased amyloid PET signal and decreased levels of CSF Aβ42 (205-208) and it remains unresolved which of these two biomarkers detects the earliest signs of amyloid accumulation. However, there are indications that the decrease in Aβ42 in the CSF precedes the earliest detectable signs of retention of PET amyloid tracers (277).

As with CSF biomarkers there are several issues concerning the clinical use of amyloid PET in AD diagnosis (266), including the fact that amyloid positivity on PET is a relatively common

occurrence in cognitively healthy individuals (270, 278, 279). These individuals might be in the preclinical stage of AD, thereby explaining the amyloid positivity, but further longitudinal studies are needed to fully address this issue. There have also been reports of amyloid negativity on PET in symptomatic FAD patients (280).

Other PET tracers have emerged in recent years, including [^{11}C]-deuterium-L-deprenyl (DED), a ligand that binds to monoamine oxidase B (MAO-B) on the outer mitochondrial membrane in astrocytes and indicates reactive astrocytosis (281, 282). Tau specific tracers, such as THK5317, THK5351, AV-1451 and PBB3, are also under development (283). These tracers hold promise in the diagnosis of AD and other neurodegenerative diseases, as well as in further elucidating the relationship between cause and effect in their pathogenesis.

The biomarkers presented above, both those that are well validated and others that carry potential, offer a unique opportunity to gain new insights into the sequence of events in preclinical AD. Cognitively asymptomatic carriers of FAD mutations are an ideal group for studying preclinical AD biomarker changes, as their expected symptom onset can be estimated and thereby the temporal relationship between biomarker changes and the onset of symptoms.

2 AIMS

The overall aim of the studies incorporated in this thesis was to shed light on AD biomarker changes in Swedish carriers of FAD mutations in the preclinical stage of the disease. Results from studies involving FAD mutation carriers are generally believed to translate onto patients with the much more common sporadic form of AD, making their implications wider than if they only applied to FAD. Preclinical biomarker changes are an important subject to study, as biomarkers are essential in characterizing the preclinical stage of AD. Disease modifying therapies that are currently in clinical trials in AD are believed to be most effective when applied as early as possible in the disease process, preferentially before the onset of the first clinically relevant cognitive symptoms. Biomarkers are crucial in identifying asymptomatic individuals with underlying cerebral AD pathology, which are believed to benefit the most from the therapeutic interventions that are under development. Biomarkers can also have a role in monitoring disease progression and treatment response and in staging disease severity.

Specific aims of each study:

- To assess levels of the core AD CSF biomarkers Aβ42, t-tau and p-tau and their correlation with years to symptom onset in asymptomatic carriers of FAD mutations, compared with non-carriers from the same families. To assess brain structure on MRI and its correlation with years to onset in the same population (paper I).

- To assess the effects of different FAD mutations on APP processing in both preclinical and clinical AD, by measuring levels of CSF sAPPα, sAPPβ, Aβ42, Aβ40 and Aβ38 in carriers of three different FAD mutations, and comparing them with non-carriers from the same families (paper II).

- To summarize and interpret long-term clinical and biomarker data from a carrier of the *PSEN1* H163Y mutation suspected of exhibiting reduced penetrance of the mutation (paper III).

- To assess the CSF levels of YKL-40 and neurogranin, reflecting glial activation and synaptic degeneration respectively, in asymptomatic carriers of FAD mutations compared with non-carriers from the same families. Also, to assess the relationship between years to symptom onset and the levels of YKL-40 and neurogranin (paper IV).

3 SUBJECTS AND METHODS

3.1 THE FAMILIAL ALZHEIMER DISEASE STUDY

The Swedish FAD study was initiated at Karolinska Institutet in 1993 and has been ongoing to this day. Initially, the participants were recruited through the Memory Clinic at the Karolinska University Hospital in Huddinge and later through the Genetics Unit of the Memory Clinic in Huddinge. The researchers involved in the study have not taken primary contact with potential participants, the participants have either contacted the Genetics Unit on their own initiative or been approached by a relative. The participants come from four families, carrying four different FAD mutations, the Swedish APP double mutation (*APPswe*) KM670/671NL (123, 284), the arctic APP mutation (*APParc*) E693G (135), the *PSEN1* H163Y mutation (164, 285) and the *PSEN1* I143T mutation (286). The clinical phenotype of AD in each family has been described in detail in previous publications (286-288). A total of 69 individuals from these four families have participated in the study, some repeatedly, amounting to 169 separate study visits.

The age at onset of the first clinically relevant cognitive symptoms in affected family members has been estimated after a retrospective review of the medical records of family members who had developed dementia before the study was initiated and a prospective clinical assessment in those who developed symptoms while enrolled in the study. Based on this the mean age at symptom onset in the families was calculated and reported to be 54 years with a standard deviation (s.d.) of ± 5 years for the *APPswe* mutation (based on 24 affected cases), 56 ± 3 years for the *APParc* mutation (based on 12 affected cases), 52 years ± 7 years for the *PSEN1* H163Y mutation (based on 9 affected cases) and 36 years ± 2 years for the *PSEN1* I143T mutation (based on 5 affected cases). These numbers, presented in paper I, changed slightly for the *APPswe* mutation carriers and the *PSEN1* H163Y carriers in papers II – IV, where the average age at onset was reported to be 54 years ± 4 years for the *APPswe* mutation (based on 19 affected cases) and 51 years ± 7 years for the *PSEN1* H163Y mutation (based on 11 affected cases). The reason for this discrepancy is that a new review of medical records was conducted between papers I and II which resulted in 5 *APPswe* mutation carriers being excluded from the age at onset calculations due to insufficient data and 2 new *PSEN1* H163Y mutation carriers being included as they had developed symptoms after the calculations for paper I were done.

The FAD study is a prospective longitudinal study involving mutation carriers from the families described above, as well as non-carriers from the same families who serve as healthy controls. The participants and researchers involved in the study are blind to the mutation status of the participants, except for those who have requested presymptomatic or diagnostic genetic testing. Each study visit involves a thorough clinical evaluation, neuropsychological assessment, MRI of the brain, EEG, skin biopsy for collection of fibroblasts and collection of CSF, blood and saliva samples. Each participant also receives genetic counseling in conjunction with the study visits. Neuroimaging of the FAD family members with PET is conducted at the Section of Nuclear Medicine & PET at the University of Uppsala, in

collaboration with a research group at the Karolinska Institutet led by Professor Agneta Nordberg, but in close temporal conjunction with the other parts of the FAD study.

3.2 SUBJECTS

The demographic characteristics of the subjects involved in the cross-sectional parts of the studies constituting this thesis are presented in table 4. See paper IV for a description of the subjects in the longitudinal part of the paper IV study. Paper III is not represented in table 4 as there were only two participants in that study. There is a slight variability in the total number of participants between studies and in the distribution between mutation carriers and non-carriers. One reason for this is that CSF samples acquired between 2006 – 2011 were included in paper I, while samples from 1993 – 2011 were included in paper II and samples from 1993 – 2015 in paper IV. The samples from the 1990's were included in papers II, III and IV after a stability assay of these frozen samples was performed and showed no signs of systematic time-dependent changes. Paper IV includes the newest samples, taken in 2012 - 2015, most of which are a part of the longitudinal analysis in paper IV, i.e. from individuals who had already undergone a previous baseline sampling. Another reason is that some of the older frozen CSF samples were depleted when it came to the later studies.

Only individuals with a first degree relative affected by FAD are included in the study, therefore all of the participants have a 50% risk of carrying a FAD mutation themselves. A small minority of the participants have opted for presymptomatic genetic testing, making a bias due to either MC or NC being more willing to participate highly unlikely. This natural "random assignment" of subjects to the MC or NC groups resulted in these groups being statistically comparable regarding the demographic variables presented in table 4; age, years to symptom onset, gender distribution and number of *APOE* ε4 carriers. It is interesting to note that the MC and NC groups included in papers I, II and IV all have a relatively high prevalence of *APOE* ε4 carriers, or around 50%, compared to the ~ 25% prevalence of *APOE* ε4 in the general population (178). The frequency of the *APOE* ε4 allele has been determined specifically in different areas of Sweden and found to be ~ 20% (289), thereby excluding the explanation that the high prevalence in the FAD family members is due to a generally high prevalence in Sweden.

	Paper I CSF		Paper I MRI		Paper II		Paper IV YKL-40		Paper IV Neurogranin	
	MC	**NC**	**MC**	**NC**	**MC**	**NC**	**MC**	**NC**	**MC**	**NC**
Number	10	12	13	20	19	17	14	17	11	14
Age	47 (9)	48 (11)	43 (12)	50 (14)	47 (12)	48 (10)	43 (10)	46 (12)	45 (8)	45 (13)
Years to onset	-7 (9)	-7 (12)	-9 (9)	-3 (12)	-8 (12)	-7 (10)	-12 (10)	-9 (12)	-10 (8)	-10 (13)
Gender (M/F)	8/2	8/4	10/3	11/9	14/5	10/7	11/3	10/7	9/2	10/4
APOE ε4 carriers	6	5	6	6	9	7	8	7	6	7
Normal cognition	10	12	13	20	13	17	14	17	11	14
MCI	-	-	-	-	3	-	-	-	-	-
AD dementia	-	-	-	-	3	-	-	-	-	-

Table 4: The demographics of the study population. Age and years to onset are presented as a mean with a standard deviation in parenthesis. CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; MC, mutation carriers; NC, non-carriers; M, male; F, female; MCI, mild cognitive impairment; AD, Alzheimer disease. There were no significant differences in any of the demographic variables when comparing the MC to the NC in any of the papers.

3.3 METHODS

The methods applied in papers I – IV are described in the respective papers in detail. In the studies involved in this thesis the main focus was on CSF biomarkers, with neuroimaging biomarkers also included in papers I and III.

3.3.1 Genetic analysis

The researchers performing the genetic analysis did not have access to any data linking DNA samples to specific subjects. Blood was drawn from each subject on two separate occasions for DNA analysis to ensure the reliability of the results.

3.3.1.1 Apolipoprotein E

The *APOE* genotyping was performed for single nucleotide polymorphisms (SNPs) rs7412 and rs429358 using TaqMan®, SNP Genotyping Assays (ABI, Foster City, CA, USA) according to manufacturer's protocol. The amplified products were run on a 7500 fast Real-Time PCR System (ABI, Foster City, CA, USA).

3.3.1.2 Mutation analysis in APP and PSEN1

Exons 16 and 17 in *APP* were sequenced to screen for the KM670/671NL (123) and the E693G mutations (135). To confirm the H163Y mutation in *PSEN1* exon 6 was sequenced (164). Finally, exon 5 was sequenced to confirm the I143T mutation in *PSEN1* (290). DNA was amplified using AmpliTaq Gold® PCR Master Mix (ABI, Branchburg, NJ, USA). Primer sequences and polymerase chain reaction (PCR) conditions are available upon request. Big Dye® terminator v3.1 Cycle sequencing Kit (ABI, Austin, TX, USA) was used for sequencing. The exons in *APP* and *PSEN1* were sequenced in both directions and analyzed on an ABI3100 Genetic Analyzer (ABI, Foster City, CA, USA).

3.3.2 Neuropsychological assessment

All of the participants underwent the same battery of 12 neuropsychological tests, employed by the same psychologist (from 1993 to the present day). The Information and Similarities tests (291) assessed verbal ability, the Block Design (291, 292) and Rey-Osterrieth copy (293) tests assessed visuospatial ability, Digit Span forward (291, 292) and Corsi Span (293) assessed immediate memory, Rey Auditory Verbal Learning total learning and 30 minutes retention as well as Rey-Osterrieth 30 minutes retention (293) tests assessed episodic memory, Trail making test part A (293) assessed attention; and Digit Symbol (291, 292) and Trail making test part B (293) assessed executive function. A measure of current global cognitive function was calculated using five tests: Information, Similarities, Block Design, Digit Span and Digit Symbol (291, 292). Premorbid global cognitive function was estimated using the Swedish New Adult Reading Test (NART) (294). All raw scores were converted to z-scores using a reference group of healthy adults from the Karolinska University Hospital at Huddinge (295).

3.3.3 CSF collection and analysis

The CSF samples were obtained in the time period between 1993 and 2015. A lumbar puncture was performed in the L3/L4 or L4/L5 interspace with the patient in a sitting position and the CSF was collected into polypropylene tubes. Most of the CSF was collected between 9:00 and 12:00, but on a few occasions, it was collected in the afternoon. Current evidence does not support a diurnal variation of either the core AD CSF biomarkers (296) or of YKL-40 and neurogranin (297). The participants received premedication with 1 g paracetamol and 5 mg diazepam prior to the procedure. Immediately after collection, the CSF was centrifuged at 3000 x g at + 4°C for 10 minutes. The supernatant was pipetted off, aliquoted into polypropylene cryotubes and stored at -80°C.

All of the biomarkers included in each of the studies were measured at the same time, using the same batch of reagents, at the Clinical Neurochemistry Laboratory at the Sahlgrenska University Hospital, Mölndal, Sweden by board certified laboratory assistants, blind to clinical data. All analytical procedures were performed according to protocols accredited by the Swedish Board for Accreditation and Conformity Assessment.

CSF Aβ42, Aβ40 and Aβ38 were analyzed by the electrochemiluminescence technology (Meso Scale Discovery, Gaithersburg, Maryland, USA), using the MS6000 Human Abeta 3-Plex Ultra-Sensitive Kit (the 6E10 version) (298). CSF total tau protein (T-tau) was determined using a sandwich enzyme-linked immunosorbent assay/ELISA (Innotest hTAU-Ag, Fujirebio Europe, Gent, Belgium) specifically constructed to measure all tau isoforms irrespective of phosphorylation status, as previously described (299), while P-tau (tau phosphorylated at threonine 181) was measured using the Innotest® phospho-tau 181P ELISA (Fujirebio Europe, Ghent, Belgium), as described previously in detail (300).

The β-secretase cleaved soluble APP (sAPPβ) and α-secretase cleaved soluble APP (sAPPα) in CSF were analyzed using the MS6000 Human sAPPalpha/sAPPbeta Kit, following the recommendations by the manufacturer, and as described previously (301). The *APPswe* mutation changes the neo-epitope recognized by the capturing antibody in the sAPPβ assay, making sAPPβ measurements unreliable in *APPswe* MC. Therefore, the *APPswe* MC were excluded from the sAPPβ analysis in paper II.

CSF neurogranin was measured using a sandwich ELISA, developed in-house at the Sahlgrenska Clinical Neurochemistry Laboratory as described previously in detail (302). CSF YKL-40 was measured using an YKL-40 ELISA kit, available from R&D Systems, Minneapolis, MN, USA.

3.3.4 Neuroimaging acquisition, processing and analysis

3.3.4.1 Magnetic resonance imaging

The MRI image data sets included in paper I were acquired on a Siemens whole-body clinical MRI 3T scanner (Magnetom Trio, Erlangen, Germany) equipped with a 12-channel phase-

array head coil. All of the participants underwent the same MRI protocol. A high-resolution 3D T1-weighted MPRAGE sequence image (T1W1) was acquired in sagittal plane (TR/TE=1780/3.42ms, inversion time=900ms, 192 sagittal slices, voxel size $1\times1\times1mm^3$, and flip angle=9°). Full brain and skull coverage was required for the MRI datasets and detailed quality control was carried out on all MR images according to previously published quality control criteria (303).

Cortical reconstruction and volumetric segmentation was performed using the FreeSurfer 5.1.0 image analysis suite (http://surfer.nmr.mgh.harvard.edu/), including removal of non-brain tissue (304), intensity normalization (305), tessellation of the boundary between gray and white matter, surface deformation following intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class (306, 307), registration to a spherical atlas (308) and creation of a variety of regional cortical and subcortical data. Results were visually inspected and manually edited if necessary in order to ensure accuracy of registration, skull stripping, segmentation and cortical surface reconstruction.

After image processing, volume measures and cortical-based measures (thickness and volume) of relevant regions of interest (see papers I and III) were selected for analysis, normalized by the subject's total intracranial volume (309). A circularly symmetric Gaussian kernel across the cortical surface was applied with a full width at half maximum (FWHM) of 15 mm to enable group analyses across the cortical mantle (paper I).

In paper III the available longitudinal MRI data was clinically rated by an experienced neuroradiologist using the MTA scale (236) on coronal reconstructions of the T1 sequence. Briefly, the degree of atrophy is scored from 0 (no atrophy) to 4 (end-stage degree of atrophy) in the hippocampus, parahippocampal gyrus, entorhinal cortex and the surrounding cerebrospinal fluid spaces. The scores were then interpreted using age-adjusted cut-offs as detailed elsewhere (243).

In this thesis, structural MRI results acquired as described above, are presented in papers I and III. Other parts of the FAD study have involved other MRI modalities, including diffusion tensor imaging and resting-state functional MRI to assess functional connectivity in the default mode network (310, 311).

3.3.4.2 Positron emission tomography

All PET examinations were performed at the Uppsala PET Centre, University of Uppsala, Sweden (see paper III for the timing of each PET examination in detail). The FDG scans from the 1990's were acquired on a GEMS 2048-15B (General Electric Medical Systems, WI, USA) scanner or a GEMS 4096-15WB scanner. All subsequent acquisitions (FDG and PiB scans) were performed on an ECAT EXACT HR+ (Siemens/CTI) or a Discovery ST PET/CT (General Electric) scanner (312). The mean injected dose was approximately 3 MBq/Kg for

FDG, and 4 MBq/Kg for PiB. Sum images were created for both FDG (30-45 min) and PiB (40-60 min), and used for subsequent image analyses.

For each modality and each participant, all the PET images were realigned and spatially normalized into a common MNI (Montreal Neurological Institute) space using a PET template (provided by SPM8 software) for FDG and a population-specific PiB-PET template (313) for PiB.

A grey matter mask was applied to a simplified probabilistic atlas (314) consisting of 12 bilateral regions of interest (ROIs). This atlas was then used for regional quantification of the PET tracers' uptake, expressed in standardized uptake value ratio (SUVr) units with the pons as reference region, as it has been found to be a reliable reference for metabolism (315) and amyloid quantification (316) both in sporadic and familial AD. All PET quantification analyses were repeated using the cerebellar grey matter as reference region. All processing steps were performed using Matlab and SPM8.

FGD- and PiB-PET data are presented in paper III of this thesis. There have been several other publications involving PET results from subjects in the FAD study, both using the aforementioned tracers and also involving the PET tracer DED (264, 312, 317).

3.3.5 Statistical analysis

The fact that FAD is a very rare disorder limits the number of individuals available for participation in studies on FAD and much of the literature on this disorder consists of small sample sizes. The study presented here is no exception, resulting in some statistical challenges due to the small number of participants.

A detailed account of the statistical analysis is presented in each paper. In short, the groups of MC and NC were compared regarding age, years to onset, gender and number of carriers of the *APOE* ε4 allele. The MC and NC groups were also compared regarding CSF biomarker levels, MRI measures and neuropsychological test measures. Finally, correlations were made between the levels of different CSF biomarkers in each group, as well as between the levels of CSF biomarkers, MRI and neuropsychological measures and years to onset.

The D'Agostino-Pearson normality test was used to assess the distribution of different variables. Normally distributed variables were compared between the MC and NC using unpaired t test, while variables that were not normally distributed were compared between the groups using the Mann-Whitney U test. Pearson correlations were applied to normally distributed data, while Spearman correlations were applied to data that was not normally distributed. Fisher exact test was used to compare categorical variables between groups. In paper IV, linear mixed models were applied to the longitudinal CSF biomarker data, as well as ANOVA to compare the longitudinal biomarker levels between the MC and NC.

Correction for multiple comparisons was made when relevant using the Benjamini-Hochberg false discovery rate (FDR) procedure (318).

3.3.5.1 Years to symptom onset calculations

Years to symptom onset is a variable which is used frequently in papers I – IV. As described previously, symptom onset is the time point when a subject develops the first clinically relevant cognitive symptoms. The mean age at symptom onset has been calculated for each of the families participating in the FAD study (see section 3.1). Using the mean age at symptom onset in the family of a given asymptomatic subject, and the actual age of the person in question, one can calculate how many years this particular subject has left to symptom onset. An example of this could be a 47-year old individual (person A) from the *APPswe* family, where the mean age of symptom onset is 54 years:

Years to onset for person A = 47 years – 54 years = - 7 years

This means that person A has 7 years left until expected onset of the first cognitive symptoms.

Another example could be a 58-year old from the *APPswe* family (person B):

Years to onset for person B = 58 years – 54 years = 4 years

Here the value for years to symptom onset is a positive one, i.e. person B is 4 years past the age of expected symptom onset.

Calculating years to symptom onset provides a timeline for disease progression with the debut of cognitive symptoms as a reference point. Using years to symptom onset allows for the estimation of when, in relation to the development clinical symptoms, a given biomarker starts to change. This makes the calculation of years to onset relevant for both MC and NC, even though one does not expect the NC to develop symptoms, as it is important to use the same timeline and point of reference for comparing biomarker changes between the NC and the MC.

3.4 ETHICAL CONSIDERATIONS

Informed written consent was acquired from all participants prior to any procedure. All study procedures were approved by the Regional Ethical Review Board in Stockholm, Sweden and adhered to the Declaration of Helsinki. Additional informed written consent was obtained for publishing the case-report presented in paper III, both from the asymptomatic MC and from the son of his brother, as the brother had already passed away from AD.

Obtaining informed consent from participants in studies on dementing illnesses can prove to be a challenge, as it can be unclear when a consent from a person with dementia is informed and when it is not. In the case of the Swedish FAD study the participants are recruited before

the onset of cognitive symptoms and can therefore be expected to understand the implications of their consent. When recruiting participants for a study on heritable diseases such as FAD, where disease modifying treatment is unavailable, it is also important to ensure that the researchers never take primary contact with possible participants, thereby making them aware of their increased genetic risk. The initiative must come from the subjects themselves, or their relatives, after they themselves have noticed that a particular illness runs in the family and want to know if there is a genetic cause. If a genetic cause is suspected, it is also important that the family members receive genetic counseling before, and in conjunction with, a search for a disease-causing mutation. The implications of finding such a mutation are wide and affect all members of the family, not just the one (or ones) seeking answers.

That researchers involved in studies on possible mutation carriers remain blind to the mutation status of the study participants is of great importance, except in instances when the participant himself/herself is aware of their status. It is also important that family members are never pressured by researchers to opt for genetic testing of any sort, i.e. the initiative must come from the person in question after having received genetic counseling. The researchers must also make sure that the mutation status of individual subjects cannot be deducted from published data, e.g. by keeping demographic data on gender and age on a group level to avoid subjects being able to identify themselves as either an MC or an NC.

Optimally, a study on individuals at risk for developing an illness such as early-onset AD should include counseling and psychosocial support for the study participants. Participants in the Swedish FAD study have all been offered such support in conjunction with their study visits. Interviews with a counselor involved in the study have emphasized the need for such support, especially for female participants, for those who have not yet passed the age at expected symptom onset and for spouses of individuals at risk (319, 320).

4 RESULTS

4.1 CSF BIOMARKERS

4.1.1 Absolute levels

The absolute levels of the CSF biomarkers included in the thesis are summarized in figure 4.

4.1.1.1 Aβ42, t-tau and p-tau

In paper I, the core AD biomarkers Aβ42, t-tau and p-tau were measured in 10 asymptomatic MC who were 47 years old on average and had a mean of 7 years left till the onset of clinical symptoms. The biomarker levels in the MC were compared to the levels of the same biomarkers in a group of 12 NC with a mean age of 48 years and a mean of 7 years left to the onset of symptoms. The levels of Aβ42 were significantly lower in the MC than the NC (729 ng/L vs. 1687 ng/L, $p = 0.0004$), while the levels of t-tau and p-tau were significantly higher in the MC than the NC (533 ng/L vs. 294 ng/L, $p = 0.03$ and 63 ng/L vs. 43 ng/L, $p = 0.03$, respectively). Here, the ratio of Aβ42 to p-tau was also compared between the MC and NC and found to be significantly lower in the MC (16 vs. 41, $p = 0.002$).

4.1.1.2 sAPPα, sAPPβ, Aβ38, Aβ40 and Aβ42

The APP processing products sAPPα, sAPPβ, Aβ38, Aβ40 and Aβ42 were measured and compared between a group of 19 MC and 17 NC in paper II. The *APPswe* mutation carriers were excluded from all the analyzes of sAPPβ, both as a part of the MC group as a whole and as an *APPswe* subgroup, due to the fact that the end-specific capture antibody in the sAPPβ assay does not react with sAPPβ modified at positions 670/671 by the *APPswe* mutation.

The MC had a mean age of 47 years and a mean of 8 years left to the onset of symptoms, while the NC were 49-years old on average and had a mean of 7 years left to symptom onset. The MC group included subjects with an MCI diagnosis (n=3) and subjects with a diagnosis of dementia due to AD (n=3). There was no difference in the levels of sAPPα, sAPPβ or sAPPα/sAPPβ when comparing the MC to the NC. However, all of the measured Aβ species, Aβ38, Aβ40 and Aβ42, were significantly lower in the MC group. Also, the Aβ2/Aβ40 ratio was significantly lower in the MC than the NC (0.05 vs. 0.11, $p < 0.0001$).

The calculations above were repeated after excluding all of the symptomatic MC, with the 13 remaining MC having a mean age of 42 years and an average of 12 years left to symptom onset. There was still no significant difference between the MC and NC on any of the demographic variables after excluding the symptomatic MC. The exclusion of the symptomatic MC did not change the biomarker results presented above, except for the levels of Aβ38 which were no longer significantly lower in the MC group.

Finally, the MC were divided into subgroups of MC by mutation, i.e. into a group of *APPswe* carriers, *APParc* carriers and *PSEN1* H163Y carriers (see table 5 for a summary of the direction

of the results). Here, the *APPswe* carriers had significantly lower levels of sAPPα and Aβ42 than the NC, but there was no difference in the levels of Aβ38 and Aβ40 between the *APPswe* carriers and the NC. The *APParc* carriers only had low levels of Aβ42 compared to the NC, but there were no differences in the levels of Aβ38 and Aβ40 when comparing the *APParc* carriers and the NC. The *APParc* carriers and the NC had comparable levels of sAPPα, but sAPPβ was significantly higher in the *APParc* carriers than in the NC. Finally, the *PSEN1* H163Y carriers had low levels of Aβ42 and Aβ38 compared to the NC, but there was no difference between the *PSEN1* H163Y carriers and the NC in the levels of Aβ40, sAPPα or sAPPβ. The ratio of Aβ42/ Aβ40 was significantly lower in all the MC subgroups than in the NC but there was no difference between any of the MC subgroups and the NC regarding the ratio of sAPPα/sAPPβ. These calculations were repeated after exclusion of symptomatic MC with no change to the results, except that the levels of Aβ40 were significantly lower in the asymptomatic *PSEN1* H163Y carriers than the NC.

The low levels of Aβ38 in the whole MC group and the *PSEN1* H163Y carrier subgroup did not survive correction for multiple comparisons and neither did the low levels of Aβ40 in the whole MC group.

	APPswe	*APParc*	*PSEN1* H163Y
sAPPα	↓	↔	↔
sAPPβ		↑	↔
Aβ38	↔	↔	↓
Aβ40	↔	↔	↔
Aβ42	↓	↓	↓

Table 5. Direction of changes in the levels of the APP processing products sAPPα, sAPPβ, Aβ38, Aβ40 and Aβ42 in the CSF of carriers of the *APPswe*, *APParc* and *PSEN1* H163Y mutations, compared to NC. Levels of sAPPβ were not available for the *APPswe* carriers. CSF, cerebrospinal fluid; NC, non-carriers.

4.1.1.3 YKL-40 and neurogranin

Paper IV included 14 MC (with a mean age of 43 years and a mean of 12 years left to symptom onset) and 17 NC (with a mean age of 46 years and a mean of 9 years left to symptom onset).

All of the participants in the study presented in paper IV were asymptomatic at baseline. There was no significant difference in the baseline levels of YKL-40 when comparing the MC to the NC. Due to depletion of some of the CSF samples included in paper IV neurogranin levels were only available from 11 MC and 14 NC. The levels of neurogranin were not significantly different between the two groups.

4.1.1.4 The outlier

During the study presented in paper I the presence of an outlier in the MC group came to our attention. This person had opted for a presymptomatic genetic test, which made his mutation status known to himself and to the researchers involved in the study. Even though he had passed the age at symptom onset in his family he was showing no signs of cognitive decline and had normal levels of the core AD biomarkers in the CSF. The statistical analyzes of biomarkers in the MC and NC in paper I and in subsequent papers were done with and without this outlier. Excluding him had no effect on the results of any of the different analyzes in any of the papers. The results in paper I are presented with and without the outlier, while the results presented in papers II and IV are only presented without the outlier.

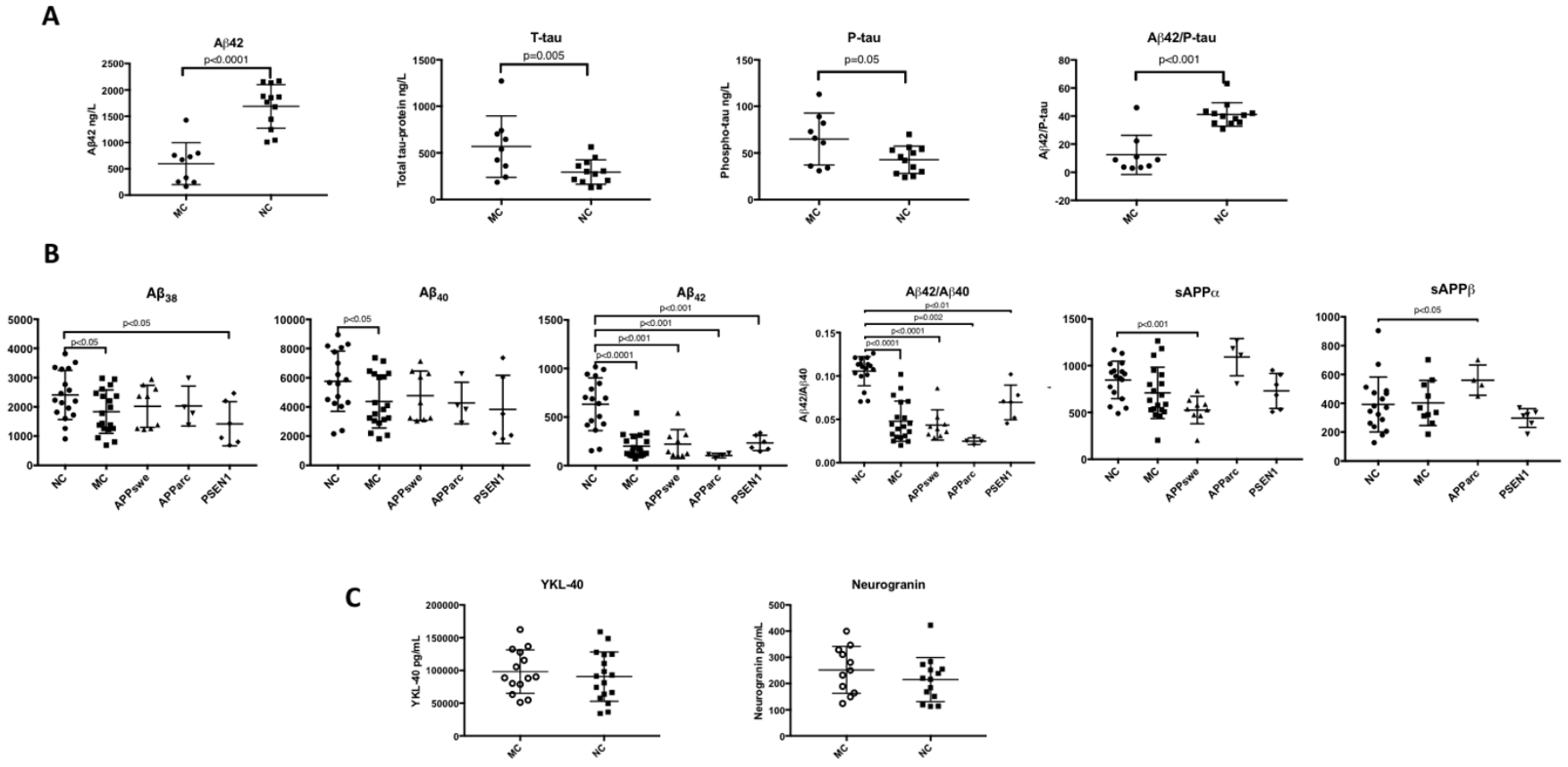

Figure 4. Absolute levels of CSF biomarkers when comparing A: Aβ42, t-tau, p-tau and the Aβ42/p-tau ratio between asymptomatic MC and NC, B: The APP processing products sAPPα, sAPPβ, Aβ38, Aβ40 and Aβ42 between MC and NC and between NC and subgroups of MC carrying either the *APPswe* mutation, *APParc* mutation or the *PSEN1* H163Y mutation, C: YKL-40 and neurogranin between asymptomatic MC and NC. CSF, cerebrospinal fluid; MC, mutation carriers; NC, non-carriers; APP, amyloid precursor protein.

4.1.2 Correlations between different biomarkers

In paper II, correlations were made between the levels of sAPPα, sAPPβ, Aβ38, Aβ40 and Aβ42. The *APParc* carriers were not included in the correlation calculations as a subgroup due to their small numbers (n=4) and as previously stated no data was available on sAPPβ in the *APPswe* carriers due to technical limitations. There was a positive and significant correlation between sAPPα and sAPPβ in both the MC and the NC, but not in the *PSEN1* H163Y subgroup. There also was a significant and positive correlation between Aβ38 and Aβ40 in all groups and between Aβ40 and Aβ42 in the MC, NC and in the *APPswe* subgroup. In the NC group there were positive correlations between sAPPα and all three Aβ species, but no such correlations were observed in the MC or the *PSEN1* H163Y subgroup. Finally, the NC group showed a positive correlation between Aβ38 and Aβ42, which was not seen in the MC or in the MC subgroups.

In paper IV, correlations were made between YKL-40 and neurogranin vs. the core AD biomarkers Aβ42, t-tau and p-tau. YKL-40 correlated positively and significantly with all three core AD biomarkers in the NC and with all but Aβ42 in the MC. The exact same pattern emerged for neurogranin, which correlated positively with Aβ42, t-tau and p-tau in the NC and with t-tau and p-tau in the MC.

All of the significant correlations presented above survived a correction for multiple comparisons.

4.1.3 Correlations with years to symptom onset

Correlations between CSF biomarkers and years to onset were made in papers I, II and IV. In paper I there was a trend of decreasing Aβ42 and Aβ42/p-tau ratio in the MC with approaching onset, while there was a trend of increasing t-tau and p-tau. The same trends were not observed in the NC. When comparing the MC to the NC, Aβ42 seemed to start decreasing in the MC around 20 years from symptom onset, the Aβ42/p-tau ratio around 15 years from symptom onset and t-tau and p-tau about 5 years from onset (see figure 5).

The correlation between the APP processing products sAPPα, sAPPβ, Aβ38, Aβ40 and Aβ42 and years to symptom onset was analyzed in paper II. There was no significant correlation between any of the aforementioned biomarkers and years to onset in either the MC group, NC group or the *PSEN1* H163Y subgroup. In the MC group however, there was a trend of sAPPα and Aβ42 decreasing with approaching symptom onset. Aβ38, Aβ40 and Aβ42 decreased significantly in the *APPswe* carriers when the onset of symptoms approached and beyond. These correlations lost their significance when *APPswe* carriers with a dementia diagnosis were excluded, apart from the decrease in Aβ42 which remained significant ($r = -0.85$, $p = 0.03$), see figure 6.

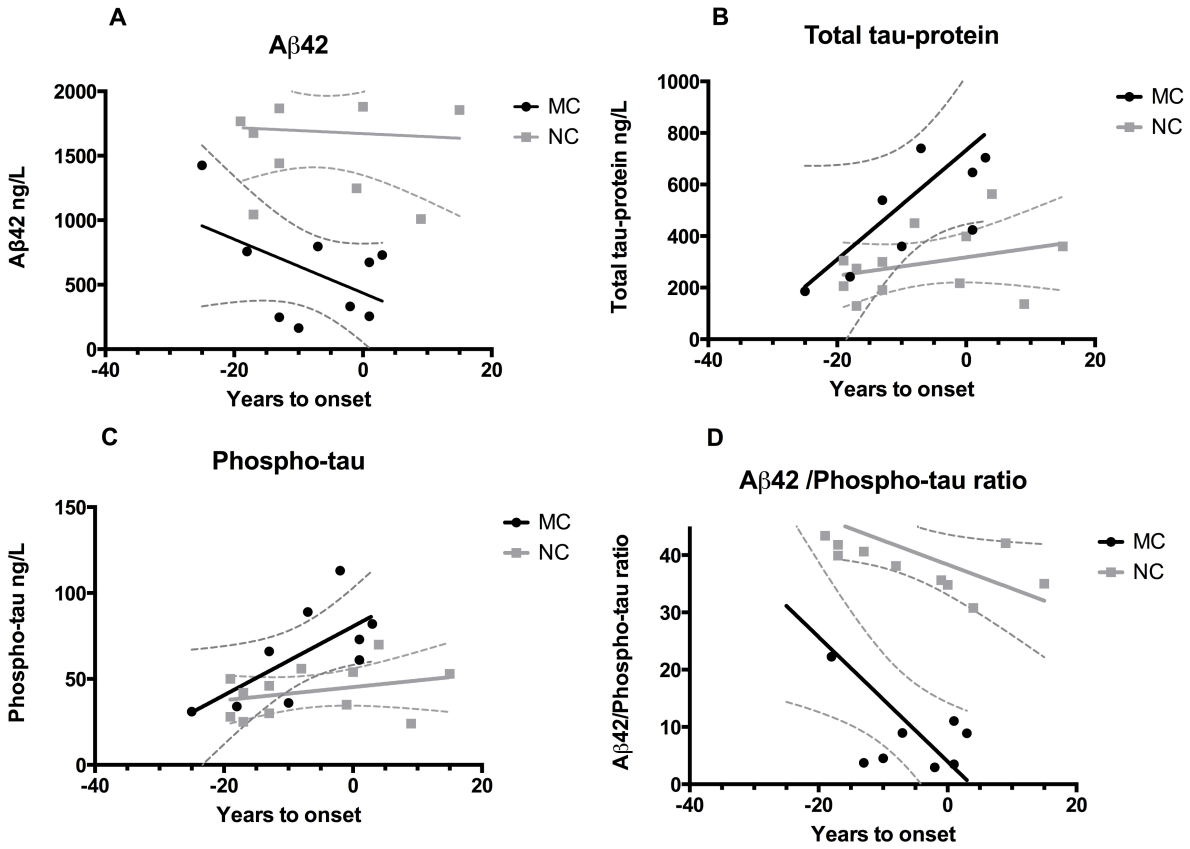

Figure 5. Relationship between the core AD biomarkers and years to symptom onset. The solid lines represent Spearman correlations, while the dashed lines represent 95% confidence intervals (CI´s) for the correlations.

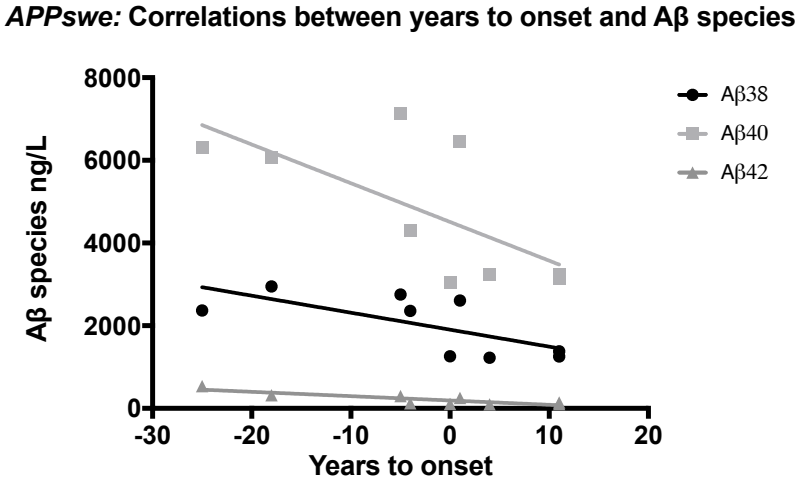

Figure 6. Correlations between years to onset and the levels of Aβ38, Aβ40 and Aβ42 (in ng/L) in carriers or the *APPswe* mutation. All three correlations were significant, $A\beta_{38}$ (r=-0.69, p=0.04), $A\beta_{40}$ (r=-0.67, p=0.05) and $A\beta_{42}$ (r=-0.86, p<0.01), decreasing in subjects sampled closer to the expected onset.

In paper IV, YKL-40 and neurogranin were correlated with years to onset in asymptomatic MC and NC. YKL-40 correlated significantly and positively with years to onset in both the MC and the NC, while no correlation was found between neurogranin and years to onset in either the MC or the NC group. These results (as the others presented above) were obtained from cross-sectional data. When the cross-sectional levels of YKL-40 and neurogranin were correlated with years to onset and plotted in a graph in the same way as the core AD biomarkers in paper I (see figure 5) it was not possible to visually gauge a separation between the MC and NC curves for YKL-40 (figure 7).

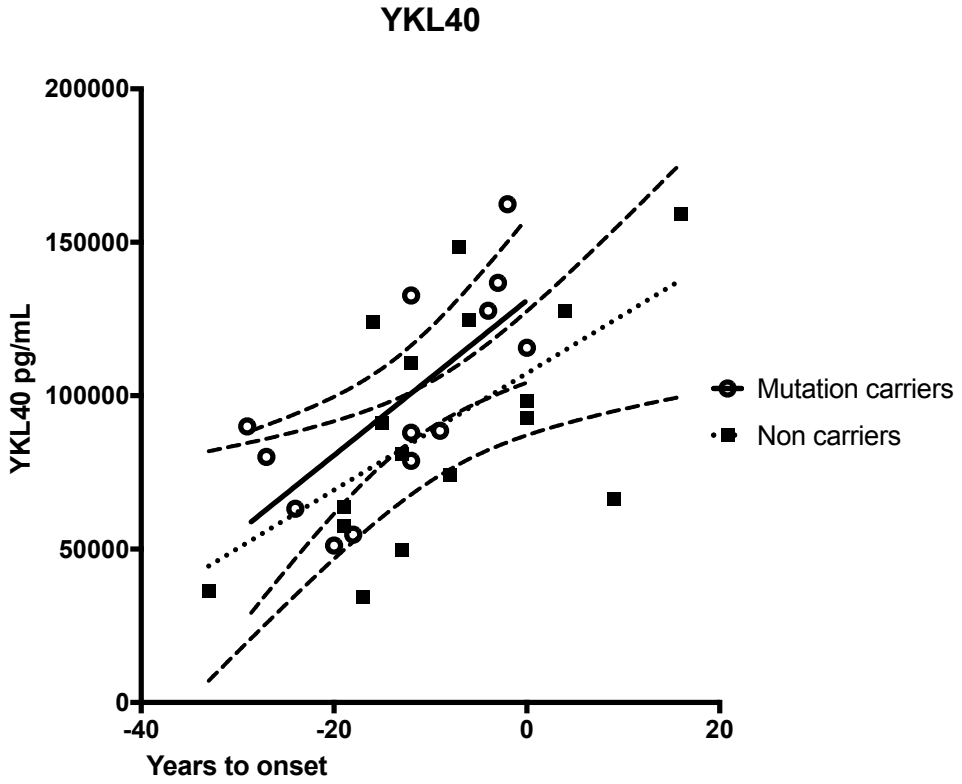

Figure 7. Correlations between YKL-40 and years to onset in MC and NC. The solid line represents the correlation between YKL-40 and years to onset in the MC ($r = 0.6817$, $p = 0.007$) while the dotted line represents the same correlation in the NC ($r = 0.6007$, $p = 0.01$). The dashed lines represent the 95% CI´s for the correlations.

4.1.4 Longitudinal analysis

Follow-up samples were available to allow for a longitudinal analysis of the CSF biomarkers YKL-40 and neurogranin in paper IV. YKL-40 was analyzed in longitudinal samples from 9 MC and 5 NC with a mean of 5 and 6 years from baseline till the first follow-up sampling respectively. Ten of these participants had one follow-up sample, while 4 participants had two follow-up samples. At the first follow-up sampling occasion four of the MC had developed MCI and at the second follow-up sampling occasion one more MC had developed MCI. Neurogranin was analyzed longitudinally in 8 of the 9 MC and in all of the 5 NC, as one of the MC samples did not suffice to analyze neurogranin as well.

There was a significant positive correlation between the longitudinal YKL-40 levels and years to onset ($F = 25.1$, $p < 0.001$) and the curve was steeper for the MC than for the NC ($F = 5.54$, $p < 0.03$).

4.2 NEUROIMAGING BIOMARKERS

MRI of the brain was included in paper I, where cortical thickness and volumetric measures were compared between a group of 13 MC and 20 NC. The mean age of the MC was 43 years, with a mean of 9 years left to symptom onset, while the mean age of the NC was 50 years, with a mean of 3 years left to symptom onset. After FDR correction for multiple comparisons there remained a significant decrease in the volume of the left precuneus, left superior temporal gyrus and left fusiform gyrus in the MC (see figure 8). There were no significant differences between the MC and NC regarding cortical thickness in either hemisphere or volumes in the right hemisphere. There were no significant correlations between the MRI variables and years to symptom onset in the MC. None of the aforementioned results changed after exclusion of the statistical outlier presented earlier in the results chapter.

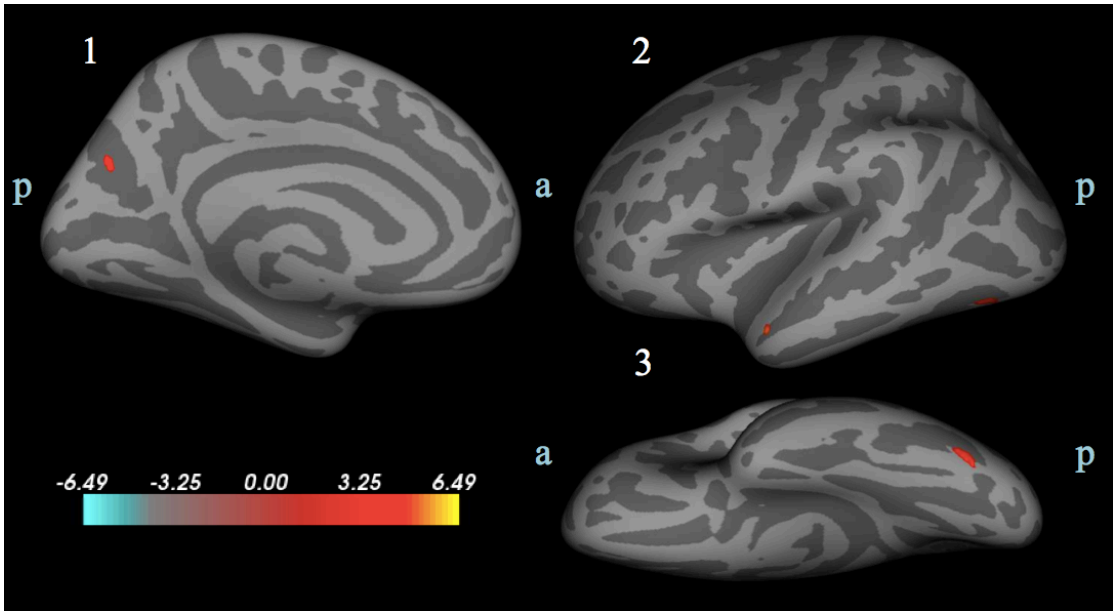

Figure 8. Regions in the left hemisphere with reduced volume in the MC are indicated in color. 1) Left hemisphere, medial surface; 2) Left hemisphere, lateral surface; 3) Left hemisphere, ventral surface; p: posterior; a: anterior. Brains are "inflated" in the three images to better represent the regions inside the sulci. Red-yellow represents less volume in the MC and blue represents more volume in the MC (see paper I for Talairach coordinates of the areas with decreased volume in the MC).

4.3 NEUROPSYCHOLOGICAL ASSESSMENT

In paper I, a neuropsychological assessment was performed on all 35 participants within 3 months of the other examinations. The z-scores of the MC in five cognitive domains; verbal, visuospatial, immediate memory, episodic memory and attention/executive function, were compared with the z-scores of the NC. There were no significant differences between the MC

and the NC in any of the five domains, however there was a trend of poorer results in the MC regarding immediate memory, episodic memory and attention/executive function (see paper I). In the MC group, there were significant and negative correlations between years to onset and three cognitive domains; visuospatial ($r = -0.54$, $p < 0.05$), episodic memory ($r = -0.70$, $p < 0.01$) and attention executive function ($r = -0.61$, $p < 0.05$). In the NC, the same significant and negative correlation was seen regarding years to onset and attention/executive function ($r = -0.50$, $p < 0.05$).

4.4 BIOMARKERS IN PAPER III

In paper III, biomarkers in the CSF (Aβ42, t-tau and p-tau) and neuroimaging biomarkers (MRI, FDG-PET and PiB-PET) as well as neuropsychological tests were combined to characterize a case of reduced penetrance of the *PSEN1* H163Y mutation. This case came to our attention during the preparation of paper I, where a carrier of the *PSEN1* H163Y mutation who was aware of his mutation status, turned out to be a statistical outlier in the MC group with normal levels of the core AD CSF biomarkers, despite having passed the mean age at symptom onset in his family. This outlier, as well as his brother who was one year older and also a mutation carrier, were a part of the FAD study over a period of 22 years. During this period both brothers underwent multiple clinical assessments and biomarker collections (see paper III). The outlier was 65 years old at the end of follow-up (in 2017), 14 years older than the mean age at symptom onset in his family, and did not show any signs of cognitive decline. He had normal levels of CSF Aβ42, t-tau and p-tau at the age of 54, no signs of atrophy in the medial temporal lobe on MRI at the age of 57 and no definite signs of increased PiB retention on PET at the age of 60. His brother was diagnosed with MCI at the age of 55 and dementia due to AD a year later, at the age of 56. He showed typical biomarker signs of AD in the CSF, on MRI, FGD-PET and PiB-PET and a curvilinear decline in cognitive function on repeated neuropsychological tests. He was admitted to a nursing home due to dependence on others for all activities of daily living and died there at the age of 64. On autopsy, he fulfilled the neuropathological criteria for definite AD.

5 DISCUSSION

5.1 PAPER I

The AD biomarker studies in papers I, II and IV all showed significant changes in CSF and neuroimaging biomarkers in the preclinical stage of FAD. In paper I, which only included presymptomatic MC, CSF Aβ42 was decreased and CSF t-tau and p-tau increased in MC who had a mean of 7 years left till the onset of symptoms. In this study the MC (with a mean of 9 years till symptom onset) also had decreased volume of the left precuneus, left superior temporal gyrus and left fusiform gyrus on MRI and a trend of poorer results than the NC on neuropsychological tests assessing immediate memory, episodic memory and attention/executive function. Despite the relatively small number of participants in the study the biomarker changes in the MC were robust enough to show a significant separation from the NC, as early as 7 – 9 years before the expected onset of symptoms.

When the CSF biomarkers were correlated with years to onset there was a trend of Aβ42 and the Aβ42/p-tau ratio decreasing and t-tau and p-tau increasing as the symptom onset approached in the MC, but not in the NC. The 95% CI's for these trends showed a separation between the Aβ42 curves of the MC and NC around 20 years before symptom onset, the 95 % CI's for the Aβ42/p-tau ratio separated around 15 years before symptom onset and around 5 years before symptom onset for t-tau and p-tau. These results are merely trends which are based on cross-sectional data and do not allow for an exact estimation of the time point at which a given CSF biomarker starts to change in MC in the preclinical stage of AD. However, they give an indication of the temporality of these changes which warrants further study.

The findings presented above have been corroborated in studies involving different populations of FAD MC. Generally, CSF Aβ42 seems to be the biomarker to show the earliest changes in preclinical FAD, followed by an increase in t-tau and p-tau some years later, sometimes years before the onset of symptoms and sometimes coinciding with them (38, 321, 322). A recent study on cognitively healthy carriers of the APOE ε4 allele also showed a decline in CSF Aβ42 starting about a decade before a similar decline in non-carriers (323). Interestingly, the studies on CSF biomarkers in cognitively healthy individuals at increased genetic risk for developing AD have not been entirely unequivocal. A study on young carriers of the *PSEN1* E280A mutation showed an *increase* in CSF Aβ42 which was observed 20 years before the expected onset of symptoms (36). This might be due to increased Aβ42 production, initially reflected in high Aβ42 levels in the CSF, which later would be expected to plummet due to aggregation of Aβ42 in the brain.

Structural MRI biomarkers in preclinical FAD have been extensively studied with somewhat variable results. One study reported a decrease in bilateral hippocampal volumes in asymptomatic MC 15 years before the expected onset of symptoms (38) while another study

found the same atrophic changes 6 years before onset (321). Hippocampal atrophy in MC starting even closer to the onset of symptoms has also been reported, with the atrophy being more pronounced on the left side (324). Yet another study found no cortical thinning or hippocampal atrophy in non-demented MC (325). Finally, a longitudinal study on hippocampal and whole brain atrophy rates in FAD found both rates to be increased in MC 5.5 and 3.5 years prior to AD diagnosis respectively (326). In the study presented in paper I we found no hippocampal atrophy when comparing the presymptomatic MC to the NC. This might be due to a true absence of atrophy in this area in our study group, but this might also be due to other factors such as the relatively small sample size. Another possible explanation could be that even though the difference in mean years to symptom onset was not significant between the MC and NC (9 years vs. 3 years), the fact that the NC were 6 years closer to symptom onset could have masked some differences between the groups.

The areas in which the MC displayed increased atrophy on MRI in paper I, the left precuneus, left superior temporal gyrus and left fusiform gyrus, are generally in agreement with areas of atrophy reported in other studies. In a study comparing early-onset AD to late-onset AD the patients with the early-onset exhibited grey matter atrophy in the hippocampus, temporal lobes, precuneus, cingulate gyrus and inferior frontal cortex, while the atrophy was most pronounced in the hippocampus, right temporal lobe and cerebellum in the patients with late-onset (327). In a longitudinal study on FAD focusing on other ROI's besides the hippocampus a decrease was reported in the cortical thickness of the precuneus in MC 4.1 years prior to AD diagnosis (328). These results are in agreement with our observation of decreased volume of the left precuneus in the presymptomatic MC in paper I. The precuneus, along with the posterior cingulate and temporoparietal regions, have been shown to have an *increased* cortical thickness and volume in carriers of FAD mutations with a mean of 16 years left to symptom onset. A longitudinal part of this same study showed an increase in the rate of cortical thickness loss in the precuneus-posterior cingulate and superior parietal, right lateral temporal and left orbitofrontal, and middle frontal regions in the presymptomatic MC (329). These results might imply an initial increase in volume and cortical thickness due to inflammation and/or accumulation of Aβ, followed by atrophy in the same areas (329, 330). Finally, the asymmetry in our findings, with the significant volume reduction in the MC being limited to the left hemisphere, both regarding the precuneus and areas in the temporal lobe, does not come as a surprise. Several studies on AD have shown earlier signs of cortical atrophy in the left hemisphere than the right, as well as faster rates of atrophy progression on the left side (331, 332).

5.2 PAPER II

In paper II we assessed CSF levels of sAPPα, sAPPβ, Aβ38, Aβ40 and Aβ42, all products of APP processing. Even though most of the MC in this study were asymptomatic we also included three MC with an MCI diagnosis and a further three with a diagnosis of dementia due to AD. We then divided the MC into subgroups, both a presymptomatic subgroup and subgroups of MC carrying the same mutation (*APPswe*, *APParc* or *PSEN1* H163Y). In contrast

to the biomarkers in paper I, which only included presymptomatic MC, the biomarkers in paper II have been much less extensively studied (apart from Aβ42) and we therefore found it of interest to also include symptomatic MC in paper II. Furthermore, dividing the MC into subgroups by mutation was more interesting in this case, as each of these three mutations is believed to affect the processing of APP differently. This was done with full awareness of the fact that each MC subgroup would be very small which would lead to the results having to be interpreted with caution. The *APParc* group only included 4 MC and therefore no correlation calculations were made solely in that group. Finally, no analysis was made on the levels of sAPPβ in the *APPswe* carriers, as the *APPswe* mutation changes the neo-epitope which is recognized by the capturing antibody of the sAPPβ assay, making the results of this assay unreliable in the case of these particular MC.

Before the analyzes of the APP processing products were made we hypothesized that each of the three mutations included in the study would have a different effect on their levels. The *APPswe* mutation is located in APP, at the cleaving site of BACE1, and is believed to increase the affinity of BACE1 for the mutated APP. Therefore, we assumed that the mutation would cause an increase in sAPPβ, Aβ38, Aβ40 and Aβ42 (all products of the amyloidogenic pathway of APP processing in which BACE1 is involved) and a decrease in sAPPα. The *APParc* mutation is located within the Aβ sequence of APP and increases the propensity of Aβ to form fibrils. This mutation would not be expected to affect the levels of sAPPα or sAPPβ, but decrease the levels of Aβ42 (and possibly also of Aβ38 and Aβ40), due to accelerated fibril formation. Figure 2 illustrates the locations of the *APPswe* and *APParc* mutations in the APP sequence. Finally, the *PSEN1* H163Y mutation is located in the third transmembrane domain of the γ-secretase and causes the γ-secretase to preferentially produce Aβ42 at the expense of Aβ40, thereby increasing the Aβ42/Aβ40 ratio, but not the overall production of Aβ. Here we would expect to see normal levels of sAPPα and sAPPβ and low levels of Aβ38 and Aβ40, due to a shift towards the production of Aβ42. It is well known that the levels of Aβ42 in the CSF are inversely related to the levels of Aβ accumulation in the brain and this makes the interpretation of CSF Aβ42 levels quite challenging. That is, low Aβ42 levels in the CSF do not necessarily reflect low Aβ42 production. This could also apply to the other Aβ species studied in paper II and should be kept in mind when interpreting the results.

When all of the MC were compared to the NC in paper II the MC had significantly lower levels of Aβ38, Aβ40 and Aβ42 than the NC. No difference was found in the levels of sAPPα or sAPPβ when comparing the MC to the NC. The results were the same after excluding the six symptomatic MC, except for the levels of Aβ38, which were no longer significantly lower in the MC. Finally, the lower levels of Aβ40 and Aβ38 in the whole MC group did not survive an FDR correction for multiple comparisons. Therefore, the whole MC group only displayed low levels of Aβ42, with no significant difference between them and the NC regarding the other APP processing products. One could argue that an FDR correction increases the risk of a type II error in this case, as the sample is very small and the number of comparisons between

the groups is limited and hypothesis driven. Therefore, it is not impossible that a true difference in Aβ38 and Aβ40 levels exists between the whole MC group and the NC. The Aβ42/Aβ40 ratio was also compared between the MC and the NC and perhaps not surprisingly was found to be significantly lower in both the MC group as a whole, and the MC subgroups, than in the NC. These significant differences remained significant after excluding the symptomatic mutation carriers, supporting the role of the Aβ42/Aβ40 ratio as a robust biomarker of early AD pathology.

When comparing only the *APPswe* carriers to the NC they had significantly lower levels of sAPPα and Aβ42 than the NC (see table 5 for a summary of the changes in the APP processing products in each subgroup of MC). The decreased levels of Aβ42 are as expected in carriers of FAD mutations in general, as Aβ42 is known to be low in the CSF early in the AD disease process. The low sAPPα levels, which have also been shown to be low in a previous study on some of the same *APPswe* carriers (333), could be due to a shift towards the amyloidogenic pathway of APP processing, at the expense of the non-amyloidogenic pathway of which sAPPα is a product. The *APParc* carriers also showed the expected decrease in Aβ42 as well as an increase in sAPPβ, but there was no difference in the levels of Aβ38 and Aβ40 when comparing the *APParc* carriers to the NC. This does not support the hypothesis that the mutated Aβ38 and Aβ40 have an increased propensity to form fibrils, in the same way as the mutated Aβ42, as Aβ38 and Aβ40 would then be expected to be scarce in monomeric form and their levels low in the CSF. The increase in sAPPβ does come as somewhat of a surprise, as the *APParc* mutation is not believed to have an effect on the balance between the amyloidogenic and non-amyloidogenic pathways of APP processing. One could speculate that a relative shortage of Aβ42, due to the accelerated fibril formation, can increase the activity of the amyloidogenic pathway causing an increase in sAPPβ production. Finally, the *PSEN1* H163Y carriers had low levels of Aβ38 and Aβ42, but normal levels of the other APP processing products. The low Aβ38 levels did not survive a correction for multiple comparisons, however there is a definite trend of Aβ38 being lower in the *PSEN1* H163Y carriers than in the other mutation carriers and the NC (see figure 4). As stated previously, the results from *in vitro* studies do not suggest that the *PSEN1* H163Y mutation affects the balance between the amyloidogenic and non-amyloidogenic pathways, making the findings of normal sAPPα and sAPPβ levels as expected. The low Aβ42 levels are also as expected and the low Aβ38 levels as well, with the latter possibly due to a loss of function of the mutated γ-secretase, causing it to be less able to cleave APP sufficiently often to produce a short Aβ species like Aβ38. Another possible explanation could be that Aβ38 is accumulating in the brain, causing a shortage in the CSF, as amyloid plaques including Aβ38 deposits have been demonstrated in FAD cases (334).

When looking at the sAPPβ/ sAPPα ratio, no significant differences emerged when comparing the MC, or the subgroups of *APParc* and *PSEN1* H163Y carriers, to the NC. The sAPPβ/ sAPPα ratio has been suggested as a biomarker of brain amyloidosis (335), but our results do not support this, at least not that the sAPPβ/ sAPPα ratio can serve as a marker of early

pathology. The correlation between sAPPβ and sAPPα was significant and positive in both the MC and the NC, which indicates that the amyloidogenic pathway and the non-amyloidogenic pathway are noncompetitive. These findings of a positive correlation between sAPPβ and sAPPα have been reported in several previous studies on subjects with MCI, FAD and SAD (335-340). The positive correlation between sAPPβ and sAPPα, and the assumed noncompetitiveness of the two APP processing pathways, can possibly explain the failure of the sAPPβ/ sAPPα ratio do differentiate between the MC and the NC. When looking at correlations between the three Aβ species on one hand and sAPPβ and sAPPα on the other hand we found that all three Aβ species were positively correlated to sAPPα in the NC. This might seem counterintuitive as sAPPα and the Aβ species are not products of the same APP cleaving pathway. However, high levels of Aβ species in the CSF, at least of Aβ42, are considered to reflect an absence of brain amyloidosis, and sAPPα could also be expected to be higher in individuals without Aβ accumulation in the brain than in patients with preclinical AD. The Aβ species were then correlated with each other, revealing a positive correlation between Aβ38 and Aβ40 and between Aβ40 and Aβ42 in both the MC and the NC. This suggests that a decrease in one Aβ species in the CSF is accompanied by a decrease in the others. Based on this one can speculate that when the threshold for Aβ accumulation in the brain is reached it involves all of the Aβ species measured here, which then aggregate in the brain parenchyma and vasculature, causing a reduction of their levels in the CSF.

In the final part of paper II, sAPPα, sAPPβ, Aβ38, Aβ40 and Aβ42 were correlated with years to onset in the MC, NC and the MC subgroups. There was no difference between the levels of sAPPα and sAPPβ in the MC and the NC and no change was seen in these markers related to proximity to symptom onset. The levels of Aβ38, Aβ40 and Aβ42 were lower in the MC than in the NC, but did not decrease as onset approached, perhaps as they had already become relatively stable at their low levels in the MC. The only significant correlations between the Aβ species and years to onset were seen in the subgroup of *APPswe* carriers, consisting of 9 individuals, of whom 2 had an MCI diagnosis and 3 had a dementia diagnosis. These correlations ceased to be significant for Aβ38 and Aβ40, but not for Aβ42, after exclusion of the subjects with dementia. These findings indicate that a decrease in Aβ38 and Aβ40 levels might be most pronounced in the symptomatic stages of AD, but only longitudinal studies involving more subjects can determine if this is in fact the case. This resonates somewhat with a study on several CSF biomarkers, including sAPPα, sAPPβ and Aβ40, which showed a decrease in these markers in patients in the advanced stages of clinical AD (341). Therefore, there is a possibility that sAPPα, sAPPβ and Aβ40, as well as Aβ38, can serve as markers of disease severity rather than as early diagnostic markers.

5.3 PAPER IV

There is a large interest in developing novel CSF biomarkers for AD, both early diagnostic markers and markers of disease progression and severity. In paper IV we included two such markers, YKL-40 and neurogranin, which represent different pathophysiological processes

believed to have an involvement in AD. YKL-40 is a glycoprotein, also known as chitinase-3-like protein, which is expressed by several cell types, including glial cells and neurons in the central nervous system (CNS) (342-344). The functions of YKL-40 have not yet been fully elucidated, but include the regulation of inflammatory responses (345), promotion of cell proliferation and migration (346) and enhancement of tumor growth, angiogenesis and macrophage infiltration (347). Neurogranin is a calmodulin-binding protein, found mainly in the dendritic spines of neurons in the association cortex of the brain (348, 349). It is a postsynaptic protein involved in synaptic plasticity and is believed to be able to serve as a synaptic marker (350, 351). Both YKL-40 and neurogranin have been shown to be able to differentiate between patients with dementia due to AD and healthy controls (352-362) as well as between subjects with stable MCI and subjects with MCI who later develop dementia (354, 358, 361-363).

The study presented in paper IV was the only biomarker study in this thesis to include longitudinal data as well as cross-sectional data. Only presymptomatic subjects were included in the cross-sectional part of the study, while five of the MC in the longitudinal part of the study developed MCI during follow-up. Due to some of the samples being low in volume, a few samples were depleted after the YKL-40 assay, resulting in YKL-40 levels being available from slightly more subjects than neurogranin levels (see table 4). The YKL-40 MC group had a mean of 12 years left till symptom onset, while the neurogranin MC had a mean of 10 years left till symptom onset. No significant differences were found when comparing the absolute levels of YKL-40 and neurogranin between the MC and the NC. Even though the MC group was quite far from expected symptom onset in this study, they still had significantly lower levels of Aβ42 and higher levels of t-tau and p-tau than the NC, indicating that they were past the initial stages of preclinical AD and had started to exhibit markers of amyloid pathology and neurodegeneration. That YKL-40 and neurogranin did not differentiate between the MC and the NC at this stage indicates that neither YKL-40 nor neurogranin can be considered to be early preclinical biomarkers of AD based on these results. The results do not contradict the results of a recent study which found no change in the levels of CSF YKL-40 in individuals with low CSF Aβ42 and normal cognition, indicating that they were in the preclinical stage of AD. The same study found high YKL-40 levels in individuals with prodromal AD, i.e. with low CSF Aβ42 and high t-tau and p-tau as well as subtle memory deficits (364). Contrary to this, neurogranin has been shown to be high in cognitively healthy individuals who later experienced a decline in cognition, as well as in healthy older subjects (with a mean age of 83 years) with low Aβ42 levels, indicative of preclinical AD (365). Why no such changes in neurogranin were observed in the study in paper IV is unclear, but the possibility exists that this is due to the small sample size.

YKL-40 and neurogranin correlated positively with t-tau and p-tau in the MC, but no correlation was observed between Aβ42 and either YKL-40 or neugranin in the MC group. Aβ42 has been shown to decrease early, and non-linearly, in preclinical AD, and stay relatively stable and low throughout the course of the disease (36-38, 366), which can explain the lack of

correlation with Aβ42. The positive correlation of YKL-40 and neurogranin with t-tau and p-tau in the MC could be indicating that these two new biomarkers are reflecting aspects related to the neurodegeneration and tau hyperphosphorylation represented by t-tau and p-tau. Interestingly, YKL-40 and neurogranin correlated positively with t-tau, p-tau *and* Aβ42 in the NC. The positive correlations with t-tau and p-tau could be explained by some of the NC finding themselves in the preclinical stage of AD, as the NC group is not exempt from developing SAD even though they do not carry a disease-causing mutation. One could thereby assume that YKL-40 and neurogranin are reflecting other pathophysiological changes related to preclinical AD in the NC group. However, the fact that YKL-40 and neurogranin correlated positively with Aβ42 as well, contrary to what one would expect in preclinical AD, makes the matter more complicated and harder to interpret.

YKL-40 correlated positively with years to symptom onset in both the MC and the NC, while no such correlation was found for neurogranin. Based on these results it is not possible to link either biomarker to early AD progression and this even suggests that YKL-40 might be an unspecific marker of the aging process. The longitudinal data on YKL-40 also showed an increase in YKL-40 levels as the age at symptom onset approached, with a steeper increase in the MC group. This suggests that YKL-40 might be a marker of a process related to normal aging which is exacerbated by a concomitant AD pathology. The same age dependent increase in CSF levels of YKL-40 has been observed previously in cognitively healthy middle aged individuals, with a sharper increase in carriers of the *APOE* ε4 allele than in non-carriers (366). An increase in YKL-40 has been reported in AD dementia as well as in frontotemporal dementia and YKL-40 has also been associated with inflammatory processes in the CNS, such as multiple sclerosis (367, 368). Finally, YKL-40 expression has been shown to be markedly increased in astrocytes in the acute phases of cerebral infarction (368), further underpinning its possible role as an unspecific marker of CNS damage and aging.

There were no signs of CSF neurogranin increasing over time in either the MC or the NC according to the longitudinal analysis. It is the rule, rather than the exception, that brains of elderly individuals exhibit multiple pathologies on autopsy, including loss of synapses (369). Here we have a young group of subjects who we suspect are mostly free of comorbidities which potentially could contribute to an increase in CSF neurogranin. From our results, it seems that preclinical AD alone is not enough to cause an increase in neurogranin, but if you add other comorbidities you might get a synergistic effect explaining the neurogranin increase seen in cognitively healthy elderly assumed to be in the preclinical stage of AD (365).

In conclusion, the study presented in paper IV does not indicate that YKL-40 or neurogranin are early preclinical biomarkers of AD. However, there is a positive correlation between the levels of YKL-40 and increasing age, which is more pronounced in patients progressing from preclinical to symptomatic AD than in healthy controls.

5.4 PAPER III

During the preparation of papers I and II the existence of a statistical outlier in the MC group came to our attention. This person, a carrier of the *PSEN1* H163Y mutation, had normal levels of the CSF biomarkers Aβ42, t-tau and p-tau, despite having passed the mean age of symptom onset in his family by 3 years. He was aware of his mutation status as he had opted for a presymptomatic genetic test and gave an informed written consent to the publication of a case report describing his clinical history and biomarker findings. In order to gain a clearer perspective on his case we included his brother, also a carrier of the *PSEN1* H163Y mutation, in the case report. His brother (referred to as brother A) was one year older than the outlier (brother B) and both brothers were enrolled in the FAD study in 1995 (at the ages of 43 and 44), undergoing regular follow-ups within the study for the next 22 years. We considered brother A to be a good control for brother B as the brothers were brought up in the same household and lived their lives in the same area. They also had similar levels of education (see paper III for a thorough description of the brothers' backgrounds and clinical history).

During the 22-year follow-up from 1995 to 2017 brother A developed dementia due to AD and finally passed away in a nursing home in 2017. Biomarkers in the CSF, on FDG-PET and PiB-PET and on MRI all pointed to an underlying AD pathology and he experienced a typical cognitive decline on neuropsychological tests. Finally, the AD diagnosis was confirmed after a brain autopsy in 2017. He received a diagnosis of MCI at the age of 55, 4 years past the mean age of symptom onset in the family and a diagnosis of AD dementia a year later. He already had abnormal levels of CSF Aβ42 11 years before the MCI diagnosis, at the age of 44. The same year he received the MCI diagnosis he had a decreased FDG uptake in the parahippocampus on PET and two years later this decrease was much more widespread. Increased PiB uptake was detected in all ROI´s except the thalamus 2 years after the MCI diagnosis (in 2008) and hippocampal atrophy on MRI 3 years after the MCI diagnosis. No presymptomatic PiB-PET examinations were available for brother A, but the widespread brain amyloidosis observed in 2008 leads to the suspicion that it had been present for some period of time before this examination.

In contrast to brother A, brother B did not develop any definite signs or symptoms of AD during the 22-year follow-up. He had normal core AD biomarker levels in the CSF at the age of 54, 3 years past the mean age at symptom onset in his family. He did not show signs of decreased FDG uptake on any of the FDG-PET examinations he underwent, the latest in 2009 (6 years past the mean age at symptom onset). Also, there was no detectable increase in PiB uptake on PET 9 years past the mean age at symptom onset (in 2012), using the grey matter of the pons as a reference. If the cerebellar grey matter was used as a reference however, there was a slightly elevated uptake of PiB in the posterior cingulate and the thalamus in 2009 and in both regions plus the anterior cingulate in 2012. This could indicate a possible incipient amyloid deposition. If that were the case one can assume the increased PiB uptake to be a very early sign of preclinical AD in brother B, as it is usually detected years or decades before the onset of the first cognitive symptoms. Brother B did not display any signs of cognitive decline on

repeated neuropsychological assessments, the last of which was made in 2017, 14 years past the expected onset of symptoms. If the PiB-PET results are a sign of the earliest stages of preclinical AD one would not expect brother B to develop symptoms for some years to come, probably not before he reaches the domain of late onset AD.

The case of brother B is very interesting as no cases of reduced penetrance of FAD mutations have been described previously in the literature with such extensive prospective longitudinal follow-up and repeated biomarker measurements. Descriptions of wide ranges of ages at symptom onset in FAD families, as well as isolated cases of suspected reduced penetrance in particular subjects, have been reported (100-103), but none with the support of longitudinal biomarker or neuropsychological data. The findings in paper III have implications for genetic counseling as well as raise question about the possible mechanisms underlying the reduced penetrance in brother B.

5.5 TEMPORAL TRAJECTORIES OF BIOMARKER CHANGES

In the studies included in this thesis one of the main goals was to map the temporal trajectories of preclinical biomarker changes in our population of Swedish FAD mutation carriers. Figure 9 summarizes the results obtained when correlating different biomarkers with years to symptom onset in this population. Some of these results are trends (CSF Aβ42, t-tau, p-tau and the Aβ42/p-tau ratio) while the others are statistically significant correlations. We feel that the trends belong in the figure as they are based on results from relatively few individuals, leading to a lack of statistical power which probably explains why the trends did not reach significance. In the figure, we have also incorporated published results from the FAD study which are not included in the papers comprising this thesis, as they involve the same subjects and are based on some of the same data. According to the summarized biomarker results it is CSF Aβ42 that becomes abnormal first in preclinical AD, around 20 years before the expected symptom onset. This is followed by increased uptake of PiB on PET (around 17 years before symptom onset) (264), a decrease in the CSF Aβ42/p-tau ratio (15 years before symptom onset), a decline in episodic memory, executive function and visuospatial function as measured on neuropsychological test (10 years from symptom onset) (370), decreased FDG uptake on PET (7 years before symptom onset) (264) and finally an increase in CSF t-tau and p-tau (around 5 years before symptom onset). These results are in partial agreement with the hypothetical model of biomarker changes proposed by Jack et al. (17, 34). According to the hypothetical model it is CSF Aβ42 and amyloid PET that become abnormal first, just as we observed in our studies. The order of the subsequent changes is somewhat different, with Jack et al. proposing that CSF tau changes next, followed by structural MRI and FDG-PET and finally by the emergence of cognitive impairment. Interestingly, we found that CSF t-tau and p-tau started to change around the same time as changes were observed on FDG-PET, suggesting that tau hyperphosphorylation and neurodegeneration have a close temporal relationship. Also, we found signs of cognitive decline on longitudinal neuropsychological tests quite early in the course of the disease. These signs of cognitive decline are subtle, reflected in a decline from baseline cognitive function on repeated tests, and usually not noticed by the patient until later.

This makes these changes of different character than the cognitive decline in the model by Jack et al. and therefore one cannot argue that these two biomarker models are in disagreement in this respect. The model by Jack et al. does not incorporate a marker such as YKL-40, which is believed to represent glial activation. In paper IV we observed changes in YKL-40 in the preclinical stage of AD, with YKL-40 increasing as the onset of symptoms approaches, more so in the MC than the NC. The data did not allow for an estimation of when the YKL-40 levels of the MC start to deviate from those of the NC. In a study involving the same population of Swedish FAD mutation carriers the MC showed high retention of DED on PET, signaling astrocytosis, very early in the preclinical stage, which then declined around the same time as an increase in PiB retention was first detected (264). This implies that inflammation might in fact precede the accumulation of amyloid. Finally, our studies did not reveal a correlation between structural changes on MRI and years to symptom onset. This suggests that structural MRI changes are downstream of the other biomarker changes presented in figure 9, or that the sample size was not large enough to detect these changes earlier on.

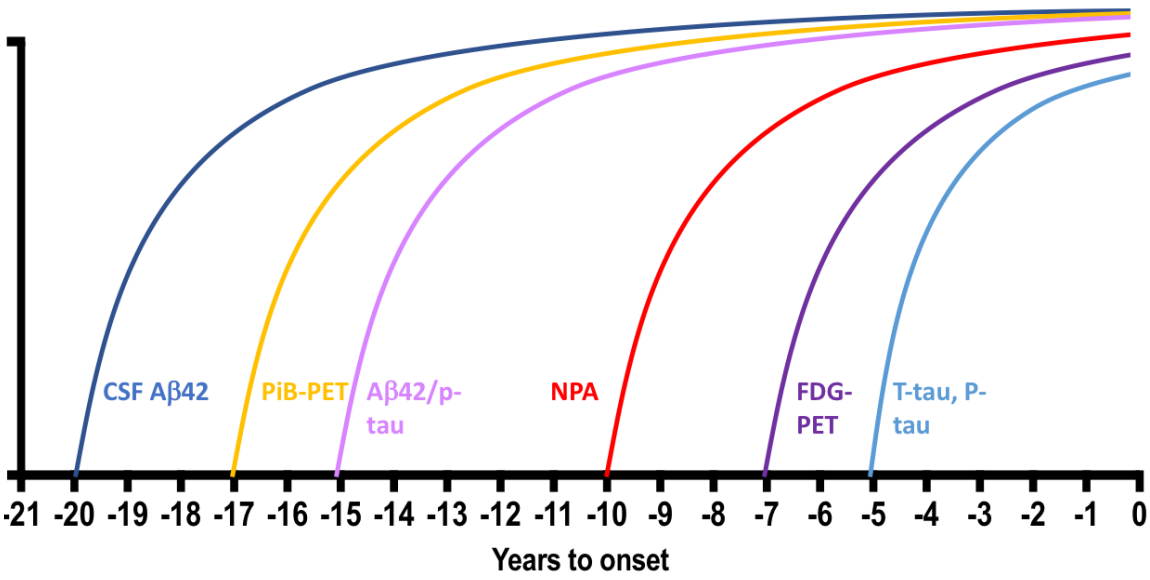

Figure 9. Hypothetical temporal trajectories of biomarker changes in the preclinical stage of AD, based on the Swedish FAD study. All of the trajectories included in the figure are derived from longitudinal data, except for the trajectories of CSF Aβ42, t-tau, p-tau and the Aβ42/p-tau ratio, which are derived from cross-sectional data. The shape of the curves presented in the figure is strictly hypothetical, with the main purpose of the curves being to provide a visual summarization of the biomarker changes. Each curve symbolizes a change in a given biomarker starting at a given point in time, which may involve an increase or a decrease of the biomarker, even though all of the curves are positive in the figure for the sake of clarity.

6 LIMITATIONS

Many of the limitations of the FAD study have already been discussed to some extent in the previous chapters of this thesis. One of the most important limitation is the relatively few participants in the study. This affects the statistical power of the study and increases the risk of significant differences between MC and NC going undetected. The small sample size does in some cases result in us having to use non-parametric statistical tests which are less likely to reveal significant differences. Also, even though correction for multiple comparisons was often necessary in our material, it causes the same problem by decreasing the statistical power. The small number of participants is a result of the rarity of FAD mutations in the general population. The Genetics unit at the Memory Clinic of the Karolinska University Hospital in Huddinge is a tertiary referral center for all Swedish cases of dementia with suspected autosomal dominant heritability. Therefore, one would not expect to find a significant amount of new FAD cases in Sweden and would have to collaborate with other countries in order to increase the number of participants. Finally, despite this sparsity of subjects, the Swedish FAD study still involves more participants than many of the other studies on FAD in the literature.

Another limitation is the cross-sectional design of the studies in papers I and II. Cross-sectional data cannot reliably account for temporal changes in biomarkers, however it can give us a valuable indication of how these changes truly evolve over time. There were also some limitations to the longitudinal data presented in paper IV. The participants were of different ages at baseline, the time from baseline to first or second follow-up was quite variable between participants and not all of the participants returned for a second follow-up. These variations cause some difficulties in the statistical analysis of the longitudinal data, deducting somewhat from the reliability of the results. The root of these problems is again the small number of subjects, the reluctance of many of them to provide repeated CSF samples (limiting us to a cross-sectional analysis) and the fact that some of the subjects who underwent a repeated LP where not inclined to do so until some years after the first one.

A lot of our results are based on relationships between different biomarkers and years to expected symptom onset. This allows us to estimate the place of a particular biomarker in the pathological cascade of AD and to compare our results to the results of other groups studying preclinical FAD. This is slightly problematic however, as different research group use different methods to define years to symptom onset. Some groups, like us, use the mean age at symptom onset of all affected members of a particular subject´s family to estimate his or her time to expected symptom onset. Other groups have used the age of the onset of symptoms in the subject´s affected parent to estimate this time. How different groups define symptom onset is also variable, some rely on the first reported cognitive symptoms by the affected individual (or an informant) while others use other reference points, e.g. the clinical diagnosis of AD. This can cause a discrepancy in how age at onset in different families is reported and in the reported time-points at which specific biomarker changes are observed.

Finally, there is always a possibility that results from studies on FAD cannot be generalized to SAD, and one must always keep that in mind when drawing conclusions from FAD studies. Also, the way different FAD mutations result in the development of AD dementia varies depending on the mutation. Thus, the composition of carriers of different mutations in each study, could possibly affect the results depending on the pathological mechanisms related to the mutation(s) in question.

7 CONCLUSIONS

An array of biomarker changes was observed in a group of Swedish FAD mutation carriers, starting years to decades before the expected onset of cognitive symptoms. These markers imply that cerebral amyloidosis, glial activation, tau-phosphorylation and neurodegeneration all sequentially appear in these subjects before the emergence of clinical symptoms. It also seems that repeated neuropsychological assessments can detect an early deviation from baseline cognition in mutation carriers before the onset of subjective cognitive decline.

These findings underpin the results of other studies pointing to similar preclinical biomarker changes, even though the exact order of events remains to be further elucidated. The results presented here are acquired from a relatively few participants, but are still highly significant in many cases, underlining the robustness of these biomarker changes. The chain of events in the preclinical stage of AD is of great interest as the root cause of AD is still under debate and biomarkers reflecting different pathological processes can shed light on cause and effect at this early stage of the disease. This in turn can be of guidance in the development of disease modifying treatments. In our results, biomarkers signaling cerebral amyloidosis were the earliest biomarkers to change in the MC, implying that amyloidosis is the event that sets the pathological cascade of AD in motion. However, the possibility remains that the biomarkers chosen in our studies fail to detect even earlier events related to other mechanisms.

Our findings also put emphasis on the fact that different FAD mutations affect APP processing differently *in vivo*, but to date most of the information we have on such effects comes from *in vitro* studies. This renders support to our previous knowledge from *in vitro* studies, as well as underlines the fact that all FAD mutations cannot be assumed to behave in exactly the same way. The statistical outlier presented in paper III is another side of the same coin, showing no signs of underlying AD pathological changes, despite being many years past the expected symptom onset. This urges us to be cautious when providing genetic counseling and also in designing clinical trials involving preclinical FAD mutation carriers, as one cannot assume complete penetrance of FAD mutations in all mutation carriers.

8 FUTURE CONSIDERATIONS

An attractive next step in studying biomarker changes in preclinical AD would be to embark on a larger multi-center longitudinal study. It would be of high interest to include greater numbers of symptom free FAD mutation carriers as well as carriers of risk alleles such as *APOE* ε4. With sufficient resources, it would also be preferential to include a cohort of cognitively healthy older individuals. The subjects would then be followed-up at previously defined intervals with repeated collection of CSF and blood and with neuroimaging and neuropsychological assessments. Through such prospective collection of data from a large number of individuals, where a substantial part of the study group is expected to develop clinical AD during follow-up, one would be able to map the temporal trajectories of preclinical biomarker changes with precision, thereby gaining valuable knowledge on the true course of events during the development of AD. This proposed longitudinal biomarker study would benefit greatly from incorporating neuropathological examinations in order to validate the biomarker changes observed *in vivo*. The only way to ascertain that *in vivo* biomarker changes are reflecting pathological changes in the brain in the way we assume is through autopsy. A few studies with a similar study design as described here are already underway, i.e. the Dominantly Inherited Alzheimer Network (DIAN), a multi-center study on individuals carrying FAD mutations (38).

Another interesting future direction would be to collect and characterize more cases of suspected reduced penetrance of FAD mutations found in other FAD studies around the globe. With a larger number of reduced penetrance cases it would be possible to pursue the underlying factor (or factors) causing these individuals to be spared from developing early-onset AD.

Finally, asymptomatic FAD mutation carriers are optimal candidates for clinical trials on potential disease modifying treatments in AD, as such treatments are believed to be most effective during the preclinical phase of the disease. Ongoing clinical trials in this population include the DIAN trials unit (DIAN-TU) (371) and the Alzheimer's preventive initiative (API) (372, 373). However, it has to be kept in mind that this involves several pitfalls, e.g. that study participants unaware of their mutation status could become aware through the development of side-effects, which presumably only affect mutation carriers as non-carriers would not receive active treatment. Also, the safety of such experimental treatments would have to be well established beforehand, as such studies would involve young, asymptomatic individuals, not patients, making the development of severe adverse events all the more devastating.

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