Alzheimer's disease heterogeneity assessment with MRI biomarkers and unsupervised statistical learning

Konstantinos Poulakis

Karolinska
Institutet

From the Division of Clinical Geriatrics
Department of Neurobiology, Care Sciences and Society
Karolinska Institutet, Stockholm, Sweden

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Konstantinos Poulakis

Stockholm 2021

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THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Konstantinos Poulakis

The thesis will be defended in public in Gene in NEO, Karolinska Institutet, Campus Flemingsberg, Huddinge.

Friday, May 28th 2021, 9.00am.

Principal Supervisor:
Eric Westman Professor
Karolinska Institutet
Department of Neurobiology,
Care Sciences and Society
Division of Clinical Geriatrics

Co-supervisor(s):
Joana B. Pereira Assistant Professor
Karolinska Institutet
Department of Neurobiology,
Care Sciences and Society
Division of Clinical Geriatrics

Daniel Ferreira Associate Professor
Karolinska Institutet
Department of Neurobiology,
Care Sciences and Society
Division of Clinical Geriatrics

Örjan Smedby Professor
KTH Royal Institute of Technology
School of Technology and Health
Division of Biomedical Engineering

Opponent:
Betty Tijms Associate Professor
Amsterdam UMC
Department of Neurology
Alzheimer Center Amsterdam

Examination Board:
Jan Marcusson Professor
University of Linköping
Department of Health, Medicine and Caring
Sciences
Division of Prevention, Rehabilitation and
Community Medicine

Pawel Herman Associate Professor
KTH Royal Institute of Technology
Department of Computer Science
Division of Computational Science and
Technology

Chengxuan Qiu Associate Professor
Karolinska Institutet
Department of Neurobiology, Care Sciences and
Society
Aging Research Center

This thesis is devoted to the Alzheimer's disease patients and their families and caregivers who accompany them during the course of dementia.

POPULAR SCIENCE SUMMARY OF THE THESIS

This thesis starts with a summary that involves no domain-specific knowledge or terminology. Dementia is a set of symptoms that include difficulties in memory, language, thinking, problem-solving and other skills. Most dementias occur in older ages and the greatest risk factor for its development is age. Few persons show the first dementia symptoms as early as in their 40s. Dementia can be caused by one or more overlapping neurodegenerative diseases. The most common diseases underlying dementia are Alzheimer's disease (AD), cerebrovascular disease, Lewy body disease, frontotemporal lobar degeneration, Parkinson's disease, and hippocampal sclerosis. In this thesis we are interested in AD which is the most common cause of dementia.

AD accounts for 60-80% of dementia cases. It is thought that the pathological changes related to it may start as much as twenty years before the dementia onset. The persons affected by AD do not notice the brain changes until pathology is enough to cause clinical symptoms. In general, dementia is associated with the accumulation or change in form of specific proteins within or around the neurons (and other related processes that the scientific community is investigating). Therefore, sometimes we say that dementia is caused by one or more proteinopathies. Specifically, two proteins are consistently reported in AD. The accumulation of beta-amyloid protein fragments (beta-amyloid plaques) outside the neurons and twisted tau proteins (tau tangles) inside the neurons, are the main pathological hallmarks in AD. The initial symptoms of dementia are expressed as memory complaints. Then, individuals show some objective symptoms of cognitive decline (mild cognitive impairment). Finally, at the dementia stage (clinical AD), the patient develops specific cognitive deficits, well documented in the diagnostic manual of neurological disorders. The main initial cognitive deficit in dementia due to AD (mild dementia stage) is the impaired episodic memory (recollection of specific events/experiences etc.). When the dementia advances, other cognitive functions such as orientation, communication, behavior, and judgement become affected (moderate dementia stage). In severe dementia due to AD, swallowing, walking, and speaking are affected and patients may become bedbound. Currently, there are no effective medications to slow down or reverse AD.

Although AD is characterized by specific pathological hallmarks and cognitive symptoms, patients demonstrate great heterogeneity in pathology, cognition and age at dementia onset among other characteristics. Recent postmortem (after death) data have shown that approximately half of the patients that present with AD pathology (amyloid-beta plaques and tau tangles) have at least one more proteinopathy that together with AD causes dementia (this can be any of the diseases mentioned in the first paragraph). Some patients are diagnosed with AD before the age of 65 and they tend to have less memory deficits (atypical AD), while other patients are diagnosed later in life and have more amnestic presentation (typical AD). Atypical AD covers an interesting set of clinical syndromes and is an active research field where new findings are reported every year. Many research groups are currently working to map the heterogeneity in AD with all the available markers that show changes due to the disease

(biomarkers). AD has a typical pattern of brain atrophy progression that follows the spread of tau tangles in the brain, which is well characterized in postmortem studies. However, recently it was shown that patients can have patterns of tau tangles that are not typical for AD. Those tau tangle patterns are followed by brain atrophy in the same brain regions. Brain atrophy (grey matter loss) can be traced using brain magnetic resonance imaging (MRI) scan during life.

In this thesis, we used advanced statistical models and MRI techniques to understand the heterogeneity during aging and in AD dementia. In studies I, II, and III we used structural MRI (cross sectional and longitudinal) to assess the brain grey matter (where neuronal cell bodies are) heterogeneity in AD dementia. Data collected from four multicenter cohorts (USA and Canada, Europe, Japan, Australia), were used to understand the grey matter changes in AD dementia. Our results showed that there are two main pathways of atrophy during the AD dementia stage (the clinical/dementia part of AD disease). The pathway that most of the AD patients have in common starts with atrophy in the temporal lobe of the brain (especially in the entorhinal cortex and hippocampus) and extends to other cortical regions (mediotemporal pathway). The other pathway, which is expressed in significantly fewer patients, starts from the cortex (especially the regions of the parietal lobe) and then involves all the brain regions (cortical pathway). Patients of the mediotemporal pathway have more memory problems, they tend to be older at the disease onset, and their disease course can be either stable or aggressive. Patients that express the cortical pathway are often younger at the disease onset, they have more atypical AD symptoms (less memory and more praxis deficits at the AD onset) and their disease course is often aggressive. Our findings can be utilized to predict atrophy and clinical progression early on during the clinical disease course of AD patients.

In study IV, we used longitudinal diffusion tensor imaging (special MRI) in a large population sample from USA to understand the different white matter (where neuronal axons are) integrity profiles that accompany individuals during the aging process and relate this to the risk of developing dementia. We discovered that some individuals have good WM integrity during aging (healthy agers) while other individuals have low WM integrity (at risk of cognitive decline). Another cluster of patients in our results, initially presented with high WM integrity, but deteriorated progressively during the years. WM integrity correlated well with cognitive health which means that damage in neuron's axons is associated with cognitive decline. Individuals with low WM integrity at the age of 60 years had a greater risk of becoming cognitively impaired. Beta amyloid in the brain of the individuals was predictive of overall WM health but did not vary between the WM clusters of individuals. WM heterogeneity in aging is probably independent of beta amyloid accumulation. However, dementia due to AD may be a product of more than one pathological processed in the brain.

In conclusion, in this thesis we focused on developing statistical methods that helped us to understand features of the heterogeneity in AD and in healthy aging. Following this approach, we envision setting the foundation for unravelling heterogeneity in AD. This will hopefully provide a platform for the future development of dementia modifying treatments and personalized prediction of biological/cognitive changes in dementia

ABSTRACT

Alzheimer's disease (AD) is the most common cause of dementia. It is characterized by loss of memory and other cognitive functions. Although it is a heterogeneous condition, it has been studied as one disease for many decades. Neuropathological data and a large body of in vivo neuroimaging literature challenge the hypothesis that AD is a single entity, supporting the hypothesis of AD as a heterogeneous disease.

In this thesis, we set out to understand some aspects of the heterogeneity in AD and aging with the help of atrophy and WM integrity markers from magnetic resonance imaging (MRI). The main aim of the thesis was to investigate the potential use of statistical and machine learning models for the assessment of heterogeneous conditions. In **Study I**, we utilized whole brain atrophy markers and cross-sectional cluster analysis to characterize the neurodegeneration variability in a large AD dementia cohort (299 amnestic AD patients). The clusters of patients that we discovered presented with distinct atrophy patterns. Some of them exist due to disease severity, but we identified topologically variable atrophy patterns too. Patients of the different clusters had distinct cognitive symptoms and clinical progression.

Then, we designed a pipeline that will help us to assess heterogeneous populations when longitudinal neuroimaging and clinical data are available (**Study II**). We tested this pipeline in atrophy data from a small dataset of AD patients to assess its usefulness in MRI data and heterogeneous conditions. The model fitted the data well and we concluded that it can be used in larger scale analyses. Moreover, larger numbers of participants with long follow-up period should increase its freedom in searching for heterogeneity in longitudinal neuroimaging trajectories. After this methodological study, we used a very large dataset that consisted of neuroimaging, cerebrospinal fluid (CSF), and clinical data. We split our data in discovery and prediction datasets. The discovery dataset included $A\beta$ positive clinically diagnosed AD dementia patients and $A\beta$ negative cognitively unimpaired individuals (CU). Based on this dataset (**Study III**), we aimed to understand whether the observed heterogeneity in AD is caused by sampling patient's data at different disease stages, or if it resembles distinct neurodegeneration subtypes. We modelled longitudinal brain atrophy data anchored to the clinical dementia onset. Our findings show that all the previously reported atrophy subtypes do exist but some of them reflect disease stages rather than subtypes. Most importantly, our modeling managed to summarize the observed heterogeneity in neurodegeneration with two unique pathways (mediotemporal and cortical). These two pathways have distinct cognitive signatures and were evaluated in a large independent AD dataset. Heterogeneity within the pathways exist and is likely caused by a complex interaction between protective/risk factors and concomitant non-AD pathologies.

Some findings indicate that WM changes may precede grey matter atrophy in AD. In **Study IV** we investigated whether more than one WM profile exists in the aging population. We wanted to understand their association with AD pathophysiological changes and relate them to the risk of developing dementia. We discovered four distinct WM integrity patterns with

different spatial WM integrity distribution in aging. Those patterns were related to different longitudinal cognitive profiles and specific white matter tracts informed about cluster assignments.

In conclusion, heterogeneity can be observed not only in AD, but also in the population including healthy individuals. In this thesis, we identified distinct pathways of brain atrophy and WM integrity. Understanding the heterogeneous patterns of the different pathophysiological markers during ageing and the course of AD, will ultimately lead to the development of disease modifying (personalized) treatments.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following original articles:

I. **Poulakis K**, Pereira JB, Mecocci P, Vellas B, Tsolaki M, Kłoszewska I, Soininen H, Lovestone S, Simmons A, Wahlund LO, Westman E. Heterogeneous patterns of brain atrophy in Alzheimer's disease. Neurobiology of Aging 2018; 65: 98–108.

II. **Poulakis K**, Ferreira D, Pereira JB, Smedby Ö, Vemuri P, Westman E. Fully bayesian longitudinal unsupervised learning for the assessment and visualization of AD heterogeneity and progression. Aging 2020; 12: 12622–12647.

III. **Poulakis K**, Pereira JB, Muehlboeck JS, Wahlund LO, Smedby Ö, Volpe G, Masters CL, Ames D, Niimi Y, Iwatsubo T, Ferreira D, Westman E. Stage vs. Subtype hypothesis in Alzheimer's disease: a multi-cohort and longitudinal Bayesian clustering study. Submitted manuscript.

IV. **Poulakis K**, Reid RI, Przybelski SA, Knopman DS, Graff-Radford J, Lowe VJ, Mielke MM, Machulda MM, Jack Jr CR, Petersen RC, Westman E, Vemuri P. Longitudinal deterioration of white-matter integrity: heterogeneity in the ageing population, Brain Communications 2021; 3: 1; fcaa238

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LIST OF ABBREVIATIONS

AD	Alzheimer's disease
CART	Classification and regression tree
CSF	Cerebrospinal fluid
CU	Cognitively unimpaired
DTI	Diffusion tensor imaging
DWI	Diffusion weighted imaging
EOAD	Early onset AD
eTIV	Estimated total intracranial volume
FA	Fractional anisotropy
FDG-PET	Fluorodeoxyglucose PET
FLAIR	Fluid attenuated inversion recovery
FTLD	Frontotemporal lobar degeneration
HPD	Highest posterior density
LOAD	Late onset AD
MCI	Mild cognitive impairment
MD	Mean diffusivity
MDS	Multidimensional scaling
MRI	Magnetic resonance imaging
MRI-PD	MRI proton density
NFT	Neurofibrillary tangles
PET	Positron emission tomography
ROI	Region of interest
SCD	Subjective cognitive decline
SUVR	Standardized uptake value ratio
TDP-43	transactive response DNA protein
WM	White matter

WMH   White matter hyperintensity

1 INTRODUCTION

Memory, both collective and individual is one of the features that has guaranteed our civilization's survival throughout the millennia. Today, people of the advanced societies have a longer expected life span than in any other time during human evolution. The biological challenges that arise from this initially positive achievement of our societies are mainly associated with the aging process of the human body. Life expectancy (at birth) increases fast and human evolution has not prepared us to cope naturally with the effects that aging has on our biology(Brown, 2015). As a result, an increasing percentage of individuals are diagnosed with diseases for which the highest risk is the aging process itself. Dementia disorder (Neurocognitive disorder) is the outcome of various age-related diseases. Alzheimer's disease (AD) is one of the underlying causes of dementia. According to the World Alzheimer's Report of 2015, 36 million people were diagnosed with dementia in 2010 and this prevalence is projected to double every 20 years and affect 115 million people by 2050 if no treatment becomes available (Dartigues, 2009). The estimated costs for dementia have also increased from 818 billion dollars in 2015 to one trillion dollars in 2018 (Wimo *et al.*, 2017). Therefore, the socioeconomic cost of AD will increase in the future with the aging population.

While the etiology of AD remains elusive, two main forms of the disease can be identified, namely, the rare early onset autosomal dominant variant (also called familial AD) and the more frequent sporadic form with no apparent familial aggregation or obvious genetic identification. The familial form of AD is characterized by mutations in genes related to the production of the beta amyloid ($A\beta$) peptide such as Amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) (Bertram and Tanzi, 2001, 2008) (see Table 1 in Bertram and Tanzi, 2008). On the other hand, the sporadic form of AD, which is the most frequent variant of the disease and is frequently characterized by a late onset, shows less obvious or no signs of genetic aggregation. The e4 allele of the *APOE* gene is the only genetic factor that has been consistently reported as a risk factor for sporadic AD (Bertram and Tanzi, 2008).

1.1   NEUROPATHOLOGY IN ALZHEIMER'S DISEASE

The main pathological hallmarks of AD include extracellular amyloid plaques and intracellular neurofibrillary tangles (NFT) (Berrios, 1990). Amyloid plaques consist of beta amyloid peptides, while NFT consist of hyper-phosphorylated microtubule-associated protein tau filaments (Shaw *et al.*, 2009). Amyloid plaques can be found in two morphological forms: diffuse plaques (diffuse extracellular beta amyloid deposits) and plaques with a dense core (Thal, 2006). Neuritic plaques are beta-amyloid plaques (dense core) surrounded by dystrophic neurites that accumulate abnormally hyper-phosphorylated tau protein (Alafuzoff *et al.*, 2009). The temporal aggregation of NFT and amyloid plaques seems to follow almost opposing topological patterns.

Amyloid plaques are initially deposited in the isocortex (Braak and Braak, 1991; Thal, 2006). The next stage involves allocortical areas such as the entorhinal region and the subiculum. At this stage the hippocampal formation, which is an instrumental brain region for the formation

of new memories, is to some extent involved. In the third phase the thalamus, hypothalamus and basal ganglia are also involved followed by the lower brainstem (medulla oblongata and midbrain) in the fourth phase. The fifth and final phase includes the part of the brainstem (pons) that was not yet involved in the fourth phase as well as the cerebellum. Amyloid pathology is said to be present as early as in the 4th decade of life in 4% of the patients (then, it increases annually) (Braak *et al.*, 2011). Therefore, a long preclinical stage of misfolded protein aggregation precedes the clinical onset of dementia.

Traditionally, it is believed that the regional distribution of NFT formation follows a trajectory that initially involves the transentorhinal cortex, followed by the entorhinal cortex, hippocampus and inferior temporal lobe and finally the isocortex (Braak and Braak, 1991). However, the initial deposition area of NFT is still controversial since new neuropathological data (Braak *et al.*, 2011) show that pretangle material is initially formed in the nuclei of the brainstem and its projections to the cerebral cortex (locus coeruleus).

Neuroinflammation and neurodegeneration are two other neuropathological hallmarks in AD. One of the recent findings on AD pathology is that immunological mechanisms in the brain together with neuronal processes (NFT and amyloid plaques) play an important role in the disease pathogenesis (Heneka *et al.*, 2015). Finally, neurodegeneration (atrophy and ventricular enlargement) is an important feature of the disease since it is typically observed in most AD patients (Whitwell *et al.*, 2018*b*).

2 FROM NORMAL AGING TO DEMENTIA

2.1 NORMAL BRAIN AGING

The aging process is accompanied by changes in the function and appearance of the brain. Cognition, as well as brain white and grey matter, shows different trajectories during adult life. Decline in working memory, information processing (speed and flow of information processing) and attention functioning are common features observed in the elderly population (Salthouse and Ferrer-Caja, 2003). A large body of literature provides evidence that decreasing white matter (WM) integrity in the frontal lobe as well as in specific WM tracts (corpus callosum) that connect different central nervous system areas (Damoiseaux *et al.*, 2009) is part of normal brain aging. Grey matter density reductions (fastest in the frontal lobe) is another widely accepted anatomical feature of normal aging (Irwin *et al.*, 2018). All together these findings show that with aging, cognitively unimpaired (CU) individuals exert slowly declining (on average) cognition, while their brains do not remain intact. It is important to mention that cognitive decline related to normal aging does not interfere with everyday life activities of the elderly individuals and therefore is not considered significant or clinically relevant. Importantly, environmental and genetic factors introduce great variability around the population average of cognitive and brain health aging trajectories.

2.2 SUBJECTIVE COGNITIVE DECLINE

Elderly individuals often report difficulties regarding their cognitive abilities. These can be either rare events or an observation of decline over time. As insignificant as these self-reports may sound to an untrained family member or observer, they may carry important early messages of an underlying neurodegenerative process. A review on subjective memory complaints has shown that increasing age is associated with more complaints (Jonker *et al.*, 2000). Moreover, at the age span 50-59 years, more than four out of ten individuals have memory complaints, while this percentage increases to six out of ten individuals over the age of 80 years.

Reports of decline in any cognitive domain (not necessarily memory) that are subjective and cannot be objectively established by cognitive testing, and are reported in more than one instance (decline over time) are known in the literature as subjective cognitive decline (SCD) (Jessen *et al.*, 2014). These complaints may be indicative or predictive of future cognitive decline that interferes with everyday life and this is why their study is important. However, normal aging, psychiatric and neurologic disorders other than dementia or even medication and substance use can orchestrate these complaints (Jessen *et al.*, 2014).

Memory complaints in SCD individuals is associated with reduction in grey matter volume (Jessen *et al.*, 2006; Schultz *et al.*, 2015). Moreover, some research on the anatomical changes in the brains of SCD individuals has also shown that they are quite heterogeneous (Jung *et al.*, 2016). Given the heterogeneity of the SCD individuals' population in terms of cognitive

complaints and their corresponding atrophy patterns, extensive research and careful follow up will shed light on the potential use of this group classification for dementia prognosis.

2.3   MILD COGNITIVE IMPAIRMENT

The intermediate condition between cognitively healthy aging and dementia is referred to as mild cognitive impairment (MCI). Individuals exhibiting objective cognitive impairment beyond the expected one for their age and educational background receive an MCI diagnosis. This diagnosis is related to objective signs of cognitive impairment in any cognitive function. However, the impairment is not enough to interfere with the everyday life activities of MCI patients to the extent that the dementia diagnosis is given. MCI can exert various phenotypes due to its heterogeneous nature. A call for standardization in the diagnosis of MCI led the First Key Symposium in 2003 to introduce a consensus that established four clinical phenotypes of MCI, namely the amnestic, non-amnestic, single and multiple domain (Winblad *et al.*, 2004). They recommended that to receive the diagnosis of MCI an individual should neither be CU nor meet the criteria for dementia syndrome that were then in use (DSM IV, ICD 10). Regarding cognitive decline, the individual should report cognitive decline and impairment in objective cognitive tasks (memory, language, visuospatial etc.). Alternatively, an informant can report the cognitive decline feature. Moreover, evident cognitive decline (over time) on objective cognitive tasks should be present. Importantly, to separate from dementia diagnosis, the individuals should have preserved basic daily activities and can have minimal impairment in complex instrumental functions.

The MCI clinical presentation can be caused by multiple etiologies, the most possible of which are: degenerative, vascular, metabolic, traumatic, or psychiatric (Winblad *et al.*, 2004). Due to the multiple candidates behind MCI not all patients progress to dementia. Some MCIs progress to dementia (approximately 15% per year), a large number remain stable in this diagnosis while approximately 26% revert to cognitively normal status (Breitner, 2014).

The strength of the MCI diagnosis is that it manages to include a large number of patients with cognitive problems for potential follow up in the future. However, the fact that only a few of these patients progress to dementia prompted the research community to investigate the use of biological markers in the attempt to further characterize individuals that receive this diagnosis. MCI was further investigated by the International Working Group (IWG) for the diagnosis of Alzheimer's disease and it was decided to call the amnestic MCI form prodromal Alzheimer's disease in the case that a supportive biological marker was in favor of that diagnosis (Dubois *et al.*, 2014). This specialized diagnosis is very important for Alzheimer's disease research since it increases the chances of early identification of patients that will likely develop Alzheimer's disease.

2.4 DEMENTIA

Dementia is the step after MCI in the dementia continuum. More formally, dementia (major neurocognitive disorder) can be defined as a group of cognitive symptoms that severely affect a person's ability to perform everyday activities caused by disease or injury. The clinical diagnosis of dementia has a clear list of criteria including ('Neurocognitive Disorders', 2013):

- Evidence of significant decline in one or more of the following cognitive functions: perceptual-motor, social cognition, language, learning and memory, executive function and complex attention. This evidence can be based on the observation of the indiviual, a knowledgeable informant, or the clinician that significant decline in cognition has occurred. Moreover, this substantial decline in cognitive skills has to be documented by standardized neuropsychological assessment or in its absence, a quantitative clinical assessment of another type.
- Disturbance/Interference of everyday activities due to reported cognitive deficits (at a minimum, assistance with complex instrumental activities such as managing medications fullfills this criterion).
- The cognitive deficits should not only occur during a delirium (organically caused disturbance/decline in attention and awareness from a previous baseline mental functioning that develops over a short period of time).
- Neurological conditions such as major depressive disorder, schizophrenia have to be excluded to arrive at the diagnosis of dementia.

Dementia is an umbrella term that includes a set of different clinico-pathological entities that are diagnosed by the aforementioned criteria. According to the diagnostic and statistical manual of mental disorders, dementia can exist due to Alzheimer's disease, frontotemporal lobar degeneration, Lewy body disease, vascular disease, traumatic brain injury, substance/medication use, HIV infection, prion disease, Parkinson's disease, Huntington's disease, multiple etiologies or even other medical conditions ('Neurocognitive Disorders', 2013). For the purpose of this thesis, five conditions (Alzheimer's disease, vascular disease, Lewy body disease, frontotemporal lobar degeneration, hippocampal sclerosis) will be further discussed.

An overview of the most common causes of dementia and their main clinical diagnostic features is presented in sections 2.4.1 and 2.4.2 to showcase their clinical similarities and the differential diagnosis challenges that arise in clinical practice.

2.4.1 Alzheimer's disease clinical diagnosis

According to the 2021 Alzheimer's disease facts and figures, approximately 60-80% of all dementia cases are estimated to be caused by Alzheimer's disease (AD). In 1983, a working group was established to define criteria for the clinical diagnosis of AD dementia by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS), and the Alzheimer's Disease and Related Disorders Association (ADRDA). It was recognized that the prevalence of AD was very high in the older population and therefore common diagnostic

guidelines were needed. The definition that was given to AD dementia was: "A brain disorder characterized by progressive dementia that occurs in middle and late life (McKhann *et al.*, 1984). Obtaining an accurate diagnosis of dementia due to AD is a clinically challenging task due to the great overlap between different types of dementias. Two groups, one in Europe (International working group, IWG) and another one in North America (National Institute on Aging – Alzheimer's Association, NIA-AA), put significant effort into revising AD diagnostic criteria since new knowledge has reconceptualized the disease diagnosis during the last two decades (Visser *et al.*, 2012). According to the DSM-5 manual, the criteria for AD diagnosis consist of: a) meeting the criteria for mild/major dementia (2.4); b) having an insidious onset that is followed by gradual progression of impairment in cognitive and behavioral symptoms; c) meeting the criteria for probable or possible AD. Those include causative evidence of AD genetic mutation (genetic testing, or autosomal-dominant family history together with autopsy confirmation, or an affected family member has a positive genetic test), clear evidence of decline over time in memory and at least one more cognitive domain, gradual decline in cognition but not acute, and no evidence of another etiology; d) the disturbance is not explained by cerebrovascular disease, some other neurodegenerative disease, another mental/neurological/systemic disorder, or the effects of a substance. We see that criteria for AD diagnosis demand an exhaustive process of exclusion of any other possibility that can explain the symptoms. Moreover, most patients with AD have multiple medical conditions that influence and complicate the diagnosis. This is the reasoning behind the most recent criteria suggested by the research community include diagnostic markers that shift the diagnosis from neuropsychological to more biological (Jack *et al.*, 2018).

In terms of symptom severity, the dementia (due to AD) continuum can be categorized in three distinct phases: 1) a mild stage when symptoms interfere with some everyday activities but most people can function independently (drive, work, leisure activities etc.), 2) a moderate stage where dementia interferes with many everyday activities (longest stage) including communicating and performing everyday tasks, 3) a severe stage where patients are likely to require care around the clock since they need assistance with daily living. Damage in the motor area of the brain often causes patients to become bed-bound and conditions related to their lack of movement may arise ('2020 Alzheimer's disease facts and figures', 2020).

2.4.2 Other causes of dementia

2.4.2.1 *Cerebrovascular disease*

When blood vessels in the brain are damaged and/or brain tissue is injured from not receiving enough blood, oxygen or nutrients, dementia due to cerebrovascular disease may be initiated ('2020 Alzheimer's disease facts and figures', 2020). It is estimated that vascular disease is the second most common cause of dementia after AD. However, the clinical differentiation between vascular disease and AD is not a trivial task. Dementia due to vascular disease can occur at any time but it increases exponentially after the age of 65 years. The diagnosis of

vascular dementia includes 1) the fulfillment of the dementia diagnosis criteria; 2) the clinical features of vascular etiology. These can be either the onset of cognitive deficits after one or more cerebrovascular events, or the evidence of significant decline of frontal-executive and complex attention functions; 3) evidence of cerebrovascular disease by neuroimaging markers and/or history, physical examination ('Neurocognitive Disorders', 2013).

2.4.2.2 *Lewy body disease*

Lewy body disease relates to the abnormal aggregations of alpha-synuclein proteins in neurons (synucleinopathy). These protein aggregates are also the neuropathological hallmark in Parkinson's disease. Similar to cerebrovascular disease, the clinical presentation of the dementia related to Lewy body disease can be challenging to distinguish from dementia due to AD, especially in late stages. However, patients with this dementia type present with some special clinical features. There are four main diagnostic features for this dementia type: 1) The main dementia diagnostic criteria are met, 2) insidious onset with gradual progression (similar to AD dementia); 3) core diagnostic features including fluctuating cognition with variations in attention and alertness, repeated detailed and well-formed visual hallucinations, spontaneous parkinsonism[1] that starts after the cognitive decline, and suggestive diagnostic features including rapid eye movement and severe neuroleptic sensitivity ('Neurocognitive Disorders', 2013; McKeith *et al.*, 2017); 4) the disturbance is not explained by cerebrovascular disease, some other neurodegenerative disease, another mental/neurological/systemic disorder, or the effects of a substance. Neuroimaging markers provide significant help in differential diagnosis between AD and Lewy body dementia (McKeith *et al.*, 2017).

2.4.2.3 *Frontotemporal lobar degeneration*

Dementia caused by this disease is rarer since it affects 2-10 per 100 000 people and normally individuals are younger (60% of cases are between 45 and 60 years old) when diagnosed with it ('Neurocognitive Disorders', 2013; 'What are frontotemporal disorders?', 2019). The term frontotemporal lobar degeneration (FTLD) is an umbrella term that includes several dementia subtypes, namely the behavioral-variant FTLD, primary progressive aphasia, Pick's disease, corticobasal degeneration and progressive supranuclear palsy. The main proteinopathies found in patients with FTLD involve tau and transactive response DNA protein (TDP-43) aggregates. This set of dementia syndromes has some common diagnostic features including: a) meeting the criteria for mild/major dementia; b) having an insidious onset that is followed by gradual progression (like AD); c) for the behavioral variant, three of the following symptoms should exist: behavioral inhibition, apathy or inertia, loss of sympathy or empathy, perseverative, stereotyped or compulsive behavior, hyperorality and dietary changes and significant decline in social cognition and/or executive abilities. For the language variant, decline in language ability (form production, word finding, object grammar, or word comprehension); d) sparing

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[1] Clinical syndrome characterized by tremor, bradykinesia, rigidity, and postural instability (Simon, R. P., Aminoff, M. J., & Greenberg, 2009)

of memory as well as perceptual motor function; e) the disturbance is not explained by cerebrovascular disease, some other neurodegenerative disease, another mental/neurological/systemic disorder, or the effects of a substance ('Neurocognitive Disorders', 2013). In late or major dementia of FTLD type, differential diagnosis is challenging while in early stages the relative memory sparing can help to distinguish it from AD.

2.4.2.4 *Hippocampal sclerosis*

Sclerosis of the brain tissue of the hippocampus can cause a distinct dementia type which is more often observed in older people than in other dementia subtypes. Since the hippocampus is related to memory formation, a main symptom of this dementia subtype is its amnestic character. The same protein as in FTLD, TPD-43, has been observed to be misfolded in histological assessment of patients (majority) with hippocampal sclerosis ('2020 Alzheimer's disease facts and figures', 2020).

3 BIOMARKERS IN ALZHEIMER'S DISEASE

A biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Working Group Biomarkers Definitions, 2001). Biomarkers can help in the differentiation between different dementias with similar clinical presentation and to identify the stage of the underlying pathophysiological process. They are also used to monitor potential drug modifying effects (Frisoni *et al.*, 2017). The new definition of AD according to NIA-AA in 2011, was "the clinical syndrome that arises as a consequence of the AD pathophysiological process" and AD pathophysiological process is defined as the "antemortem biological changes that precede the postmortem neuropathological diagnosis of AD as well as the neuropathological substrate"(McKhann *et al.*, 2011). To monitor those changes, biomarkers that have been used in AD research include fluid and in vivo imaging measures of AD pathophysiology (McKhann *et al.*, 2011; Visser *et al.*, 2012; Frisoni *et al.*, 2017).

3.1 NEUROPSYCHOLOGICAL ASSESSMENT

Neuropsychological assessment is a valuable and commonly used clinical tool for the diagnosis of dementia. It consists of a set of specialized tests that help to assess specific cognitive domains that may be affected in the different dementia disorders. The normative levels of functionality in the populations can be objectively quantified and therefore reduced scores signify cognitive deficits in the respective domains. It is important to understand that environmental factors such as sex, education and social class can modify the levels of cognition in some domains. Therefore, individual cognitive scores of suspected cognitively impaired persons should be compared to environmental background matched data to discover potential cognitive deficits.

3.2 BRAIN IMAGING IN HEALTHY AGING AND ALZHEIMER'S DISEASE

Imaging markers include various measures from computed tomography (CT), magnetic resonance imaging (MRI) and molecular imaging such as positron emission tomography (PET). CT can measure atrophy in vivo, while MRI, among other things, can measure atrophy and WM changes. Amyloid and phosphorylated Tau accumulation can be quantified using PET. Glucose metabolism can be quantified by FDG PET.

3.2.1 MRI

MRI has proven to be of substantial importance in medical research and clinical practice since it provides in vivo markers of different biological processes occurring in the brain. Several MR sequences have been implemented. Some MRI sequences are used in hospitals or in specialized clinics, while other sequences are still used for research only. MRI sequences can be broadly grouped into structural, that we use to understand the structural properties of different brain regions (the most common sequences are T1, T2, PD) and functional (fMRI), that we use to understand brain activity. Many other MRI sequences are used to understand specific properties (calcium, CSF flow etc.) of the brain (Grover *et al.*, 2015).

3.2.2 Structural

Structural MRI (sMRI) is a non-invasive method that reveals the structure of various tissue types without any exposure to ionizing radiation. A combination of numerous magnetic fields causes hydrogen atoms to emit radio frequencies and then receiving coils record them. Variations in the acquisition parameters produce images where the WM, grey matter and cerebrospinal fluid have different intensities and can be distinguished. In this way the spatial specificity of the images can be used to identify pathological findings (tumors, hemorrhages, injuries, aneurysms, etc.) as well as brain atrophy due to neurodegenerative diseases.

3.2.3 Diffusion tensor imaging

DWI, which is another MR method, utilizes water diffusion properties to generate contrasts in MR images. DWI maps the diffusion process of water molecules in different tissues of the brain and can be used to map WM tracts in the brain. The loss of neuronal connections due to loss of integrity in WM tracts can be studied with this type of contrast, which may have clinical implications (Chua *et al.*, 2008). An example application of diffusion tensor imaging (DTI, special DWI technique to study WM structures) is the study of fractional anisotropy (FA) and mean diffusivity (MD) for the assessment of WM integrity in the pathophysiological continuum of AD (Kantarci, 2014).

3.2.4 Positron emission tomography

PET is an imaging technique that can measure metabolic processes in the brain. The research on particular PET tracers that can identify $A\beta$ and tau protein aggregates expanded significantly during recent decades, resulting in tracers that show very good agreement with post-mortem neuropathological evidence of abnormal protein accumulation (Saint-Aubert *et al.*, 2017).

3.3 CEREBROSPINAL FLUID

The interstitial fluid that forms a cushion around the central nervous system, both in the brain and spinal cord is called cerebrospinal fluid (CSF). This fluid has several functions that serve the CNS, including buoyancy of the brain parenchyma and support against traumas caused by rapid movements of the body and head, flow of important nutrients and cleaning of the CNS from harmful substances that were released from the brain tissue (Wright *et al.*, 2012). CSF is mostly produced by the choroid plexus, but also from the ventricular ependyma, brain tissue and arachnoidal membrane.

The extraction and analysis of CSF has advanced our knowledge of the AD pathophysiology. The levels of $A\beta$ and tau that could only be validated postmortem in the past, can now be quantified through CSF extraction. The levels of those proteins correlate very well with postmortem pathology but their relationship to amyloid plaques and NFT during the aging process is an active research field. Currently validated AD specific fluid markers include CSF $A\beta1-40$, $A\beta1-42$, Total tau (tTAU) and Phosphorylated tau ($pT181$). These markers can indirectly measure the ongoing amyloid and phosphorylated tau accumulation. Cerebrospinal

fluid markers are used in Studies 2, 3 and 4 to assess $A\beta$ status of the identified groups of patients and CU individuals.

4 HETEROGENEITY IN ALZHEIMER'S DISEASE

The first patient who was diagnosed with AD dementia presented with cognitive deficits that did not follow the typical amnestic presentation that is commonly used to describe AD. Early clinical onset (EOAD), together with atypical cognitive deficits and the presence of AD-related neuropathology, indicated that from the early stages of AD history, this type of disease is characterized by heterogeneous symptoms. In 1968 it was recognized that the neuropathological findings of early and late onset AD[2] cases were quantitatively similar (Blessed *et al.*, 1968). The merging of these two age-related forms of dementia together with the recognition that AD and senile dementia are the same disease was an important step in the clinical diagnosis (Katzman, 1976). Unfortunately, the first NINCDS-ADRDA criteria defined AD dementia as a progressive memory decline, which only referred to the amnestic presentation of AD (more common among late onset cases), leaving all the atypical non-amnestic presentations in the dark until researchers revisited the topic to characterize the syndrome better, in lack of any disease modifying treatments (McKhann *et al.*, 1984, 2011).

Increasing attention has been drawn to the non-amnestic presentations of AD and some of the main research findings until 2013 are summarized in two review articles (Mendez, 2012; Lam *et al.*, 2013). The heterogeneity of the syndrome of AD dementia can be described in terms of three key features: age of onset, genetic profile, and comorbidities.

4.1 CLINICAL SYNDROMES

Regarding the different clinical syndromes in relation to the AD diagnosis, and the NIA-AA criteria of 2011, a probable dementia diagnosis can be made based on amnestic cognitive deficits, non-amnestic cognitive deficits, or both. Probable non-amnestic AD dementia includes: 1) A language presentation with deficits in word finding, 2) a visuospatial presentation, with deficits in spatial abilities including object agnosia, impaired face recognition, simultanagnosia, and alexia, and 3) an executive presentation dysfunction with impaired reasoning, judgement and problem solving. All the non-amnestic categories should include at least one cognitive deficit that is not specific to their atypical presentation. However, as mentioned earlier in 2.4.1, the latest 2018 criteria are shifting away from the diagnosis based on syndromes and no specific description of criteria for the atypical presentations has been incorporated (Jack *et al.*, 2018).

4.2 CLINICAL SYNDROMES AND AGE OF ONSET

Regarding the age of onset and related clinical syndromes:

- Late-onset AD (LOAD), refers to the diagnosis of AD after the age of 65, is characterized predominantly by memory impairment.

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[2] Early onset AD refers to the diagnosis of dementia due to AD before 65 years of age.

- o Prototypical or typical AD, the most common AD phenotype with LOAD, is associated with bilateral hippocampal, temporal and parietal atrophy of diffuse form. Clinically more than one cognitive domain apart from the episodic memory shows decline.
  - o Temporal (or limbic predominant) variant of AD is the syndrome characterized by atrophy predominantly in the limbic areas, late-onset (even later than typical AD) and isolated episodic memory decline.
- EOAD (before 65 years of age) can be either autosomal dominant (around 5% of the cases) with genetic mutations (see paragraph for familial and sporadic AD) or sporadic AD (Mendez, 2012; Graff-Radford *et al.*, 2021).
  - o Left language variant AD, logopenic primary progressive aphasia (EOAD in most cases).
  - o Posterior cortical atrophy (PCA) variant. See (Crutch *et al.*, 2013, 2017) for a review in PCA.
  - o Right visuoperceptive variant AD.
  - o Frontal-executive variant AD.

Under the diagnosis of probable AD, heterogeneity in clinical presentation is prominent as is pointed out by Scheltens and colleagues (Scheltens *et al.*, 2017).

4.3 NEUROPATHOLOGICAL EVIDENCE

Recent evidence suggests that several patients with a definite diagnosis of AD do not present the typical spatial pattern of neurofibrillary tangles described in the literature, indicating that different pathological subtypes within AD might exist. Based on the distribution of NFT in the brain, Murray and colleagues described three distinct pathological patterns (Murray *et al.*, 2011) characterized by predominance of NFT counts in the hippocampus, in the cortex (middle frontal, superior temporal, and inferior parietal) or both. The clusters that were found included a group of subjects with NFTs predominantly in the limbic areas (limbic predominant), a group of subjects with diffuse NFT both in the hippocampus and in the cortex (typical), and a group with more NTFs in the cortex (hippocampal sparing). Followed by this classification, Whitwell and colleagues investigated the grey matter atrophy patterns of these neuropathologically defined subtypes and found good spatial agreement between the two (Whitwell *et al.*, 2012). Although other groups have tried to assess AD heterogeneity using neuroimaging (Shiino *et al.*, 2006; Karas *et al.*, 2007), the study by Whitwell et al (2012) was the first to use structural MRI to investigate AD subtypes defined pathologically with post-mortem data. One of the main claims of Whitwell's study is that hippocampus to cortex grey matter volume ratio can be used to classify patients into the three subtypes: typical AD, limbic-predominant, and hippocampal-sparing AD. After these two studies, the literature seems to be divided in two directions.

4.4 BIOMARKERS

4.4.1 Heterogeneity in MRI markers

Some studies have focused on understanding the differences in decline and the clinical syndromes of the different subtypes defined by MRI, while others have studied the subtypes with structural imaging or did both if clinical and imaging data were available. For a complete review that covers the majority of published studies in AD subtypes to date you can refer to Habes and colleagues who summarized the findings of those studies from a methodological perspective (Habes *et al.*, 2020), and Ferreira and colleagues who systematically reviewed the same topic from a more bio-clinical perspective and also suggested a framework for heterogeneity in AD (Ferreira *et al.*, 2020).

The studies that used structural MRI can be further subdivided in two main classes:

- The first class can be called supervised, since the criteria for grouping the subjects into clusters are based on prior knowledge. Examples of this approach are the classification of subjects according to: post-mortem pathological features (Whitwell *et al.*, 2012), ratio of neuroimaging features such as hippocampal to cortical ROIs ratio (Byun *et al.*, 2015; Planche *et al.*, 2019), atrophy scales assessed in MR images by experienced radiologists (medial temporal lobe atrophy, posterior atrophy and global atrophy)(Ferreira *et al.*, 2017).
- The second class can be called data-driven, and both unsupervised and supervised machine learning methods are used to identify homogeneous groups of subjects. The next lines focus on the methodological background of the data-driven methods.

The class of studies that assessed AD subtypes with unsupervised or semi-supervised multivariate methods used surface or voxel-based morphometry. The advantage of these approaches is that, by considering all the features simultaneously, one can explore the multivariate dependencies between them, which is of instrumental importance for brain structure and function. This makes the data modeling closer to the organization of the brain, since we assume that dependencies do exist instead of using only a subset of features or a single feature of the brain and disregarding the rest of the information available. One of the attributes that might reduce the comparability of studies that are vertex-based to the ones that are voxel-based, is the exclusion of subcortical regions in the case of the former analysis-type category.

In chronological order, Noh and colleagues used agglomerative hierarchical clustering to group subjects with AD into different subtypes (Noh *et al.*, 2014). The statistical problems that arise from the analysis of the almost 80 000 variables for a group of 152 subjects is well described in the clustering literature (Babu B Hari, Chandra N Subash, 2011). The same method was used later in 2016, in another study (Hwang *et al.*, 2016) of the same research group, and the classification results of the Noh and colleagues study were used in the investigation of the clinical progression of the hippocampal-sparing subtype (Na *et al.*, 2016). Nevertheless, these studies identified clusters of atrophy similar to the ones described by (Whitwell *et al.*, 2012). The study by Dong and colleagues is a paradigm of an investigation that used more than one

diagnosis in the same model to reveal heterogeneity features in the patients from the Alzheimer's Disease Neuroimaging Initiative (ADNI)(Dong *et al.*, 2017). Mild cognitive impairment (MCI) and AD subjects were both used in the same model, which introduced an important bias. MCI cases that did not progress to AD might have introduced noise, which the statistical model has no specific features to separate and estimate. Moreover, as an example of a study that used both 1.5 and 3 Tesla scans, another issue might make the interpretation of the models hard. The introduction of a variable (fixed effect, etc.) to account for field strength might not be enough, because the relationship between field strengths might have a different sign for different brain regions. Two studies have taken into account some of the important issues that were mentioned above in terms of high dimensionality (Park *et al.*, 2017; Corlier *et al.*, 2018). The exclusion of subcortical regions in these vertex-wise studies did not allow exploring features of the covariance between hippocampus and other subcortical regions with the cortex. One study that should not be disregarded is the one by Varol and colleagues (Varol *et al.*, 2017), where support vector machines were used for the identification of AD subtypes, as well as incorporating cognitively unimpaired subjects in the modeling. That study used a low feature number, which allowed for increased interpretability but not all the patterns that were found in previous studies were identified (a minimal atrophy cluster which is characterized by low levels of atrophy in the medial temporal lobe is not observed and the hippocampal sparing cluster also has extended medial-temporal lobe atrophy). This could be due to the small sample of the study or the inclusion of controls and MCI subjects in the same model as the AD patients, which might have limited the ability to identify all AD subtypes.

4.4.2 Heterogeneity in other imaging markers

The pathophysiological processes in AD are not limited to structural brain images. In 2020, two systematic reviews summarized the current findings in the AD heterogeneity field (Ferreira *et al.*, 2020; Habes *et al.*, 2020). Different imaging modalities such as DTI, Tau PET and amyloid-PET studies have shown some interesting preliminary results. Sui and colleagues, in a sample of 48 AD dementia patients from the ADNI cohort found three latent microstructural integrity factors (temporofrontal, long fiber bundle including the corpus callosum and superior longitudinal fasciculus and parietal) with DTI imaging (Sui and Rajapakse, 2018). Amyloid-PET has not shown very heterogeneous patterns so far (Jeon *et al.*, 2019). Tau PET has been a very interesting and rapidly evolving biomarker. In one study from the Mayo Clinic cohorts, three clusters of dementia and MCI patients including mediotemporal, temporoparietal and frontal patterns of Tau in a sample of 86 individuals in total are reported (Lowe *et al.*, 2018). In another study with data from the same cohorts, a minimal, a more cortical, and a more diffuse pattern of tau were identified (Whitwell *et al.*, 2018*a*). The clusters of patients with higher cortical tau uptake were also reported to have the lowest prevalence of APOE e4 allele carriers. Finally, a study by Vogel and colleagues in 2020 showed four patterns of tau accumulation including limbic predominant and mediotemporal sparing patterns as well as posterior and lateral temporal patterns (Vogel *et al.*, 2020). This study included predominantly preclinical AD, and therefore it is hard to compare its results with the findings of Murray and colleagues (Murray *et al.*, 2011).

4.4.3 Heterogeneity in CSF markers

Under the AD dementia diagnosis, some heterogeneous patterns of CSF markers were also identified, showing that extreme variability can also be identified in non-imaging data (Duits *et al.*, 2021). More specifically, a study of a large group of patients diagnosed with AD dementia or AD dementia with Lewy bodies showed that five groups of different CSF (ubiquitin, tau, $A\beta$) exist within AD: low $A\beta$, high incidence of APOE e4 carriers, and late dementia onset; low $A\beta$, high tau and early dementia onset; low $A\beta$, high tau, high ubiquitin, recent dementia diagnosis; high $A\beta$ and recent dementia diagnosis; low $A\beta$ late dementia onset and included most of the AD with Lewy bodies cases (Iqbal *et al.*, 2005). A recent study based on CSF proteomics and unsupervised analysis has identified three clusters of AD dementia $A\beta$ positive patients including a group with high neuronal hyperpasticity and high beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) levels (largest cluster in the sample), an innate immune activation group and a group with low BACE1 levels and blood brain barrier dysfunction (Tijms *et al.*, 2020).

5 STATISTICAL METHODS AND APPROACHES

5.1 UNSUPERVISED APPROACHES

Since the focus of this thesis is the assessment of heterogeneity in AD, unsupervised learning is the main tool that we use to understand the different cross sectional and longitudinal patterns of disease imaging biomarkers (all patients have the same theoretical disease label).

Unsupervised learning is the branch of data analysis that facilitates, 1) clustering and 2) association analyses. The word unsupervised is intended to indicate that the dataset under assessment has no target or so-called response variable. As the exact opposite to supervised learning (regression or classification), this type of analysis aims to find a response vector (put observations into classes), so that similar observations fall into the same class. In this review I will focus on clustering analysis. A cluster is a "collection of objects which are similar between them and dissimilar to other clusters objects" (Jiawei Han, 2001). The similarity between observations can be calculated with a distance measure. The selection of the distance measure is very important and affects the clustering result. Different types of variables will need different types of distance measures if we desire meaningful clusters. Popular measures of distance are Euclidean distance, Mahalanobis distance and Manhattan distance.

Clustering can be subdivided into two branches, distance-based and model-based.

- Distance-based clustering explicitly uses some distance measure to define distance and then an iterative algorithm is applied to optimize a criterion, which aims to minimize the within cluster distances of observations (intra-cluster) and maximize the between cluster distance of observations (inter-cluster) (Figure 3). Popular methods include the k-means and hierarchical clustering methods.
- Model-based clustering uses statistically defined distributions to describe the clusters. That is, the clusters are assumed to have the shape of a distribution. In this way, we can characterize a cluster if we know the parameters that describe its distribution. Thereafter, clustering turns into a problem of parameter estimation and common likelihood approaches can be utilized to find clusters. A famous model-based clustering approach is the Gaussian mixture model clustering. In this model, we predefine the number of clusters that we want and the optimization procedure will find the means, standard deviations of the Gaussian clusters and also the probabilities of each observation belonging to any of the clusters (the clustering part). Different clusters might have different shapes. Gaussian clustering can help to identify their shape with the estimation of covariance between the variables. Distance is also used in model-based clustering during the model optimization process.

5.1.1 Clustering in high dimensional data.

The rapid growth of data availability, especially with new applications in bioinformatics and neuroimaging, has brought the data analysis community to the fore of a new challenge, called high dimensional data analysis. Here I refer to wide datasets that include many more measurements (variables) than observations. In the context of neuroimaging, an example vertex-wise study might result in more than three hundred thousand variables that correspond to cortical thickness measures and 100-200 subjects. This will result in a dataset where the variables are thousand times more than the observations. The specification of distance measures for high dimensional data is a challenging task and a topic of ongoing research (Babu B Hari, Chandra N Subash, 2011). Some of the issues arising from an analysis in these datasets are listed here:

- The clustering tendency will decrease with the increasing amount of noisy variables.
- As the number of attributes or dimensions increases, the distance measures become increasingly meaningless and the empty space (dimensional neighbourhoods with no observations) increases exponentially. The resulting clusters tend to be equidistant from each other (Aggarwal *et al.*, 2001).

Some of the ideas behind the engineering of high dimensional clustering methods include (Babu B Hari, Chandra N Subash, 2011):

- Dimensionality reduction: Projection methods that effectively reduce the initial dimensions of the dataset. This leads to a dataset representation in a dimension much lower than the initial one, where clustering will not suffer from optimization issues. Principal component analysis (PCA) is such a method.
- Subspace clustering: Clustering that effectively discards variables or reduces the importance of some variables. This method also leads to a more manageable dataset in terms of clustering. The optimization searches for dimension neighbourhoods where the data "live".
- Bayesian modeling. In terms of model-based clustering, Bayesian modeling can introduce bias to the estimation of the cluster parameters. In this way we can explicitly add power to the statistical parameters so that we can increase the information provided to the clustering algorithm from a study sample perspective.

A number of models have been proposed in the literature to cluster data referred to as n<<p (number of observations is much lower than the number of parameters)(Laurent. Bergé, Charles. Bouveyron, 2012).

5.1.2 Longitudinal clustering: Ideas and models

Longitudinal data clustering refers to the clustering of observations/subjects where each or some of the variables are measured repeatedly over time. Subjects with similar trajectories for each variable's measurements over time are selected to be clustered together. This will eventually yield to homogeneous sub-populations in terms of trajectories. Different methods

were proposed in the literature, including model-based classification based on mixture models (models that have both fixed and random effects) (James and Sugar, 2003; Luan and Li, 2003; Komárek and Komárková, 2013, 2014) variants of k-means (Tarpey and Kinateder, 2003; Genolini *et al.*, 2013, 2016), and PCA like methods (Greven *et al.*, 2010). These methods use similarity like cross sectional data analysis. However, in most of the available longitudinal clustering algorithms in contrast to cross sectional clustering two subjects are considered similar:

- If they have similar values at each time point. In this way we account for local similarities between subjects. It is intuitive in that, 1) data with all observations for all the time points are needed to calculate such similarities, or 2) we define a time neighbourhood around which all data points should be similar in terms of their features (to incorporate subjects with missing time points).
- If their trajectories shape is similar. This case can also include data that are irregularly sampled (Komárek and Komárková, 2013). Model-based clustering with mixed effect will estimate intercepts and slopes to define the shape of each subject trajectory and the clustering will be based on this feature.

5.2 SUPERVISED APPROACHES

Formally, supervised learning is the term used by the machine learning community to describe statistical classification and regression. This set of methods have a complementary yet important role in this thesis. After the investigation of potential grouping labels for patients and CU individuals with clustering, supervised learning was used to understand how the clusters that we created associate with features other than the ones used in the clustering. More information on the classification and regression methods that were used in this thesis will be provided in the section "participants and methods".

6   AIMS OF THE THESIS

The overall aim of this thesis is to utilize neuroimaging markers together with unsupervised statistical learning methods to define and characterize subtypes of Alzheimer's disease (AD). The specific aims of the different studies included in the thesis are specified below.

In **paper I**, we study subtypes of AD based on patterns of atrophy in grey matter using cluster analysis. Further, we describe the clinical characteristics and biomarker patterns in the different subtypes defined with atrophy markers.

In **paper II**, we classify longitudinal atrophy trajectories, while assessing the effects of different confounders using Bayesian unsupervised learning in the AD subtypes defined in paper 1. Further we present brain atrophy maps of central tendency and dispersion through the same model without additional postprocessing. Moreover, we study the convergence of the clustering algorithm.

In **paper III**, we investigate whether the previously defined subtypes are truly distinct subtypes or just groups of patients at different stages of AD. To do this we train a model similar to the one from paper 2 where trajectories of atrophy after the clinical onset of the disease are estimated in a large multiethnic dataset.

Finally, in **paper IV** we extend our investigation to white matter and the general population. We explore how white matter integrity varies between groups of individuals over time and also its potential associations with rate of cognitive decline. Additionally, we discover differences between white matter integrity aging groups in terms of other pathologies (white matter hyperintensities and amyloid burden), cognition and demographical characteristics.

7 PARTICIPANTS AND METHODS

This section is devoted to the description of the cohorts used in this thesis, the inclusion criteria for participants in the studies, a short presentation of the statistical methods that were utilized and the study designs of the four papers.

7.1 Ethical considerations

All studies in this thesis were conducted according to the reviewed Helsinki Declaration. The ADNI study was conducted in multiple centers. Thus, ethical approvals were separately granted in each center. The AIBL study was approved by the institutional ethics committees of Austin Health, St Vincent's Health, Hollywood Private Hospital and Edith Cowan University. AddNeuroMed is a public AD cohort collected in six sites across Europe, with ethical review board approval obtained by each local institution. Approval for the JADNI study was obtained from the local ethics committees or institutional review committees at the 38 participating clinical sites, including the principal investigator's site (The University of Tokyo). The MCSA study was conducted in Rochester, Minnesota, and the ethical board of Mayo Clinic has approved the collection and analysis of the data. Informed written consent for the study participation was obtained from all participants/relatives at each clinical site.

7.2 PARTICIPANTS

7.2.1 Cohorts

ADNI

The ADNI is an initiative that started in 2003 and is still ongoing. It has gone through three phases (ADNI, ADNI GO, and ADNI 2) of data collection and renewal of its aims and selection criteria and now the 4th phase (ADNI 3) is ongoing. It was launched as a private public partnership. The overall goals of ADNI can be summarized as: to test if different imaging modalities together with other biomarkers of the human central nervous system, as well as neuropsychological criteria and clinical information can help to understand and predict progression from MCI to AD. However, many additional aims were added over the years and the accumulation of new knowledge in the fight against neurodegenerative disorders. More specifically, ADNI strives to reveal new sensitive biomarkers that will help to monitor disease progression and treatment and reduce the expenditures of clinical trials. For more information visit http://adni.loni.usc.edu/

AddNeuroMed

AddNeuroMed is a pan-European cohort and part of the innovative Medicines Initiative (Funded by the EU sixth framework programme). Similar to ADNI and highly harmonized with it, the architects of AddNeuroMed set out to understand AD and discover sensitive biomarkers of disease progression. Extensive neuropsychological examination and blood testing was recorded for most participants, while MRI sampling was applied to a subset of participants (followed up for 12 months). The participating institutions that acquired MRIs

which were used in this thesis were the following: University of Perugia (Italy), Aristotle University of Thessaloniki (Greece), University of Kuopio (Finland), King's College London (United Kingdom), and University of Toulouse (France). For more information visit https://doi.org/10.7303/syn22252881

JADNI

The Japanese equivalent of ADNI was a public-private multicenter initiative that started in 2007 to collect data from many centers around Japan under the leadership of Principal Investigator Takeshi Iwatsubo. Extensive neuropsychological data, MRI, and PET (glucose metabolism and $A\beta$) were collected in more than one instance for each participant. This study aimed to understand similar features of AD as the designers of ADNI and AddNeuroMed but in the context of the Japanese populations. For more information visit https://humandbs.biosciencedbc.jp/en/hum0043-v1.

AIBL

The Australian Imaging, Biomarkers and Lifestyle study of Ageing is a large longitudinal study that spans from healthy aging to dementia. Its aims are similar to those of ADNI and JADNI. The contribution of this study in AD research is great since it adds information about the variability of healthy and dementia data that help to conclude the strength and generalizability of potential imaging biomarkers in the fight against AD. The study has assessment intervals of 18 months, uses MRI, $A\beta$ PET, blood samples, extensive neuropsychological testing, and lifestyle questionnaires. More information can be found in https://aibl.csiro.au/.

MCSA

The Mayo Clinic Study of Aging aims through the conduction of research to promote healthy aging, the development of prediction models for the assessment of risk for cognitive impairment, and the prediction and prevention of dementia. It is a community-based study (Olmsted County, Minnesota) and as such one of its ultimate goals is to follow up many individuals to identify those who will develop cognitive impairment early on and follow them up to understand the timeline of pathophysiological changes underlying dementia. Among other markers, they collect MRI (structural, functional), PET ($A\beta$, tau, glucose metabolism), CSF blood and neuropsychological data. For more information visit https://www.mayo.edu/research/centers-programs/alzheimers-disease-research-center/research-activities/mayo-clinic-study-aging/overview.

7.2.2 Studies I and II participants

Data from ADNI and AddNeuroMed were used to address the aims of the first study (Table 1). Since AddNeuroMed MR protocols are harmonized in order to be used together with ADNI participants from both studies were pooled together (Westman *et al.*, 2011).

Table 1. Demographics of ADNI and AddNeuroMed in Study I.

	AD	CU
N	299	328
Female/Male	164/135	163/165
Age*	76 (71-80.3)	74.5 (71.5-78.3)
Years of education*	12 (8-16)	14 (11-16)
MMSE*	23 (21-25)	29 (29-30)
CDR global**	0.9 (0.4)	0 (0)
ADAS word recall **	6.32 (1.49)	3.7 (1.3)

*median (Q1-Q3) where Q1=1st quartile, **mean (sd), MMSE: Mini Mental State Examination, CDR: Clinical Dementia Rating, ADAS: Alzheimer's Disease Assessment Scale.

The second study included a small subsample of the first study. The inclusion criteria were very strict to include AD patients from ADNI that were used in the first study, with no missing data in the time interval that was used in the study. Moreover, only $A\beta$ negative in CSF CU individuals who were CU during all their future assessments and had no missing MRI timepoints (the same as the AD patients) were included (Table 2).

Table 2. Demographics of Study II where a subset of ADNI was included.

	AD	CU
N	72	31
Females/Male	34/38	15/16
Age at first visit**	76 (7.4)	74 (4.4)
Age at disease onset*	71 (8.9)	-
Years of education *	16 (3)	16 (3)
MMSE**	24 (1.5)	29 (0)
CDR global*	0.72 (0.25)	0 (0)
APOE e4 allele carrier N (%)	50 (69.4%)	3 (9.7%)
CSF $A\beta_{1-42}$*	137.38 (24)	234.11 (21)
CSF pTau $_{181}$*	37.5 (12.6)	18 (4.45)
ADAS word recall**	6.17 (1.43)	2.81 (0.95)

*median (mad), **mean (sd), MMSE: Mini Mental State Examination, CDR: Clinical Dementia Rating, ADAS: Alzheimer's Disease Assessment Scale.

7.2.3 Study III participants

For Study III, data from ADNI, JADNI, AIBL, and AddNeuroMed were used. All four datasets are harmonized to have similar parameters in the MRI scanners. The complete dataset was split

into discovery and prediction datasets for the purpose of the analysis. In the discovery dataset all CU individuals were $A\beta$ negative, they had at least two MRI measurements and their cognitive status remained CU during all the available visits recorded in the four cohorts. As for the AD patients of the discovery dataset, they were $A\beta$ positive and had at least two MRI measurements (Table 3). The prediction dataset included AD patients that were not $A\beta$ positive/ had no $A\beta$ information/ or had at most one MRI available (Table 4).

Table 3. Demographics of the discovery set in Study III.

	CU			AD		
	ADNI	JADNI	AIBL	ADNI	JADNI	AIBL
N	158	62	85	207	90	23
Females/Males	77/81	34/28	40/45	116/91	38/52	10/13
Age at first visit*	73.5 (5.7)	67.5 (5.8)	70.2 (7.4)	75.7 (7.1)	75 (8.6)	72.6 (9.9)
Age at disease onset*	-	-	-	72 (7.4)	73 (7.4)	72 (7)
Education class**	3.65 (0.7)	3.15 (0.9)	2.91 (1)	3.36 (1)	2.57 (1)	2.61 (1)
APOE e4 allele carrier***	26 (16%)	8 (13%)	26 (31%)	155 (75%)	55 (61%)	17 (74%)
APOE e2 allele carrier***	30 (19%)	5 (8%)	17 (20%)	8 (4%)	4 (4%)	0 (0%)
MMSE*	30 (0)	30 (0)	29 (1.5)	23 (3)	23 (1.5)	24 (4.5)
CDR**	0 (0)	0.01 (0.1)	0.03 (0.1)	0.79 (0.3)	0.64 (0.2)	0.67 (0.2)

*median (mad), **mean (sd), ***n(%), MMSE: Mini Mental State Examination, CDR: Clinical Dementia Rating, ADAS: Alzheimer's Disease Assessment Scale. Education years are categorized in 4 classes (1 =< 0-8 years; 2 = 9-13 years; 3 = 13-15 years, 4 > 15 years).

Table 4. Demographics of the prediction set in Study III.

	AD			
	ADNI	JADNI	AIBL	AddNeuroMed
N	216	168	67	120
Females/Males	119/97	70/98	29/38	41/79
Age at first visit*	76.4 (7.6)	76.2 (5.6)	76.7 (7.1)	76 (6.6)
Age at disease onset*	74 (8.9)	75 (5.9)	74.9 (7.4)	72 (5.9)
Education class**	3.26 (0.91)	2.57 (0.91)	2.5 (1.06)	1.54 (0.82)
APOE e4 allele carrier***	115 (53.2%)	74 (44%)	24 (35.8%)	59 (49%)
APOE e2 allele carrier***	85 (39.4%)	59 (35.1%)	23 (34.3%)	48 (40%)
MMSE*	23 (3)	22 (2.9652)	22 (4.4)	22 (5.9)
CDR**	0.81 (0.35)	0.71 (0.25)	0.84 (0.46)	1.18 (0.5)

Aβ positive***	99 (46%)	16 (10%)	22 (39%)	-
Aβ negative***	27 (13%)	14 (8%)	2 (3%)	-

*median (mad), **mean (sd), ***n(%), MMSE: Mini Mental State Examination, CDR: Clinical Dementia Rating, ADAS: Alzheimer's Disease Assessment Scale. Education years are categorized in 4 classes (1 =< 0-8 years; 2 = 9-13 years; 3 = 13-15 years, 4 > 15 years). Aβ status was assessed either with PET or CSF sampling.

7.2.4 Study IV participants

In Study IV a subset of the MCSA cohort included participants with repeated measurements DTI. In contrast to the other studies here CU, MCI, and AD were used. Table 5 includes an overview of the data used.

Table 5. Demographics and baseline data of MCSA subset used in Study IV.

	Age group at the baseline scan		
	60-70	**70-80**	**80+**
N	247	188	118
Females/Males	119/128	90/98	44/74
Age at first visit*	65.00 (3.0)	74.90 (3.7)	84.50 (4.1)
Years of education**	16.00 (2.97)	14.00 (2.97)	14.50 (3.71)
APOE e4 allele carrier***	71 (29)	59 (31)	32 (27)
APOE e2 allele carrier***	40 (16)	32 (17)	12 (10)
Gait speed*	121.00 (15.9)	114.00 (18.1)	100.70 (20.8)
CU***	230 (93.1)	161 (85.6)	95 (80.5)
Global cognition*[1]	0.47 (0.8)	0.05 (1.0)	-0.34 (0.9)
Memory*[1]	0.45 (0.9)	0.00 (1.2)	-0.36 (1.4)

*median (mad), **mean (sd), ***n (%), CU: cognitively unimpaired, 1: cognitive functionality, normative z-values where higher values correspond to better cognitive score.

7.3 METHODS

The following section is devoted to the description of the cohorts.

7.3.1 Diagnostic criteria

The diagnosis criteria for dementia as well as some other key features of every cohort that were included in the thesis are briefly described in Table 6. We will not describe the diagnosis of CU or MCI since it is not vital to the studies presented here.

Table 6. Main characteristics of the cohorts used.

	ADNI	JADNI	AIBL	AddNeuroMed	MCSA
Study design	Longitudinal multicenter cohort	Longitudinal multicenter cohort	Longitudinal multicenter	Longitudinal	Longitudinal
Data collection period	2004-present	2018-2014	2006-present	2004-2010	2004-present
Country	USA and Canada	Japan	Australia	Finland, France, Greece, Italy, Poland, Sweden and United	USA
Dementia inclusion criteria	MMSE (20-26); CDR (0.5-1); Age (>65); NINCDS/ADRDA criteria for probable AD; GDS <6	MMSE (20-26); CDR (0.5-1); Age (>65); NINCDS/ADRDA criteria for probable AD; GDS <6	MMSE (18-26); CDR (>0.5); NINCDS-ADRDA criteria for probable AD, ICD-10	MMSE (12-28); Age (>60); NINCDS/ADRDA criteria for probable AD and DSM-IV	CDR>1 (dementia) or CDR>0.5 (further evaluation); DSM IV for dementia
Follow-up	3-12 months	6-12 months	18 months	12 months	Variable (1-3
MRI field strength	1.5T and 3T	1.5 T	1.5 and 3T	1.5 T	3T
Biomarkers	yes	yes	yes	no	yes
CSF $A\beta$ platform cut-off	Elecsys, <880pg/ml(Hansson et al., 2018)[*]	Luminex , <333 pg/mL(Iwatsubo et al., 2018a)	-	-	-
PET cut-off	FBB SUVR >1.08 AV45 SUVR >1.11[**]	AV45 SUVR >1.11; PiB SUVR > 1.48(Yamane et al., 2017)(Iwatsubo et al., 2018a)	PiB SUVR >1.5(Rowe et al., 2010)	-	PiB SUVR >1.48 (centiloid 19)(Jack et al., 2017)

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The cohort information stated here regards this thesis and specific biomarkers used here. MMSE: Mini mental state examination; CDR: Clinical dementia rating: CDR: CDR sum of boxes; GDS; Geriatric depression scale; NINCDS/ADRDA (National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association; ICD-10 Classification of Mental and Behavioural Disorders; standardized uptake value ratio (SUVR), AV45 (florbetapir), PiB (Pittsburgh compound B), FBB (Florbetaben), *The UPENN biomarker batch 9 was used to quantify ADNI CSF positivity. **Composite SUVRs for FBP $\geq$1.11 or FBB $\geq$1.08 were defined as positive as described on the ADNI website (https://adni.bitbucket.io/reference/docs/UCBERKELEYFBB/UCBerkeley_FBB_Methods_04.11.19.pdf).

7.3.2 Neuroimaging

The imaging markers that were used for the purpose of this thesis are listed in table 7.

Table 7. Imaging modalities used in the four studies.

	Study I	Study II	Study III	Study IV
T1 sMRI	Single*	Repeated*	Repeated*	-
T2 sMRI	-	-	-	Repeated**
PET	-	-	Single**	Repeated**
DTI	-	-	-	Repeated*

*Main analysis, **secondary analysis

Morphometry and image processing

Morphometry characterizes the shape and size of the brain and its structures. Voxel-based morphometry (VBM) is a method that segments MRI images into different brain tissues (white matter, grey matter, CSF), normalizes them to a template and applies smoothing (Whitwell, 2009). Furthermore, some brain atlas provides regional volumes (ROIs) that can be further used in the investigation of hypotheses. Surface-based morphometry (SBM) extracts the surface between CSF and grey matter and the surface between grey and WM (Dale *et al.*, 1999; Fischl *et al.*, 1999). Then we can calculate the distance between the two surfaces and estimate the thickness of the cortex in any region to obtain regional values of thickness. Modern software with user-friendly programming environments is implemented for both VBM: Statistical Parametric Mapping (SPM, www.fil.ion.ucl.ac.uk/spm), FSL, (www.fmrib.ox.ac.uk/fsl) and SBM: Freesurfer (http://surfer.nmr.mgh.harvard.edu), Brain Voyager (brainvoyager.com).

In this thesis, we are using structural magnetization-prepared rapid acquisition gradient echo scans MRI (T1) as a tool, for Studies 1, 2 and 3 in our attempt to address the AD heterogeneity question from an atrophy perspective. More specifically, we use Freesurfer 5.3 (Study I) and 6.0 (Studies II and III) pipelines to extract a set of volume and thickness measurements of the cortex and deep grey matter structures which we use later in our analyses. As it is recommended, we visually inspect the structural MRIs for artefacts and also the output of the Freesurfer pipeline for possible issues in the segmentation of the grey matter, WM and CSF. In Study IV, we use T2-FLAIR MRI to investigate the correlation between WM hyperintensities (WMH) in the aging population with groups that have different WM integrity trajectories over the years.

Diffusion MR

After the DTI acquisition, the data from the images were reconstructed, underwent preprocessing, and were debiased, denoised and the skull was stripped out (Vemuri *et al.*, 2018). They were further preprocessed with the FSL and BrainSuite software and finally diffusion tensors were fitted to extract FA and MD. Lastly, the WM Eve atlas was applied to the data to extract regional values (Poulakis *et al.*, 2021). After some cleaning for voxels that mostly represented air or CSF, ROIs were used in further analysis. In this thesis we used only FA measured by diffusion tensor imaging (DTI) at a ROI level, to assess longitudinal heterogeneity in the integrity of WM tracts in cognitively unimpaired and demented elderly individuals (in Study IV).

Other markers

$A\beta$ PET was used to quantify $A\beta$ pathology in Studies III and IV. Based on standardized uptake value ratios for AV45 (florbetapir), PiB (Pittsburgh compound B), and FBB (florbetaben) PET, $A\beta$ positivity (dichotomized variable) was established in Study III (Table 6). In Study IV $A\beta$ was used as a continuous measure to quantify associations with WM tracts and WM integrity clusters of individuals. CSF from Aβ was used as a continuous variable in Study II and as a dichotomous variable in Study III (Table 6).

7.3.3 Analyses preceding the main modeling.

Before the main analysis we applied transformations of the data so that our results were not confounded by batch effects related to specific cohort sampling and design biases. In Study I, the data from ADNI and AddNeuroMed were corrected for the effects of age, sex, education, and estimated intracranial volume (eTIV). We utilized the residual method, assuming a linear effect of the aforementioned confounders in ROI volumes (Voevodskaya *et al.*, 2014). In Study II, we accounted for the effects of age, sex, years of education, eTIV, and baseline CSF values of pTau181 and $A\beta_{1-42}$. Moreover, in Study II, z-values were calculated for the AD patients for CU brain aging, based on a follow-up time matched sample of CU individuals. In Study IV a similar approach was followed where all data were z-value transformed based on the first visit of each participant. Confounders that we accounted for during the main analysis in Study IV included APOE e4 carriership and sex. Age was not accounted for since it was the main variable of interest around which the study design was tailored.

Study III has the most complicated design of data pretreatment among the four studies. A sample of $A\beta$ negative CU individuals from three cohorts was used to estimate mixed effect models where ROI brain data were used as dependent variables, age was used as fixed effect and cohort assignment was used as random effect (together with individual specific random effects). Through these estimations we managed to acquire a mean value of healthy anatomy at a given age and cohort as well as the variation around this mean. We also managed to predict within and between cohort variabilities. For each AD patient and each brain ROI from the discovery and prediction cohort of Study IV, a specific atrophy value corresponding to his/her age and cohort was subtracted and the result was divided by the model's standard deviation. The resulting value reflects the number of standard deviations below the normative CU value

for a specific cohort and the age to which the participant's brain data corresponds. In other words, the question that we answer through this pre-treatment is "how much is the atrophy caused by the disease and not by cognitively healthy anatomical aging or cohort?".

7.3.4 Unsupervised

The main methodological part of the four studies consists of clustering algorithms of variable difficulty. In Study I, random forest was used to understand the similarity of brain atrophy between pairs of AD patients in our cross-sectional dataset. Although the same statistical model was utilized in the other three studies (II, III and IV), the methodological differences between their designs are substantial, and the interpretations of the results in the three studies are completely different. In this section, a short presentation of the methodological designs of each study will reveal their similarities and differences.

Study I

The main features of this study include its cross-sectional nature, the data used in the clustering (Table 1), and the atlas that was applied to the sMRI (Destrieux *et al.*, 2010) data in order to consider both sulci and gyri in the model.

Figure 1. Flowchart of Study I.
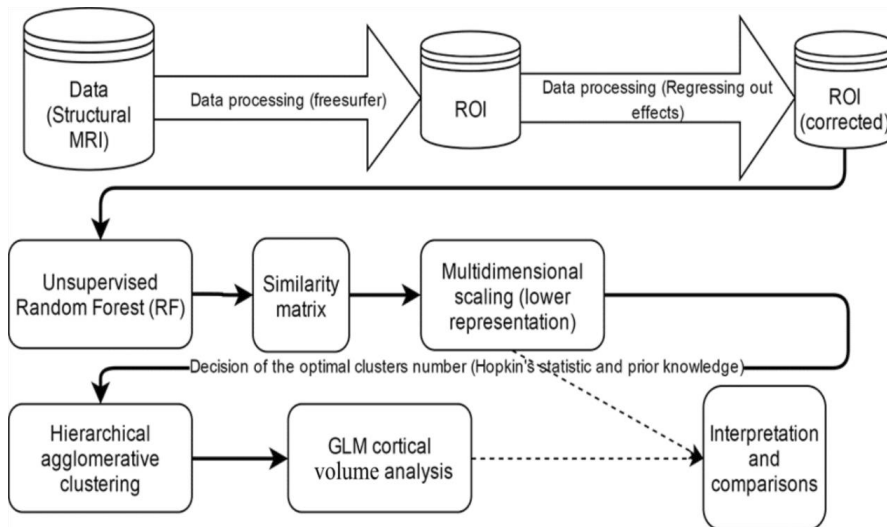
The study flowchart (Figure 1) shows that after correction for confounders (see section 7.3.3), the next step is the calculation of a similarity matrix trough the random forest method. Random forest is no more than an ensemble classifier that consists of several classification and regression trees (CART). In the case of clustering, random forest turns into a classification model that aims to classify correctly between artificial data (class 1) and the data that we feed it with (class 2)(Shi and Horvath, 2006). If the classification is successful, this means that the data have an interesting covariance that also makes them potentially clusterable. The similarity (proximity) matrix that is produced by random forest informs us on how similar an observation

(patient) is to any other observation, considering the brain volumes with which we provided it. Then, multidimensional scaling (MDS) summarizes the similarity matrix into few components. This is where all the dataset is reduced to a 2D/3D scatter plot that resembles a map where each patient is close to another patient if their brain patterns are alike and far away if their brain patterns are completely different. A allegory to better understand how MDS works follows: imagine that we have a matrix where the kilometric distance of any capital city of Europe to any other capital city of Europe is recorded. If we calculate the MDS of this distance matrix and then plot it, an approximate map of Europe will be produced.

Clustering is applied to the reduced data (MDS components) and the number of clusters is established. After that step, voxel wise maps for the comparison between each cluster of AD patients and the group of CU individuals was produced for the purpose of our analysis.

Study II

In Study II our efforts were focused on understanding how longitudinal clustering works and adapting it to brain atrophy data.

Figure 2. Flowchart of Study II.
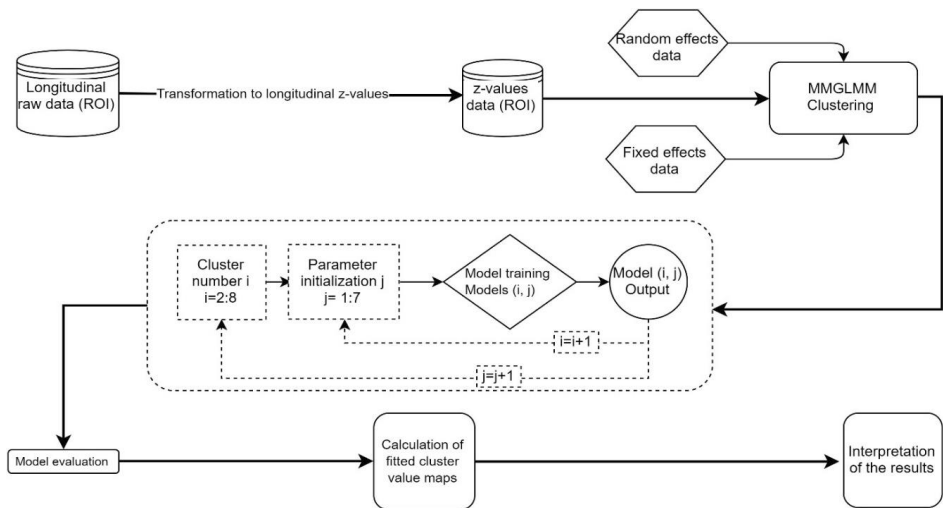
The design of the study was established so that there would be no additional need for analysis to visually assess cluster differences post-clustering as in Study I. The longitudinal raw thickness (cortical) and volume (subcortical) data of the AD patients were z-transformed with a CU group as a reference level of atrophy on which the mean and standard deviations were based (Table 2). Subsequently, fixed and random effects as they were described in section 7.3.3 were added.

The model that was used attempted to reconstruct patient's brain atrophy trajectories given a small set of available timepoints and is described in broad terms in section 5.1.2. To increase the chances of a successful trajectory reconstruction, the similarity of patients through

clustering was also estimated. Through the sequential procedure where the trajectories of patients and the patient's assignments into clusters were estimated, we managed to estimate trajectories for each patient by comparing him/her with other patients that fell into the same cluster. The ultimate goals of the algorithm were to calculate the chances for each patient to be in each cluster and to estimate cluster atrophy trajectories over time.

Clustering of each patient can be achieved in two different ways:

- The first method involves the recall of all the simulated chances that a patient belongs to each cluster. Then the average chance that a patient belongs to a cluster is calculated. The cluster to which the patient is allocated is the one to which the patient has the highest average chance to belong to. This is called the maximum probability rule. This method disregards clustering uncertainty that can be calculated with Bayesian methods.
- The second method utilizes the highest porterior density (HPD) credible intervals (intepreted as confidence intervals in frequentistic statistics) of the distribution that assesses the probability that a patient belongs to any of the clusters. For example, in a three-cluster solution a patient will have three HPD intervals, where each one will inform us on how well he or she is bunched to each cluster. If none of them shows high chances of allocation (for example the HPD takes values higher than 50%), then our certainty on the patient cluster assignment is low.

Figure 3. Longitudinal data presentations under three different timescales.
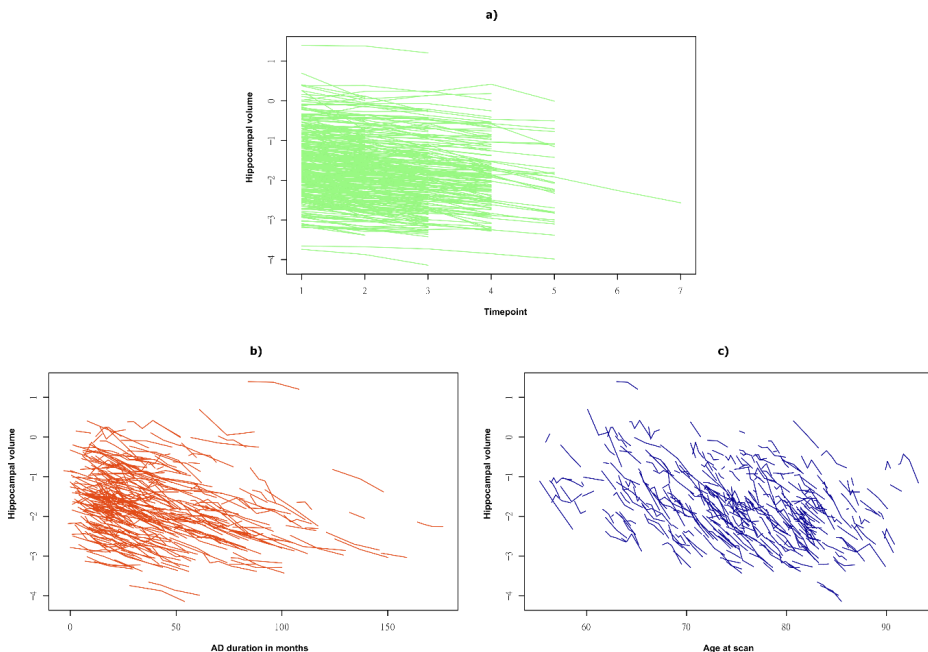
Three approached for modeling longitudinal AD data. In a), the timepoint approach (green) which is commonly used; b) disease duration approach; c) age as a timescale approach. All data are normalized for the effects of aging and cohort.
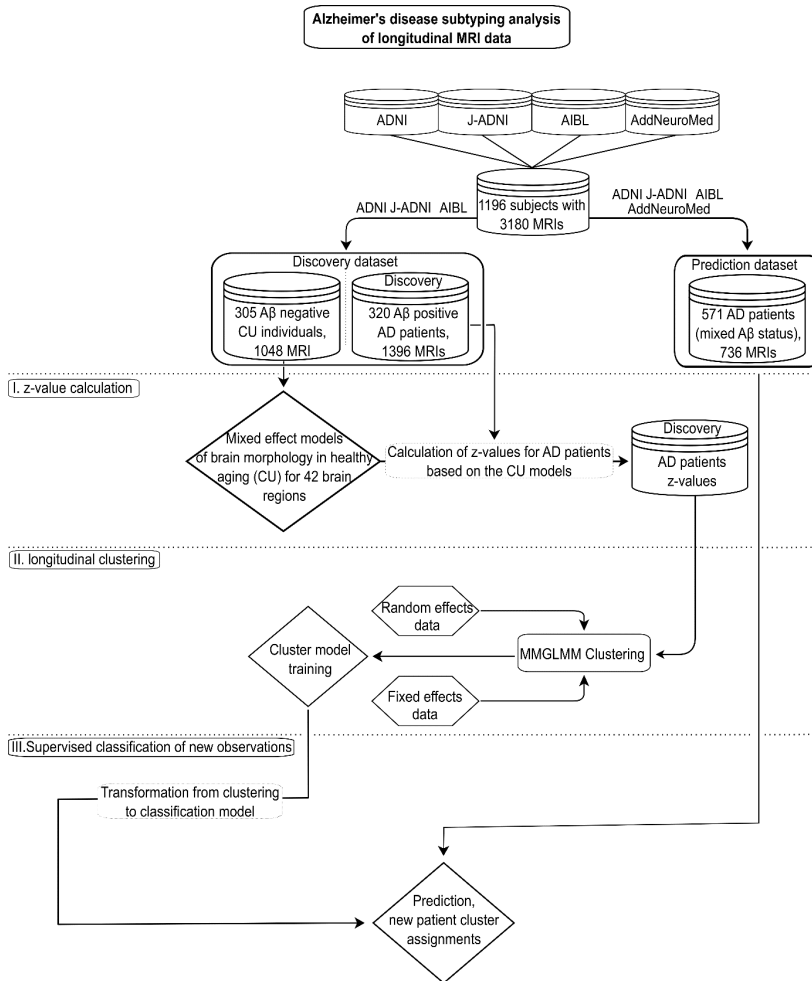
The algorithm that we used (Komárek and Komárková, 2013) included many parameters requiring estimation. To achieve an acceptable learning of the parameters we decided to give "hints" to the algorithm, by initializing it with different potentially informative clustering solutions. Practically, this was achieved by initializing the cluster intercepts with 1) baseline data clustering including k-means and hierarchical clustering, 2) results from Study I that included our current knowledge on brain atrophy levels in AD, 3) uninformative default values of the computational packages that were used. Many models were trained (seven different models for 2-8 number of clusters solutions = 49 models), and their performance was assessed to conclude the best clustering solution. Finally, fitted values were calculated to visually assess the average atrophy profile for each cluster. Moreover, to assess cluster-specific variance, dispersion fitted values were also calculated.

Study III

Since the main aim of Study III was to understand the effect of disease staging on the observed heterogeneity of AD neurodegeneration patterns, disease duration was modeled using the same approach as in Study II (for actual data used see section 7.2.3). However, cohort time point in Study II was not used here. Instead, the time from clinical dementia onset was used as random effect. This introduced a complete change in the methodological approaches of Studies I and II. In those studies, for the sake of simplicity, we assumed that disease duration is a common linear population effect for all AD patients. In Study III we changed our methodological approach and modeled disease trajectories independently and non-linearly for each potential neurodegeneration trajectory. Our approach was motivated by the preexisting knowledge that some dementia patients have more aggressive disease course than others. The timescale of the model that was trained for the purpose of Study III informs us on how atrophy evolves from clinical AD onset and onwards. For a very good methodological presentation on how different timescales can affect atrophy pattern estimations, the reader should read a recent study by Dicks and colleagues (Dicks *et al.*, 2019).

The differences between three timescales in modelling longitudinal atrophy data is presented in figure 3. An example of the timepoint approach that was used in studies I and II but with hippocampal atrophy data that were used in study III (table 3 AD data) shows that information on trajectories can be seen there too (Figure 3a, green). However, all the temporal profiling is lost since the assumption that age at scan or disease duration are population wide effects was made. Figure 3c (blue) shows how hippocampal volume reduces with age, assuming that disease has the same effect in all AD patients. Finally, figure 3b (red) shows hippocampal atrophy in AD patients over 150 months from the disease onset, assuming that age is a population wide effect.

Figure 4. Flowchart of Study III.

Alzheimer's disease subtyping analysis
of longitudinal MRI data

ADNI   J-ADNI   AIBL   AddNeuroMed

ADNI J-ADNI AIBL   1196 subjects with   ADNI J-ADNI AIBL
3180 MRIs   AddNeuroMed

Discovery dataset   Prediction dataset

Discovery

305 Aβ negative   320 Aβ positive   571 AD patients
CU individuals,   AD patients,   (mixed Aβ status),
1048 MRI   1396 MRIs   736 MRIs

I. z-value calculation

Mixed effect models   Discovery
of brain morphology in healthy   Calculation of z-values for AD patients
aging (CU) for 42 brain   based on the CU models   AD patients
regions   z-values

II. longitudinal clustering

Random effects
data

Cluster model   MMGLMM Clustering
training

Fixed effects
data

III. Supervised classification of new observations

Transformation from clustering
to classification model

Prediction,
new patient cluster
assignments

Study IV

In this study, longitudinal unsupervised learning (similar to Figure II) was used to assess changes in WM integrity as it can be approximated by FA in DTI. The longitudinal modeling of the data for the purpose of this study was different from that of Studies II and III, since a population and not a disease-specific sample was assessed. Looking at the DTI marker independently of clinical diagnosis did not allow for the use of disease duration as the main timescale. Instead, age at scan was chosen (Figure 3c). In this way, the resulting clusters present groups of individuals with differential WM aging in the population.

Through this kind of modeling three linear trajectory alternatives are most likely to be discovered: a) Individuals with the average WM aging will represent the most prevalent

patterns of WM aging in the population; b) individuals with low WM integrity for their age and c) individuals with high WM integrity for their age. WM integrity can of course be compromised by disease and this was assessed later in the data analysis.

Regarding nonlinear modeling alternatives, our analysis allowed for the discovery of clusters of patients which will show a different trajectory in one brain region that in another. This could mean for example that one cluster of individuals deteriorates first in frontal brain regions and then in posterior, while another cluster does the opposite. This modeling approach allowed for the discovery of new patterns that have not previously been reported in the literature where population-wise approaches are only present for longitudinal data (Gunning-Dixon *et al.*, 2009).

7.3.5 Supervised

Study I

After the clustering was completed the most important variables from the random forest were exported and were used to predict (with a CART model) cluster allocation based on the clustering results. The goal of this approach was to find the most important brain regions that contribute to the heterogeneity in AD neurodegeneration patterns without overfitting the CART model.

Study II

Supervised analysis was not applied to the data of Study II.

Study III

Discriminant modeling

To further characterize our clustering model's ability (section 7.3.4, study III) to generalize in unseen data, we utilized its components to build a supervised classifier (Figure 4). Using an already published methodology we "turned" the longitudinal unsupervised model into a multivariate longitudinal discriminant model (Hughes *et al.*, 2018). Given cross-sectional or longitudinal brain data of an AD patient, the new model calculates the chances that the patient belongs to any of the longitudinal clusters that were discovered by the longitudinal clustering model. Moreover, the data do not need to be included in the cohorts that were used in the training of the model. This feature increases the generalizability of the study.

Graph theory

Graph theory, a set of methodologies to differentiate properties of a network was also utilized in Study III. During the clustering, brain data were utilized as a network since we calculated output covariance matrices for cluster intercepts and slopes (random effects). To further understand the properties of every discovered cluster, we used these covariance matrices to calculate a strength measure for each brain region at a network level. Our data were longitudinal, so the cluster slope covariance matrix resembled patterns of atrophy over time.

We used one graph theory measure, node strength, for the sake of simplicity. Node strength equals the sum of the correlations between one brain region and any other region connected to it (Mårtensson *et al.*, 2018). In that way, it summarized the extent to which one brain region atrophies are similar to all other regions.

Study IV

In Study IV, after the clustering procedure, our next goal was to understand which WM tracts were mostly involved in the discrimination between FA clusters. To do this we used Bayesian generalized linear mixed effect models with shrinkage priors (Piironen and Vehtari, 2017). We used these priors in order to keep only a small set of variables that were most informative for the discrimination between clusters. After optimizing models for all cluster combinations, an odds ratio system was used to eliminate the most irrelevant WM tracts. Then we combined the WM tracts that were important for all the models. That set of regions is important not only in separating FA clusters but also in understanding which regions deteriorate in aging in general.

Finally, we focused on understanding those features that differentiate the various FA clusters. To do this, we used the highly discriminative WM tracts from the previous step and trained a multivariate multioutput model. WM tracts were used as output and demographic characteristics and biomarkers were used as input in the model. This model helped us to understand which characteristics differ between clusters over and above WM integrity.

7.3.6 Other statistical analyses

Other statistical analyses include a) corrections for multiple comparisons; b) conventional methods for the assessment of differences between groups of individuals; estimations of group level cross-sectional or longitudinal changes in non-imaging markers. These methods are included in the individual papers and they will not be described here.
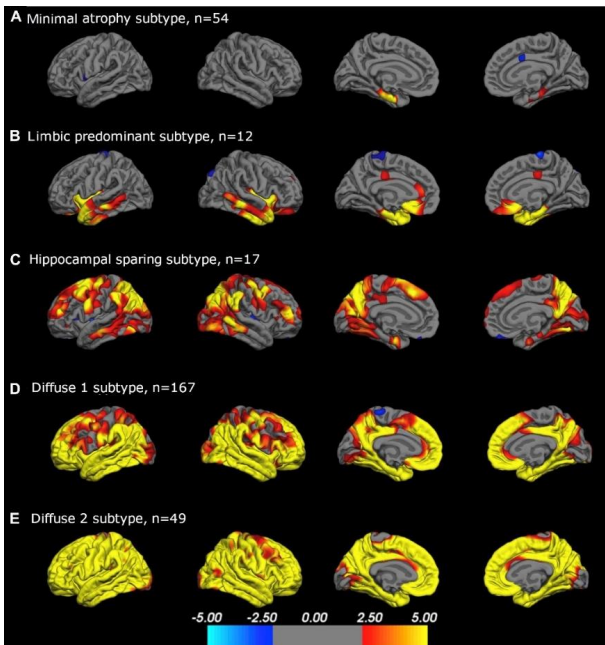
8 RESULTS

This section presents a summary of the most important results from each study. To make things clearer in terms of the literature and our findings the following terms will be used in the results and discussion sections. We will refer to groups of individuals found in the literature as subtypes of (followed by the type of marker that was assessed in the respective study); we will refer to the term cluster for groups discovered in the studies of this thesis; we will refer to pattern when trying to describe a cluster or subtype biomarker pattern.

8.1 HETEROGENEITY IN ALZHEIMER'S DISEASE, A CROSS SECTIONAL PERSPECTIVE

Heterogeneity in the clinical presentation of AD and the proteinopathies that characterize it is a topic of great interest because of its implications in clinical practice and in the development of precision medicine approaches for the treatment of AD. The analysis of sMRI cross-sectional data from north American and European data has shown that heterogeneous patterns of atrophy do exist under the diagnostic umbrella of dementia due to AD. We discovered five subgroups of patients with patterns of atrophy that are summarized in Figure 5.

A cluster of patients characterized with little brain atrophy compared to controls was named minimal atrophy (MA, n ≈ 18%) (Figure 5). Another cluster of patients had temporal lobe, cingulate cortex, and the medial orbitofrontal cortex atrophy and was described as predominantly limbic (LP, n= 4%). Next, two clusters

Figure 5. Discovered patterns of atrophy in study I.

Cortical volume patterns in different AD patient clusters. Significant cortical volume loss in the minimal atrophy (A), the limbic-predominant (B), the hippocampal-sparing (C), the diffuse 1 (D), and the diffuse 2 (E) clusters compared to the cognitively unimpaired individuals (at a group level). Higher values (yellow color) indicate stronger differences between groups, threshold at p = 0.001. The color bar denotes −log (p value).

with diffuse atrophy in the cortex and subcortical regions were identified. One of them was less severe (D1, n ≈ 56%), than the other (D2, n ≈ 16%). Finally a cluster with predominantly cortical atrophy was named hippocampal sparing (HS, n ≈ 6%) to maintain the same

terminology as that of the existing literature (Whitwell *et al.*, 2012). The main brain regions that showed differential patterns of volumes between clusters of patients were the precuneus, middle occipital, superior parietal, and orbital gyri. Differences between age of onset and patterns of atrophy were also identified in our sample. The HS cluster included patients of younger dementia onset than the other clusters.

Patients of the different clusters exhibited significantly different clinical picture and progression. Patients of the HS cluster showed worse baseline praxis than the MA cluster, while patients of the D1, D2 and HS clusters showed worse clinical progression than the MA and LP clusters.

8.2 A FRAMEWORK TO ASSESS HETEROGENEOUS PATTERNS WITH LONGITUDINAL INFORMATION

In Study II we assessed the ability of a Bayesian hierarchical mixture model to cluster irregularly sampled longitudinal neuroimaging data of AD diagnosed patients. Three main longitudinal patterns were identified: a typical AD pattern (same as diffuse atrophy in Study I) (n=47.2%), an MA (n=31.9%) and a HS (n=47.2%) pattern (Figure 7). Some patients were excluded from the final analysis as outliers (n=4.2%, clusters with fewer than three patients) or as patients that had no certain classification to any of the clusters (n= 4.2%, patients that can belong to more than one clusters given the highest posterior density intervals of the probability to belong in each of the clusters).

Since in this study we assessed the chance that each patient belongs to any of the clusters, two measures of clustering were examined (Figure 6). In figure 6A patients are colored based on maximum probability classification (MDS components 1, 2 and 3). In figure 6B patients are colored based on HPD intervals classification. In comparison to figure 6A, in figure 6B we added the uncertain classification with orange color (Two subject from cluster 2 and one subject from cluster 7 cannot be classified to any cluster with high certainty). In figure 6C colors are the same as in figure 6B, but we excluded from the plot the HPD uncertain classification subjects: orange and the outlier clusters 7: black and 8: yellow. Finally, in figure 6D the patients are colored exactly as in 6C but the MDS

Figure 6. Comparison of maximum probability and HPD interval classifications of AD patients in Study II.
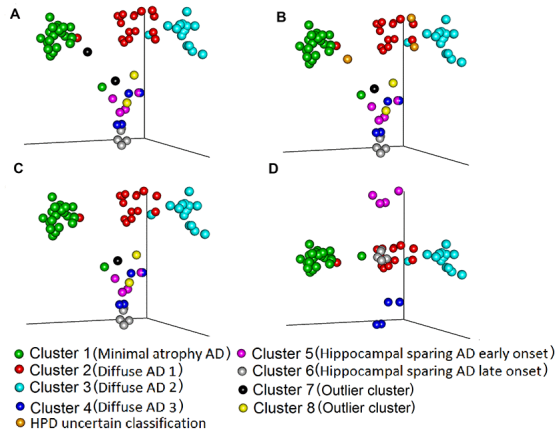

Three-dimensional representation of (Multidimensional scaled (MDS)) component-individual probabilities matrix (this matrix includes the probability of each subject being in any of the clusters). The scatter plots represent subjects and are colored according to the clustering based on two approaches, maximum probability and highest posterior density intervals (HPD).

components 1, 2 and 5 are plotted, to showcase the separation between cluster 4, 5, and 6. The names in parenthesis after the cluster numbers refer to Figure 7.

The patterns of atrophy that we discovered in this sample (at baseline) are like the ones found in Study I. However, some more patterns that mainly show atrophy severity differences were found. Also, more years of education and steep cognitive decline (CDR, MMSE) were related to the HS pattern of atrophy. Constructional praxis decline was also worse for the HS cluster. However, we avoided doing intensive comparisons between clusters of patients due to the low sample sizes (no statistical tests are reported).

On a methodological note, the results of the optimization process showed that the model is indeed capable of adapting to the sMRI data when "smart" initial values are provided. The clustering solution that was chosen as most appropriate for interpretation was based on initialization of the longitudinal model with the results from Study I. Thus, an initialization close to a local optimal (data specific) can guarantee sufficient optimization for the longitudinal model.

Figure 7. Fitted values for cortical thickness and subcortical volumes for the different patterns of atrophy in Study II.
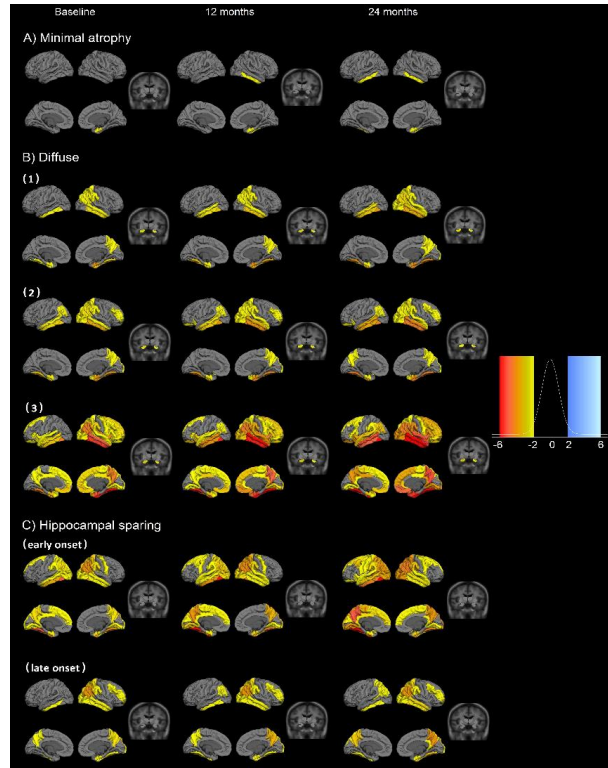
Each row presents the median fitted values of atrophy of the six components for three time points (baseline, 12 and 24 months from the first measurement). Since the data from right and left hemisphere were considered separately, each component and timepoint has left/right, medial/lateral cortical and one coronal subcortical visualization. The data are presented as cognitively unimpaired group z-scores. Fixed effects: Intracranial volume = average Intracranial volume, Sex = female, Age = 75 years, Time from onset of dementia = 5 years, Education = 16 years, CSF pTau 181P = 50 pg/ml, CSF Aβ1-42 = 100 pg/ml. Data are presented as standard deviations below the estimated mean of the cognitively unimpaired population.

8.3 PATHWAYS OF NEURODEGENERATION IN ALZHEIMER'S DISEASE

In Study III the sample size and longitudinal follow-up was much greater than in the previous two studies. The results of this study should be interpreted from the perspective of clinical disease duration since this is the timescale that was used. We identified five brain atrophy

trajectories that expressed the 320 *Aβ* positive AD dementia diagnosed patients (discovery dataset, Table 3). Each patient was well clustered to one of the five patterns (Figure 8).

Figure 8. Fitted values for cortical thickness and subcortical volumes for the different longitudinal patterns of atrophy from AD onset in Study III.
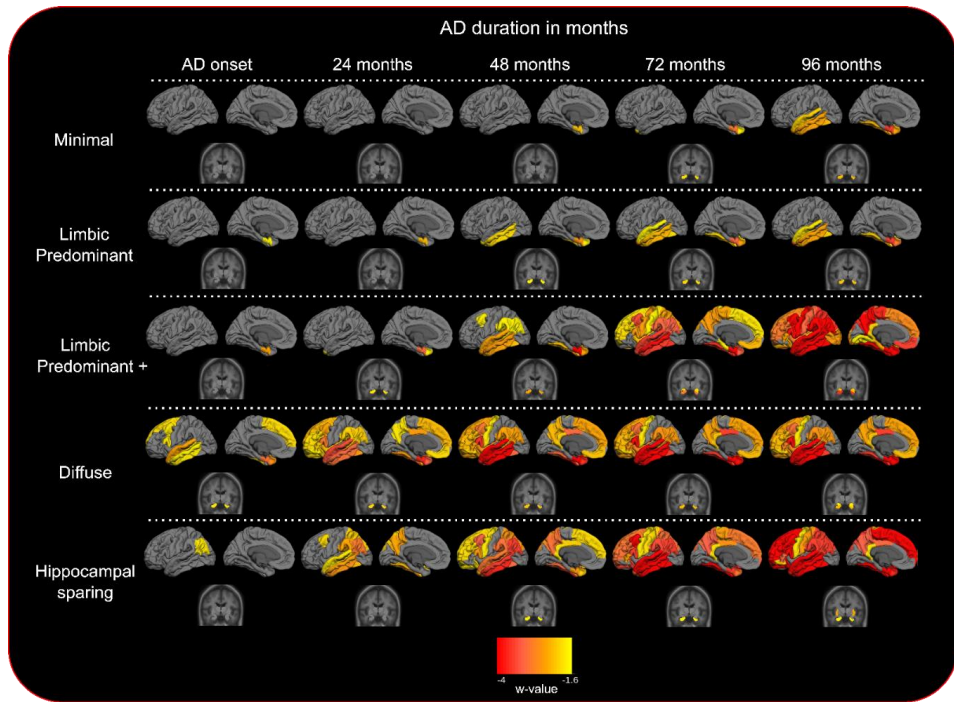
Atrophy fitted values from clinical AD onset. Each row represents one cluster of patients with the corresponding pattern of atrophy. The color scale illustrates cortical thinning and subcortical volume loss compared to Aβ negative, cognitively unimpaired (CU) individuals. Data are w-value transformed and therefore colors represent standard deviations below the CU group controlled for aging. Fitted values are fixed for intracranial volume and MRI scanner field strength.

The most prevalent cluster of patients (MA, n=59.1%) progressed slowly from no observable to medial and then lateral temporal lobe atrophy. The second largest cluster (LPA, limbic predominant atrophy, n=29.1%) showed little entorhinal atrophy in the AD onset and spread to the whole temporal lobe later, during the disease course. Next, a cluster similar to LPA but with somewhat more atrophy in the AD onset (LPA+, n = 7.2%), swiftly developed atrophy in most cortical areas during the disease course. A cluster with widespread atrophy at the disease course was also identified (diffuse atrophy, DA, n= 1.6%). Patients of that cluster showed steep atrophy trajectories that swiftly involved most of the cortex. Finally, an initially cortical atrophy cluster of patients was also discovered (HS, n = 3.1%).

The cluster covariance matrices showed that the DA cluster had higher nodal strength than the MA (both intercept and slope covariance) for few medial (frontal, occipital, temporal) regions, while the HS had higher nodal strength than the MA (only intercept covariance matrix) for

some medial temporal and ventromedial prefrontal regions. Otherwise, the MA had greater nodal strength than the other clusters.

The five clusters of patients showed differences in APOE e4 carriership, with the MA cluster showing the highest prevalence of carriers while the HS showed the lowest. Moreover, patients of the LPA+ and HS cluster had the highest premorbid intelligence (cognitive reserve proxy) and showed the steepest decline in MMSE over time across the whole sample. AD specific neuropsychological testing showed that patients of the HS cluster presented with worse language and praxis skills, while patients of the LPA+

Figure 9. Cluster specific cognitive trajectories after the clinical onset of dementia in Study III.
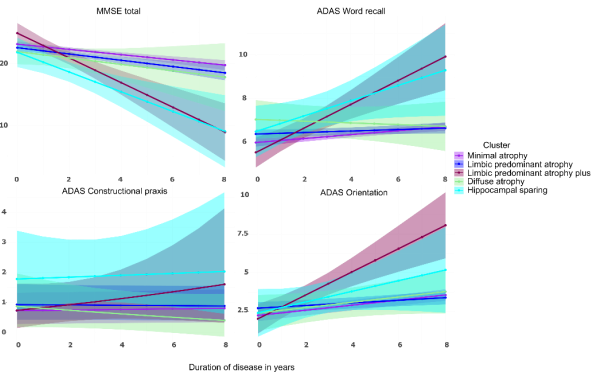
The trajectories are estimated with mixed effect models to account for intra subject and cohort variability. MMSE: Mini mental state examination; ADAS: Alzheimer's disease assessment scale

cluster showed worse orientation at the AD onset (Figure 9). The five atrophy trajectories include all the AD neurodegeneration subtypes that are reported in the literature (minimal atrophy, limbic predominant, typical AD, hippocampal sparing). However, we managed to summarize all subtypes into two neurodegeneration pathways, a cortical and a mediotemporal one. The MA, LPA, and LPA+ clusters belong to the mediotemporal pathway (although they have different atrophy dynamics) and the HS cluster belongs to the cortical one. As for the DA cluster, we cannot conclude its pathway since we "observed" the patients in a disease stage when atrophy was already prominent.

8.4   WHITE MATTER HETEROGENEITY IN THE AGING POPULATION

In Study IV our investigation of heterogeneity in WM aging yielded four distinct clusters of individuals. The best solution in terms of quality criteria included only two patterns but that solution separated the sample into low and high FA patients (marker severity separation). This solution was excluded since our initial goal was to understand if non-linear WM integrity patterns with spatially different FA distribution exist in the population. The next best solution divided the sample into four clusters with evident differences in longitudinal and spatial FA distributions which was further interpreted. Two approaches (maximum probability rule and HPD intervals) were used to address the clustering allocation issue (section 7.3.4, Study II). The HPD intervals method has shown that several individuals did not get well clustered to any of the four clusters. However only a few individuals had a high chance of being clustered in

very different clusters which implies that the trained model represents the whole sample (Figure10).

Figure 10. Cluster–subject probability classification in Study IV.
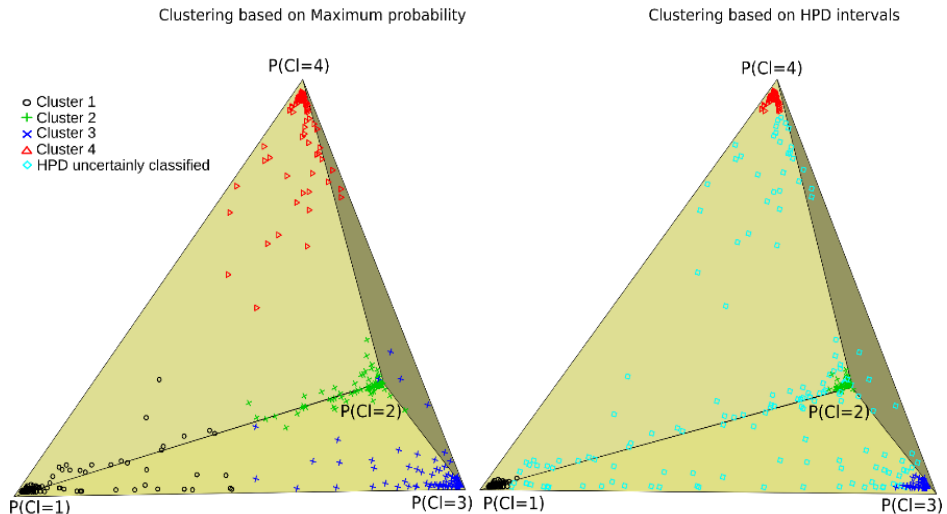

The individual-cluster matrix was visualized with the MDS method. Each corner of the 3D pyramid represents one cluster. The closer a dot is to a corner, the more certain a classification is. More individuals fall between clusters 1 and 2 than clusters 1 and 4 which means that the latter clusters represent very different WM tract patterns.

In order of prevalence, cluster 1 (45.5%) had relatively spared WM tracts at baseline (60 years of age), while its longitudinal progression was the slowest across the sample (healthy WM agers).

Cluster 4 (21%) showed very low baseline FA (Figure 11). It also reached the lowest values of the sample at 90 years of age. Clusters 2 (15.5%) and 3 (18%) presented with intermediate WM integrity profiles although some prominent differences existed between them. Cluster 2 started at baseline with FA values lower but close to cluster 1. Over time and especially after the age of 75 this reduced in FA significantly. Conversely, cluster 3 started with spatially variable FA (low FA in internal capsule but high in many other regions). Over time cluster 3 showed FA decline rates similar to cluster 4. A fine difference between clusters 2 and 3 is that cluster 3 ended up with very low FA values in the splenium of the corpus callosum, while cluster 2 declined more in anterior tracts over time. The cluster specific cognitive profiles followed the trends of WM integrity, with cluster 4 occupying the worst baseline values and cluster 3 following it, especially in cognitive decline rates. As for biomarkers of WM health and amyloidosis, WMH developed faster in clusters 2, 3, and 4 than in cluster 1 and amyloid load was not significantly different between clusters. Some WM tracts including the anterior internal capsule, posterior corona radiata, and genu of the corpus callosum were strong discriminants between the identified WM clusters.

Figure 11. Main findings in Study IV.
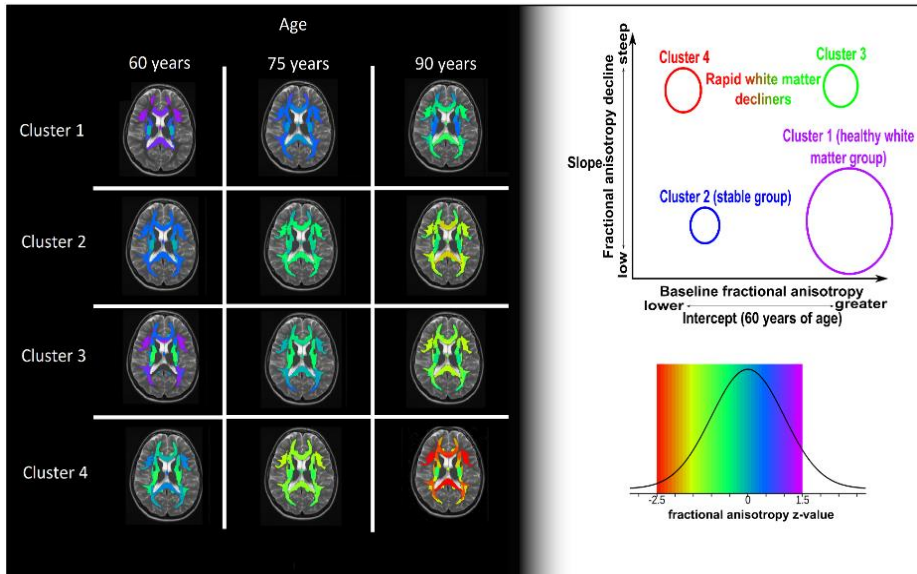
Horizontal planes of fitted FA values (tract and region specific) per cluster, corrected for APOE status and sex at different ages (left panel). Relationship between cluster WM intercepts and slopes where circle diameter is proportional to cluster frequency in the sample (right top panel). Purple to red color spans higher to lower FA z-values, respectively (right bottom).

9 DISCUSSION

This thesis was an effort to combine different statistical methodological approaches with neuroimaging data of several types to shed light on the heterogeneity of the most common cause of dementia, Alzheimer's disease (AD). This section will focus on the connection between the four studies of this thesis in terms of aims, methods and results.

In a recent study, Jack and colleagues suggested a new diagnostic framework for AD, that is based on the knowledge that we have about the existence of pathophysiological changes in the triad of AD, amyloid, tau, and neurodegeneration (AT(N))(Jack *et al.*, 2018). It is clearly stated in their manuscript that this framework does not aim to replace the clinical diagnosis but more focuses on the use of pathophysiological markers when they are available to increase the certainty of correct disease classification. Nonetheless, it introduces a new perspective to the diagnosis of AD where the typical AD clinical presentation is not the most important feature. In Study I we showed that heterogeneous patterns of neurodegeneration can be found even within the amnestic presentation of AD. This means that heterogeneity in AD is not only clinical, in the sense that the amnestic versus non-amnestic AD presentations will define their neurodegeneration pattern.

Going back to the main results of Study I, all AD patients had predominantly memory deficits. However, our unsupervised classification based on cross-sectional brain atrophy, showed that some clusters of patients had a pattern of atrophy that was not "typical" for AD, coupled with some specific differences in cognitive status from most patients (who were grouped in other clusters by our analysis). This observation leads me to the speculation that in AD, a patient's specific atrophy pattern is usually coupled with region-specific cognitive deficits that are hard to disentangle from the main memory component that is usually observed, since the memory component is so prominent that it takes over in the diagnostic procedure. An example from our results is that some patients had clear memory deficits, but also deficits in praxis (Koedam *et al.*, 2010; Whitwell *et al.*, 2012; Lam *et al.*, 2013; Park *et al.*, 2017; Young *et al.*, 2017). This group of patients showed excess parietal lobe atrophy (hippocampal sparing, HS) and less hippocampal atrophy than another group with a more typical AD pattern of atrophy (Ferreira *et al.*, 2020; Habes *et al.*, 2020). In Study I, we also found a cluster with very subtle brain atrophy (minimal atrophy, MA) in comparison to the other patients. That group of patients was indeed diagnosed with AD, but the signature atrophy of AD had not fully developed at the time that they were assessed (Ferreira *et al.*, 2017; Habes *et al.*, 2020).

Murray and colleagues investigated the distribution of tau in neuropathologically defined AD patients with at least NFT Braak stage IV. The results showed that from a NFT distributional perspective, three groups of patients exist: a group with more tau tangles in the cortex than in the hippocampus (HS), a group with the opposite trend (Limbic predominant, LP) and a group with high proportions of tangles both in the hippocampus and cortex (Typical AD) (Murray *et al.*, 2011). Those data were correlated with the atrophy that the patients had antemortem and the patterns were similar in terms of atrophy distributions at group level (Whitwell *et al.*, 2012).

Those atrophy subtypes were reported in numerous studies (see (Habes *et al.*, 2020) for a review). They also suggested that the hippocampus to cortex (specific regions, not all cortex) atrophy ratio will help to separate neuropathological subtypes. From a neuropathological perspective, the three groups describe the sample of Murray and colleagues well. However, patients can be at any disease stage when observed antemortem. Therefore, it is possible that they may not have a fully developed atrophy signature (Whitwell *et al.*, 2012) for any of the neuropathological subtypes described by Murray and colleagues. As a result, the MA cluster (Ferreira *et al.*, 2020) of patients cannot be separated clearly from the typical AD with the ratio of hippocampus to cortex. This brings into light the issue of disease staging when assessing potential biomarkers of AD patients during life. How can we understand and order patients in terms of which disease stage they belong to (in each potential biomarker), so that we can increase our knowledge about the disease pathophysiological heterogeneity antemortem? This was the question that we focused on in Studies II, III, and IV of this thesis.

Considering that patients can be at any stage of the disease when we sample their data, we decided to focus on understanding how a research design that incorporates such data can be modeled statistically. In Study II, we altered some features from Study I but deliberately kept some others unchanged. The main difference between Studies I and II is that the later was more methodological. The focus was on the development of a pipeline that would help us utilize longitudinal data to understand AD without losing too much information from the data. Moreover, we wanted a model that is appropriate for the data in question in order to avoid methodological pitfalls of previous studies in the AD literature (Feczko *et al.*, 2019). The main features that we changed from Study I when developing Study II were the inclusion of longitudinal data, z-value transformation to account for aging in atrophy data (the exact same timepoints from the ADNI study design were used both for CU individuals and AD patients), the reduction of the sample to a very restricted dataset that had $A\beta$ and tau from CSF biomarkers and the exact same timepoints. However, we kept the timescale of Study I to be able to directly compare the longitudinal with the cross-sectional results, without unrealistic assumptions. The results of Study II are not very far from those of Study I, though we could do more evidence-based longitudinal interpretations. At baseline we identified all the atrophy patterns that were found earlier whilst some finer atrophy profiles were also captured. For example, the HS pattern of atrophy was expressed in two forms, a late and an early clinical onset one. The diffuse atrophy pattern was expressed in three different forms of severity instead of two in Study I. I reserve myself from further interpretations of Study II due to the small sample size, as the study was mainly a proof of concept. It is of great importance to stress that both Studies I and II were built on a methodological assumption that has been utilized by many computational studies in AD up until now (Peng *et al.*, 2016; Zhang *et al.*, 2016; Dong *et al.*, 2017; Ferreira *et al.*, 2017; Fiford *et al.*, 2018; Poulakis *et al.*, 2018). That is, the duration of AD has the same effect in the population. This is not an assumption that is mentioned in the studies but taking a closer look at the analyses that are done in the field of AD heterogeneity they show that disease duration (or the estimated time that has passed from the AD clinical onset) is considered to be a population effect that the studies account for (Peng *et al.*, 2016;

Zhang *et al.*, 2016; Dong *et al.*, 2017; Ferreira *et al.*, 2017; Fiford *et al.*, 2018; Poulakis *et al.*, 2018). I speculate that the reason that most of the studies in the field find common group level patterns of atrophy is because we tend to simplify our analyses with that assumption. Some other studies do not even consider disease duration as a factor that may bias the results (Noh *et al.*, 2014; Byun *et al.*, 2015; Dong *et al.*, 2016, Gamberger *et al.*, 2016a; Hwang *et al.*, 2016; Na *et al.*, 2016; Park *et al.*, 2017; Risacher *et al.*, 2017; Varol *et al.*, 2017; Sui and Rajapakse, 2018). Since we have few published studies (in the field of AD heterogeneity) on the effect of the disease duration in the different AD atrophy subtypes apart from the common finding, that EOAD patients tend to have a more aggressive disease course (Mendez, 2017), we normally assume the effect to be a fixed effect. Our lack of knowledge on biomarker-specific trajectories during the disease course for every AD subtype, is the result of longitudinal data absence in the past studies (2010 until approximately 2020)(Ferreira *et al.*, 2020; Habes *et al.*, 2020).

Nowadays, many cohorts all around the world gather data systematically that can show the course of atrophy for each AD patient on a whole brain level. Based on the availability of those systematically collected MRIs, we decided to approach the aims of Study III from a different perspective namely, where the disease duration would be the main feature of interest. The collection of longitudinal MRI data from four continents led us to that study (Ellis *et al.*, 2009; Saykin *et al.*, 2010, Iwatsubo *et al.*, 2018b; Birkenbihl *et al.*, 2020). We utilized the methodological approach of Study II but edited the pipeline in such a way that the aging effect would be clearly accounted for and the disease duration would be the main timescale of the model. In Study II the patient's data were adjusted so that they would be expressed as z-values corrected for the decline in volume and thickness due to aging (slope of atrophy in CU aging). The aging effect itself (for example, how much the specific brain region average atrophy is at the age of 70 years in the healthy population), was considered to be a fixed effect in the longitudinal clustering model that included the AD patients. To improve the methodology and make a strict model that would account for the aging effect, we calculated regional-specific mixed effect models adjusted for cohort (ADNI, JADNI, and AIBL), that estimated atrophy at any age after 55 in Study III. In that way we answered the question, "what is the average regional atrophy in the brain of an $A\beta$ negative CU individual at a specific age?". Given those estimations, we calculated AD patient-specific z-values corrected for the aging effect. This methodological difference between Study II and Study III became evident when we calculated cluster specific atrophy maps. For example, in Study II, the cluster specific atrophy map of the MA cluster shows the patterns of atrophy at the first timepoint (corrected linearly for aging). On the other hand, in Study III the same atrophy map for the MA cluster corresponds to the disease onset estimated atrophy pattern. The two images have different atrophy patterns, and they are not interpreted in the same way. Dicks and colleagues show how different anatomical patterns yield from different timescales in AD (Dicks *et al.*, 2019). Regarding the disease duration feature, this is a variable that added a longitudinal sense of timing in the study of AD subtypes.

We are aware of the drawbacks that the AD diagnosis as a time reference may have. Sex, socioeconomic status, location, and even more AD related issues (Mendez, 2017) may

compromise the accurate estimation of the dementia due to the AD diagnosis event. Other variables, such as age and MMSE have been used to account for the longitudinal effect in studies of AD patients (see also Figure 2)(Dicks *et al.*, 2019). However, I believe that MMSE is not a specific marker for disease duration in AD. As for the age at scan, it could serve very well in the assessment of brain changes during the life of the patients. Unfortunately, both MMSE and age have a non-linear relationship to atrophy during the disease course. Moreover, if we had used them as model timescales, then it would have been necessary to add the disease duration in the model as a population effect (fixed), and this would have cancelled all the motivation for Study III.

The results of Study III show a clearer picture than those of Study II from the point of interpretation. The identified patterns of atrophy are not very different, but in Study III it is easier to put them in the context of disease duration and connect them with the stage before the clinical diagnosis of AD and the neuropathological results. By this I mean that 1) since we have obtained estimations of the atrophy patterns at the clinical disease onset for the first time, we can connect our study results with the studies in prodromal AD, which is the disease stage before clinical AD(ten Kate *et al.*, 2018); 2) since our estimations span from the AD onset onwards (eight years after AD onset), they come close to the perimortem brain "image" that reflects the neuropathological assessment of AD in terms of proteinopathies. We assumed that eight years (after the disease onset) of modeling would be sufficient, since in a very recent study (9230 dementia diagnosed patients) where time to institutionalization/death from recorded dementia diagnosis has been estimated, an average of five years of survival (interquartile range = 2.2 years) after diagnosis was reported (Joling *et al.*, 2020). Moreover, the cognitive follow-up data that we modeled a-posteriori in Study III led us to conclusions about the patterns of atrophy that have the most aggressive clinical course. The inclusion of multi-ethnic data from AD patients allows us to draw more generalizable conclusions. By this, I mean that the more data from different samples we include in our models, the more variability we include in them. As a result, our statistical estimates (error) better approximate the true heterogeneity in the population. The main atrophy patterns that we observed in Study III included a mediotemporal pathway of atrophy that was expressed by three clusters of patients and was the largest in the dataset and a cortical pathway that was limited in patient number. The results were not unexpected if one takes the EOAD literature into account. Patients that expressed the cortical pathway had clearly earlier onset of dementia than the ones that expressed the mediotemporal pathway. This is something that we also found in Study I (HS cluster) and in study II (cluster HS early onset) at group level (by group level, I mean the group average age at clinical onset of dementia). The difference is that in Study III, the early onset is a much clearer finding in terms of age at onset dispersion at group level. More specifically, the distribution of ages at disease onset of the HS cluster of patients in Study III has lighter tails than the respective distributions in Studies I and II. Moreover, Study III is the first one that let us see the estimated atrophy levels at the disease onset. If we associate the cognitive deficits (Alzheimer's disease assessment scale) as they are estimated at the disease onset with the atrophy distribution at the disease onset, the correlations are very clear. We can conclude that

atrophy development follows two main pathways in AD, namely mediotemporal and cortical pathways.

As mentioned before, variability may still be present within the two pathways of atrophy. The results from Study II showed that two different HS patterns exist depending on the age of dementia onset. This result was not captured in Study I or III. I believe that this arose due to the longitudinal information included in the model of Study II in comparison to Study I. The stable atrophy trajectory of the late onset HS cluster (compared to the steep one in early onset HS cluster) and its lesser initial atrophy (compared to the early onset HS cluster), separated the two clusters. Regarding the differences between the HS clusters in Studies II and III, I believe that in Study III the algorithm was optimised to identify the most prominent components of atrophy based on disease duration and it may have overlooked the potential existence of the late onset HS since its signal may not be as "strong" as for the other atrophy trajectories. Be that as it may, it is important to state that variability may still exist within the five clusters that were identified in Study III. The algorithm discovered the "strongest" signal in a separation into five clusters, but this does not mean that clusters such as the MA (MA at baseline; n = 189 in the discovery set and n = 313 in the prediction set) cluster are not heterogeneous within themselves in morphological terms. This feature is already discussed in Study I where the diffuse 1 cluster was the most heterogeneous in terms of atrophy patterns. Future studies may further investigate the patients of each cluster in Study III to understand whether more atrophy trajectories can be identified.

One finding that was unique in Study III compared to the previous studies is the separation of the mediotemporal pathway in smooth and steep atrophy trajectories. We discovered a cluster of AD patients where initial atrophy was limited to the medial temporal lobe, but it developed very swiftly to cover all the cortex and hippocampus (in the typical AD manner). It is important to recognize that even within the amnestic subtype of AD (also typical in terms of atrophy), patients can have very different trajectories of biomarkers and clinical presentation (Vermunt *et al.*, 2019).

The connection between our findings in AD subtypes and the previous literature can be summarized regarding some main features of heterogeneity in AD. The age at dementia diagnosis (onset of clinical dementia) is a feature that has been studied since the early years of AD research. EOAD refers to AD patients with onset earlier than 65 years of age (about 5% of the AD cases), while late onset AD (LOAD) refers to AD patients with onset later than 65 years of age (Mendez, 2017). On a historical note, although the pathophysiological and pathological features of AD and senile dementia were similar, the diagnosis of dementia due to AD, was initially given to patients under 65 years of age (Katzman, 1976). On the other hand, the diagnosis of senile dementia was given to patients over 65 years of age. To connect our data with these features, we should use some early MRI findings that related the age at dementia onset with a specific sMRI signature. A study from 2007, concluded that age of onset modulates the cortical involvement (atrophy) in AD (Karas *et al.*, 2007). More specifically, it was observed that the younger the onset of AD dementia is, the more prominent precuneus atrophy

is observed (Karas *et al.*, 2007; Scheltens *et al.*, 2017). Moving on to the progression of early onset cases, I have already pointed out that EOAD is related to a more aggressive disease course (Mendez, 2017). Moreover, a study from 2016 showed that on a group level, patients with a hippocampal sparing pattern of atrophy (more cortical atrophy) had a greater decline rate in all cognitive functions than those with typical AD or LP patterns of atrophy (Na *et al.*, 2016). Our results in Study I evaluated the HS pattern and showed that it exists in the AD population with precuneus atrophy as the main feature. In Studies II and III, longitudinal data were added and showed that the HS pattern is among the most rapidly progressive atrophy patterns in AD. The cognitive features that were reported in the literature (Young *et al.*, 2017; Ferreira *et al.*, 2020; Habes *et al.*, 2020) were re-estimated in all three studies (I, II, and III), and showed that patients that have this pattern of atrophy initially show more non-amnestic cognitive features than most AD patients, while their deterioration over time in all cognitive domains is among the steepest (observed in AD) (Scheltens *et al.*, 2018).

The MA pattern of atrophy observed in Studies I, II, and III, is similar to other studies in the literature (Habes *et al.*, 2020). In the second and third study, this specific atrophy pattern occupied some time span, followed by mild atrophy development over time. This pattern was also observed in other MRI studies (Dong *et al.*, 2017; Ferreira *et al.*, 2017, Kate *et al.*, 2018*b*) but not in the study by Whitwell and colleagues (Whitwell *et al.*, 2012). Probably, as we discuss in Paper III of the thesis, this distinct pattern of atrophy reflects earlier stages of neurodegeneration more than a distinct AD subtype. The LPA subtype that has been identified consistently in the body of the literature (Whitwell *et al.*, 2012; Ferreira *et al.*, 2020; Habes *et al.*, 2020) is, in my opinion, the hardest pattern to understand given the current published data. Some studies (Ferreira *et al.*, 2017; Persson *et al.*, 2017; Corlier *et al.*, 2018, Kate *et al.*, 2018*a*) like the first study of this thesis, show that this atrophy pattern is distinct in the population of AD and includes more patients with LOAD. In contrast, in Study III we show that this atrophy stage can be observed in the mediotemporal pathway of atrophy. On the other hand, Whitwell and colleagues have shown that this atrophy pattern correlates with the distribution of neurofibrillary tangles in the brain (like, or above Braak stage IV). The term LATE (Limbic-predominant age-related TDP-43 encephalopathy (LATE): consensus working group report), was established by a working group in 2019 to describe the neuropathological findings that characterize a late life dementia with distinct stages (TDP-43 proteinopathy first in amygdala, then hippocampus, and then middle frontal gyrus)(Nelson *et al.*, 2019). This consensus shows the clinical need to characterise a type of dementia that may include hippocampal sclerosis pathological findings, frontotemporal lobar degeneration, and AD genetic characteristics, and also an amnestic presentation similar to AD. With our atrophy data we cannot say whether the LPA patterns in Studies I, II, and III are pure AD as defined by Murray and colleagues (Murray *et al.*, 2011) or a more mixed type as in LATE. It is of worth to say that our LPA is predominantly an amnestic cluster of probable AD dementia patients with slow atrophy progression and cognitive deterioration. Here it is important to mention that LOAD plays a role, since LATE and our LPA patterns of atrophy are associated with late onset dementia diagnosis.

The differences between EOAD and LOAD are of great interest since they show a source of variability within AD that may lead to the etiologies behind it. Two features led us to Study IV. Firstly, the knowledge that WM changes may occur before grey matter changes in the AD brain. High concentrations of $A\beta_{1-42}$ in the CSF correlate well to WM changes in DTI for sporadic and familial AD patients (Li *et al.*, 2014, 2015). Regarding the sporadic AD cases, this correlation extended to the MCI and even subjective cognitive impairment individuals. Moreover, WM changes are more prominent in EOAD than in LOAD patients (Canu *et al.*, 2013; Caso *et al.*, 2015; Daianu *et al.*, 2016). In addition, these changes have been associated with posterior WM tracts in most studies (Canu *et al.*, 2013; Caso *et al.*, 2015; Daianu *et al.*, 2016). Driven by these results and being predominantly interested in the AD heterogeneity, we decided to assess WM at a population level (include CU, MCI, and dementia). We chose to look at the aging population instead of only AD patients, to observe the general trajectories of WM health and to understand which of those are more related to AD pathology and dementia risk even before grey matter atrophy is prominent. Study IV builds on Study II in the sense that the statistical pipeline used is the same, but with some changes in the study design to adapt to the population level study. The participants had a much greater age span than the ones in Study II. If we had used the timepoint timescale (visit 1, visit 2 etc) that was used in Study II, we would have assumed (implicitly) the population ages in the same linear manner in terms of WM integrity, which is not true. Moreover, age from dementia onset (Study III) was impossible to use since most of the participants were CU. Thus, we decided to use as a timescale for our longitudinal model the age at scan, and modeled between 60 and 90 years of age. With this timescale we managed to assess whether different WM profiles exist in the aging population, which includes healthy and cognitively impaired individuals.

The results of Study IV show that four different WM trajectories can summarize the population's WM integrity aging. There is a cluster of healthy WM agers that represents the majority of the population (cluster 1). This cluster does not remain unaffected by aging, but WM integrity deterioration in those individuals is limited. On the other extremity, we discovered a group that accounted for around 20% of the sample and showed low initial WM integrity and steep decline over time (cluster 4). WM integrity was clearly associated with cognitive status and the cluster with steep declines in FA had the highest percentage of cognitively impaired individuals. It is important to mention that corpus callosum was a region that deteriorated with aging in all individuals within our sample. Between those two clusters, two more WM clusters were identified. They where almost equal in sample representation and initial FA values. One of them (cluster 2) deteriorated similarly to cluster 1, while the other (cluster 3) deteriorated similarly to cluster 4. All clusters contained MCIs but the MCIs of cluster 3 had the steepest memory decline over time. This makes cluster 3 an interesting group of individuals for the understanding of the FA signature that shows increased risk of dementia development with affected memory as a predominant feature. Regarding the association fibers (brain WM fibers connecting regions of the same brain hemisphere) that are related to AD dementia, we found baseline differences in the parahippocampal part of the cingulum between

clusters, which may explain the differences in memory decline between them (Wang *et al.*, 2012; Ito *et al.*, 2015).

There are two interesting results from Study IV that may help to connect it with Studies I-III. Amyloid burden as it is measured by PiB PET, was not different between the clusters as defined by longitudinal WM integrity markers. This probably means that WM deterioration is associated with cognitive decline and relates to dementia but independently of $A\beta$ pathology. I believe that dementia is a result of independent and dependent pathologies. In this special case it seems that $A\beta$ is one pathology path and WM integrity decline is another, in the sense that a more aggressive WM health profile increases the risk of dementia but is not associated with more $A\beta$ in the brain. Importantly, overall $A\beta$ load is associated with FA decline, but not with different FA trajectories. The second interesting result that connects WM health clusters with the heterogeneity in AD lies in the anterior-posterior WM health gradient that we found. Consistent with the literature (Grieve *et al.*, 2007; Gunning-Dixon *et al.*, 2009), we found that anterior WM tracts decline more with aging than the posterior ones. This finding is in line with a hypothesis supported by many, that the late myelinated WM tracts decline first with aging and disease (Brickman *et al.*, 2012). The interesting finding however, is that clusters 3 and 4 had greater WM integrity decline in posterior projection and brainstem fibers than clusters 1 and 2. Nevertheless, cluster 4 also had significant decline in anterior fibers. This makes cluster 3 a group of individuals with a selective decline in basal-posterior fibers as well as steep cognitive decline. If we relate our finding to the literature where it is supported that EOAD patients are affected more in posterior WM regions (Canu *et al.*, 2013; Daianu *et al.*, 2016), then we can speculate that the profile of WM integrity in more atypical AD (higher percentage of EOAD) is probably similar to that of cluster 3 in our study (Sui and Rajapakse, 2018). Again, this is a population level study and direct connections with dementia other than the percentage of cognitively impaired individuals in each study may be farfetched. However, through cluster 3 we may be seeing the first changes that lead to more atypical AD phenotypes or EOAD. More data on the progression of the patients that fall into cluster 3 may help to untangle this hypothesis. I believe that it is very hard to discuss the existence of different WM profiles in each AD subtype, since subtypes of AD may be partially associated with pathologies other than the ones that affect WM integrity. However, our findings in Study IV show that some characteristics of the heterogeneity observed in AD can also be potentially traced in WM health (Ferreira *et al.*, 2018).

Until now, in the discussion of the results of this thesis, I have not discussed anything about ante mortem tau glucose metabolism (FDG-PET) or tau concentration findings in PET in the AD heterogeneity field. More results are becoming available and every year a handful of studies are added to the literature. However, as mentioned above, in this thesis we focused on grey matter atrophy and WM integrity brain markers. Moreover, we had a strong focus on the developments of analytical methodologies (not statistical models) that would allow for the analysis of those markers. Therefore, the discussion is also focused on the above-mentioned features. Although we know that the correlation between tau PET, FDG-PET and atrophy is

close in AD, more research should be done to understand the relationship of the three markers in the context of disease heterogeneity.

I take this opportunity to write a methodological note. To date, very few statistical models can cope with many features, such as vertex/voxel/region-based brain imaging data from more than one modality, or with a combination of imaging and non-imaging data. When we see publications with such data, only summary markers are available from each modality to reduce the optimization problem (Gamberger *et al.*, 2016*b*; Young *et al.*, 2017). If we try to understand what the assumptions and challenges of modelling full-scale data with unsupervised statistics are, we will most probably come to the conclusion that it is almost impossible to do so with the tools currently available. Therefore, I personally believe that before we set out to answer multifactorial questions, such as the heterogeneity of psychiatric/neurological disorders, we should first develop methods that can take the right input (the data of interest, even if they consist of thousands of variables) and acquire an output that we can interpret in depth (no black box models), so that we can draw biologically plausible conclusions.

The studies of this thesis have weak and strong points depending on from which angle we examine them. The statistical approaches that we used to model the data in each of the four studies, assume theoretical conditions that may not adapt perfectly to the data. However, as in each statistical model that examines our world's phenomena, assumptions need to be made for the models to hold their properties. We did our best to adapt the models as well as possible to the designs of the four studies. Moreover, we interpreted our findings according to the assumptions and limitations of our modeling approaches in order to reduce scientific bias. In the future, as we did in this thesis, we envision working on further developing our models, so that we can increase our adaptation to real life data.

10 CONCLUSIONS AND FUTURE DIRECTIONS

In this thesis, we investigated how neuroimaging markers can help us to understand heterogeneous neurodegeneration processes in aging and AD. To realize this study plan, we employed statistical models that helped us to test the existence of subpopulations (defined with neuroimaging) in cohorts from Europe (AddNeuroMed), North America (ADNI, MCSA), Japan (JADNI), and Australia (AIBL).

Our results help to answer some relevant questions about WM aging and the heterogeneity in AD. Given the amount of data that I have worked with and all the methodological approaches that we used, we can now say that the variability in atrophy trajectories in AD dementia is evident, may have different etiologies, and is predictive of future atrophy and cognition. Two main pathways of atrophy are expressed within AD (A cortical pathway and a mediotemporal pathway). These pathways converge if the disease course is long enough. Moreover, they are associated, among other features, with the disease onset of AD patients and their cognitive reserve. EOAD patients have a higher chance of following the cortical pathway, while LOAD have a higher chance of following the mediotemporal one. WM health introduces even more variability in this phenomenon, as we discovered groups of individuals with healthy and at-risk WM aging profiles. WM heterogeneity is not directly correlated with the accumulation of $A\beta$ in the brain of individuals, but AD heterogeneity may be a result of both traditional AD pathology (NFTs and $A\beta$ plaques) as well as differential WM changes in the aging brain.

Future research in AD heterogeneity should focus on assessing how markers of brain pathophysiology, that can be obtained during or even before the clinical dementia stage, can be merged with our existing knowledge in atrophy pathways. Following this scientific "trail" without predispositions and biases, one day we may complete the puzzle of AD heterogeneity and understand its underlying etiologies.

11 ACKNOWLEDGEMENTS

Dorota (and others). The discussions, lunches, conferences, department meetings and other forms of social interactions with you, made my life at KI a beautiful experience.

Moreover, I want to thank some people from Mayo Clinic in Rochester, MN, USA. **Prashanthi Vemuri**, thank you for the warm welcome to your research group and the time that you spent to introduce me to the aging research. **Robert Reid**, thank you for all the help with the DTI data and for introducing me to diffusion imaging and tractography. **Scott Squires** and **Scott Przybelski,** thank you for your help with IT and data management.

I also want to thank my family for, among other things, supporting my decisions in life and believing in me more than myself. Without you, this thesis would not have been possible. My **friends**, thank you for your support during my studies. **Elektra**, thank you for your support during the PhD studies and also for the cover of the thesis.

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