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Valtteri Julkunen

Cortical Thickness Analysis in Early Diagnostics of Alzheimer's Disease

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VALTTERI JULKUNEN

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ABSTRACT

The main role for conducting imaging in the diagnostics of Alzheimer's disease (AD) has been to exclude other reasons for the cognitive symptoms. Morphological changes in the brain which are characteristic of AD have been assessed with visual atrophy scales and manual volumetric methods. However, manual methods are laborious, rater-dependent, and need *a priori* decision of the region of interest. Therefore automatic analysis methods are of interest. This thesis assessed the alterations in cortical thickness (CTH) by using automated image analysis methods in a spectrum of subjects ranging from healthy controls to AD patients.

In the first publication, subjects with mild cognitive impairment (MCI) were assessed with magnetic resonance imaging (MRI) at the baseline and followed clinically up to 7 years. The subjects who progressed to AD (P-MCI) during the follow-up demonstrated significantly reduced CTH at the baseline in several areas of frontal, temporal and parietal cortices compared to those MCI subjects who remained as MCI (S-MCI). Cortical thinning in these areas was also associated with worse cognitive performance at the baseline.

In the second publication, the CTH analysis was expanded with larger study groups encompassing also healthy controls and AD patients. Differences in CTH between the MCI groups were located similarly as in the previous study, and were partly preserved even after adjusting for various confounging variables. Compared to healthy controls, the AD group displayed significantly reduced CTH in several areas of frontal and temporal cortices of the right hemisphere. Higher education and lower MMSE scores were correlated with reduced CTH in the AD group.

The third publication focused on the relationship between education and CTH in a multicenter study containing healthy controls, MCI and AD patients. Higher education was associated to thicker regional cortex in temporal, insular and cingulated cortices among the controls. In the AD group, the subjects with more education years displayed reduced CTH in temporal, parietal and occipital cortices.

In the fourth publication, the MRI scans of the open-access database ADNI were assessed with CTH analysis, tensor-based morphometry, manifold-based learning and hippocampal volumetry. This comprehensive MRI analysis was found to distinguish the AD patients from the controls with an accuracy of 89% and to predict the progression from MCI to AD with an accuracy of 68%.

National Library of Medical Classification: WT 155, WM 220, WN 185
Medical Subject Headings: Alzheimer Disease; Dementia; Magnetic Resonance Imaging; Mild Cognitive Impairment

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TIIVISTELMÄ

Perinteisesti kuvantamista on käytetty AT:n diagnostiikassa sulkemaan pois muut mahdolliset syyt oireille. Lisäksi visuaalisia arviointiasteikoilla sekä manuaalisilla tilavuudenmittausmenetelmillä on voitu arvioida AT:lle tyypillisiä aivojen rakenteellisia muutoksia, mutta manuaaliset menetelmät ovat työläitä, tekijäriippuvaisia ja vaativat rajoittumista tiettyihin rakenteellisiin alueisiin. Siksi automaattiset analyysimenetelmät ovat kiinnostavia. Tässä neljästä osajulkaisusta koostuvassa väitöskirjatyössä tutkittiin automaattisella laskentamenetelmällä aivokuoressa tapahtuvia muutoksia AT:ssa.

Ensimmäisessä osatyössä tutkittiin, onko AT:iin sairastuvilla henkilöillä muutoksia aivokuorenpaksuudessa jo lievän kognitiivisen heikkenemisen vaiheessa (MCI). Tulosten mukaan aivokuoressa voitiin havaita ohenemista ohimo-, otsa- ja päälaenlohkojen alueilla niillä henkilöillä, jotka myöhemmin seurannassa sairastuivat AT:iin. Aivokuoren oheneminen oli myös yhteydessä huonompaan kogntiviseen tasoon CDR-SB asteikolla mitattuna.

Toisessa julkaisussa aivokuorenpaksuusanalyysi tehtiin suuremman MCI-joukon lisäksi terveille verrokeille ja AT-potilaille. AT:iin sairastumista ennakoiva aivokuorenpaksuuden oheneminen MCI-vaiheessa sijoittui samoille alueille kuin 1. osatyössä, mutta tulokset säilyivät tilastollisesti merkittävinä myös useiden sekoittavien tekijöiden vakioimisen jälkeen. Verrokki- ja AT-ryhmän väliset erot aivokuorenpaksuudessa sijaitsivat oikean aivopuoliskon otsa- ja ohimolohkojen alueilla. Lisäksi ohuempi aivokuori korreloi AT-potilailla pidemmän koulutuksen ja huonomman muistin kanssa.

Kolmas osatyö keskittyi koulutusvuosien ja aivokuorenpaksuuden väliseen yhteyteen. Aineistona perustui kansainväliseen monikeskustutkimukseen. Terveillä verrokeilla pidempi koulutus oli yhteydessä paksumpaan aivokuoreen ohimolohkon, insulan ja pihtipoimun alueilla. AT-ryhmässä pidempi koulutus korreloi ohuemman aivokuoren kanssa useilla alueilla ohimo-, päälaen- ja takaraivolohkojen aivokuorella.

Neljännessä osatyössä tutkittiin kansainvälisen ADNI-tietokannan kontrolli, MCI ja AT henkilöiden magneettikuvia neljällä eri aivokuoren ja aivojen syvien osien rakenteita mittaavilla laskentamenetelmillä. Yhdistelemällä tietoa eri menetelmistä voitiin erottaa terveet verrokit AT-potilaista 89% tarkkuudella. Ennustetarkkuus AT:iin sairastuvuudelle MCI-vaiheessa oli 68%.

Yleinen Suomalainen asiasanasto: Alzheimerin tauti; neurologia; magneettitutkimus; aivokuori; markkerit; muistihäiriöt

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Valtteri Julkunen

List of the original publications

This thesis is based on the following original publications, referred by their Roman numbers in the text.

I Julkunen V, Niskanen E, Muehlboeck S, Pihlajamäki M, Könönen M, Hallikainen M, Kivipelto M, Tervo S, Vanninen R, Evans AC, Soininen H. Cortical thickness analysis to detect progressive mild cognitive impairment — a reference to Alzheimer's disease. *Dementia and Geriatric Cognitive Disorders 28: 404-12, 2009.*

II Julkunen V, Niskanen E, Koikkalainen J, Herukka S-K, Pihlajamäki M, Hallikainen M, Kivipelto M, Muehlboeck S, Evans AC, Vanninen R, Soininen H. Differences in cortical thickness in healthy controls, subjects with mild cognitive impairment and Alzheimer disease patients – a longitudinal study. *Journal of Alzheimer's Disease 21: 1141-51, 2010.*

III Liu Y*, Julkunen V*, Paajanen T, Westman E, Wahlund L-O, Aitken A, Sobow, T, Mecocci P, Tsolaki M, Vellas B, Muehlboeck S, Spenger C, Lovestone S, Simmons A, Soininen H, and AddNeuroMed Consortium. Education increases reserve against Alzheimer's disease—evidence from structural MRI analysis. *Neuroradiology 54: 929-38, 2012. * Authors had equal contribution to this study*

IV Wolz R*, Julkunen V*, Koikkalainen J, Niskanen E, Zhang DP, Rueckert D, Soininen H, Lötjönen J, the Alzheimer's Disease Neuroimaging Initiative. Multi-method analysis of MRI images in early diagnostics of Alzheimer's disease. *PLoS One 6:e25446, 2011. * Authors had equal contribution to this study.*

Contents

ABBREVIATIONS

Aβ	Amyloid β
AD	Alzheimer's disease
ADNI	Alzheimer's disease neuroimaging initiative
ANM	AddNeuroMed
APOE	Apolipoprotein E
CCR	Correct classification rate
CDR	Clinical dementia rating
CDR-SOB	CDR sum of boxes
CSF	Cerebrospinal fluid
CT	Computer tomography
CTH	Cortical thickness
DBM	Deformation-based morphometry
DSM	Diagnostic and Statistical Manual of Mental Disorders
EV	Entorhinal cortex volume
FDG	Fluorodeoxyglucose
FDR	False discovery rate
FTD	Frontotemporal dementia
GDS	Global deterioration scale
GM	Grey matter
HC	Healthy control
LBD	Lewy body disease
LDA	Linear discriminant analysis
MBL	Manifold-based learning
MCI	Mild cognitive impairment
MMSE	Mini-Mental State examination
MPRAGE	Magnetization-prepared rapid acquisition gradient echo
MR	Magnetic resonance
MRI	Magnetic resonance imaging
NFT	Neurofibrillary tangle
NP	Neuropshycological tests
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke, and the Alzheimer's Disease and Related Disorders Association
PET	Positron emission tomography
PiB	Pittsburgh compound B
P-MCI	Progressive mild cognitive impairment
ROI	Region of interest
S-MCI	Stable mild cognitive impairment
SD	Standard deviation
SE	Sensitivity
SP	Specificity
STAND	Structural abnormality index
SVM	Support vector machine
TBM	Tensor-based morphometry
VaD	Vascular dementia
VBM	Voxel-based morphometry

1 INTRODUCTION

The most common cause for dementia, Alzheimer's disease (AD), is a degenerative brain disease leading to cognitive deterioration, impairments in activities of daily living and eventually to death. Other common reasons for dementia include vascular dementia (VaD), frontotemporal dementias (FTD), Parkinson's disease and Lewy body disease (LBD), but AD alone accounts for over half of all the dementia diagnoses (Jellinger et al. 1990, Neuropathology Group MRC CFAST 2001). In 2006, approximately 27 million people were living with AD worldwide, but that number is estimated to quadruple by 2050 (Brookmeyer et al. 2007). The socio-economical burden will be thus a major challenge to all societies in the future. On the other hand, it has been estimated that if the onset of AD could be postponed by a mere five years, then the prevalence would decline by 50 % (DeKosky and Marek 2003), and even a modest delay of one year would decrease the amount of new AD cases by 9 million during the next four decades (Brookmeyer et al. 2007). The medication available at the moment is not able to cure or even slow the development of AD, but disease-modifying therapies are under frenetic research. However, so far there has been no breakthrough, partly because the subjects in the clinical trials might have progressed too far in the disease development and thus have already suffered unrecoverable damage in the brain. Consequently it has been proposed that the medication would be most efficient when applied in the early stages of AD (Cummings et al. 2007). This means that one crucial issue in the AD research currently is to find a sensitive and specific marker that would allow us to make the diagnosis earlier. Some AD biomarkers might also help in monitoring treatment effects and provide individual data about the disease state and prognosis.

For the last decade AD research has been largely focusing on mild cognitive impairment (MCI) (Petersen 2001, Petersen 2004). An individual with MCI suffers from a mild memory or other cognitive impairment, but does not have abnormal difficulties in daily life nor does he/she fulfill the criteria of dementia. In subjects with MCI, the annual rate of conversion to AD is approximately 6-25 % which is substantially higher compared to the rate of 0.2-4 % in the healthy population (Petersen 2001). However, MCI has various outcomes in addition to AD, including reverting to a normal state of cognition (Gauthier et al. 2006, Larrieu et al. 2002). This underlines the need for developing methods to pinpoint those subjects who will convert to AD in the future.

Biomarkers in cerebrospinal fluid (CSF), assessment by either positron emission tomography (PET) or magnetic resonance imaging (MRI) have shown greatest potential in the early diagnostics of AD. At present, neuroimaging with computer tomography (CT) and MRI is being used in the differential diagnostics of neurodegenerative disorders as well as in excluding other reasons for the cognitive defect such as tumors or normal pressure hydrocephalus. However, MRI provides better resolution and contrast compared to CT. MRI is also non-invasive and reasonably widely available.

The development of MRI-based markers for earlier diagnosis of AD has been rapid during the last years. The research field has moved from the use of visual rating scales (Scheltens et al. 1992) on to manual volumetry of the hippocampus (Boccardi et al. 2011) and further to explorative automatical methods assessing group-wise differences in the whole brain. The most recent methods assess multiple areas from both cortical and sub-cortical structures and these allow extraction of potential AD markers based on statistical analyses and anatomical labels at a single-subject level (Koikkalainen et al. 2011, Lerch and Evans 2005, Lötjönen et al. 2010, Wolz et al. 2010b). These novel MRI features can be used to aid the early diagnostics of AD in an automated and evidence-based way.

Although the pathological changes of AD are known to start years before the clinical onset, the diagnosis has been based on the presence of dementia and severe clinical symptoms referring to AD, exclusion of other diseases and insidious onset (McKhann et al. 1984). Now the recent development in the field of biomarkers has led to revision of the diagnostic criteria for AD (Dubois et al. 2007, Dubois et al. 2010, McKhann et al. 2011). The essential change is that the new criteria for prodromal AD proposed by Dubois et al (2010) are based solely on a positive biomarker finding in addition to the core feature of memory impairment thus allowing a substantially earlier possibility for intervention (Dubois et al. 2010). However, the search for the most useful, precise and reliable biomarkers is still ongoing and especially biomarker validation in the diagnostics at the single-subject level still needs further confirmation. This need for further validation is emphasized especially in the American version of the new diagnostic guidelines that regard the new biomarkers merely as factors which increase the certainty that the basis of the clinical dementia syndrome is the AD pathophysiological process (McKhann et al. 2011).

This study assessed the alterations in cortical thickness (CTH) with automated imaging analysis methods in a spectrum of subjects ranging from healthy controls (HC) to AD patients with a special focus on the MCI subjects. Correlations between CTH and several demographic and clinical factors were also investigated. Finally, the predictive power of the CTH analysis was compared to other computational state-of-the-art MRI analysis methods. This study was carried out partly within the EU funded project PredictAD (www.predictad.eu) and the pan-European study AddNeuroMed (www.innomed-addneuromed.com).

2 REVIEW OF THE LITERATURE

2.1 Dementia and Alzheimer's disease (AD)

2.1.1 General diagnosis of dementia and AD

Dementia is a clinical syndrome characterized by severe impairment in multiple cognitive domains such as memory, reasoning, judgment and abstract thinking (American Psychiatric Association 2000). The level of cognitive defects leads to loss of general functioning and the ability to perform activities of daily life and inevitably into a need for constant care. The dementia syndrome can be caused by several different organic reasons such as neurodegenerative disorders, brain tumors, hypothyroidism, vitamin B_{12} deficiency, hepatic encephalopathy and syphilis (Knopman et al. 2001). The currently used criteria for dementia according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV-TR) are displayed in Table 1.

Table 1 DSM-IV-TR criteria for dementia (American Psychiatric Association 2000)

The development of multiple cognitive deficits manifested by both
A1: memory impairment
A2: at least one of the following cognitive disturbances: aphasia, apraxia, agnosia, disturbance in executive functioning
The cognitive deficits in criteria A1 and A2 each cause significant impairment in social or occupational functioning and represent a significant decline from a previous level of functioning
The deficits do not occur exclusively during the course of a delirium

The diagnosis of AD is commonly based on the DSM-IV-TR criteria for the dementia of the Alzheimer's type and/or the criteria by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) work group (McKhann et al. 1984). In the clinical environment, the diagnosis is usually based on the NINCDS-ADRDA criteria and is a probabilistic definition of either probable or possible AD, which can be further verified to definite diagnosis by autopsy, or rarely by brain biopsy. The NINCDS-ADRDA criteria are presented in Table 2. In general, they require a gradual onset between ages 40-90, symptoms of dementia syndrome affecting memory and other cognitive functions and the absence of any other reason for the cognitive decline. In the research setting, the diagnosis of AD is most often a two-step process based on the presence of dementia by the DSM-IV-TR criteria and fulfillment of the criteria for probable AD of the NINCDS-ADRDA work group.

Table 2 NINCDS-ADRDA clinical criteria for Alzheimer's disease (AD), applied from McKhann et al. (1984)

Probable AD	Possible AD	Definite AD
Dementia established by clinical examination and documented by MMSE or a similar cognitive scale, and confirmed by neuropsychological tests	May be made on the basis of the dementia syndrome, in the absence of other neurologic, psychiatric, or systemic disorders sufficient to cause dementia, and in the presence of variations in the onset, in the presentation, or in the clinical course	The clinical criteria for probable Alzheimer's disease
Deficits in two or more areas of cognition	May be made in the presence of a second systemic or brain disorder sufficient to produce dementia, which is not considered to be *the* cause of the dementia	Histopathologic evidence obtained from a biopsy or autopsy
Progressive worsening of memory and other cognitive functions	Should be used in research studies when a single, gradually progressive severe cognitive deficit is identified in the absence of other identifiable cause	
No disturbance of consciousness		
Onset between ages 40 and 90, most often after age 65		
Absence of systemic disorders or other brain diseases that in and of themselves could account for the progressive deficits in memory and cognition		

MMSE = Mini-Mental State Examination

2.1.2 Neuropathology of AD

AD is a neurodegenerative disease that is thought to result mainly from incorrect processing of proteins leading to accumulation of extracellular β-amyloid (Aβ) plaques and intraneuronal neurofibrillary tangles (NFTs) followed by neuronal and synaptic loss (Braak and Braak 1991, Khachaturian 1985, Mirra et al. 1991). Aβ plaques are end products originating from proteolysis of Aβ precursor protein located in the cell membranes. The Aβ plaques detected in AD are formed predominantly of the most insoluble and self-aggregating form of the Aβ peptide family, $A\beta_{42}$.

Tau protein is an important ingredient in the microtubules of neurons. For some unknown reason, in AD tau displays a tendency to hyperphosphorylate abnormally and form neurofibrillary tangles that disrupt the function of the neurons. These characteristic findings of AD have been shown to develop in a specific pattern starting from medial temporal lobe and slowly progressing to neocortical areas through the limbic system (Braak and Braak 1997, Delacourte et al. 1999). The clinical symptoms of the disease are especially linked to the distribution of tau pathology and progress as new areas in the brain are affected. The following staging has been proposed to describe the relationship between the

pathological findings of neurofibrillary tangle depositions (Figure 1) and clinical representation of AD (Braak and Braak 1997):

1. Transentorhinal stages (I-II)
   - No symptoms
2. Limbic stages (III-IV)
   - Clinical symptoms
3. Neocortical stages (V-VI)
   - Fully developed AD

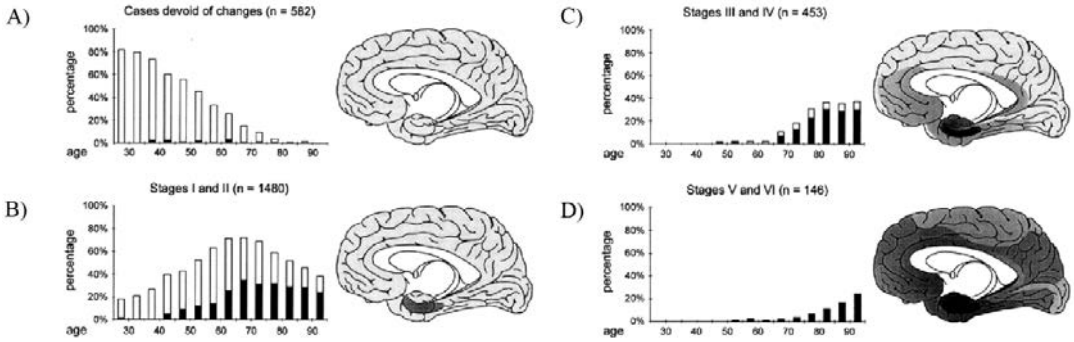

Figure 1 Progression of pathological neurofibrillary tangles in the brain during the course of Braak's stages. A) Frequency of cases devoid of changes in relation to the total number of cases in the various age categories. B – D) Evolution of the AD-related accumulation of neurofibrillary tangles during stages I-VI. The dark parts of the columns represent the subgroups displaying amyloid deposits. AD = Alzheimer's disease. Reprinted from Braak and Braak (1997) with permission from Elsevier.

In addition to the core features of Aβ plaques and neurofibrillary tangles also many other pathological processes such as chronic inflammation, oxidative stress, mitochondrial dysfunction, cholesterol dyshomeostasis, and impaired neurotransmission have been associated with AD (Nimmrich and Ebert 2009, Pereira et al. 2005). Their role in the pathogenesis of AD is not completely clear, but there are hopes that these findings might provide new targets for therapeutic interventions.

2.1.3 Risks and protective factors

Several factors including high age, positive family history for AD, the shared risks with cardiovascular diseases, low education, lack of social contacts, dietary and life-style factors, depression, brain injuries and stroke have been associated with a higher risk of developing AD (Coley et al. 2008, Eskelinen et al. 2009, Eskelinen et al. 2011, Kivipelto et al. 2001, Kivipelto et al. 2002, Peters et al. 2008a, Peters et al. 2008b, Rovio et al. 2005). Different factors modifying the risk for AD have been summarized in Table 3.

Table 3 Risks and protective factors for Alzheimer's disease

Risks	Protective factors
Inherent	
Age	
Positive family history	
APOE ε4 allele carrier	
Social	
Lack of social network	Socially active
Low education	High education
Cardiovascular	
Low physical activity	Aerobic exercise
Saturated fatty acids	Omega-3-fatty acids, anti-oxidants
Smoking	Reasonable use of alcohol
High cholesterol level at midlife	Acetylsalicylic acid and non-steroidal anti-inflammatory drugs
High blood-pressure at midlife	Treatment of high blood-pressure
Diabetes, metabolic syndrome	
Other	
Stroke and brain injury	Coffee
Depression	Hormone replacement therapy
Overuse of alcohol	

APOE = apolipoprotein E

Recently a dementia risk score for late life AD risk based on midlife vascular risk factors has been proposed (Kivipelto et al. 2006). However, although the associations between different risks, protective factors and AD have been revealed in epidemiological studies, it is not clear how successfully the risk can be modified by an intervention. For example, negative results concerning omega-3-fatty acids and polyunsaturated fatty acids (Devore et al. 2009, Kröger et al. 2009) as well as lowering of cholesterol levels with statin drugs (McGuinness et al. 2009) have been reported. In addition, the benefits of hormone replacement therapy in post-menopausal women has proved questionable (Hogervorst et al. 2009, Lethaby et al. 2008). Furthermore, there are conflicting findings regarding treatment of hypertension and AD with some studies showing a decreased risk for both AD and VaD (Forette et al. 2002) as well as stroke-related AD dementia (Tzourio et al. 2003), while others (Applegate et al. 1994, Lithell et al. 2003, Peters et al. 2008a) have found no significant difference between the active treatment and placebo group on the incidence of dementia. There can be many reasons for these discrepancies in the literature, such as heterogeneity of the study populations in different studies, different inclusion / exclusion criteria and varying follow-up times. In order to assess the efficacy of an intervention on multiple risk factors simultaneously in a prospective fashion, a study aiming to prevent cognitive impairment, dementia and disability was launched recently in Finland (Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability, FINGER). The 2-year multi-domain life-style intervention involves nutritional guidance, exercise, cognitive training, increased social activity, and intensive monitoring and management of metabolic and vascular risk factors. The study is ongoing and no results have been reported thus far, for up-to-date information see http://clinicaltrials.gov/ct2/show/NCT01041989.

2.1.4 Imaging in diagnostics of dementia and AD

According to the original NINCDS-ADRDA criteria (McKhann et al. 1984), the diagnosis of AD requires exclusion of other, possibly treatable reasons for the dementia syndrome with sufficiently broad scale of clinical examination, cognitive tests, blood samples and imaging. Until recently, the most important role of imaging in memory disorders and dementia has been to rule out treatable diseases that can cause similar symptoms as AD (normal pressure hydrocephalus, brain tumors and haematoma) (Knopman et al. 2001, Scheltens et al. 2002). However, finding treatable causes in the routine neuroimaging for all patients in the diagnostics of dementia might not be as common as one might think. In a study of Farina et al. (1999), a potentially reversible cause of dementia was detected in only 7.2 % of 362 demented patients in CT and there were no findings that had not been discovered clinically in any patient. Chui and Zhang (1997) concluded that imaging found reversible disease rarely, but occasionally re-directed the diagnosis and thus had an influence on the care of the patient. In a systematic review on the use of CT in dementia, the most cost-effective approach was scanning of all patients under 65 years of age and treatment of only those with subdural haematoma (Foster et al. 1999). Furthermore, it was found that the treatment of normal-pressure hydrocephalus actually reduced quality-adjusted survival.

Although the amount of additional information gained by traditional routine neuroimaging seems somewhat limited, it is regarded as a useful tool also in the differential diagnostics of AD from other dementia causing diseases such as FTD (Chan et al. 2001), Creutzfeldt-Jakob disease (Schröter et al. 2000) and VaD (Roman et al. 1993). Moreover, a systematic review concluded that although finding a treatable cause that had not been suspected with clinical prediction rules is not very common, relying only on clinical examination may miss patients with potentially reversible causes of dementia (Gifford et al. 2000). As a result, the routine neuroimaging is recommended by the current guidelines of diagnosis and management of Alzheimer's disease and other disorders associated with dementia (Waldemar et al. 2007). In addition, the above-mentioned studies were performed using CT which is known to provide inferior spatial resolution and contrast compared to MRI. MRI has also the advantage of being completely non-invasive in terms of radiation. MRI has been the method of choice in the recent development in the field of AD imaging biomarkers that are discussed in more detail in Section 2.4.

2.1.5 Current clinical practice in Finland

Current clinical practice and diagnostics of AD and other memory disorders in Finland is based on the National guidelines provided by a workgroup of Finnish experts in the field of neurology, geriatrics and psychiatry (www.kaypahoito.fi).

The diagnostic procedure begins usually in the primary health care with screening tests for memory functions performed by a nurse specialized in memory disorders and a clinical examination conducted by a general practicine. A careful medical history is taken from the patient and optimally also supplemented with information from a relative or a caregiver. Blood samples and electrocardiography are taken in order to exclude secondary causes for memory problems and as a general physical examination. Symptom severity of cognitive decline and abilities to perform daily activities as well as psychiatric symptoms are assessed by using different clinical rating scales. Usually the cognitive deficits are evaluated with the Finnish version of The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) test battery including also the MMSE test. Clinical Dementia Rating (CDR) is a scale measuring general symptom severity including impairment of memory, orientation, judgment as well as difficulties in daily activities. In addition, Alzheimer's Disease Co-operative Study - Activities of Daily Living (ADCS-ADL) inventory and Global Deterioration Scale / Functional Assessment and Staging scale are used to describe the

patient's state and functional abilities in a structured fashion. All the basic examinations are conducted in primary healthcare while neuroimaging, comprehensive neuropsychological tests and profound differential diagnostics are completed in specialized neurological and geriatric departments or dedicated memory clinics. The diagnosis of AD is usually based on the NINCDS-ADRDA criteria (McKhann et al. 1984), while in some cases, special tests such as CSF biomarkers can be supplemented into the test battery. For a summary concerning the diagnostic procedures please see Table 4.

Table 4 Summarization of diagnostic guidelines of memory disorders in Finland, modified from the Käypä Hoito recommendations (www.kaypahoito.fi).

Primary healthcare
Medical history from the patient and interview of a realative/caregiver
General examination done by physician
Evaluation of memory functions, screening for depresison and general assessment of functioning • MMSE, CERAD • Neuropsychiatric Inventory , Geriatric Depression Scale • CDR, GDS/FAST, ADCS-ADL
Blood samples • Blood count, electrolytes, liver, kidney and thyroid function, B12 vitamin, lipid profile, others if needed
Electrocardiography
Specialized healthcare
Specialist consultations • Mild symptoms, possibly early neurodegenerative disease • Differential diagnostics • Statements regarding juridicial problems, ability to work, drivers licence • Medication for memory disorders
Neuroimaging • MRI or CT with a memory protocol • Visual assessment for intracranial reasons for memory disorder, evaluation of global and hippocampal atrophy, vascular lesions and white matter changes
Neuropsychiatric examination • Special situations such as working-age patients, neuropsychiatric differential diagnostics or unusual symptoms
Other tests • CSF • PET and SPECT • Genetic tests

ADCS-ADL = Alzheimer's Disease Co-operative Study - Activities of Daily Living inventory, CERAD = The Consortium to Establish a Registry for Alzheimer's Disease, CDR = Clinical Dementia Rating, CSF = Cerebrospinal fluid, GDS/FAST = Global Deterioration Scale / Functional Assessment and Staging, MMSE = Mini-Mental State Examination, PET = Positron emission tomography, SPECT = Single-photon emission computed tomography

All the patients with memory disorders undergo neuroimaging, preferably MRI. If MRI is not possible due to a medical condition (i.e. presence of a pacemaker) or limited access to MRI, neuroimaging is recommended to be done by a multidetector-row CT. MRI memory protocol includes T2-weighted axial slices, fluid attenuated inversion recovery (FLAIR) and 3D T1-weighted sequences with preferably 1 mm slice thickness. Image quality and possible non-degenerative lesions (i.e. tumor, haematoma, and focal pathologies) are assessed visually as well as findings suggestive of atypical findings for dementia such as brain stem atrophy or abnormal signals in the basal ganglia. Global atrophy is evaluated according to a four-step scale from 0 (no atrophy) to 3 (severe atrophy) (Pasquier et al. 1996). Hippocampal atrophy is graded according to the Scheltens scale described in chapter 2.4.1.1 (Scheltens et al. 1992, Scheltens et al. 1995). White matter changes are described using the four-step stageing devised by Fazekas and colleagues (Fazekas et al. 1987).

According to the current Finnish guidelines (www.kaypahoito.fi) medication should be considered for all patients with a new AD diagnosis. In mild and moderate AD, the drug of choise is an acetylcholinesterase inhibitor (rivastigmine, galantamine, donepezil). An NMDA inhibitor, memantine, can be used if an acetylcholinesterase inhibitor is not suitable for the patient. The combination of acetylcholinesterase inhibitor and memantine is recommended for the later stages of AD. There is no evidence that these medications can reverse the course of AD or improve the memory of the patient. However, in mild AD, they can be used to stabilize the patient's cognitive symptoms and in the later stages they reduce behavioral symptoms and maintain the ability to manage daily activities independently.

2.2 Mild cognitive impairment (MCI)

Cognitive problems relating to normal aging and abnormal impairment of memory reaching beyond normal boundaries have been recognized for a long time. The state describing these possibly pathological symptoms has been endowed with numerous names during the last decades and terms such as malign senescent forgetfulness, ageing-associated cognitive decline, age-associated memory impairment, mild neurocognitive disorder, age-related cognitive decline, mild cognitive disorder and mild cognitive impairment (MCI) have been used extensively in the literature (Crook et al. 1986, Kral 1962, Levy 1994, Petersen et al. 1995, Petersen et al. 1999, Smith et al. 1996, WHO 1992). During the last ten years the term MCI has become the most commonly used term to describe an individual with an objectively measurable impairment in cognitive functions that exceeds the borders of benign absent-mindedness but does not justify a diagnosis of AD or any other dementia disorder (Petersen et al. 2009). The original MCI criteria published by the Mayo Clinic Alzheimer's Disease Research Center included: 1) memory complaint by the patient, family, or physician, 2) normal activities of daily living, 3) normal global cognitive function, 4) objective impairment in memory or in one other area of cognitive function as evident by scores >1.5 standard deviations (SD) below the age-appropriate mean, 5) clinical dementia rating (CDR) (Berg 1988) score of 0.5 and 6) absence of dementia (Petersen et al. 1995, Smith et al. 1996). In 2004, a major revision was done to the MCI criteria with the addition of the clinical phenotypes of amnestic MCI and non-amnestic MCI and their subtypes of single and multiple domain classifications (Petersen 2004, Winblad et al. 2004). Single domain MCI refers to a state where only one area of cognition is impaired, whereas a subject with multidomain MCI performs inadequately in several areas of cognition (i.e. reasoning, judgment, memory). A flow-chart describing the diagnosis and classification of MCI subtypes according to Winblad et al. (2004) is presented in Figure 2.

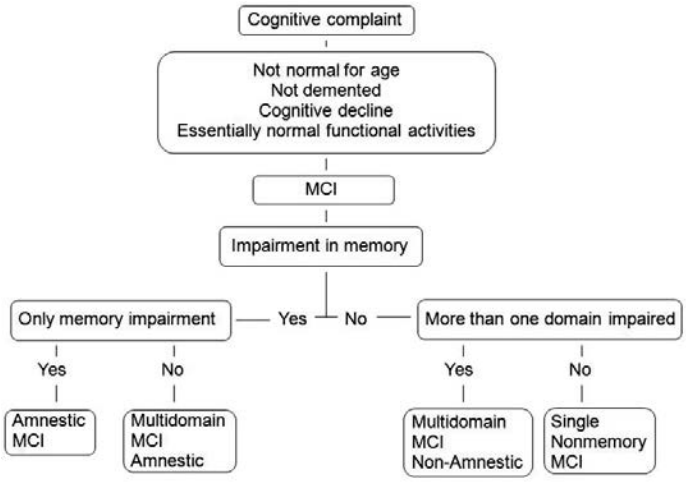

Figure 2 Flow-chart of the guidelines in diagnosis and classification of the mild cognitive impairment (MCI) subtypes, modified from Winblad et al. (2004)

According to epidemiological studies, in the elderly the prevalence of MCI varies between 5-23 % depending on the MCI criteria, assessed population and study design (Busse et al. 2006, Hänninen et al. 2002, Lopez et al. 2003, Palmer et al. 2008, Unverzagt et al. 2001). The stratification into MCI subtypes is considered important since the different subtypes are hypothesized to originate from various background pathologies such as degenerative, vascular and psychiatric disorders (see Figure 3). The subtypes have a different prognosis with some involving the development of AD or another memory disorder, some remaining in a stable state or even reverting to normal cognition (Gauthier et al. 2006, Larrieu et al. 2002). Especially subjects with multiple-domain and amnestic MCI seem to develop AD more often than those with the other subtypes, whereas the non-amnestic multiple domain MCIs are more likely to progress to a non-AD dementia (Busse et al. 2006, Palmer et al. 2008).

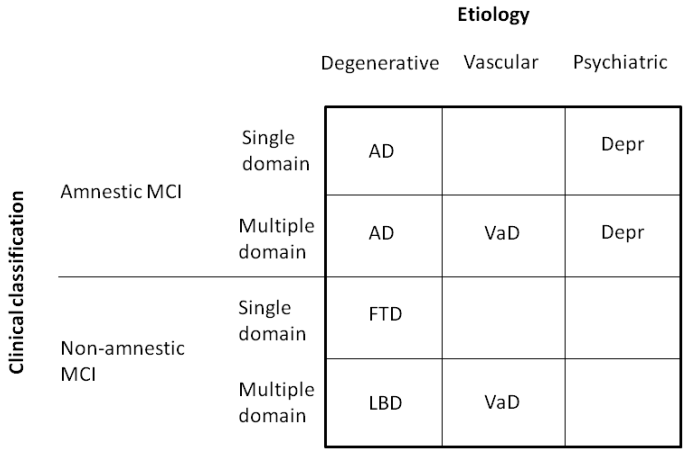

Figure 3 Stratification of mild cognitive impairment (MCI) subtypes according to clinical phenotype and hypothesized etiology. AD = Alzheimer's disease, VaD = Vascular dementia, Depr = depression, FTD = Frontotemporal dementia, LBD = Lewy body disease. Modified from Petersen et al. (2009).

In studies by Mayo Clinic (Petersen et al. 1999, Roberts et al. 2008), it was shown that the annual rate of progression of MCI to dementia was 12-15 % which is substantially higher compared to the rate of 1-2 % encountered in normal healthy controls (Petersen and Morris 2003). In conclusion, MCI multiplies the risk of developing AD (Petersen 2001). However, the source of the subjects in these studies seems to had an impact on the conversion rates, since the participants from memory clinics present higher rates of about 10-15 % (Farias et al. 2009) compared to 4-10 % in community-based studies (Busse et al. 2006, Larrieu et al. 2002, Solfrizzi et al. 2004). One of the reasons for this difference is probably the more heterogeneous background of the underlying pathologies behind the MCI syndrome in the community based studies, whereas reports based on memory clinic cohorts might have a higher prior probability for suffering from an underlying memory disorder.
Based on the epidemiological knowledge on the prevalence and prognosis of MCI presented above, MCI is regarded as a high risk "pre-dementia" state which most commonly leads to AD. Although no curative treatment for AD exists, the disease-modifying drugs – once they are discovered – are hypothesized to be most effective before the damage in the brain is non-recoverable and the person has become demented (Cummings et al. 2007). This underlines the importance of the concept of MCI as it offers the chance for early intervention.

2.3 Revision of the definition of AD

Since the publication of the original diagnostic criteria for AD (McKhann et al. 1984), the knowledge about AD pathology has increased tremendously. The new imaging and biochemical analysis methods now make it possible to assess these changes already before the dementia phase or autopsy. It has been also noted that the diagnosis based on the DSM-IV-TR and the NINCDS-ADRDA criteria are not convergent with the neuropathological diagnosis in a large proportion of subjects in community-based studies (sensitivity 65-83 %) (Lim et al. 1999, Petrovitch et al. 2001)). The specificity against other neurodegenerative disorders, such as FTD, can be as low as 23% (Varma et al. 1999). In addition, the inability of the current criteria to detect AD with high specificity before the dementia-phase has been stressed as one of the reasons for the failures in drug development (Greig et al. 2005). Furthermore, the clinical criteria for MCI allow that there may be a variety of background pathologies behind the mild symptoms. Even among the amnestic subtypes which are regarded as the most probable early-AD subjects, it is fairly common to have reasons other than AD behind the syndrome. In the study of Jicha et al. (2006), only 71% of those amnestic MCI subjects who progressed to dementia actually presented AD pathology at autopsy. In the same study, neither demographic variables nor cognitive measures had any predictive value in determining which patients diagnosed with MCI would develop the neuropathologic features of AD.

Consequently these issues led to a proposition of new diagnostic criteria for AD for use in research (Dubois et al. 2007). The new criteria have been built around the core feature of episodic memory impairment accompanied by a positive biomarker or genetic finding and exclusion of other reasons for the symptoms. The criteria devised by Dubois et al. 2007 are presented in Table 5.

Table 5 Alzheimer's disease (AD) criteria for research, modified from Dubois et al. (2007)

Probable AD
Core feature
Presence of an early and significant episodic memory impairment
• Gradual and progressive change in memory

- Objective evidence of significantly impaired episodic memory on testing
- The episodic memory impairment can be isolated or associated with other cognitive changes at the onset of AD or as AD advances

Supportive features

Presence of medial temporal lobe atrophy
Abnormal cerebrospinal fluid biomarker
Specific pattern on functional neuroimaging with PET
Proven AD autosomal dominant mutation within the immediate family

Exclusion criteria

History: sudden onset or early occurrence of gait disturbances, seizures, behavioural changes
Clinical features: Focal neurological features or early extrapyramidal signs
Other medical disorders severe enough to account for memory and related symptoms

Definite AD
Both clinical and histopathological (brain biopsy or autopsy) evidence of the disease
Both clinical and genetic evidence (mutation on chromosome 1, 14, or 21) of AD

The new criteria revised the diagnostic procedure of AD significantly by moving them from the dementia-phase to the time of early memory problems. Besides the exclusion of other diseases, the diagnosis is also based on a positive biomarker-finding showing biochemical, structural or metabolic changes characteristic of AD. In addition to making the early diagnostics possible, the new criteria based on quantitative biomarkers will possibly allow a better definition of the disease state, individual prognosis and measurement of drug effects.

However, the revised criteria have also attracted criticism. Oksengard and colleagues (2010) tested the Dubois criteria in a cross-sectional study by re-classifying subjects from a memory clinic sample originally diagnosed using the NINCDS-ADRDA criteria (Oksengard et al. 2010). They reported that out of 23 AD patients diagnosed as having full-blown Alzheimer dementia according to the current NINCDS-ADRDA criteria, the proposed new criteria for Alzheimer's disease identified only 12 patients. The investigators speculated that the discrepancy regarding the AD diagnoses could be due to the fact that the norms for biomarker "abnormality" are difficult to establish so that they would generalize well from one cohort to another, which limits their usage in a clinical setting at present, i.e. there are no universally accepted cut-off values. Schneider et al. (2010) assessed the benefit of CSF biomarkers to increase the power of clinical trials compared to enrolling amnestic MCI subjects without requiring the biomarker criteria by examining 400 MCI subjects from the Alzheimer's Disease Neuoimaging Initiative (ADNI) database (Schneider et al. 2010). Their conclusion was that although the subjects meeting the "probable AD" criteria with the positive CSF finding displayed slightly more evidence of cognitive impairment and showed a greater decline compared to the subjects with negative CSF, the requirement of biomarker-positive patients might not result in more efficient clinical trials, but in fact trials would take longer because fewer patients would be available. It is also not clear how the new biomarker-based criteria should be applied in the clinic since it is not known in any detail which of the proposed biomarkers are most sensitive and specific to AD, and which of them provide the best cost-efficiency when combined. The methods that are used to acquire the biomarkers have not been standardized nor has there been a consensus about the optimal thresholds in different age-groups. The standardization of the biomarkers is ongoing and it will be one of the major challenges for the future.

Additionally, some atypical variants of AD such as posterior cortical atrophy (Pantel and Schroder 1996) and frontal atrophy (Larner 2006) were not included in these criteria.

Motivated by the critique and accumulating knowledge about the performance of the biomarkers Dubois and colleagues (2010) published a new position paper revising the definition of AD (Dubois et al. 2010). New definitions – encompassing prodromal AD, different types of AD and preclinical stages of AD – that would better describe the relationship between the AD pathology and the diagnosis were introduced (Table 6).

Table 6 The new lexicon for Alzheimer's disease (AD), modified from Dubois et al. (2010)

AD

Clinical disorder that starts with the onset of the first specific clinical symptoms of the disease, encompasses both the prodromal and dementia phases
Diagnosis based on specific memory changes and in-vivo markers of Alzheimer's pathology
The clinical phenotype can be typical or atypical

Typical AD

AD, which is characterized by an early significant and progressive episodic memory deficit that remains dominant in the later stages of the disease
Is followed by or associated with other cognitive impairments
The diagnosis is further supported by one or more in-vivo positive biomarkers of AD pathology

Atypical AD

Primary progressive non-fluent aphasia, logopenic aphasia, frontal variant of AD, and posterior cortical atrophy

Mixed AD

AD and brain imaging/biological evidence of other comorbid disorders such as cerebrovascular disease or Lewy body disease

Prodromal AD (early symptomatic, predementia phase of AD)

Episodic memory loss, not demented
Positive biomarker evidence

AD dementia

Phase of AD during which cognitive symptoms are sufficiently severe to interfere with social functioning and instrumental activities of daily living

Preclinical states of AD

Asymptomatic stage between the earliest pathogenic events/brain lesions of AD and the first appearance of specific cognitive changes, includes

- Asymptomatic at-risk state for AD: positive biomarker in PET or CSF
- Presymptomatic AD: can be ascertained only in families that are affected by rare autosomal dominant monogenic mutations known to lead to AD

MCI

Measurable mild cognitive impairment, no significant effect on activities of daily living
There is no disease to which MCI can be attributed
Memory symptoms that are not characteristic of AD or biomarker negative

The essential changes from the previous version are the heavy reliance on the biomarker evidence of AD pathology, shifting from probabilistic diagnosis to typical / atypical / prodromal AD diagnosis and the replacement of "definite AD" with "neuropathologically confirmed AD", which also underlines the role of a positive biomarker finding as evidence of AD pathology. The preclinical states of AD were also introduced, referring to asymptomatic subjects with either positive AD biomarker or rare autosomal dominant monogenic mutations known to lead to AD. However, it should be underlined that the preclinical asymptomatic stages do not justify a *diagnosis* of AD. Dubois and colleagues have proposed that in the future, autopsy could be used mainly when diagnosing comorbidities of AD, not as the final proof of diagnosis as is currently the case. Furthermore, the role of MCI changed, since the subjects with mild cognitive symptoms are diagnosed with prodromal AD due to a positive biomarker finding while a negative finding would point the diagnosis towards reasons other than AD. It is also worth noting that *in theory* even an individual without any symptoms could be diagnosed with AD based on a positive finding on PET or CSF biomarker ("preclinical AD"), although Dubois and colleagues emphasize that in the clinical setting, the diagnosis should be made only on symptomatic subjects. This novel way of defining AD is based mainly on the findings concerning the behavior of different biomarkers in the AD continuum suggesting that the earliest pathological signs of amyloid accumulation can be detected even in the asymptomatic phase of AD (Jack et al. 2009). According to several groups (Ingelsson et al. 2004, Jack et al. 2009) the pathophysiological markers (CSF $A\beta_{42}$ and PET Aβ imaging) reveal the earliest changes followed by the markers of neurodegeneration (CSF tau, fluorodeoxyglucose (FDG)-PET, structural MRI) in the MCI phase (De Santi et al. 2001, Vemuri et al. 2009a, Vemuri et al. 2009b). This temporal progression of different markers has been summarized in a hypothetical model published by Jack et al. (2010) and displayed in Figure 4.

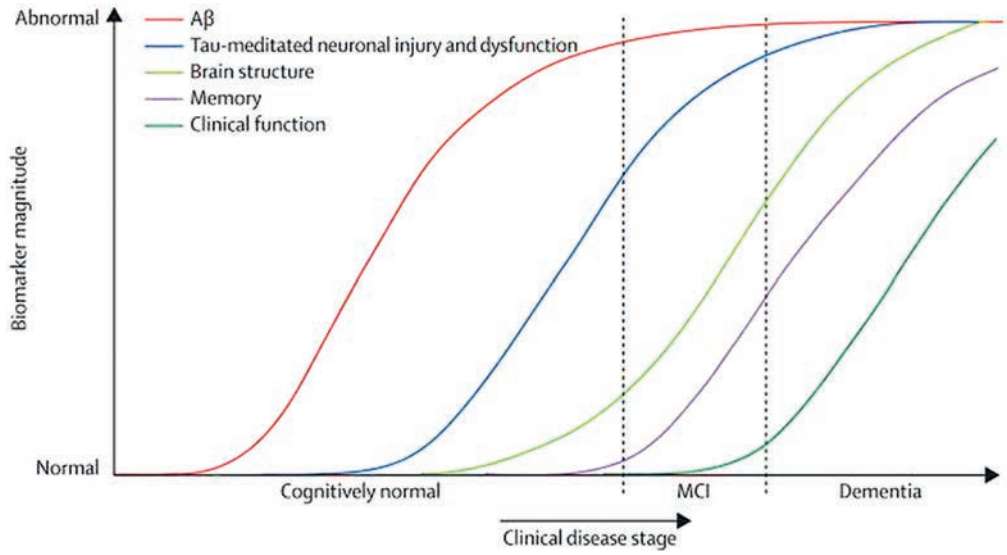

Figure 4 Hypothetical dynamic model of biomarker behavior in the Alzheimer's disease (AD) continuum. Aβ = β-amyloid detected by positron emission tomography (PET) amyloid imaging or from cerebrospinal fluid (CSF), alterations in the amount of tau protein can be assessed by CSF sample or fluorodeoxyglucose (FDG)-PET and changes in the brain structures by MRI. Reprinted from The Lancet Neurology (Jack et al. 2010) with permission from Elsevier.

According to this model, the amyloid markers could be used to place the diagnosis even in the asymptomatic phase. On the other hand, these markers seem to become saturated quite early in the MCI phase meaning that their value in predicting the time to conversion from MCI to AD is limited, as is their correlation with the clinical severity of the disease or their usage in measuring disease progression or treatment effects. However, the markers of neurodegeneration (CSF tau, FDG-PET, structural MRI) seem to correlate well with disease severity and could be thus most useful after a person has developed MCI. It has also been shown that 10-21% of cognitively normal subjects present levels of amyloid in the brain characteristic of AD without suffering any cognitive problems (Aizenstein et al. 2008, Mintun et al. 2006). It has been speculated whether this finding is an indication of early AD pathology or a sign that the amyloid in the brain might be only a non-specific bystander without any significant impact on the AD development. Nevertheless, this probably means that the use of topographical markers could be used to decrease the number of false positive diagnoses in the MCI phase as they are more closely related to the progression of the symptoms and clinical staging of AD severity.

There are also other open questions relating to making the AD diagnosis in cognitively normal persons. Even if the pathophysiological markers could really reveal the earliest AD patients, why would one test an asymptomatic subject in the first place? One possibility would be screening of all people from a certain age onwards, but such an approach would require that there would have to be an effective curative treatment with careful evaluation regarding the possible side-effects and cost benefit analyses. There is the danger of a circular logic if we consider all positive amyloid markers as a sign of AD just because amyloid can be found in AD. Furthermore, according to the most recent knowledge, the behaviour of the major biomarkers of AD (CSF Aβ42 and tau, amyloid and fluorodeoxyglucose positron emission tomography (PET) imaging, and structural MRI) seem to be more complex than the hypothetical model presented above suggests (Jack et al. 2012, Mouiha and Duchesne 2012). For example, individual characteristics such as age, gender, APOE genotype and the amount of amyloid plaques in the brain seem to have a significant impact on the biomarker levels and the effects of these variables are not linear (Jack et al. 2012). The creation of truly reliable and evidence-based models of AD biomarker behavior will thus require significant additional longitudinal data in individual subjects. It is generally believed that although the proposed new criteria still have some major issues that need to be solved before they can be widely accepted, the trend in this direction is worth continuing and the reliance of new biomarkers in future AD diagnostics will probably become the standard practice.

In the United States, the accumulating knowledge regarding AD lead to the publication of new diagnostic guidelines for AD (McKhann et al. 2011), MCI due to AD (Albert et al. 2011) and a framework paper describing the preclinical stages of AD for research purposes (Sperling et al. 2011) in 2011. The role of the new biomarkers in these diagnostic guidelines for AD (McKhann et al. 2011) is more cautious than those in the Dubois criteria (Dubois et al. 2010) as they regard the biomarkers as evidence that *may* increase the certainty that the basis of the clinical dementia syndrome is the AD pathophysiological process**.** Furthermore, AD biomarker tests are not recommended for routine diagnostic purposes at the present time but they could be used in investigational studies, clinical trials, and as optional clinical tools for use where available and when deemed appropriate by the clinician (McKhann et al. 2011). Guidelines regarding MCI due to AD are also rather conservative and follow closely the current definition of MCI (Petersen 2004) with the exception that AD biomarkers of Aβ deposition, neuronal injury or associated biochemical changes could be used in research or specialized clinical settings to 1) supplement standard clinical tests to help determine possible causes of MCI symptoms and 2) help determine the likelihood of cognitive and functional progression and the likelihood that this progression will occur

within a defined period (Albert et al. 2011). Thus the term "preclinical AD" was established solely for research purposes to provide a common rubric to advance the study of preclinical AD and to aid the field in moving toward earlier intervention at a stage of AD when some disease-modifying therapies may be most efficacious (Sperling et al. 2011).

2.4 Imaging biomarkers of AD

2.4.1 Traditional structural imaging in AD

As stated above, neuroimaging of the brain is recommended in the diagnosis of AD according to the current guidelines (Waldemar et al. 2007). Although surgically treatable reasons can be found with CT, MRI provides better spatial resolution and does not involve exposure to ionizing radiation. In addition, MRI can be used to increase the sensitivity and specificity of the clinical diagnosis through a variety of techniques such as visual rating scales of brain atrophy, manual and automatic segmentation and volumetry of regions of interest (ROIs) as well as explorative approaches which map the whole brain and identify an AD-type signature or "fingerprint". These techniques will be presented in the following chapters.

Although MRI is superior to traditional CT in many ways, certain limitations such as the presence of pacemakers, anxiety of the patient and restricted availability in some hospitals can negate the use of MRI in the diagnostics of memory disorders. Therefore, 64-detector row CT has been suggested as an improvement over the traditional CT scanning (Wattjes et al. 2009). According to Watties et al. (2009) the 64-detector row CT can be used to reliably assess the amount of global cortical atrophy, medial temporal atrophy as well as white matter changes in the brain. The results of visual assessment were comparable to those obtained with MRI (Wattjes et al. 2009). The key difference between traditional CT and multidetector-row CT (64 or even more detector-rows) is that in addition to axial slices a spiral CT done with a multidetector-row scanner can provide also coronal reconstruction images where the amount of atrophy is easy to evaluate. Although the novel automated image analysis methods are designed for MRI, the 64-detector row CT could represent the second best option if MR imaging is not possible.

Additionally to excluding surgically treatable reasons (e.g. tumor, haematoma, and hydrocephalus) and allowing the assessment of atrophy, neuroimaging can provide information concerning differential diagnostics of memory disorders and other possible comorbidities. Essential aspects are characterization of white matter changes, infarcts and microbleedings (Vernooij and Smits 2012). Typically the white matter lesions are evaluated with T2-weighted MRI or FLAIR sequence or diffusion tensor imaging, micro bleedings with T2* or susceptibility weighted imaging in MRI, and infarcts with either CT or structural MRI (Vernooij and Smits 2012).

2.4.1.1 Visual rating of atrophy

Brain atrophy caused by AD can be assessed visually from the MRI images. Especially in AD the hippocampal area is degenerated and one of the most frequently used visual scales describing hippocampal atrophy is that published by Scheltens et al. (1992). The scale was developed by arranging the MR images of 21 healthy controls and 21 AD patients into groups with various degrees of atrophy, with the atrophy being scored on a scale from 0 (no atrophy) to 4 (severe atrophy) from six oblique slices parallel to brain stem axis. The amount of atrophy was determined by the width of the choroid fissure and temporal lobe as well as the height of the hippocampal formation. An example of the rating based on the scale of Scheltens and colleagues (1992) is presented in Figure 5.

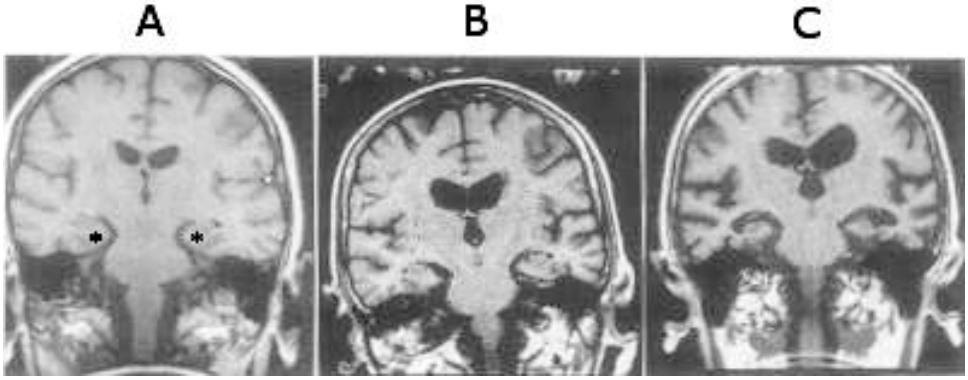

Figure 5 Example magnetic resonance imaging (MRI) scans displaying the different severity of hippocampal atrophy according to the Scheltens scale. A = 0, B = 2 and C = 4 scores. Hippocampus is marked in the A image with an asterisk. Reproduced from Scheltens et al. (1992) with permission from BMJ Publishing Group Ltd.

The scale was found to be helpful in the clinical environment where a quick judgment about the presence of medial temporal lobe atrophy was needed. However, the inter-rater reliability of the scale left room for improvement with a complete agreement of only 37% in the rating scores and even the judgment of whether an image was rated as being free of atrophy (score 0) or not (score 1-4) was uniform in only 70% of the cases (Scheltens et al. 1995). Nevertheless, the visual scaling seemed to provide high sensitivity and specificity of over 90% between healthy controls and AD patients and was found to be comparable or more accurate than the manual volumetry of the medial temporal lobe (Desmond et al. 1994, Wahlund et al. 2000). It should be noted though that also lower sensitivity/specificity values of 70/76% have been reported using the same scale (Scheltens et al. 1997). The Scheltens scale was recently compared with more novel methods for assessing regional brain volumes and cortical thicknesses in a multivariate analysis as well as manual hippocampal volumetry (Westman et al. 2011). Hippocampal volumetry and the multivariate analysis provided better accuracies of 83-89% compared to 81% of visual rating in healthy controls versus AD patients. In predicting conversion from MCI to AD at one year follow-up the multivariate analysis provided 11% units better sensitivity, 79%, compared to the Scheltens scale and hippocampal volumetry at a fixed specificity of 68%.

A more accurate visual rating system to be used to score the severity of medial temporal atrophy encompassing 8 regions was also recently developed (Shen et al. 2011, Urs et al. 2009). The aim of these studies was to expand the scope and utility of the Scheltens method as well as to make the scoring more standardized by providing a series of reference images. This new method could not distinguish the MCI subjects from the AD patients, which must be viewed as a disadvantage considering its possible usage in the early diagnostics of AD.

Although the visual rating scales are convenient in clinical use, the downsides concerning the inter-rater variability, semi-quantitative scaling of the atrophy and subjective nature of the visual assessment have stimulated the development of more sophisticated methods for early MRI diagnostics of AD.

2.4.1.2 Manual tracing of hippocampus

Hippocampal atrophy detected by MRI is one of the key AD biomarkers according to the new proposed criteria for AD (Dubois et al. 2007, Dubois et al. 2010, McKhann et al. 2011). Manual tracing has been regarded as the golden standard in the assessment of the hippocampal volume. This can be done in various ways and there are no generally accepted

guidelines on how to properly undertake the outlining. Recently Konrad et al. (2009) reviewed a total of 71 different published protocols for delineating the hippocampus. An even a more comprehensive review was done by Geuze et al. (2005) who examined 423 data-driven papers on hippocampal volumetry. The manual tracing protocols published so far differ in several factors including technical aspects of MR imaging (magnetic field volumes, slice thicknesses, corrections of orientation and volumes) as well as in how to define the anatomical borders of the hippocampus. As a result, the mean volume of a "normal" hippocampus seems to vary between 2-5.3 cm^3 in different studies (Geuze et al. 2005). This major variance in the volumetric protocols hinders the comparison of results across studies and complicates the incorporation of manual hippocampal volumetry into drug trials and clinical work. Therefore an initiative to harmonize the different protocols into a standard guideline including the 12 most cited and comprehensively described protocols was launched (Boccardi et al. 2011). Figure 6 illustrates the differences among the protocols used to delineate the hippocampus.

Figure 6 Manual tracing of the hippocampus. The first line shows histological figures, the corresponding magnetic resonance imaging (MRI) slices from the same region are presented in the same columns. The second line shows MR images without tracings, third and fourth display the same images outlined with different manual protocols. The figure demonstrates how different protocols lead to varying delineations of the hippocampus. This means that also the hippocampal volumes measured from the same image differ between the protocols. Reprinted from Boccardi et al. (2011) with permission from IOS Press.

The current knowledge indicates that manual hippocampal volumetry is able to distinguish between healthy controls, subjects with MCI and AD patients and is predictive of future cognitive deterioration in MCI at the group level (Dickerson et al. 2001, Jack et al. 1999, Killiany et al. 2000, Killiany et al. 2002, Tapiola et al. 2008). Although the manual volumetry of the hippocampus is time-consuming, and subject to inter-rater variability requiring a harmonized protocol in order to improve its validity, it is regarded as an accurate way of measuring one of the best established AD imaging marker, the extent of hippocampal atrophy.

2.4.2 New automatical methods

The most recent structural MRI analysis techniques were planned to fulfill the requirements of being i) fully automatic, ii) able to assess specific ROIs or whole-brain in an explorative fashion and iii) able to provide individualized data. A fully automated method eliminates the inter-rater variability since no human intervention is required, which is desirable as it makes comparison of different studies using same technique easier and measurements more objective. The discovery of possible new disease specific markers and investigation of disease related structural changes is convenient when a method assesses the whole brain simultaneously instead of choosing particular areas (i.e. anatomical areas) *a priori*. On the other hand, new automated methods fulfilling the above-mentioned aims focusing on known relevant structures, such as the hippocampus, are also needed. In attempt to meet this need, several novel MRI analysis methods described below have been developed for better imaging markers of AD.

2.4.2.1 Voxel-based morphometry

In addition to the structures of medial temporal lobe, AD is known to alter other brain areas including the neocortex of frontal and parietal lobes (Braak and Braak 1991). In order to overcome the limitations of laborious manual segmentation of brain structures and the need to decide *a priori* which structures of interest are to be assessed, an automatic method called the voxel-based morphometry (VBM) was developed (Ashburner and Friston 2000, Wright et al. 1995). In VBM, the MR images are registered into a standard stereotactic space, grey matter (GM) is automatically extracted from other tissues and statistical differences in grey matter density between the groups of interest are calculated at the voxel-level. As the result, a statistical parametric map is generated revealing those regions where grey matter concentration differs significantly between the groups (Ashburner and Friston 2000). VBM offers an explorative approach to neuroimaging by investigating the whole brain in a rater-independent fashion. An MR image with extracted gray matter using VBM is presented in Figure 7.

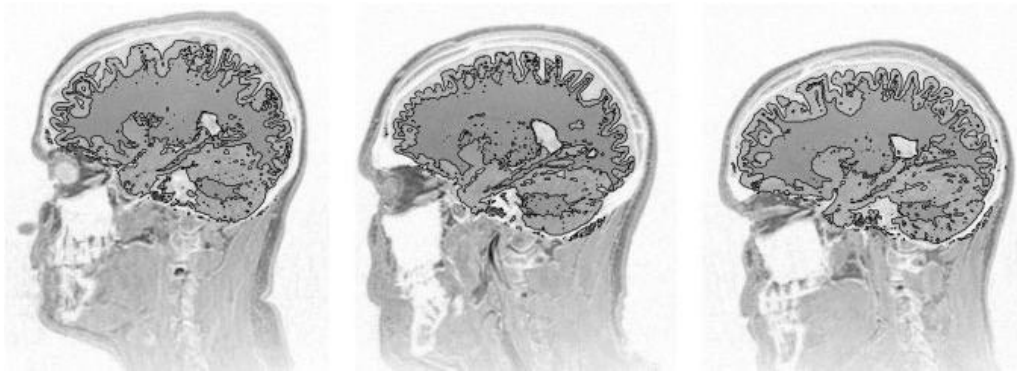

Figure 7 An example of magnetic resonance imaging (MRI) scans where the grey and white matter has been extracted with voxel-based morphometry (VBM). The segmentations have been superimposed on the sagittal slices. Reprinted from Ashburner and Friston (2000) with permission from Elsevier.

VBM has been used to assess the differences between healthy controls, MCI and AD patients. The resulting statistical maps show patterns of GM loss similar to the Braak stages describing the development of AD pathology during the disease and are predictive of AD in MCI (Baron et al. 2001, Bozzali et al. 2006, Chetelat et al. 2005, Hämäläinen et al. 2007b, Karas 2004, Karas et al. 2003). In particular, atrophy in the left hippocampus and

parahippocampal gyrus has been shown to be the most consistent finding predicting conversion from amnestic MCI to AD (Figure 8) (Ferreira et al. 2011).

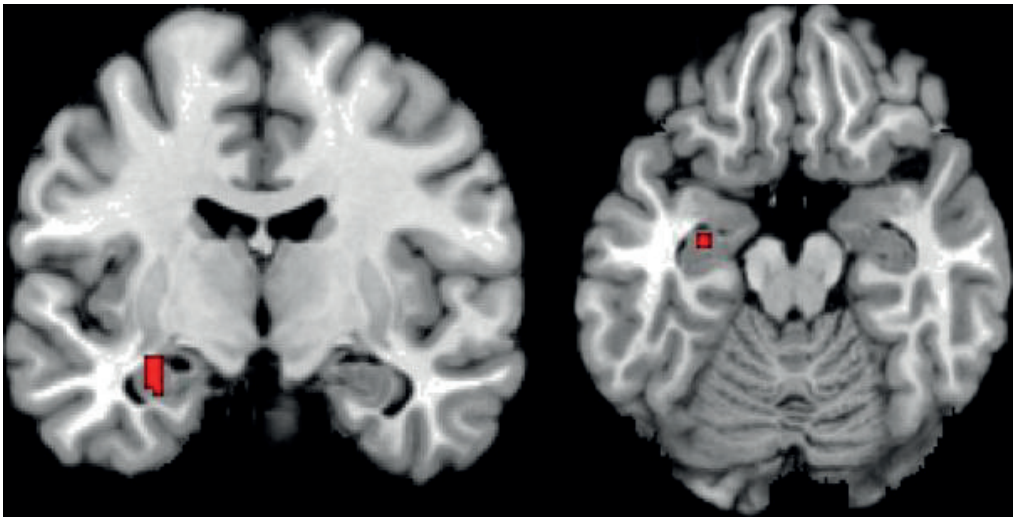

Figure 8 Red area in the images demonstrates the region of the left hippocampus and parahippocampal gyrus where loss of grey matter at the baseline predicted later conversion to Alzheimer's disease among subjects with amnestic mild cognitive impairment. Reprinted from Ferreira et al. (2011) with permission from Elsevier.

A recent study with follow-up time of 3 years demonstrated how GM loss accelerates also in the temporal neocortex and cingulated gyrus of those MCI subjects who develop AD (progressive MCI, P-MCI) compared to those who remain in the MCI state (stable MCI, S-MCI) (Spulber et al. 2012).

Although VBM has been a widely used technique that allows whole-brain explorative analyses without the need of manual tracings, it has been criticized because of it might overestimate some structure differences or detect false-positive regions due to unequalization of the image histograms of different subjects during the registration process. According to a critical article published in 2001 the effects found in VBM studies were caused mainly by differences in registration accuracy rather than by neurobiological differences in the local atrophy (Bookstein 2001). Therefore, new techniques based on VBM are under construction. A recently published paper reported a decreased amount of false positive findings with an enhanced VBM-algorithm focusing on fixing the problem regarding the unequalization of image histograms (Li et al. 2010a). Another modified VBM-technique has revealed abnormalities resembling closely the distribution of amyloid plaque deposition in AD by focusing on T2-weighted MRI scans (Diaz-de-Grenu et al. 2011). The investigators claimed that T2-weighted VBM could detect signal changes due to histopathology above those attributable to atrophy (Diaz-de-Grenu et al. 2011). Regardless of such improvements, it should be also noted that these VBM techniques are designed to investigate between-group differences and are not suitable for detecting abnormalities in individual cases, although variations of the VBM algorithms allowing also assessment of individual morphometry have been developed.

2.4.2.2 Hippocampal volume and atrophy

Several automated techniques assessing the volume of the hippocampus (Chupin et al. 2009, Collins and Pruessner 2009, Leung et al. 2010, Lötjönen et al. 2010, Lötjönen et al.

2011, Morra et al. 2008, Wolz et al. 2010a) as well as the change in the volume as a function of time (i.e. atrophy) (Leung et al. 2010, Wolz et al. 2010c) has been developed. In all these techniques, the aim has been to measure the volume or atrophy of hippocampus accurately without the need for manual intervention. This requires segmentation of the hippocampal formation from the MRI which can be done in various ways. Atlas-based segmentation is a frequently used technique where an intensity template, or "atlas", is registered to the MRI scan that is being measured. The resulting transformation is used to propagate tissue class and /or information of anatomical structures in each voxel of the template into the target MR image. This procedure can be done by choosing one atlas or by using multiple atlases and selecting those voxel-labels that the majority of all warped atlases propose for each voxel. The multi-atlas segmentation seems to be more accurate compared to using a single atlas (Babalola et al. 2008), while its downside has been the long computational time of several hours (van der Lijn et al. 2008). However, recent work by Lötjönen and colleagues (2011) showed that the volume of the hippocampus can be measured automatically using multi-atlas segmentation in about 2 minutes on a basic laptop computer (Lötjönen et al. 2010, Lötjönen et al. 2011). This method is illustrated in Figure 9.

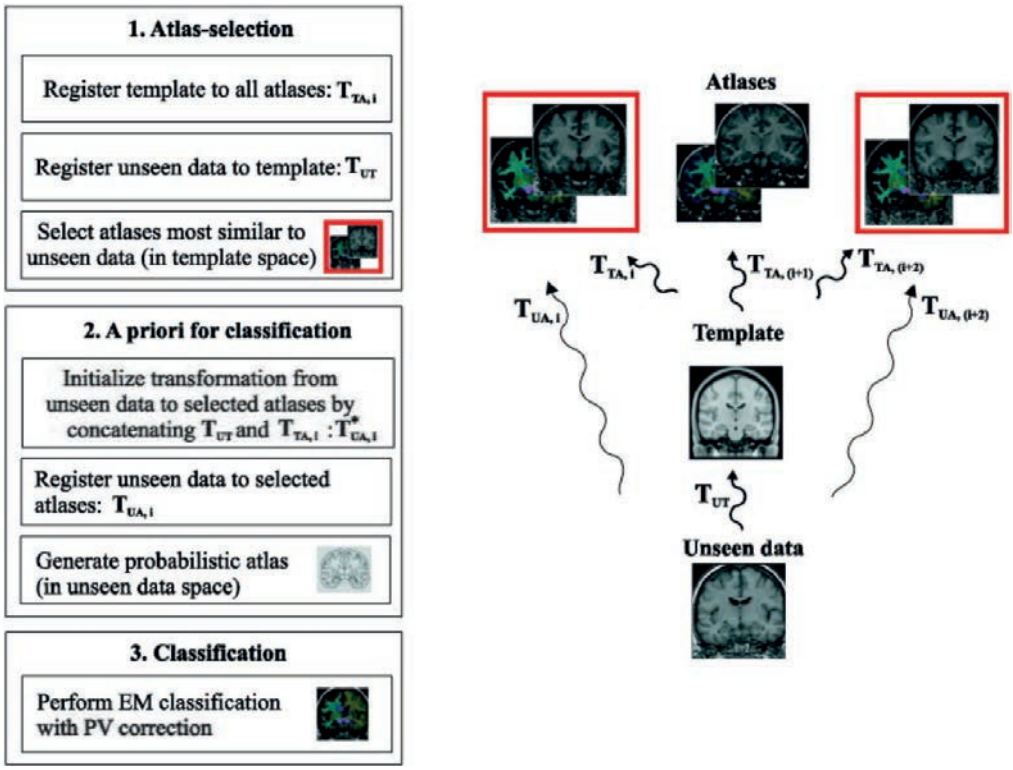

Figure 9 Automatical measurement of hippocampal volume via multi-atlas segmentation, reprinted from Lötjönen et al. (2011) with permission from Elsevier.

These automatic methods measuring hippocampal volume have been shown to attain a similar accuracy as can be achieved with manual segmentations done by different raters (Leung et al. 2010, Lötjönen et al. 2011, Morra et al. 2008, van der Lijn et al. 2008, Wolz et al. 2009). However, intense atrophy, such as encountered in some AD patients, can lower the segmentation quality (Chupin et al. 2009). In addition to the measurement accuracy, an important feature for an imaging marker is the ability to differentiate between healthy and

pathological conditions. Using automatic hippocampal segmentation results for controls versus AD classification range between 76-83 % and 64-67 % for predicting future conversion to AD in MCI at the single-subject level (Chupin et al. 2009, Lötjönen et al. 2011).

Automatic measurement of hippocampal atrophy during follow-up has been postulated to provide more information on the disease development of individual subjects and thus achieve a better classification between the healthy controls, MCI and AD (Leung et al. 2010, Wolz et al. 2010c). Wolz et al. (2010c) acquired a consistent and atrophy-sensitive measurement via simultaneous segmentation of baseline / follow-up scans using 4D images (time-coordinate added to the regular 3D coordinates x,y,z) for each subject. Leung et al. (2010) measured change in the hippocampal volume by applying a boundary shift integral to the segmentations generated in the baseline scan. Both studies reported accelerating hippocampal atrophy in those MCI subjects who later converted to AD.

2.4.2.3 Manifold-based learning

Manifold-based learning (MBL) is a novel machine learning approach where non-linear dimensionality reduction estimates the low-dimensional representation of a set of input images based on a similarity graph that is defined through pair-wise image similarities (Wolz et al. 2010b). The manifold is a coordinate embedding of all images of a given dataset. In the manifold embedding, each vertex represents an image and all pair-wise similarities between images are used to define the edge weights in the graph. Pair-wise similarities can be measured as the intensity similarity between the images or the amount of deformation needed to make the images similar, or as a combination of the two (Wolz et al. 2010a). Figure 10 demonstrates a coordinate embedding of 30 brain atlases and 796 images from elderly dementia patients and age-matched control subjects.

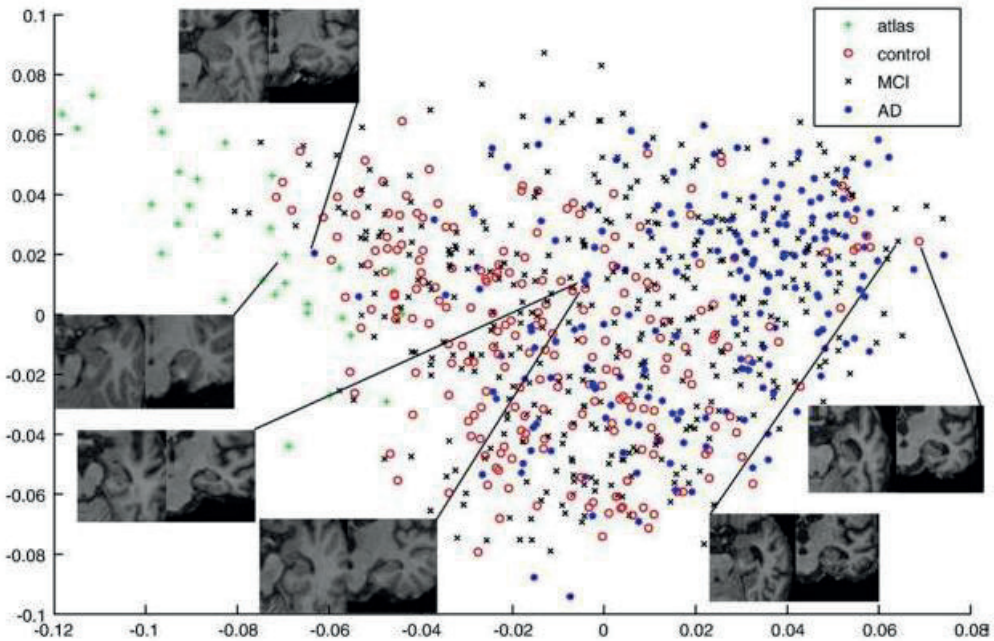

Figure 10 Coordinate embedding, or a manifold, representing 30 atlases based on healthy subjects and 796 images from elderly dementia patients and age-matched control subjects. Distances between images in the coordinate system embedding represent pair-wise image similarities in terms of hippocampal appearance. Reprinted from Wolz et al. (2010a) with permission from Elsevier.

This low-dimensional representation is a compact technique to capture the variability in the MR images in a dataset. An enhanced algorithm was recently published allowing also the incorporation additional information, such as demographics of patients, into the manifold (Wolz et al. 2011). Wolz et al. (2011) showed that by assessing pairwise image similarities evaluated over a region of interest around the hippocampus as described in Wolz et al. (2010a) and adding non-image data (Apolipoprotein E (APOE) genotype, CSF $A\beta_{42}$) to the manifold correct classification rates of 84 % and 64 % were obtained in controls / AD and S- / P-MCI classifications.

2.4.2.4 Tensor- and deformation-based morphometry

Tensor- and deformation-based morphometry (TBM and DBM) are methods used to measure differences in brain shapes by assessing the parameters derived from deformation fields which are generated from registration of the MR image into a standard space (Ashburner et al. 1998, Hua et al. 2008a, Hua et al. 2008b, Koikkalainen et al. 2011). The measure in these techniques describing the shape is usually the determinant of the Jacobian matrix of the deformation field. The template where the target images are registered can be a single MRI scan or a general template achieved by averaging multiple images. Similarly to the multi-atlas segmentation used in automated hippocampal volumetry (Lötjönen et al. 2011), novel TBM techniques utilize the multi-atlas approach when measuring the deformation fields, which have been reported to lead to more accurate structural measurements as well as improved classification accuracy of controls versus AD and S-MCI versus P-MCI subjects (Klein et al. 2005, Koikkalainen et al. 2011, Lötjönen et al. 2010). Figure 11 illustrates an example of a group-wise comparison of brain shapes between controls versus AD and S- / P-MCI subjects.

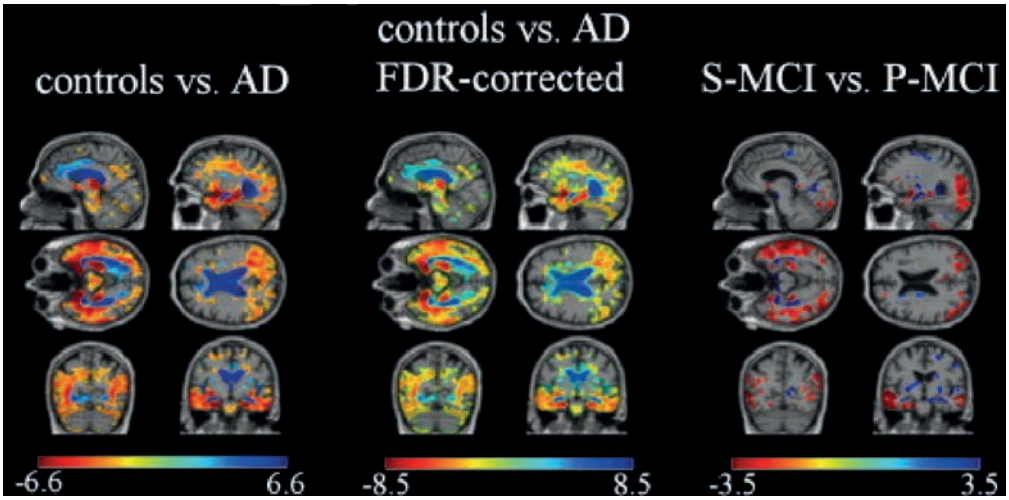

Figure 11 Statistically significant ($p < 0.05$) differences in brain volumes in group comparisons of healthy controls, MCI subjects and AD patients. The color-scales represent statistically significant T-values obtained with multi-template tensor-based morphometry. AD = Alzheimer's disease, S-MCI = stable mild cognitive impairment, P-MCI = progressive MCI, FDR = false discovery rate. Reprinted from Koikkalainen et al. (2011) with permission from Elsevier.

In addition to assessing group-level differences in the AD continuum (Hua et al. 2008a, Hua et al. 2008b), TBM and DBM have been used to arrange classifications of controls versus AD and S-/P-MCI subjects individually. The accuracies in the control/AD

classification have been 83-86 % and 72-73 % in the S-/P-MCI classification depending on the analysis methods and study sample (Koikkalainen et al. 2011, Teipel et al. 2007).

2.4.2.5 Cortical thickness analysis

The above presented techniques (visual inspection, hippocampal volumetry, VBM, TBM, DBM, MBL) can be all used to estimate the volumetric atrophy of different brain structures during the continuum from healthy aging to AD. In particular, the human cortex has interested neuroscientists for over 100 years (Brodmann 1909). Manual assessment of cortical thickness is an extremely laborious task and very vulnerable to measurement errors due to the necessity of creating a correct cut or slices perpendicular to the cortical surfaces. Therefore, various automated methods for measuring CTH from MRI scan have been proposed as novel imaging markers for AD (Fischl and Dale 2000, Jones et al. 2000, Kabani et al. 2001, Kim et al. 2005, Lerch and Evans 2005, MacDonald et al. 2000). Usually these methods work in a similar fashion by extracting the cortical mantle via automated segmentation of GM, white matter (WM) and CSF, creating a deformable polygon mesh on the WM and pial surfaces and finally estimating the CTH in tens of thousands of nodes throughout the entire cortical mantle with sub-millimeter accuracy. The advantage of this kind of procedure is that the cortical morphology is measured as a simple distance between two surfaces providing accurate and meaningful qualitative data that can be used in group-wise comparisons as well as in single-subject level predictive analyses. In addition to the explorative approach where the whole cortex is assessed simultaneously, these methods allow hypothesis-driven experiments based on anatomical regions obtained via parcellation of the cortex with algorithms that label the cortical points according to pre-defined atlases (Collins et al. 1995, Desikan et al. 2006, Fischl et al. 2004). Figure 12 presents CTH models produced with different techniques.

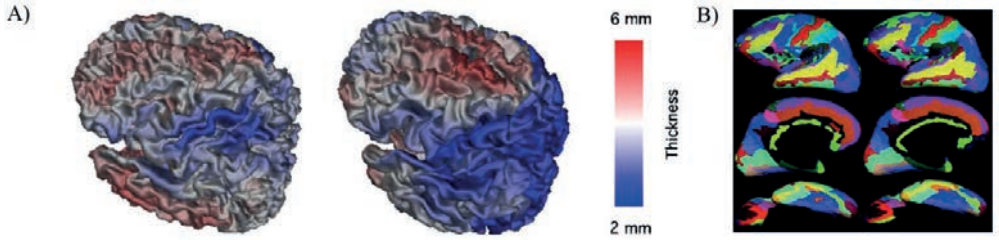

Figure 12 A) Cortical thickness models illustrating thickness profiles of a healthy control (left) and a patient with Alzheimer's disease (AD) (right). The thickness values are colour-labeled from 2 mm (blue) to 6 mm (red). The image demonstrates clearly thinner cortex in several regions in the case of AD patient compared to a healty control subject. Reprinted from Lerch et al. (2008) with permission from Elsevier. B) Automatically achieved anatomical parcellation map of cortical regions. Colours in the brain model demonstrate the different anatomical regions. Reprinted from Fischl et al. (2004) by permission of Oxford University Press.

CTH analysis has been used to detect cortical thinning at the group-level between healthy controls, MCI and AD in a pattern closely resembling the accumulation of AD-type pathological changes starting from medial temporal lobe and extending to the parietal and frontal cortices through the lateral temporal lobe as the disease progresses (see Figure 13) (Dickerson 2009, Fennema-Notestine et al. 2009, Im et al. 2008, Lerch et al. 2005, Lerch et al. 2008, Seo et al. 2007, Singh et al. 2006).

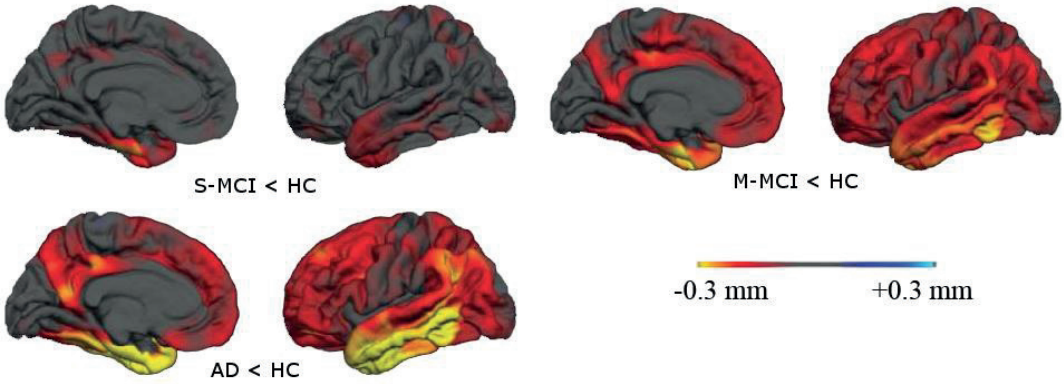

Figure 13 Group differences in average cortical thickness displayed for left hemisphere in comparisons between healthy controls (HC) versus stable MCI (S-MCI), HC versus multi-domain MCI (M-MCI) and HC versus Alzheimer's disease (AD). The color scale represents thickness differences ranging from <−0.3 (yellow) to >+0.3 (cyan) mm. Reprinted from Fennema-Notestine et al. (2009) with permission from John Wiley & Sons Inc.

At the single-subject level, the CTH analysis has shown sensitivity / specificity of 69-94 %/ 77-95 % and 62-83 % / 59-65 % in differentiating between controls and AD and predicting AD in MCI (S-/P-MCI classification), respectively (Bakkour et al. 2009, Cuingnet et al. 2011, Lerch et al. 2008, Liu et al. 2010b, Liu et al. 2011).

It has been reported that different demographical factors such as age, gender, education and APOE genotype might also have an impact on CTH (Filippini et al. 2009, Im et al. 2006, Querbes et al. 2009, Seo et al. 2011), but all the results are not consistent. Negative findings between the connection of GM volume and APOE genotype in AD (Drzezga et al. 2009) have been reported, too.

In addition to these surface based methods, techniques measuring cortical morphology based on novel voxel-based approach (Acosta et al. 2009), fractal analysis (King et al. 2009, King et al. 2010) as well as "4D CTH analysis" assessing the longitudinal change in CTH (Li et al. 2010b) have been developed. Although each of these techniques has been proposed to improve the performance compared to the earlier CTH methods presented above, they represent mainly a rather theoretical framework. Some preliminary results with only 15-40 subjects from the ADNI database have been reported.

2.5 Other biomarkers of AD

In addition to the above presented MRI techniques, also methods based on PET and CSF have been included as viable biomarkers in the proposed new criteria for AD (Dubois et al. 2007, Dubois et al. 2010, McKhann et al. 2011). However, obtaining CSF biomarkers requires an invasive procedure (lumbar puncture) and the availability of PET is not common. Therefore, it would be desirable to have a biomarker that could be obtained from a simple blood sample. In addition to structural and molecular imaging, researchers have also used techniques based on functional imaging in assessing brain changes characteristic of AD. Methods describing AD biomarkers based on PET, functional imaging, CSF and peripheral blood will be described briefly in the following chapters.

2.5.1 Positron emission tomography

The two most frequently used PET techniques are based on fluorine 18-fluorodeoxyglucose-ligands (18F-FDG) and imaging of amyloid deposits in the brain with

Pittsburgh compound B (N-methyl-[^{11}C]2-(4'-methylaminophenyl)-6-hydroxybenzothiazole, PiB). 18F-FDG-PET can be used to assess the glucose metabolism of the cerebral cortex *in vivo*, and a pattern of focally decreased cerebral glucose metabolic rate occurring in AD patients has been identified. The regions presenting AD-type hypometabolism have usually included neocortical association areas bilaterally in parietotemporal regions and the posterior cingulum, and the extent of hypometabolism has been correlated with the severity of cognitive impairment (Coleman 2005, Mazziotta et al. 1992) (Figure 14). Sensitivities and specificities of about 86 % in discriminating healthy controls from AD have been reported, but there is substantial variation in these values (95% CI: 76-93% in sensitivity and 72-93% in specificity) and factors such as cognitive reserve (i.e. the level of education) might also have significant impact on the findings obtained with 18F-FDG-PET (Patwardhan et al. 2004, Stern et al. 1992). 18F-FDG-PET has also been reported to possess value in predicting future conversion to AD in MCI at the group-level (Desikan et al. 2010, Herholz et al. 2011, Landau et al. 2010, Mosconi et al. 2004), whereas the sensitivity and the specificity at the single-subject level prognosis were not very high (sensitivity 57 %, specificity 67 %) (Herholz et al. 2011).

Studies utilizing PiB-PET have shown accumulation of amyoid deposits in the brain of AD patients and MCI subjects compared to controls in a pattern matching the known pattern of AD pathology (Kemppainen et al. 2006, Kemppainen et al. 2007, Klunk et al. 2004) (Figure 14).

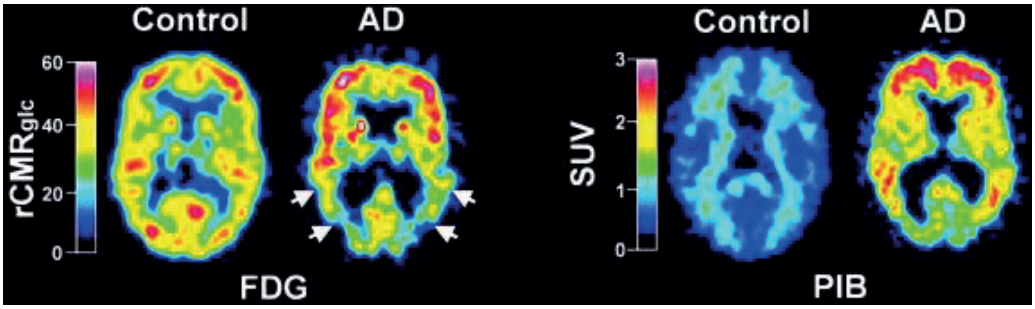

Figure 14 Examples of fluorine 18-fluorodeoxyglucose positron emission tomography (18F-FDG-PET) (left) and Pittsburgh compound B (PiB) uptake in a 67-year-old control subject and a 79-year-old Alzheimer's disease (AD) patient. FDG-PET shows reduced glucose metabolism (arrows) and PiB-PET increased PiB uptake in the AD patient compared to the healthy control. rCMRglc = glucose metabolic rate in µmol/min/100ml, SUV = standardized uptake value. Reprinted from Klunk et al. (2004) with permission from John Wiley & Sons Inc.

On the other hand, there are studies revealing AD-like PiB uptake also among the healthy elderly meaning that either the technique is not specific to AD or that PiB is able to identify the very earliest AD-type pathological changes even in the asymptomatic phase (Mintun et al. 2006). According to recent studies, the increased amount of PiB uptake seems to correlate with low CSF $A\beta_{42}$ among healthy controls (Fagan et al. 2006) and worse cognitive performance in control and MCI groups (Pike et al. 2007) suggesting that these subjects might truly be displaying underlying AD pathology. However, only a long follow-up trial will be able to clarify this issue.

One limitation to PiB-PET from clinical point of view is its short radioactive decay half-life of 20 minutes, which essentially means that the ligand needs to be manufactured in the same site where it is going to be used. As an improvement, the ^{18}F-FDDNP (2-(1-(6-[(2-[(18)F[fluoroethyl)(methyl)amino]-2-naphthyl)ethylidene)malononitrile) ligand with half-life of 110 minutes was developed (Agdeppa et al. 2001, Small et al. 2006). ^{18}F-FDDNP-PET

has been shown to be able to distinguish the control, MCI and AD groups with good accuracy and correlate with cognitive performance similarly to PiB-PET (Small et al. 2006). In addition, ^{18}F-FDDNP was found to bind also to neurofibrillary tangles raising hopes for even better early detection of the pathological changes of AD compared to PiB-PET (de Leon et al. 2007). The half-life of 110 minutes is still a major issue considering the clinical environment, and the diagnostic accuracies and prognostic value of PiB-PET and ^{18}F-FDDNP-PET at the single-subject level in asymptomatic phase or MCI is currently unknown. However, in order to overcome the limitations of current amyloid tracers, various new compounds, such as [(18)F]3'-F-PiB (Flutemetamol), (18)F-AV-45 (Florbetapir), and (18)F-AV-1 (Florbetaben), are being investigated (Vallabhajosula 2011, Vandenberghe et al. 2010).

2.5.2 Functional imaging

In addition to providing an accurate structural image of the central nervous system, MRI can be also used to assess brain function (functional MRI, fMRI). fMRI is based on fast continuous image sequencing of the brain during an activating task. The stimulating task induces neuronal activation in local areas leading to changes in the local blood flow of the brain. This activation can be visualized in real-time by applying a blood-oxygen-level-dependent (BOLD) contrast to the MR image (Kwong et al. 1992). Studies using the fMRI technique and visual learning paradigms have revealed that the brain activity of AD patients is significantly decreased or even completely missing in the region of the medial temporal lobe as compared to healthy controls (Dickerson et al. 2005, Kato et al. 2001, Sperling et al. 2003).

In MCI, the results regarding brain activation during various fMRI paradigms are more heterogeneous. Some investigators have reported increased activation in the medial temporal lobe (Dickerson et al. 2005, Hämäläinen et al. 2007a) whereas others observed decreased neuronal function in the same area as compared to healthy controls (Johnson et al. 2006, Machulda et al. 2003). Not only these changes in the temporal regions but also alterations in parietal and retrosplenial cortices have been reported to reflect abnormal activation levels during cognitive tests in MCI (Poettrich et al. 2009). Taking into account the possible heterogeneity of the MCI background and the small number of subjects (10-20 in each diagnostic group) examined in these studies, the variance in the results is not surprising. It should be also noted that the fMRI paradigms were not the same in all studies which also complicates the comparison between the results. One possible explanation for the discrepancy regarding medial temporal lobe activation levels in MCI is that in early MCI, the individual might use compensation mechanisms in order to remain cognitively normally leading to an abnormal increase in neuronal activity, while later in the disease development, this compensation capacity fails and the activation levels decrease permanently. However, more research with larger and more accurately defined study groups will be needed to verify this hypothesis.

Neuronal function and brain activation can also be investigated in a more straightforward fashion be either measuring directly the electrical activity of the brain with electroencephalography (EEG) or by assessing the weak magnetic fields induced by the changing electric currents during neuronal signaling by using magnetoencephalography (MEG). EEG is a relatively old and somewhat overlooked technique in the current AD research, and it has not been included in the recently published new AD criteria (Dubois et al. 2007, Dubois et al. 2010) or the American guidelines (McKhann et al. 2011). Studies utilizing EEG have claimed that there are detectable changes in various EEG signals obtained from MCI and AD patients as compared to healthy controls, and the magnitude of these changes correlates with the severity of memory impairment and hippocampal

atrophy (Gianotti et al. 2007, Grunwald et al. 2001, Liddell et al. 2007). Despite these significant group-level differences between healthy controls, MCI and AD, there is a considerable amount of overlap between groups in the EEG measures of these studies. Furthermore, raw EEG data can be rather complicated and it is difficult to convert it into simple and meaningful biomarkers, which may be one of the reasons for the reluctance to utilize EEG in the field of AD research. However, a recent paper described a computational approach where MCI to AD conversion was predicted by replacing the raw EEG data with the connection weights of a nonlinear auto-associative artificial neural network trained to reproduce the recorded EEG tracks (Buscema et al. 2010). Buschema and colleagues (2010) reported an accuracy of 86 % in predicting which MCI subjects would convert to AD during a 1-year follow up. Although the result is promising, it should be noted that they used a complicated computational model with a total of 56 classification features in a set of few dozens of subjects, and the majority of their results were substantially poorer. This means that the prediction accuracy would probably diminish severely from the peak value of 86 % if the neural network model was tested with a different dataset.

MEG is a more novel technique and offers some improvements over EEG. It is less sensitive to signal errors induced by the intervening tissues between the brain and the sensor (i.e. skull and scalp) and measures covering the whole brain with numerous sensors are more convenient as MEG does not require placement of electrodes into the scalp. Studies utilizing MEG in the diagnostics of AD use a variety of measures such as fuctional connectivity, median frequency of measured signals, source and spectral analysis (Stam 2010). Common findings are general slowing of background activity, decreased reactivity to eye opening and a lower mean or median frequency (Stam 2010). The accuracy of MEG in detecting patients with AD from healthy controls varies between 66-88 % (Escudero et al. 2008, Fernandez et al. 2006a, Gomez et al. 2007, Poza et al. 2007, Poza et al. 2008).

The possibilities of MEG in predicting which MCI subjects will progress to AD are largely unknown. Some preliminary results with 17 MCI subjects described an increase of 350% in the relative risk of developing AD in those MCI subjects with high left parietal delta dipole density scores (Fernandez et al. 2006b). However, proper follow-up studies with sufficiently large groups and validation procedures will be needed to verify this finding.

Although MEG has some advantages over EEG, this novel technology also has its downsides. MEG systems are expensive and they require a magnetically shielded room, which places considerable limitations on the availability, usability and mobility.

2.5.2 Cerebrospinal fluid

CFS enfolds the central nervous system and pathological processes in the brain are reflected in the molecular profile of CSF. The most widely studied CSF biomarkers of AD are the amounts of total tau (t-tau), hyperphosphorylated tau (P-tau) and $A\beta_{42}$ proteins. In AD, the CSF concentration of $A\beta 42$ is low and t-tau is high compared to healthy controls (Motter et al. 1995) and their level correlates to the amyloid load and the presence of neurofibrillary pathologic abnormalities in the brain in the post-mortem assessment (Tapiola et al. 2009). Combinations of CSF $A\beta_{42}$ and t-tau concentrations have been shown to distinguish AD patients from controls with high sensitivities (85–94 %) and specificities (83–100 %) (Blennow and Hampel 2003). Abnormal levels of these CSF markers have been shown to be predictive of future conversion to AD from MCI at the group-level and varying sensitivities and specificities of 83-95 % / 72-83 % in predicting AD in MCI at the single-subject level have been reported (Hansson et al. 2006, Herukka et al. 2005, Herukka et al. 2007, Mattsson et al. 2009, Parnetti et al. 2006). However, the results regarding prediction of progression of MCI to AD in the ADNI cohort have been lower with correct classification rates of 60-65 %

(Cui et al. 2011, Ewers et al. 2012, Westman et al. 2012). It should be noted that the cut-off values for what are considered as abnormal CSF values vary considerably in different studies, which can be attributed at least partly to different sample analysis methods and heterogeneities in the study samples across the different studies. Acquiring CSF requires a lumbar puncture which is regarded as an invasive operation, can be uncomfortable for the patient and does not always provide successful sample. For example in the study of Hansson et al. (2006), 24 % of the MCI subjects could not be used in the analyses since they were unwilling to undergo the procedure or had inadequate sample quality. However, severe adverse effects relating to lumber punctures are very rare (Peskind et al. 2005). There has been also debate about how significantly age impacts on the diagnostic value of CSF biomarkers. Schmand et al. (2012) reported that CSF biomarkers lose their predictive power in predicting AD in MCI after the age of 75 years (Schmand et al. 2012). Mattson et al. (2012) found that although the diagnostic accuracies for AD decreased with age, the predictive values for a combination of biomarkers remained essentially stable (Mattsson et al. 2012).

2.5.3 Peripheral blood

Peripheral blood would be an ideal source for a biomarker since obtaining blood samples is not very invasive, instead it is widely acceptable and cheap compared to obtaining CSF-based markers or imaging with MRI or PET. Measurements based on metabolomics and proteomics, altered levels of plasma Aβ40, Aβ42 and homocysteine as well as changes in inflammatory markers such as interleucine 8 have been shown to associate to AD (Faux et al. 2011, Orešič et al. 2011, Sato et al. 2012, Seppälä et al. 2010, Sundelöf et al. 2008).

The evidence regarding most proteins or metabolites evaluated in peripheral blood suggests that they are – at best – biological correlates of AD with statistically significant findings in AD versus controls in some cohorts, but they seem to lack sensitivity or specificity for diagnosis or for tracking the response to therapy (Irizarry 2004). The search for reliable biomarkers for AD in peripheral blood is very challenging because of difficulties with the standardization of the methods of analysis and the low reproducibility of the results. For example, very conflicting results regarding the use of plasma $A\beta_{42}$ and $A\beta_{40}$ have been reported varying from promising results (Graff-Radford et al. 2007, van Oijen et al. 2006) to the conclusion that plasma levels of Aβ do not seem to be useful biomarkers for AD (Lopez et al. 2008, Tamaoka et al. 1996). Although some studies haw described positive findings at the group-level, it is hard to find studies that report sensitivities and specificities at the single-subject level. A recent study is an exception in that it reported sensitivity / specificity of 77 / 70 % in predicting AD in a MCI sample of 143 subjects based on the analysis of serum metabolic profiles combined with a rigorous cross-validation process (Orešič et al. 2011). The AD patients were separated from the controls with sensitivity / specificity of 64 / 70 % and these numbers were further improved to 67 / 76 % by adding age into the diagnostic model. Although the results are interesting and promising, they will need to be validated in other sufficiently large cohorts.

2.6 Predicting AD

2.6.1 Methodological aspects

Making the diagnosis of AD reliably in the phase of only mild cognitive problems entails that one must rely on biological tests or imaging as stated in the new AD lexicon (Dubois et al. 2010). There would be clear advantages associated with the identification of biomarkers that would be both sensitive and specific for AD pathology and thus justify the diagnosis before dementia – or predicting those progressing to AD in the MCI phase since AD is still currently diagnosed by the presence of dementia. These biomarkers could help in defining

the disease state and its prognosis, act as surrogate markers of disease development in drug trials, and reduce the economical burden of AD significantly once disease-modifying therapies become available (Brookmeyer et al. 2007, Cummings et al. 2007). Clinical trials could be faster, easier and cheaper since biomarkers could be used to enrich the study populations with subjects of AD pathology (Hampel et al. 2010).

The numerous studies and techniques presented above have been used to detect differences between healthy controls, MCI subjects and AD patients. Usually studies have reported statistically significant differences at the group-level between these populations and claim that they have identified a possible new AD marker. Some studies go further and organize a follow-up for the MCI subjects, divide them into S- and P-MCI groups according to their clinical status after a given time and then undertake a statistical analysis between the groups in order to identify those measures that differentiate the stable subjects from those with "prodromal AD" at the baseline. Although such group-level information is useful in finding new potential biomarkers and in testing hypotheses, the true clinical benefit of candidate markers and methods needs to be validated at a single-subject level.

Several approaches can be used to assess the predictive value of a candidate biomarker at the single-subject level. The traditional technique is to establish cut-off values that differentiate between S- and P-MCI subjects and then apply the cut-offs to the study sample and calculate classification accuracy, sensitivity, specificity and other relevant values. The optimal cut-off is commonly found by using a receiver operating characteristic curve analysis. Although this approach can be successfully used to assess the *optimal* performance of a given marker in a selected dataset, it reveals little about how well the cut-off would perform in another population that has not been used to optimize the cut-off value. The only way to truly test the performance is to obtain another cohort where the same cut-offs are then applied. Most studies do not report this kind of validation in another cohort. Such an approach is, however, important when one considers the vast heterogeneity of the MCI subjects.

The technique allowing a more profound assessment of generalizability and performance of a candidate biomarker is called cross-validation. In cross-validation, the study sample is randomly divided into training and testing sets. The optimal performance of a candidate marker is learned in the training set while the figures measuring performance (sensitivity, specificity, etc.) are calculated *in the testing set.* Then the whole sample is divided again randomly into different training / testing sets and the performance figures are calculated for the new test sample. This procedure is repeated, for example 100 times, and the classification performance of the candidate marker is commonly reported as the mean of all 100 correct classification rates (CCRs), sensitivities and specificities.

The use of cross-validation makes it possible to estimate the distribution of biomarker performance – not only of the most optimistic model – and thus provides a reasonable estimate of how the model may perform in an independent validation setting. This is crucial when the candidate biomarker is under consideration for use in the clinical environment. For example it has been shown that the prediction accuracy of hippocampal volume in a MCI sample of 371 cases from the ADNI dataset can vary anywhere between 56-71% depending on how the training and testing sets are chosen (Lötjönen et al. 2011). In other words, if the value of hippocampal volume was assessed only by the traditional approach by finding the best cut-off and sensitivity / specificity with receiver operating characteristic analysis, or even applying the optimized cut-off only once into a validation set, one might end up overestimating the accuracy in the clinical environment or to claim that hippocampal atrophy is a useless biomarker in AD, depending on the dataset picked by chance.

Even the use of cross-validation does not ensure that the behavior of a candidate marker is thoroughly evaluated. In order to become fully validated, the candidate marker for AD

should be naturally assessed in several different cohorts that include both memory clinic and population-based datasets.

2.6.2 Studies predicting AD

Numerous studies have assessed the use of different AD biomarkers in predicting the outcome of MCI. The majority of the studies have focussed on a single modality but also trials combining multiple biomarkers have been published. A summary of prediction studies in MCI using MRI alone and combined with other biomarkers is presented in Table 7.

Table 7 Studies using biomarkers in predicting progression to Alzheimer's disease (AD) in mild cognitive impairment (MCI). Stable MCI refers to subjects who remain in MCI while progressive MCI subjects are diagnosed as converting to AD during a given follow-up time. The number of subjects (N) is presented as the number of S-MCI/P-MCI subjects. "Validation" refers to the use of cross-validation or separate cohorts as the training and testing sets when calculating correct classification rate (CCR, sensitivity (SE) and specificity (SP). Studies marked with an asterisk (*) use the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort.

Study	Method	N	Follow-up (years)	CCR/SE/SP	Validation
MRI studies					
Bakkour et al. (2009)	CTH, HV, EV	29/20	2.5	CTH: -/83/65 HV: -/83/50 EV: -/72/65	No
Chupin et al. (2009)*	HV	134/76	1.5	64/60/65	Yes
Costafreda et al. (2011)	Hippocampal shape analysis	ANM: 70/33	1	80/80/77	Yes
Cuingnet et al. (2011)*	10 MRI methods	134/76	1.5	CTH: -/32/91 Voxel-STAND:-/57/78 Voxel-COMPARE: -/62/67 HV: -/62/69	Yes
Duchesne et al. (2010)	Multidimensional structural MRI	11/20	5.6	81/70/100	Yes
Ferrarini et al. (2009)	Hippocampal shape analysis	15/15	2.8	80/80/80	Yes
Koikkalainen et al. (2011)*	TBM	215/154	1.5 (max 3)	72/77/71	Yes
Korf et al. (2004)	Visual rating	75/37	3	69/70/68	No
Liu et al. (2010b)	CTH, regional volumetry	79/21	1	69/76/68	No
Lötjönen et al. (2011)*	HV	ADNI: 155/189 Kuopio: 64/42	ADNI: 2 Kuopio: 3.1	ADNI: 63/-/- Kuopio: 66/-/-	Yes
Misra et al. (2009)*	Regional volumetry	76/27	2	55-82/-/-	Yes
Querbes et al. (2009)*	CTH	50/72	2	73/75/69	Yes

Teipel et al. (2007)	DBM	15/9	2.3	80/67/93	No
Westman et al. (2011)*	Regional CTH and volumes	ADNI: 256/62 ANM: 97/22	1	ADNI: 59/74/56 ANM: 70/64/71 Combined: 62/71/60	Yes
Westman et al. (2011)	Multivariate (MV) MRI, manual HV, Visual rating	ANM: 82/19	1	MV MRI: -/79/68 Manual HV: -/68/68 Visual rating: -/68/68	Yes
Wolz et al. (2010c)*	Hippocampal atrophy	167/112	2	66-70/62-66/64-72	Yes
Multimodal studies					
Davatzikos et al. (2011)*	MRI SPAREAD-index, CSF	170/69	1	MRI: 56/95/38 CSF: 44-57/47-90/23-61 Combination:60/84/50	Yes
Fleisher et al. (2008)	HV, NP	76/53	3	HV: 60/-/- NP: 79/-/-	Yes
Schmand et al. (2012)*	Structural MRI, CSF, NP, FDG-PET	94/81	2.7	MRI: 66/-/- CSF: 63/-/- NP: 64/-/- Combination: 65/-/-	No

ANM = AddNeuroMed, CSF = cerebrospinal fluid, CTH = cortical thickness, DBM = deformation-based morphometry, EV = Entorhinal cortex volume, FDG-PET = fluorodeoxyglucose positron emission tomography, HV = hippocampal volume, NP = neuropsychological tests, MRI = magnetic resonance imaging, STAND = structural abnormality index, TBM = tensor-based morphometry

According to the studies presented in Table 7, prediction of MCI to AD progression using MRI during a follow-up time of few years can be made with CCR/sensitivity/specificity of about 54-82/47-95/38-93, respectively. Usually these numbers are quite well balanced and are in the region of 60-70 with the few exceptions where sensitivity is very high at the cost of low specificity (Bakkour et al. 2009, Davatzikos et al. 2011), or vice versa (Cuingnet et al. 2011, Duchesne et al. 2010, Teipel et al. 2007). The best results have been achieved with a very limited number of subjects (Duchesne et al. 2010, Ferrarini et al. 2009, Teipel et al. 2007) but this raises questions about the generalizability of the results. There is also considerable variation in the results even within the ADNI cohort regardless of which MRI feature is used. For example, hippocampal volume seems to predict the conversion to AD in MCI with varying sensitivities and specificities of 60-83 and 50-69, respectively. It was recently shown that depending on the sub-population used in the ADNI, the results can easily vary by 10-20 % units in the final classifications, which complicates the comparison of the results between different studies since it is rare that these studies would use exactly the same populations (Lötjönen et al. 2011). Therefore, it is also difficult to state with confidence which MRI markers provide the most predictive information, as only very few studies have assessed different MRI features simultaneously. Studies that do not report classification accuracy or sensitivity/specificity are not included in Table 7 as their results are even harder to compare with each other. There is some evidence that the combination of biomarkers assessing different pathological aspects of AD might be useful (Davatzikos et al. 2011, Eckerström et al. 2010, Furney et al. 2011), but also negative findings have been reported (Schmand et al. 2012). Furthermore, it has been suggested that the MRI-based

markers might be more useful than cellular and metabolic measures as predictors of the clinical decline in MCI (Desikan et al. 2010).

ADNI has been an essential source in the development of new AD biomarkers and has already resulted in hundreds of publications assessing the challenges of earlier AD diagnostics. However, one of the key questions for the future is to investigate how well the methods developed in the ADNI will translate to the community or general clinic setting when they enter wider diagnostic use (Weiner et al. 2012).

3 AIMS OF THE STUDY

This research aims to assess the alterations in CTH with automated pipelining methods examining a spectrum ranging from healthy controls to AD patients with a special focus on MCI subjects. CTH analysis is also used to investigate the correlation of cortical morphology and different clinical and neuropsychological parameters. Finally, the predictive power of CTH analysis in MCI is compared to other computational state-of-the-art MRI analysis methods. More specifically, this thesis aims to find answers to the following questions:

1. How does the CTH profile change in AD compared to healthy aging?

2. Can CTH analysis be used to detect the changes typical to AD already before severe clinical symptoms, i.e. in MCI?

3. How does cortical thinning relate to the progression of clinical symptoms?

4. Do certain demographical factors, such as education, associate to CTH in the AD continuum?

5. How accurately can the structural analysis of brain alterations be used to separate healthy elderly subjects from those with AD at the single-subject level, and how accurate is the prediction of future conversion to AD in MCI?

4 SUBJECTS AND METHODS

4.1 Subjects

In studies I and II, the subjects were gathered from the database of Kuopio University Hospital. This database consists of subjects who originally participated in population-based epidemiological studies (Hänninen et al. 2002, Kivipelto et al. 2001). Study II was based on the population of study I supplemented with healthy controls, AD patients and more MCI subjects from the population-based database of Kuopio University Hospital (Hänninen et al. 2002, Kivipelto et al. 2001). Informed written consent was acquired from all the subjects according to the Declaration of Helsinki. All the studies were approved by the Ethics Committee of Kuopio University Hospital.

The subjects in study III are participants of the pan-European study AddNeuroMed (www.innomed-addneuromed.com). AddNeuroMed is a prospective, longitudinal multicenter study aiming at discovering biomarkers that would allow more accurate and earlier diagnosis of AD, prediction of cognitive deterioration and monitoring of disease progression. Data for the AddNeuroMed was collected from six medical centers across Europe: University of Kuopio, Finland; University of Perugia, Italy; Aristotle University of Thessaloniki, Greece; King's College London, United Kingdom; Medical University of Lodz, Poland; and University of Toulouse, France. AddNeuroMed is funded by the European Union and members of the European Federation for Pharmaceutical Industries and Associations (EFPIA). Informed consent was obtained for all subjects and protocols and procedures were approved by the relevant Institutional Review Board at each data acquisition site and the data coordination site.

Study IV investigates subjects in the ADNI database. The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations, as a 60 million dollar, 5-year public-private partnership. The primary goal of ADNI has been to test whether serial MRI, PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and AD. ADNI is the result of the efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 adults, ages 55 to 90, to participate in the research -- approximately 200 cognitively normal older individuals to be followed for 3 years, 400 people with MCI to be followed for 3 years, and 200 people with early AD to be followed for 2 years. For detailed information see www.adni-info.org. The ADNI study has been approved by the Institutional Review Boards of all of the participating institutions. Informed written consent was obtained from all the participants at each site.

An overview of the subjects in studies I-IV is presented in Table 8. More detailed demographical and clinical data for these subjects in each study are presented in the Appendix (Table 1 in each study I-IV). The follow-up time was considered as the time from the baseline / screening to last available examination (S-MCI subjects) or to the diagnosis of AD (P-MCI subjects) in all studies.

Table 8 Healthy control (HC), stable mild cognitive impairment (S-MCI), progressive MCI (P-MCI) and Alzheimer's disease (AD) subjects used in studies I-IV. Follow-up time (mean ± standard deviation) for P-MCI subjects was considered as time from baseline to the diagnosis of AD.

Study	Cohort	N	Follow-up time
I	Kuopio		
	• S-MCI	45	3.8 ± 1.3
	• P-MCI	15	1.9 ± 1.3
II	Kuopio		
	• HC	26	
	• S-MCI	68	3.4 ± 1.4
	• P-MCI	30	2.5 ± 1.7
	• AD	21	
III	ANM		
	• HC	113	
	• MCI	121	
	• AD	121	
IV	ADNI		
	• HC	231	
	• S-MCI	238	2.1 ± 1.1
	• P-MCI	167	1.5 ± 0.8
	• AD	198	

ADNI = the Alzheimer's Disease Neuroimaging Initiative, ANM = AddNeuroMed

4.1.1 Controls

HC subjects were used in studies II, III and IV. In study II, the HC subjects were volunteers from population-based cohorts in Kuopio and the methods used for the identification of control subjects have been described in a previous study (Kivipelto et al. 2001). Briefly, the controls had no history of neurological or psychiatric diseases and showed no impairment in the detailed neuropsychological examination. There were 26, 113 and 231 HC subjects in studies II, III and IV, respectively (Table 8). Complete demographic and clinical data for these subjects are presented in the Appendix (Table 1 in studies II, III and IV).

In study III, the controls did not have any neurological or psychiatric disorders and were not taking any psychoactive medication. The classification of the controls and MCI subjects was based on CDR score and clinician's judgment, rather than on cognitive tests.

In study IV, all HC subjects were participants in the ADNI project (www.adni-info.org). In the ADNI, the HC subjects have Mini-Mental state examination (MMSE) scores between 24–30, a CDR score of 0, are non-depressed, have no memory complaints, and are non-demented. Delayed recall of one paragraph from the Logical Memory II subscale of the Wechsler Memory Scale–Revised (maximum score of 25) (Wechsler 1987) was used as the memory criterion with cutoff scores based on education for HC. Furthermore, the HC subjects were matched with other subjects of the same age (Petersen et al. 2010).

4.1.2 MCI subjects

MCI subjects were included in studies I-IV (Table 8).

Study I consisted initially of 81 MCI subjects. They were followed for a maximum of 7 years (mean 3.8 years for S-MCI, mean time to conversion for P-MCI 1.9 years). MRI scans were conducted annually until the end of 4 years of the follow-up time or until the diagnosis of AD. After the 4-year period, the subjects were clinically examined on a regular

basis. All patients had MCI defined by the presence of cognitive symptoms, a global CDR (Hughes et al. 1982) stage of 0.5 and a score of less than 9 in the New York University delayed paragraph recall test (Kluger et al. 1999) and by not meeting the diagnostic criteria for AD. Exclusion criteria were: depression, other neurological or psychiatric disease or drug treatment potentially affecting cognition or clinically significant other disease affecting the subject's participation in the long-term follow-up, any known primary neurodegenerative disease, any severe unstable medical condition that could interfere with the assessment of cognition, a score of more than 4 on the modified Hachinski ischaemic scale, and any severe or unstable cardiovascular disease or asthmatic conditions (Feldman et al. 2007). A total of 21 subjects had to be excluded from the study either because of screening failure in the clinical assessment, inadequate quality of the MRI image or missing data of cognitive outcome in the follow-up. Of the remaining 60 participants, 15 were classified as P-MCI and 45 as S-MCI subjects. The definition of P-MCI included conversion to AD according to NINCDS-ADRDA criteria (McKhann et al. 1984).

In study II, 98 MCI patients were pooled from a population-based database (Hänninen et al. 2002, Kivipelto et al. 2001). MCI was diagnosed using the criteria originally proposed by the Mayo Clinic Alzheimer's Disease Research Center (Petersen et al. 1995, Smith et al. 1996). At the time this study population was recruited, the MCI criteria designated were as follows: 1) memory complaint by patient, family, or physician; 2) normal activities of daily living; 3) normal global cognitive function; 4) objective impairment in memory or in one other area of cognitive function as evident by scores >1.5 SD below the age-appropriate mean; 5) CDR score of 0.5; and 6) absence of dementia. All the MCI subjects were considered as having the amnestic subtype of the syndrome. A total of 30 MCI subjects progressed to AD during the follow-up.

The MCI population of study III consisted of 121 subjects from the AddNeuroMed database. All the MCI subjects were diagnosed as having amnestic MCI by meeting the following criteria: 1) memory complaint by patient, family, or physician, 2) normal activities of daily living, 3) normal global cognitive function as measured by the MMSE (score range between 24–30), 4) Geriatric Depression Scale score less than or equal to 5, 5) subject aged 65 years or above, 6) CDR memory score of 0.5 or 1, and 7) absence of dementia. None of the subjects had alcohol/substance misuse or other diseases or medical conditions affecting cognition.

The MCI subjects in study IV were participants in the ADNI study. They had MMSE scores between 24–30, a memory complaint, objective memory loss measured by education adjusted scores on Wechsler Memory Scale Logical Memory II, a CDR of 0.5, absence of significant levels of impairment in other cognitive domains, essentially preserved activities of daily living, and an absence of dementia (Petersen et al. 2010). All the subjects were considered as having amnestic MCI. Of the 405 MCI subjects, a total of 167 progressed to AD during 1.5 ± 0.8 years (mean ± standard deviation) (Table 8).

4.1.3 AD subjects

AD subjects participated in studies II, III and IV (Table 8). In all these studies, the diagnosis of dementia was based on the DSM-IV/DSM-IV-TR criteria (American Psychiatric Association 1994, American Psychiatric Association 2000) and the diagnosis of AD on the NINCDS-ADRDA criteria (McKhann et al. 1984). The severity of the cognitive decline was graded according to the CDR scale. For a detailed description of the demographics and clinical data see the appendix (Table 1 in all studies).

4.2 MRI acquisition

MR images for the Kuopio cohort in studies I and II were acquired using one of the two 1.5-T MR scanners at the Department of Clinical Radiology, Kuopio University Hospital. All anatomical high-resolution T1-weighted images were acquired using a 3D MPRAGE (3-dimensional magnetization-prepared rapid acquisition gradient echo) sequence. There was minor variation in the imaging parameters across the different subjects in study II (for details see Appendix, study II, chapter "MRI acquisition"). All the HC and AD subjects were scanned with the first scanner and the first imaging parameter set. Twenty of the S-MCI and 7 of the P-MCI subjects' MR images were obtained with the second scanner. The imaging parameter set 2 was used for 37 S- MCI and 14 P-MCI subjects.

In study III, the image acquisition was conducted with six different 1.5T MRI scanners using a custom high- resolution sagittal 3D T1-weighted MPRAGE sequence specifically designed for the ADNI study to ensure compatibility across scanners. Quality-control was done in three steps including 1) usage of a test phantom, 2) scanning of volunteers for reference images to ensure compatibility of the data across different sites, and 3) applying a continuous supervision of data quality. For a detailed description of the MRI methods in AddNeuroMed see (Simmons et al. 2009).

In study IV, the imaging was conducted according to the MRI protocols and the preprocessing steps of the ADNI. The MRI scans were standard 1.5T screening/baseline T1-weighted images obtained using volumetric 3D MPRAGE protocol with varying resolutions. For detailed information of the MRI protocols and preprocessing steps see Jack et al. (2008).

4.3 Imaging analysis methods

4.3.1 Cortical thickness analysis

The CTH measurements from the MR images were obtained using two different automatic software pipelines. In studies I, II and IV, a method developed at the McConnell Brain Imaging Centre, Montreal Neurological Institute (Lerch and Evans 2005) was used. The first step in this pipeline was to register each MRI into the stereotaxic ICBM152 template (Mazziotta et al. 2001) and correct intensity non-uniformities (Sled et al. 1998). In the second phase, masks for WM, GM and CSF were created in order to achieve a primary segmentation (Smith 2002). These brain masks were fine-tuned by estimating the partial volume effect in each voxel (Tohka et al. 2004). The final segmentation was then achieved using the intensity-normalized stereotaxic environment for classification of tissues - algorithm (Zijdenbos 1998). The inner (WM/GM intersection) and outer (GM/CSF intersection) surfaces were then modelled with a deformable mesh by applying the constrained Laplacian-based automated segmentation with the proximities algorithm to the segmented image (Kim et al. 2005). Thus, both created polygon mesh surfaces consisted of 81,920 polygons and 40,962 nodes per hemisphere. CTH in millimeters was then calculated as the distance between two concentrically linked surface maps (Lerch and Evans 2005). Finally, a 20 mm full width at half maximum diffusion smoothing kernel was applied to the cortical thickness maps in order to reduce the impact of imperfect alignment between cortices and to increase the signal-to-noise ratio and statistical power (Chung and Taylor 2004, Lerch and Evans 2005).

The CTH measurement of every study subject was checked manually one by one from the quality control images produced by the CTH analysis software. In study IV, the described toolbox did not achieve satisfactory results on some study subjects because of i) failure in tissue segmentation and brain masking (48 subjects) and ii) failure in partial

volume effect estimation (59 subjects). In addition, the cortical model of 31 subjects was completely deformed and thus unusable. For these subjects, the CTH features were considered as missing values.

In study III an automated software called "FreeSurfer" was used (Fischl and Dale 2000). FreeSurfer estimates CTH in a similar fashion as the Montreal pipeline by performing skull stripping, transformation of the image data to a standard Talairach space, applying of intensity normalization followed by extaction of the cortical mantle based on intensity gradients. Similarly to the Montreal pipeline, the CTH maps produced are not restricted to the voxel resolution of the original data and are thus capable of detecting submillimeter differences between groups. However, in contrast to the Montreal method, the FreeSurfer measures also volumes of the subcortical structures (hippocampus, amygdala, caudate, putamen, ventricles), provides cortical parcellation based on anatomical areas as well as cortical volumes for these anatomical regions (Fischl et al. 2004).

4.3.2 Hippocampal volume

Hippocampal volume (HV) in study IV was measured using an approach based on fast and robust multi-atlas segmentation (Lötjönen et al. 2010, Lötjönen et al. 2011). The method is based on multi-atlas segmentation combined with atlas selection. First, a set of hippocampus atlases is selected from a pool of atlas images according to image similarity with the query image. After registering all atlases into the query image, a spatial prior is generated from the multiple label maps. This spatial prior is then used to obtain a final segmentation based on an expectation maximization segmentation algorithm (Van Leemput et al. 1999) and the volume is calculated based on the segmentation of the hippocampal formation.

4.3.3 Manifold-based learning

In MBL, a non-linear dimensionality reduction with Laplacian eigenmaps (Belkin and Niyogi 2003) is used to learn features to discriminate between different subject groups. Laplacian eigenmaps estimate the low-dimensional representation of a set of input images based on pairwise image similarities (Belkin and Niyogi 2003). Pairwise image similarities are estimated from the intensity appearance in a region around the hippocampus and amygdala. All images are aligned in a template space using a coarse non-rigid registration (10 mm B-spline control-point spacing, (Rueckert et al. 1999)). Such a coarse non-rigid alignment ensures that the corresponding brain structures are aligned but still makes it possible to measure subject-specific differences. After performing dimensionality reduction, the first 20 dimensions of the resulting manifold are used as features to perform classification with a method of choice. Detailed information on the theory and the application of MBL technique is available in (Wolz et al. 2010c, Wolz et al. 2011). Figure 15 presents an example of a 2D embedding of a set of ADNI images acquired from healthy controls and subjects with AD.

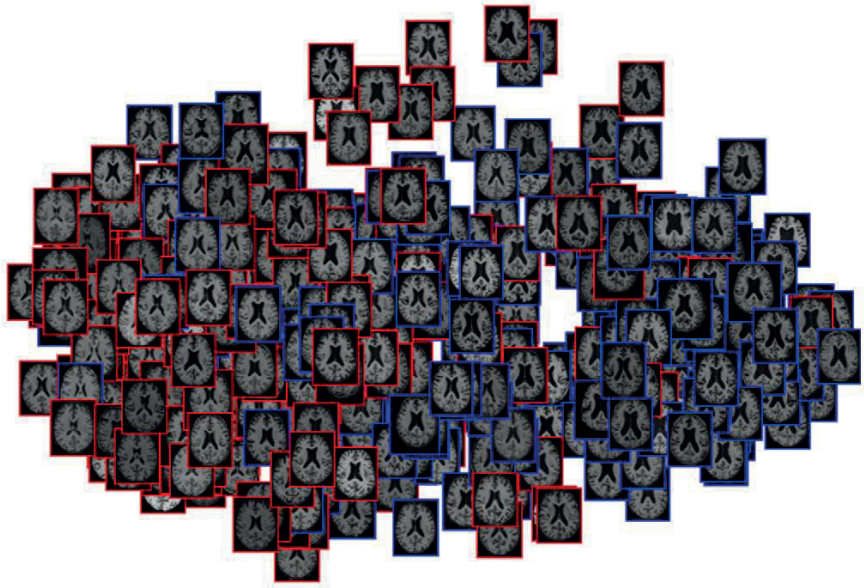

Figure 15 2D manifold embedding of a set of images acquired from healthy controls (red) and subjects with AD (blue) from the ADNI database. The images are aligned in a 2-dimensional coordinate system based on a similarity graph that is defined with pairwise image similarities.

Figure 15 demonstrates that even an embedding with two dimensions provides a relatively good separation between both groups. In study III, a higher dimensional space was used for a more accurate discrimination of the study groups.

4.3.4 Tensor-based morphometry

In TBM, a template has been registered non-rigidly to multiple database cases and typically the determinant of the Jacobian matrix ('the Jacobian') of the deformation is used as a measurement of the morphometry. In this study, the standard TBM analysis was extended to a multi-template approach (Brun et al. 2009, Koikkalainen et al. 2011) in a similar fashion as atlas-based segmentation is extended to multi-atlas segmentation. The template images were a set of 30 randomly selected images (10 controls, 10 MCIs, and 10 ADs) from the ADNI database. Each template image was registered to a study image, and Jacobian maps were computed for each template image. To combine the results of multiple templates, all template images were registered to the mean anatomical template generated from the 30 images, and all the results were normalized to this reference space (Koikkalainen et al. 2011). The combination of the results was performed by averaging the ROI-wise feature values of all the templates as described in detail below.

4.4 Statistical analysis

4.4.1 Demographics and clinical data

Statistical tests were performed with SPSS (SPSS Inc., Chicago, Ill., USA) and the level of statistical significance was set to $p < 0.05$ in all the statistical analyses in this thesis, unless otherwise stated. Independent samples t-tests were used to compare differences of continuous variables with normally distributed values. The chi-squared test was used to assess the differences in gender, scanner/voxel volume as well as APOE allele distribution across the study groups. The Mann-Whitney U test was applied to assess the difference of

CDR sum of boxes (CDR-SOB) and global deterioration scale (GDS) scores between the MCI groups in study I and the difference of CDR-SOB scores and follow-up times in study II.

4.4.2 Imaging data

To examine statistical differences in CTH between the study groups in studies I, II and IV, a t-test was performed in every cortical node in both hemispheres using in-house scripts in Matlab (Mathworks Inc., Natick, MA, USA). A correction for multiple comparisons was done using the false discovery rate (FDR)-correction method (Genovese et al. 2002). Age and gender were used as nuisance variables in all CTH analyses according to the guidelines (Barnes et al. 2010). In studies I and II, the scanner type/voxel volume were also used as nuisance variables. Furthermore, in study II the analysis between all the groups was conducted also with education as a nuisance, and S-/P-MCI analysis with also the number of APOE ε4 alleles, MMSE and CDR-SOB scores as well as follow-up time as additional nuisance variables. Differences in brain volumes (CSF, GM, WM) in study II were tested by using analysis of covariance with gender taken into account. Pairwise comparison of brain volumes between the study groups was done with Post Hoc tests and Bonferroni correction for multiple comparisons.

In study I, the correlation between CDR-SOB and cortical thickness at the group level was tested in every node by examining if the variation in cortical thickness correlated with the variation in CDR-SOB. The correlation analysis was performed both across all subjects and within the study subgroups. A correction for multiple comparisons was performed using the FDR-correction method (Genovese et al. 2002). Age, gender and scanner type were used as nuisance variables in the analysis. Cortical thickness analysis between the two groups was also done with CDR-SOB set as a nuisance variable to eliminate its effect on the results.

In study II, the correlation analyses were conducted similarly to study I, but the analyses were done only separately within the study groups. In study II, the effect of the APOE ε4 allele carrier status to CTH was also assessed by analyzing the differences in CTH in each group between APOE ε4 carriers (at least one ε4 allele) and non-carriers (no ε4 alleles).

In study III, the correlation between years of schooling and regional cortical thicknesses/volumes was tested with the partial correlation coefficient for each diagnostic group. Since education may not correlate linearly with regional thickness/volume, all diagnostic groups were further dichotomized into higher and lower education groups with a threshold education of 9 years (median from the data). Analysis of covariance was used in the statistical tests between the low/high education groups. The intracranial volume (for volume measures), country of origin, MMSE scores, age, and gender as covariates in the partial correlation analysis and analysis of covariance. Because of multiple comparisons, FDR-correction (Benjamini and Hochberg 1995, Storey et al. 2004) was used to control for type 1 error in the correlation analysis and analysis of covariance with the q value software package (http://genomics.princeton.edu/storeylab/). A q value of < 0.05 was considered as statistically significant in the FDR correction.

4.4.3 Feature selection for classification

In study IV, features based on automated structural MRI analysis were used to make an automated classification of the study subjects into the diagnostic groups when considering the baseline characteristics only. CTH and TBM analyses produce local point-wise information based either on cortical thickness or the volume of specific areas. The number of original features is thus tens of thousands meaning that feature reduction techniques are needed to make the classification more efficient and robust. In order to reduce the number

of features used in the classification experiments, a ROI-based approach utilizing an anatomical atlas of 83 structures (Gousias et al. 2008, Hammers et al. 2003) was selected. Both the CTH nodes and the TBM voxels were mapped to ROIs based on these anatomical regions, and the CTH and the Jacobian values were averaged within each ROI. In order to enhance the classification power, the data points within the ROIs were weighted based on statistical analysis based on group level differences. For a detailed description of this procedure see the appendix (study IV, supplementary material). In addition to CTH and TBM, features based on hippocampal volume and MBL were also used in the classification experiments. The features used in study IV are displayed in Table 9.

Table 9 Features used in classification of healthy controls (HC), stable mild cognitive impairment (S-MCI), progressive MCI (P-MCI) and Alzheimer's disease (AD) in study IV

Method	Number of features	Description
HV	1	Total volume of left and right hippopcampus
CTH	9 (HC vs AD)	Average CTH within a ROI defined based on group-level statistical analysis
	7 (HC vs P-MCI)	
	8 (S-MCI vs P-MCI)	
TBM	84	Average Jacobian of atrophic voxels within a ROI, weighted based on voxel-wise p-values
MBL	20	Coordinates of a subject in a low-dimensional manifold space learned from pairwise image similarities

CTH = cortical thickness, HV = hippocampal volume, MBL = manifold-based learning, TBM = tensor-based morphometry

4.4.4 Classification and validation procedures

In study IV, two different subsets from the ADNI database were used: 1) All 834 available baseline images described in the subjects Section, Table 8, and 2) 509 baseline images used by Cuingnet et al. (2011) and detailed in their publication

In order to perform the study using cross-validation in the full dataset, it was divided into three equally sized parts. Part I was used to perform the statistical tests for the CTH and TBM features while parts II and III were left for evaluation of the classification performance. This was repeated three times so that each part was once used to perform the statistical tests. Each time 95% of the subjects in the evaluation set (parts II and III) were used to train a classifier which was then applied to the test set (the remaining 5%). This was repeated 100 times, each time selecting randomly the test set subjects, with the results of the 100 repetitions being averaged. The classification evaluation was performed using 3 x 100 = 300 repetitions, and the results presented are the average values of all these classifications.

In dataset II, the statistical ROIs for CTH and TBM feature extraction were calculated from the 325 baseline images that are not part of dataset II. In order to allow direct comparison of classification accuracy with the work by Cuingnet et al. (2011), separate

training and testing sets for the different comparisons were defined using the exact sub-groups reported in their manuscript.

The classification was done using linear discriminant analysis (LDA) and support vector machines (SVM). LDA is a widely used technique which helps find a linear combination of features that best separates several classes (Krzanowski 1988). In this work, LDA was used as implemented in the classify function in Matlab with a multivariate normal density model with uninformative priors ($p = 0.5$). SVM use training data to find a separating hyperplane in the n-dimensional training space that best separates two subject groups (Cortes and Vapnik 1995). Test subjects are then classified according to their position relative to the defined hyperplane in the n-dimensional feature space. The analysis was performed by using the libSVM library. The radial basis function kernel was selected based on the guidelines provided by the libSVM library (Software available 2.3.2011 at http://www.csie.ntu.edu.tw/cjlin/libsvm).

5 RESULTS

5.1 Study I

The purpose of study I was to determine whether CTH analysis could be used to detect cortical thinning among those MCI subjects who will later develop AD. The correlation between cortical thickness and clinical symptom severity as measured by CDR-SOB scores was also investigated.

Differences in clinical and demographic data are presented in the appendix, study I, Table 1. As compared to S-MCI, the P-MCI subjects performed significantly worse in Alzheimer's Disease Assesment Scale-cognitive subscale, CDR-SOB and GDS scores compared to the S-MCI group. The groups did not differ in terms of age, gender, scanner type distribution or MMSE scores.

The CTH analysis revealed pronounced cortical thinning in the P-MCI group bilaterally in the areas of superior and middle frontal gyri, superior temporal sulci, middle and inferior temporal gyri, fusiform and parahippocampal gyri and retrosplenial cortices as compared to the S-MCI group. A similar significant difference in CTH was also found in the right precuneus, paracentral lobe as well as in the right anterior and left posterior cingulate gyrus (Figure 16). Overall, the differences in cortical thickness between the P-MCI and S-MCI groups were more pronounced in the right hemisphere.

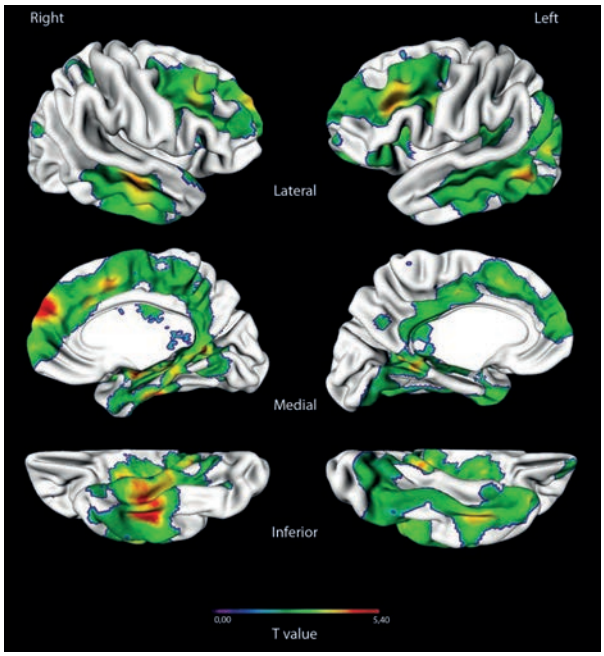

Figure 16 Brain regions demonstrating significantly ($p < 0.05$, FDR-corrected) thinner cortex at baseline MR imaging in the progressive compared to stable mild cognitive impairment (MCI) subjects in study I. Differences are illustrated with color-labeled t values scaled from 2.18 and 2.19 (threshold t values corresponding to $p = 0.05$ for the left and right hemispheres, respectively) to the maximum $t = 5.40$.

In the correlation analysis encompassing the whole study population (n = 60), the higher CDR-SOB scores were correlated with a lower CTH in brain regions largely corresponding to the regions demonstrating CTH differences between the P-MCI and S-MCI subjects. In contrast to the CTH differences, the correlation findings were slightly more prominent in the left hemisphere (Figure 17).

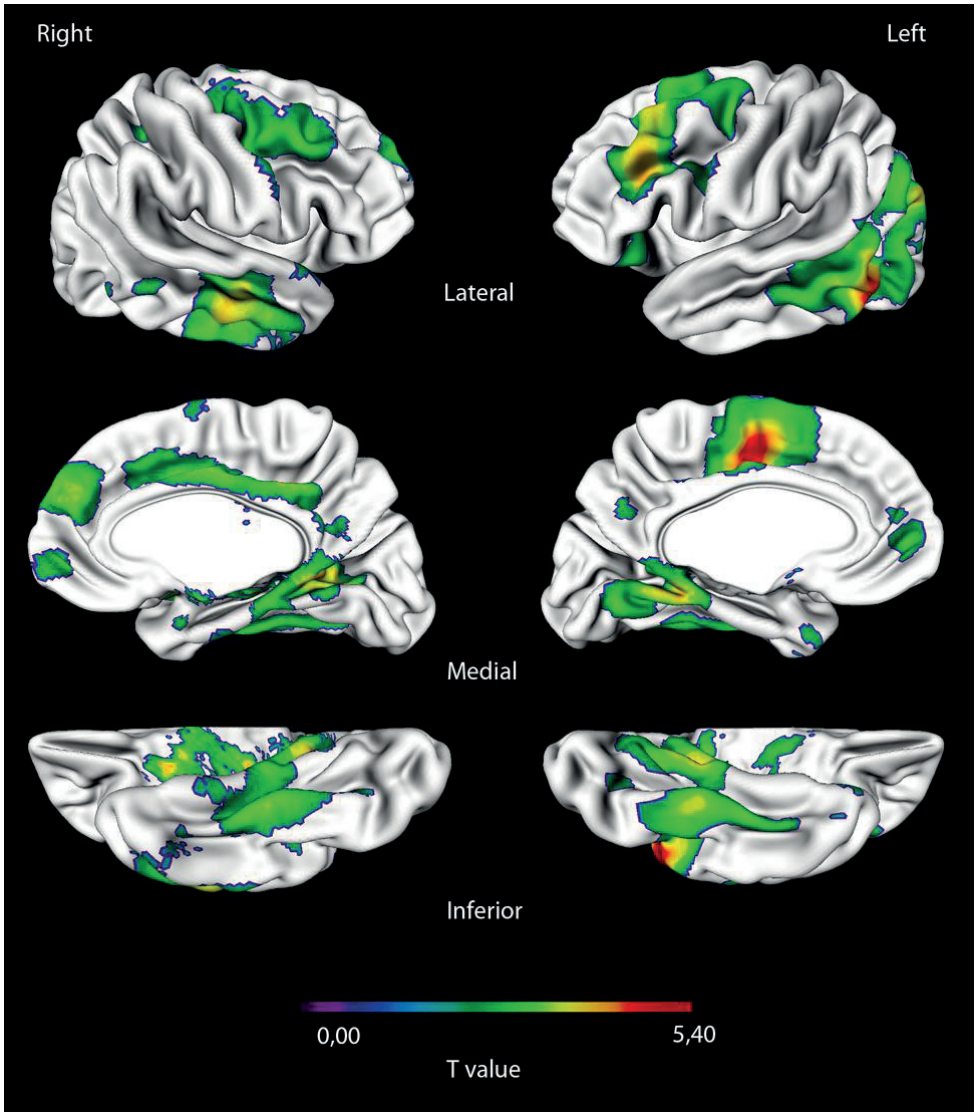

Figure 17 Brain regions showing correlation between Clinical Dementia Rating Sum-of–Boxes score (CDR-SOB) and cortical thickness (CTH) across all study subjects in study I presented as color-labeled t values.

When the two groups were analysed separately the findings within the P-MCI group largely resembled the results of the whole group analysis, whereas the S-MCI group showed no correlation between CDR-SOB scores and CTH values.

5.2 Study II

Study II was designed to verify the findings of study I in a larger dataset and to expand the study population to encompass also healthy controls and AD patients. Furthermore, the association analyses between CTH and clinical variables were done with several additional features (education, APOE genotype) besides clinical disease severity.

When comparing the clinical and demographical characteristics between the study groups, the MMSE scores differed significantly between the HC, S-MCI, P-MCI and AD groups being, as expected, highest in the control and lowest in the AD group. CDR-SOB scores differed similarly between controls, S-MCI and P-MCI groups. CDR-SOB scores were not available for the AD group. The AD subjects were older than the MCI subjects while the PMCI subjects had the highest number of education years. There were no statistically significant differences in the APOE ε4 carrier or gender distributions between the study groups. In addition, the scanner and imaging parameter set distributions did not differ significantly between the two MCI groups. Detailed demographics and clinical data of the subjects in study II can be found in the appendix, study II, Table 1.

Results of the CTH analyses between HC, MCI and AD groups are displayed in Figure 18.

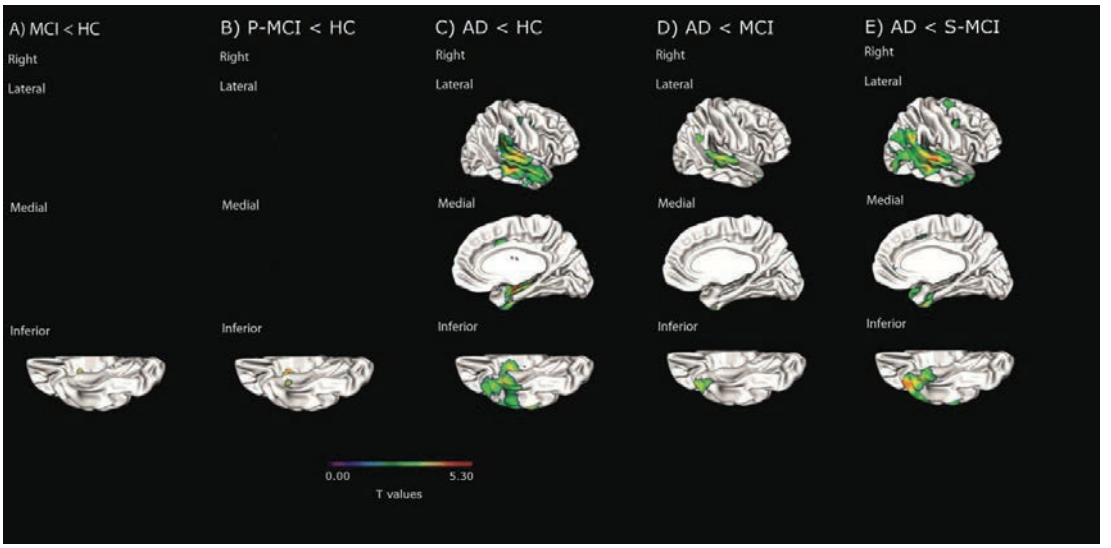

Figure 18 Statistical differences ($p < 0.05$, FDR-corrected) in cortical thickness (CTH) in study II between healthy controls (HC) and A) all subjects with mild cognitive impairment (MCI), B) progressive MCI (P-MCI) and (C) Alzheimer's disease (AD); CTH differences between AD and D) MCI group and E) stable MCI (S-MCI) group.

When comparing the whole MCI group (n = 98) to the controls (n=26) a small area of thinner cortex in MCI was found on the right posterior parahippocampal gyrus (Figure 18A). When the S-MCI (n = 68) and PMCI (n = 30) subjects were compared to controls separately, only the P-MCI group presented a small area of thinner cortex in the anterior right parahippocampal gyrus (Figure 18B). Compared to controls, the AD group (n = 21) demonstrated significantly thinner cortex in the cingulate sulcus, the structures of medial temporal lobe (parahippocampal and fusiform gyri), and lateral temporal lobe (inferior, middle and superior temporal gyrus) of the right hemisphere (Figure 18C).

The AD group had a thinner cortex in the temporal gyri of the right hemisphere as compared to the whole MCI group (Figure 18D).When comparing separately to the S-MCI subjects, the areas of significant difference enclosed major parts of medial and lateral temporal lobes as well as areas in the frontal cortex and cingulated sulcus (Figure 18E). All of the results were observed on the right hemisphere. There were no statistically significant differences in CTH between the P-MCI and AD groups. Taking into account the level of education as years of schooling did not have any significant influence on the results in the CTH analyses between the study groups.

A closed comparison of the P- and S-MCI groups revealed distinct differences in CTH in large brain areas almost identically to study I. Again, the areas of thinner cortex were more widespread and the t values were higher on the right hemisphere, especially in the region of medial temporal lobe (Figure 19A). However, setting MMSE, CDR-SOB, follow-up time and APOE genotype as nuisance variables – to examine the differences in CTH distinct from all other factors than the progressive nature of the disease in the P-MCI subjects – diminished the differences between the groups markedly (Figure 19B).

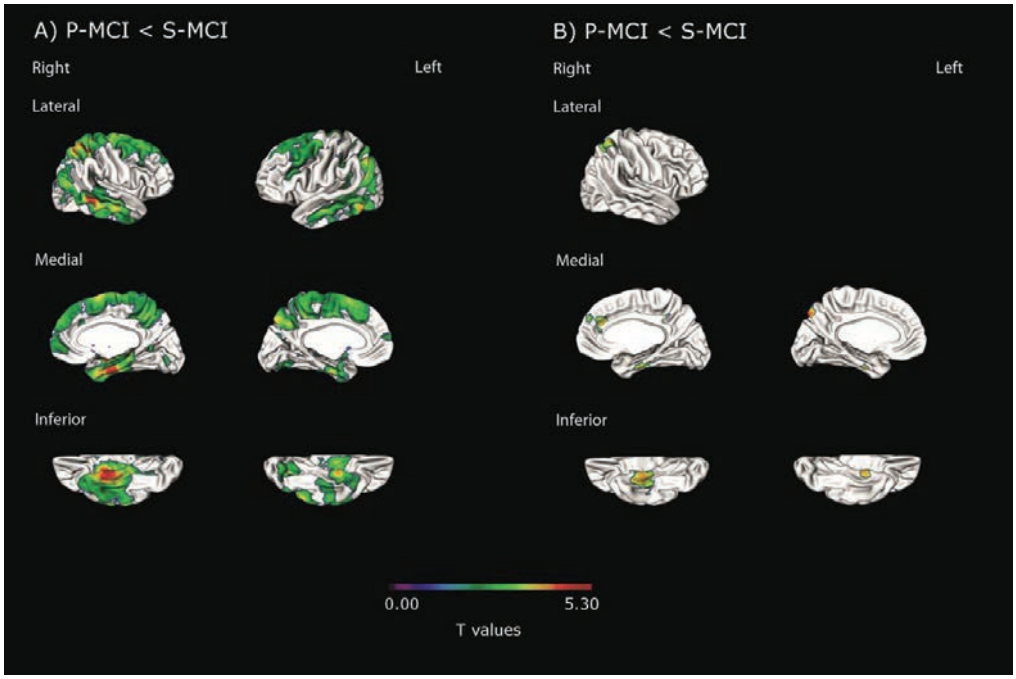

Figure 19 Differences ($p < 0.05$, FDR-corrected) in cortical thickness (CTH) in study II between subjects with A) stable mild cognitive impairment (S-MCI) and progressive MCI (P-MCI) (nuisances: age, gender, scanner, and voxel volume) and B) S-MCI and P-MCI groups with also follow-up time, education, number of apolipoprotein E (APOE) ε4 alleles, Mini-Mental State Examination (MMSE), and Clinical Dementia Rating Sum-of–Boxes score (CDR-SOB) added as nuisances.

The correlation found between CTH and CDR-SOB could not be repeated in study II with a different MCI population compared to study I. The only significant findings in study II were the associations between CTH and education as well as CTH and MMSE scores in the AD group. Longer education was associated with thinner cortex in the superior and middle frontal gyri as well as in the posterior, middle and superior temporal cortices,

occipital cortices and left precuneus (Figure 20A). Worse performance in MMSE was associated with thinner cortex in multiple frontal and parietal regions (Figure 20B).

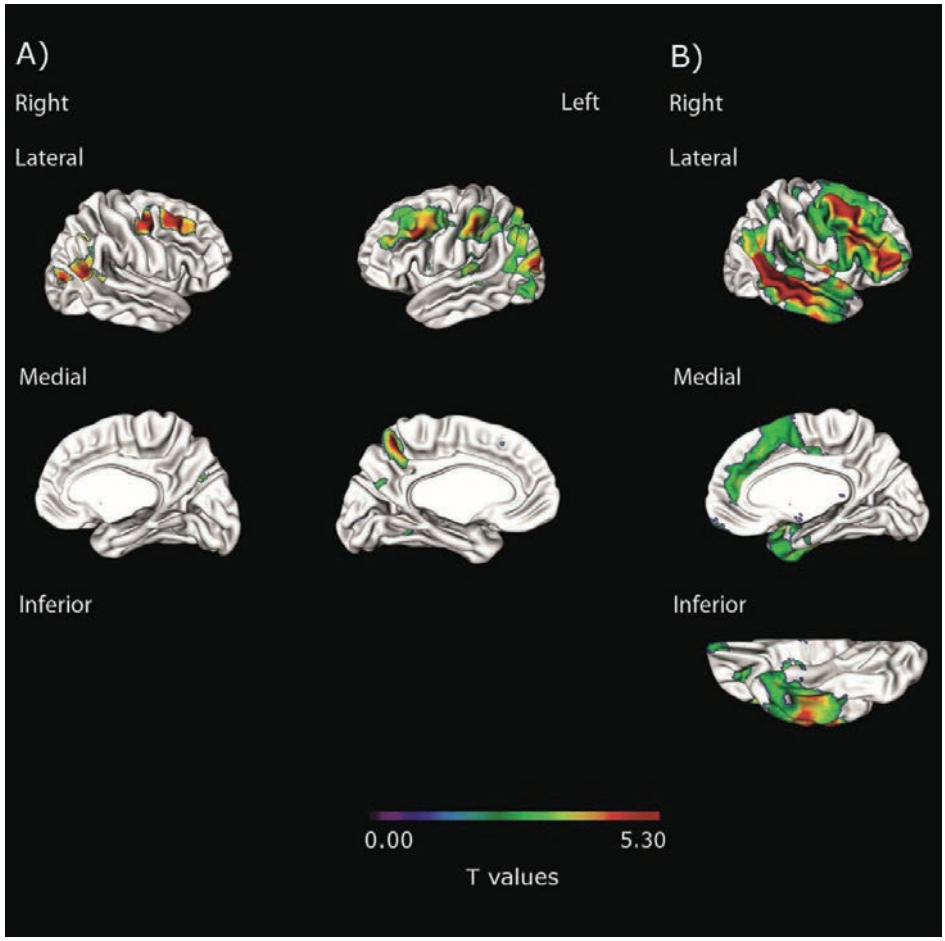

Figure 20 Areas where reduced cortical thickness (CTH) were associated to A) longer education, B) worse Mini-Mental State Examination (MMSE) scores in the Alzheimer's disease (AD) group in study II. Areas of significant correlation ($p < 0.05$, FDR-corrected) are illustrated.

5.3 Study III

Study III focused on elaborating on the connection between education and CTH. All the CTH analyses were done using the AddNeuroMed consortium data and the FreeSurfer analysis toolbox as described above.

The HC subjects in the higher education sub-group (mean 14 ± 3 years of schooling) demonstrated a significantly thicker cortex in the regions of temporal pole, transverse temporal gyrus, and isthmus of cingulate cortex as compared to the subjects in the lower education sub-group (education 6 ± 2 years) (Figure 21).

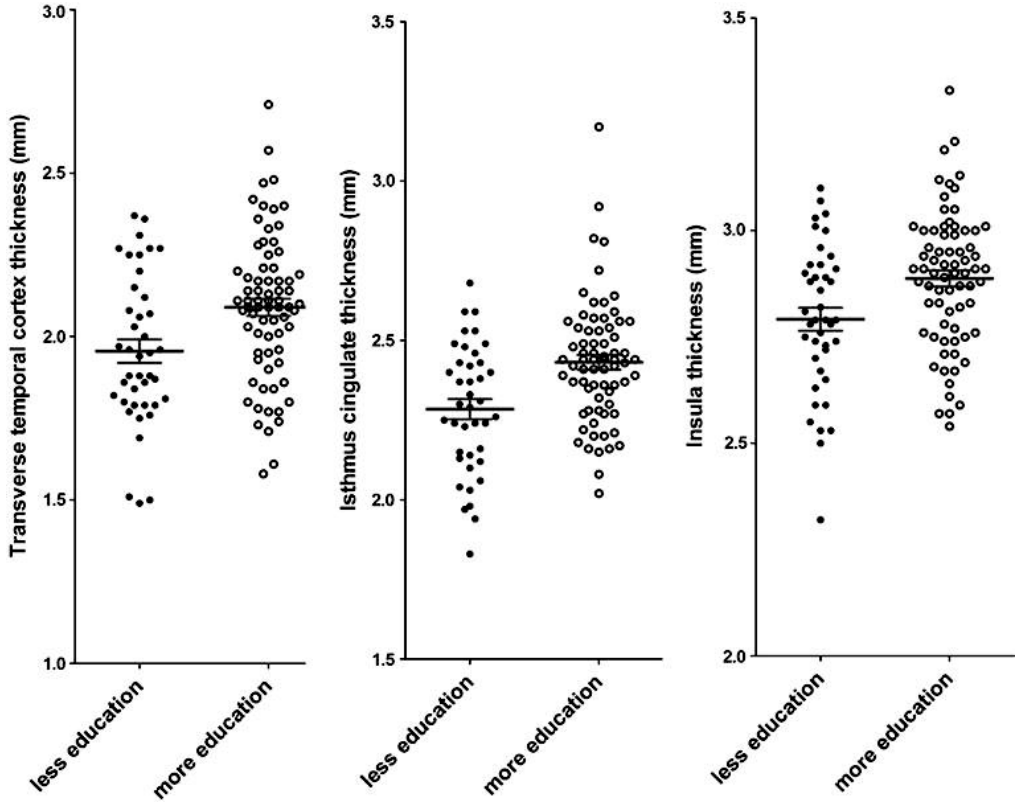

Figure 21 Boxplots comparing regional mean cortical thicknesses in the healthy controls with education over 9 years compared to those with less than 9 years of education in study III. The brain areas other than transverse temporal, isthmus of cingulated and insular cortex did not display statistically significant differences.

There were no statistically significant associations between education and brain morphology in the MCI group. In the AD group, longer education was correlated with thinner regional cortex in several areas of temporal, frontal parietal and occipital cortices (Figure 22).

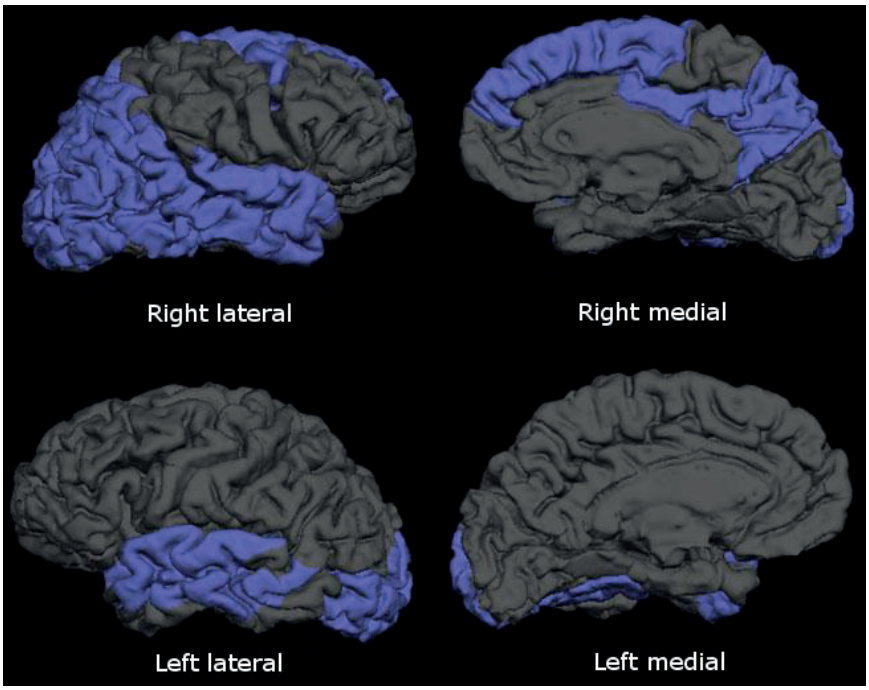

Figure 22 In the AD group of study III, the years of schooling were inversely correlated with regional cortical thicknesses of the bilateral lateral occipital cortex, middle and superior temporal gyri, left fusiform gyrus, and right caudal middle and superior frontal gyri, inferior and superior parietal gyri, inferior temporal gyrus, posterior cingulate cortex, and precuneus cortex.

Furthermore, the AD sub-group with higher education (12 ± 3 years) exhibited significantly thinner cortex in the areas of temporal, parietal and occipital cortices compared to the low education (5 ± 2 years) sub-group (appendix, study III, table 2).

5.4 Study IV

Study IV aimed to assess the value of CTH analysis at the single-subject level in the diagnostics of AD and compared this technique to other state-of-the-art methods of neuroimaging: automated hippocampal volumetry, manifold-based learning and tensor-based morphometry. The study was performed using all the 834 (231 HC, 238 S-MCI, 167 P-MCI, 198 AD) subjects of the large multicenter study ADNI. For a direct comparison with the work by Cuingnet and colleagues (2011) a subset of 509 baseline images were also analysed in a separate experiment.

The morphological brain changes between HC, MCI and AD groups were mapped and visualized with CTH and TBM methods. Figures 23 and 24 display the statistically significant differences between the study groups by using color-labeled t-value maps on brain models.

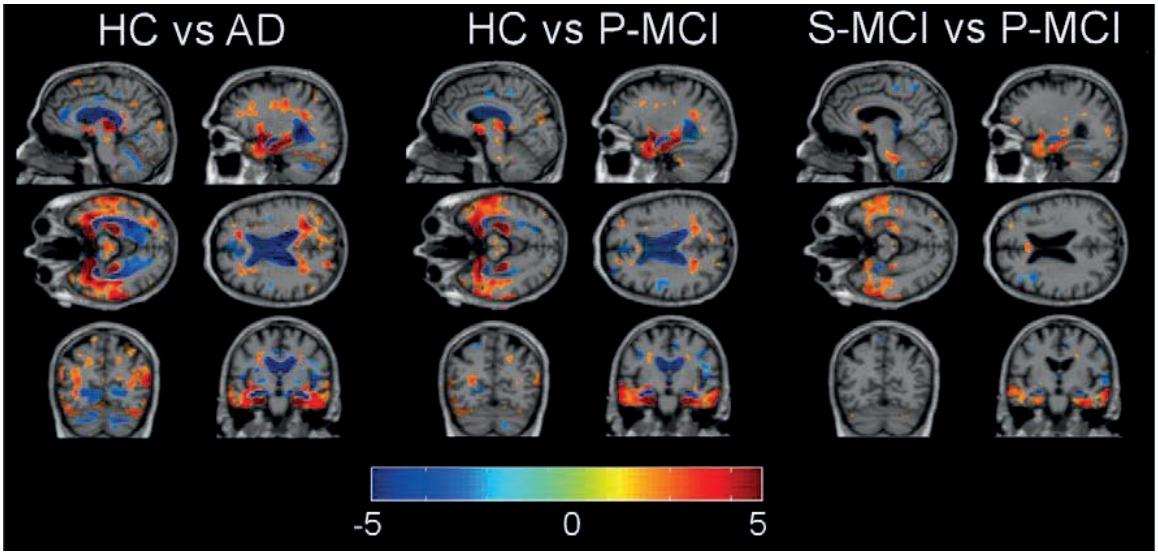

Figure 23 Volumetric differences in the tensor-based morphometry (TBM) analysis between healthy controls (HC), stable mild cognitive impairment (S-MCI), progressive MCI and AD in study IV. The t-value color scale illustrates larger volumes with blue and smaller with red hues. AD and P-MCI groups present atrophic changes in temporal, occipital and parietal regions as well as in the ventricles when compared to the HC group. The main differences between the two MCI groups are located in the temporal lobe.

The AD patients demonstrated widespread cortical atrophy compared to the controls sparing only the areas of sensorimotoric and visual cortices (Figure 24). Differences between the stable and progressive MCI groups in the ADNI cohort were located roughly in the same regions as seen in studies I and II, with the exception that there were visually no differences in the distribution of statistically significant areas between the hemispheres (Figure 24).

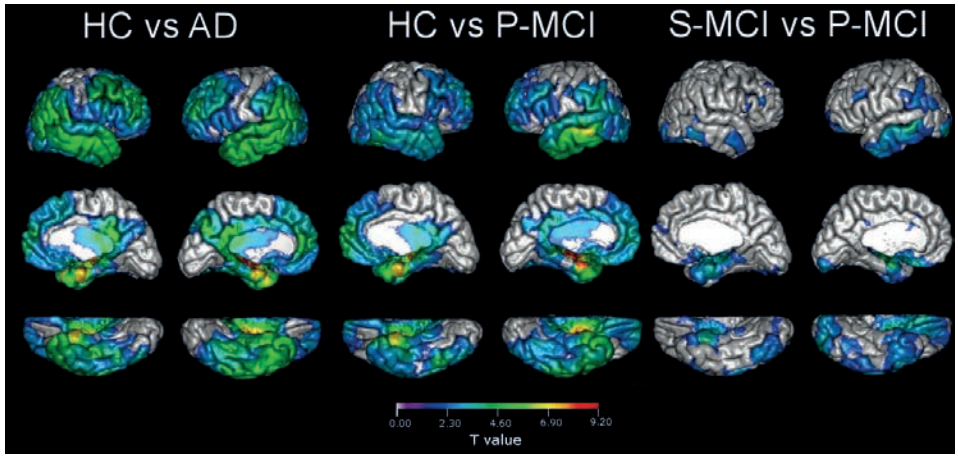

Figure 24 Results for t-tests for statistically significant group differences based on cortical thickness (CTH) measurements in study IV. CTH differences are presented as color-labeld t values. AD = Alzheimer's Disease, HC = healthy control, P-MCI = progressive mild cognitive impairment, S-MCI = stable MCI.

Results for the single-subject level classification experiments are summarized in Table 10. Classification of S-/P-MCI subjects was done with CCR values of 62-65% (HV, MBL, TBM, LDA classifier). CTH performed worse with CCR of 56%. Sensitivities and specificities were about 63-67%, except for CTH (specificity 45%). Combination of all methods improved CCR/sensitivity/specificity significantly to 68/67/69%. The SVM classifier acquired better sensitivity values at the cost of lower CCR and specificity (appendix, study IV, table 5).

Table 10 Results or the classification experiments in study IV. All the results were obtained with linear discriminant analysis (LDA) classifier and represent average % numbers of 300 cross-validation iterations. The classification rates obtained with the combination of all techniques was significantly better ($p < 0.05$) than any single technique used independently. The dataset consists of all the subjects in the ADNI database (231 HC, 238 S-MCI, 167 P-MCI, and 198 AD subjects).

Technique	HC vs AD			HC vs P-MCI			S-MCI vs P-MCI		
	CCR	SE	SP	CCR	SE	SP	CCR	SE	SP
MBL	85	87	83	78	81	75	65	64	66
HV	81	81	79	76	77	76	65	63	67
CTH	81	89	71	77	85	65	56	63	45
TBM	87	90	84	79	82	76	64	65	62
All	89	93	85	84	86	82	68	67	69

AD = Alzheimer's disease, CCR = correct classification rate, CTH = cortical thickness analysis, HC = healthy control, HV = hippocampal volumetry, MBL = manifold-based learning, P-MCI = progressive mild cognitive impairment, SE = sensitivity, S-MCI = stable MCI, SP = specificity, TBM = tensor-based morphometry

Detection of "prodromal AD" (i.e. the controls versus P-MCI classification) was done with CCRs of 76-79% with the different methods individually (LDA classifier). The combination of all methods improved the CCR significantly to 84%. SVM produced similar CCRs as LDA, but the sensitivity/specificity values were more unstable (appendix, study III, table 4).

As a reference, a HC versus AD classification was also performed. CCRs of 81-87% were obtained with single methods while combinations provided the most accurate results with CCR of 89% (LDA classifier) (Table 10).

In terms of CCR, TBM and MBL provided the best results in the HC versus AD classification, whereas there were no substantial differences in favor of any single method in the other classification experiments. The best results were achieved with the combination of all features in all the study experiments.

Dataset II displayed more variation in the results, but generally the sensitivities/specificities were in the range of 80-93/69-76% (HC versus AD), 75-90/59-92% (HC versus P-MCI) and 55-72/35-76% (S-MCI versus P-MCI) (appendix, study III, table 6).

6 DISCUSSION

6.1 Cortical thinning in the AD continuum (studies I, II and IV)

One of the primary objectives of this research was to assess the alterations in cortical morphology in the continuum from healthy aging to AD via the MCI phase. This was done by assessing CTH in the whole brain between each of these diagnostic groups in studies I, II and IV. Since MCI is recognised as being a heterogeneous state with only a part of the subjects having AD pathology underpinning the symptoms, the MCI populations in these studies were further divided into sub-groups of S- and P-MCI subjects according to their progression to AD during a follow-up of several years. The subjects in studies I and II were collected from population-based cohorts in Kuopio, while study IV was based on the widely used openly available cohort, the ADNI. CTH was measured using an automated pipelining method that provides submillimeter level information about the morphology of the cortical mantle in tens of thousands of nodes in an explorative fashion.

The analyses in study I indicated that there was significant thinning of the cortex at the baseline in frontal, temporal and parietal cortices in those MCI subjects who later progressed to AD during the follow-up. A similar finding was confirmed in study II with a larger population of MCI subjects. The main difference between these studies was that in study II there were statistically significant findings even after adjusting the analysis for numerous confounding variables (age, gender, MMSE, CDR-SOB, education, APOE genotype, follow-up time and imaging parameters). Additionally, the CTH analyses in study II were extended to encompass also HC and AD groups. These analyses revealed that the S-MCI subjects present a similar cortical profile with the HC group, whereas the P-MCI population seems to have developed an AD type pattern of cortical thinning even at the time of early memory problems. In studies I and II, the CTH differences were located mainly in the right hemisphere. The main limitations in these studies were the small number of subjects in the diagnostic groups. Especially in study I, the number of P-MCI subjects and in study II, the size of HC and AD groups is a limiting factor. Thus, the CTH difference analyses were further continued in the ADNI cohort with a total of 834 subjects. In the ADNI, it was found that the AD group presents widely spread cortical thinning in almost the whole brain compared to the healthy elderly subjects. The CTH profile of the P-MCI subjects was again strikingly similar to the AD group. In line with studies I and II, the baseline CTH differences of the "prodromal AD" subjects (i.e. subjects with P-MCI) versus the S-MCI were located in the regions of temporal, parietal and frontal lobes.

These findings are in line with the known pattern of the progression of NFT pathology and clinical symptoms in AD (Braak and Braak 1991, Braak and Braak 1997) as well as with the existing literature of MRI studies in MCI and AD. Singh et al. (2006) investigated CTH profiles across control, MCI and AD groups. They reported cortical thinning compared to controls mainly in the medial temporal lobe in MCI while in AD the lateral regions of temporal lobe and larger neocortical structures were alo affected. Studies conducted with other CTH analysis methods verify the same pattern of advancing atrophyas a characteristic of subjects with mild AD or individuals with the progressive form of MCI (Bakkour et al. 2009, Dickerson 2009).

Furthermore, by using the VBM-method, various studies have reported that those patients close to conversion from MCI to AD, who usually are categorized either as having P-MCI or multiple domain amnestic MCI, suffer atrophy not only in the structures of

medial temporal lobe but also in posterior and lateral temporal lobes, neocortical areas and the region of precuneus (Bell-McGinty et al. 2005, Chetelat et al. 2005, Hämäläinen et al. 2007b, Karas 2004, Whitwell et al. 2007a, Whitwell et al. 2007b). The results concerning the localization of atrophy between the hemispheres in the course of developing AD are somewhat conflicting. According to Singh et al. (2006) atrophy was more prominent in the left hemisphere in contrast to the findings of studies I and II, while Dickerson et al. (2009) reported distinct atrophy in the right precuneus while the same area in the left hemisphere remained intact. There were no substantial differences detected between the hemispheres in study IV.

Several factors such as the heterogeneity of the MCI subjects and the small group sizes could at least partly explain these differences. Also the definition of MCI varies across the cohorts. Furthermore, it is known that the prior probablility of having AD pathology as the reason for MCI is different in population-based studies and publications based on samples from memory-clinics. It is thus possible that the proportion of AD pathology versus other background pathologies behind the symptoms in MCI varies across studies, and this may account for some of the variablility in the results in publications with virtually similar study designs. In addition, some studies (Bell-McGinty et al. 2005, Chetelat et al. 2005, Hämäläinen et al. 2007b, Jack et al. 2004, Jack et al. 2008, Seo et al. 2007, Tapiola et al. 2008, Whitwell et al. 2007a) have dichotomized the MCI groups into sub-groups according to the profile of the cognitive deficts (single versus multiple domain MCI) or the outcome after a follow-up (P-MCI and S-MCI) whereas other groups have used only one general MCI definition (Apostolova et al. 2007, Davatzikos et al. 2008, Singh et al. 2006, Uotani et al. 2006, Whitwell et al. 2007b). In summary, the heterogeneity and lack of standardization in MCI definition and study designs leave room for speculation regarding the details concerning structural findings typical to MCI and developing AD. However, the most commonly reported pattern is that the cortical thinning in the AD continuum seems to start from the medial temporal lobe and it progresses gradually through the lateral temporal regions into larger neocortical areas as the disease develops.

The strengths of this research include the use of large number of MCI subjects collected both from a population-based database as well as the major multi-site cohort ADNI. Relatively long follow-up times, especially in the Kuopio cohort, combined with similar results across different MCI samples matching the existing literature and known pathological changes increase the reliability of the results.

The small P-MCI group in study I as well as the number of HC and AD subjects in study II is a limiting factor in these studies. The diagnosis of AD was not confirmed with autopsy in any of the studies I-IV meaning that some of the AD subjects might not actually be suffering from AD pathology, which could induce error into the results.

In addition, the Montreal CTH pipeline encountered problems in studies I and IV with MRI scans of low quality or where there was extensive atrophy. For these 116 subjects (9 in study I, 107 in study IV) there were no CTH measurements available. A more robust CTH pipeline would be desirable especially considering the use of this kind of technique in the clinical environment. For example, the FreeSurfer method seems to be less sensitive to image quality issues, although no true comparative studies concerning the reliability and robustness of different automatical MRI analysis methods exist at the moment. An optimal MRI analysis method should be easy to install and use, it should match the segmentation accuracy of an experienced radiologist and perform stellarly regardless of low image quality or heavy atrophy. The computational time should be also as short as possible to ensure smooth usability.

Another problem relating to the MR images in studies I-IV is the use of multiple scanners and imaging parameters. Naturally this same issue concerns the whole field of research, as there are no standardized imaging protocols that are universally accepted.

Variation in the image acquisition techniques is a confounding factor which could potentially lead to erroneous results in the automatic MRI analysis methods. In studies I and II, this problem was resolved by setting different scanners and imaging parameters as nuisance variables in the analyses. Utilization of this technique does not mean that the problem is completely compensated, but there are no guidelines or published methods on how to correct for the issue more reliably. This issue was examined in a recent VBM study which tested the effect of six different MRI scanners on the morphometric results in a sample of 62 AD patients and 74 HC subjects (Stonnington et al. 2008). The authors concluded that the scanner differences were substantially smaller than the group differences and only significant in the thalamus. There was no significant interaction of the scanner with disease group and the results were not confounded by scanner differences, which is encouraging considering the possibilities of pooling subjects scanned with varying imaging parameters in order to achieve larger group sizes. The MRI protocols in studies III (AddNeuroMed) and IV (ADNI) were planned in an identical way and much effort was expended to minimize the downsides of using multiple scanners in these cohorts (Jack et al. 2008, Simmons et al. 2009). Considering that the MRI protocols and methods in Stonnington et al. (2008) were not even designed to minimize the possible error caused by the use of different acquisition techniques and yet they did not have any significant effect on the results, it was decided not try to statistically adjust the experiments for the imaging parameters in studies III and IV.

6.2 Correlation of cortical thickness with clinical and demographical factors (studies I-III)

The severity of the symptoms in MCI as measured by the CDR-SOB scores was associated to thinner cortex in the same regions where disease-related cortical thinning was present in study I. In addition to CDR-SOB, several other scales (GDS and Alzheimer's Disease Assesment Scale-cognitive subscale) showed also significant differences between the MCI groups in study I. Thus, it seems that cortical thinning is associated with declines in multiple areas of cognition as well as with disease development to AD in MCI. Similar findings have been reported also by other groups (Bakkour et al. 2009, Dickerson et al. 2009, Vemuri et al. 2009a). Dickerson et al. (2009) reported that the higher CDR-SOB scores, indicative of more severe clinical symptoms, were associated with thinner cortex in multiple regions of the temporal, frontal and parietal lobes in very mild AD (Dickerson et al. 2009). Using a stepwise multiple linear combination analysis, they showed that a linear combination of medial temporal, inferior temporal, and inferior frontal regions was the best predictor of CDR-SOB scores (Dickerson et al. 2009). Bakkour et al. (2009) demonstrated in a sample of 29 S-MCI and 20 P-MCI subjects that the CRD-SOB scores were associated with thinner cortex in medial and inferior temporal as well as superior parietal cortex, and the mean thickness of the whole cortex. Lower MMSE total scores were associated with thinner cortex widths in frontal and parietal areas of the MCI subjects. Volumetric measures were correlated with CDR-SOB and MMSE only in the entorhinal region (Bakkour et al. 2009). Vemuri et al. (2009a) found that Structural Abnormality Index (STAND) scores, which reflect the degree of AD-like anatomic features on MRI, were correlated with both CDR-SOB and MMSE scores in amnestic MCI (n = 192) and AD (n = 98) .

Interestingly, the CSF biomarkers in that study were not associated with either of these cognitive measures. This finding regarding the lack of correlation between CSF biomarkers and cognitive decline has been reported also in AD in a cross-sectional study (Stefani et al. 2006). A longitudinal study found that whole-brain atrophy rates are linked to changes in MMSE scores, but this is not the case with the CSF biomarkers (Sluimer et al. 2010). A

conflicting result was found in study II, where the cognitive/clinical measures exhibited no correlation to CTH in the MCI group while worse performance measured by MMSE scores was associated with thinner cortex only in the AD group. One reason for this discrepancy could be that the MCI population in study II was slightly different from that examined in study I. It is possible that the scales measuring cognitive capabilities (MMSE and CDR-SOB) are not very sensitive in early stages of the disease, and thus even a small change in the study groups could obliterate the association between the MRI findings and these scores.

With the exception of some discrepancy in these results, the relationship between atrophic changes in MRI, cognitive decline in MCI and AD as well as progression to AD in MCI seems quite strong. Furthermore, atrophy in MRI has been reported to correlate with Braak NFT stage and NFT load (Gosche et al. 2002, Jack et al. 2002) linking these findings directly to the pathological changes of AD. It is somewhat surprising that the indication of neuronal injury in CSF (that is elevated levels of tau) is not as strongly related to the cognitive and clinical changes. On the other hand, it has been shown that the elevation in the CSF tau value precedes the structural damage observed as atrophy in MRI (Price et al. 2001). Thus, the CSF tau level might rise abnormally before there is any detectable structural damage and clinically observable symptoms leading to non-significant findings in the correlation analyses. This means that MRI might be a more stable measure of disease development since it starts to detect the pathological changes only after there is neuronal loss involved.

In studies II and III, longer education was associated with a thinner cortex in several brain regions in AD. However, in study III, the HC subjects with higher education displayed thicker regional cortices compared to the "low education" HC group. The phenomenon of finding lower CTH in AD patients with higher education could be explained with the theory of cognitive and/or brain reserve and similar results have been reported also by other groups (Querbes et al. 2009, Seo et al. 2011). Cognitive reserve refers to the ability of the brain to combat damage by using pre-existing cognitive processing approaches or recruiting compensatory approaches (Stern 2002). Brain reserve means that those individuals with larger brain volumes can sustain more brain damage before reaching a threshold when they will suffer clinical symptoms (Satz 1993). In study III, the CTH analysis between high and low education groups was adjusted for MMSE which should decrease the possibility that the significant finding would be explained by more severe cognitive impairment in the group with less education. The finding strongly supports the concept of education being a protective factor against cognitive decline and it fits well also to the reports stating that there is more rapid disease progress in those highly educated AD subjects (Stern et al. 1995). Once the damage is sufficiently severe the compensatory capabilities fail leading to rapid progression of the disease. At the time AD is diagnosed, there is already a substantial volumetric loss of cortical structures thus accounting for the correlation results found in studies II and III.

The finding regarding thicker cortex values in the high education sub-group of HC subjects is more controversial. No such correlation could be detected in study II, and also opposite results have been reported where non-demented people with more education or better socioeconomic status present increased atrophy (Coffey et al. 1999, Fotenos et al. 2008). On the other hand, in one study, individuals with a high early life intelligence quotient had larger brains than people with a low intelligence quotient in a group of healthy elderly subjects (Sole-Padulles et al. 2009). However, it should be noted that the significant finding in study III was present only in the groupwise CTH difference analysis. This, and the non-significant finding in study II, could mean that the connection between education and brain morphology is non-linear and thus not easily detectable by regular correlation analysis. There are also numerous other confounding factors such as differences in the schooling systems, heterogeneity in the study samples across studies and varying

MRI analysis methods that complicate any comparison of results from different studies. The number of years of schooling may not be the best way to evaluate the level at which an individual has "exercised" his/her brains cognitively during their life and developed cognitive/brain reserve. Information on later occupational life could clearly be helpful when dividing subjects into low- or high education groups. The finding is important though, as it means that the MRI based AD biomarkers could be especially useful among the highly educated subjects where AD-like morphology at the time of earliest memory problems could be interpreted as evidence of a higher risk for developing AD.

The APOE ε4 allele is one of the best established risk genes for AD. In study II, the AD subjects with an APOE ε4 allele had thinner cortex values in the right temporal gyrus compared to the non-carriers, while no other significant results relating to APOE status and CTH were found in the other groups. In some studies, the possession of an ε4 allele has been related to a more vulnerable cortex in the temporal lobe (Filippini et al. 2009, Pievani et al. 2009) as well as increased atrophy in various other brain structures (Liu et al. 2010a), while others have failed to find any connection between the presence of the risk gene and brain morphology (Drzezga et al. 2009). A recent study in the AddNeuroMed cohort showed that the possession of the ε4 allelle relates to smaller structures in several brain regions in HC, MCI and AD subjects, but the effects vary according to gender and the number of ε4 alleles (Liu et al. 2010a). The structural differences between the ε4 carriers vs. non-carriers were significant in all diagnostic groups (HC / MCI / AD) in females, while in men there were significant findings only in the MCI group. It should be noted though that some brain structures were generally smaller in male subjects compared to females, which makes it more difficult to detect the damage, leading to fewer findings in men groups. Thus, Liu and colleagues concluded that the effects of APOE genotype on brain morphology might be concealed in studies using unmatched APOE ε4 allele frequencies and gender between case and control groups (Liu et al. 2010a). Other confounding factors including the differences in the imaging analysis methods complicate even further the comparison of the results. Taken together, although the literature concerning APOE genotype and brain morphology is somewhat conflicting, it is probable that the possession of the APOE ε4 allele alters the brain in such a way that it is susceptible to more severe atrophy in AD.

6.3 Classification of the study subjects and prediction of AD in MCI (study IV)

Brain atrophy detected on MRI has been included in the new diagnostic criteria of prodromal AD for research purposes (Dubois et al. 2007), in the new "AD lexicon" (Dubois et al. 2010) and is a core biomarker in the new US guidelines (McKhann et al. 2011). Amyloid-based measures like the CSF-peptide Aβ and the uptake of the PiB tracer on PET may show the earliest AD-type changes (Hampel et al. 2008, Hampel et al. 2010, Jack et al. 2010). However, there is evidence that the amyloid biomarkers reach their saturation levels already by the time that patients have clinically apparent symptoms of cognitive impairment (Gomez-Isla et al. 1997, Hyman et al. 1993), whereas atrophy, neuronal loss, synaptic loss, and the number of tangles continue to increase with the severity of the disease (Bakkour et al. 2009, Dickerson et al. 2009, Ingelsson et al. 2004, Jack et al. 2005, Jack et al. 2010, Vemuri et al. 2009a). This is an important feature for a biomarker since the findings in MRI might thus have more predictive value especially in the group of subjects with mild cognitive problems.

The predictive capabilities of different MRI features in MCI have been assessed in various studies and cohorts (see Table 7, section 2.6.2). However, the variation in the study

samples complicates the comparison of results across studies. Little is known if the combination of several structural measures could improve the predictive power. In study IV, a multi-method approach including four state-of-the-art MRI analysis methods was used to automatically classify HC, S-MCI, P-MCI and AD subjects into the diagnostic groups according to baseline data. The best CCRs achieved with individual features in study IV were 87% (HC/AD classification), 65% (S-MCI/P-MCI) and 79% (HC/P-MCI). The combination of all MRI features improved the results to 89%, 68% and 84% in HC/AD, S-/P-MCI and HC/P-MCI classifications, respectively. These results reveal how a combination of different MRI-based features can improve results based on only one measurement, achieving a more powerful and stable classifier. Although TBM seemed to be the best single method in some experiments, the results strongly support the approach of applying several MRI analysis methods simultaneously. The only downside of such an approach is the increasing computational time. However, running all the MRI analysis methods used in study IV on a single MR image still takes only a few hours on a modern computer, which is hardly a restriction considering the clinical environment.

Recent publications have demonstrated that biomarkers derived from MR imaging are able to distinguish healthy controls from AD with an accuracy ranging from 76% to 94% (Chupin et al. 2009, Gerardin et al. 2009, Liu et al. 2011, McEvoy et al. 2009, Querbes et al. 2009, Wolz et al. 2010c). The present results are at the same level with the most accurate results reported earlier. However, the goal of improving *early* diagnostics is to achieve distinguishing between those MCI subjects who will convert to AD (P-MCI) in the near future from those who will remain stable or even revert back to normal cognition (S-MCI). Several studies have tried to resolve this challenge using the ADNI cohort (Chupin et al. 2009, Cuingnet et al. 2011, Lötjönen et al. 2011, Querbes et al. 2009, Wolz et al. 2010c), independent cohorts (Bakkour et al. 2009, Devanand et al. 2007, Fleisher et al. 2008, Korf et al. 2004) as well as other multi-center cohorts similar to the ADNI, such as the AddNeuroMed (Liu et al. 2010b). The CCRs seem to vary considerably between 54-82% most being in the range of 60-70% (see Table 7). The highest accuracies are usually obtained with a very low number of subjects (Duchesne et al. 2010, Ferrarini et al. 2009, Teipel et al. 2007) or without using any validation in the classification process (Bakkour et al. 2009, Liu et al. 2010b, Teipel et al. 2007). The present results are in line with these findings. In order to obtain a more direct comparison of different methods the analyses were also conducted in the same subset from ADNI that was used by Cuingnet et al. (2011). Our results using the combined feature set outperform the majority of the ten methods tested by Cuingnet et al. (2011). This direct comparison shows that the present results compare favorably to other established methods currently availabe in neuroimaging.

It has also been postulated that biomarkers measuring different aspects of AD pathology (CSF, MRI, PET) might improve the classification accuracy over a single biomarker. The combination of MRI and PET has been found to slightly improve the HC/AD classification over either method alone (Hinrichs et al. 2009). Adding CSF measures to MRI was reported to provide better accuracy in the separation of P-MCI subjects from a combined HC/S-MCI group (Eckerström et al. 2010). In the study of Eckerström et al. (2010), no validation in the classification calculations was used, and the study sample was quite small (n = 68) leading to questionable generalizability of the results. In addition, purely negative findings regarding the benefits of using multiple biomarkers or the combination of MRI to neuropsychological tests have been published (Fleisher et al. 2008, Kohannim et al. 2010, Schmand et al. 2012). Considering solely the classification accuracies of the present study and those reported in the literature, a combination of different features extracted from a single MRI seems to provide results that are comparable to those obtained with other or multiple biomarkers. The finding is interesting as it suggests that a single MRI scan provides not only help in the differential diagnostics of cognitive impairment, but it also

reliably describes an individual's position on the HC/AD continuum. MRI is also widely available, non-invasive and often useful in the differential diagnostics of memory problems thus making it a compelling option as the first biomarker that would be examined in a patient with mild memory problems. The comprehensive differential diagnostics between AD and non-AD cognitive impairments will still require the assessment of various different biomarkers.

6.4 Future studies

The findings regarding the morphological changes in the cortex during the AD continuum at the group level seem quite uniform across the research field despite some minor inconsistensies. Similarly, the correlation between structural changes in MRI and the clinical decline has strong support based on the studies in this thesis and the existing literature.

The effects of education on the brain/cognitive reserve require further validation in larger study samples, preferably with more multifaceted data on how an individual has exercised his/her brains cognitively later in the life.

Several important issues remain to be resolved considering the use of MRI based biomarkers in the clinical environment. Although single-subject level information about the predictive power of different MRI features now exists, most of the classification techniques are "black boxes" that provide only the classification results without any clinically usable cut-off values or decision rules. Such cut-off values would be useful when comparing the results from different studies as well as when creating standardization protocols for different MRI equipment and imaging parameters. One of the key questions in the future will be how well the imaging markers developed in the large research cohorts, such as the ADNI and the AddNeuroMed, will perform when tested especially in population-based cohorts with even more heterogeneous MCI samples with various background pathologies which were excluded in these multi-site databases. It is also questionable if the prediction accuracy of MCI to AD conversion (about 65-70 % in the current thesis and recent literature) is high enough to be usable in the clinical decision making, especially as it is possible that the accuracy might decrease when applying these imaging markers outside the strictly defined research cohorts.

7 CONCLUSIONS

This thesis focused on structural brain imaging and the use of automated MRI analysis methods in the early diagnostics of AD. Based on the results, the following conclusions are made:

1. CTH is decreased in almost all brain areas excluding the sensomotoric and visual cortices in AD as compared to healthy aging

2. Those individuals who later progress to AD demonstrate cortical thinning in temporal, parietal and frontal cortices already at the time of mild cognitive impairment, several years before the AD diagnosis. The profile of this thinning resembles closely the pattern characteristic of the changes seen in AD

3. The cognitive decline and the progression of the clinical symptoms in MCI and AD are associated with cortical thinning in the brain regions typically altered in AD

4. Education may act as a protective factor against AD by providing both structural reserve as well as compensatory mechanisms which help the individual to remain cognitively intact even though there has been brain damage inflicted by the disease

5. Structural MRI analysis with automated methods can be used at the individual level to separate the healthy elderly from AD patients with an accuracy of about 89%. The future progression from MCI to AD during follow-up can be predicted with an accuracy of about 68%

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Valtteri Julkunen

Cortical Thickness Analysis in Early Diagnostics of Alzheimer's Disease

The diagnostic criteria of Alzheimer's disease (AD) are under revision. The proposed new guidelines aim at earlier detection of the disease, which could allow more efficient interventions. This study assessed the relationship between disease state and cortical morphology measured using MRI, and evaluated the power of automated image analysis methods in the early diagnostics of AD. The results revealed that cortical thinning characteristic of AD can be observed even years before the appearance of severe symptoms. In addition, education seems to provide both a structural and a compensatory reserve against the damage inflicted by the disease.

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