Alzheimer's disease through the MR-eye; novel diagnostic markers and the road to clinical implementation

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Alzheimer's disease through the MR-eye

Novel diagnostic markers and
the road to clinical implementation

Lies Clerx

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PROEFSCHRIFT

Ter verkrijging van de graad van doctor aan de Universiteit Maastricht,
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voor Marie-Lou en Maurice,
From here to the moon and back,
Who else in this world will love you like that?
Forever and always, I'll be where you're at
From here to the moon and back,
From here to the moon and back,

CONTENTS

Chapter 1	General introduction	11
 Part I: Grey matter atrophy in (early) AD		
Chapter 2	The added value of MRI-biomarkers to neuropsychological test performance for the prediction of AD in subjects with MCI <i>Submitted</i>	27
Chapter 3	Measurements of medial temporal lobe atrophy for prediction of AD in subjects with MCI <i>Neurobiol Aging. 2013 Aug;34(8):2003-13</i>	49
Chapter 4	Sensitivity of different MRI-techniques to assess grey matter atrophy patterns in Alzheimer's disease is region-specific <i>Curr Alzheimer Res. 2013 Nov;10(9):940-51</i>	89
 Part II: Structural connectivity in (prodromal) AD		
Chapter 5	New MRI markers for Alzheimer's disease: a meta-analysis of diffusion tensor imaging and a comparison with medial temporal lobe measurements <i>J Alzheimers Dis. 2012;29(2):405-29</i>	127
Chapter 6	Reduced callosal white matter integrity surpasses cerebrospinal fluid and atrophy markers as predictor of cognitive decline in subjects with MCI: a combined volumetry and DTI study <i>Submitted</i>	189

Part III: Robustness of structural and functional abnormalities in AD

Chapter 7	Consistency of FreeSurfer regional atrophy measurements compared to manual measurements in Alzheimer's disease <i>Submitted</i>	219
Chapter 8	The stability of resting state networks over time in healthy old and young subjects and its relevance for early memory dysfunction <i>Submitted</i>	249
Chapter 9	General discussion Future directions	275 284
Appendix	Summary Nederlandse samenvatting Curriculum vitae List of publications Dankwoord	295 301 307 309 313

Chapter 1

General Introduction

Purpose and motivation

Due to the aging of the population, Alzheimer's disease (AD) is a growing socio-economic problem. The underlying changes associated with this neurodegenerative disease start years before clinical manifestation [1]. On a pathological level, AD-patients show an accumulation of amyloid plaques and neurofibrillary tangles which are associated with loss of neurons [2]. Potential disease modifying therapies should be given in the earliest stage of the disease, to prevent further neuronal loss. To date, no single diagnostic marker is available with sufficiently high predictive accuracy rates to provide an accurate diagnosis in a single patient. Consequently, there is a need for novel markers to detect the disease at a preclinical stage. Magnetic Resonance Imaging (MRI) provides a framework to visualize structural and functional changes associated with neurodegenerative diseases *in vivo*. The use of neuroimaging in clinical practice has shifted from exclusion of other diseases that may cause the cognitive impairment (e.g. tumor, hemorrhage) to early detection (e.g. AD versus healthy aging) and differential diagnosis (e.g. AD versus frontotemporal dementia).

The current thesis aims to expand insight into the use of both conventional (e.g. hippocampal volumetry) and novel (e.g. diffusion tensor imaging) MR imaging markers for early detection, diagnosis and prognosis of AD. Associations with clinical and cerebrospinal fluid (CSF) biomarkers are explored to further disentangle the underlying nature and course of the disease.

GENERAL INTRODUCTION

Alzheimer's disease and MCI

Alzheimer's disease (AD) is a progressive neurodegenerative disorder affecting approximately 250.000 people in the Netherlands, and 35 million individuals worldwide [3]. Despite numerous research, AD is currently incurable and the number of patients is expected to exceed 115 million by 2050. The term mild cognitive impairment (MCI) has been introduced to describe patients who do not fulfill clinical criteria for dementia, but who do have objective evidence of memory deficits without notable interference with daily life activities [4]. MCI patients carry an increased risk of developing AD, with about 10-15% progressing to AD per year in academic

clinical settings. In these subjects, MCI is considered to be a transitional phase. However, not all MCI-patients progress to AD: some develop another type of dementia, while others improve or remain stable [5]. The course of AD is also variable: not all patients progress at the same rate and the factors influencing or predicting progression are not well known [6]. Better modeling of disease progression in individual subjects will allow us to predict when cognitive abilities may break down and when social and behavioral disturbances may arise. Staging models based on dynamic biomarkers (Figure 1)[7] will furthermore allow us to evaluate treatment effects of novel drugs in clinical trials based on the effects of a specific drug in slowing down or stopping transition from one stage to the next.

Figure 1. Hypothetical model of imaging biomarkers in Alzheimer's disease [7].

Diagnosing AD

Pathophysiology

The neuropathological hallmarks of AD are extracellular senile plaques consisting of amyloid-beta ($A\beta$) and intracellular neurofibrillary tangles consisting of hyperphosphorylated tau, both associated with neuronal loss. $A\beta$ is a peptide produced by cleavage of the $A\beta$ precursor protein (APP) [8] and deposition of amyloid plaques is widely distributed throughout the cortex, starting in the posterior association cortices, and showing a relative sparing of the medial temporal lobe (MTL) [2]. The 'amyloid cascade hypothesis'

was recently postulated in which A β starts to accumulate approximately 15 years before the onset of clinical symptoms [7, 9]. Formation of neurofibrillary tangles on the other hand, is postulated to start in the MTL and spreads into adjacent lateral temporal and association parietal cortices, followed by prefrontal cortices and other neocortical areas (e.g. motor and sensory areas) in later stages of the disease [10]. Plaques and tangles are associated with regional neuronal loss, cortical atrophy and cognitive decline [2, 10]. Both hallmarks of AD can most accurately be quantified post-mortem, or *in vivo* by means of CSF analysis or beta-amyloid Positron Emission Tomography (PET) imaging [11].

Clinical assessment

A clinical diagnosis of MCI or dementia involves assessing cognitive functions as well as the degree of interference in daily life activities, psychological well-being and burden of care. These elements can be evaluated by means of an interview with both patient and caregiver(s), and neuropsychological assessment. The latter one plays a major role in determining the nature and extent of the cognitive dysfunction. Additional investigations are needed to clarify the underlying cause of the cognitive impairment. Some causes can be identified by means of a blood test or neurological examination, but for most neurodegenerative disorders imaging or CSF assessment are needed.

Macrostructural neuroimaging

MRI or computed tomography (CT) are routinely used to exclude other diseases that can cause the cognitive impairment. They can also provide positive evidence for AD. Such studies mainly focussed on the MTL, which is affected early in the disease process. However, atrophy of the MTL is non-specific for AD [12] and tissue loss is not limited to this region [13]. To evaluate the degree of atrophy in the MTL, clinicians prefer the use of visual rating scales because these instruments are readily available, quick and easy to perform [14]. However, visual rating scales show lower predictive accuracy rates and higher inter and intraindividual variability effects [15] compared to volumetric (hippocampal) measurements. Manual volumetry is still considered gold standard in research practice [16-18], but is time consuming which thereby limits routine clinical or large-scale research use. Automated hippocampal measurement needs much less rater time and shows no interrater variability effects, but might be susceptible to scanner and scan protocol variability.

Novel imaging markers

Novel imaging markers have now been developed to evaluate changes in gray (GM) and white matter (WM), both on a structural (e.g. diffusion tensor imaging (DTI)) and functional level (e.g. resting state functional MRI). These markers can be used to test structural and functional connectivity between brain regions. Structural markers are able to visualize physical connectivity between brain regions, while functional connectivity markers display functional connections between brain regions that are not necessarily directly physically connected. In early AD, both structural and functional connectivity are altered [19-24] and there is some evidence that these measures become abnormal before macroscopic atrophy is evident. Thus, by means of these techniques, structural disconnection may be detected at a stage where macrostructural changes such as hippocampal atrophy may not have become visible yet, and are linked with neuropsychological performance [25, 26].

Structural connectivity markers

DTI measures the random motion of water molecules within tissue, reflecting structural organization. Diffusion anisotropy (FA) is often regarded as an index reflecting microscopic tissue integrity and correlates with the integrity of the axonal membrane [27]. Reduction of FA values suggests a reduction in axon number, an impairment of axonal flow, or both. An index of the magnitude of diffusion independent of the direction is mean diffusivity (MD). An increase in MD is thought to reflect enlargement in the extracellular space due to altered cytoarchitecture (i.e. loss of neurons, axons, and dendrites), suggesting immaturity or degeneration of the tissue leading to elevated water diffusivity within these regions [28, 29]. Alterations in FA or MD could also be due to diffusion changes either parallel or perpendicular to the principal direction of the tensor. These measurements are referred to as axial (AXD), and radial diffusivity (RAD), respectively. An increase in RAD is thought to signify increased space between fibers (suggesting demyelination or dysmyelination) [30] whereas increases in AXD suggest axonal damage [31].

Functional connectivity markers

Functional connectivity can be tested using the BOLD signal in task-related functional or resting-state MRI paradigms [32-34]. Functional connections can be derived from their temporal pattern in neurophysiologic change or activation during task-related

or task-free research environments [35]. Task-related decreases are found in certain regions which remain active in an organized fashion during the resting state of the brain [36, 37]. One of these ‘resting state networks’ is the default mode network which is particularly relevant for aging and dementia since its vulnerability to atrophy, deposition of the amyloid protein, and reduced glucose metabolism [38].

Biomarkers in cerebrospinal fluid (CSF)

CSF biomarkers are increasingly used to detect brain changes associated with AD *in vivo*. In CSF, decreases in levels of beta-amyloid 1-42 ($A\beta$ 1-42) are found, together with increases in total (t-tau) and phosphorylated tau (p-tau). As the presence of these protein levels reflects AD pathology, these CSF biomarkers have been shown to differentiate patients with AD from control subjects [39] and to predict conversion rate in MCI-patients [40]. Both MRI and CSF are valuable markers of disease [41], however, the relation between these markers has been less extensively studied.

To cure or not to cure?

Despite tremendous research, AD is currently incurable. Cholinesterase inhibitors are most widely used to reduce the behavioural disorders associated with the disease [42]. Non-pharmalogical interventions are often additionally implemented to further reduce behavioural disturbances, and to reduce caregiver burden [43, 44].

Aims and outline

MR imaging provides more information than macrostructural atrophy and vascular abnormalities. However, the added value and exact position of novel imaging techniques in the diagnostic field of AD remains unclear. We will investigate whether MRI investigation is necessary in the diagnostic process of AD and which MRI measurements can incrementally contribute to the early and specific diagnosis of Alzheimer disease. The overall aim of this thesis is to evaluate the clinical value of novel imaging markers for AD in relation to current (gold standard) methods (i.e. hippocampal volumetry, visual rating scale). The first part of the thesis evaluates well-known imaging techniques which assess gray matter atrophy in the brain. In the second part we will examine the discriminative and predictive value of a novel imaging marker for AD: Diffusion Tensor Imaging (DTI). In the third part, we will investigate the robustness of structural and functional abnormalities in AD.

1. Macrostructural imaging

The current approach in most of Dutch memory clinics consists of cognitive examination of patients by means of a neuropsychological test battery, together with a neurological investigation, (hetero)anamnesis, a blood test and an MRI scan. This clinical protocol forms the starting point of the thesis:

1. ‘What is the added value of volumetric MRI to neuropsychological test performance?’

In *Chapter 2*, we investigate if MR imaging markers increase predictive accuracy for AD relative to a model that only includes demographical and neuropsychological information. Furthermore, the importance of MTL atrophy in AD allows us to formulate a second question:

2. ‘Which measurement of MTL atrophy is the most sensitive to predict AD-conversion in subjects with MCI?’

Chapter 3 investigates the predictive value of four different MTL atrophy measurements for conversion to AD in subjects with MCI. Two questions result from recent evidence stating that AD pathology is not restricted to the MTL:

3. ‘Which regions in the brain can discriminate between controls, MCI and AD?’

&

4. ‘Are different techniques assessing gray matter atrophy in the brain leading to the same pattern of abnormalities throughout the same dataset?’

Chapter 4 describes the discriminative value of several techniques measuring GM atrophy in the brain, both in and outside medial temporal lobe regions.

2. Connectivity markers

The second part of the thesis examines the discriminative and predictive value of a novel imaging marker for AD: DTI. The main questions are:

5. 'What is the added value of DTI in discriminating healthy controls, MCI and AD?'

&

6. 'Is DTI superior to MTL measurements?'

Chapter 5 will assess the value of DTI as imaging marker for AD in relation to MTL measurements by means of a meta-analysis. The same question will be addressed in chapter 6 by means of a case-control study. Differences in both gray matter atrophy and WM integrity are evaluated in a multi-center cohort of healthy old subjects, MCI and AD-patients. The predictive value of both volumetry and DTI for cognitive decline at 2 years follow-up is furthermore investigated.

3. Methodological issues

The third and last part of the thesis investigates the robustness of structural and functional abnormalities in AD. The main question is:

7. 'Are structural and functional imaging techniques influenced by internal (e.g. patients age) and external (e.g. software, hardware) factors?'

Chapter 7 examines the consistency of FreeSurfer, an automated segmentation tool, to detect structural abnormalities under varying processing conditions, and in relation to manual volumetry.

Chapter 8 investigates the validity of resting state fMRI as a marker for disease, by evaluating the stability or test-retest reproducibility of resting state networks over time in a healthy population.

Chapter 9 presents our overall conclusions and puts the main findings presented in this thesis in the context of our current knowledge about AD.

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Part I

Grey matter atrophy in (early) AD

Chapter 2

The added value of MRI-biomarkers to neuropsychological test performance for the prediction of AD in subjects with MCI

Lies Clerx, Eva Dierckx, Laura van de Pol, Ineke van Rossum, Frans Verhey, Pauline Aalten, Frederik Barkhof, Robin Wolz, Daniel Rueckert, Giovanni B. Frisoni, Magdalini Tsolaki, Flavio Nobili, Yvonne Freund-Levi, Asa Wallin, Lutz Frölich, Harald Hampel, Hilka Soininen, Philip Scheltens and Pieter Jelle Visser

ABSTRACT

Background. Neuropsychological tests and magnetic resonance imaging (MRI) measures can identify Alzheimers's disease (AD) in the stage of mild cognitive impairment (MCI). We aimed to investigate whether measurements of medial temporal lobe (MTL) atrophy could increase the predictive accuracy of neuropsychological tests for AD-type-dementia.

Methods. We selected 184 MCI-subjects from DESCRIPA and from the Alzheimer Dementia cohort of the VU University Medical center in Amsterdam. Neuropsychological tests for attention, memory, fluency, executive functioning and visuoconstruction were included. MTL atrophy measurements consisted of a visual rating scale and an automated hippocampal measurement. Outcome measure was the odds ratio for progression to AD-type dementia in 2 years.

Results. Among neuropsychological tests the odds ratio was highest for the wordlist delayed recall (3.59). The odds ratio was 4.14 for the MTA-score and 7.78 for hippocampal volume. Hippocampal volume but not MTA-score increased the predictive accuracy of delayed recall score for AD-type dementia after 2 years (AUC = 0.81; $p < .001$). Predictive accuracy was increased both in subjects with normal and abnormal delayed recall scores at baseline. In the subgroup of subjects with an MMSE score >27 , both delayed recall and MRI at baseline were predictors of conversion. In the group of subjects with a normal MTA-score (<3), only volumetric measurements predicted AD.

Conclusion. These results provide further support for the clinical use of MRI biomarkers for identification of prodromal AD among MCI-patients. Assessment of hippocampal volume together with a delayed recall test may be sufficient for short-term prediction of AD in subjects with MCI.

INTRODUCTION

Due to the aging population, the prevalence and concomitant costs of neurodegenerative diseases including Alzheimer's Disease (AD) are expected to increase dramatically [1]. Before entering the phase of dementia, subjects with AD experience a period in which they have so called mild cognitive impairments (MCI) without encountering interference in daily life activities. Identification of subjects with MCI at risk for AD is crucial, as these subjects are useful candidates for drug trials which aim to slow down disease progression.

New criteria for Alzheimer's disease (AD) recommend the use of cerebrospinal fluid (CSF), magnetic resonance imaging (MRI) or positron-emission tomography (PET) biomarkers for the diagnosis of AD in subjects with MCI [2, 3]. However, in non-academic settings evaluation of AD pathology by means of CSF and PET biomarkers is often not feasible. In these settings diagnosis is primarily based upon neuropsychological evaluation, anamnestic information and MRI measurements. Despite numerous studies evaluating MRI measurements as a candidate biomarker for AD, the added value of MRI measures over neuropsychological tests is not clear yet.

Neuropsychological tests that can best predict AD-type dementia in subjects with MCI are typically episodic memory tasks, such as immediate and delayed recall [4, 5]. However, according to some studies, assessing other cognitive domains such as categorical fluency [6], visuoconstruction [7] and tasks of executive functioning [4, 8] may also be useful in predicting AD-type dementia in MCI-patients. MRI is routinely used to exclude other diseases or other causes of dementia [9] and medial temporal lobe (MTL) atrophy as assessed on structural MRI has furthermore proven to be an effective clinical aid in the prediction of AD-type dementia in subjects with MCI [10, 11].

Previous studies that examined the added value of MRI over neuropsychological tests are inconclusive in nature. In one study, hippocampal and entorhinal cortex volumes showed limited added value to memory and measures of functioning [12], whereas in other studies [13, 14] was found that a combination of both memory and MRI increased predictive accuracy over each measurement alone. In a study

of Zhou et al. [15] was found that the combination of the ADAS-Cog with the CDR-sum of boxes had a slightly better predictive accuracy than the ADAS-Cog with cortical thickness of the right temporal lobe (conversion rate 92.7% and 88.8% respectively). Another study found that neuropsychological tests and MRI were the most informative techniques (compared to Positron Emission Tomography (PET)), with 84% and 82% correct classifications [16].

The aim of the present study was to evaluate the added value of MRI over neuropsychological tests for the prediction of AD in subjects with MCI in a memory clinic population. Atrophy of the MTL was assessed using an automated atlas-based hippocampal measurement (LEAP) [11, 17] and a qualitative visual rating scale (MTA-score) [18]. We also investigated whether hippocampal volume had predictive value for AD-type dementia in a group of subjects with a high score on the Mini-Mental State Examination (MMSE) or with a normal MTA score. Test sequence analyses were performed to determine the optimal order of cognitive tests and hippocampal volume for the prediction of AD-type dementia [19].

MATERIALS AND METHODS

Subjects

Subjects with MCI were selected from DESCRIPA (Development of Screening Guidelines and Clinical Criteria for Predementia AD) [20]. The VUmc center, one of the DESCRIPA centers, contributed an additional sample of subjects that were seen outside the DESCRIPA inclusion period. Inclusion criteria were: age 54 years or older and diagnosis of MCI. Exclusion criteria were diagnosis of dementia at baseline or any somatic, psychiatric or neurological disorder (e.g. epilepsy) that might have caused the cognitive impairment [20]. For the present study, participants were selected from 9 of the 20 participating centers as only in those centers both neuropsychological data and MRI measurements were available. Of the 270 subjects with MCI in these sites, 204 had MRI data available and, 184 of those patients also had complete data for the neuropsychological tests (see below) and completed at least 1 follow-up. There were no differences between included and excluded subjects with respect to age, gender, educational level, hippocampal volume, and cognitive test scores, except for the MMSE-score (included subjects: 27.47 (2.08) vs. excluded subjects: 26.29 (2.78); $p < .001$).

Clinical assessment and follow-up

All participants underwent a standard diagnostic workup, including clinical history, medical and neurological examination, clinical chemistry, functional evaluation using the Clinical Dementia Rating scale (CDR) [21], the MMSE, and rating scales for depression and neuropsychiatric symptoms. Baseline diagnosis of MCI was made according to the criteria of Petersen et al. [22]. Cognitive impairment was defined as a z-score <-1.5 SD on any of the following tests: the learning measure or delayed recall of a word list learning test or equivalent memory test, the trail making test (TMT) part A, TMT part B, categorical fluency, Rey figure copy test or an equivalent test [23]. Outcome measure was conversion to AD-type dementia after 2 years. AD diagnosis was made according to the DSM-IV [24] and NINCDS-ADRDA criteria, [25].

Cognitive testing

A neuropsychological battery was carried out in order to evaluate performance in several cognitive domains. In each center a primary test for verbal memory was chosen that was identical or at least similar to tests used in the other participating centers (memory: Rey Auditory Verbal Learning Test (n=136), Consortium to Establish a Registry for Alzheimer's Disease (CERAD, n=28) or Buschke Selective Reminding Test (n=20); attention and executive functioning: all TMT-A and TMT-B (n=184), categorical fluency animals (n=123) or fluency animals, fruits or cartypes (n=61); visuoconstruction: Rey Figure copy test (n=136), CERAD copy figures (n=28), Copy figures from the Mental Deterioration Battery (n=20)) [20]. Raw scores on neuropsychological tests were corrected for age, education, and sex, in accordance with locally collected or published normative data and expressed as z-scores, which were used for further analysis.

MRI acquisition and analysis

At each site, patients were scanned according to the routine MRI-protocol. Scanners and protocols at different sites varied but all scanning was performed at 1.0 or 1.5 Tesla. All scans included a three-dimensional T1 weighted gradient echo (3DT1) sequence and a fast fluid attenuated inversion recovery (FLAIR) sequence. Two measurements of MTL atrophy were included: an automatically measured hippocampal volume based on atlas registration (LEAP) [17] and a qualitative visual rating scale [18]. Elaborate descriptions of these MRI protocols and measurements

are stated in a previous publication [11].

APOE genotype

APOE genotype was determined by polymerase chain reaction of genomic DNA extracted from EDTA anticoagulated blood in 154 subjects. Subjects were classified as APOE- ϵ 4 carriers or non-carriers.

Statistical analysis

Statistical analyses were performed with IBM SPSS version 20.0 (Chicago, IL, USA) and statistical software package R (R foundation, Vienna, Austria). Differences between groups were analyzed using an independent sample t-test for continuous variables and chi-square test for categorical variables. Area under the curve (AUC) for AD-type dementia after 2 years follow-up was calculated using a time-dependent ROC curve in R [26]. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and odds ratio (OR) for conversion to AD-type dementia after 2 years were calculated using data-driven cut-points based on a time dependent ROC. Cut-points were determined by maximizing the Youden Index (sensitivity+specificity-1) for prediction of AD-type dementia at 2-years follow-up. Significance was set at $p < 0.05$ and 95% confidence intervals (CI) were calculated. Data for each neuropsychological test and MRI-measurement were dichotomized into separate variables according to these cut-points. Univariate and multivariate logistic regression analyses were performed for dichotomized variables to assess whether a combination of markers was more predictive for AD than each of these measurements alone. Demographic variables and MMSE score were added in the first step, univariate significant cognitive test scores in the second step and hippocampal volume or MTA score in the third step. After each step we only retained statistically significant predictors in the model. Sensitivity and specificity values were calculated based on the results of the regression model. To assess whether the model improved after each step, the increase in χ^2 was tested. A predicted value of 0.5 assumed presence of the disease.

RESULTS

Subject characteristics

From the total group of MCI-patients included in this study 40% were non-amnesic

MCI (74% single-domain and 26% multi-domain) and 60% amnesic MCI (45% single-domain and 55% multi-domain). Subject characteristics at baseline are shown in Table 1. Subjects who converted to AD-type dementia at 2-years follow up were older, had lower baseline scores on the MMSE, the immediate and free delayed recall, the TMT-B and categorical fluency task compared to those who did not progress, and were often carrier of the APOE-e4 allele. Baseline hippocampal volumes were lower and MTA scores higher in converters compared to non-converters.

Table 1. Subject characteristics.

	Non-converters	Converters
N	148	36
Age	69.4 (7.9)	72.9 (6.8)**
Female (%)	57	60
Years education	9.1 (4.1)	9.7 (4.3)
MMSE score	27.7 (2.1)	26.6 (1.9)**
Z-score wordlist (free delayed recall)	-1.17 (1.20)	-1.91 (0.82)***
Z-score wordlist (immediate free recall)	-1.03 (1.04)	-1.42 (0.85)*
Z-score Fluency	-0.96 (0.91)	-1.20 (0.72)
Z-score TMT-A	-1.09 (1.56)	-0.69 (1.25)
Z-score TMT-B	-1.45 (1.96)	-1.44 (1.71)
Z-score Rey Complex Figure	-0.08 (1.23)	-0.03 (1.16)
ApoE-e4-carrier (%)	42	59*
Hippocampal volume (mm3)	6016 (747)	5291 (656)***
MTA-score	2.5 (1.5)	3.5 (1.5)**
Average FU (year)	2.37 (0.79)	1.51 (0.58)***
Average time to AD (year)	-	1.38 (0.55)

All volumetric measurements are corrected for intracranial volume. Values are mean (SD). Abbreviations: MMSE = Mini-Mental state examination; TMT: Trial-making test; ApoE = apolipoprotein E genotype; LEAP: learning embeddings for atlas propagation; MTA: medial temporal lobe atrophy; FU = follow-up; AD = Alzheimer's disease.

* $p < .05$, ** $p < .01$, *** $p < .001$ for differences between groups.

Univariate predictive accuracy

The AUC for prediction of AD-type dementia at 2 years follow-up was around 0.70 for the MRI measurements, the MMSE and the delayed recall score, while it was

below 0.61 for the other neuropsychological measurements (0.52-0.61) (Table 2). When scores were dichotomised based on a cut-point that maximised the Youden index, the overall predictive accuracy (odds ratio) was highest for hippocampal volume (OR=7.8), followed by MTA-score (OR=4.1) and the free delayed recall test (OR=3.6), the MMSE-score (OR=2.9), the immediate free recall test (OR=2.5), categorical fluency (OR=2.3) and TMT-B (OR=1.9). TMT-A and Rey complex figure test did not predict AD conversion. When MRI-measurements were investigated for both hemispheres separately, it was found that the predictive accuracy was somewhat higher for the left hippocampus compared to the right hippocampus. Differences in odds ratio for left and right MTA-score were negligible (Table 2).

Multivariate predictors

Neuropsychology

When age, MMSE score and neuropsychological tests were entered together, only age and delayed recall score independently predicted AD conversion (Table 3).

Neuropsychology combined with MRI-markers

When age, MMSE score and delayed recall score were combined with MRI measurements, hippocampal volume (change in $\chi^2 = 16.57$; $df = 1$; $p < .001$) but not the MTA-score ($p = .06$) increased the likelihood of conversion (Table 3). Both left and right hippocampal volume increased predictive accuracy (left: $\chi^2 = 18.47$ $df = 1$; $p < .001$; right: $\chi^2 = 8.90$ $df = 1$; $p < .001$) (Table 3). The increase of predictive accuracy was due to an increase in both sensitivity and specificity.

Test sequence

Figure 1 shows probability plots of AD-type dementia for different sequences of delayed recall test and hippocampal volume in which the change in probability of AD-conversion is visualized after adding the result of each individual test. If hippocampal volume was analyzed first, assessment of the delayed recall task increased predictive accuracy in subjects with a normal hippocampal volume (OR=4.3, $p = 0.02$) but not in subjects with abnormal hippocampal volumes (OR=1.3, $p = 0.7$) (Figure 1A). If the delayed recall task was administered first, assessment of hippocampal volume increased predictive accuracy for AD-type dementia both in subjects with a normal delayed recall score (OR=16.4, $p < 0.001$) and in subjects with an abnormal delayed recall score (OR=5.0, $p < 0.001$) (Figure 1B).

Table 2. Predictive accuracy of neuropsychological and MRI measurements for AD-type dementia after 2 years

		AUC	p-value	Cut-off	OR (CI)	p-value	Sens	Spec	PPV	NPV
Neuropsychological assessment										
-	MMSE	0.62	.00	27	2.98 (1.79-4.97)	.00	0.47	0.77	0.35	0.85
-	Wordlist free delayed recall	0.65	.00	-1.31	3.59 (2.07-6.24)	.00	0.75	0.54	0.30	0.89
-	Wordlist immediate free recall	0.61	.002	-1.21	2.54 (1.50-4.27)	.00	0.69	0.53	0.28	0.87
-	Fluency	0.60	.01	-0.90	2.32 (1.36-3.94)	.01	0.72	0.48	0.27	0.86
-	TMT-A	0.50	.95	-0.11	1.02 (0.60-1.73)	.93	0.68	0.33	0.21	0.79
-	TMT-B	0.57	.06	-0.42	1.90 (1.09-3.32)	.02	0.75	0.38	0.24	0.86
-	Rey Complex Figure Test	0.52	.62	-0.65	1.21 (0.64-2.28)	.56	0.32	0.65	0.19	0.78
MRI biomarkers										
-	MTA-score	0.67	.001	≥ 3	4.14 (2.00-8.58)	.00	0.73	0.60	0.32	0.90
	○ Left	0.64	.004	≥ 1	3.10 (1.57-6.12)	.001	0.62	0.65	0.32	0.87
	○ Right	0.63	.008	≥ 1	2.87 (1.45-5.68)	.002	0.64	0.61	0.30	0.87
-	Hippocampal volume	0.72	.00	< 5374	7.78 (3.56-17.1)	.00	0.62	0.83	0.46	0.90
	○ Left	0.73	.00	< 2655	7.70 (3.50-16.9)	.00	0.68	0.79	0.43	0.91
	○ Right	0.66	.002	< 2664	5.17 (2.38-11.3)	.00	0.42	0.85	0.43	0.87

Neuropsychological test scores are expressed as Z-scores and corrected for age, education and sex. All scores are dichotomised based on the cut-point that optimised the Youden index [11]. Sens = Sensitivity; Spec = Specificity; PPV = positive predictive value; NPV = negative predictive value; AUC = area under the curve; OR = Odds Ratio; CI = confidence interval.

Table 3. Multivariate logistic regression

Model	Change in deviance ^b	Significant predictors in multivariate model ^a	Overall accuracy (%)	Sens (%)	Spec (%)
<i>First step</i>					
1. Age + MMSE	$\chi^2=11.06$, $df=2$, $p=.004$		67	0	80
<i>Second step + Neuropsychological tests</i>					
2. 1+DR	$\chi^2=8.99$, $df=1$, $p=.003$	age, DR	74	33	81
<i>Third step + MRI measures</i>					
3a. 2 +MTA	NS	DR	75	42	82
3b. 2 +total hippocampal volume	$\chi^2=16.57$, $df=1$, $p=.000$	DR, HCV	81	59	86

*Change in deviance is the change in deviance from maximum deviance (step 1) or the previous step (steps 2 and 3); MMSE = Mini Mental State Examination; DR = free delayed recall; MTA = medial temporal lobe atrophy; NS = not significant; Sens = sensitivity; Spec = specificity. ^a Increase in predictive accuracy as expressed by χ^2 . ^b Predictors independently predicting conversion after 2 years.

Figure 1. Probability of Alzheimer's disease (AD)-type dementia in 2 years related to the test sequence of the delayed recall test (DR) and hippocampal volume (HCV). The change in AD probability is shown according to test scores. The starting point represents the AD conversion rate in the total mild cognitive impairment (MCI) group. Dotted lines represent negative (or normal) test results and full lines represent positive (or abnormal) test results. (A) First HCV then DR; (B) first DR then HCV. Thirty-seven subjects had both abnormal DR scores and HCV (with 18 converters to AD-type dementia), 63 subjects had an abnormal DR score and a normal HCV (10 converters), 12 subjects had a normal DR score and an abnormal HCV (5 converters), 72 subjects had both a normal DR score and HCV (3 converters). * $p < 0.001$: difference in AD probability in 2 years between subjects with normal and abnormal test results after second assessment.

Predictive value in subjects with a a MMSE or MTA score above cut-off

If subjects with an MMSE-score >27 were selected, (n=106, of which 14 converted) both delayed recall and MRI measurements at baseline predicted AD-type dementia at follow-up (Table 5). In subjects with a normal MTA-score (summed left and right score <3) (n=105, of which 12 converted), hippocampal volume but not delayed recall or MMSE score predicted progression (Table 5).

Table 4. Univariate predictive accuracy in cases with MMSE>27 or MTA-score<3

Measure	OR (CI)	p	X ²	df	p
A. Predictive accuracy MMSE >27*					
DR	9.17 (1.94-43.40)	0.005	11.14	1	0.001
Hippocampal volume	5.87 (2.40-14.30)	0	14.9	1	0
MTA	2.71 (1.16-6.34)	0.02	5.44	1	0.02
B. Predictive accuracy MTA<3**					
MMSE-score	2.45 (0.71-8.52)	0.16	1.88	1	0.17
DR	2.04 (0.56-7.46)	0.28	1.21	1	0.27
Hippocampal volume	6.53 (2.84-14.98)	0	19.59	1	0

Data-selection based on A. MMSE >27 or B. MTA-score below 3. OR = odds ratio; CI = confidence interval; df = degrees of freedom; DR = free delayed recall score; MMSE = mini-mental state examination score. *Baseline MMSE > 27 and AD at follow-up (n=14); **Baseline MTA < 3 and AD at follow-up (n=12).

DISCUSSION

Our main finding is that hippocampal volume but not MTA-score increases the predictive accuracy of the delayed recall score for the prediction of AD-type dementia after 2 years. Predictive accuracy was increased both in subjects with normal and abnormal delayed recall scores at baseline. In the subgroup of subjects with an MMSE score >27, both delayed recall and MRI measurements at baseline were predictors of conversion at follow-up. In the group of subjects with a normal MTA-score (<3), only volumetric measurements predicted AD.

Neuropsychological predictors

In line with previous studies, delayed recall appeared the best neuropsychological predictor [27-31]. We found that categorical fluency and TMT-B had a moderately high predictive accuracy for AD. Previous studies also found that the TMT-B task predicted AD in amnesic MCI [32, 33] and that categorical fluency was the second most sensitive test in the detection of cognitive changes due to prodromal AD [34]. Important to note however is that other disorders may cause low performance on delayed recall tests. Disorders which can impair attentional processes (e.g. depression, anxiety) may result in poor registration and encoding of information [35] and disorders of executive functioning may moreover result in retrieval problems [36]. The same pattern can be identified in our data, whereas a cut-point of -1.31 (Z-score) on the delayed recall task shows a high sensitivity together with a low specificity. Cued recall techniques on the other hand are thought to minimize the effect of attentional deficits and thus be more specific for AD [5, 35, 37].

MRI predictors

In line with previous studies, it was found that visual rating scales show lower predictive accuracy compared to volumetric measurements [11]. The present study furthermore confirmed that in a group of subjects with a normal MTA-score (<3), volumetric measurements still independently predicted AD-conversion, indicating that hippocampal volume is more sensitive for AD than a visual rating scale. Our finding of left>right is in line with one study investigating a large ADNI cohort stating that left hippocampal volume at baseline was the best single predictor of AD-conversion [38]. Another study in cognitively normal subjects reported that left hippocampal volume was a predictor for verbal memory function [39].

Neuropsychology and MRI-measurements

The present study found that hippocampal volume increased predictive accuracy relative to a model with only neuropsychological information. Furthermore, test sequence analysis found that assessment of hippocampal volume increased predictive accuracy both in subjects with a normal and abnormal delayed recall score. In line with these findings, a recent study found the highest predictive accuracy for a model that combined a delayed recall test, functional measures and

hippocampal and entorhinal cortex volumes with age and MMSE-score [12]. Unlike hippocampal volume, visual MTA-ratings did not increase the predictive accuracy for conversion in a model with the delayed recall task. In line with the added value of hippocampal volume in subjects with normal memory scores, hippocampal volume also predicted conversion in subjects with a normal MMSE-score. These findings indicate the necessity of adding volumetric measurements of the hippocampus to standard neuropsychological investigation in order to increase the certainty that the MCI syndrome is due to AD-pathology.

Limitations

We determined cut-offs within a study population that also included subjects from the present analyses. This could have led to an overestimation of the predictive accuracy. However, it is unlikely that it influenced our findings with respect to the differences in predictive accuracy between measurements, as the same method was used to define the cut-point for each measurement. The diagnosis of AD at follow-up was not validated neuropathologically which may have possibly led to a misclassification of some cases. Follow-up data with AD diagnosis for all subjects was only available for relatively short follow-up intervals (2 years). A recent meta-analysis found that predictive accuracy for MTL atrophy decreased when follow-up was longer [40]. Still, short-term prognosis may be important for selection of subjects for trials. Recent studies point out the importance of cued recall tasks as specific marker for AD-conversion [5, 29, 35, 41, 42]. One could suggest using free delayed recall tasks for diagnosing MCI, and cued recall tasks to predict which MCI patients are likely to convert, and thus represent a group of prodromal AD-patients [35, 42]. A recent study found biological evidence for the role of cued recall as neuropsychological marker for early AD [41]. Unfortunately, in our study no cued recall task was available. Candidate tests which can be considered in future studies are the Visual Association Test (VAT) [43] and the Memory Impairment Screen (MIS) [44]. A recent study found that the MISplus showed highest predictive accuracy values in predicting future AD-conversion in subjects with MCI [5]. Scanners with different field strengths (1T and 1.5T) were used, reflecting real-life situations in which scanners and magnetic field strengths do vary. A previous study showed that it is unlikely that a small difference in field strength has a major impact on volumetric measurements [11]. It can be

considered a strength that a population from a memory clinic setting was used for this study. Conversely, findings may not be readily generalised to community populations.

Implications for clinical practice

Our findings indicate that a combination of neuropsychological data, especially the administration of delayed recall tasks, together with hippocampal volume is preferred. Sensitivity and specificity increases when hippocampal volume is added to a model which only includes neuropsychological data. In addition, structural MRI assessment is useful to rule out specific causes of cognitive impairment (e.g. stroke, tumor) [12]. The added value of performing a visual rating scale on top of neuropsychological testing is however limited in terms of sensitivity and specificity (no increase in overall predictive accuracy). Although a combination of both neuropsychology and volumetry is preferred, predictive accuracy values of hippocampal assessment alone are comparable. Since the likelihood to detect MCI patients at risk for AD-conversion is highest for volumetric measurements, volumetric assessment of MTL atrophy together with a delayed recall task may be sufficient for the diagnostic investigation of MCI-patients. In addition, one could consider adding elaborate neuropsychological investigation in subjects with normal hippocampal volumes.

Assessing disease progression by means of episodic memory tests (delayed recall) and MMSE-score is an alternative at the cost of a lower sensitivity and specificity. For example, subject may progress to AD but still perform relatively well on cognitive tests (low sensitivity). Moreover, not all subjects with memory impairment progress to AD (low specificity). Free recall is moreover dependent on retrieval mechanisms, motivational and attentional processes. In line with recent criteria, however, one should additionally assess whether free recall deficits can be normalized with cueing. Failure to recall words after the presence of cues is indicative of prodromal AD [3]. When applying a more extensive neuropsychological test battery one should consider including MMSE-score, a verbal wordlist test (including immediate and free delayed recall), a fluency test and a test for executive functioning (e.g. TMT-B task), which are all shown to be independent predictors of AD-conversion in subjects with MCI.

We previously showed that CSF markers further improved predictive accuracy over hippocampal atrophy by increasing the sensitivity [21]. Combined use of both CSF and imaging biomarkers is highly accurate for either early diagnosis or exclusion of AD in patients with MCI [21, 32, 39-41]. Future studies could add both MRI and CSF measurements to neuropsychological data in order to further increase predictive accuracy.

CONCLUSION

The present study found that volumetric measurements are the best predictors of AD-conversion after 2 years in a group of MCI-subjects. Assessment of hippocampal volume together with a delayed recall test may be sufficient for short-term prediction of AD in subjects with MCI.

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Chapter 3

Measurements of medial temporal lobe atrophy for prediction of AD in subjects with MCI

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ABSTRACT

Our aim was to compare the predictive accuracy of four different medial temporal lobe measurements for Alzheimer's disease (AD) in subjects with mild cognitive impairment (MCI). Manual hippocampal measurement, automated atlas based hippocampal measurement (LEAP), a visual rating scale (MTA-score), and lateral ventricle measurement were compared. Predictive accuracy for AD 2 years after baseline was assessed by ROC analyses with area under the curve (AUC) as outcome. Annual cognitive decline was assessed by slope analyses up to 5 years after baseline. Correlations with biomarkers in cerebrospinal fluid (CSF) were investigated. Subjects with MCI were selected from the DESCRIPA multicenter study (n=156) and the single-center VU medical center (n=172). At follow-up, AUC was highest for LEAP (0.71) and manual hippocampal measurement (0.71), while lower for MTA-score (0.65) and lateral ventricle (0.60). Slope analysis yielded similar results. Hippocampal measurements correlated with CSF total tau (t-tau) and phosphorylated tau (p-tau), not with beta-amyloid (A β)1-42. MTA-score and lateral ventricle volume correlated with CSF A β 1-42. We can conclude that volumetric hippocampal measurements are the best predictors of AD conversion in subjects with MCI.

INTRODUCTION

Alzheimer's disease (AD) is the leading cause of dementia in the elderly affecting more than 27 million people worldwide. Early detection of AD may prevent irreversible damage by enabling preventative treatment [1, 2]. A primary focus of research in this area is identifying which biomarkers are clinically useful for the early diagnosis of AD.

Medial temporal lobe (MTL) atrophy as assessed on structural MRI has proven to be an effective clinical aid in the early diagnosis of AD [3], and this method predicts AD in subjects with mild cognitive impairment (MCI) [3-7]. There are several ways to determine the degree of MTL atrophy in the brain including manual delineation, (semi-) automated techniques to measure hippocampal volume, qualitative ratings of MTL atrophy (MTA-score), and assessment of lateral ventricular volume [8-18]. Each of these methods has its strengths and limitations.

Manual volumetry is considered the gold standard [19-21] but is time consuming, limiting routine clinical or large-scale research use. Automated measurements are quick and widely applicable, but may be susceptible to scanner and scan protocol variability. Volumetric measurements of the lateral ventricle require a minimum of rater time with robust automatic segmentations but show a lot of variability and asymmetry between subjects [14]. Qualitative ratings are quick to perform but sensitive to interrater variability and show lower accuracy rates compared to volumetric analysis [22, 23]. Visual rating scales are furthermore insensitive to detect atrophy progression over time [24].

A number of studies have examined differences between various techniques to measure atrophy of the MTL, mostly comparing manual with automated hippocampal volumetry [25-27] or volumetric hippocampal measurements to a visual rating scale [15, 24, 28-30]. These studies typically evaluate the diagnostic accuracy of different MRI techniques by comparing AD patients with healthy control subjects. Most studies found that manual hippocampal measurement shows similar performance as automated hippocampal segmentation [25, 31]. However, the performance of automated techniques may be less precise when applied in AD patients suffering from moderate to severe brain atrophy and/or white matter

hyperintensities which may lead to false allocations of gray matter, white matter or cerebrospinal fluid (CSF) [32-34]. One author stated that visual rating of MTL atrophy is a quick and clinically useful technique for differentiating AD from controls and is quicker and more accurate than volumetry [29].

To our knowledge, no study has compared the diagnostic performance of manual and atlas-based hippocampal segmentation, lateral ventricle volume and a qualitative rating. Moreover, no comparative studies have been performed on the predictive accuracy of these different methods to predict AD in subjects with MCI, their relation with CSF biomarkers of AD, and the of multi-center settings on diagnostic performance.

The aim of the present longitudinal study was to compare the predictive accuracy of four different MTL measurements for the progression to AD-type dementia in patients with MCI over a 2-year follow up period. Atrophy of the MTL was assessed using manual measurement of the hippocampus, automatically measured hippocampal volume based on atlas registration (LEAP), volumetric measurement of the expansion of the lateral ventricle and a largely used qualitative rating scale. Since subjects may convert at a later point in time, slope analyses were additionally performed with annual cognitive decline up to 5 years as outcome measure. The correlation of MTL measures with AD biomarkers in CSF was also investigated and the predictive accuracy was tested both in a multicenter study with different scan protocols and in a single-center study.

MATERIALS AND METHODS

Subjects

We selected participants with MCI from the DESCRIPA (Development of Screening Guidelines and Clinical Criteria for Predementia AD) study and the Alzheimer Center of the VU University Medical center (VUmc) in Amsterdam. DESCRIPA is a multicenter prospective cohort study from the European Alzheimer's Disease Consortium (EADC) aimed at developing clinical criteria and screening guidelines for predementia AD [35]. For this study participants were selected from 9 of the 20 participating centers where MRI scanning was performed as part of clinical practice or as research protocol. The VUmc cohort in the present study included the VUmc subjects enrolled in the DESCRIPA study and an additional sample of subjects that

were seen outside the DESCRIPA inclusion period. (Appendix S1).

Inclusion criteria for both cohorts were: age 54 years or older, diagnosis of MCI and availability of results for each MRI measure and outcome at follow-up. Exclusion criteria were diagnosis of dementia at baseline or any somatic, psychiatric or neurological disorder (e.g. epilepsy) that might have caused the cognitive impairment [35]. At baseline scans were available for 456 subjects. Visually rated MTL atrophy was available for all subjects. Of these, 54 had no FU data and were excluded. Of the remaining 402 subjects, scans were not available in digital format for 21 subjects. Of the remaining 381 scans, manual segmentation of the hippocampus could be performed on 341 scans (reason missings: technical problem volumetric measurement [n=5], technical problem automated intracranial volume estimation [n=25] and logistics [n=10]), LEAP based volumetry on 357 scans (reason missings: technical problem volumetric measurement [n=11] and logistics [n=13]), and lateral ventricle volumetry on 335 scans (reason missings: technical problem volumetric measurement [n=37], and logistics [n=9]). Data for all four medial temporal lobe measurements was available for 328 subjects, 156 from DESCRIPA and 172 from VUmc. There were no differences between included and excluded subjects with respect to age, gender, educational level and cognitive test results.

Clinical and cognitive assessment

All participants underwent a standard diagnostic workup, including clinical history, medical and neurological examination, clinical chemistry, functional evaluation using the Clinical Dementia Rating scale (CDR) [36], the Mini-Mental State Examination (MMSE), and rating scales for depression and neuropsychiatric symptoms. A neuropsychological battery was performed to evaluate performance in several cognitive domains. In each center a primary test for verbal memory, language, attention, executive functioning, and visuoconstruction was chosen that was identical or similar to tests used in other centers [35]. Raw scores on neuropsychological tests were corrected for age, education, and sex, in accordance with locally collected or published normative data and expressed as z-scores, which were used for further analysis. Baseline diagnosis of MCI was made according to the criteria of Petersen et al. [37, 38]. MCI was defined as a z-score <-1.5 SD on any of the following tests: the learning measure or delayed recall of a word list learning test or equivalent memory test, the trail making test (TMT) part A, TMT

part B, verbal fluency, Rey figure copy test or an equivalent test [39]. We calculated a composite score as the average z-score of the 6 tests if scores were available for at least 3 tests [40].

Follow-up was conducted annually for up to 5 years. The primary outcome measure was conversion to AD-type dementia after 2 years. AD diagnosis was made according to the DSM-IV [41] and NINCDS-ADRDA criteria, [42]. Secondary outcome measures were annual cognitive decline on the MMSE and the cognitive composite score.

MRI acquisition and image analysis

Scan protocol

At each site, patients were scanned according to the routine MRI-protocol. Scanners and protocols at different sites varied but all scanning was performed at 1.0 or 1.5 Tesla (Appendix S1). All scans included a three-dimensional T1 weighted gradient echo (3DT1) sequence and a fast fluid attenuated inversion recovery (FLAIR) sequence.

MRI-measurements

MTL atrophy was assessed using manual measurement of the hippocampus, automatically measured hippocampal volume using multi-atlas segmentation (LEAP), volumetric measurement of the expansion of the lateral ventricle and a qualitative rating. All volumetric measurements were corrected for intracranial volume (ICV). The LEAP measurement was ICV corrected by means of a scaling factor from MNI (Montreal Neurological Institute), the manual hippocampal measurement and the lateral ventricle measurement were ICV corrected by means of a scaling factor derived from FSL software. The total rating time of each method can be found in Supplementary Table S1. Rater time needed for analysis was lowest for LEAP hippocampal measurement (4 minutes) followed by the qualitative rating (5 minutes). Rater time was 30 minutes for lateral ventricle measurement and 150 minutes for manual hippocampal volumetry.

For the manual segmentation of the hippocampus, the baseline 3DT1-weighted volume sequence was reformatted in 2-mm slices (in-plane resolution: 1x1 mm) and oriented perpendicular to the long axis of the left hippocampus [21]. Regions of interest (ROIs) of the hippocampus were constructed by manual delineation of hippocampal borders on both sides on the reformatted slices, using the in-house–

developed software package Show_Images 3.7.0 (VU University Medical Center, Amsterdam, The Netherlands). Delineation of the hippocampus was performed using previously described criteria [43-45] by 3 trained technicians (coefficients of variation: interrater <8%, intrarater <5%) blinded to diagnosis. ROIs included the dentate gyrus, cornu ammonis, subiculum, fimbria, and alveus. Baseline hippocampal volume was calculated by multiplying the total area of all ROIs of each hippocampus by slice thickness. Baseline hippocampal volumes were adjusted for intracranial volume (ICV), using the scaling factor derived from SIENAX (FSL, FMRIB, Oxford, UK) [46].

Automated hippocampal volumetry was performed using the Learning embeddings for atlas propagation (LEAP) method [17]. In this method, multi-atlas registration is applied to a cohort of brain images after representing the whole population together with an initial set of atlases. The initial set is propagated to a number of unlabeled images in their local neighbourhood which are used to label them. Images labeled in this way become atlases themselves and are, consequently, further propagated throughout the whole dataset. In this way, each image is labeled using a number of atlases in its close vicinity, which has been shown to perform more robust on diverse datasets than other multi-atlas registration techniques [17]. A brief visual inspection of the segmented hippocampi was performed in order to identify clear failures of the automated method. Except for technical failures listed below, no subjects were excluded after this inspection and no manual correction was performed.

Measurement of the lateral ventricle was executed with an extension of SIENAX [47, 48], part of FSL [49]. SIENAX starts by extracting brain and skull images from the single whole-head input data [50]. The brain image is then affine-registered to MNI152 space [51, 52] using the skull image to determine the registration scaling. This is primarily in order to obtain the volumetric scaling factor, to be used as a normalisation for head size. Next, tissue-type segmentation is carried out [53] in order to calculate total volume of brain tissue (including separate estimates of volumes of grey matter, white matter, peripheral grey matter and ventricular CSF). After the tissue segmentation, a registered mask is used to exclude the CSF on the outer side of the brain. The resulting ventricular structure is manually edited when the segmentation or the mask did not take the whole ventricle into account or did contain CSF pixels not belonging to the ventricles (CSF found outside the ventricles). The visual rating of MTL atrophy was performed using a qualitative scale [15]. Rating was performed on coronal T1-weighted images using a 5-point visual scale (MTA-

scores), ranging from 0 (no atrophy) to 4 (severe atrophy) based on the height of the hippocampal formation and the surrounding CSF spaces. In the analysis, the sum of both sides (left and right) was used. All visual rating was performed at VUmc by a number of trained raters. Scans from VUmc were rated by a group of 3 raters supervised by a neuroradiologist (intrarater weighted Cohen $\kappa > 0.80$; interrater weighted Cohen $\kappa > 0.80$) [54]. The DESCRIPA scans were rated by a single rater from VUmc (intrarater weighted Cohen $\kappa = 0.68$) [45].

CSF analyses

CSF was collected by lumbar puncture and levels of beta amyloid ($A\beta$)1-42, total tau (t-tau) and phosphorylated tau (p-tau) in CSF were measured using commercially available sandwich ELISAs (Innotest β -amyloid 1-42; Innotest hTAU-Ag; Innogenetics, Ghent, Belgium), specially constructed to measure $A\beta$ 1-42 and t-tau [55, 56] by experienced technicians at the lab in Gothenburg for the DESCRIPA cohort and in Amsterdam for the VUmc cohort. CSF was available for 147 subjects. We corrected for interlaboratory ELISA differences by analyzing 33 samples at both labs and we adjusted VUmc values to those of DESCRIPA using the following equating formula: Gothenborg = average Gothenborg + (SD Gothenborg/SD VUmc)*(VUmc - average VUmc) [57]. Abnormal values for CSF measures were a concentration ≤ 550 pg/mL for $A\beta$ 1-42, >52 pg/mL for p-tau and >375 pg/mL for t-tau [58].

APOE genotype

APOE genotype was determined by polymerase chain reaction of genomic DNA extracted from EDTA anticoagulated blood in 262 subjects. Subjects were classified as APOE- ϵ 4 carriers or non-carriers.

Statistical analysis

Statistical analysis was performed with IBM SPSS version 19 (Chicago, IL, USA) and statistical software package R (R foundation, Vienna, Austria). Correlations between the different methods for MTL assessment and between MTL and other biomarkers (CSF, APOE- ϵ 4 allele) were analyzed by the Pearson's correlation coefficient for continuous data, and the Spearman rank test for correlations including the MTA-score. Intraclass correlation coefficient (ICC) and paired t-test were performed to investigate the agreement between MTL measurements.

The main outcome measure was the area under the curve (AUC) for AD-

type dementia after 2 years follow-up, calculated using a time-dependent ROC curve in R [59]. Differences in AUC between methods were tested as described elsewhere [60]. We calculated the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), odds ratio (OR) and hazards ratio (HR) for AD-type dementia at 2- year follow-up using data-driven cut-points based on a time dependent ROC. First we calculated the cut-point that maximized the Youden Index (sensitivity+specificity-1) for predicting AD-type dementia after 2 years. Second, we selected cut-points that predicted AD-type dementia with a sensitivity of 85%. Cut-points were calculated in the whole sample and in each cohort separately. Spline analyses were performed to determine AD-free survival after 2 year as a function of each MRI-measurement in the total cohort. Slope analyses with mixed models were performed to investigate whether MTL atrophy was associated with change on the MMSE and a cognitive composite score at follow-up. The analyses included the baseline score and available follow-up scores up to 5 years after baseline and were corrected for age and education. An unstructured covariance structure with center as a random effect was used as this model provided the best -2 log likelihood compared to models with simpler covariance structures [40].

RESULTS

Subject characteristics

From the total group of MCI-patients included in this study 37% were non-amnestic MCI (73% single-domain and 27% multi-domain) and 63% amnestic MCI (44% single-domain and 56% multi-domain). Baseline and follow-up characteristics are shown in table 1. Both cohorts were comparable for age and APOE-e4 status. The DESCRIPA cohort included more females and education was lower compared to the VUmc cohort. Scores on the MMSE and a delayed recall task were lower in the VUmc cohort. At follow-up, ninety-one subjects were diagnosed with probable Alzheimer type dementia (28%). Conversion rate was higher in the VUmc cohort (35.5%) than in the DESCRIPA cohort (19.2%), $p < .001$. Twelve subjects converted to a different type of dementia at follow-up: 4 subjects converted to frontotemporal dementia, 6 to dementia with Lewy bodies, one to vascular dementia and one to another form of dementia. These subjects were included in the no-AD group. The follow-up length was slightly higher in the VUmc cohort. Characteristics of subjects with and without AD-type dementia at FU in each cohort are shown in supplementary table S2.

Table 1. Subject characteristics.

	Descripta cohort	VUmc cohort	Combined cohort
N	156	172	328
Age	70.3 (7.9)	70.9 (7.3)	70.6 (7.6)
Female (%)	59.6	44.2 **	51.5
Years education	8.5 (3.9)	11.2 (3.3)***	10 (3.8)
MMSE score	27.2 (2.3)	26.6 (2.6)*	27.0 (2.5)
Z-score wordlist (delayed recall)	-1.23 (1.20)	-1.58 (1.10)*	-1.38 (1.20)
ApoE-e4-carrier (%)	45	56	51
Homo/heterozygous ApoE-e4-carriers (%)	38/7	38/18	38/13
Manual hippocampus (mm ³)	7874 (1294)	7462 (1097)**	7657 (1211)
LEAP hippocampus (mm ³)	5897 (798)	5446 (632)***	5661 (749)
MTA-score	2.8 (1.7)	2.3 (1.7)**	2.5 (1.7)
Lateral ventricle (mm ³)	53409 (26105)	57374 (28042)	55488 (27170)
CSF A β 1-42, pg/ml	561 (256)	604 (283)	594 (277)
Abnormal CSF A β 1-42 (%)	49	52	51
CSF t-tau, pg/ml	418 (298)	558 (347)*	525 (351)
Abnormal CSF t-tau (%)	51	68	64
CSF p-tau, pg/ml	64 (33)	80 (47)	77 (45)
Abnormal CSF p-tau (%)	54	71	67
CSF Ratio A β 1-42/t-tau	2.2 (2.2)	1.6 (1.5)	1.8 (1.6)
Outcome at last FU (%)			
- No AD	80.8	64.5	72
- AD	19.2	35.5***	28
Average FU non-demented subjects (year)	2.35 (0.84)	2.67 (1.31)*	2.47 (1.09)
Average time to AD (year)	1.51 (0.71)	2.29 (1.39)**	2.07 (1.28)

All volumetric measurements are corrected for intracranial volume. Values are mean (SD). Abbreviations: MMSE = Mini-Mental state examination; ApoE = apolipoprotein E genotype;

A β 1-42 = beta amyloid 1-42; t-tau = total tau, p-tau = phosphorylated tau; FU = follow-up; AD = Alzheimer's disease. * $p < .05$, ** $p < .01$, *** $p < .001$ for differences between cohorts.

Correlations between MTL-measures

Manual and automated LEAP hippocampal volumes correlated highly (Pearson's r : 0.71, $p < .001$) with intermediate agreement (ICC: 0.38, $p < .001$, Bland-Altmanplot (Supplementary Figure S2)). Paired t-test showed significant differences between the two techniques (t : 42.27, $p < .01$) with LEAP hippocampal measurement showing lower volumes. Using the cut-point based on the Youden index, the kappa and the overlap between manual and LEAP hippocampal volume were 0.60 and 0.80 respectively.

The correlation between the MTA-score and the manual and LEAP hippocampal volume was -0.36 and -0.27 respectively ($p < .01$). Using the cut-point based on the Youden index, the kappa was 0.29 and the overlap between scores 0.69 for manual vs MTA-score and 0.37 (kappa) and 0.65 (overlap) for LEAP vs MTA-score. The lateral ventricle measurement strongly correlated with MTA-score ($r = 0.60$, $p < .001$) and showed weak correlations with both manual ($r = -0.20$, $p < .01$) and LEAP hippocampal volume ($r = -0.20$, $p < .01$).

Correlations with AD biomarkers

Manual and LEAP hippocampal volume significantly correlated with CSF t-tau, p-tau and A β /t-tau ratio (all $p < .01$, Table 2). The MTA-score correlated with A β 1-42 ($p < .05$) and the ratio A β /t-tau ($p < .01$). The lateral ventricle volume correlated with A β 1-42 ($p < .01$) and p-tau ($p < .05$). Only the LEAP hippocampal volume at baseline was associated with APOE-e4 allele status (LEAP volume APOE-e4 carriers 5516 mm³ vs 5852 mm³ for APOE-e4 non-carriers, $t = 2.72$, $p < .001$).

Table 2. Correlation with CSF measurements.

	A β 1-42	T-tau	P-tau	A β /t-tau
Manual hippocampus	0.09	-0.28**	-0.23**	0.29**
LEAP hippocampus	0.05	-0.32**	-0.27**	0.33**
MTA-score	-0.20*	0.11	0.06	-0.24**
Lateral ventricle	-0.30**	0.12	0.16*	-0.1

Shown are Pearson correlation coefficients or Spearman-rank correlation coefficients (correlations with MTA score). Abbreviations: A β 1-42 = beta amyloid 1-42; t-tau = total tau; p-tau = phosphorylated tau; MTA = medial temporal lobe atrophy. * $p < .05$, ** $p < .01$.

Predictors of AD-type dementia in combined sample

In the total sample, the AUC for prediction of AD-type dementia at 2 years follow-up was highest for the LEAP hippocampal measurement (0.71) and manual hippocampal measurement (0.71), while it was substantially lower for the MTA-score (0.65) and lateral ventricle (0.60) (Figure 1). If measurements were dichotomised using the cut-point that maximised the Youden Index, the overall predictive accuracy for AD after 2 years was best for the manual and LEAP hippocampal volumetric measurements (OR 6.4-6.5, HR 4.4-4.5), and lowest for the qualitative rating and lateral ventricle volume (OR <4, HR <3) (Table 3). Figure 2 shows the 2-year dementia risk according to baseline MTL score based on spline analysis. The x-axis shows the degree of atrophy and the y-axis depicts the risk for AD at follow-up associated with the degree of atrophy. Both manual and LEAP hippocampal volume had a non-linear relation with dementia risk indicating that the risk for dementia did not change much with very large or very small volumes. The optimal cut-point based on the Youden index for these measures was found halfway through the linear part of the plot.

If measurements were dichotomised using the cut-point that provided a sensitivity of 85% similar results were obtained. These analyses also showed that the specificity was higher for manual and LEAP volumetric measurements of hippocampal volume than for MTA-score and lateral ventricle volume.

Predictors of cognitive decline

Since subjects with MCI may convert to AD-type dementia after the 2-years follow-up, we performed slope analyses with annual cognitive decline up to 5 years as outcome measure. Subjects with abnormal scores for manual and LEAP hippocampal measurement and MTA-score at baseline declined 2 times as fast on the MMSE ($p<.001$) and declined 2.6 to 4 times as fast on the cognitive composite score ($p<.01$) compared to subjects with normal MTL scores (Table 4, Figure 3). The lateral ventricle volume only predicted decline on the cognitive composite score ($p<.01$) (Table 4, Figure 3).

Multicenter versus single-center cohort

In the DESCRIPA multicenter cohort, AUC analysis showed best predictive accuracy values for LEAP (0.74) followed by manual hippocampus volume (0.71). In the single-center cohort, manual hippocampus was the best predictor for AD (AUC: 0.69) followed by LEAP (AUC: 0.68) and MTA-score (AUC: 0.65) (Figure 1). In order to investigate whether optimal cut-points for predicting AD-type dementia differed between the multicenter and single center cohort, we calculated within each cohort the cut-point that maximised the Youden index and the cut-point that provided a sensitivity of 85% for the prediction of AD-type dementia after 2 year (Table S3). These analyses showed that the optimal cut-points for the manual and LEAP hippocampal measurement were similar between the multicenter and the single-center cohort with relative differences of less than 2.5% for the cut-points based on the Youden index and less than 5.5% for the cut-points that provided a sensitivity of 85%.

Fig. 1. Area under the curve (AUC) of a ROC-curve for MRI-measurements for A) total sample, B) DESCRIPA cohort and C) VUmc cohort. All volumetric measurements are corrected for intracranial volume. Abbreviations: ROC = receiver operating characteristics; MTA = medial temporal lobe atrophy.

Fig. 2. Spline analyses showing AD-free survival after 2 year as function of MRI-measurement in total cohort for A) Manual hippocampal volume, B) LEAP hippocampal volume, C) MTA-score and D) Lateral ventricle volume. Red line indicates cut-point that maximised the Youden index. All volumetric measurements are corrected for intracranial volume. Abbreviations: AD = Alzheimer's disease; MTA = medial temporal lobe atrophy.

Fig. 3. Slope analyses showing annual cognitive decline on A) the MMSE and B) a cognitive composite score as predicted by baseline MTL measurements. Abnormal MRI-values at baseline were defined as manual hippocampal volume $<7559 \text{ mm}^3$, LEAP hippocampal volume $<5374 \text{ mm}^3$, MTA-score ≥ 3 and lateral ventricle volume $\geq 58491 \text{ mm}^3$. Figure A+B: the solid line indicates the subjects with normal values. The dotted line indicates subjects with abnormal values. Abbreviations: MAN = manual hippocampal volume; LEAP = LEAP hippocampal volume; MTA = medial temporal lobe atrophy score; LATVEN = lateral ventricle volume; MMSE = Mini-Mental State Examination. All volumetric measurements are corrected for intracranial volume. The cognitive composite score = average z-score of learning or delayed recall of a word list learning test or equivalent memory test, the trail making test (TMT) part A, TMT part B, verbal fluency, Rey figure copy test or an equivalent test.

Table 3. Predictive accuracy for AD-type dementia after 2-years.

Cut-point based on Youden index	Cut-off	Sens	Spec	PPV	NPV	OR	HR
Manual hippocampus	7559	0.78	0.65	0.46	0.88	6.47	4.55
LEAP hippocampus	5374	0.66	0.77	0.52	0.85	6.4	4.43
MTA-score	3	0.66	0.64	0.41	0.83	3.4	2.77
Lateral ventricle	58491	0.53	0.68	0.39	0.79	2.36	2.01
Cut-point for a sensitivity of 85%							
Manual hippocampus	8379	0.88	0.37	0.35	0.89	4.22	3.15
LEAP hippocampus	5901	0.87	0.42	0.36	0.89	4.72	3.34
MTA-score	2	0.86	0.3	0.32	0.85	2.57	2.03
Lateral ventricle	34859	0.84	0.27	0.31	0.81	1.87	2

All volumetric measurements are corrected for intracranial volume. Cut-off in mm³ for volumetric measurements. Abbreviations: Sens = sensitivity; Spec = specificity; PPV= positive predictive value; NPV = negative predictive value; AUC = area under the curve; OR = odds ratio; HR = hazard ratio; LEAP = Learning Embeddings for Atlas Propagation; MTA = Medial temporal lobe atrophy.

DISCUSSION

The present study showed that volumetric measurements of the MTL are better predictors for AD-type dementia in subjects with MCI than a qualitative rating or the assessment of the lateral ventricle volume.

This is to our knowledge the first study comparing four different measures for MTL atrophy in a large sample of MCI-subjects from a memory clinic population. Furthermore this is the first study to compare the predictive accuracy of these measurements in single-center and multicenter settings and to investigate the correlations between these measurements and other AD biomarkers.

Comparison of MTL assessment methods

Predictive accuracy

The degree of neurodegeneration in MTL structures is the best MRI marker of imminent conversion to AD, with decreased hippocampal volume being the most robust structural MRI feature [61].

Table 4. Annual cognitive decline over 5 years of follow-up.

		MMSE score		Cognitive composite score	
		Baseline score	Slope	Baseline score	Slope
Manual hippocampus	Normal	27.6 (0.47) ^a	-0.45 (0.09) ^{a,b}	-0.90 (0.08)	-0.05 (0.02) ^{c,d}
	Abnormal	26.7 (0.47)	-1.04 (0.10) ^e	-1.00 (0.09)	-0.17 (0.03) ^e
LEAP hippocampus	Normal	27.4 (0.42) ^c	-0.54 (0.09) ^{a,b}	-0.92 (0.07)	-0.07 (0.02) ^{b,c}
	Abnormal	26.5 (0.46)	-1.13 (0.12) ^e	-1.01 (0.09)	-0.18 (0.04) ^e
MTA-score	Normal	27.4 (0.43) ^f	-0.49 (0.08) ^{a,e}	-0.84 (0.07) ^c	-0.05 (0.02) ^{a,b}
	Abnormal	26.9 (0.43)	-1.14 (0.11) ^e	-1.05 (0.08)	-0.20 (0.03) ^e
Lateral ventricle	Normal	27.4 (0.46)	-0.70 (0.09) ^e	-0.96 (0.07)	-0.01 (0.03) ^c
	Abnormal	27.0 (0.48)	-0.94 (0.13) ^e	-1.00 (0.09)	-0.22 (0.05) ^{c,e}

Data are mean (SD). Slope refers to annual change on the test. A negative slope indicates cognitive decline. Scores dichotomised based on Youden cut-offs.

Key: MMSE=Mini-Mental State Examination; MTA = medial temporal lobe atrophy

^a $p < 0.001$ for baseline score or slope compared with the abnormal biomarker group.

^b $p < 0.01$ for slope different from 0 (=a statistically significant change over time in test score).

^c $p < 0.01$ for baseline score or slope compared with the abnormal biomarker group.

^d $p < 0.05$ for slope different from 0 (=a statistically significant change over time in test score).

^e $p < 0.001$ for slope different from 0 (=a statistically significant change over time in test score). ^f $p < 0.05$ for baseline score or slope compared with the abnormal biomarker group.

In our study, predictive accuracy of both volumetric hippocampal measurements for AD-type dementia was indeed higher than that of the qualitative rating and lateral ventricle measure. This is in line with current evidence stating that manual

and automated volumetric methods show similar performances in diagnosing AD [25, 31, 62, 63] and that qualitative rating scales or lateral ventricle measurements are less accurate predictors than volumetric methods [24, 28, 30].

The slope analyses of cognitive decline over 5 years again yielded similar results with LEAP and manual hippocampal volume at baseline predicting cognitive decline on both the MMSE and a cognitive composite score. Slope analysis for the MTA-score predicted cognitive decline equally well as the volumetric measures. This is again in line with previous findings showing that automatically measured volume change in the hippocampus is correlated with decline of performance on the MMSE [64] and that manually measured hippocampal volume reduction is correlated with the severity of impairment on neuropsychological tests [65]. Another study found that performance on the MMSE was directly correlated with hippocampal volume [66].

Relation with other AD-biomarkers

Manual and LEAP hippocampal volume significantly correlated with CSF t-tau and p-tau but not with A β 1-42. The correlation of hippocampal volumetric measurements with CSF tau but not with CSF A β is in line with previous studies conducted in subjects with MCI [67, 68] and subjects with prodromal AD or AD [69]. It may be explained by the observation that antemortem hippocampal volume significantly correlated with the density of neurofibrillary tangles at autopsy [70, 71] but not with amyloid beta plaque load [70]. The qualitative MTA-score and lateral ventricle volume correlated with CSF A β 1-42. This correlation may indicate that these MTL measures in part reflect generalised brain atrophy as previous studies showed that lower A β 1-42 levels but not t-tau levels were associated with total brain atrophy and ventricular volume [72].

Overlap between measurements

Although the LEAP and manual hippocampal measurement correlated highly ($r=0.71$) and scores showed 80% overlap, the intraclass correlation coefficient (ICC) of 0.38 indicated a moderate agreement. This might be due to the fact that the LEAP volume was consistently lower, since the ICC is sensitive for absolute sizes. A previous study that compared LEAP with manual measurements found a much higher ICC of 0.89 [17]. Although in this study LEAP volume was systematically lower

than the manual volumetric measurement, this difference was smaller than in our study. Differences in absolute volumes may be due to the use of different borders for hippocampal delineation. Major parts of the hippocampus are included in both volumetric methods with the only difference that the LEAP method misses some of the hippocampal head, some of the alveus and some of the fimbria, and ends a few slices earlier than manual hippocampal outlining. Despite the differences in raw volume, both methods have a comparable diagnostic accuracy indicating that these parts are less important for AD pathology. It is also likely that the cut-off points for each hippocampal method likely reflected a similar degree of abnormality. Namely, if we defined the cut-off that provided a sensitivity of 0.85, the specificity of each method was equal. When the cut-off was used that optimised the Youden index, small differences in sensitivity and specificity were found but the combination of sensitivity and specificity expressed as odds ratio was the same. Differences in absolute volumens between different measurements protocols may be reduced in the future as efforts are made towards the harmonization of an MRI segmentation protocol for hippocampal delineation [20]. Since manual volumetry is used as the standard against which automated segmentation algorithms are assessed, future synchronization and comparison of both techniques will be facilitated. Correlations between manual and LEAP volumetric measurements and MTA-score were low with a moderate overlap between both methods. The same pattern was found for correlations and overlap between both volumetric hippocampal measurements and expansion of the lateral ventricle.

The findings above indicate, in line with previous studies, that volumetric and qualitative measures of MTL atrophy measure different aspects of AD pathology [73, 74]. Manual and LEAP hippocampal volume correlated with each other, both showed high predictive accuracy values and both correlated with t-tau and p-tau but not with A β 1-42. In contrast, visually rated MTA-score and expansion of the lateral ventricle correlated highly with each other but not with the volumetric measures. They showed lower predictive accuracy for AD-conversion than the volumetric measures and correlated highly with amyloid beta in CSF rather than CSF tau. As discussed above, it is possible that both the MTA-score and lateral ventricle volume reflect a widening of ventricular spaces i.e. the temporal horn, which may be indicative of generalised atrophy rather than atrophy of the hippocampus alone.

Comparison of cohorts

None of the MTL measures showed major differences in predictive accuracy between the single and multicenter cohort. When optimal cut-points for each measure were calculated for each cohort separately these cut-points only slightly differed between the cohorts for the volumetric hippocampal measurements, which shows that volumetric measurements display stable cut-points across different cohorts. Some differences were noted between the cohorts on cut-points for the MTA-score and lateral ventricle, suggesting that MTA-score and lateral ventricle are more sensitive for cohort differences.

Technical considerations

In general automated measurements are more susceptible to scanner and scan protocol variability. In our study, no significant differences between both methods were found with LEAP showing similar or even slightly better performance than manual volumetry. This suggests that automated measurements can be performed in multi-centre studies without strict standardisation of scan protocols. The percentage of technical failures was small for manual hippocampal measurement (0.07 %) and LEAP hippocampal measurement (0.03 %). All failures were observed in 2 DESCRIPA sites that apparently used scan frequencies which occasionally led to technical errors during the pre-processing phase (e.g. intensity problems), making these particular scans unsuitable for applying subsequent volumetric measurements. SIENAX related measurements (ICV measure and lateral ventricular volume) yielded a higher technical failure rate (1% for lateral ventricle) and were observed across all sites. These were due to calculation errors or a combination of software and image quality problems. These findings indicate that the lateral ventricle measurement is more sensitive for specific differences in image quality than a manual or automated hippocampal measurement. It should be noted that scan sequences were designed for routine clinical practice and not for automated measurements and it is likely that failure rate could be reduced by using protocols optimised for automated measurements.

Limitations

The present study had several limitations. There were differences in subject characteristics between the single-center and multicenter cohort. Subjects from

the single-center VUmc cohort were more severely impaired and had lower baseline MMSE scores, more severe memory impairment, a lower hippocampal volume, higher CSF tau, and a higher conversion rate to dementia. Despite these differences, however, the predictive accuracy was similar in each cohort. Another limitation is that the cut-offs of all MRI measurements were determined within a study population that also included the subjects from the present analyses. This could have led to an overestimation of the predictive accuracy. However, it is unlikely that it influenced our findings with respect to the differences in predictive accuracy between MRI measurements, as we used the same method to define the cut-point for each measurement. Follow-up data with AD diagnosis for all subjects was only available for relatively short follow-up intervals (2 years). For clinical trials, however, short-term prognosis may be important. For those subjects of whom long-term follow-up data was available, predictive accuracy for annual cognitive decline up to 5 years was additionally investigated. The diagnosis of AD at follow-up was not validated neuropathologically which may have possibly led to the misclassification of some cases. We used scanners with different field strengths (1T and 1.5T) which reflected real-life situations in which scanners and magnetic field strengths do vary. However, these differences may have introduced bias. We therefore compared the hippocampal volume between subjects scanned on a 1.0T scanner ($n=127$) and a 1.5T scanner ($n=201$) after correction for age, gender, educational level, baseline MMSE score and follow-up diagnosis. The difference in volume between 1.0 and 1.5 T scanners was 0.2% for the LEAP method ($p = 0.9$, [75]), 0.9% for manual hippocampal volume ($p = 0.6$), and 1.5% for the lateral ventricle volume ($p = 0.8$). We also tested whether field strength modulated the effect of the volumetric measures on conversion to AD-type dementia. For none of the measures the interaction between field strength and volumetric measure was statistically significantly associated with conversion ($p > 0.15$). A recent study also found a limited effect of fieldstrength on hippocampal volume. This study compared the hippocampal volume measured by the LEAP method between subjects scanned both on a 1.5T and 3T scanner (Wolz 2013, under review). This study found a very high correlation between the measurements on each scanner ($r=0.98$). The volumes measured on 3T were on average 24.4mm^3 or 1.17% larger than on 1.5T (Wolz 2013, under review). This variability was similar to that for volumes rescanned on scanners with the same field strength (1.5%) (Wolz 2013, under review). Taken together, it is unlikely that the small difference in field strength in our study had a major effect

on the volumetric measurements in our study. Different methods were used for ICV correction of both hippocampal measurements (see 2.3.2). Both correction methods were thoroughly compared and showed high correlations (ICC: 0.93 for LEAP with MNI versus FSL scaling and 0.95 for manual volumetric measurement with MNI versus FSL scaling) and similar results regarding predictive accuracy. As a result of this analysis, ICV correction of each individual method was applied. It can be considered a strength that for this diagnostic study a population from a memory clinic setting was used. A consequence however is that these findings may not be applicable to other settings, including the general population.

Clinical implications

Volumetric measurements of the MTL are the best predictors for AD-type dementia in subjects with MCI. Both volumetric measurements strongly correlate with CSF markers of neuronal injury (CSF t-tau and p-tau), are able to predict cognitive decline and show consistent cut-off values between different cohorts. LEAP hippocampal volume has the advantage over manual volumetry that it needs much less rater time and shows no interrater variability effects. In addition, LEAP has a low technical failure rate. Visual rating scales are also quick and easy to perform but show lower predictive accuracy rates and higher inter and intraindividual variability effects [23] compared to LEAP volumetric measurement. Another disadvantage of visual rating scales, although outside the scope of this study, is that they cannot detect subtle atrophy progression and are thus insensitive to change over time [24, 28]. Since the hippocampus is among the first areas affected by the disease [76, 77], repeated measurement of its volume is clinically important. LEAP hippocampal measurement is suitable for implementation in clinical practice with on average four minutes control time on a standard computer. Cut-points that maximised the balance between sensitivity and specificity as expressed by the Youden index or that provided a sensitivity of 85% were defined. The cut-point based on the Youden index may be preferred since it has shown to be more consistent between the multi and single centre cohort (Supplementary Table S3). Depending on the clinical needs also other cut-points may be chosen, that for example maximize the positive or negative predictive value [78]. Any choice for a specific cut-point has a trade-off between the chance of missing the disease (false negative rate, 1-sensitivity) or incorrectly diagnosing someone as having the disease (false positive rate, 1-specificity). The choice will therefore depend on its clinical use. For example, if a treatment for MCI

due to AD would be available the choice may depend on the safety profile of the treatment. If a treatment has many or severe side-effects, high specificity is more important than a high sensitivity and the opposite is true for treatments with little side-effects. In the situation that treatment is not available and biomarkers are used for diagnosis, one may prefer to use a cut-point with a low false positive rate as an incorrect diagnosis of AD may have a major negative impact on the patient.

Future directions

Future MRI-studies need to investigate abnormalities in AD signature regions in and outside the MTL. A recent study found that subjects with future cognitive impairment (preclinical AD and MCI) also showed reduced brain volume in posterior cingulate/precuneus and orbitofrontal cortex, at least 4 years before any cognitive symptoms [79]. Other structural MR studies also found abnormalities in AD or MCI outside the MTL region such as the corpus callosum [80-83], cingulum [84-86], parietal [87], temporal lobe other than MTL [88] and frontal lobe [89]. Future MRI-studies need to investigate abnormalities in AD signature regions in and outside the MTL.

CONCLUSION

Volumetric hippocampal measurements are the best predictors of conversion to AD-type dementia in subjects with MCI after 2 years follow-up and are able to predict annual cognitive decline. For the limited rater time, LEAP automated hippocampal measurement may be preferred.

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SUPPLEMENTARY DATA

Table S1. Measurement logistics MRI.

	Rater time	Computer calculation time
Manual hippocampal measurement	150	-
ICV measurement*	10	-
Segmentation with SIENAX**	-	75
LEAP hippocampal measurement	4	250
MTA-score	5	-
Lateral ventricle	30	-

Time in minutes per scan. *ICV measurement time should be added to manual hippocampal measurement. ** Segmentation time should be added to time needed for lateral ventricle measurement. ICV: intracranial volume, LEAP: learning embeddings for atlas propagation, MTA: medial temporal lobe atrophy.

Table S2. Differences between AD (AD+) and non-AD (AD-) in both cohorts.

	Descripta		VUmc	
	AD – (1)	AD + (2)	AD – (3)	AD + (4)
N	126	30	111	61
Age	69.4 (55.2-89)	74.1 (63-90)**	70.4 (54-87)	72 (56-81)
Female (%)	60.3	56.7	38.7	54.1
Years education	8.4 (3-18)^^	8.9 (3-22) †	11 (6-18)	11 (6-17)
MMSE score baseline	27.5 (18-30)	26 (21-29)**	27 (19-30)	26 (18-30)**
Z-score wordlist – delayed recal	-1.07 (-4.06;1.96)	-1.9 (-3.16;-0.21)***	-1.43 (-3.16;2.35)	-1.94 (-3.46;-0.05)*
% ApoE 4	43.3	53.8	50	68.2
Manual hippo-campus	8107 (5318-12882)^^	6891 (5054 – 9149)***	7714 (5454-10455)	7003 (4850-9892)***
Leap hippocampus	6033 (3684-8281) ^^^	5324 (4297-7254)***	5601 (4088-7061)	5162 (3327-6487) ***
MTA-score	2.5 (0-6) ^^^	3.9 (0-7)*** †	1.8 (0-6)	3 (0-8) ***
Lateral ventricle	51446 (12128-126853)	61652 (25330-144640)	53474 (10786-162353)	64470 (8587-167163) *

All volumetric measurements are corrected for intracranial volume.

AD: Alzheimer's disease, MMSE: Mini-Mental State Examination score, APOe4: apolipoprotein E4, LEAP: Learning Embeddings for Atlas Propagation, MTA: Medial temporal lobe atrophy. Differences between converters and non-converters in each separate cohort (group (1) vs group (2) and group (3) vs group (4): * $p < .05$, ** $p < .01$, *** $p < .001$.

Differences between non-converters (group (1) vs group (3)): ^^ $p < .01$, ^^^ $p < .001$.

Differences between converters (group (2) vs group (4)): † $p < .05$.

Table S3. Cut-off points.

	Youden			Sensitivity 85%		
	Descripa	VUmc	Both	Descripa	VUmc	Both
Manual HC	<7427	<7590	<7559	<8647	<8173	<8379
LEAP HC	<5374	<5431	<5374	<6043	<5794	<5901
MTA-score (L+R)	≥3	≥3	≥3	≥3	≥1	≥2
Lateral ventricle	≥58491	≥45045	≥58491	≥31494	≥38763	≥34859

All volumetric measurements are corrected for intracranial volume and presented in mm³.
HC: hippocampus, LEAP: Learning Embeddings for Atlas Propagation, MTA: Medial temporal lobe atrophy, L: left, R: right.

Fig. S1. Image of hippocampal LEAP segmentation. This figure shows an example of an image of hippocampal volume segmentation for horizontal, coronal, and sagittal sections using the atlas-based LEAP method.

Fig. S2. Bland-Altman Plot displaying the agreement between both volumetric hippocampal measurements (values are log-transformed). Both measures are corrected for intracranial volume.

Appendix S1. Scan parameters and MRI protocols used at each center.

DESCRIPA

CENTER HUDDINGE

Siemens Avanto 1.5 T, 21 slices, FOV 220 mm, FOV phase 87.1, distance factor 30, phase R>L, slice thickness 5.0 mm, TE: 96 ms, TR: 4000 ms, flip angle 150°, number of averages 1.

Siemens Symphony 1.5 T, 21 slices, FOV 220 mm, FOV phase 75.0, distance factor 30, phase R>L, slice thickness 5.0 mm, TE: 99 ms, TR: 4100 ms, flip angle 150°, number of averages 2.

CENTER KUOPIO

Siemens Vision 1.5 T, T1 3D-scan, MPRAGE OBL; COR>TRA, FOV 250, mat 256x256, 128 slices, TR 9.7ms, TE 4 ms, Slice th 2.0mm, no slice gap, flip angle 12°

CENTER MALMO

Siemens Sonata 1.5 T, MPRAGE + Impr-cor, 144 slices, FOV 250 mm, phase R>L, TR 1970, TE 3.93, distance factor 50, slice thickness 1.5 mm, flip angle 15°.

CENTER MUNICH

Siemens Magnetom Vision; 1.5 T; MPRAGE; Slice thickness 1.05 mm, TR 11.4, TE 4.4, TI 300; FOV 256*256; Flip angle 8 graden; number of averages 1.

CENTER THESSALONIKI

Siemens Expert Plus unit 1.0 T, 3D-MPR: 15 (TR) ,7 TE ,8 FLIP ANG., 250 (Slabth), 1,49Ef thick, 168 Partitions, 250 FOV,256x192 Matrix, 1 Aquis. ,ACQ TIME 10,21min

CENTER BRESCIA

Philips Gyroscan PG 1.0 Tesla: sagittal 3D T1 scan: TR= 20 ms, TE= 5 ms, flip angle= 30°, field of view= 220 mm, acquisition matrix 256 × 256, number of slices= 100/130, slice thickness 1,3 mm.

CENTER GENOA

Philips Intera 1.5 Tesla: Sagittal MP RAGE : t1w/3d/tfe, TR 8.5, TE 3.9, Flip 8, thickness 1, no overcontiguous, no gap, matrix 100% 256, fov 256, reconstructed voxel size 1.00/1.00/1.00, rfov 100%, T1 t1w/se, TR 580, TE 15, flip 69, slices 22, thick 5mm, matrix 80% 240, fov 240, rfov 75%, pre (tra)- and postGD-GDPA (tra, sag, cor)

CENTER MAASTRICHT

Philip NT, 1.5 T Gyroscan: T1-weighted images obtained in the coronal plane using a 3D-gradient fast field echo (FFE) sequence. TR = 35 ms, TE = 7 ms, FA= 35, FOV= 240 mm, slice thickness = 1.5 mm, matrix size = 256x256, voxelsize = 0.94mm x 0.94mm x 1.5 mm.

CENTER MANNHEIM

Siemens Medical solution Magnetom, Vision plus 1.5 Tesla: T1 MPR 30, TR=11.4 ms, TE=4.4 ms, flipangle=15, FoV=256mm, format 8/8, slices=162, no gap.

CENTER VUmc (VUmc data from DESCRIPA + an additional sample)

Siemens Magnetom Impact Expert 1.0 T, 3D scan, 168 slices, FOV 250 mm, matrix 256 × 256; slice thickness 1.5 mm, TE: 7 ms, TR: 15 ms, TI 300 ms, flip angle 15°.

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Chapter 4

Sensitivity of different MRI-techniques to assess gray matter atrophy patterns in Alzheimer's disease is region-specific

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ABSTRACT

The present study compares four different structural magnetic resonance imaging techniques used to measure gray matter (GM) atrophy in Alzheimer's disease (AD): manual and automated volumetry, cortical thickness (CT) and voxel-based morphometry (VBM). These techniques are used interchangeably in AD research and thus far it is unclear which technique is superior in detecting abnormalities early in the disease process. 18 healthy participants without any memory impairment, 18 patients with MCI, and 17 patients with mild AD were included and between-group differences were investigated in AD signature regions (areas in the prefrontal cortex (PFC), medial temporal lobe (MTL) and posterior parietal cortex (PPC)). Both manual volumetric measurements and VBM were able to detect GM atrophy in the early stages (differentiation controls and MCI), mainly in the MTL. In the early phase, automated volumetric measurements showed GM differences in the PPC but not in the MTL. In our sample, CT measurements were not sensitive for group differences in the early stages. PFC regions showed abnormalities in the later stages (controls vs AD) when manual volumetric measurements or VBM are employed. Manual volumetric measurements together with VBM are preferred techniques for assessing GM differences showing abnormalities in most of the investigated regions, with a predominance of the MTL in the early phase. Automated FreeSurfer volumetric measurements show similar performances in the early phase, displaying group differences in the PPC but not in MTL regions. Measurements of CT are less sensitive in the MCI stage and its sensitivity is restricted to the MTL and PPC regions in later stages of the disease (AD).

INTRODUCTION

Since the prevalence of Alzheimer's disease (AD) is expected to increase rapidly in the following decades, early detection and intervention in persons who are at risk for developing AD is an important public health goal. Clinical and pathological evidence indicates that older individuals who have significant memory impairment but are not demented may be in a transitional phase between normal aging and AD, a state often referred to as 'mild cognitive impairment' (MCI) [1]. A large part of the patients with MCI have a high likelihood of progressing to probable AD within a few years [2-4]. Brain imaging can aid the early diagnostic process, ultimately by detecting neurodegenerative abnormalities at an earlier stage than the standard neurological examination or before objective signs are present [5, 6]. Imaging can furthermore help to select MCI-subjects for AD-trials by detecting differences in an early stage and is able to monitor disease progression. Within the domain of magnetic resonance imaging (MRI), the major focus lies on manual segmentation of structures known to be involved in dementia, especially the parahippocampal gyrus and the hippocampus [7-9].

The present study compares four different structural MRI techniques used to assess gray matter (GM) atrophy in AD. Manual volumetry, still considered gold standard [10-12], is however time-consuming, making it difficult to implement in routine clinical practice or for large-scale research use. To overcome the disadvantages of manual volumetry, techniques to perform automated (whole brain) analyses have been designed, e.g. Voxel based morphometry (VBM), automated segmentation methods (e.g. FreeSurfer) and cortical thickness (CT). Automated measurements are widely implemented and fast, but may underestimate atrophy status and thus lead to false negatives results [13]. CT analysis in AD patients has revealed cortical thinning in several brain regions known to be affected in AD [14-16] and this thinning has furthermore shown to be related to the severity of AD, even in early stages [17]. The main disadvantage of the method is its inaccuracy in medial cortical areas (e.g. parahippocampal gyrus) and the impossibility to determine cortical thickness in the hippocampal region.

Another widely used technique to study brain atrophy in-vivo is voxel-based morphometry (VBM), a method to calculate voxel-wise gray matter concentration of

brain areas based on a whole-brain analysis [18, 19]. Volume changes of GM, white matter (WM) and cerebrospinal fluid (CSF) are examined separately. The automated computational procedures of VBM are unaffected by inter-rater differences and anatomical boundary shifts are minimized by the utilization of statistical maps and segmentation techniques [20]. VBM can be applied as a first-pass strategy, to generate hypotheses [21]. Disadvantages of VBM can be found in the pre-processing phase, when warping and smoothing of MRI data is applied. Warping algorithms may not always succeed in matching the anatomical characteristics across participants and smoothing furthermore reduces the individual variability of gyrus and sulcus features [22]. Especially when degenerated brains are studied, these steps might induce both type I and II errors in the outcome. VBM furthermore does not provide information of brain atrophy at a single-subject level, it only permits group-level analysis, limiting its diagnostic employability in the clinical setting.

The four techniques mentioned above are used interchangeably in AD research and thus far it is unclear which technique is superior in detecting abnormalities early in the disease process. Since manual volumetric measurements of the medial temporal lobe (MTL) are hard to implement in everyday clinical practice, possible alternatives to measure GM atrophy in AD are evaluated. It is furthermore known that AD pathology is not restricted to the MTL lobe and that prefrontal [23] and posterior parietal regions [24] also show abnormalities early in the disease process. In this study, regions were selected based on their relevance for AD.

This is, to our knowledge, the first study directly comparing four techniques which measure gray matter atrophy in a group of healthy controls, patients with MCI and patients suffering from mild AD. We evaluated which technique is superior in detecting abnormalities early in the disease process. In addition, we investigated whether atrophy of prefrontal cortex (PFC), posterior parietal cortex (PPC) or medial temporal lobe (MTL) regions is most indicative of (preclinical) AD.

MATERIALS AND METHODS

Subjects

Three groups of older male participants were included: 20 healthy participants

without any objective memory impairment (controls), 20 patients with amnesic MCI, and 20 patients with mild AD. Controls were recruited by means of an advertisement in local newspapers. An extensive neuropsychological test battery was administered and subjects were included when their performance did not deviate from normal on the Verbal Learning Test [25, 26]. Patients with MCI and mild AD were recruited from the Memory Clinic of the Maastricht University Hospital. Diagnosis was made according to the Petersen criteria for MCI (with at least an impairment in the memory domain) [27, 28], and the DSM-IV [29] and NINCDS-ADRDA criteria for AD [30]. Diagnosis was based on medical history, co-morbidity, course, and MRI scanning. The MRI scan was only acquired to exclude neurosurgical lesions vascular pathology; levels of atrophy were not used for the diagnosis of MCI and AD.

Exclusion criteria were: MRI-contraindication, abuse of alcohol and drugs, other past or present psychiatric or neurological diseases or serious system diseases, and structural abnormalities in the brain that could account for the cognitive decline. Two participants in the control group were excluded because a brain infarct was detected on the MRI scans. Two participants in the MCI group, and three in the AD group were excluded because their MRI images showed motion artefacts. The study was approved by the local Medical Ethics Committee of the Maastricht University Medical Centre. Written informed consent was obtained from all participants and from the primary caregiver of the AD patients in accordance with the committee's guidelines and with the Declaration of Helsinki [31].

Image acquisition and analysis

MRI scans were acquired with a 3 Tesla Gyroscan NT MRI scanner (Philips, Best, The Netherlands). Structural T1 images were acquired in the sagittal plane using an MPRAGE sequence (TR=8 , TE= 3.7 msec, FA= 8°, FOV= 240 X 240, matrix size= 240 x 240, number of slices= 180). Regions of interest were selected in the prefrontal cortex (inferior prefrontal cortex, orbitofrontal cortex), posterior parietal cortex (precuneus, posterior cingulate cortex) and medial temporal lobe (hippocampus, parahippocampal gyrus). For each technique, left and right side of each selected region were analyzed separately.

Manual volumetry

For the manual tracing we used GIAN (General Image Analysis Tools; [32]), a customized software program which allows tracing of regions of interest (ROI) in a triplanar and rotatable 3D surface-rendered view, and calculation of GM volumes of interest. Boundaries of selected frontal and temporal structures were set according to criteria described in a previous publication [33] (Supplementary Table S1). Boundaries of the posterior cingulate and precuneus cortex were adapted from Jones et al. [34] and Ryu et al. [35] respectively (Supplementary Table S1).

Both raters (LC, CE) were blind to the demographic and cognitive characteristics of the participants. Intra-rater reliability was determined by the Intraclass Correlation Coefficient (ICC) [36]. To account for age-related cortical shrinkage we measured the intracranial volumes (ICV) with the FSL Brain Extraction Tool [37]. Our analyses were corrected for ICV, which partials out individual variability in maximal total brain volume achieved in adulthood independent from possible brain atrophy at later ages (Supplementary Table 1). To control for Type I errors, we performed the false discovery rate (FDR) controlling procedure, a correction for multiple comparisons [38]. This method differs from the classical Bonferonni approach in that it is less stringent and as such also reduces the increased probability of making Type II errors.

Automated volumetry

In accordance with Gronenschild et al. [39], a Macintosh with OSX 10.5.8. was used for analysis. Cortical reconstruction and volumetric segmentation was performed with FreeSurfer version 4.5.0, which is freely available (<http://surfer.nmr.mgh.harvard.edu>). In this approach, brain areas are segmented using a nonlinear template matching [40]. After linearly registering the test image to the template, the algorithm estimates the nonlinear transformation between a given MRI and a probabilistic atlas of the selected brain structure constructed from a cohort of 14 young and middle-aged subjects using a maximum likelihood criterion. Probabilistic labels are warped back to the individual MRI using the inverse of this transform. The final segmentation is accomplished by maximizing the a posteriori probability in the Bayes formula at each voxel. Voxel-wise probabilistic labels and their predicted image intensities serve as the prior term, while the intensity similarity between the target image and the template serves as the likelihood term.

In this study, FreeSurfer voxel volumes were used (not the tabulated volumes corrected for partial volume effects) in order to obtain a proper comparison with the manual volumetric measurement. The borders of the FreeSurfer ROI of the isthmus fits best with our definition of the posterior cingulate cortex as adopted for the manual segmentation and were subsequently used. The posterior cingulate cortex ROI included in the FreeSurfer automated measurement is more rostral compared to our definition.

Cortical thickness (CT)

The FreeSurfer CT pipeline has been described and validated in previous publications (Dale et al., 1999; Fischl et al., 1999; Fischl and Dale, 2000; Han et al., 2006). To summarize, processing involves intensity normalisation, registration to Talairach space, skull stripping, segmentation of WM, tessellation of the WM boundary, smoothing of the tessellated surface and automatic topology correction. The tessellated surface is used as the starting point for a deformable surface algorithm to find the WM and then the pial boundary. For each point on the tessellated WM surface, the CT is calculated as the average of the distance from the WM surface to the closest point on the pial surface and from that point back to the closest point on the WM surface [41]. The cortex of the brain was automatically subdivided into gyral-based regions of interest (ROIs) [42]. To accomplish this, a registration procedure was used that aligns the cortical folding patterns and probabilistically assigns every point on the cortical surface to one of the 32 ROIs. For the purposes of this study, we focused on 6 ROIs bilaterally. For each ROI the mean cortical thickness was extracted for subsequent statistical analysis.

For our second approach, a vertexwise analysis, we mapped the thickness measures on the inflated surface of each participant's reconstructed brain. This allows visualization across the surface without interference from cortical folding. Maps were smoothed using a circularly symmetric Gaussian kernel with a full width half maximum (FWHM) of 20 mm and averaged across participants using a non-rigid high-dimensional spherical method to align cortical folding patterns to a template supplied by FreeSurfer. This procedure provided accurate matching of morphologically homologous cortical locations among participants on the basis of each individual's anatomy while minimizing metric distortion, resulting in a mean measure of cortical thickness at each vertex on the reconstructed surface.

Statistical comparisons between the surface maps were generated by computing a

general linear model of the group effects (corrected for age centered) on thickness at each vertex in the cortical mantle. Maps were created using a statistical threshold of $p = 0.05$. A cluster-wise procedure was performed to correct for multiple comparisons. This method utilizes a simulation to get a measure of the distribution of the maximum cluster size under the null hypothesis. Z-maps are synthesized and smoothed using a residual FWHM, and then thresholded at $p = 0.05$. Next, areas of maximum clusters are recorded, under these specifications, and the procedure is repeated for 5000 iterations. Once the distributions of the maximum cluster size are obtained, correction for multiple comparisons is performed by finding clusters in the statistical maps using the same threshold as was given in the simulation procedure. For each cluster, the p value is the probability of seeing a maximum cluster of that size, or larger, during the simulation. Clusters remaining in similar areas of significance as in the original cortical thickness maps would indicate that the result is not likely due to chance. For each cluster, maximum, minimum, mean and standard deviation of the p -values were extracted.

Voxel-based morphometry (VBM)

Structural data was analyzed with FSL-VBM, a voxel-based morphometry style analysis carried out with FSL tools [43]. First, structural images were brain-extracted using BET [37]. Next, tissue-type segmentation was carried out using FAST4 [44]. The resulting GM partial volume images were then aligned to MNI152 standard space using the affine registration tool FLIRT [45, 46] followed by nonlinear registration using FNIRT [47, 48], which uses a b-spline representation of the registration warp field [49]. The resulting images were averaged to create a study-specific template, to which the native gray matter images were then non-linearly re-registered. The registered partial volume images were then modulated (to correct for local expansion or contraction) by dividing by the Jacobian of the warp field. The modulated segmented images were then smoothed with an isotropic Gaussian kernel with a sigma of 2 mm. Finally, voxelwise general linear modeling (GLM) was applied using permutationbased nonparametric testing (5000 permutations). [50] ROI's for displaying significant volume loss were selected based on the Harvard-Oxford cortical and subcortical atlas, which is also integrated in FSLview for inspection and identification. The following regions were selected: precuneus, hippocampus, parahippocampal gyrus, posterior cingulate gyrus, inferior frontal gyrus (sum of pars opercularis and pars triangularis) and frontal orbital cortex.

Statistical analysis

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 19.0. Demographical and cognitive group differences were compared with ANOVA. The ROI-based measures (manual volumetric measurement, FreeSurfer automated measurement and mean CT) were compared between the 3 groups by means of a pairwise ANCOVA for each separate technique (manual volumetric measurement, FreeSurfer automated measurement and CT-ROI measurement), with volume as dependent variable, group as independent variable (fixed factor), and intracranial volume (ICV) and centered age as covariates. For the vertex-wise CT-analysis, statistical analysis was carried out by means of FreeSurfer (see above, paragraph 2.2.3). For VBM, voxel-wise GLM was applied using permutation-based non-parametric testing, including a correction for multiple comparisons across space. Corrected p -values are stated in the result section (p_{corr}).

RESULTS

Subject characteristics

The three groups significantly differed with respect to age (MCI<AD, $p=0.03$; CON<AD, $p=0.02$), Mini-Mental state examination (MMSE) score (CON>MCI, $p=0.03$; MCI>AD, $p<.001$; CON>AD, $p<.001$) and score on the delayed recall task (CON>MCI, $p<.001$; MCI>AD, $p<.05$; CON>AD, $p<.001$), but not with respect to educational level. Subject characteristics are shown in table 1 and volumetric comparisons between the three groups are shown in supplementary table S2 (manual segmentation) and supplementary table S3 (automated segmentation).

Group differences

Manual volumetric measurement

Manual volumetric measurements of the right parahippocampal gyrus (PhG) were able to differentiate between controls and MCI patients ($F=15.93$, $p_{corr}<.001$). In later stages of the disease (controls versus AD) differentiation was possible in the following structures: left hippocampus ($F=5.14$, $p_{corr}<.05$), right hippocampus ($F=6.86$, $p_{corr}<.05$), left PhG ($F=29.87$, $p_{corr}<.001$), right PhG ($F=21.21$, $p_{corr}<.001$), left orbitofrontal cortex (OFC) ($F=20.24$, $p_{corr}<.001$), right orbitofrontal cortex (OFC) ($F=11.28$, $p_{corr}<.01$), left posterior cingulate cortex (PCC) ($F=4.90$, $p_{corr}<.05$), left

precuneus (PC) ($F=7.50$, $p_{\text{corr}} < .01$) and right PC ($F=15.88$, $p_{\text{corr}} < .001$). Discrimination between early and later stages of the disease (MCI versus AD) was possible by manual measurements of the left PhG ($F=17.20$, $p_{\text{corr}} < .001$), left inferior prefrontal cortex (IPFC) ($F=11.11$, $p_{\text{corr}} < .01$), left OFC ($F=10.66$, $p_{\text{corr}} < .01$) and right PC ($F=6.48$, $p_{\text{corr}} < .01$) (Table 2).

Table 1. Subject characteristics

	Con	MCI	AD
N	18	18	17
Age	64.56 (3.4)	65.11 (4.5)	70.59 (9.1)
Educational level	4 (1.4)	4 (1.8)	4 (1.9)
MMSE score	28.89 (0.9)	27.61 (2.3)	21.18 (3.9)
15 WLT learning	37.50 (7.6)	26.06 (9.8)	23.47 (11.7)
15 WLT memory	8.56 (1.9)	3.67 (2.8)	1.73 (2.4)
Fluency animals	23 (5.3)	21 (5.4)	13.93 (4.7)
Manual hippocampus volume L/R (mm ³)	4656 (308)/ 4758 (637)	4410 (482)/ 4308 (796)	3883 (817)/ 3807 (876)
ICV (ml)	1492 (100)	1539 (121)	1574 (125)

All volumetric measurements are corrected for intracranial volume. Values are mean (sd). MMSE: Mini-Mental state examination; WLT: wordlist; Con: controls; MCI: mild cognitive impairment; AD: Alzheimer's disease.

Automated volumetric measurement

FreeSurfer automated volumetric measurements were able to differentiate between controls and MCI-patients in the left PCC ($F=7.82$, $p_{\text{corr}} < .01$) and right PC ($F=6.78$, $p_{\text{corr}} < .05$). As the disease progresses, differences between controls and AD were most notably in the left hippocampus ($F=11.54$, $p_{\text{corr}} < .01$), right hippocampus ($F=13.35$, $p_{\text{corr}} < .001$), left PCC ($F=14.04$, $p_{\text{corr}} < .001$), right PCC ($F=16.34$, $p_{\text{corr}} < .001$) and right PC ($F=12.69$, $p_{\text{corr}} < .001$) regions. MCI and AD patients could be distinguished by automated measurements of the right PCC ($F=9.85$, $p_{\text{corr}} < .01$).

Cortical thickness

Quantification of cortical thinning by atlas-based ROI-analysis

Differences in CT were only present in the comparison between controls and AD-patients in the right PCC ($F=9.27$, $p_{\text{corr}} < .01$) and right ERC (Table 2).

Vertex-wise group comparison with clusterbased correction

Vertex-wise comparison of CT between controls and AD revealed one cluster in the left hemisphere and three clusters in the right hemisphere that demonstrated significantly reduced cortical thickness in AD (Figure 1, Table 3). These clusters mainly comprised temporal and parietal cortical areas. Two clusters in the left hemisphere, entirely situated in the frontal lobe, showed increased cortical thickness between controls and AD. For the comparison between MCI and AD, thinning was found in AD patients compared to MCI in the left hemisphere in parietal and temporal regions. In the right hemisphere thinning was found in parietal, frontal and temporal regions (Figure 2, Table 3).

VBM

The comparison between controls and MCI or AD showed several regions of significant GM loss compared with controls ($p < 0.05$, TFCE-corrected). VBM measurements of the left and right hippocampus were able to differentiate between controls and MCI patients ($p < 0.05$ and $p < .001$ respectively, TFCE corrected). In later stages (controls versus AD), differentiation was possible in the following structures: left ($p < .001$, TFCE corrected) and right ($p < .001$, TFCE corrected) hippocampus, left ($p < .001$, TFCE corrected) and right PhG ($p < .001$, TFCE corrected), right IPFC ($p < .001$, TFCE corrected), left ($p < .01$, TFCE corrected) and right OFC ($p < .01$, TFCE corrected), left ($p < .001$, TFCE corrected) and right PCC ($p < .001$, TFCE corrected) and left ($p < .01$, TFCE corrected) and right PC ($p < .001$, TFCE corrected). Discrimination between early and later stages of the disease was found for the left ($p < .001$, TFCE corrected) and right ($p < .001$, TFCE corrected) hippocampus, left ($p < .001$, TFCE corrected) and right PhG ($p < .001$, TFCE corrected), left ($p < .01$, TFCE corrected) and right IPFC ($p < .01$, TFCE corrected), left ($p < .01$, TFCE corrected) and right OFC ($p < .001$, TFCE corrected), right PCC ($p < .05$, TFCE corrected), and left (($p < .01$, TFCE corrected) and right PC ($p < .05$, TFCE corrected)) (Table 4).

Table 2. Overview of significant gray matter group differences for each separate technique.

	Manual volumetry [‡]			Automated volumetry [‡]			Mean cortical thickness in ROI [§]		
	CON vs MCI	CON vs AD	MCI vs AD	CON vs MCI	CON vs AD	MCI vs AD	CON vs MCI	CON vs AD	MCI vs AD
Hippocampus L		*			**		n.a.	n.a.	n.a.
Hippocampus R		*			***		n.a.	n.a.	n.a.
ERC L		n.a.	n.a.						
ERC R		n.a.	n.a.				***		
PhG L		***	***						
PhG R	***	***							
IPFC L			**						
IPFC R									
OPFC L		***	**						
OPFC R		**							
PCC L		*		**	***				
PCC R					***	**		**	
PCL		**							
PCR		***	*	*	***				

Con: controls; MCI: Mild cognitive impairment; AD: Alzheimer's disease; ROI: region of interest; L: left; R: right; ERC: entorhinal cortex; PhG: parahippocampal gyrus; IPFC: inferior prefrontal cortex; OPFC: orbital prefrontal cortex; PCC: posterior cingulate cortex; PC: precuneus; n.a.: not available. * p<.05; ** p <.01; ***p <.001. [§]: corrected for age (centered), †: corrected for intracranial volume (ICV). Cortical-thickness analyses were not corrected for ICV.

Table 3. Vertex-wise Cortical Thickness (CT) analysis.

Comparison	Lobe	Region(s)	Cluster size (in vertices)	Mean p-value	SD	Peak
Con vs AD	L	Inferiorparietal (14.57%), inferioriortemporal (10.97%), supramarginal (10.91%), fusiform (10.18%), superioritemporal (9.75%), PC (8.57%), middle temporal (6.61%), lingual (5.23%), bankssts (5.11%), PCC (3.93%), superiorparietal (3.33%), PhG (2.42%), ERC (2.01%)	36954	0.007	0.71	Left posterior cingulate
	R	Superiortemporal (15.52%), middletemporal (12.18%), inferioritemporal (9.97%), fusiform (9.92%), supramarginal (9.67%), insula (6.08%), PCC (5.08%), lingual (4.23%), bankssts (3.66%), PhG (3.03%), PC (2.72%), postcentral (2.44%)	38968	0.004	0.25	Right fusiform
	R	Superior parietal (97%), postcentral (3%)	3639	0.02	0.02	Right superior parietal
	R	Inferior parietal (100%)	3588	0.01	0.01	Right inferior parietal

Significant cluster analyses for CT measurement. Con: controls; MCI: Mild cognitive impairment; AD: Alzheimer's disease; SD: standard deviation; L: left; R: right; ERC: entorhinal cortex; PhG: parahippocampal gyrus; IPFC: inferior prefrontal; bankssts: banks of the superior temporal sulcus . ** posterior cingulate and * isthmus cingulate as defined by Desikan borders.

Continuation Table 3. Vertex-wise Cortical Thickness (CT) analysis.

Comparison	Lobe	Region(s)	Cluster size (in vertices)	Mean p-value	SD	Peak
MCI vs AD	L	Inferior parietal (20.79%), superior parietal (15.64%), supramarginal (15.04%), inferior temporal (11.01%), fusiform (10.82%), middle temporal (5.65%), bankssts (5.34%), lingual (5.04%), lateral occipital (4.72%), superior temporal (1.67%), PhG (1.63%), PCC (1.38%)	29116	0.01	0.25	Left fusiform
	L	PC (70.47%), middle cingulate** (17.24%), PCC* (10.49%), paracentral (1.80%)	4988	0.02	0.57	Left precuneus
	R	Inferior parietal (66.99%), superior parietal (31.59%), supramarginal (0.79%), postcentral (0.64%)	29116	0.03	0.25	Right superior parietal
	R	Superior temporal (19.07%), supramarginal (15.36%), inferior temporal (9.11%), fusiform (8.74%), bankssts (5.31%), postcentral (5.04%), pars opercularis (4.50%), insula (4.27%), pars triangularis (4.21%), PC (3.92%), pars orbitalis (1.54%)	4988	0.007	0.72	Right fusiform

Significant cluster analyses for CT measurement. Con: controls; MCI: Mild cognitive impairment; AD: Alzheimer's disease; SD: standard deviation; L: left; R: right; ERC: entorhinal cortex; PhG: parahippocampal gyrus; IPFC: inferior prefrontal; bankssts: banks of the superior temporal sulcus . ** posterior cingulate and * isthmus cingulate as defined by Desikan borders.

Figure 1. Cortical-thickness (CT) analyses for the differentiation between controls and AD-patients as measured by FreeSurfer. *A. Left hemisphere lateral view, B. Left hemisphere medial view, C. Right hemisphere lateral view and D. Right hemisphere medial view.* Vertex-wise comparison showed one cluster in the left hemisphere and three clusters in the right hemisphere displaying significantly reduced cortical thickness in AD (A, B, C and D: temporal and parietal cortical areas; blue) compared to controls. Two clusters in the left hemisphere, entirely situated in the frontal lobe showed increased cortical thickness between controls and AD (A and B: red-yellow).

Figure 2. Cortical-thickness analyses for the differentiation between MCI and AD-patients as measured by FreeSurfer. *A. Left hemisphere lateral view, B. Left hemisphere medial view, C. Right hemisphere lateral view and D. Right hemisphere medial view.* Vertex-wise comparison showed thinning patterns in AD patients compared to MCI in the left hemisphere in parietal and temporal regions (A and B). In the right hemisphere thinning was found in parietal, frontal and temporal regions (C and D).

Table 4. Vertex-wise Voxel Based Morphometry (VBM) analysis.

Comparison	Lobe	Cluster size (in voxels)	Region(s)	Cluster size	Mean p-value	SD	MNI space coordinates (X/Y/Z)
Con vs MCI	L	1	Hc	69	0.04	0.006	58/57/27
	L	2	Hc	7	0.04	0.006	50/43/39
	R	1	Hc	173	0.03	0.011	32/56/28
	R	2	Hc	15	0.04	0.002	33/45/33
	R	3	Hc	4	0.05	0.001	27/50/32
Con vs AD	L	1	Hc	978	0.002	0.005	57/58/21
	R	1	Hc	1043	0.001	0.005	32/58/21
	L	1	PhG	174	0.004	0.007	60/48/23
	R	1	PhG	188	0.004	0.009	31/48/24
	R	2	PhG	1	0.05	0.000	37/45/34
	R	1	IPFC	416	0.01	0.01	16/67/43
	R	2	IPFC	54	0.03	0.006	66/71/49
	L	1	OPFC	44	0.02	0.02	59/67/27
	R	1	OPFC	20	0.04	0.005	35/73/25
	R	2	OPFC	25	0.02	0.01	31/67/27
	R	3	OPFC	1	0.05	0.000	23/79/29
	R	4	OPFC	3	0.04	0.003	31/76/33
	L	1	PC	204	0.03	0.000	45/36/50
	R	1	PC	579	0.01	0.01	40/36/39
	R	2	PC	3	0.05	0.001	38/42/61

Continuation Table 4. Vertex-wise Voxel Based Morphometry (VBM) analysis.

Comparison	Lobe	Cluster number	Region(s)	Cluster size (in voxels)	Mean p-value	SD	MNI space coordinates (X/Y/Z)
Con vs AD	L	1	PCC	704	0.01	0.01	47/47/53
	R	1	PCC	865	0.01	0.01	43/36/48
MCI vs AD	L	1	Hc	688	0.008	0.01	57/58/21
	R	1	Hc	911	0.003	0.01	32/58/21
	L	1	PhG	191	0.005	0.01	61/50/23
	R	2	PhG	184	0.005	0.01	30/51/23
	L	1	IPFC	72	0.03	0.01	70/66/49
	L	2	IPFC	97	0.02	0.01	66/71/49
	R	1	IPFC	380	0.02	0.01	16/69/45
	L	1	OPFC	53	0.02	0.02	60/67/27
	R	1	OPFC	272	0.02	0.01	38/67/28
	L	1	PC	156	0.03	0.01	49/26/51
	L	2	PC	16	0.03	0.01	52/42/60
	R	1	PC	231	0.03	0.01	41/33/42
	R	1	PCC	29	0.04	0.01	44/36/51
	R	2	PCC	161	0.03	0.01	42/54/52
	R	3	PCC	11	0.04	0.003	39/41/53

Significant cluster analyses for VBM measurement. Con: controls; MCI: Mild cognitive impairment; AD: Alzheimer's disease; SD: standard deviation; L: left; R: right; ERC: entorhinal cortex; PhG: parahippocampal gyrus; IPFC: inferior prefrontal; bankssts: banks of the superior temporal sulcus . ** posterior cingulate and * isthmus cingulate as defined by Desikan borders.

DISCUSSION

The present study showed that four different techniques used to assess gray matter atrophy in the brain show different results in the same dataset. When comparing controls with MCI-patients, manual volumetric and VBM measurements show abnormalities in the MTL region whereas automated volumetric measurements show abnormalities in PPC but not in MTL regions. In AD, abnormalities in the PPC regions (manual volumetric measurements, CT and VBM) and less pronounced in PFC regions (manual volumetric measurements, CT and VBM), become apparent. Differences between early (MCI) and late (AD) phases of the disease were most noticeable in PFC regions (manual volumetric measurements and VBM).

This is to our knowledge the first study directly comparing four different measures to assess gray matter atrophy in the brain. Previous studies assessing gray matter atrophy patterns in AD mainly focused on MTL regions [51-55]. This study evaluates the discriminative power of each of these measurements, in and outside the MTL.

Differences between techniques

In the early phase of the disease when differentiation between controls and MCI is valuable, manual volumetric measurements and VBM were both able to detect differences in the MTL (PhG and Hc respectively) whereas automated FreeSurfer segmentation was sensitive to differences in the posterior parietal lobe. This is in line with current evidence stating that both manual volumetric measurements [10, 56, 57] and VBM are sensitive in an early stage, with a predominance of MTL regions [58-60]. A recent study found that brain volume differences measured by VBM were already present at an early presymptomatic stage of AD, when subjects had no memory complaint or measurable cognitive impairment that would allow a clinical diagnosis of MCI or dementia according to current criteria [50]. It is furthermore known from the literature that automated segmentation techniques such as FreeSurfer are less suitable for hippocampal segmentation due to a frequent overestimation of hippocampal tissue volume leading to false-negative results [61]. This method seems however accurate in differentiating between controls and MCI in the parietal lobe [62, 63], which is again in line with our findings.

In later stages of the disease, manual and VBM measurements showed significant group differences in MTL, PPC and PFC regions. Automated segmentation

and, less pronounced, cortical thickness measurements were sensitive to differences in MTL and PPC, but not in PFC regions.

For differential diagnosis and to monitor disease progression, it is also relevant to investigate which techniques were able to detect affected regions between subjects with MCI and AD. Manual volumetry and VBM were able to differentiate MCI and AD patients in the PhG (manual volumetry), hippocampus (VBM), IPFC, OPFC and PC. Automated volumetry was able to differentiate MCI and AD in the right PCC, and cluster based CT-analysis in the left precuneus.

Regional abnormalities

Our study showed that regional distribution of gray matter abnormalities was technique-dependent. Manual volumetric segmentation and VBM first showed abnormalities in the MTL regions, followed by PPC and PFC regions in the later stages. Automated volumetric measurements first showed abnormalities in the PPC region, followed by MTL regions. Abnormalities in cortical thickness became apparent later in the disease process, mainly in MTL and PPC regions. The increased cortical thickness found in the comparison between controls and AD was found in middlefrontal and orbitofrontal cortex areas, regions previously reported to show increased volume in older subjects [64]. The nature of this increase however remains unclear. In later stages of the disease only manual volumetric and VBM measurements were able to detect differences between controls and AD in PFC regions.

Atrophy progression in Alzheimer's disease

Based on the current literature and our findings a sequence of brain abnormalities in AD can be formulated. Gray matter atrophy initially starts in the anterior parahippocampal gyrus (entorhinal cortex region), then spreads to other medial temporal lobe (hippocampus) and posterior parietal regions (posterior cingulate and precuneus regions) [50, 65-67]. In later stages of the disease, pathology extends to prefrontal cortex regions [68]. This pattern is consistent with the spread of AD pathology evidenced by histopathology.

Limitations

The present study had several limitations. Since only cross-sectional data were available, no evaluation of predictive value for early Alzheimer's disease can be

made for each of these techniques. Only males were included in this study in order to reduce interindividual variation. Generalisation of our findings to females should therefore be done with caution. Previous studies did however not reveal substantial genderrelated differences with respect to atrophy in AD. In this study, efforts were made towards an equality of anatomical boundaries for all of the investigated techniques. Although differences between ROI's were small, borders of manual, automated and VBM measurements were not entirely overlapping. This could have led to some bias in the dataset. Furthermore, all techniques assessed gray matter atrophy but approaches of each technique differed. For example, the comparison of VBM with manual and automated volumetric measurements is not straightforward since VBM analyzes the brain by a voxel-wise analysis compared to the ROI-approach in volumetric measurement. The same is true for the CT ROI-measurement where the mean thickness of gray matter in a ROI is analyzed compared to total cortical volume of a ROI in the volumetric approaches. However, the power of each technique in discriminating between groups was analyzed in a similar statistical manner (with a same amount of statistical corrections). Finally, in this study only FreeSurfer automated volumetry was evaluated. Other (atlas-based) automated measurements, which are outside the scope of this study, are also valuable for evaluation of gray matter atrophy in AD [12, 69, 70].

Clinical implications

Manual volumetry can still be considered as the gold standard for the evaluation of gray matter atrophy in AD [10, 56, 57]. The discriminative power of manual volumetric measurements is however restricted to the MTL in the early phase. Since medial temporal lobe regions such as the parahippocampal gyrus are among the first areas affected by the disease [7], measurement of its volume is clinically important and manual volumetric measurements, however time-consuming, could complement standard clinical practice by reporting GM abnormalities at the single subject-level. Despite the methodological considerations discussed in the introduction, VBM is also sensitive in the early stage. VBM is however less suitable for clinical implementation due to its incapability for reporting reduced GM volume at the single subject-level. A recent study [71] showed that the predictive accuracy for AD-conversion in subjects with MCI is similar for automated (LEAP segmentation) [70] and manual hippocampal measurement. However, the automated measurement used in our study (FreeSurfer), seems less suitable for

evaluation of medial temporal lobe atrophy in the early phase, but can complement the diagnostic process by assessing the degree of (posterior) parietal atrophy [72]. Evaluation of both medial temporal and posterior parietal regions in the early phase can strengthen the diagnosis of underlying AD pathology.

CONCLUSION

We conclude that the discriminative ability of different techniques measuring gray matter atrophy in the brain is region-specific. Manual volumetric measurements together with VBM are preferred showing abnormalities in most of the investigated regions, with a predominance of the MTL in the early phase. Automated volumetric measurements show similar performances in the early phase of the disease, displaying significant differences in the PPC but show limited discriminative power in MTL regions. Automated measurements of cortical thickness are less sensitive in the MCI stage and its sensitivity is furthermore restricted to the MTL and PPC regions in later stages of the disease (AD). Our data furthermore suggests, in line with the literature, that patients suffering from MCI first show abnormalities in the MTL (PhG and hippocampus) and PPC (posterior cingulate and precuneus) followed by abnormalities in the PFC (orbitofrontal and inferior prefrontal cortex) in the later stages.

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SUPPLEMENTAL MATERIAL

Supplementary Table 1. Anatomical boundaries of investigated structures

Brain structure (Brodmann area)	Anatomical boundaries manual segmentation	Rater	ICC (range)	Anatomical boundaries FreeSurfer automated segmentation
Parahippocampal gyrus	Tracing of the parahippocampal gyrus was performed on every coronal slice on which the hippocampus was visible. The subiculum was taken as the dorsal border and the collateral sulcus as the ventral border (Insausti et al., 1998; Visser et al., 1999).	C.E.	0.84-0.98	The rostral and caudal boundaries of the parahippocampal gyrus were the posterior end of the entorhinal cortex and the caudal portion of the hippocampus (where it could be identified inferomedial to the trigone of the lateral ventricle), respectively. The medial boundary was designated as the medial aspect of the temporal lobe and the lateral boundary was the collateral sulcus.
Hippocampus (28)	The volume of the hippocampus included the hippocampus proper (including the dentate gyrus), the alveus, and the subiculum. The anterior and posterior borders were based on both sagittal and coronal sections of the brain.	C.E.	0.91-0.99	The volume of the hippocampus included the hippocampus proper, the alveus, the uncus, the dentate, the subiculum, and retrosplenial gyri. It was defined superolaterally by the interface with the inferior horn of the lateral ventricle, inferolaterally by white matter, and inferomedially by a secondary border connecting the medial tip of the subadjacent white matter with the subiculum-parahippocampal gyrus interface [73].

Continuation Supplementary Table 1. Anatomical boundaries of investigated structures

Brain structure (Brodmann area)	Anatomical boundaries manual segmentation	Rater	ICC (range)	Anatomical boundaries FreeSurfer automated segmentation
Inferior prefrontal cortex (44/45)	<p>The ventral-anterior border was the anterior horizontal ramus of the lateral (cq. Sylvian) fissure and the dorsal border was the fundus of the inferior frontal sulcus.</p> <p>Posteriorly, we used the fundus of the precentral sulcus as the border.</p> <p>The anterior border was formed by the frontal pole region. Since no clear marker indicates the borders with the dorsolateral prefrontal cortex, the inferior and the orbital prefrontal cortex, a pragmatic cut-off was used, viz. a vertical plane was drawn from the middle between the anterior top of the cingulate sulcus and the end of the anterior tip of the olfactory sulcus to set the borders of the frontal pole [74].</p>	L.C.	0.84-0.98	<p>The whole of the inferior frontal gyrus was labelled volumetrically, and then was subdivided on the surface. The rostral boundary was the rostral extent of the inferior frontal sulcus, and the caudal boundary was the precentral gyrus. The medial and lateral boundaries were the lateral bank of the inferior frontal sulcus and the medial bank of the lateral orbital sulcus and/or the circular insular sulcus, respectively (a) Pars opercularis. The first gyrus from the precentral gyrus.</p> <p>(b) Pars triangularis. The second gyrus from the precentral gyrus.</p>

Continuation Supplementary Table 1. Anatomical boundaries of investigated structures

Brain structure (Brodmann area)	Anatomical boundaries manual segmentation	Rater	ICC (range)	Anatomical boundaries FreeSurfer automated segmentation
Orbitofrontal cortex (47/11/12)	Based upon cytoarchitecture (HBMU), the posterior border was defined by a straight line that was drawn downwards from the inner curvature of the corpus callosum. The anterior border consisted of the frontal pole region. For the posterior boundary of the frontal pole, we selected a vertical plane drawn from the middle between the anterior tip of the cingulate sulcus and the anterior tip of the olfactory sulcus, which allows us to exclude a majority of area 10 [74]. Medially, this region was bounded by the ventral part of the anterior cingulate region. The lateral border was anteriorly the horizontal ramus of the Sylvian fissure and posteriorly the circular sulcus of the insula.	L.C.	0.84-0.98	(a) Lateral division. The rostral boundary of the lateral division of the orbitofrontal cortex was the rostral extent of the lateral orbital gyrus (where it appears with the frontomarginal sulcus), and the caudal boundary was the caudal portion of the lateral orbital gyrus. The medial and lateral boundaries were the midpoint of the olfactory sulcus and the lateral bank of the lateral orbital sulcus and/or the circular insular sulcus, respectively. (b) Medial division. The rostral boundary of the medial division of the orbitofrontal cortex was the rostral extent of the medial orbital gyrus, and the caudal boundary was the caudal portion of the medial orbital gyrus/gyrus rectus. The medial and lateral boundaries were the cingulate cortex on the inflated surface and the medial bank of the superior frontal gyrus (or the cingulate gyrus when visible), respectively.

Continuation Supplementary Table 1. Anatomical boundaries of investigated structures

Brain structure (Brodmann area)	Anatomical boundaries manual segmentation	Rater	ICC (range)	Anatomical boundaries FreeSurfer automated segmentation
Posterior cingulate cortex (23/29/30)	Posterior cingulate cortex (PCC) borders were marked sagittally using predefined borders. First the most caudal coronal slices on which the anterior commissure (AC) was visible and the most caudal coronal slice on which the posterior commissure (PC) was visible were used to subdivide the cingulate gyrus. The PC line was used as the anterior border of the PCC. Hereafter, a line was drawn in the parieto-occipital sulcus (POS) to define the posterior border of the PCC. All slices between the PC and POS were subsequently traced on coronal slices. The retrosplenial cortex (RS), which corresponds with Brodmann areas 29 and 30, was not objectively divided from the posterior part of the cingulate gyrus and was included in our definition of the PCC.	L.C.	0.96-0.98	The rostral and caudal boundaries were the posterior division of the cingulate cortex and the parahippocampal gyrus, respectively. The medial and lateral boundaries were the medial wall (area unknown) and the precuneus, respectively.

Continuation Supplementary Table 1. Anatomical boundaries of investigated structures

Brain structure (Brodmann area)	Anatomical boundaries manual segmentation	Rater	ICC (range)	Anatomical boundaries FreeSurfer automated segmentation
Precuneus cortex (7)	Precuneus borders were set coronally at the level where both the marginal ramus and subparietal sulcus can be identified (anterior border) and were the parieto-occipital sulcus disappears (posterior border). As superior border we used the superior margin of the brain, as inferior border the subparietal sulcus. The outline of the precuneus was traced manually on each sagittal slice.	L.C.	0.99	The rostral boundary was the posterior extent of the paracentral lobule, and the caudal boundary was the lingual gyrus. The medial and lateral boundaries were the parieto-occipital fissure and the superior parietal gyrus, respectively.

Supplementary Table 2. Volumetric differences between groups as measured by manual segmentation.

Structure	Controls (n=18)	MCI (n=18)	AD (n=17)
L Hippocampus	4656.17 (308.46)	4410.89 (482.34)	3882.65 (817.08)
R Hippocampus	4757.61 (637.47)	4308.28 (795.99)	3806.82 (875.62)
L Parahippocampal gyrus	2707.17 (353.38)	2548.33 (449.67)	1831.94 (383.76)
R Parahippocampal gyrus	2823.11 (347.69)	2299.89 (438.82)	1980.35 (443.64)
L Posterior cingulum cortex	2428.39 (384.92)	2291.83 (441.22)	2041.00 (527.06)
R Posterior cingulum cortex	2348.39 (363.36)	2371.39 (452.24)	2029.88 (457.14)
L Precuneus cortex	9353.11 (1689.59)	8949.72 (1294.82)	7828.23 (1709.58)
R Precuneus cortex	9624.89 (1566.74)	8666.72 (1294.82)	7550.71 (1377.01)
L Inferior prefrontal cortex	4694.94 (1047.90)	5084.22 (941.53)	3733.35 (1167.05)
R Inferior prefrontal cortex	4650.61 (1279.52)	4716.83 (1114.54)	4192.76 (1399.28)
L Orbitofrontal cortex	7637.56 (1067.35)	7291.33 (1855.00)	4822.65 (1780.15)
R Orbitofrontal cortex	7332.28 (1149.51)	6910.67 (1715.06)	5118.12 (1646.07)

Results are shown as mean (standard deviation). Values are in mm³. L: left, R: right, MCI: mild cognitive impairment, AD: Alzheimer’s disease.

Supplementary Table 3. Volumetric differences between groups as measured by FreeSurfer automated segmentation.

Structure	Controls (n=18)	MCI (n=18)	AD (n=17)
L Hippocampus	3959.83 (300.96)	3657.67 (641.96)	3350.06 (586.00)
R Hippocampus	3952.22 (288.18)	3773.33 (769.96)	3268.94 (604.55)
L Parahippocampal gyrus	2422.83 (319.85)	2365.50 (391.91)	2190.82 (355.63)
R Parahippocampal gyrus	2231.89 (442.95)	2089.61 (367.29)	1959.18 (377.09)
L Entorhinal cortex	1753.83 (424.37)	1670.72 (628.05)	1570.53 (448.11)
R Entorhinal cortex	1813.56 (424.37)	1504.05 (522.00)	1386.53 (402.17)
L Posterior cingulum cortex	2441.89 (374.45)	2195.89 (291.75)	2076.65 (290.69)
R Posterior cingulum cortex	2254.11 (395.45)	2084.94 (287.22)	1849.47 (317.70)
L Precuneus cortex	9061.56 (1201.80)	9088.94 (1080.63)	8512.00 (1374.91)
R Precuneus cortex	10284.55 (1320.47)	9525.67 (1127.67)	8780.41 (1262.93)
L Inferior prefrontal cortex	8026.17 (1336.90)	7741.67 (1249.68)	7639.12 (1710.98)
R Inferior prefrontal cortex	7551.72 (1301.51)	7896.56 (1646.70)	7427.12 (1334.26)
L Orbitofrontal cortex	10786.22 (1205.57)	10793.33 (1440.10)	10836.22 (1205.57)
R Orbitofrontal cortex	10711.89 (1298.58)	11080.17 (1406.77)	10574.71 (1239.31)

Results are shown as mean (standard deviation). Values are in mm³. L: left, R: right, MCI: mild cognitive impairment,

AD: Alzheimer's disease.

Chapter 5

New MRI markers for AD: a meta-analysis of diffusion tensor imaging and a comparison with medial temporal lobe measurements

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ABSTRACT

The aim of the present study is to evaluate the diagnostic value of diffusion tensor imaging (DTI) for early Alzheimer's disease (AD) in comparison to widely accepted medial temporal lobe (MTL) atrophy measurements. A systematic literature research was performed into DTI and MTL atrophy in AD and mild cognitive impairment (MCI). We included seventy-six studies on MTL atrophy including 8122 subjects and fifty-five DTI studies including 2791 subjects. Outcome measure was the effect size (ES) expressed as Hedges g . In volumetric studies, atrophy of the MTL significantly differentiated between AD and controls (ES 1.32-1.98) and MCI and controls (ES 0.61-1.46). In DTI-Fractional anisotropy (FA) studies, the total cingulum differentiated best between AD and controls (ES=1.73) and the parahippocampal cingulum between MCI and controls (ES=0.97). In DTI-Mean diffusivity (MD) studies, the hippocampus differentiated best between AD and controls (ES=-1.17) and between MCI and controls (ES=-1.00). We can conclude that in general, the ES of volumetric MTL atrophy measurements was equal or larger than that of DTI measurements. However, for the comparison between controls and MCI-patients, ES of hippocampal MD was larger than ES of hippocampal volume. Furthermore, it seems that MD values have somewhat more discriminative power than FA values with higher ES in the frontal, parietal, occipital and temporal lobe.

INTRODUCTION

Alzheimer's disease (AD), the most common cause of dementia, affects about 33.9 million people worldwide. Over the next forty years, the prevalence of AD is expected to triple due to the aging population [1]. This creates an urgent need for early diagnosis of the disease to enable treatment at an early stage. Many AD-related research has focused on mild cognitive impairment (MCI), a transitional state between normal aging and early AD [2, 3]. Estimates of annual conversion from MCI to AD show a great variability, from 5% to 40% per year [4], depending primarily upon the setting in which the studies were carried out (clinical versus population-based studies). Medial temporal lobe (MTL) atrophy as assessed on structural magnetic resonance imaging (MRI) has proven to be an effective clinical aid in the early diagnosis of AD [5], and this method could predict AD in subjects with MCI [6-8]. Still, the diagnostic accuracy of MTL atrophy for AD is only moderately high [9].

Recently, MRI techniques have become available which can measure the integrity of cerebral white matter (WM). It has been shown that WM integrity is decreased in subjects with MCI and AD suggesting a potential diagnostic value that may possibly help to improve the diagnostic accuracy of MTL atrophy measurements [10, 11]. Integrity of WM tracts can be assessed using diffusion tensor imaging (DTI), which measures the diffusion of water molecules in neural tissue. By means of DTI one can detect whole-brain diffusional abnormalities at a stage where macrostructural changes may not have become visible yet. Hence, DTI may be an early marker for neurodegeneration. DTI-studies mostly use fractional anisotropy (FA) and mean diffusivity (MD) measurements in a priori defined regions of interest (ROI) or whole-brain voxel wise analyses to evaluate the integrity of WM in subjects with AD and MCI. FA is a measure of anisotropic water diffusion and reflects the degree of directional diffusion in cellular structures within the fiber tracts. By using FA one can evaluate fiber density, axonal diameter and myelination in WM [12]. A decrease in FA indicates a loss of fiber tract integrity and thus WM damage [13]. MD is a measure of diffusion in the noncolinear direction of free diffusion (translational diffusion). MD reflects the average diffusion within a voxel [13, 14]. An increase in MD represents a loss of anisotropy due to an increase in free water diffusion [15]. Abnormally decreased FA and increased MD in subjects with MCI and AD compared to controls suggests tissue damage which is consistent with neuropathological data

demonstrating partial loss of myelin, axons, and oligodendrial cells in white matter [16].

To date there have been several descriptive reviews of DTI studies of AD and MCI [15, 17, 18] and one review recently quantified the diagnostic value of DTI in subjects with MCI and AD [19]. The aim of the present study was to evaluate the potential diagnostic value of DTI for early AD in comparison to widely accepted MTL atrophy measurements by using a meta-analysis approach. We tested the ability of MTL and DTI measurements to discriminate between controls, subjects with MCI, and subjects with AD-type dementia. This meta-analysis is the first to quantify the potential diagnostic value of DTI in comparison with widely used MTL atrophy measurements. Furthermore, it is the first time that effect size (ES) measures between subjects with MCI and AD are compared with each other. This is important from a clinical point of view, as it indicates the degree of overlap between MCI and AD.

METHOD

Search strategy

An online literature search of the database PUBMED was conducted by one rater (LC) up to August 2011. The keywords used for searching volumetric MTL and DTI studies are listed in Table 1. Abstracts of the retrieved articles were screened for relevance. Searching the references of relevant papers identified further articles for inclusion.

Data selection

Inclusion criteria were: (i) the study included subjects with AD and/or MCI; (ii) the data on average DTI and/or MTL values and standard deviation were provided; (iii) the article was limited to humans; and (iv) the article was written in English, Dutch, German, or French. If raw scores were not presented in the article, authors were contacted and resulting data were added to the meta-analysis. Since ES was calculated with correction for sample size (see below), studies with a relatively small sample size in one of the groups ($n < 10$) were also included. We excluded MTL-studies that did not correct for intracranial volume (ICV), post mortem studies and studies using patient subgroups of ‘vascular’ MCI or AD. For

the DTI articles, limitation of the search to the time period of the last 10 years ensured that the scanning techniques met current standards. We included DTI studies that used different methods of extracting DTI data: manual segmentation, (semi-) automated ROI or tractography. Studies using whole-brain voxelwise analyses (VBM) and tract-based spatial statistics (TBSS) were also included.

Data extraction and analysis

One rater (LC) recorded the mean and standard deviation of all the investigated structures for each subgroup (controls, MCI, AD) by means of a data extraction form using Microsoft Excel (Microsoft Corporation, Redmond, USA). Data on different MTL structures (hippocampus, entorhinal cortex and parahippocampal gyrus) were extracted separately. Values in ROI were taken from the average of left and right hemisphere. Articles that reported the visually rated MTA score [20] were also included. Diffusional data were classified in the same way, according to region and DTI modality. DTI- measurements of the following regions were included in the meta-analysis: frontal, parietal, temporal, or occipital lobe white matter (WM), hippocampus, parahippocampal gyrus, anterior, middle, posterior, parahippocampal and total cingulum, anterior and posterior corpus callosum (CC), uncinate fasciculus (UF), superior longitudinal fasciculus (SLF), and internal capsule. To correct for small sample size, ES was calculated using Hedge's g , which is the difference between group means divided by the pooled standard deviation [21]. Effect sizes between 0.2 and 0.5 were classified as small, between 0.5 and 0.8 as medium, and over 0.8 as large [22]. The following group comparisons were made: subjects with AD versus controls, subjects with MCI versus controls and subjects with AD versus subjects with MCI. We only performed pooled analyses if at least 3 independent studies contributed data for a specific combination of region and technique [23].

Heterogeneity was assessed with Cochrane's Q , which is calculated as the weighted sum of squared differences between individual study effects and the pooled effect across studies [24]. Since there was a significant level of heterogeneity between the studies, pooled Hedge's g was calculated using a random effects model [25]. Basic assumption of the random effects model is that the effects being estimated in the different studies are not identical, but follow some distribution around a mean global effect. We performed meta-analysis to identify possible explanations for heterogeneity. We tested the effect of subject characteristics (age, Mini Mental

State Examination (MMSE) and Clinical Dementia Rating Scale (global score) (CDR)) and data acquisition parameters (MTL: field strength and slice thickness; DTI: field strength, b-value, voxel size, number of directions and number of excitations (NEX)) on Hedge's g using random effects meta-regression. Analyses were only performed if at least 6 studies provided data for the analysis.

Publication bias or the tendency of researchers to handle the reporting of experimental results that are positive differently from those that are negative (or inconclusive), leads to bias in the overall published literature [26]. This bias may lead to an overestimation of observed effects. We tested for this with the Begg and Mazumdar adjusted rank correlation test [27] and with funnel plots.

RESULTS

MTL atrophy

In total 2544 articles assessing MTL structures in AD and/or MCI were found. Seventy-six studies investigated atrophy in the MTL region and were subsequently included with a total of 8122 participants: 2431 subjects with AD, 2515 subjects with MCI and 3176 controls. Subject characteristics of the included MTL atrophy studies are provided in Table 2. Technical specifications of the MTL atrophy studies can be found in Supplementary Table 1.

MTL measurements

For the comparison between subjects with AD and controls, ES Hedge's g was significant for all 4 measures studied (Table 3). Effect sizes were all large, ranging from 1.32 (parahippocampal gyrus) to 1.98 (entorhinal cortex). When comparing subjects with MCI and controls, Hedge's g was again significant in all 4 measures investigated (Table 3). ES was large for entorhinal cortex volume (1.46), MTA-score (1.11) and hippocampal volume (0.89) and medium for parahippocampal gyrus volume (0.61). The comparison of subjects with MCI and AD was significant for all 4 measures (Table 3). ES was large for entorhinal cortex (1.17), hippocampus (0.93) and parahippocampal gyrus (0.92) and medium for MTA-score (0.74).

Heterogeneity and meta-regression

Nine of 12 effect sizes showed a significant level of heterogeneity (Table 3). Fixed effects meta-regression showed no significant associations between ES and subject

characteristics or data acquisition parameters.

Publication bias

Four of 12 group comparisons (controls vs AD; entorhinal cortex and hippocampal volume, controls vs MCI; entorhinal cortex, MCI vs AD; hippocampal volume) displayed a significant level of publication bias as measured by Begg and Mazumdar's rank correlations (Table 3).

DTI

In total 415 articles on DTI in AD and/or MCI were found. Fifty-five studies were included in the meta-analysis with a total of 2791 participants: 819 AD, 715 MCI and 1257 controls. Subject characteristics of the included DTI studies are provided in Table 4. Technical specifications of the DTI studies can be found in Supplementary Table 2.

Fractional anisotropy

For the comparison between subjects with AD and controls, the ES was significant in 10 of the 15 investigated regions (Table 5). A large ES was found in the total cingulum (1.73), parahippocampal cingulum (1.17), splenium of the corpus callosum (CC) (1.10), uncinate fasciculus (1.03) and posterior cingulum (0.84). Medium effect sizes were found in the SLF (0.77), frontal lobe (0.70), genu CC (0.63), temporal lobe (0.57) and middle cingulum (0.42).

Between subjects with MCI and controls, Hedge's *g* was significant in 5 of the 12 regions studied (Table 5). A large ES was found in the parahippocampal cingulum (0.97) and a medium effect size in the posterior cingulum (0.65). A small ES was detected in splenium CC (0.44), genu CC (0.41) and temporal lobe (0.38). The comparison between subjects with MCI and AD was significant in 5 of the 8 regions studied: splenium CC (0.51), posterior cingulum (0.37), genu CC (0.35), frontal lobe (0.29) and temporal lobe (0.27). Effect sizes were all small (Table 5).

Mean diffusivity

For the comparison of AD and controls, the ES was significant in all the 10 investigated regions (Table 6). A large ES was found in the hippocampus (-1.17), parietal lobe (-1.03), splenium CC (-0.94) and temporal lobe (-0.89). A medium ES was detected in the frontal lobe (-0.75), posterior cingulum (-0.74), UF (-0.72), genu

CC (-0.67), occipital lobe (-0.67) and internal capsule (-0.66). When comparing MCI with controls, Hedge's g was significant in 7 of the 9 investigated regions (Table 5). A large ES was found in the hippocampus (-1.00) and a medium ES in the parietal lobe (-0.69). A small ES was found in splenium CC (-0.46), genu CC (-0.43), temporal lobe (-0.40), frontal lobe (-0.32) and posterior cingulum (-0.26). The comparison between subjects with MCI and AD was significant in 3 of the 5 regions studied: posterior cingulum (-0.40), genu CC (-0.32) and splenium CC (-0.04). All effect sizes were small (Table 5).

Heterogeneity and meta-regression

Twenty of 35 effect sizes for FA (Table 5) and 10 of 24 effect sizes for MD (Table 6) showed a significant level of heterogeneity. To investigate possible sources of heterogeneity, fixed effect regression was performed using patient characteristics (age, MMSE, CDR) and acquisition details (field strength, b-value, voxel size, number of directions and number of excitation). Meta-regression found some evidence that the ES was dependent on disease severity. In the comparison between controls and subjects with AD ES increased with severity of the disease: ES of parietal lobe FA ($B=-0.1$, $p=0.04$) and genu CC MD ($B=-0.2$, $p=0.01$) increased with lower MMSE-scores. Comparing controls and MCI-patients: ES of occipital lobe MD ($B=-0.18$, $p<0.001$) and hippocampal MD ($B=-0.5$, $p=0.02$) increased with lower MMSE-scores (Figure 1). Meta-regression furthermore indicated that a number of technical parameters were associated with ES (Supplementary Table 3).

Publication bias

Seven of 59 group comparisons for DTI displayed a significant level of publication bias, measured by Begg and Mazumdar's rank correlations. For FA, publication bias was present at comparisons between controls and AD in frontal WM, genu CC, splenium CC and uncinate fasciculus, and between controls and MCI in splenium CC. For MD, the parietal lobe (controls vs AD) and the hippocampus (controls vs MCI) showed a significant level of publication bias (Tables 5 and 6, Figure 2).

DISCUSSION

The aim of the present study was to compare the value of structural MTL measurements with DTI modalities in discriminating controls, subjects with MCI and AD. Volumetric MTL atrophy measurements showed large effect sizes in all MTL regions investigated. Besides volumetric assessment of MTL regions, also diffusivity measurements seem to be sensitive for group differences in distinct brain regions, especially in temporal, parietal, cingulate and callosal regions.

Group differences within and between MRI modalities

For all group comparisons, the absolute ES of volumetric MTL atrophy measurements was somewhat larger than for the DTI measurements. However, for the comparison between controls and MCI-patients, ES of hippocampal MD was larger than ES of hippocampal volume. This is in line with Müller [28] who stated that elevated diffusivity (MD) in hippocampal regions, particularly on the left side, was the strongest independent predictor of poor verbal memory performance in the total group of MCI patients and controls whereas hippocampus size could explain rather low proportions of variation in memory function.

As expected, for both structural and DTI measurements, ES for the differences between AD and controls were larger than the ES for the differences between MCI/controls, and MCI/AD.

The largest difference in ES between MTL atrophy and DTI measurements was seen in the comparison between MCI and AD. In this comparison, the DTI measurements had smaller ES than MTL atrophy measurements, indicating that DTI measurements showed more overlap between subjects with MCI and AD than MTL atrophy measurements. This could mean that in subjects with cognitive impairment loss of WM integrity precedes macroscopic atrophy. It could also be due to the fact that the difference in average MMSE score between subjects with MCI and AD was slightly larger in the MTL atrophy studies (5.10) compared to the DTI studies (4.45), which may increase the ES for the MTL atrophy studies (see below).

There may be several explanations for the differences in ES between MTL atrophy and DTI measurements. First, there are more studies available investigating MTL atrophy compared to DTI, resulting in somewhat smaller ES and larger standard

deviations for the DTI studies. Second, the ES is dependent on both the difference between the groups and the standard deviation and thus it is possible that in some regions DTI modalities have a somewhat lower ES due to a larger variability between subjects. Third, as mentioned above, subjects with MCI or AD included in MTL atrophy studies might have more severe cognitive impairment than subjects with MCI and AD included in DTI studies. However, we found no significant differences in severity of cognitive impairment in subjects with AD between MTL atrophy and DTI studies, as measured by the MMSE. For the MTL studies mean (sd) MMSE score for AD-patients was 21.41 (2.38) compared to 21.39 (3.04) for the AD-patients in the DTI studies ($p=0.85$). Although it is possible that for specific comparisons there may be differences in disease severity (see above), it seems unlikely that the differences in ES between MTL and DTI studies are in general attributable to differences in disease severity between the MTL atrophy and DTI studies.

Topographic differences

DTI abnormalities were found in many different regions. FA and MD changes seem to follow a slightly different anatomical pattern. Highest ES (relative to controls) for DTI-FA were typically seen in cingulate and callosal areas (total cingulum, posterior cingulum, parahippocampal cingulum, splenium CC and uncinate fasciculus). For DTI-MD the largest differences were seen in the hippocampus, followed by the parietal lobe, splenium CC and temporal lobe. In general effect sizes of DTI-FA were comparable to those of DTI-MD. Smaller effect sizes in DTI-FA compared to DTI-MD were most notable in frontal, parietal, occipital and temporal lobe. This could be due to the fact that in regions with a more complex architecture, FA values are more variable due to a decrease in crossing fibers or other non-parallel organization [15]. It furthermore seems that the discriminative power of DTI-MD is slightly higher than for DTI-FA, with a broader set of regions showing significant white matter abnormalities. This finding is in line with the current literature stating that DTI-FA is a less sensitive marker for decreased white matter integrity than DTI-MD [29-31].

MTL atrophy was assessed in different ways and data suggest that entorhinal cortex (ERC) volume is the best volumetric discriminator as ERC showed the largest ES for all group comparisons. These results are in line with previous histological studies, which showed that the earliest neuropathological changes in AD appear in the ERC [32-34]. Furthermore, several MRI studies have suggested that volumetric measurements of

the ERC are more sensitive than hippocampal volume for predicting AD in subjects with MCI [35-37]. Structural MR studies also revealed abnormalities in AD or MCI outside the MTL region. Callosal atrophy [38-41], cingulum atrophy [33, 42, 43], parietal [44] and frontal atrophy [45] have all been reported to be abnormal in MCI or AD.

Clinical implications

Our findings suggest that DTI may have the potential to be used in clinical practice in a similar way as MTL measurements. However, it is less likely that DTI will soon replace MTL atrophy assessment. First, there is limited information on how well DTI can discriminate subjects with AD from subjects with other types of dementia. At present there are insufficient studies available to provide pooled estimates for these differences. Preliminary findings suggest that FA values of the anterior WM are decreased in vascular dementia as well [46]. Another study [47] found that subjects with Lewy Body Dementia showed abnormalities in the corpus callosum, pericallosal areas and the frontal, parietal, occipital and, less prominently, in temporal white matter. Subjects with frontotemporal dementia (FTD) had greater reductions of FA in frontal brain regions relative to AD, whereas no other region in AD showed greater reductions of FA when compared to FTD [48]. Second, longitudinal DTI-studies on the prediction of AD in non-demented subjects are currently lacking. In the case of MTL measurements, studies indicate that MTL atrophy is a strong predictor for AD in subjects with MCI with an ES ranging from 0.54 to 2.25 [9]. Third, to date DTI measurements generally provided group differences rather than dichotomized values that can be used in clinical practice. Sensitive cut-off scores for different DTI-modalities in distinct brain regions need to be determined and validated.

Limitations

The present study had several limitations. With regard to some brain structures our meta-analysis was based on relatively few studies, which reduced the accuracy of the parameter estimates. Moreover, as analyses of the different techniques were based on different studies, we cannot exclude the possibility that differences in ES resulted from differences in sample selection and other study characteristics. We frequently observed statistically significant heterogeneity between the studies. Meta-regression could only explain part of this heterogeneity. Moreover, for some parameters, the number of studies available for meta-regression analyses was small,

which limited statistical power. In general slice thickness in DTI studies was higher than in MTL studies. This factor might have reduced sensitivity of DTI markers. The only outcome measure calculated in this study is the ES. Accuracy measurements such as sensitivity or specificity could not be taken into account since dichotomous data of the included studies was not available. One author [30] reported a sensitivity up to 89% for MD and 78% for FA measurements for the detection of hippocampal abnormalities in subjects with MCI. For a number of regions and comparisons, data had been published in less than 3 studies and could therefore not be included in the meta-analysis. For example, only a few studies investigated DTI in the entorhinal cortex or the perforant pathway. Furthermore, it was only possible to examine FA differences in all cingulum regions between controls and subjects with AD. Superior longitudinal fasciculus, uncinate fasciculus and internal capsule could also not be examined for all group comparisons. Our results may have been affected by publication bias and by the fact that not all studies presented means and standard deviations for the regions studied or did not reply to our request for data. However, it is unlikely that publication bias substantially influenced our results since Begg and Mazumdar's rank correlation was only significant ($p < .05$) for 4 group comparison in the MTL studies and 7 group comparisons in the DTI studies. Furthermore, a number of these significant comparisons could be due to multiple testing effects since only one of these comparisons was significant at $p < .01$. We limited our meta-analysis to FA and MD. However, it has recently been suggested that the examination of directional diffusivities such as axial (DA) and radial diffusion (DR) may yield important information about the underlying neuropathology driving differences in FA as well [49-52]. One author suggested that DA and DR may be even more reliable markers of degeneration than FA [29]. Still, there are not enough studies to conduct a separate meta-analysis of these modalities. It has to be noted that in many studies FA values in the hippocampal region, which mainly consists of gray matter, are usually ignored. Mean FA values in the hippocampus are generally lower than 0.2, which is close to the noise level. Hippocampal FA therefore needs to be interpreted with caution.

CONCLUSION

Despite the fact that one study suggested that DTI measurements seem to be more sensitive than volumetric measurements [30] or that measures of microstructure provide unique information not obtainable with volumetric mapping in regions known to be crucial in AD [53], our review does not support the notion that DTI is superior to structural MTL assessment in detecting early stage AD. Nonetheless, based on the current literature [19] and our findings, we can conclude that DTI is a sensitive method to detect white matter changes in subjects with MCI and AD. It seems that in widespread brain regions diffusivity is already impaired early in the disease process, with more severe white matter disruptions with increasing disease severity. There is a need of additional studies that test the predictive accuracy of DTI measurements for AD in subjects with MCI and that test differences in DTI measurements between AD and other dementias. In order to better understand the development of WM integrity loss in relation to grey matter loss and the diagnostic potential of DTI measurements, there is a particular need for longitudinal MRI studies that combine structural with DTI assessments. Such studies will advance our understanding of the effects of neurodegeneration on gray matter, white matter, and their interaction and will enhance our ability to detect subtle brain changes early in the disease process. Since diagnostic criteria for prodromal AD require the presence of episodic memory impairment in combination with at least one AD biomarker [5, 54], future studies should compare the use of DTI makers for the diagnosis of prodromal AD with that of other AD biomarkers such as CSF markers [55, 56], PET imaging markers [57] or familial genetic mutations [58].

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Table 1. Search terms used to search the databases PUBMED and ISI WEB OF KNOWLEDGE

Search terms
("medial temporal lobe") and ("magnetic resonance imaging") and ("Alzheimer*" or "mild cognitive impairment")
("hippocampus") and ("magnetic resonance imaging") and ("Alzheimer*" or "mild cognitive impairment")
("entorhinal cortex") and ("magnetic resonance imaging") and ("Alzheimer*" or "mild cognitive impairment")
("parahippocampal gyrus") and ("magnetic resonance imaging") and ("Alzheimer*" or "mild cognitive impairment")
("diffusion tensor imaging") and ("Alzheimer*" or "mild cognitive impairment")

Table 2. Subject characteristics medial temporal lobe studies

Study	N	Age	MMSE	CDR	Regions	Diagnostic criteria
Pitkänen et al. (1996) [59]					HC	1
Controls	76	59.7 (19.9)				
AD	55	69.9 (8.3)	No data	No data		
Convit et al. (1997) [60]					HC	5
Controls	27	69.3 (8.3)	29.3 (5.5)			
MCI	22	74.1 (7.3)	28.3 (5.4)			
AD	27	72.3 (7.4)	18.3 (4.3)	No data		
Kaye et al. (1997) [61]					HC, PHG	1
Controls	18	86.8 (1.9)	28.4			
AD	12	90.4 (5.2)	26.9	No data		
Mori et al. (1997) [62]					HC, PHG	1
Controls	12	66.2 (4.9)	≥ 28			
AD	46	70.3 (7.1)	19.6 (3.5)	No data		
Jack et al. (1998) [63]					HC	1

Controls	24	81.04 (3.78)	28.79 (1.28)			
AD	24	80.42 (4.02)	20.74 (4.60)	0.85 (0.43)		
Juottonen et al. (1998) [64]					ERC	2
Controls	32	72 (4.1)	28.3 (1.4)			
AD	30	70.3 (8.5)	20.7 (3.7)	1.53 (0.51)		
Krasuski et al. (1998) [65]					HC, PHG	1
Controls	21	69.3 (6.8)	29.7 (0.2)			
AD	13	71.2 (8.3)	23.7 (2.7)	No data		
Laakso et al. (1998) [66]					HC	1
Controls	42	72.0 (4.0)	28.0 (1.0)			
MCI	42	70.0 (5.0)	28.0 (2.0)			
AD	55	70.0 (8.0)	22.0 (4.0)	No data		
Visser et al. (1999) [8]					HC, PHG, MTA	1
- Con	18	76.8 (4.0)	27.1 (2.8)			
- AD	7	79.6 (4.9)	16.6 (6.0)	No data		
Barber et al. (2000) [67]					HC, PHG	1
- Con	26	76.2 (5.0)	28.1 (2.0)			
- AD	22	77.3 (5.0)	16.5 (4.0)	No data		
de Toledo-Morrell et al. (2000) [68]					HC, PHG	1
Controls	30	72.4 (64-84)	≥ 28			
AD	18	68.6 (49-84)	24.1 (20-28)	No data		
Laakso et al. (2000) [69]					HC, ERC	2

Con	30	69.0 (9.0)	29.0 (1.0)			
AD	30	73.0 (9.0)	20.0 (4.0)	1.27 (0.83)		
Xu et al. (2000) [70]					HC, ERC	1
Controls	30	78.9 (6.2)	28.5 (1.6)			
MCI	30	78.4 (6.4)	25.7 (2.8)			
AD	30	78.5 (6.3)	20.6 (5.0)	No data		
De Santi et al. (2001) [71]					HC	1; 5
Controls	11	76.5 (5.4)	29.1 (1.5)			
MCI	15	74.6 (6.7)	28.5 (1.9)			
AD	12	76.1 (7.0)	20.3 (7.2)	No data		
Du et al. (2001) [72]					HC, ERC	1
Controls	40	75.1 (4.3)	29.0 (0.9)			
MCI	36	75.1 (8.2)	25.8 (3.6)			
AD	29	75.8 (5.1)	17.7 (5.7)	No data		
Goncharova et al. (2001) [73]					ERC	1
Controls	34	70.3 (6.6)	29.2 (0.7)			
AD	16	71.4 (9.1)	27.3 (1.1)	No data		
Wolf et al. (2001) [74]					HC	1
Controls	17	78.5 (3.1)	28.3 (1.3)			
MCI	12	78.5 (2.2)	25.7 (1.1)			
AD	10	78.2 (3.0)	22.9 (2.0)	No data		
Bottino et al. (2002) [75]					HC, PHG	1

Controls	20	69.1 (4.84)	29.1 (0.7)			
AD	39	73.1 (7.21)	20.2 (3.6)	No data		
Hsu et al. (2002) [76]					HC	1
Controls	20	74.0 (6.2)	29.0 (1.3)			
MCI	20	74.2 (6.7)	27.7 (2.7)			
AD	20	74.5 (6.2)	22.7 (3.5)	No data		
Killiany et al. (2002) [77]					HC, ERC	2
Controls	28	71.8	29.2			
MCI	94	72	29.1			
AD	16	69.8	24.2	No data		
Mega et al. (2002) [78]					HC	3
Controls	10	73.0 (7.4)	29.2 (1.0)			
MCI	10	71.0 (5.2)	28.9 (1.3)	No data		
Pantel et al. (2002) [79]					HC, PHG	1
Controls	22	65.3 (0.8)				
MCI	21	65.2 (0.9)				
AD	12	63.7 (7.4)	18.75 (4.0)	No data		
Du et al. (2003) [80]					ERC	1
Controls	23	76.5 (7.9)	29 (1.0)			
AD	21	74.5 (6.7)	22 (7.0)	No data		
Du et al. (2004) [81]					HC, ERC	1
Controls	25	76.8 (7.8)	29.0 (1.0)			
AD	20	75.3 (7.2)	21.0 (7.2)	No data		
Pennanen et al. (2004) [37]					HC, ERC	1; 5

Controls	59	72.7 (4.3)	27.3 (1.8)			
MCI	65	72.8 (4.5)	24.0 (2.5)			
AD	48	71.1 (8.1)	21.4 (3.5)	No data		
Testa et al. (2004) [82]					HC	2
Controls	25	70.0 (8.0)	29.0 (1.0)			
AD	27	74.0 (9.0)	21.0 (4.0)	No data		
Müller et al. (2005) [28]					HC	3
Controls	18	66.9 (9.0)	28.7 (1.0)			
aMCI	18	67.3 (8.7)	25.2 (2.2)	No data		
Bastos-Leite et al. (2006) [83]					MTA-score	2
Controls	15	68.9 (8.0)	27.9 (1.9)			
AD	21	69.3 (10.9)	18.8 (4.5)	No data		
Kalus et al. (2006) [84]					HC, ERC, PHG	1
Controls	10	70.8 (5.6)	29.0 (27-30)			
MCI	10	76.0 (11.5)	22.4 (19-28)			
AD	10	73.9 (9.8)	19.2 (6-27)	No data		
Teipel et al. (2006) [85]					HC, ERC, PHG	2
Controls	22	61.5 (8.9)	29.4 (0.7)			
AD	34	69.0 (8.0)	23.1 (4.1)	No data		
Uotani et al. (2006) [86]					HC	5
Controls	20	65.7 (8.7)	29.6 (0.8)			

MCI	7	73.2 (8.6)	26.9 (2.1)			
AD	33	70.9 (8.3)	14.1 (8.1)	No data		
van de Pol et al. (2006) [87]					HC, MTA-score	1
Controls	73	75.0 (8.0)	No data			
AD	103	71.0 (9.0)	No data	1.0 (0.5-3)		
Barnes et al. (2007) [88]					HC	7
Controls	11	56.0 (14.3)	29 (1)			
AD	19	56.3 (10.6)	20 (7)	No data		
Meyer et al. (2007) [89]					HC, ERC	1, 4
Controls	52	65.6 (11.0)	29.50 (0.73)			
MCI	30	77.2 (8.52)	26.27 (1.80)			
AD	19	78.74 (5.05)	16.84 (9.66)	No data		
Ridha et al. (2007) [90]					HC, MTA-score	2
Controls	47	65.5 (11.4)	29.5 (0.7)			
AD	26	65.7 (11.5)	19.4 (4.0)	No data		
Slavin et al. (2007) [91]					HC	1; 5
Controls	17	70.2 (3.6)	28.2 (1.3)			
aMCI	18	74.4 (7.7)	26.8 (1.3)	No data		
Colliot et al. (2008) [92]					HC	1; 3
Controls	25	64.0 (8.0)	No data			
aMCI	24	74.0 (8.0)	27.2 (1.4)			

AD	25	73.0 (6.0)	24.4 (2.7)	No data		
Delano-Wood et al. (2008) [93]					HC	3
Controls	20	78.14 (7.06)				
MCI	20	78.00 (7.32)	No data	No data		
Duara et al. (2008) [94]					MTA-score	1; 3
Controls	117	71.7 (5.7)	29 (1.2)			
aMCI	91	76.4 (6.0)	26.6 (2.3)			
AD	53	79.9 (6.0)	22.9 (3.8)	No data		
Kenny et al. (2008) [95]					ERC	1
Controls	37	75.4 (6.8)	28.1 (1.6)			
AD	26	78.1 (5.0)	18.7 (4.4)	No data		
Appel et al. (2009) [96]					MTA-score	1; 3
Controls	40	71.0 (5.6)	29.3 (0.9)			
(a)MCI	118	74.7 (6.3)	26.9 (2.3)			
AD	34	76.6 (6.5)	22.7 (2.8)	No data		
Bai et al. (2009) [97]					HC	3
Controls	23	70 (5.0)	28.3 (1.5)			
aMCI	39	71 (5.0)	27 (1.6)	No data		
Cho et al. (2009) [98]					MTA-score	1
Controls	27	No data	No data			

AD	33	74.4 (7.8)	16.55 (4.40)	1.7 (0.81)		
Feczko et al. (2009) [99]					ERC	1
Controls	47	76.2 (6.8)	29.0 (1.3)	0		
AD	29	73.8 (11.7)	22.9 (3.8)	0.88 (0.22)		
Ferrarini et al. (2009) [100]					HC	1
Controls	50	70.8 (5.8)	28.1 (1.4)			
MCI	30	71.9 (6.2)	26.7 (1.3)			
AD	50	71.3 (7.7)	18.5 (3.4)	No data		
Henneman et al. (2009) [101]					MTA-score	2; 3
Controls	19	66 (9.0)	29 (1.0)			
MCI	25	71 (6.0)	25 (3.0)			
AD	31	67 (8.0)	23 (4.0)	No data		
Henneman et al. (2009) [102]					HC, MTA-score	2; 3
Controls	34	67 (9.0)	28 (2.0)			
MCI	44	71 (6.0)	26 (3.0)			
AD	64	67 (9.0)	22 (5.0)	No data		
Hyun et al. (2009) [103]					MTA-score	1
Controls	27	No data	No data			
AD	33	74.4 (7.8)	16.55 (4.40)	1.7 (0.81)		
Jauhiainen et al. (2009) [104]					HC, ERC	2; 3

Controls	21	71.2 (4.9)	27.7 (2.0)			
aMCI	14	72.4 (7.3)	25.6 (3.1)			
AD	15	73.1 (6.7)	21.7 (3.7)	0.8 (0.3)		
Lee et al. (2009) [105]					HC	2; 4
Controls	95	72.7 (7.1)	29.7 (10.5)			
MCI	73	73.6 (7.5)	27.2 (12.9)			
AD	47	77.2 (7.2)	21.2 (12.9)	No data		
Loewenstein et al. (2009) [106]					MTA	6
Controls	108	72.23 (5.4)				
aMCI	78	76.27 (6.4)				
AD	48	77.08 (6.2)	No data	No data		
Morra et al. (2009) [107]					HC	1; 3
Controls	100	76.6 (4.8)	29.1 (0.9)	0.0 (0.9)		
MCI	200	75.4 (7.0)	26.9 (1.9)	1.5 (0.8)		
AD	100	75.9 (7.2)	23.4 (1.9)	4.5 (1.6)		
Rogalski et al. (2009) [108]					HC, ERC, PHG	3
Controls	14	73.6 (6.7)	29.4 (0.8)			
aMCI	14	76.8 (7.0)	26.9 (2.0)	No data		
Bird et al. (2010) [109]					MTA	1
Controls	25	65.3 (7.6)				
aMCI	6	65.3 (11.0)	27.0 (1.5)			

AD	7	66.6 (7.5)	26.1 (2.8)	No data		
Bouwman et al. (2010) [110]					MTA	1
Controls	138	60.0 (10.0)	28.3 (1.8)			
MCI	65	70.0 (8.0)	26.3 (2.7)			
AD	145	68.8 (8.0)	21.5 (4.6)	No data		
Cherubini et al. (2010) [111]					HC	2; 3
Controls	30	67.9 (7.4)	28.97 (1.24)			
aMCI	30	66.8 (6.0)	27.57 (2.10)			
AD	30	68.7 (7.8)	21.33 (4.03)	No data		
Choo et al. (2010) [33]					HC, ERC	2; 5
Controls	18	70.7 (5.2)	No data			
MCI	19	71.6 (7.1)				
AD	19	71.1 (5.1)		0.95 (0.44)		
Desikan et al. (2010) [112]					HC	1; 3
Controls	208	76.0 (4.9)	29.1 (1.0)	0		
MCI	353	74.5 (7.4)	27.0 (1.8)	1.6 (0.9)		
AD	163	74.9 (7.5)	23.3 (1.9)	4.2 (1.6)		
Jhoo et al. (2010) [113]					HC	2; 4; 5
Controls	17	70.8 (5.4)	28.4 (1.9)			
aMCI	17	70.8 (7.0)	24.4 (3.7)			
AD	17	70.7 (5.8)	16.9 (3.8)	0.79 (0.25)		
Johnson et al. (2010) [114]					HC	1

Controls	32	70.3 (6.3)	29.4 (0.84)			
AD	27	73.0 (6.5)	27.5 (2.25)	0.5		
Lehmann et al. (2010) [115]					HC, ERC, PHG	2
Controls	10	59.7 (6.3)	29.8 (0.4)			
AD	10	60.0 (7.6)	20.4 (5.8)	No data		
Liu et al. (2010) [116]					HC	2; 3
Controls	94	74.0 (5.0)	29.0 (1.0)			
MCI	79	74.0 (6.0)	27.0 (2.0)			
AD	118	75.0 (6.0)	21.0 (5.0)	1.3 (0.5)		
Luckhaus et al. (2010) [117]					HC	2
Controls	12	65.7 (4.1)	28.0 (1.53)			
MCI	30	64.1 (9.5)	27.5 (1.88)	No data		
AD	15	68.7 (7.6)	23.47 (1.88)			
Mueller et al. (2010) [118]					HC, ERC	1; 3
Controls	53	69.5 (7.3)	29.3 (1.1)			
aMCI	20	73.5 (7.1)	28.0 (2.1)			
AD	18	69.1 (9.6)	21.6 (5.1)	No data		
Pengas et al. (2010) [119]					HC	2
Controls	28	64.9 (61-73)	29.3 (0.8)			
aMCI	24	67.6 (63-72)	26.8 (1.4)	No data		
Ryu et al. (2010) [120]					HC	2
Controls	14	70.6 (5.2)	24.1 (2.3)			

AD	23	74.4 (6.3)	17.6 (4.8)	No data		
Sanchez-Benavides et al. (2010) [121]					HC, ERC	1; 5
Controls	34	71.7 (5.9)	28.9 (1.2)			
MCI	24	74.3 (6.2)	26.0 (2.3)	No data		
AD	20	75.3 (6.6)	21.4 (3.4)			
Schott et al. (2010) [122]					HC	1; 3
Controls	199	76.0 (5.1)	29.1 (1.0)	0.03 (0.11)		
MCI	334	74.9 (7.2)	27.0 (1.8)	1.58 (0.89)		
AD	144	75.2 (7.3)	23.5 (1.9)	4.19 (1.55)		
Westman et al. (2010) [123]					HC	1; 5
Controls	100	73.0 (6.0)	29.0 (1.0)	0		
MCI	100	75.0 (5.0)	27.0 (3.0)	0.5		
AD	100	76.0 (6.0)	21.0 (5.0)	1.2 (0.5)		
Yakushev et al. (2010) [124]					HC	1
Controls	18	69.0 (6.7)	28.9 (1.0)			
AD	20	69.8 (7.4)	25.7 (1.7)	0.63 (0.22)		
Zarei et al. (2010) [125]					HC	2
Controls	22	70.7 (6.0)	28.7 (1.4)			
AD	16	69.5 (6.7)	22.9 (3.2)	No data		
Echavarri et al. (2011) [126]					HC, PHG	1; 3
Controls	18	64.5 (3.3)	28.8 (27-30)			
MCI	18	65.1 (4.5)	27.6 (22-30)			

AD	18	72.2 (9.7)	21 (10- 28)	No data		
Jacobs et al. (2011) [44]					HC, PHG	5
Controls	35	69.1 (7.7)	28.2 (1.5)			
MCI	30	69.2 (8.1)	26.3 (2.0)			
AD	9	73.8 (4.3)	24.8 (3.1)	No data		
Prestia et al. (2011) [127]					HC	1
Controls	19	72.5 (7.8)	29.1 (1.0)			
AD	20	72.7 (9.1)	22.0 (4.3)	No data		
Zhang et al. (2011) [128]					HC	3
Controls	243	77.8 (4.5)				
MCI	146	78.8 (4.6)	No data	No data		

Data are mean (sd) or mean (range). Volumetric measurements of HC: hippocampus, ERC: entorhinal cortex, PHG: parahippocampal gyrus. MMSE: Mini-mental state examination; CDR: Clinical Dementia rating scale (AD group); MTA: Medial temporal lobe atrophy; Con: healthy controls; MCI: mild cognitive impairment, subtypes not specified or all subtypes included; aMCI: amnesic-type MCI ; AD: Alzheimer's disease. Main diagnostic criteria: (1) NINCDS-ADRDA: probable and/or possible AD = McKhann et al., 1984 [129]; (2) NINCDS-ADRDA: probable AD – McKhann et al., 1984 [129]; (3) MCI according to Petersen et al., (1999) [3] or (2001) [130]; (4) MCI according to Winblad et al., 2004; (5) Diagnostic and Statistical Manual of Mental Disorders (DSM) Fourth edition; (6) National Alzheimer Coordinating Center guidelines (NACC) – Washington.; (7) Based on genetic or pathological analysis.

Table 3. Effect size volumetric medial temporal lobe studies

		Effect size	p-value	Heterogeneity	p-value	Publication Bias	p-value
		Hedges g (CI)		Cochrane's Q		K-tau	
Controls vs AD	N studies (N Controls/N AD)						
MTA score	10 (580/505)	1.79 (1.46; 2.13)	<0.001	41.08	<0.0001	0.24	0.37
Hippocampus	53 (2085/1920)	1.74 (1.55; 1.93)	<0.001	307.02	<0.0001	0.22	0.02
Entorhinal cortex	19 (605/440)	1.98 (1.56; 2.41)	<0.001	136.63	<0.0001	0.57	0.001
Parahippocampal gyrus	14 (273/262)	1.32 (0.96; 1.63)	<0.001	40.14	<0.0001	0.36	0.08
Controls vs MCI	N studies (N Controls/N MCI)						
MTA score	6 (447/344)	1.11 (0.56; 0.65)	<0.001	51.51	<0.0001	0.20	0.71
Hippocampus	40 (1914/2106)	0.89 (0.72; 1.05)	<0.001	195.24	<0.0001	0.09	0.38
Entorhinal cortex	11 (359/283)	1.46 (0.94; 1.98)	<0.001	80.08	<0.0001	0.49	0.04

Continuation **Table 3.** Effect size volumetric medial temporal lobe studies

Parahippocampal gyrus	6 (110/108)	0.61 (0.34; 0.89)	<0.001	2.96	0.71	0.33	0.45
		Effect size		Heterogeneity		Publication Bias	
		Hedges g (CI)	<i>p</i> -value	Cochrane's Q	<i>p</i> -value	K-tau	<i>p</i> -value
<i>MCI vs AD</i>	<i>N studies (N MCI/N AD)</i>						
MTA score	7 (350/318)	0.74 (0.47; 1.00)	<0.001	12.91	0.05	0.14	0.77
Hippocampus	32 (1822/1334)	0.93 (0.71; 1.16)	<0.001	209.26	<0.0001	0.32	0.01
Entorhinal cortex	10 (269/224)	1.17 (0.65; 1.69)	<0.001	59.24	<0.0001	0.02	0.99
Parahippocampal gyrus	5 (94/61)	0.92 (0.51; 1.33)	<0.001	5.36	0.25	0.40	0.46

CI: Confidence interval; K-tau: Kendalls tau; MCI: subjects with mild cognitive impairment;
AD: subjects with Alzheimer's disease; MTA: Medial Temporal Lobe atrophy.

Table 4. Subject characteristics diffusion tensor imaging studies

Study	N	Age	MMSE	CDR	DC	Mod	Regions studied										PHG	HC	IC
							FL	PL	OL	TL	GCC	SCC	ACg	MCg	PCg	PHg	TCg	UF	SLF
Rose et al. (2000) [131]	9	72 (0.7)	>28	No data	1	FA						X					X		X
	11	70 (0.6)	6-21																
Bozzali et al. (2002) [132]	10	66.1 (61-75)	28.6 (26.8-30.7)	No data	2	MD FA	X	X	X										X
	16	69.6 (64-85)	19.4 (9.3-25.4)																
Stahl et al. (2003) [133]	10	60.5 (37-78)	29.0 (0.5)	No data	2	FA	X	X	X	X	X	X							X
	9	63.7 (48-78)	23.7 (4.5)																
Fellgiebel et al. (2004) [134]	10	62 (6.80)	28.6 (1.3)	No data	2; 3	MD FA	X	X	X	X	X	X							X
	14	68.2 (9.2)	24.6 (2.7)																
	19	69.6 (8.3)	18.3 (4.8)																
	19	69.6 (8.3)	18.3 (4.8)																
Head et al. (2004) [135]	25	77 (5)	28.9 (1.2)	0.72 (0.25)	1	MD FA	X	X	X	X	X	X							
	25	77 (6)	22.9 (4.7)																
Sugihara et al. (2004) [46]	10	65.4	No data	No data	1; 6	FA					X	X							
	20	69.2																	
Choi et al. (2005) [136]	10	75.57 (4.98)	29.50 (1.08)	No data	1; 6	MD FA	X			X	X	X							
	10	76.98 (6.79)	25.20 (2.49)																
Fellgiebel et al. (2005) [137]	21	67.7 (8.5)	28.7 (1.1)	No data	2; 3	MD FA									X				
	17	67.5 (8.9)	25.3 (2.2)																
	25	69.5 (7.7)	18.9 (4.6)																
	25	69.5 (7.7)	18.9 (4.6)																
Duan et al. (2006) [138]	12	64.35 (9.15)	28 (27-30)	No data	2	MD FA	X	X	X	X	X	X							X
	16	65.22 (8.58)	13 (8-22)																
Naggara et al. (2006) [139]	12	73.40 (4.83)	29.80 (0.40)	No data	2	MD FA	X	X	X	X	X	X							X
	12	76.80 (4.01)	27.00 (2.75)																
Rose et al. (2006) [140]	17	73.59 (9.06)	28.24 (1.25)	No data	1; 3; 6	FA									X				
	17	73.58 (8.96)	26.12 (2.21)																

Continuation **Table 4.** Subject characteristics diffusion tensor imaging studies

Study	N	Age	MMSE	CDR	DC	Mod	Regions studied												IC					
							FL	PL	OL	TL	GCC	SCC	ACg	MCg	PCg	PHg	TCg	UF		SLF	PHG	HC		
Taoka et al. (2006) [141] - Con - AD	15	72 (62-81)	15 (4-22)	No data	6	MD																		
	15	74 (63-87)				FA																		
Chen et al. (2007) [142] - Con - AD	10	70	No data	No data	2	MD																		
	10	71				FA																		
Firbank et al. (2007) [143] - Con - AD	15	75 (8)	28.3 (2.1) 19.1 (4.3)	No data	2	MD	X	X	X													X		
	15	76 (7)				FA																		
Huang and Auchus (2007) [144] - Con - aMCI - AD	6	71.17 (5.74)	29.50 (0.84) 26.63 (2.56) 24.75 (1.50)	No data	1; 3	FA	X	X	X															
	8	74.75 (8.55)																						
		73.75 (6.08)																						
	4																							
Müller et al. (2007) [30] - Con - aMCI	18	66.9 (9.0)	28.7 (1.0) 25.2 (2.2)	No data	3	MD																		
	18	67.3 (8.7)				FA																		X
Stahl et al. (2007) [145] - Con - aMCI - AD	19	63.9 (10.3)	30 (27-30) 27 (23-29) 25 (15-29)	No data	2; 3	FA	X	X	X	X	X												X	
	16	68.9 (9.3)																						
		68.8 (10.8)																						
	15																							
Zhang et al. (2007) [146] - Con - MCI - AD	18	71.6 (9.2)	29.5 (0.8) 27.9 (2.0) 22.1 (4.0)	1.0 (0.4)	2; 3; 7	MD				X	X												X	
	17	73.1 (7.4)				FA																		
		77.1 (8.8)																						
	17																							
Cho et al. (2008) [147] - Con - MCI	11	70.64 (2.88)	29.7 (0.8) 24.9 (2.4)	No data	3	MD	X	X	X	X	X												X	
	11	72.64 (7.33)				FA																		
Delano-Wood et al. (2008) [93] - Con - MCI	20	78.14 (7.06)	No data	No data	3	MD																		
	20	78.00 (7.32)				FA																		
Ding et al. (2008) [148] - Con - AD	20	71.9 (6.8)	28.3 (1.0) 19.5 (2.9)	0.5 (0.2)	2	MD																		
	17	67.4 (8.9)				FA																		

Continuation **Table 4.** Subject characteristics diffusion tensor imaging studies

Study	N	Age	MMSE	CDR	DC	Mod	Regions studied										IC				
							FL	PL	OL	TL	GCC	SCC	ACg	MCg	PCg	PHg		TCg	UF	SLF	PHG
Fujie et al. (2008) [149] - Con - aMCI	16	70.9 (4.0)	29.2 (1.2)	No data	3	FA											X				
	16	71.7 (7.1)	27.2 (2.3)																		
Parente et al. (2008) [150] - Con - MCI - AD	16	68.0 (7.7)	29.4 (0.9)	No data	6	FA															
	25	72.0 (9.3)	28.0 (1.1)																		
	20	76.0 (8.6)	18.3 (5.1)																		
Shim et al. (2008) [151] - Con - MCI	17	68.8 (3.6)	28.7 (1.1)	No data	5	MD FA	X	X	X	X	X										
	21	73.0 (6.9)	24.5 (2.5)																		
Ukmar et al. (2008) [152] - Con - aMCI - AD	18	59.50 (6.91)	29.94 (0.23)	No data	3; 6: mild AD	FA	X	X	X	X	X										
	15	72.26	28.84 (0.83)																		
	14	81.00 (5.01)	23.45 (2.76)																		
Yasmin et al. (2008) [153] - Con - AD	19	69.0 (5.3)	28.5 (1.4)	No data	2	MD FA											X				
	19	71.1 (8.1)	21.6 (5.3)																		
Bai et al. (2009) [154] - Con - aMCI	22	72.0 (5.4)	28.4 (1.5)	No data	3	FA															
	22	72.0 (4.4)	26.8 (1.7)																		
Chen et al. (2009) [155] - Con - aMCI - AD	16	69.0 (8.4)	No data	No data	2; 3	MD FA				X	X										
	13	73.2 (9.3)																			
	11	76.7 (8.5)																			
Chen et al. (2009) [39] - Con - aMCI - AD	20	70.1 (7.1)	No data	1.43 (0.57)	2; 3	MD FA				X	X										
	10	71.0 (9.1)																			
	30	73.8 (10.7)																			
Kiuchi et al. (2009) [156] - Con - MCI - AD	16	71.9 (7.2)	27.4 (2.0)	No data	2; 8	MD FA											X				
	16	72.8 (9.1)	25.3 (0.9)																		
	17	74.5 (6.5)	21.7 (1.0)																		
Lee et al. (2009) [105] - Con - MCI - AD	95	72.7 (7.1)	29.7 (10.5)	No data	2; 5	FA	X	X	X	X											
	73	73.6 (7.5)	27.2 (12.9)																		
	47	77.2 (7.2)	21.2 (12.9)																		

Continuation **Table 4.** Subject characteristics diffusion tensor imaging studies

Study	N	Age	MMSE	CDR	DC	Mod	Regions studied												IC				
							FL	PL	OL	TL	GCC	SCC	ACg	MCg	PCg	PHg	TCg	UF		SLF	PHG	HC	
Liu et al. (2009) [157]	19	75.0 (6.0)	28.0 (1.0)	1.21 (0.47)	2; 3	FA				X	X												
	27	75.0 (6.0)	26.0 (2.0)																				
	17	76.0 (7.0)	22.0 (5.0)																				
Mielke et al. (2009) [158]	25	74.3 (7.1)	28.8 (1.2)	1.1 (0.4)	2; 8	FA					X	X			X								
	25	75.8 (5.3)	26.6 (2.1)																				
	25	75.6 (7.0)	22.4 (3.1)																				
Nakata et al. (2009) [159]	18	69.4 (4.7)	28.8 (1.2)	No data	2	MD FA									X								
	23	72.0 (8.1)	21.9 (4.0)																				
Rogalski et al. (2009) [108]	14	73.6 (6.7)	29.4 (0.8)	No data	3	MD FA														X			
	14	76.8 (7.0)	26.9 (2.0)																				
Stricker et al. (2009) [160]	14	77.4 (8.1)	29.3 (0.7)	No data	1	FA																X	
	16	77.3 (9.0)	24.4 (3.5)																				
Wang et al. (2009) [11]	10	70.1 (7.7)	29.7 (0.5)	No data	3	MD FA	X			X	X												
	10	72.2 (7.1)	26.6 (2.1)																				
Zarei et al. (2009) [161]	22	70.7 (6.0)	28.7 (1.4)	0.81 (0.44)	2	FA			X	X													
	16	69.5 (6.7)	22.9 (3.2)																				
Zhang et al. (2009) [48]	19	61.5 (10.6)	29.5 (0.5)	No data	1	FA					X	X	X		X								
	18	62.8 (7.1)	21.7 (5.8)																				
Catheline et al. (2010) [162]	15	74.6 (4.6)	28.2 (1.1)	No data	1	FA									X	X							
	15	70.4 (9.1)	19.2 (5.5)																				
Cherubini et al. (2010) [111]	30	67.9 (7.4)	28.97 (1.24)	No data	2; 3	MD																X	
	30	66.8 (6.0)	27.57 (2.10)																				
	30	68.7 (7.8)	21.33 (4.03)																				

Continuation **Table 4.** Subject characteristics diffusion tensor imaging studies

Study	N	Age	MMSE	CDR	DC	Mod	Regions studied											IC				
							FL	PL	OL	TL	GCC	SCC	ACg	MCg	PCg	PHg	TCg		UF	SIF	PHG	HC
Hong et al. (2010) [163]	15	70.07 (3.83)	28.2 (1.21)	0.97 (0.48)	2	FA																X
	15	71.67 (9.36)	19.73 (2.96)		MD																	
Jhoo et al. (2010) [113]	17	70.8 (5.4)	28.4 (1.9)	0.79 (0.25)	2; 5; 6	FA										X						
	17	70.8 (7.0)	24.4 (3.7)																			
	17	70.7 (5.8)	16.9 (3.8)																			
Johnson et al. (2010) [114]	32	70.3 (6.3)	29.4 (0.84)	0.5	1	FA	X	X		X		X						X	X	X		X
	27	73.0 (6.5)	27.5 (2.25)		MD																	
Kantarci et al. (2010) [164]	60	73 (54-86)	No data	SOB 0 (0) 4 (2-10)	1	FA								X								
	30	74 (48-89)			MD																	
Pievani et al. (2010) [165]	15	69.8 (6.0)	28.8 (1.5)	No data	1; 3	MD																
	19	68.5 (7.9)	26.0 (1.2)			FA																
	25	75.3 (8.7)	19.1 (5.8)																			
Pitel et al. (2010) [166]	32	69.4 (5.6)	27.8 (1.6)	No data	2	FA				X		X										
	13	78.2 (9.5)	22.1 (2.4)		MD																	
Scola et al. (2010) [167]	20	65.2 (56-75)	27.4 (1.3)	No data	2	MD	X	X	X	X	X	X			X							X
	19	68.2 (52-79)	25.5 (1.7)		FA																	
	21	69.9 (56-79)	20.4 (6.3)																			
Sexton et al. (2010) [31]	8	73.0 (7.5)	28.8 (1.0)	No data	1; 6	MD										X						
	8	77.1 (4.6)	28.1 (1.8)			FA																
	7	68.1 (9.6)	26.4 (3.1)																			
Sjöbeck et al. (2010) [168]	15	76.5 (1.79)	29.3 (0.28)	No data	2	FA	X		X													
	15	77.5 (1.64)	23.5 (1.09)																			

Continuation **Table 4.** Subject characteristics diffusion tensor imaging studies

Study	N	Age	MMSE	CDR	DC	Mod	Regions studied																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
							FL	PL	OL	TL	GCC	SCC	ACg	MCg	PCg	PHg	TCg	UF	SLF	PHG	HC	IC																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																		
Yakushev et al. (2010) [124] - Con - AD	18	69.0 (6.7)	28.9 (1.0)	0.63 (0.22)	1	MD																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																		</

Data are mean (sd) or mean (range). MMSE: Mini-Mental State Examination; CDR: Clinical Dementia Rating scale (AD group); DC: diagnostic criteria; Mod: Modality (FA and or MD); FL: Frontal lobe; PL: parietal lobe; OL: occipital lobe; TL: temporal lobe; GCC: genu corpus callosum; SCC: splenium corpus callosum; ACg: anterior cingulum; MCg: middle cingulum; PCg: posterior cingulum; PHg: parahippocampal cingulum; TCg: total cingulum; UF: uncinate fasciculus; SLF: superior longitudinal fasciculus; PHG: parahippocampal gyrus; HC: hippocampus; IC: internal capsule; Con: healthy controls; MCI: mild cognitive impairment, subtypes not specified or all subtypes included; aMCI: amnesic-type MCI ; AD: Alzheimer's disease. Main diagnostic criteria: (1) NINCDS-ADRDA: probable and/or possible AD – McKhann et al., 1984 [129]; (2) NINCDS-ADRDA: probable AD – McKhann et al., 1984 [129]; (3) MCI according to Petersen et al., (1999) [3] or (2001) [130]; (5) MCI according to Winblad et al., 2004 [170]; (6) Diagnostic and Statistical Manual of Mental Disorders (DSM) Third or Fourth edition; (7) AD Cooperative Study criteria (ADCS) [171](8) Petersen, 2004. MD: mean diffusivity; FA: fractional anisotropy;

Table 5. Effect size DTI-fractional anisotropy studies

		Effect size		Heterogeneity		Publication Bias	
		Hedges g (CI)	p-value	Cochrane's Q	p-value	K-tau	p-value
Controls vs AD	N studies (N Controls /N AD)						
Frontal Lobe	15 (309/265)	0.70 (0.35;1.04)	0.001	48.13	<0.001	0.56	0.004
Parietal Lobe	13 (284/240)	0.26 (0.13;0.66)	0.19	51.04	<0.001	0.38	0.08
Occipital Lobe	14 (298/244)	0.11 (-0.09;0.32)	0.29	10.11	0.52	-0.39	0.09
Temporal Lobe	18 (357/314)	0.57 (0.29;0.84)	<0.001	45.0	<0.001	0.24	0.17
Hippocampus	4 (63/70)	1.36 (-0.16;2.88)	0.08	40.88	<0.001	0.67	0.31
Total cingulum	5 (84/90)	1.73 (0.22;3.23)	0.02	63.33	<0.001	0.80	0.09
ACg	3 (59/58)	1.37 (-0.30;3.04)	0.11	30.18	<0.001	0.33	1.00
MCg	3 (43/44)	0.42 (-0.006;0.85)	0.05	0.12	0.94	-0.33	1.00
PCg	10 (186/196)	0.84 (0.63;1.05)	<0.001	7.11	0.63	-0.16	0.60
PHg	7 (134/128)	1.17 (0.40;1.95)	0.003	22.88	<0.001	0.80	0.09
Genu CC	19 (417/361)	0.63 (0.42;0.83)	<0.001	31.29	0.03	0.39	0.02
Splenium CC	22 (467/417)	1.10 (0.65;1.55)	<0.001	178.92	<0.001	0.34	0.03
SLF	7 (125/136)	0.77 (0.15;1.39)	0.01	31.54	<0.001	0.62	0.07
UF	5 (84/94)	1.03 (0.48;1.58)	<0.001	11.68	0.02	1.00	0.03
Internal capsule	9 (136/139)	0.09 (-0.19;0.37)	0.54	1.81	0.94	0.14	0.76
Controls vs MCI	N studies (N Controls /N MCI)						
Frontal Lobe	10 (444/345)	0.26 (-0.11;0.62)	0.17	37.26	<0.001	0.02	1.00
Parietal Lobe	8 (196/177)	0.02 (-0.26;0.29)	0.90	10.31	0.17	0.50	0.11
Occipital Lobe	9 (434/335)	0.18 (-0.13;0.49)	0.25	5.47	0.37	-0.47	0.26
Temporal Lobe	14 (517/412)	0.38 (0.11;0.65)	0.006	37.34	<0.001	0.30	0.15
Hippocampus	6 (310/247)	0.55 (-0.16;1.26)	0.12	42.12	<0.001	0.33	0.45
PHG	3 (270/189)	0.06 (-0.12;0.25)	0.52	0.39	0.84	1.00	0.30
PCg	10 (402/327)	0.65 (0.32;0.98)	<0.001	29.47	<0.001	0.38	0.15
PHg	4 (68/68)	0.97 (0.61;1.32)	<0.001	0.09	0.99	0.00	1.00
Genu CC	16 (573/466)	0.41 (0.18;0.64)	<0.001	37.74	<0.001	0.27	0.16
Splenium CC	17 (602/504)	0.44 (0.25;0.64)	<0.001	31.97	0.01	0.40	0.03
SLF	4 (285/214)	0.66 (-0.02;1.34)	0.06	17.45	<0.001	1.00	0.09
UF	3 (47/51)	0.26 (-0.28;0.81)	0.35	3.71	0.16	1.00	0.30
MCI vs AD	N studies (N MCI/N AD)						
Frontal Lobe	6 (145/120)	0.29 (0.05;0.54)	0.02	2.67	0.75	0.07	1.00
Parietal Lobe	6 (167/146)	-0.09 (-0.32;0.13)	0.40	1.35	0.93	0.13	0.85
Occipital Lobe	6 (145/120)	-0.07 (-0.40;0.26)	0.69	1.65	0.80	-0.1	1.00
Temporal Lobe	10 (212/195)	0.27 (0.03;0.51)	0.03	11.90	0.22	-0.11	0.72
PCg	6 (120/123)	0.37 (0.12;0.63)	0.004	4.88	0.43	0.07	1.00
PHg	4 (68/68)	0.46 (-0.14;1.06)	0.13	8.44	0.04	-0.67	0.31
Genu CC	10 (224/211)	0.35 (0.16;0.54)	<0.001	4.69	0.86	-0.2	1.00
Splenium CC	12 (274/256)	0.51 (0.11;0.91)	0.01	51.01	<0.001	0.06	0.84

CI: Confidence interval; MCI: mild cognitive impairment; AD: Alzheimer's disease. Data shown in bold are statistically significant. ACg: anterior cingulum; MCg: middle cingulum; PCg: posterior cingulum; PHg: parahippocampal cingulum; UF: uncinate fasciculus; SLF: superior longitudinal fasciculus, PHG: parahippocampal gyrus.

Table 6. Effect size DTI-mean diffusivity studies

		Effect size		Heterogeneity		Publication Bias	
		Hedges g (CI)	p-value	Cochrane's Q	p-value	K-tau	p-value
Controls vs AD	N studies (N Controls /N AD)						
Frontal Lobe	10 (165/176)	-0.75 (-1.24;-0.27)	0.002	38	<0.001	-0.47	0.07
Parietal Lobe	9 (155/166)	-1.03 (-1.58;-0.49)	<0.001	38.73	<0.001	-0.61	0.03
Occipital Lobe	8 (123/139)	-0.67 (-1.00;-0.33)	<0.001	11.81	0.11	0.14	0.71
Temporal Lobe	12 (187/207)	-0.89 (-1.30;-0.48)	<0.001	37.76	<0.001	-0.27	0.24
Hippocampus	7 (125/131)	-1.17 (-1.81;-0.53)	<0.001	31.35	<0.001	-0.62	0.07
PCg	9 (156/164)	-0.74 (-0.97;-0.51)	<0.001	5.57	0.70	-0.11	0.75
Genu CC	12 (226/216)	-0.67 (-0.98;-0.35)	<0.001	26.79	0.005	-0.30	0.19
Splenium CC	12 (226/216)	-0.94 (-1.53;-0.35)	0.002	81.10	<0.001	-0.36	0.12
UF	4 (65/76)	-0.72 (-1.27;-0.17)	0.01	7.32	0.06	0.00	1.00
Internal capsule	7 (118/118)	-0.66 (-1.19;-0.13)	0.01	21.66	<0.001	-0.43	0.23
Controls vs MCI	N studies (N Controls /N MCI)						
Frontal Lobe	7 (325/249)	-0.32 (-0.62;0.001)	0.05	10.88	0.09	-0.24	0.55
Parietal Lobe	5 (77/786)	-0.69 (-1.09;-0.28)	0.01	6.12	0.19	0.00	1.00
Occipital Lobe	6 (315/244)	-0.19 (-0.55;-0.17)	0.30	11.49	0.04	0.20	0.71
Temporal Lobe	10 (379/289)	-0.40 (-0.56;-0.24)	<0.001	7.41	0.60	-0.24	0.37
Hippocampus	7 (344/276)	-1.00 (-1.62;-0.40)	0.001	46.74	<0.001	-0.81	0.02
PHG	3 (270/189)	-2.52 (-6.62;1.58)	0.23	246.13	<0.001	0.33	1.00
PCg	7 (342/257)	-0.26 (-0.43;-0.10)	0.002	5.16	0.52	-0.62	0.07
Genu CC	10 (379/289)	-0.43 (-0.69;-0.17)	0.001	14.96	0.09	-0.02	1.00
Splenium CC	10 (379/289)	-0.46 (-0.76;-0.15)	0.004	20.79	0.01	-0.21	0.53
MCI vs AD	N studies (N MCI/N AD)						
Temporal Lobe	6 (89/113)	-0.17 (-0.45;0.12)	0.25	3.74	0.59	0.13	0.85
Hippocampus	3 (68/70)	-0.33 (-0.77;-0.11)	0.14	3.39	0.18	1.00	0.30
PCg	5 (88/99)	-0.40 (-0.70;-0.11)	0.007	2.12	0.70	0.00	1.00
Genu CC	6 (89/113)	-0.32 (-0.61;-0.03)	0.03	2.61	0.76	0.07	1.00
Splenium CC	6 (89/113)	-0.04 (-0.07;0.003)	0.03	8.54	0.13	0.00	1.00

CI: Confidence interval; MCI: mild cognitive impairment; AD: Alzheimer's disease. Data shown in bold are statistically significant. PCg: posterior cingulum, UF: uncinate fasciculus; PHG: parahippocampal gyrus.

Figure 1. Meta-regression with MMSE as predictor and absolute effect size as outcome measure. (A) FA values of the parietal lobe between controls and subjects with Alzheimer's disease (AD); $B = -0.1 \pm 0.04$, $p = 0.04$. (B) MD of the genu corpus callosum (CC) between controls and subjects with AD; $B = -0.2 \pm 0.05$, $p = 0.01$.

Figure 2. Funnel plots of standard errors plotted against effect sizes in order to identify publication bias. (A) Comparison between controls and subjects with Alzheimer’s disease (AD) for frontal lobe fractional anisotropy (FA). Begg and Mazumdar tau = 0.56, $p = 0.004$. (B) Comparison between controls and AD for FA of the genu corpus callosum (CC). Begg and Mazumdar tau = 0.39, $p = 0.02$. (C) Comparison between controls and AD for FA of the splenium CC. Begg and Mazumdar tau = 0.34, $p = 0.03$. (D) Comparison between controls and AD for FA of the uncinate fasciculus. Begg and Mazumdar tau = 1.00, $p = 0.03$. (E) Comparison between controls and subjects with MCI for FA of the splenium CC. Begg and Mazumdar tau = 0.40, $p = 0.03$. (F) Comparison between controls and AD for mean diffusivity (MD) of parietal WM. Begg and Mazumdar tau = -0.61, $p = 0.03$. (G) Comparison between controls and subjects with MCI for MD of the hippocampus. Begg and Mazumdar tau = -0.81, $p = 0.02$.

SUPPLEMENTAL MATERIAL

Supplementary table 1. Technical specifications medial temporal lobe studies. Technical details of MTL studies included in the meta-analysis.

Study	Total N	Field strength (Tesla)	Slice thickness (in mm)	Volumetric measurement of MTL structures
Pitkänen et al. (1996) [1]	131	1.5	1.5	Manual tracing
Convit et al. (1997) [2]	76	1.5	4	Manual tracing
Kaye et al. (1997) [3]	30	1.5	2.5	Manual tracing
Mori et al. (1997) [4]	58	1.5	1.5	Semi-automated tracing
Jack et al. (1998) [5]	48	1.5	1.6	Manual tracing
Juottonen et al. (1998) [6]	62	1.5	1.75	Manual tracing
Krasuski et al. (1998) [7]	34	1.5	0.5	Manual tracing
Laakso et al. (1998) [8]	139	1.5	1.5	Manual tracing
Visser et al. (1999) [9]	25	0.6	5	Manual tracing
Barber et al. (2000) [10]	48	1	1	Manual tracing
de Toledo-Morrell (2000) [11]	48	1.5	5	Manual tracing
Laakso et al. (2000) [12]	60	1.5	2	Manual tracing
Xu et al. (2000) [13]	90	1.5	1.6	Manual tracing
Du et al. (2001) [14]	105	1.5	1.4	Manual tracing
De Santi et al. (2001) [15]	38	1.5	1.3	Manual tracing
Goncharova et al. (2001) [16]	50	1.4	5	Manual tracing
Wolf et al. (2001) [17]	39	1.5	1.5	Manual tracing
Bottino et al. (2002) [18]	59	1.5	0.6	Manual tracing
Hsu et al. (2002) [19]	60	1.5	1	Manual tracing
Killiany et al. (2002) [20]	138	1.5	1.5	Manual tracing
Mega et al. (2002) [21]	20	1.5	1.5	Manual tracing
Pantel et al. (2002) [22]	55	1.5	-	Manual tracing
Du et al. (2003) [23]	44	1.5	1.4	Manual tracing
Du et al. (2004) [24]	45	1.5	1.4	Manual tracing
Pennanen et al. (2004) [25]	172	1.5	2	Manual tracing
Testa et al. (2004) [26]	52	1.5	1.3	VBM + manual tracing
Müller et al. (2005) [27]	36	1.5	1	Manual tracing
Bastos-Leite et al. (2006) [28]	36	1.5	1.5	N.A.
Kalus et al. (2006) [29]	30	1.5	1	Manual tracing
Teipel et al. (2006) [30]	56	1.5	1.2	Manual tracing

Study	Total N	Field strength (Tesla)	Slice thickness (in mm)	Volumetric measurement of MTL structures
Uotani et al. (2006) [31]	60	-	5	Manual tracing
van de Pol et al. (2006) [32]	176	1.5	1.5	Manual tracing
Barnes et al. (2007) [33]	30	1.5	1.5	Manual tracing
Meyer et al. (2007) [34]	101	1.5	-	Manual tracing
Ridha et al. (2007) [35]	73	1.5	1.5	Manual tracing
Slavin et al. (2007) [36]	35	4	1.5	Manual tracing
Colliot et al. (2008) [37]	74	1.5	1.5	Automated segmentation
Delano-Wood (2008) [38]	40	1.5	3.8	Manual tracing
Duara et al. (2008) [39]	261	1.5	1.5	N.A.
Kenny et al. (2008) [40]	63	1.5	1.6	Manual tracing
Appel et al. (2009) [41]	192	1.5	1.5	N.A.
Bai et al. (2009) [42]	62	1.5	2	VBM + semi-automated tracing
Cho et al. (2009) [43]	60	1.5	-	N.A.
Feczko et al. (2009) [44]	76	1.5	1.25	Automated segmentation
Ferrarini et al. (2009) [45]	130	1	1.3	Manual tracing
Henneman et al. (2009) [46]	75	1.5	1.5	N.A.
Henneman et al. (2009) [47]	142	1.5	1.5	Automated segmentation
Hyun et al. (2009) [48]	60	1.5	-	N.A.
Jauhiainen et al. (2009) [49]	50	1.5	1	Manual tracing
Lee et al. (2009) [50]	215	1.5	-	Semi-automated tracing
Loewenstein et al. (2009) [51]	234	1.5	1.5	N.A.
Morra et al. (2009) [52]	400	1.5	1.2	Automated segmentation
Rogalski et al. (2009) [53]	28	1.5	1.6	Manual tracing
Bird et al. (2010) [54]	38	1.5	1.5	N.A.
Bouwman et al. (2010) [55]	348	1	1.5	N.A.
Cherubini et al. (2010) [56]	90	3	1	Automated segmentation
Choo et al. (2010) [57]	56	3	1.4	Manual tracing
Desikan et al. (2010) [58]	724	1.5	-	Automated segmentation
Jhoo et al. (2010) [59]	51	3	1.4	Manual tracing
Johnson et al. (2010) [60]	59	3	1	Automated segmentation
Lehmann et al. (2010) [61]	20	1.5	1.5	Manual tracing
Liu et al. (2010) [62]	291	1.5	1.2	Automated segmentation
Luckhaus et al. (2010) [63]	57	1.5	1.8	Manual tracing
Mueller et al. (2010) [63]	91	4	2	Manual tracing

Study	Total N	Field strength (Tesla)	Slice thickness (in mm)	Volumetric measurement of MTL structures
Pengas et al. (2010) [64]	52	1.5	1.8	Manual tracing
Ryu et al. (2010) [65]	37	1.5	1	Automated segmentation
Sanchez-Benavides et al. (2010) [66]	78	1.5	1.5	Semi-automated tracing
Schott et al. (2010) [67]	677	1.5	-	Semi-automated tracing
Westman et al. (2010) [68]	300	1.5	1.2	Manual tracing
Yakushev et al. (2010) [69]	38	1.5	1	Manual tracing
Zarei et al. (2010) [70]	38	1.5	1	Automated segmentation
Echavarri et al. (2011) [71]	54	3	1	Manual tracing
Jacobs et al. (2011) [72]	74	1.5	1.5	Semi-automated tracing
Prestia et al. (2011) [73]	39	1	1.3	Manual tracing
Zhang et al. (2011) [74]	389	3	1	Automated segmentation

MTL: Medial Temporal Lobe; VBM: Voxel Based Morphometry. N.A.: not applicable (only qualitative MTA-rating was provided in the study).

Supplementary table 2. Technical specifications diffusion tensor imaging studies
Technical details of DTI studies included in the meta-analysis.

Study	Total N	Field strength (Tesla)	Number of directions	Voxel size (mm)	b-value (s/mm ²)	NEX	DTI modality
Rose et al. (2000) [75]	20	1.5	6	1.2 x 2.3 x 6.0	875	1	Manual placement of standard ROI's
Bozzali et al. (2002) [76]	26	1.5	8	2.0 x 2.0 x 5.0	1044	1	Automated segmentation
Stahl et al. (2003) [77]	19	1.5	6	1.8 x 1.8 x 3.6	1000	10	VBM
Fellgiebel et al. (2004) [78]	43	1.5	6	Slice thickness 5.0	900	6	Manual placement of standard ROI's
Head et al. (2004) [79]	50	1.5	7	2.5 x 2.5 x 4.0	1005	4	Manual tracing
Sugihara et al. (2004) [80]	30	1.5	6	Slice thickness 5.0	1000	1	Manual placement of standard ROI's
Choi et al. (2005) [81]	20	1.5	6	1.9 x 1.9 x 5.0	1000	4	Manual placement of standard ROI's
Fellgiebel et al. (2005) [82]	63	1.5	6	1.8 x 1.8 x 5.0	900	4	Tractography
Duan et al. (2006) [83]	28	1.5	25	1.9 x 1.9 x 5.0	1000	1	Manual placement of standard ROI's
Naggara et al. (2006) [84]	24	1.5	25	1.8 x 1.8 x 3.0	1000	2	Manual placement of standard ROI's
Rose et al. (2006) [85]	34	1.5	6	Slice thickness 2.5	1100	1	VBM
Taoka et al. (2006) [86]	30	1.5	6	1.8 x 1.8 x 6.3	1000	6	Tractography
Chen et al. (2007) [87]	20	1.5	25	1.9 x 1.9 x 3.0	1000	1	Automated segmentation
Firbank et al. (2007) [88]	30	1.5	6	2.6 x 1.9 x 6.0	4000	1	Manual placement of standard ROI's
Huang and Auchus (2007) [89]	18	1.5	12	1.9 x 1.9 x 3.0	1000	1	Manual placement of standard ROI's
Müller et al. (2007) [90]	36	1.5	6	1.8 x 1.8 x 5.0	900	1	Manual placement of standard ROI's
Stahl et al. (2007) [91]	50	1.5	6	1.8 x 1.8 x 3.6	1000	10	Manual placement of standard ROI's
Zhang et al. (2007) [92]	52	1.5	6	2.3 x 2.3 x 5.0	1000	1	Manual placement of standard ROI's
Cho et al. (2008) [93]	22	1.5	25	2.0 x 2.0 x 4.0	1000	1	Manual placement of standard ROI's
Delano-Wood (2008) [38]	40	1.5	42	3.8 x 3.8 x 2.8	1990	7	Tractography + manual placement of ROI's
Ding et al. (2008) [94]	37	1.5	25	1.9 x 1.9 x 5.0	1000	1	Manual placement of standard ROI's
Fujie et al. (2008) [95]	32	3	12	1.8 x 1.8 x 3.0	700	1	Tractography

Study	Total N	Field strength (Tesla)	Number of directions	Voxel size (mm)	b-value (s/mm ²)	NEX	DTI modality
Parente et al. (2008) [96]	61	1.5	6	1.8 x 1.8 x 6.5	1000	3	Manual placement of standard ROI's
Shim et al. (2008) [97]	38	1.5	25	2.0 x 2.0 x 5.0	1000	1	Manual placement of standard ROI's
Ukmar et al. (2008) [98]	47	1.5	6	2.5 x 1.9 x 6.0	1000	1	Manual placement of standard ROI's
Yasmin et al. (2008) [99]	38	1	12	1.8 x 1.8 x 3.0	700	6	Tractography
Bai et al. (2009) [100]	44	1.5	25	1.9 x 1.9 x 4.0	1000	2	Manual placement of standard ROI's
Chen et al. (2009) [101]	40	1.5	25	1.9 x 1.9 x 5.0	1000	2	Manual placement of standard ROI's
Chen et al. (2009) [102]	60	1.5	25	1.9 x 1.9 x 5.0	1000	2	Manual placement of standard ROI's
Kiuchi et al. (2009) [103]	49	1.5	6	1.8 x 1.8 x 3.0	1000	6	Tractography
Liu et al. (2009) [104]	63	1.5	30	1.7 x 1.7 x 5.0	1000	1	TBSS + manual placement of ROI's
Mielke et al. (2009) [105]	75	3	32	2.2 x 2.2 x 2.2	700	2	Tractography + manual tracing
Nakata et al. (2009) [106]	41	1	12	1.8 x 1.8 x 3.0	700	6	Tractography
Rogalski (2009) [53]	28	1.5	24	2.0 x 2.0 x 3.0	800	6	Manual placement of standard ROI's
Stricker et al. (2009) [107]	30	3	15	1.9 x 1.9 x 3.0	1500	4	TBSS + manual placement of ROI's
Wang et al. (2009) [108]	20	3	16	1.9 x 1.9 x 2.0	1000	1	Manual placement of standard ROI's
Zarei et al. (2009) [109]	38	1.5	60	2.0 x 2.0 x 2.0	700	1	TBSS, tractography
Zhang et al. (2009) [110]	37	4	6	2.0 x 2.0 x 3.0	800	4	Tractography
Catheline et al. (2010) [111]	30	1.5	6	2.4 x 2.4 x 2.5	800	1	Tractography
Cherubini et al. (2010) [56]	90	3	12	1.5 x 1.5 x 2.0	1000	1	Automated segmentation
Choo et al. (2010) [57]	56	3	25	0.94 x 0.94 x 0.94	1000	1	Tractography, manual placement of VOI's
Hong et al. (2010) [112]	30	1.5	25	Slice thickness 4.0	1000	1	Manual placement of standard ROI's
Jhoo et al. (2010) [59]	51	3	25	0.94 x 0.94 x 0.94	1000	1	Tractography, manual placement of VOI's

NEX: Number of excitations; ROI: Region of interest; VOI: Volume of Interest; TBSS: Tract-based Spatial Statistics.

Supplementary table 3. Significant univariate regressions for DTI effect sizes and technical parameters

Meta-regression indicated that a number of technical parameters were associated with effect size (ES). An increasing number of directions was associated with a higher ES. A possible explanation for this finding is that with an increasing number of directions contrast of FA/MD maps improves. The contrast-to-signal variance ratio between the main WM and the surrounding regions significantly increases as number of directions increases allowing a better delineation of the gray–white matter junction and thus a more precise FA determination [120, 121]. The ES of splenium FA seemed to increase with decreasing b-value and increasing field strength (MCI vs AD). This could be explained by the fact that a higher field strength provides better spatial and contrast resolution compared to a lower field strength. In one study FA values were significantly higher at 3.0 Tesla compared with 1.5 Tesla [122]. In general, a higher b-value leads to lower signal-to-noise levels and higher FA values [89, 124]. Our finding is therefore somewhat counterintuitive. However, there seems to be a lot of variability between studies regarding the effect of b-value on FA, since other studies found that FA values were not dependent on changes in b-value [123, 124]. The ES of parietal MD (controls vs MCI) and genu CC MD (MCI vs AD) seems to increase with increasing voxel size. The ES of hippocampal MD on the contrary, seems to increase with a decrease in voxel size. A possible explanation is that in broader structures (e.g. corpus callosum) a higher voxel size can clearly reveal possible differences whereas in smaller structures (e.g. hippocampus) a smaller voxel size is needed for better resolution. Current evidence indicates that in general, mean diffusivity values are not affected by voxel size [125].

Supplementary table 3

		Effect size	<i>p</i> -value	Significant univariate regressions	
		Hedges <i>g</i> (CI)		DTI parameter	<i>p</i> -value
FA					
Controls vs AD	<i>N</i> studies (<i>N</i> Controls /<i>N</i> AD)				
Occipital Lobe	14 (298/244)	0.11 (-0.09;0.32)	0.29	Directions	0.05
Total cingulum	5 (84/90)	1.73 (0.22;3.23)	0.02	Field strength	0.008
ACC	3 (59/58)	1.37 (-0.30;3.04)	0.11	Directions B-value (-)	0.04 0.04
Splenium CC	22 (467/417)	1.10 (0.65;1.55)	<0.001	Directions	0.008
Controls vs MCI	<i>N</i> studies (<i>N</i> Controls /<i>N</i> MCI)				
PCC	10 (402/327)	0.65 (0.32;0.98)	<0.001	Directions	0.006
MCI vs AD	<i>N</i> studies (<i>N</i> MCI/<i>N</i> AD)				
Splenium CC	12 (274/256)	0.51 (0.11;0.91)	0.01	Field strength B-value (-)	0.03 0.01
MD					
Controls vs AD	<i>N</i> studies (<i>N</i> Controls /<i>N</i> AD)				
Frontal Lobe	10 (165/176)	-0.75 (-1.24;-0.27)	0.002	Directions	0.053
Parietal Lobe	9 (155/166)	-1.03 (-1.58;-0.49)	<0.001	Directions	0.009
Splenium CC	12 (226/216)	-0.94 (-1.53;-0.35)	0.002	Directions	0.04
Internal capsule	7 (118/118)	-0.66 (-1.19;-0.13)	0.01	Directions	0.02
Controls vs MCI	<i>N</i> studies (<i>N</i> Controls /<i>N</i> MCI)				
Parietal Lobe	5 (77/786)	-0.69 (-1.09;-0.28)	0.01	Voxel size (+)	0.04
MCI vs AD	<i>N</i> studies (<i>N</i> MCI/<i>N</i> AD)				
Hippocampus	3 (68/70)	-0.33 (-0.77;-0.11)	0.14	Field strength Voxel size (-)	0.06 0.06
Genu CC	6 (89/113)	-0.32 (-0.61;-0.03)	0.03	Voxel size (+)	0.01

CI: Confidence interval; MCI: mild cognitive impairment; AD: Alzheimer's disease. Data shown in bold are statistically significant. PCC: posterior cingulum; PHC: parahippocampal cingulum; UF: uncinate fasciculus; SLF: superior longitudinal fasciculus.

Regression: + or – = polarity of regression coefficient/slope.

Chapter 6

Reduced callosal white matter integrity surpasses cerebrospinal fluid and atrophy markers as predictor of decline in subjects with MCI : a combined volumetry and DTI study

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ABSTRACT

Background: Microstructural damage of white matter (WM) tracts is a prominent feature of Alzheimer's disease (AD). To date, it is still unknown whether subtle abnormalities in WM tissue microstructure precede volumetric alterations in gray matter (GM) and how alterations in both tissue-types are related to each other.

Methods: In 3 memory clinics, we recruited 57 patients with mild cognitive impairment (MCI) and 35 patients with mild AD, and combined these with data of 18 healthy controls (Con). The discriminative ability of atrophy and diffusion tensor imaging (DTI) was explored in 6 regions-of-interest: hippocampus, parahippocampal gyrus, posterior cingulum, isthmus of the cingulum and genu and splenium of the corpus callosum. Effect sizes (ES) were calculated in order to detect the measurement with the highest diagnostic potential. The value of both DTI and volumetry for predicting decline in MCI patients at 2 years follow-up was additionally investigated.

Results: Compared to controls, MCI-patients had lower GM volumes in the parahippocampus (ES=1.34), posterior cingulum (ES=1.29) and isthmus (ES=1.03) and AD-patients showed lower baseline volumes in the hippocampus (ES=1.48), parahippocampus (ES=1.33) and isthmus cingulate (ES=0.72). All investigated ROIs showed group differences in WM integrity with highest ES found for parahippocampal (ES>3.0) and hippocampal diffusivity (ES>2.5), followed by posterior cingulum (ES±2.5), isthmus (ES±2.0) and genu (ES±1.8). No differences were found between MCI/AD. Smaller hippocampal volumes in the patient group (MCI+AD) were associated with higher hippocampal diffusivity. Baseline axial and mean diffusivity values in the genu were able to discriminate MCI-stable and MCI-decline.

Conclusions: DTI metrics are more sensitive for group differences than atrophy measurements. Our results suggest that diffusion changes precede and occur independent of GM atrophy. DTI metrics in the genu are able to predict cognitive decline in MCI.

INTRODUCTION

Medial temporal lobe (MTL) atrophy as assessed on structural MRI has proven to be an effective clinical aid in the early diagnosis of AD [1]. MTL atrophy is however not entirely specific for AD since it can also be found in other forms of dementia [2]. It is furthermore known that AD pathology is not restricted to the MTL, also posterior parietal and prefrontal regions are involved [3, 4]. Apart from GM atrophy, it has been shown that white matter (WM) integrity is decreased in subjects with MCI and AD [5], suggesting a potential diagnostic value on a microstructural level that may help to improve the diagnostic accuracy of volumetric AD biomarkers. The integrity of WM can be assessed using diffusion tensor imaging (DTI) which measures the diffusion of water molecules in neural tissue [6]. In general, WM integrity of the following regions seems to be affected: hippocampal gyrus, temporal white matter, genu and splenium of the corpus callosum and posterior cingulum [6-9].

The underlying mechanism responsible for these WM changes is not yet known. It is presumed that there is a local variation in cell density, oligodendrocytes, myelination and amyloid plaques causing altered diffusion of water molecules in certain brain regions. In the majority of DTI studies of AD, the investigation of WM abnormalities was only performed assessing changes in fractional anisotropy (FA) and mean diffusivity (MD). However, contributions to FA and MD alterations could be due to changes of diffusion either parallel or perpendicular to the principal direction of the tensor. These measurements are referred to as axial (AXD), and radial diffusivity (RAD) respectively. An increase in RAD is thought to signify increased space between fibers (suggesting demyelination or dysmyelination) [10] whereas increases in AXD suggest axonal damage [11]. Alterations in diffusion metrics which reflect the integrity of WM tracts not only may give important cues to elucidate the pathophysiological cascade, but could also have diagnostic potential and thus possibly serve as an early marker for AD.

The aim of this study is to investigate the pattern of gray and white matter alterations in MCI and AD-patients in a multicenter setting. To date, no study has compared the diagnostic value of both volumetry and DTI-markers in a multicenter setting in both MCI and AD-patients [12]. Multicenter study designs are important since they show greater generalizability of findings than single-center studies. First, we

explored the ability of automated GM atrophy measurements and DTI metrics to discriminate between healthy controls (Con), subjects with MCI, and subjects with AD in 6 regions of interest (ROI). These ROIs were selected based on their pivotal role in AD and consisted of the hippocampus, parahippocampal gyrus, posterior cingulum, isthmus of the cingulum and genu and splenium of the corpus callosum. Effect sizes (ES) were calculated in order to quantify and compare the potential diagnostic value of both DTI and atrophy measurements. In the second place we investigated associations between the degree of GM loss and WM integrity in the selected ROIs. Third, we investigated the value of these markers for predicting cognitive decline at 2 years follow-up in MCI patients.

METHODS

Subjects

Healthy controls were recruited by means of an advertisement in local newspapers. An extensive neuropsychological test battery was administered and subjects were included when their performance did not deviate from normal on the Verbal Learning Test [13, 14]. Patients with MCI and AD were selected from LeARN, a multicenter study investigating the clinical value of novel biomarkers for AD. Four academic memory clinics (Leiden University Medical Centre, Maastricht University Medical Centre, Radboud University Nijmegen Medical Centre and VU University Medical Centre) specialized in the diagnosis and treatment of memory disorders participated in the study. For the present study, participants were selected from 3 of the 4 participating centers where diagnosis based on expert panel judgments was available. Inclusion and exclusion criteria are described in a previous paper [15]. The MCI-group consisted of both amnesic and non-amnesic subjects, in order to obtain a representative sample of subjects visiting a memory clinic. An overview of clinical and biomarker characteristics of the AD-group can be found in Appendix 1.

Clinical and cognitive assesment

Baseline clinical assessment

All participants underwent a standard diagnostic workup, including clinical history taking, medical and neurological examination, clinical chemistry, functional evaluation using the Clinical Dementia Rating scale (CDR) [16], rating scales for depression and neuropsychiatric symptoms, a neuropsychological test battery and neuroimaging. Cerebrospinal fluid (CSF) was collected for a subsample of the total

population. The MMSE [17] was administered as a general cognitive screening instrument. Subjects were invited annually for follow-up assessment during 2 years, including questionnaires, MMSE and cognitive tests. Diagnosis was made by an expert panel which consisted of a neurologist, a psychiatrist and a geriatrician with at least 5 years of clinical experience. An adjusted Delphi method was applied starting with the assessment of each case by each expert individually, followed by solving all diagnostic discrepancies during a group discussion meeting. Outcome measure was decline (both on a cognitive and/or functional level), which was again determined by the three clinical experts. They were asked to individually judge observed decline by answering the question “In your opinion, what was the course of cognitive or daily functioning during the 2-year follow-up?”. The study was approved by the Medical Ethics Committee of each participating center and written informed consent was obtained from all participants and from the primary caregiver of the AD patients in accordance with the committee’s guidelines and with the Declaration of Helsinki [18].

Neuropsychological examination

A neuropsychological test battery was performed to evaluate performance in several cognitive domains. An overview of both patient and informal caregiver assessments at baseline and follow-up is presented in a previous publication [15].

MRI acquisition and imaging analysis

Scan protocol

At each site, patients were scanned according to the routine MRI-protocol. Scanners and protocols at different sites varied but all scanning was performed at 3.0 Tesla (See Appendix S1 for a detailed description of the scan protocols). Before the start of the study, a dummy procedure was carried out in order to optimize the homogeneity of scan quality in each center. All scans included a three-dimensional T1 weighted gradient echo (3DT1) sequence and diffusion weighted images (DTI) (See Tables S1 and S2 for a detailed description of the scan protocols).

Preprocessing of diffusion data

The DTI data were processed with the freely available MR diffusion toolbox ExploreDTI version 4.8.3 (www.exploredti.com) [19]. Briefly, in a sequence of pre-processing steps the diffusion-weighted images were corrected for subject motion

and eddy current induced distortions, incorporating the B-matrix rotation to preserve the diffusion gradient orientation information correctly [20]. Correction for echo-planar imaging (EPI) distortion was performed using T1 data as reference [21]. The diffusion tensor was estimated with “RESTORE”, a robust non-linear least-squares method [22]. From this diffusion tensor, the DTI metrics FA, MD, RAD and AXD were derived.

FreeSurfer automated volumetry

Cortical reconstruction and volumetric segmentation was performed with FreeSurfer version 5.1.0 (<http://surfer.nmr.mgh.harvard.edu>). The precise technical details of these procedures are described elsewhere [23, 24]. In short, each dataset is corrected for intensity non-uniformities, skull-stripped, and then segmented to identify the boundaries between GM and WM and to create a surface representation of the cortical WM. Finally, the cerebral cortex is parcellated into units based on its gyral and sulcal structure [25]. According to probabilistic information estimated from a reference atlas, a neuroanatomical label is assigned to each vertex of the surface model and the corresponding measures (i.e., volume) are calculated for each section. All procedures with FreeSurfer are conducted in native space. The following GM regions have been chosen for further analysis because they all share an early vulnerability to AD pathology: hippocampus, parahippocampal gyrus, posterior and isthmus cingulate (See supplemental figure S1).

Template based ROI-analyses of DTI metrics

Regional WM integrity was assessed using the DTI metrics FA, MD, RAD and AXD. ROIs were mainly adopted from the cortical parcellation analysis, now focussing on WM regions just below the cortical mantle. Because of the importance of diffusivity changes in callosal regions in early AD, we additionally investigated diffusion abnormalities in the genu and splenium of the corpus callosum. Bilateral ROIs were created based on a template (called ‘Bert’) provided by FreeSurfer. Because the results of ExploreDTI were in native T1 space, the FA, MD, AXD, and RAD volumes were mapped to this participant on the basis of the registration of each T1 to this participant using FSL’s flirt and fnirt from the FSL toolbox (version 5.0.4, see <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki> [26]). Within the selected WM ROIs the average of each DTI metrics was computed.

CSF analyses

CSF was obtained by lumbar puncture between the L3/L4 or L4/L5 intervertebral space, and collected and aliquoted into polypropylene tubes. CSF samples were stored at -80°C at each center and samples of Maastricht and Nijmegen were transported on dry ice to Amsterdam for analysis after up to 2.5 years. The commercially available INNOTEST enzyme-linked immunosorbent assay was used (ELISAs; Innogenetics, Ghent, Belgium) to quantify CSF Ab1-42, performed by experienced laboratory technicians.

Statistical analysis

Analysis was performed with the Statistical Package for Social Sciences (SPSS version 20). Between-group differences on clinical, demographic and cognitive characteristics were analyzed using the independent sample t-test for continuous and χ^2 for categorical variables (significant values set at $p < 0.05$). Univariate analysis of covariance (ANCOVA) was used to test the discriminative ability of CSF markers, gray matter volume and average DTI metrics. Group differences were investigated in the splenium and genu of the corpus callosum, left and right hippocampus, parahippocampal gyrus, posterior and isthmus cingulum. Gender, demeaned age and demeaned center code were included as covariates to account for the potential effects of age, gender and multicenter settings on group differences. Effect sizes were calculated using Hedge's g , which is the difference between the group means divided by the pooled standard deviation. Effect sizes between 0.2 and 0.5 were classified as small, between 0.5 and 0.8 as medium, and over 0.8 as large [27]. In order to investigate the relation between GM and WM tissue abnormalities, correlation analysis (one-tailed) were conducted within the patient group (MCI and AD). To detect whether WM abnormalities were independent of GM abnormalities, GM volumes were entered as a covariate in one of the statistical models. When volumetric data were analysed, intracranial volume (ICV) was added as a control variable. In order to detect which markers (CSF, atrophy or DTI) at baseline were able to predict decline at follow-up, we investigated which markers differed between the groups of MCI-decline and MCI-stable.

Correction for intracranial volume (ICV)

To account for age-related cortical shrinkage we measured the intracranial volumes

(ICV) with the FSL Brain Extraction Tool [28].

Correction for multiple comparisons

To control for Type I errors, we performed the false discovery rate (FDR) controlling procedure in all cases, a correction for multiple comparisons [29]. This method differs from the classical Bonferroni approach in that it is less stringent and as such also reduces the increased probability of making Type II errors.

RESULTS

Subject characteristics

MCI and AD-patients differed from healthy controls in terms of age (Con vs MCI: $p < .01$, Con vs AD: $p < .001$), gender ($p < .001$), MMSE ($p < .001$), delayed recall ($p < .001$) and MTA-score (Con vs MCI: $p < .05$, Con vs AD: $p < .001$) (Table 1). AD-patients showed lower MMSE ($p < .001$) and lower delayed recall scores ($p < .05$) compared to MCI-patients. CSF values of t-tau ($p < .05$) were higher in AD-patients compared to MCI. Values of CSF A β 1-42 and the degree of MTL atrophy as assessed by a visual rating scale (MTA-score) did not differ between both patient groups (Table 1).

Patterns of gray matter atrophy

Relative to controls, MCI-patients had significantly lower GM volumes in the left and right parahippocampus ($p < .001$), right posterior cingulum ($p < .05$), and left ($p < .01$) and right isthmus of the cingulum ($p < .001$) (Table 2). After correction for multiple comparisons, only left ($p < .01$) and right parahippocampal gyrus volume ($p < .01$) significantly differed between Con and AD. No significant GM atrophy differences were found between MCI and AD-patients in the investigated regions (Table 2).

Loss of WM integrity

MCI-patients showed lower FA values in the bilateral isthmus and posterior cingulate, parahippocampal gyrus (all $p < .001$), hippocampus (left: $p = .013$, right: $p = .002$) and genu ($p < .001$) and splenium ($p < .05$) of the corpus callosum compared to healthy controls. Radial and mean diffusivity of the isthmus, parahippocampal gyrus, hippocampus and genu were higher in MCI-patients compared to controls (all $p < .001$). Diffusivity differences between controls and MCI were also found in the splenium ($p = .003$ for MD and $p = .004$ for RAD). Values of axial diffusivity were increased in MCI-patients relative to controls in the posterior and isthmus cingulum,

parahippocampal gyrus, genu (all $p<.001$), splenium ($p=.028$) and hippocampus (left: $p=.01$, right: $p<.001$) (Table 3). Patients with AD showed lower FA values in the bilateral isthmus (left: $p<.001$, right: $p<.01$), posterior cingulum ($p<.001$), parahippocampal gyrus ($p<.001$), left hippocampus ($p=.036$), and genu ($p<.001$) of the corpus callosum compared to healthy controls. Axial, radial and mean diffusivity of the genu, isthmus, posterior cingulum, parahippocampal gyrus, and hippocampus were higher in AD-patients compared to controls (all $p<.001$). Mean diffusivity of the splenium ($p=.035$) was higher in AD-patients compared to controls. (Table 3). Similar to the GM atrophy results, no DTI metrics significantly differed between MCI and AD-patients.

Table 1. Subject characteristics.

	Controls	MCI	AD
N	18	57	35
Age (years)	64.6 (3.4)‡¶	68.5 (8.5)	71.80 (9.3)
Male (%)	100†¶	71	66
Years education	5.9 (2.3)	6.0 (2.8)	5.2 (2.7)
MMSE score	28.9 (1.0)†¶	25.8 (3.0)¶	22.6 (3.2)
Wordlist delayed recall	8.6 (1.9) †¶	3.7 (3.0)^	2.3 (3.3)
MTA-score	1.1 (0.9)◇¶	2.1 (2.8)	2.7 (2.0)
CSF Aβ1-42	-	684.3 (250.7)	598.6 (230.8)
CSF t-tau (pg/mL)	-	471.9 (285.6)^	644.8 (348.6)
CSF p-tau (pg/mL)	-	54.6 (26.4)	68.2 (35.3)

NOTE: Values are mean (SD). Abbreviations: MMSE = Mini-Mental state examination; MTA = medial temporal lobe atrophy; Aβ1-42 = beta amyloid 1-42; t-tau = total tau, p-tau = phosphorylated tau; MCI = mild cognitive impairment; AD = Alzheimer's disease.

† $p<0.001$ compared with the MCI group. ‡ $p<0.01$ compared with the MCI group. ◇ $p<0.05$ compared with the MCI group. ¶ $p<0.001$ compared with the AD group. § $p<0.01$ compared with the AD group. ^ $p<0.05$ compared with the AD group.

Table 2. Regional gray matter volumes (in mm³) of controls and patients with MCI or AD.

	Controls	MCI	AD
Hippocampus			
<i>Left</i>	3833 (288)	3460 (857)	3147 (590)
<i>Right</i>	3837 (286)	3589 (773)	3195 (655)
Parahippocampus			
<i>Left</i>	1314 (170) †§	951 (342)	957 (340)
<i>Right</i>	1357 (295) †§	965 (305)	978 (383)
Posterior cingulate			
<i>Left</i>	4465 (344)	3795 (652)	3835 (541)
<i>Right</i>	4106 (427) ◇	3644 (581)	3512 (592)
Isthmus cingulate			
<i>Left</i>	3872 (521) ‡	3320 (552)	3485 (550)
<i>Right</i>	3558 (436) †	2982 (494)	3201 (562)

NOTE: All analyses stated in the table are corrected for ICV, age, gender and center. Values are mean (SD). Abbreviations: MCI = mild cognitive impairment; AD = Alzheimer's disease. Results are corrected for multiple comparisons [29]. † $p < 0.001$ compared with the MCI group, ‡ $p < 0.01$ compared with the MCI group. ◇ $p < 0.05$ compared with the MCI group, § $p < 0.01$ compared with the AD group.

Quantification and comparison of effect sizes of volumetry and DTI-metrics

In order to quantify and compare the potential diagnostic value of DTI and atrophy measurements, effect sizes were calculated. Highest ES (>3.0) was found for diffusivity measurements in the parahippocampal gyrus (Con vs AD, Con vs MCI) and hippocampus (Con vs MCI, Con vs AD), followed by diffusivity measurements in the posterior and isthmus cingulate and genu (all Con vs MCI, Con vs AD). Volumetric measurements showed somewhat lower effect sizes ranging from 0.7 (hippocampus, Con vs MCI) to 1.48 (hippocampus, Con vs AD) (supp Table S3).

Table 3. Between group differences in regional white matter integrity (corrected for gender, age and center).

	Controls				MCI				AD			
	FA	MD	RAD	AXD	FA	MD	RAD	AXD	FA	MD	RAD	AXD
Hippocampus												
• Left	.28(.02)†π	.82(.06)†¶	.70(.06)†¶	1.06(.08) ‡¶¶	.27(.03)	1.19(.42)	.97(.13)	1.61(.15)	.26(.02)	1.16(.15)	1.00(.14)	1.49(.20)
• Right	.27(.01)†\$	.84(.06)†¶	.72(.06)†¶	1.09(.08)†¶	.26(.02)	1.12(.15)	1.15(.26)	1.44(.20)	.27(.01)	1.15(.14)	1.00(.12)	1.47(.18)
PHG												
• Left	.41(.04)†¶	.64(.04)†¶	.49(.04)†¶	.93(.05)†¶¶	.32(.03)	.90(.12)	.74(.11)	1.22(.16)	.33(.03)	.88(.09)	.73(.09)	1.20(.11)
• Right	.38(.04)†¶	.66(.03)†¶	.52(.04)†¶	.96(.05)†¶¶	.33(.04)	.89(.11)	.73(.10)	1.22(.13)	.34(.04)	.88(.09)	.71(.09)	1.21(.11)
PCC												
• Left	.57(.05)†¶	.70(.09)†¶	.45(.09)†¶	1.22(.10)†¶	.46(.04)	.93(.09)	.67(.09)	1.47(.11)	.48(.05)	.93(.09)	.65(.09)	1.47(.09)
• Right	.56(.05)†¶	.72(.08)†¶	.46(.08)†¶	1.24(.09)†¶	.45(.04)	.94(.09)	.68(.09)	1.47(.11)	.46(.05)	.93(.10)	.66(.10)	1.47(.11)
Isthmus												
• Left	.57(.03)†¶	.76(.06)†¶	.49(.05)†¶	1.32(.09)†¶	.51(.05)	1.09(.31)	.65(.13)	1.56(.17)	.53(.04)	.92(.08)	.62(.08)	1.53(.11)
• Right	.54(.04)†¶	.75(.06)†¶	.49(.05)†¶	1.27(.08)†¶	.51(.05)	1.06(.30)	.63(.11)	1.51(.13)	.52(.05)	.90(.09)	.61(.09)	1.49(.11)
Corpus callosum												
• Genu	.59(.04) ‡¶	.91(.10) ‡¶	.57(.08) ‡¶	1.60(.16) ‡¶¶	.52(.08)	1.14(.16)	.78(.18)	1.86(.14)	.53(.07)	1.16(.16)	.80(.16)	1.90(.17)
• Splenium	.61(.05)‡	.97(.06) ‡π	.59(.07)‡	1.75(.11) ‡	.62(.07)	1.04(.11)	.63(.13)	1.86(.14)	.63(.06)	1.01(.08)	.60(.09)	1.83(.11)

NOTE: All analyses stated in the table are corrected for age, gender and center. Values are mean (SD).

Abbreviations: PHG = parahippocampal gyrus; PCC = posterior cingulum; MCI = mild cognitive impairment; AD = Alzheimer's disease;

FA = fractional anisotropy; MD = mean diffusivity; RAD = radial diffusivity; AXD = axial diffusivity. Results are corrected for multiple comparisons [29]. †p<0.001 compared with the MCI group. ‡p<0.01 compared with the MCI group, ‡p<0.05 compared with the MCI group. ¶p<0.001 compared with the AD group. §p<0.01 compared with the AD group. πp<0.05 compared with the AD group.

Associations between gray and white matter abnormalities

In the patient group (MCI/AD, n=92) was found that smaller hippocampal volumes were associated with higher values of hippocampal diffusivity (MD, RAD, AXD), especially in the left hemisphere (Fig. 1, supplemental Table S4).

Group differences in WM diffusivity did not change when analyses were additionally corrected for GM volumes indicating that WM diffusivity differences were independent of GM atrophy patterns.

MCI decline vs MCI stable

None of the GM volumes of the investigated structures or CSF values differed between MCI-patients showing decline at follow-up compared to MCI-patients that remained stable (Table 4). With respect to diffusivity, values of MD ($p=.013$) and AXD ($p=.001$) of the genu were higher in MCI-subjects who clinically declined compared to MCI-subjects without decline (Table 4, Fig. 2).

Table 4. Differences between MCI-stable and MCI-decline.

	MCI-stable	MCI-decline
N	26	31
Age	65.2 (9.0)**	71.3 (7.0)
Male (%)	65	74
Years education	5.8 (3.0)	6.1 (2.7)
Baseline MMSE score	25.7 (2.5)	25.7 (3.3)
Baseline wordlist delayed recall	4.4 (3.0)	3.2 (2.9)
Hippocampus (mm ³)		
<i>Left</i>	3765 (555)	3203 (771)
<i>Right</i>	3844 (674)	3373 (796)
Parahippocampus (mm ³)		
<i>Left</i>	1002 (315)	907 (361)
<i>Right</i>	1010 (298)	926 (310)
Posterior cingulum (mm ³)		
<i>Left</i>	3909 (501)	3700 (751)
<i>Right</i>	3841 (472)	3477 (619)
Isthmus of the cingulum		
<i>Left</i>	3282 (463)	3351 (595)
<i>Right</i>	3043 (486)	2930 (501)
Genu corpus callosum		
<i>FA</i>	.54 (.09)	.51 (.08)
<i>MD</i>	1.07 (.14)*	1.20 (.15)
<i>RAD</i>	.72 (.17)	.84 (.17)
<i>AXD</i>	1.78 (.11)**	1.92 (.15)
MTA-score	1.2 (1.6)	2.7 (3.3)
CSF A β 1-42	733.4 (283.5)	637.5 (211.4)
CSF t-tau	403.8 (345.4)	536.9 (201.9)
CSF p-tau	47.1 (30.9)	61.8 (19.3)

NOTE: Volumetric measurements are corrected for intracranial volume. Values are mean (SD). Abbreviations: MMSE = Mini-Mental state examination; MTA = medial temporal lobe atrophy; FA = fractional anisotropy; MD = mean diffusivity; RAD = radial diffusivity; AXD = axial diffusivity; A β 1-42 = beta amyloid 1-42; t-tau = total tau, p-tau = phosphorylated tau; MCI = mild cognitive impairment; AD = Alzheimer's disease. Results are corrected for multiple comparisons [29]. * $p < .05$, ** $p < .01$ for differences between groups. Analyses were corrected for age and center (no gender differences were present).

Fig. 1. Associations between volumetric and mean diffusivity values in hippocampal regions. (A, B) Group differences between controls, MCI and AD for left (A) and right (B) hippocampal mean diffusivity (*all $p < .001$), (C) Associations between diffusivity in the left hippocampus ($r = -.52$; $p < .01$) and (D) Associations between left hippocampal volume and mean diffusivity in the right hippocampus ($r = -.19$; $p < .05$). The bilateral hippocampus was the only structure, among those considered in the present study, where associations were found between GM atrophy and WM integrity. NOTE: MCI = mild cognitive impairment; AD = Alzheimer's disease; MD = mean diffusivity.

Fig. 2. Diffusivity differences in anterior and posterior parts of the corpus callosum in MCI-stable vs MCI-decline (2 years follow-up). The anterior part of the corpus callosum (CC) (genu) is depicted in green and the posterior part of the CC is depicted in blue (splenium.) *Differences between MCI-stable and MCI-decline were found in the genu: mean and axial diffusivity metrics (MD: $p = .013$, AXD $p = .04$). No differences in anisotropy (FA) or radial diffusivity (RAD) were found between MCI-stable and MCI-decline. NOTE: MCI = mild cognitive impairment; FA = fractional anisotropy; MD = mean diffusivity; RAD = radial diffusivity; AXD = axial diffusivity.

DISCUSSION

The present study applied a combined volumetric MRI and DTI method on selected regions of interest to obtain complementary information on both GM atrophy and microstructural WM alterations in brain tissue of MCI and AD-patients in a multicenter setting. The concomitant measurement of both GM volume and WM diffusion measures allowed us to detect the brain tissue effects of underlying AD-pathology. Together with the expert panel diagnosis, the novelty in this study was the investigation of the potential of DTI to discriminate between progressive and stable MCI-patients in relation to volumetry, CSF and neuropsychological data.

It was found that diffusivity measurements are more sensitive for group differences (higher ES) than atrophy measurements. DTI metrics are furthermore not significantly influenced by the effects of ageing, gender and center in apriori selected ROIs. Group differences in WM diffusivity did not change when analyses were additionally corrected for GM volumes indicating that WM diffusivity differences were independent of GM atrophy patterns. Baseline axial and mean diffusivity values in the genu were able to discriminate MCI-stable and MCI-decline.

Previous findings showed a widespread pattern of GM atrophy in AD and a more restricted GM atrophy pattern in MCI patients [30]. In our study, a similar pattern of GM differences was found in the bilateral parahippocampal gyrus (Con/MCI and Con/AD), isthmus of the cingulum (Con/MCI) and right posterior cingulum (Con/MCI). Other structures were more susceptible to ageing (hippocampus) effects or did not survive the correction for multiple comparisons (hippocampus, left posterior cingulum, isthmus of the cingulum (Con/AD)). Group differences in diffusivity were evident in all the investigated structures. Highest ES was found for the parahippocampal gyrus, followed by the hippocampus, posterior and isthmus cingulate, and genu (Con/AD). For the discrimination between Con/MCI, ES was highest for the parahippocampal gyrus, followed by the hippocampus and posterior cingulate. In line with previous studies we found that changes in absolute diffusivity (MD, RAD and AXD) were more sensitive for group differences than changes in anisotropy (FA) [31]. This could be explained by the fact that if both AXD and RAD are increased in AD, MD is increased and therefore FA can remain relatively unperturbed [32]. Important to note is that DTI group differences were

less influenced by the effects of ageing, gender and scanner differences than GM atrophy differences.

Predictors of decline

Only baseline diffusivity (MD and AXD) in the genu of the corpus callosum differed between MCI-decline and MCI-stable. These differences were furthermore independent of age, gender or site differences. No demographic, CSF or volumetric measurements were able to predict cognitive decline after 2 years in the group of MCI patients. Several studies found an early involvement of the genu in early and preclinical stages of AD [33, 34]. A possible explanation for our findings is that the genu contains the highest density of small-diameter fibers, which are known to be most susceptible to neurodegeneration [35]. As a result, WM damage in the genu becomes more evident with disease progression, and diffusion metrics in this region thus allow discrimination between decliners and non-decliners. Secondly, with disease progression, inter-hemispheric connections of prefrontal regions become abnormal, a process in which the genu is known to play a central role [36]. We indeed found differences between MCI-stable and MCI-decline on the verbal fluency test, a test known to rely on prefrontal regions during active retrieval of semantic information ($p=.02$). Other authors however state that the splenium of the CC is the site of maximum damage in early AD [32]. Despite the fact that diffusivity in the splenium differed between both patient groups and controls, the splenium did not show any predictive value for decline in the group of MCI-subjects.

Loss of WM integrity is independent of GM atrophy

The associations between the degree of GM atrophy and elevated values of absolute diffusivity (MD, RAD, AXD) in the bilateral hippocampus are in line with previous studies [37] and suggest the presence of Wallerian degeneration of connecting fibers, secondary to GM atrophy in this region [38]. However, our results did not change when analyses were additionally corrected for GM volume, suggesting that WM diffusivity differences were independent of GM atrophy patterns and that Wallerian degeneration is not the primary mechanism of WM change in AD. The lack of a significant correlation between diffusivity and volumetric measurements in regions outside the hippocampus strengthens the idea that DTI and MR-based volumetry measures represent different aspects of underlying pathology [39]. These findings are in line with previous work [33, 40] and neuropathological evidence indicating

that WM changes in AD are not solely due to Wallerian degeneration [41].

Similarities between MCI and AD-patients

MCI and AD patients differed with respect to MMSE and delayed recall scores (MCI>AD) in the absence of GM or WM differences. Values of diffusivity (MD, RAD, AXD) in some ROIs were even lower in AD compared to MCI patients. These inconsistencies are likely to be due to differences in the clinical stage of the recruited patients. Since MCI patients represent a heterogeneous group of subjects, their profile of GM and WM abnormalities may be similar to that of AD patients, without major consequences in daily life. Another possibility is that the majority of loss of WM integrity occurs at the time of disease manifestation, while further progression to dementia is accompanied by lower rates of WM change. Previous studies found the same pattern of overlap between MCI and AD [42-44]. The similarity of *in vivo* volumetric and diffusivity measures in the AD and MCI groups, together with a different clinical status, could be explained by the fact that the AD group has less cognitive reserve which could have led to an earlier transition to AD compared to an extended MCI status in the group with less impaired global and episodic memory impairment. Except for the higher preservation of cognitive functioning, subjects with MCI did not differ from AD patients with respect to educational level or head size, both direct indicators of cognitive reserve [45]. Other, more standardized measures of cognitive reserve were however unavailable in the present study. The group of MCI-patients in our study consisted of both amnesic and non-amnesic subtypes. The heterogeneous nature of this sample allowed us to investigate decline in a representative sample of subjects visiting a memory clinic. The same pattern of diffusion abnormalities was furthermore found in MCI-decline vs MCI-stable when only amnesic MCI (aMCI) subjects were included in the statistical analyses (n=32) (data not shown). Diffusivity abnormalities in the genu were again the only predictors of decline in the group of aMCI.

Strengths and Limitations

It can be considered a strength that for this diagnostic study a memory clinic population was used as we aim to generalize our findings to these subjects who are eligible candidates for clinical trials. As a consequence, however, our findings may not be applicable to other settings, including the general population. The

use of different MR imaging units and acquisition protocols could have potentially introduced bias. Apart from uniformity during the preprocessing, we additionally corrected for the effect of site differences in all analyses. The present study adopted an automated ROI-analysis based on apriori hypotheses. An advantage is that typical problems associated with voxel-based methods (e.g. alignment) or manual ROI definition (e.g. subjective, time-consuming) are avoided. A disadvantage of the automated atlas-based ROI approach is that changes are only revealed in pre-selected areas, lacking whole brain volumetric and diffusion information.

Clinical implications

Despite the clinical relevance of current DTI-findings in understanding the underlying pathology of AD, many of these findings are not yet readily transferrable to clinical practice. The Alzheimer's Disease Neuroimaging Initiative (ADNI) and ADNI-2, did not adopt DTI as a core protocol due to the uncertain long-term test-retest precision [46], questionable relevance to clinical trials, and absence of an established calibration method [47]. These pitfalls should be thoroughly investigated and resolved before DTI can be successfully implemented in everyday clinical practice. In order to implement DTI data as a biomarker for AD, future studies need to establish and validate cut-offs, and standardize automated techniques for use in clinical practice. After overcoming these methodological issues, a quantitative metric of absolute diffusivity (MD, RAD or AXD) could be extracted from posterior parietal (e.g. PCC) or temporal regions (e.g. parahippocampal gyrus or hippocampus) in order to evaluate the presence of neurodegenerative processes (diagnosis). For tracking disease progression, one could measure absolute diffusivity in the anterior part of the corpus callosum (genu) to evaluate which MCI-patients are prone to decline within 2 years (prognosis). The clear boundaries of the corpus callosum make this major WM tract easy to delineate, in comparison to WM portions in medial temporal structures.

CONCLUSION

The present study demonstrated significant GM and WM differences between AD, MCI and healthy controls in a multicenter setting. Diffusivity measurements are more sensitive for group differences (higher ES) than volumetric measurements and are less influenced by the effects of ageing, gender and center in apriori selected ROIs. Our results suggest that diffusion changes precede and also occur independently of

GM atrophy. DTI metrics in medial temporal, posterior parietal and callosal regions may serve as an early marker for degeneration, even before atrophy is detectable. Diffusivity differences in the genu of the corpus callosum are able to predict decline in a group of MCI-subjects. Translation of the current findings to clinical practice remains to be demonstrated.

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SUPPLEMENTAL MATERIAL

Supplementary Table S1. T1 scan protocol parameters^a

Center	Mode	TR (ms)	TE (ms)	Flip angle (°)	Matrix	Slices	Voxel size (mm ³)
MUMC	3D FFE	8.2	3.7	8	240 x 240	180	1 x 1 x 1
VUMC	3D FFE	7.8	3	12	256 x 256	176	0.9375 x 0.9375 x 1
NUMC	3D FFE	2300	4.7	12	256 x 256	192	1 x 1 x 1

^a All acquisitions were made in the sagittal plane

Supplementary Table S2. DTI scan protocol parameters^a

Center	TR (ms)	TE (ms)	Matrix	Slices	Voxel size (mm ³)	B0 ^b	Directions	b value (s/mm ²)
MUMC	8250	80	128 x 128	70	2 x 2 x 2	1	61	1000
VUMC	13000	94	256 x 256	45	1 x 1 x 2.4 ^c	5	30	1000
NUMC	13000	102	128 x 128	81	2 x 2 x 2	1	30	1000

^a All acquisitions were made in the axial (transversal) plane

^b Number of B0 acquisitions

^c Acquisition was made with a voxel size of 2 x 2 x 2.4 mm³ and then interpolated.

Supplementary Table S3. Effect sizes (Hedge's g) of volumetry and DTI metrics.

Measure	Group	Hedge's g	CI 95-	CI 95+
RAD PHG	Con/AD	-3.45	-4.32	-2.57
MD PHG	Con/AD	-3.45	-4.32	-2.57
AXD PHG	Con/AD	-3.16	-4	-2.32
RAD PHG	Con/MCI	-3.02	-3.74	-2.3
MD HC	Con/AD	-2.98	-3.79	-2.17
MD PHG	Con/MCI	-2.91	-3.62	-2.2
AXD HC	Con/AD	-2.82	-3.61	-2.03
RAD HC	Con/AD	-2.79	-3.57	-2
RAD HC	Con/MCI	-2.67	-3.35	-1.98
AXD PCC	Con/AD	-2.63	-3.39	-1.86

Measure	Group	Hedge's g	CI 95-	CI 95+
MD PCC	Con/MCI	-2.56	-3.23	-1.88
MD PCC	Con/AD	-2.56	-3.31	-1.8
FA PHG	Con/MCI	2.55	1.87	3.22
AXD PHG	Con/MCI	-2.45	-3.11	-1.78
RAD PCC	Con/MCI	-2.44	-3.11	-1.78
FA PCC	Con/MCI	2.43	1.77	3.09
AXD PCC	Con/MCI	-2.38	-3.03	-1.72
MD isthmus	Con/AD	-2.26	-2.98	-1.54
FA PHG	Con/AD	2.26	1.54	2.98
RAD PCC	Con/AD	-2.22	-2.94	-1.51
AXD isthmus	Con/AD	-2.09	-2.79	-1.39
RAD isthmus	Con/AD	-1.95	-2.63	-1.27
MD genu	Con/AD	-1.87	-2.55	-1.2
RAD genu	Con/AD	-1.82	-2.49	-1.15
AXD genu	Con/AD	-1.82	-2.49	-1.15
FA PCC	Con/AD	1.8	1.13	2.47
AXD isthmus	Con/MCI	-1.76	-2.37	-1.16
AXD genu	Con/MCI	-1.73	-2.33	-1.13
MD genu	Con/MCI	-1.72	-2.32	-1.12
MD isthmus	Con/MCI	-1.48	-2.06	-0.9
HC volume	Con/AD	1.48	0.84	2.11
FA isthmus	Con/MCI	1.46	0.88	2.04
PCC volume	Con/AD	1.39	0.76	2.02
PHG volume	Con/MCI	1.34	0.77	1.92
PHG volume	Con/AD	1.33	0.7	1.95
PCC volume	Con/MCI	1.29	0.72	1.85
MD HC	Con/MCI	-1.23	-1.8	-0.67
FA isthmus	Con/AD	1.13	0.52	1.74
FA genu	Con/MCI	1.11	0.55	1.67
FA genu	Con/AD	1.05	0.45	1.66
Isthmus volume	Con/MCI	1.03	0.47	1.58
FA HC	Con/AD	1	0.4	1.6
AXD splenium	Con/MCI	-0.87	-1.42	-0.33
AXD HC	Con/MCI	-0.83	-1.38	-0.29
RAD isthmus	Con/MCI	-0.81	-1.35	-0.26
MD splenium	Con/MCI	-0.79	-1.34	-0.24

Measure	Group	Hedge's g	CI 95-	CI 95+
AXD splenium	Con/AD	-0.73	-1.31	-0.14
Isthmus volume	Con/AD	0.72	0.14	1.31
RAD genu	Con/MCI	-0.72	-1.26	-0.18
HC volume	Con/MCI	0.68	0.14	1.22
MD splenium	Con/AD	-0.57	-1.14	0.01
FA HC	Con/MCI	0.39	-0.14	0.93
RAD splenium	Con/MCI	-0.38	-0.92	0.15
FA splenium	Con/AD	-0.36	-0.93	0.21
FA splenium	Con/MCI	-0.16	-0.69	0.37
RAD splenium	Con/AD	-0.12	-0.69	0.44

NOTE: Only left side of the brain was taken into account.

CI = confidence interval; RAD = radial diffusivity; AXD = axial diffusivity; MD = mean diffusivity; FA = fractional anisotropy; PHG = parahippocampal gyrus; PCC = posterior cingulum; Con = Controls; MCI = mild cognitive impairment; AD = Alzheimer's disease.

Supplementary Table S4. Associations between GM atrophy and WM integrity

Structure	MD L	MD R	RAD L	RAD R	AXD L	AXD R
Hippocampus L	-.52**	-.19*	-.23*	-0.15	-.52**	-.23*
Hippocampus R	-.45**	-0.1	-0.12	-0.07	-.48**	-0.14

NOTE: Differences in anisotropy (FA) were not associated with hippocampal volume.

MD = mean diffusivity; RAD = radial diffusivity; AXD = axial diffusivity; L = left, R = right.

* $p < .05$, ** $p < .01$

Supplemental Fig. S1. Regions-of-interest selected for the present study. The following structures were selected for further analysis based on their pivotal role in Alzheimer's disease: hippocampus (blue), parahippocampal gyrus (red), posterior cingulum (green), and isthmus of the cingulum (yellow). (A) Sagittal view showing the hippocampus and parahippocampal gyrus, (B) Coronal view showing the posterior cingulum, hippocampus and parahippocampal gyrus and (C) Sagittal view showing the isthmus and posterior part of the cingulum.

Appendix 1. Clinical characteristics of the Alzheimer group

Alzheimer diagnosis was made by an expert panel which had access to clinical, functional and neuropsychological data, together with quantitative MRI information (e.g. vascular pathology (infarcts, microbleeds), degree of medial temporal lobe atrophy (MTA) as measured by the visual rating scale [48]). Since no other biomarker (e.g. CSF) apart from MTA was included in the expert panel diagnosis, some AD-cases a posteriori showed a non-AD-like CSF profile (n=14). We further investigated this group and excluded 2 subjects from the initial group of 37 AD-subjects since both patients showed a neuropsychological profile with mainly executive problems together with an MTA-score below cut-off [1], probably due to vascular dementia. From the remaining AD-subjects with a non-AD-like CSF profile (n=12), 6 patients were categorized as 'SNAP' [49] showing abeta 1-42 values just above the cut-off (>550 in stead of the suspected ≤ 550) together with elevated values of t-tau (>375) and p-tau (>52) [50]. Of the 6 remaining subjects, 2 showed severe MTA as assessed by the visual rating scale (left+right side, >6), 3 cases showed severe episodic memory problems matching an Alzheimer profile and 1 patient showed severe memory problems together with highly impaired activity of daily living (ADL).

The background of the slide features three overlapping film reels. The reels are light beige with white rectangular frames. Some frames contain black text and arrows, such as 'UP FOR PROJECTOR' and the number '2'. The reels are positioned in the top right, bottom left, and bottom right corners, creating a circular pattern around the central text.

Part III

Robustness of structural and
functional abnormalities in AD

Chapter 7

Can FreeSurfer compete with manual volumetric measurements in Alzheimer's disease?

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ABSTRACT

Alzheimer's disease (AD) results in tremendous structural and functional changes in the brain. The performance of automated brain segmentation techniques may be less precise when applied in AD-patients, possibly leading to false allocations of gray matter, white matter or cerebrospinal fluid. FreeSurfer has been shown to operate as an accurate and reliable instrument to measure cortical thickness and volume of neuroanatomical structures. Considering the principal role of FreeSurfer in the imaging field of AD, the present study aims to investigate the robustness of FreeSurfer to capture morphological changes in the brain against varying processing variables in comparison to manual measurements (the gold standard). T1-weighted MRI scan data were used pertaining to a sample of 53 individuals (18 healthy participants, 18 patients with mild cognitive impairment, and 17 patients with mild AD). Data were analyzed with different FreeSurfer versions (v4.3.1, v4.5.0, v5.0.0, v5.1.0), on a custom-built cluster (LINUX) and a Macintosh (UNIX) workstation. Group differences across versions and workstations were most consistent for both the hippocampus and posterior cingulate, regions known to be affected in the earliest stages of the disease. Furthermore, it was found that later versions of FreeSurfer were more sensitive for group differences, and corresponded best with the gold standard. In conclusion, later versions of FreeSurfer were more accurate than earlier versions, especially in medial temporal and posterior parietal regions. This development is very promising for future applications of FreeSurfer in research studies, and encourages the future role of FreeSurfer as a candidate marker in clinical practice.

INTRODUCTION

Considering the increase of the aging population in our society and age being the greatest risk factor for the development of dementia, there is a growing interest in understanding and treating dementia. Currently, Alzheimer's disease (AD) is estimated to affect 35 million patients worldwide (or 0.5% of the global population) and this number is estimated to increase to 115 million by 2050 [1]. On a brain level, Alzheimer's disease (AD) pathology results in excessive structural and functional damage, secondary to processes such as accumulation of amyloid-beta and tau proteins, neuroinflammation and neuronal death [2]. Structural and functional imaging measurements are currently evaluated for clinical use in predicting or diagnosing AD [3, 4]. An indispensable part of this effort is the development of a robust method to measure morphological and pathological changes in the brain [5]. Manual volumetric measurements are still regarded gold standard for evaluation of local brain atrophy [5, 6], however, clinical settings require diagnostic instruments that are quick, reliable and easy to implement. FreeSurfer (Athina A. Martinos Center for Biomedical Imaging, Boston) comprises a popular and freely available set of tools for deriving neuroanatomical volume and cortical thickness measurements by means of automated brain segmentation (<http://surfer.nmr.mgh.harvard.edu>). At present, more than 80 studies are published using FreeSurfer to investigate structural changes in the brain of (early) AD patients (Source: PubMed, <http://www.ncbi.nlm.nih.gov/pubmed/>).

Due to the variation in software and hardware environments, both in research and clinical practice, an equally important question related to measuring brain atrophy concerns the power and robustness of automated techniques to capture (small) morphological and pathological changes in the brain against these varying processing conditions. Since the pathological events seen in AD affect the morphology of the brain, it is conceivable that with such fundamental changes in brain structure, minor changes in processing conditions could affect the segmentation process and thus observed group differences. In a previous study we systematically evaluated how the morphometric results derived from FreeSurfer may be affected by the following processing variables: FreeSurfer version (v4.3.1, v4.5.0, and v5.0.0), workstation type (Macintosh and Hewlett-Packard), and Macintosh Operating System version (OSX 10.5 and OSX 10.6) [7]. Results revealed significant differences between

FreeSurfer version v5.0.0 and the two earlier versions ranging between $8.8 \pm 6.6\%$ (range 1.3 – 64.0%) (volume) and $2.8 \pm 1.3\%$ (1.1 – 7.7%) (cortical thickness). About twice as small differences were found between either the two workstation types or between OSX 10.5 and OSX 10.6. These differences almost peer with effect sizes reported in neurodegenerative studies. Our previous study investigated changes in healthy young subjects and patients suffering from psychotic disorder [7]. Brain changes in these groups are less pronounced than those found in aging and neurodegenerative diseases. In view of the fact that FreeSurfer is extensively used in studies of ageing, a next step is a validation of FreeSurfer in a neurodegenerative population. The novelty in this study is that a comparison with manual volumetry is included as reference method, which is required in order to validate the accuracy of the segmentation results and thus subsequently detect which abnormalities are due to neurodegeneration and which can be classified as error. Such validation studies require a comparison with manual segmentations or even post-mortem assessments (see [8] for a discussion on the limitations of both reference methods). The accuracy of the cortical thickness measures is more difficult to validate and requires mainly post-mortem (histological) measurements [9]. Only few studies directly compared FreeSurfer with manual volumetric measurements, mainly focusing on the medial temporal lobe in AD [10, 11], major depressive disorder [12], and temporal lobe epilepsy [13, 14]. These studies generally suggest that manual volumetry is slightly superior or equally sensitive to FreeSurfer automated volumetric measurements.

The aim of the present study is to investigate whether the sensitivity of FreeSurfer to detect group differences is consistent over different software versions and operating systems despite tremendous morphological changes typically seen in AD-patients. Stability of group differences across various processing conditions is investigated, and in order to understand which processing condition fits best with the 'reference' group differences, FreeSurfer's group differences are compared with manual volumetry, the gold standard in research practice [6, 15]. To the best of our knowledge, this is the first study assessing group differences across various processing conditions and validating these findings against gold standard measurements. Based on the literature, six well-established AD signature regions were chosen. Hippocampal atrophy is known to play a major role in the development of AD [16], but is however not specific for AD [17]. Since other medial temporal lobe (e.g. parahippocampal gyrus)[18], prefrontal (e.g. inferior prefrontal

and orbitofrontal cortex) [19] and posterior parietal regions (e.g. posterior cingulate and precuneus) [20] have shown to be altered during the disease process, these regions are additionally evaluated.

METHODS

Participants

Three groups of male participants were included: 18 healthy participants without any subjective memory impairment (CON), 18 patients with amnesic MCI (aMCI), and 17 patients with mild AD [18, 21]. Mean age (years $\pm$ sd) of the included individuals was 64.6 ± 3.4 , 65.1 ± 4.5 , and 70.6 ± 9.1 , respectively. The study was approved by the ethics committee of the Maastricht University Medical Center and all participants gave written informed consent in accordance with the committee's guidelines and with the Declaration of Helsinki [22].

MRI acquisition

MRI scans were administered with a 3T whole-body MR system release 2.0 (Philips Achieva, Philips Medical Systems, Best, The Netherlands) equipped with an eight-element head coil (SENSE, factor 2). Anatomical T1 images were acquired with a gradient echo sequence with TR = 8.0 ms, TE = 3.7 ms, FA = 8°, FOV = 240 x 240 mm², matrix = 240 x 240, number of slices = 180, voxel size = 1.0 x 1.0 x 1.0 mm³.

FreeSurfer

Automated volumetry

Cortical reconstruction and volumetric segmentation was performed with FreeSurfer, which is freely available (<http://surfer.nmr.mgh.harvard.edu>). The technical details of these procedures have been described previously (for a recent overview see: [9]). Briefly, in this approach, brain areas are segmented using a nonlinear template matching [23]. After linearly registering the test image to the template, the algorithm estimates the nonlinear transformation between a given MRI and a probabilistic atlas of the selected brain structure constructed from a cohort of 40 subjects aged 19-87 years using a maximum likelihood criterion [24]. Probabilistic labels are warped back to the individual MRI using the inverse of this transform. The final segmentation is accomplished by maximizing the a posteriori probability in the Bayes formula at each voxel. Voxel-wise probabilistic labels and their predicted

image intensities serve as the prior term, while the intensity similarity between the target image and the template serves as the likelihood term. In this study, both voxel and tabulated volumes (corrected for partial volume effects) were used. Important to note is that voxel volumes are most suited to obtain a proper comparison with manual volumetry, because of the absence of partial volume correction.

Cortical thickness (CT)

The FreeSurfer CT pipeline has been described and validated in previous publications [25-28]. To summarize, processing involves intensity normalisation, registration to Talairach space, skull stripping, segmentation of WM, tessellation of the WM boundary, smoothing of the tessellated surface and automatic topology correction. The tessellated surface is used as the starting point for a deformable surface algorithm to find the WM and then the pial boundary. For each point on the tessellated WM surface, the CT is calculated as the average of the distance from the WM surface to the closest point on the pial surface and from that point back to the closest point on the WM surface [26]. The cortex of the brain was automatically subdivided into gyral-based regions of interest (ROIs) [24]. To accomplish this, a registration procedure was used that aligns the cortical folding patterns and probabilistically assigns every point on the cortical surface to one of the 34 ROIs. For the purposes of this study, we focused on 6 ROIs bilaterally. For each ROI the mean cortical thickness was extracted for subsequent statistical analysis. This technique is referred to as CT-parcellation.

For our second approach, a vertex-wise analysis, the thickness measures were mapped on a spherically inflated surface of each participant's reconstructed brain. This allows visualization across the surface without interference from cortical folding. By means of a combination of linear and non-linear transformations, the spherical cortical folding patterns were aligned to a spherical template provided by FreeSurfer. This technique, called "surface- based intersubject registration" [29], provides an accurate matching of morphologically homologous cortical locations across participants on the basis of each individual's anatomy while minimizing metric distortions. The resulting CT map was smoothed by a circularly symmetric Gaussian filter with a full width half maximum (FWHM) set to 20 mm in order 1) to compensate for residual misalignments; 2) to increase the signal-to-noise ratio; and 3) to make the data more normally distributed. This technique is referred to as

CT-vertex henceforth.

Quality control was performed after each step of the automated FreeSurfer pipeline (volumetry and cortical thickness) to account for possible errors (e.g. misregistrations, outliers).

Manually defined ROIs

For the manual tracing we used GIANT (General Image Analysis Tools; [30]), a software program which allows tracing of regions of interest (ROI) in a triplanar and rotatable 3D surface-rendered view, and calculation of GM volumes of interest. Boundaries of the selected frontal and temporal structures were set according to criteria described in a previous publication [31]. Boundaries of the posterior cingulate and precuneus cortex were adapted from [32] and [33], respectively. Both raters (LC, CE) were blind to the demographic and cognitive characteristics of the participants. Intra-rater reliability was determined by the Intraclass Correlation Coefficient (ICC) [34]. ICC's for each region can be found in a previous publication [35] (Supplementary Table S1).

Regions of interest (ROI) selected from the Desikan atlas

For the purpose of this study, we used the Desikan atlas and focused on one subcortical ROI and five cortical ROIs bilaterally: the hippocampus, the parahippocampal gyrus (PhG), the inferior prefrontal cortex (IPFC), the orbital frontal cortex (OPFC), the posterior cingulate cortex (PCC), and the precuneus (PC) (see Appendix for more details on which FreeSurfer ROIs [24]) were selected and merged). FreeSurfer ROIs were chosen in accordance with the anatomical borders of the manually defined ROIs: e.g. FreeSurfer's definition of the isthmus was most consistent with our definition of the posterior cingulate cortex as adopted for the manual segmentation and was subsequently used. The posterior cingulate cortex ROI included in the FreeSurfer automated measurement is more rostral compared to our definition.

Intracranial volume

The ICV was calculated from the inner skull contours produced by the FSL Brain Extraction Tool [36] and visually checked.

Processing conditions

Workstations

Several workstations and corresponding operating systems (OS) were used: an iMac with OSX 10.5, a MacPro with OSX 10.6 (called “iMac2” and “MacPro2” in Gronenschild et al. 2012, respectively), and a custom-built cluster equipped with Intel i7 quad-cores (3.20 GHz) running under Scientific Linux 6.2 (called “RadCluster” henceforth). Both Macintosh (Mac) workstations were used in 32 bits mode and the RadCluster in 64 bits mode.

Software versions

Four versions of FreeSurfer were used: v4.3.1, released on 19 May 2009, version v4.5.0, released on 11 August 2009, version v5.0.0, released on 16 August 2010, and version v5.1.0, released on 24 May 2011. For the Mac workstations these are 32 bits versions, whereas for the RadCluster these are 64 bits versions. An additional remark with respect to v5.1.0 should be made: we used an intermediate version of the processing pipeline in order to resolve issues around the order of the correction for intensity non-uniformity stage and Talairach stage in the pipeline (see also <https://surfer.nmr.mgh.harvard.edu/fswiki/TalFailV5.1>).

Statistical Analysis

Group analysis of the segmentation results was performed in two ways. In the first approach, analysis was performed with IBM SPSS version 19 (Chicago, IL, USA) on Macintosh version 19.0. FreeSurfer-based volumes, manual volumes and CT-parcellations were compared between the three groups by means of univariate pair-wise ANCOVA with either volume or CT-parcellation as dependent variable, group as independent variable and centered age as covariate. For both volumetric measurements, intracranial volume (ICV) was taken as an additional covariate. To correct for multiple comparisons, we applied the false discovery rate (FDR) controlling procedure [37].

Our second approach was a vertex-wise analysis of CT using FreeSurfer tools. Statistical comparisons between the CT maps were generated by computing a general linear model (GLM) of the CT group differences (corrected for centered age) at each vertex in the cortical mantle, with a statistical threshold set to $p = 0.05$. A cluster-wise procedure was performed to correct for multiple comparisons [38]. This

method utilizes a simulation to get a measure of the distribution of the maximum cluster size under the null hypothesis. Z-maps are synthesized and smoothed using a residual FWHM, and then thresholded at $p = 0.05$. Next, areas of maximum clusters are recorded, under these specifications, and the procedure is repeated for 5000 iterations. Once the distributions of the maximum cluster size are obtained, correction for multiple comparisons is performed by finding clusters in the statistical maps using the same threshold as was given in the simulation procedure. For each cluster, the p value is the probability of seeing a maximum cluster of that size, or larger, during the simulation. Clusters remaining in similar areas of significance as in the original cortical thickness maps would indicate that the result is not likely due to chance. For each cluster, maximum, minimum, mean and standard deviation of the p -values were extracted.

To quantify the differences of the results of the above vertex-wise analyses across FreeSurfer versions or workstations, the measure of spatial overlap (Dice coefficient, [39]) of the respective clusters was computed. Its range is between 0 (no overlap) and 1 (complete overlap, i.e., exactly similar). It is generally accepted that a value larger than 0.7 indicates a good agreement [40].

RESULTS

Subject characteristics can be found in Table 1. A complete overview of the comparison CON vs. AD for each selected ROI is illustrated in Fig. 1 (the group comparisons CON vs. MCI and MCI vs. AD are shown in the supplemental material, Figs. S1-S2 respectively). Each cell is color-coded according to its p -value after correction for multiple comparisons. In case of CT-vertex we have taken the minimal p -value in each ROI.

Table 1. Subject characteristics

	Con	MCI	AD
N	18 ^a	18 ^c	17
Age	64.56 (3.4)	65.11 (4.5)	70.59 (9.1)
Educational level	4 (1.4)	4 (1.8)	4 (1.9)
MMSE score	28.89 (0.9) ^{b,d}	27.61 (2.3) ^f	21.18 (3.9)
15 WLT learning	37.50 (7.6)	26.06 (9.8)	23.47 (11.7)
15 WLT memory	8.56 (1.9) ^{d,e}	3.67 (2.8) ^c	1.73 (2.4)
Fluency animals	23 (5.3)	21 (5.4)	13.93 (4.7)
Manual hippocampus	4656 (308)/4758	4410 (482)/4308	3883 (817)/3807
volume L/R (mm3)	-637	-796	-876
ICV (ml)	1492 (100)	1539 (121)	1574 (125)

All volumetric measurements are corrected for intracranial volume. Values are mean (sd). MMSE: Mini-Mental state examination; WLT: wordlist; Con: controls; MCI: mild cognitive impairment; AD: Alzheimer's disease.

^a $p < 0.05$ for difference between CON and AD

^b $p < 0.05$ for difference between CON and MCI

^c $p < 0.05$ for difference between MCI and AD

^d $p < 0.001$ for difference between CON and AD

^e $p < 0.001$ for difference between CON and MCI

^f $p < 0.001$ for difference between MCI and AD

Fig. 1 Overview of statistical significances across FreeSurfer versions and workstations for the comparison of controls versus AD. Each cell is color-coded according to its p-value in four categories, see coding scale at right. The first set of four columns refers to the MacOSX 10.5 workstation, the second set to the MacOSX 10.6 workstation, and the third set to the custom-built cluster workstation (called RadCluster). In each set, the columns present the results for version v4.3.1*, v4.5.0, v5.0.0, and v5.1.0, respectively. Finally, the last column refers to the p-values based on the manual outlining. Only the results after correction for multiple comparisons are shown. Four separate FreeSurfer measurements were used: 1) number of voxels within a segmented ROI (called voxel volume); 2) tabulated ROI volume (equal to voxel volume but corrected for partial volume effects); 3) mean cortical thickness (CT) within an ROI (called CT-parcellation-based); 4) vertex-wise CT cluster (called CT-vertex). Abbreviations are as follows: HIPPO = hippocampus; ERC = entorhinal cortex; PhG= parahippocampal gyrus; IPFC = inferior prefrontal cortex; OPFC = orbital prefrontal cortex; PCC = posterior cingulate cortex; PC = precuneus; L = left; R = right.

Fig. 2 Significance of cortical thickness differences between controls and ADs obtained from the vertex-wise analysis before and after correction for multiple comparisons (left hemisphere). The color scale at the right depicts the range of p-values shown (expressed in 10-x). The colors yellow-red indicate cortical thickening, the color blue indicate cortical thinning. The results for four FreeSurfer versions are displayed, from top to bottom: v4.3.1*, v4.5.0, v5.0.0, and v5.1.0, all obtained on a MacOSX 10.6 workstation.

Robustness of FreeSurfer across workstations and software versions

Generally, it can be noted that FreeSurfer derives more significant results for later versions in case of volumetric measurements (either voxel or tabulated). In addition, the CT-vertex method produces consistent results through versions as well as workstations, especially for MTL regions. With respect to cross-workstation differences, it can be observed that MacOSX 10.6 is most similar to RadCluster, in particular for v5.1.0. For both voxel and tabulated volumetric measurements, group differences between workstations and software versions were most consistent in hippocampal and posterior cingulate regions. Group differences in the PhG, OPFC R and PC L were only found in FreeSurfer v5.0.0 and/or v5.1.0, across all workstations. The CT-vertex results for MacOSX 10.6 and all FreeSurfer versions are

illustrated in Fig. 2 for the left hemisphere (comparison CON vs. AD, both corrected and uncorrected results are displayed). Cortical thinning (negative effects, blue colored) was mainly observed in temporal and parietal cortical areas. Cortical thickening (positive effects, red-yellow colored) was apparent in the frontal lobe only for versions v4.5.0 and v5.1.0. For the other versions, these positive clusters did not survive the correction for multiple comparisons. For the comparison CON vs. MCI, no significant clusters were found.

The Dice coefficients for the agreement of the CT surface clusters (positive and negative effects taken together) are summarized in Tables 2 and 3. In most cases the agreement was good to excellent, and better between workstations than between FreeSurfer versions. In order to detail these findings, worst and best agreements for the cross-workstation comparisons are shown in Fig. 3. Vertices depicted in green indicate the presence of the clusters in the results of both workstations. Red and yellow indicate the presence of only one of the respective workstations. The corresponding Dice coefficients were 0.68 and 0.97, respectively. A complete disagreement was found for the posterior cingulate cortex (see left medial view). Similarly, the worst and best results for the cross-version comparisons are shown in Fig. 4, with corresponding Dice coefficients of 0.58 and 0.94, respectively. A disagreement was found in the frontal lobe, and posterior cingulate cortex (see left medial view).

Table 2. Cortical thickness surface cluster similarities (Dice coefficients) across workstations

MCI vs. AD	MacOSX10.6	RadCluster
MacOSX10.5	0.80 – 0.94	0.75 – 0.92
MacOSX10.6	-	0.78 – 0.90
CON vs. AD		
MacOSX10.5	0.82 – 0.96	0.75 – 0.96
MacOSX10.6	-	0.68 – 0.97

CON: controls; MCI: Mild cognitive impairment; AD: Alzheimer’s disease.

Table 3. Cortical thickness surface cluster similarities (Dice coefficients) across FreeSurfer versions

MCI vs. AD	v4.5.0	v5.0.0	v5.1.0
v4.3.1*	0.78 – 0.93	0.69 – 0.80	0.78 – 0.85
v4.5.0	-	0.66 – 0.82	0.73 – 0.85
v5.0.0	-	-	0.78 – 0.89
CON vs. AD			
v4.3.1*	0.75 – 0.89	0.61 – 0.78	0.70 – 0.86
v4.5.0	-	0.58 – 0.75	0.63 – 0.82
v5.0.0	-	-	0.68 – 0.94

CON: controls; MCI: Mild cognitive impairment; AD: Alzheimer’s disease.

Fig. 3 CT Cluster similarities for the worst (top row) and best (bottom row) agreement found between workstations. The worst agreement was found for FreeSurfer v5.0.0 between MacOSX 10.6 (yellow) and RadCluster (red) and left hemisphere: SI = 0.68. The best agreement was found for FreeSurfer v5.0.0 between MacOSX 10.6 9 (yellow) and RadCluster (red) and right hemisphere: SI = 0.97. Vertices colored green indicate the presence of the clusters in the results of both workstations.

Comparison of FreeSurfer group differences with gold standard measurements

When comparing AD-patients with controls, a good agreement was found between later versions of FreeSurfer and manual measurements, especially in MTL and posterior parietal regions. It is however remarkable that compared to the highly significant effect found for manual volumes, no group differences were found in the left OPFC for the automated measurements except in one instance, i.e., CT-vertex approach on MacOSX 10.5, version v4.5.0. For the right PCC, the opposite pattern was found where manual volumetric measurements did not show group differences in the right PCC compared to the automated measurements (all versions and workstations except for the CT-vertex approach). Compared to manual volumetric measurements, FreeSurfer seems less sensitive for detecting differences in prefrontal regions (both volume and CT). For the FreeSurfer volumetric and CT-parcellation measurements, group differences between CON vs. MCI were most apparent in posterior parietal regions (PCC and PC) whereas for the manual volumetric measurements, differences in the MTL were found (right PhG). The CT-vertex technique was unable to detect group differences between CON vs. MCI. When MCI and AD-patients were compared, group differences were mainly found in posterior parietal regions for the FreeSurfer volumetric measurements, compared to differences in MTL and prefrontal regions found with the manual volumetric measurement. For the comparison MCI vs. AD, the CT-vertex technique corresponded best with manual volumetric measurements and was even more sensitive for group differences than manual volumetry in posterior parietal regions. The CT-parcellation technique was unable to detect group differences between MCI vs. AD.

Fig. 4 CT Cluster similarities for the worst (top row) and best (bottom row) agreement found between FreeSurfer versions. The worst agreement was observed for RadCluster between versions v4.5.0 (yellow) and v5.0.0 (red) and left hemisphere: SI = 0.58. The best agreement was observed for MacOSX between versions v5.0.0 (yellow) and v5.1.0 (red) and right hemisphere: SI = 0.94. Vertices colored green indicate the presence of the clusters in the results of both workstations. The colors yellow and red denote the clusters found in only one of the workstations.

DISCUSSION

Alzheimer's disease pathology has a massive impact on both the structure and function of the ageing brain. Performance of automated brain segmentation techniques may be less precise when applied in these patients due to fundamental changes in certain brain structures. FreeSurfer has been shown to operate as an accurate instrument to measure brain atrophy in neurodegenerative diseases [35, 41-44], emphasizing the need for both validation and comparison of the FreeSurfer pipeline with manual volumetry in a cohort of subjects with and without Alzheimer's disease pathology. To the best of our knowledge, this is the first study evaluating the robustness of FreeSurfer to capture morphological and pathological changes against varying processing conditions, both in healthy old subjects and subjects suffering from (early) AD. For direct comparison, atrophy was also measured by

means of manual volumetry, nowadays regarded as gold standard.

Overall, consistent results across versions and workstations were obtained for the hippocampus and PCC in case of volumetry. These structures seem less affected by changes in processing parameters, a finding of particular relevance for the imaging field of AD since these structures are known to be primarily involved in the disease process [4, 45]. With respect to CT vertex-wise analyses, consistency was observed across versions and workstations for the PhG, left PCC, and left PC. In addition, the CT-vertex technique was much more sensitive than the CT-parcellation technique. This finding is in line with previous work which found that CT-vertex was more sensitive than CT-parcellation in discriminating AD and controls [46]. The partial disagreement between CT-parcellation and manual volumetry may be related to the fact that effective validation of the CT technique requires postmortem investigations, and that manual volumetric measurements are thus not the most appropriate source of comparison. Furthermore, although differences between ROI's were small, borders of manual and FreeSurfer measurements were not entirely overlapping.

The FreeSurfer v5.1.0 results for MacOSX 10.6 are almost identical to those for RadCluster; only the CT-vertex results for the right PCC and PC deviate. In addition, a higher level of agreement was found between MacOSX 10.6 and Radcluster than between MacOSX 10.5 and 10.6. This supports the hypothesis in our previous paper, namely that most probably the number of bits under which the operating system is running (either 32 bits or 64 bits) is an important factor [7]. Although both MacOSX 10.5 and 10.6 were used in 32 bits mode, our results may suggest that the underlying UNIX shell for MacOSX 10.6 nonetheless uses mathematical libraries with 64 bits precision.

Only few studies reported on the capability of FreeSurfer to discriminate groups in comparison to manual volumetric measurements [10-12, 14, 47]. In general, these studies suggest that manual volumetry is slightly superior or equally sensitive to FreeSurfer automated volumetric measurements. While the previous studies focussed on one ROI (e.g. hippocampus), the present study extends these findings by examining all cortical signature regions in AD, and thereby increasing the diagnostic relevance and generalizability.

For the comparison CON vs. AD, the present study found that manual volumetry and later versions of FreeSurfer (v5.0.0 and v5.1.0) were both sensitive in detecting hippocampal and parahippocampal volume reduction. Group differences in the posterior parietal lobe were less consistent between FreeSurfer and manual volumetry and it was found that later versions of FreeSurfer (v5.0.0 and v5.1.0) corresponded best with gold standard measurements. Furthermore, FreeSurfer was less sensitive for group differences in prefrontal regions compared to manual volumetric measurements (comparison CON vs. AD and MCI vs. AD). In the early phase of the disease (CON vs. MCI), FreeSurfer is more sensitive for group differences in posterior parietal regions compared to the MTL which is most pronounced in manual volumetry.

Limitations

One limitation may be related to the composition of the study population that comprised 18 healthy controls, 18 patients with amnesic MCI, and 17 patients with mild AD. Only males were included in this study in order to reduce interindividual variation. Generalization of our findings to females should therefore be done with caution. Previous studies did however not reveal substantial gender related differences with respect to atrophy in AD [13]. The sample size of the present study is relatively small, which may possibly limit the ability to detect differences between groups that might become apparent when sample sizes are larger. Nonetheless, data was normally distributed and based on the inclusion of a time-intensive technique such as manual volumetry and on our hypothesis, sample size was fair enough to effectively address our research questions. Neurodegenerative processes in AD do not occur randomly, but likewise do not follow the boundaries set out by atlases. This is in line with the notion that locations of neurodegeneration may not coincide with predefined ROIs and thus such ROIs may harbor intrinsically less discriminative power, i.e., local effects are averaged out. On the other hand, the discriminative power of the manually defined ROIs was high in all cases, except for the IPFC and right PCC. Obviously, differences in the locations of the ROI boundaries between FreeSurfer and manual outlining may play a role, leading to overestimation of the volumes by the automated technique [8, 10, 47, 48]. To correct for multiple comparisons, we performed the FDR controlling procedure instead of the classical Bonferonni approach because the former is less stringent and as such reduces the increased probability of making Type II errors [49, 50].

As a consequence, correction for multiple comparisons was done over 14 comparisons for the FS automated volumetry and 12 for manual volumetry, which is slightly in favor of the manual technique. The two latest versions of FreeSurfer (5.2.0 and 5.3.0) were not included in the present analyses. However, a literature search (PUBMED) showed us that near half of the published FreeSurfer papers in the field of AD thus far used one of the versions evaluated in this paper (n=34). 31 papers did not specify the version used, 3 used newer versions and 17 studies used earlier releases (e.g. version 3.0). This finding clearly emphasizes the importance of the results reported in the present study.

Implications for research and clinical practice

From the present study follows that despite the large morphological changes on a brain level, FreeSurfer can reliably be used to investigate structural brain alterations in AD. Another important finding is that, compared to manual volumetry, later versions of FreeSurfer are more accurate than earlier versions. This development is very promising for future applications of FreeSurfer in scientific studies on neurodegenerative diseases, and encourages the future role of FreeSurfer in clinical settings. While FreeSurfer is created for use in research settings, the potential of FreeSurfer for use in clinical practice is promising. Based on our findings, we would suggest focusing on automated volumetry of posterior parietal regions in the early phase, followed by segmentation of MTL regions and measurements of CT in the later stages. The potential of CT-vertex measurements as a marker of disease progression should be further investigated in large-scale longitudinal studies. Prior work of our group has shown that mixing up versions, workstations or operating systems might lead to significant differences in cortical thickness or volumetry in schizophrenic patients [7]. However, the current findings are much more optimistic, showing that the latest version of FS produces much more stable results, even in patients with heavily atrophied brains. While this is an important scientific finding, we agree with the FreeSurfer developers that it is still advised to try to keep the research environment stable

CONCLUSION

The present study showed that FreeSurfer is a robust method to measure morphological and pathological changes in both healthy and atrophied brains.

Overall, consistent results across versions and workstations were obtained particularly for the hippocampus and PCC in case of volumetry. Later versions of FreeSurfer (v5.0.0 and v5.1.0) corresponded best with gold standard measurements (manual volumetry), especially in MTL regions. In addition, the CT-vertex technique was much more sensitive than the CT-parcellation technique. The continuous efforts of the developers of FreeSurfer to improve its performance are very promising for AD research and the neuroimaging field in general, and thus potentially also for clinical practice.

APPENDIX

The analysed cortical and subcortical ROIs were composed of the following FreeSurfer segmentation IDs with their corresponding structure names:

HIPP: 17 (left hippocampus) / 53 (right hippocampus)

PHG: 1016 (left parahippocampal) / 2016 (right parahippocampal)

IPFC: 1018 (left pars opercularis) / 2018 (right pars opercularis)

1020 (left pars triangularis) / 2020 (right pars triangularis)

OPFC: 1012 (left lateral orbitofrontal) / 2012 (right lateral orbitofrontal)

1014 (left medial orbitofrontal) / 2014 (right medial orbitofrontal)

PCC: 1010 (left isthmus cingulate) / 2010 (right isthmus cingulate)

PC: 1025 (left precuneus) / 2025 (right precuneus)

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SUPPLEMENTAL MATERIAL

For **Supplemental Table 1**. ‘Anatomical boundaries of investigated structures’ please see Chapter 4, page 126-130.

Supplementary Figure. 1 Overview of statistical significances across FreeSurfer versions and workstations for the comparison of controls versus MCI. Each cell is color-coded according to its p-value in four categories, see coding scale at right. The first set of four columns refers to the MacOSX 10.5 workstation, the second set to the MacOSX 10.6 workstation, and the third set to the custom-built cluster workstation (called RadCluster). In each set, the columns present the results for version v4.3.1*, v4.5.0, v5.0.0, and v5.1.0, respectively. Finally, the last column refers to the p-values based on the manual outlining. Only the results after

correction for multiple comparisons are shown. Four separate FreeSurfer measurements were used: 1) number of voxels within a segmented ROI (called voxel volume); 2) tabulated ROI volume (equal to voxel volume but corrected for partial volume effects); 3) mean cortical thickness (CT) within an ROI (called CT-parcellation-based); 4) vertex-wise CT cluster (called CT-vertex). Abbreviations are as follows: HIPPO = hippocampus; ERC = entorhinal cortex; PhG = parahippocampal gyrus; IPFC = inferior prefrontal cortex; OPFC = orbital prefrontal cortex; PCC = posterior cingulate cortex; PC = precuneus; L = left; R = right.

Supplementary Figure. 2 Overview of statistical significances across FreeSurfer versions and workstations for the comparison of MCI versus AD. Each cell is color-coded according to its p-value in four categories, see coding scale at right. The first set of four columns refers to the MacOSX 10.5 workstation, the second set to the MacOSX 10.6 workstation, and the third set to the custom-built cluster workstation (called RadCluster). In each set, the columns

present the results for version v4.3.1*, v4.5.0, v5.0.0, and v5.1.0, respectively. Finally, the last column refers to the p-values based on the manual outlining. Only the results after correction for multiple comparisons are shown. Four separate FreeSurfer measurements were used: 1) number of voxels within a segmented ROI (called voxel volume); 2) tabulated ROI volume (equal to voxel volume but corrected for partial volume effects); 3) mean cortical thickness (CT) within an ROI (called CT-parcellation-based); 4) vertex-wise CT cluster (called CT-vertex). Abbreviations are as follows: HIPPO = hippocampus; ERC = entorhinal cortex; PhG = parahippocampal gyrus; IPFC = inferior prefrontal cortex; OPFC = orbital prefrontal cortex; PCC = posterior cingulate cortex; PC = precuneus; L = left; R = right.

Chapter 8

The stability of resting state networks in aging: relevance for early memory dysfunction

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ABSTRACT

Reduced functional connectivity (FC) and atrophy in hubs of the default mode network (DMN) is a consistent and early finding in Alzheimer's disease (AD). In order to use FC changes in the DMN as candidate biomarker for AD, its stability in normal aging needs to be assessed. Test-retest-reliability of RSNs was investigated both in healthy young (HY) and healthy old (HO) subjects. Twenty HY and twenty HO subjects underwent three scanning sessions on a 3.0 Tesla MRI. Differences in FC were investigated in eight RSNs. Within-group analyses showed no change over time (intraclass correlation coefficients: 0.53-0.85). Between-group comparisons showed more FC in the DMN and auditory system in HY compared to HO subjects. Inverse correlations were found between instability of specific DMN areas and neuropsychology: more variability in precuneal connectivity was linked with lower performance. As expected, FC in the DMN and auditory system decreases with aging. Activity in major RSNs remains stable over time, and instability in key hubs of the DMN could become a predictor for future memory problems, supporting its potential as a future biomarker.

INTRODUCTION

Brain activation in the past has mainly been studied using the BOLD signal in task-related fMRI paradigms, aimed at localizing a variety of memory functions including age associated effects [1-3]. The last decade, task-related decreases are found in certain regions which remain active in an organized fashion during the resting state of the brain [1, 2]. One of these so-called 'resting state networks' (RSNs) is the default mode network (DMN), particularly relevant for aging and dementia since this network is vulnerable to atrophy, deposition of the amyloid protein, and show a reduced glucose metabolism [3]. Furthermore, the DMN comprises several areas that are also affected early on in Alzheimer's disease (AD), such as the posterior cingulate cortex, medial and lateral prefrontal cortex, medial inferior parietal lobe, precuneus, and the medial temporal lobe (Hafkemeijer, et al., 2012; Jacobs, et al., 2013). Because of this reason, the DMN is considered to hold great potential as clinical biomarker. Besides the DMN, the brain consists of a number of other low frequency RSNs, such as the visual and auditory networks [4]. The potential effects of normal aging on these functional hubs have not thoroughly been investigated [5].

The first step toward a biomarker is assuring that age-effects do not influence the reliability of RSNs. In view of a number of studies demonstrating the utility of RSNs [6, 7], it remains to be investigated if rs-fMRI is a stable and reliable technique for identifying and characterizing abnormalities in a variety of diseases. As it is widely known that pathophysiological processes of neurodegenerative diseases (e.g. AD) begin many years prior to clinical symptoms, efforts currently aim at detecting AD-pathology in clinically (still) asymptomatic individuals. The ultimate goal of establishing these so-called 'biomarkers' is the identification of and intervention in individuals at the preclinical stage to delay and possibly even prevent onset of the clinical syndrome.

The present study aimed at investigating the test-retest reliability (i.e. stability) of RSNs within and between healthy old and healthy young subjects over 3 different time points. Test-retest reliability was investigated both on a voxel-wise basis (FSL) and by means of the intraclass correlation coefficient (ICC) (SPSS). As the DMN is the most investigated RSN and thus far has the closest link to AD, our second aim was to investigate whether individual changes in stability in certain hubs of the DMN are

associated with cognitive performance. If instability over time is linked with subtle cognitive alterations in healthy old subjects, this finding would be of particular relevance for early diagnosis and intervention in at-risk individuals.

METHODS AND MATERIALS

Subjects

Forty healthy subjects were included and gender-matched: 20 elderly subjects (HO) (age between 54-66 years) and 20 young subjects (HY) (age between 23-29 years). Subjects were recruited for the RIMCAD study (Retroactive Interference during Memory Consolidation in Aging and Dementia) by means of advertisement. Exclusion criteria were significant medical (e.g. cancer, thyrotoxicosis), neurological (e.g. epilepsy), or other psychiatric disorders (e.g. schizophrenia, depression, Parkinson's disease); current use of medication which is known to influence cerebral function (e.g. anti-depressants); pregnancy or any other contraindication against MRI-scanning (e.g. metal implants, claustrophobia). One subject in the elderly group was excluded because MRI images were not available for all three time points. The Ethical Review Board of the University Hospital of Cologne had approved the study, and the study was accomplished in compliance with the latest revision of the Declaration of Helsinki. Written informed consent was provided by all subjects.

Neuropsychological assessment and study design

All subjects received neuropsychological examination to confirm their healthy status, including Mini-mental state examination (MMSE) [8], immediate and delayed recall of the German version of the Verbal Learning and Memory test [9], Trail Making Test (TMT) part A and B [10] and Rey-Osterrieth Complex Figure test [11]. Three separate experimental sessions were organized, with on average two weeks in between each session. At the time of the first visit subjects were tested neuropsychologically whereafter the first MRI examination was performed. At the second and third visit (2 weeks and 4 weeks from baseline, respectively) only MRI scanning was performed.

MRI data acquisition

All imaging was performed using a 3.0 Tesla Siemens Trio MRI scanner (Siemens, Erlangen, Germany) equipped with a standard head coil for radio frequency

transmission and signal reception. For each participant, 3 resting-state scans were acquired (one per visit) (191 continuous T2*-weighted echo planar imaging (EPI) volumes; repetition time = 2200 ms ; echo time = 30 ms; flip angle = 90°; 36 axial slices; matrix 64 x 64; voxel size 3.1 x 3.1 x 3.0 mm; scan time = 7 minutes). During the scans, participants were instructed to keep their eyes closed, think of nothing in particular, and not to fall asleep. In addition, a high-resolution T1 anatomical image was obtained for each subject using a three-dimensional magnetization-prepared, rapid acquisition gradient echo sequence. To control for cerebral abnormalities (e.g. tumor, subclinical stroke) and macroangiopathy, T2-weighted FLAIR and Time-of-Flight measurements were acquired in the older subjects only.

Data preprocessing and analyses

Data analysis was carried out using FMRIB's Software Library (FSL version 4.1.9; www.fmrib.ox.ac.uk/fsl). Preprocessing was carried out with MELODIC and consisted of motion correction, removal of nonbrain tissue (using the Brain Extraction Tool), spatial smoothing using a 5 mm full-width-at-half-maximum Gaussian kernel, and high-pass temporal filtering equivalent to 100 seconds (0.01 Hz). After preprocessing, fMRI volumes were registered to the subject's high-resolution T1-weighted scan using affine registration (FLIRT) and subsequently to standard space (MNI152) images using nonlinear registration (FNIRT) with a warp resolution of 10 mm.

Assessment of functional connectivity

Functional connectivity of RSNs was defined in terms of the similarity of the BOLD fluctuations in each brain voxel in relation to characteristic fluctuations in eight predefined components (RSNs of interest). For the selection of these components an existing template was used containing a weighted mask of networks that are most reliably (and reproducibly) identified from a model-free analysis of the spatio-temporal structure of resting-state BOLD fluctuations [12]. These predefined components include over 80% of the total brain volume and comprise: medial and lateral visual systems (component 1 and 2, respectively), auditory and somato-sensory system (component 3), sensori-motor system (component 4), default mode network (component 5), executive control (component 6), and right and left visuo-spatial and working memory networks (component 7 and 8, respectively). Components accounting for cerebrospinal fluid (CSF) and total brain white matter (WM) were furthermore included to capture activations for both sources of noise.

To investigate FC, a voxel-wise comparison of the resting state signal was carried out using a regression technique referred to as the “dual-regression” method [13, 14]. This technique is based on first extracting the temporal pattern of resting state signal fluctuations within a RSN (represented by each component in the predefined template) or CSF or WM, for each individual rs-fMRI dataset separately (spatial regression). Matrices describing the temporal dynamics for each component and subject separately were created and subsequently used in a linear model fit against the subject’s fMRI data set (temporal regression) in order to estimate subject-specific correlation maps. After this dual regression, spatial maps of all subjects were concatenated into single 4-dimensional files for each RSN. The regions that showed differences in FC between groups were visualized by means of FSLview and subsequently used to extract mean Z-values from each individual spatial map (FWE-corrected $p < 0.05$). These values represent connectivity to the given RSN with higher absolute z-values reflecting stronger connectivity to a RSN.

Statistical analysis

To investigate our first aim, non-parametric permutation tests (5000 permutations) were used in order to detect statistically significant differences between the groups within the boundaries of the spatial maps defined by the predefined template (See Figure 6 in Beckmann et al., 2005 [12]). A family-wise error (FWE) correction for multiple comparisons was performed, implementing threshold-free cluster enhancement (TFCE) using a significance threshold of $p < 0.05$ [15]. First, main effects of group (old versus young), time (first, second and last time point) and the interaction group by time were examined. (Normalized) gray matter volume was added as an additional covariate in all permutation tests in order to correct for the effect of gray matter differences between subjects [16] (together with demeaned ‘age’ and/or ‘education’; depending on the research question). Our second aim was to detect subtle differences in FC of the DMN within HO and HY subjects over time, and to correlate these individual changes in FC over time with neuropsychological test performance. For these additional statistical analyses on a subject-level, masks of selected regions-of-interest (ROI) were created by means of FSLview. Regions in the medial temporal lobe (hippocampus), posterior parietal lobe (precuneus and posterior cingulate gyrus) and frontal lobe (anterior cingulate gyrus) were selected based on their relevance for AD [17, 18]. ROIs were placed on the T1_MNI152_2mm template by using the Harvard-Oxford (sub)cortical

structural atlases, provided within FSL and resulting masks were subsequently used for extracting mean Z-scores for further analysis.

(Non-imaging) statistical analysis

To quantify test-retest reliability (or measurement reproducibility), intraclass correlation coefficients (ICC) were calculated for each individual resting state network [19, 20]. First, the template containing each of the eight resting state networks was split into separate masks, one for each network, by means of `fslsplit`. Mean Z-scores of functional connectivity in the resulting masks (i.e. separate resting state networks) were extracted for each time point and were subsequently exported to SPSS (version 19.0; SPSS, Chicago, IL, USA). The ICC was calculated over the three different time points (SPSS – ICC, two-way mixed effects model for consistency). Different guidelines exist for the interpretation of ICC, but one reasonable scale is that an ICC value of less than 0.40 indicates poor reproducibility, ICC values in the range 0.40 to 0.75 indicate fair to good reproducibility, and an ICC value of greater than 0.75 shows excellent reproducibility [21].

ROI-based statistical analyses were performed using SPSS whereas correlation analyses were performed within both groups to assess relationships between regional FC within the DMN and neuropsychological test performance. Mean Z-scores of FC between a certain ROI and the rest of the DMN were extracted and transferred to SPSS. The absolute difference in FC between the first and the last time point was calculated (since we expected to find most variability between both utmost points of time) for each separate region with a higher value indicating larger differences between both time points, and thus more instability of FC over time for a given subject in a particular structure. To control for Type I errors, a false discovery rate (FDR) controlling procedure was performed in order to correct for multiple comparisons [22].

RESULTS

Demographics and cognitive performance

Subject characteristics are shown in Table 1. As expected from our study design, mean age of the old group differed significantly from that of the young group ($p < .001$). In the old group, education was lower compared to the younger group ($p < .01$). Differences in cognitive performance between old and young subjects were

found for the delayed recognition trial of the VLMT memory test ($p = .008$) and the delayed recall of the Rey-Osterrieth Complex Figure test ($p = .04$).

Identification of FC differences between HO and HY subjects across all time points

After correcting for GM and education, increased FC in older compared to younger subjects was found in all networks except the default mode network and the left dorsal visual stream (Table 2, Figure 1). Regions of increased FC in older subjects were mainly situated in frontal and occipital regions (Table 2, Figure 1). After correcting for GM and education, increased FC in younger compared to older people was found in all components except the lateral visual cortex (Table 2, Figure 2). These regions of increased FC in younger subjects were mainly situated in frontal and parietal regions (Table 2, Figure 2). For the whole group, no significant differences over time were found in each of the eight investigated networks. Likewise, no significant differences were found for the time x group interaction.

Table 1. Subject characteristics.

	Old (n=19)	Young (n=20)
Age (in years)	59.2 (6.1)***	24.6 (2.8)
Education (in years)	12.4 (3.3)**	15.6 (2.7)
Female (in %)	47	50
MMSE score	29.4 (0.7)	29.5 (0.9)
VLMT learning	50.9 (9.3)	56.4 (8.8)
VLMT memory		
*delayed recall (number of words)	10.7 (2.8)	11.6 (2.9)
*delayed recognition (number of words)	11.7 (3.1)**	13.8 (1.3)
TMT-A (sec.)	26.0 (13.0)	20.6 (9.6)
TMT-B (sec.)	53.4 (29.0)	39.2 (17.9)
CF Rey score IR	35.9 (0.3)	35.5 (0.9)
CF Rey score DR	22.5 (6.3)*	27.0 (4.5)

Values are mean (sd). MMSE: Mini-Mental state examination; VLMT: verbal learning and memory test; TMT: trail making test; CF Rey: Rey complex figure test. Differences between both age groups significant at *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$.

Quantification of test-retest reliability by means of ICC

Test–retest reliability, expressed by means of the intraclass correlation coefficient (ICC), was investigated for both groups separately, and for each individual RSN. In HY subjects, ICCs varied from 0.53 (sensory-motor system) to 0.81 (medial visual system and auditory system). All networks, except the lateral visual system (0.66) and sensory motor system (0.53), showed excellent reproducibility in HY subjects (Table 3). For HO subjects, ICCs varied between 0.54 (executive control) and 0.85 (right dorsal visual stream). The medial visual cortex (0.82), auditory system (0.78) and dorsal visual stream (left/right) (0.77/0.85) showed excellent reproducibility whereas the lateral visual cortex (0.73) and the default mode network (0.73) showed good reproducibility. The sensory-motor system (0.57) and executive control networks (0.54) showed fair reproducibility over time (Table 3). A visualization of test-retest reliability in the DMN is shown in Figure 3. Since the individual variability of FC within the DMN was most evident in HO subjects (subject number 1-19), the effect on neuropsychological test performance was further investigated.

Table 2. Functional connectivity over time in healthy old and young subjects (all time points).

Resting state network (component number)	p-value [^] before correction	p-value [^] after correction for GM and education	Total clustersize significant at $p < .05$	MNI coordinates peak p-value			Location of clusters showing increased connectivity*
				x	y	z	
a) Old > Young	- Medial visual system	.0010	318	62	16	16	Inferior frontal gyrus, pars
	- Lateral visual system	.0040	2268	4	-74	-30	opercularis
	- Auditory system	.0010	65	36	30	32	Cerebellum
	- Sensory-motor system	.0002	13393	-30	-54	44	Middle frontal gyrus, frontal pole
	- Executive control	.0050	18	-28	38	-20	Superior parietal lobule, angular gyrus, supramarginal gyrus, lateral occipital cortex
	- Dorsal visual stream right	.0002	190	-50	-68	28	Frontal pole, orbitofrontal cortex
	- Dorsal visual stream left	.0160	n.s.				Lateral occipital cortex, angular gyrus
							Lateral occipital cortex, angular gyrus

Continuation **Table 2.** Functional connectivity over time in healthy old and young subjects (all time points).

b) Young > Old									
-	Lateral visual system	.0006	n.s.		5003	0	8	40	Anterior cingulate gyrus, paracingulate gyrus,
-	Auditory system	.0002	.0004						juxtapositional lobule cortex
-	Sensory-motor system	.0006	.0200		29	-6	56	-16	Frontal pole, frontal medial cortex
-	Default mode network	.0002	.0002		40329	0	-70	30	Precuneus
-	Executive control	.0120	.0020		2936	8	2	72	Superior frontal gyrus, juxtapositional lobule cortex
-	Dorsal visual stream right	.0002	.0002		1190	-48	52	2	Frontal pole
-	Dorsal visual stream left	.0130	.0004		2901	-4	-78	42	Precuneus, cuneus

^ *p*-values refer to the peak of all significant voxels. All *p*-values < .05 are family-wise error (FWE)-corrected. MNI: Montreal Neurological Institute; n.s.: not significant; DMN: default mode network. (a) Decreased functional connectivity in young compared to old (b) Decreased functional connectivity in old compared to young.

* The following atlas was used to localize the clusters (after correction) in FSLview: Harvard-Oxford (sub)cortical structural atlas.

Table 3. Intraclass correlation coefficient (ICC) analysis for healthy old vs. healthy young subjects.

	Old (n=19)	Young (n=20)
Medial visual system	0.82	0.81
Lateral visual system	0.73	0.66
Auditory system	0.78	0.81
Sensory-motor system	0.57	0.53
Default mode network	0.73	0.75
Executive control	0.54	0.8
Dorsal visual stream right	0.85	0.76
Dorsal visual stream left	0.77	0.77

Values are depicted for each separate resting state network.

Figure 1. Regions showing increased functional connectivity in older subjects ($p < .05$ family-wise error [FWE]-corrected). (A) lateral visual cortical areas in yellow, sensory-motor system in red (B) right dorsal visual stream in green (C) medial visual system in brown, auditory system in blue, (D) executive control in dark blue. All images have been co-registered into the space of the MNI 152 template. The left hemisphere of the brain corresponds to the right side of the image.

Figure 2. Regions showing increased functional connectivity in younger subjects ($p < .05$ family-wise error [FWE]-corrected). (A) auditory system depicted in red/yellow, sensory-motor system in blue (B) default mode network depicted in green, executive control in blue (C) right dorsal visual stream depicted in purple, left dorsal visual stream in yellow. All images have been coregistered into the space of the MNI 152 template. The left hemisphere of the brain corresponds to the right side of the image. The image shows sagittal, coronal and axial views.

Figure 3. Quantification of test-retest reliability within the default mode network (DMN) by means of the intraclass correlation coefficient (ICC). Functional connectivity values within the DMN are depicted for each subject and time point separately. Subjects 1-19 belong to the group of healthy old subjects, subjects 20-39 belong to the group of healthy young subjects. The ICC reflecting test-retest reliability over all time points was 0.75 for healthy young subjects compared to 0.73 for healthy old subjects.

Associations between stability of RSNs and NP test performance

On a group level, no differences in stability were found over time. Next, in line with the findings above, we investigated on a subject-level whether stability differences in key hubs of the default mode network were linked with performance on neuropsychological tests.

Lower FC in older compared to younger subjects was mainly found in the DMN. ROIs in the DMN were selected based on their relevance for AD. Correlation analyses in older subjects were performed in order to investigate if changes in FC between a ROI and the DMN over time were linked with neuropsychological test performance at baseline. Variability over time in HO subjects was assessed for each separate ROI and was expressed as absolute difference between time point 1 and 3 (Figure 4). It was found that higher variability over time in the precuneus was associated with a decrease in MMSE score ($r=-0.56$, $p < 0.01$), lower immediate ($r=-0.56$, $p < 0.01$) and delayed recall ($r=-0.55$, $p < 0.05$), and recognition scores

($r=-0.49$, $p < 0.05$) (Figure 5). After correcting for multiple comparisons, associations between precuneal connectivity and MMSE score, and between precuneal connectivity and immediate recall score remained significant ($p < 0.05$) (Figure 5). No associations were found between variability in the hippocampus or cingulate cortex (ACC/ PCC) and neuropsychological test performance.

Figure 4. Subject-specific variability in functional connectivity in hubs of the DMN. Individual data-points per subjects are depicted on the X-axis whereas the length of the coloured rectangle corresponds with the degree of variability in a certain region of interest (longer rectangle = more variability between different data-points). Absolute differences between the first and the last time point (scansession) are depicted on the Y-axis.

Figure 5. Associations between variability in functional connectivity in hubs of the default mode network and neuropsychological performance. (A) Higher variability in precuneal connectivity was associated with a decrease in MMSE score ($B = -0.18 \pm 0.06$; $p = 0.012$), (B) lower immediate recall scores ($B = -2.42 \pm 0.86$; $p = 0.012$), (C) lower delayed recall scores ($B = -0.71 \pm 0.27$; $p = 0.019$) and (D) lower recognition scores ($B = -0.71 \pm 0.30$; $p = 0.032$).

* p-values significant after correction for multiple comparisons.

DISCUSSION

The present study evaluated the potential of RSNs as a biomarker for disease, by investigating its test-retest reliability in healthy aging. To the best of our knowledge, this is the first study assessing the stability of 8 RSNs over time in both HO and HY subjects by means of rs-fMRI. Thus far studies mainly focused on HO [23, 24] or HY subjects [25, 26], compared both groups cross-sectionally [27] or investigated the stability of resting state networks (RSNs) by means of other imaging modalities (e.g. PET) [28]. Test-retest reliability in the present study was investigated both on

a voxel-wise basis and by means of intraclass correlation coefficients. Second, we correlated regional DMN instability in older individuals with neuropsychological test performance in order to identify people who might carry an increased risk for future cognitive impairment.

Age-effects

A decrease in FC with aging was found in the auditory system and the DMN. Even after correction for GM atrophy and education, decreases in FC in HO subjects remained significant, indicating that age-related changes in connectivity are not solely due to reductions in gray matter volume or cognitive reserve. Previous studies show similar results, *viz.*, a negative association between age and FC of the DMN [29, 30]. Small clusters of increased FC in older subjects were found in the sensory-motor system. These differences were mainly found in the precentral gyrus which forms the posterior border of the frontal lobe, and could possibly be explained by the posterior-anterior shift in aging [31]. Normal aging seems to induce a connectivity disruption within the DMN, more specifically along the anterior-posterior axis of this network [32], showing reduced deactivations in occipitotemporal areas and increased deactivations in bilateral frontal areas (e.g. precentral gyrus) in HO compared to HY [33].

In line with our findings, an age-related FC reduction has been reported within superior and middle frontal PCC/precuneus, middle temporal, superior parietal, and medial areas such as medial prefrontal, anterior cingulate, and hippocampal regions [34]. On the other hand, older subjects were also found to show greater activity at rest, or greater deactivations, mainly in anterior brain areas. This increased activity at rest in frontal DMN regions of elderly adults has been interpreted as an attempt to compensate for the decrease of resting-state activity in posterior DMN areas [33].

Stability of resting state networks over time

No significant differences in FC over time were found in each of the eight investigated networks for the total group of healthy subjects. Furthermore, intraclass correlation coefficients between different time points mainly showed good to excellent reproducibility. Previous studies with a different design (e.g. one age group) report similar results, in particular in the DMN. One study in HO subjects showed a relative

stability of rest-specific activity over time in regions commonly associated with the default-mode [28]. Other studies found that DMN coactivation patterns are reproducible in HY subjects (Meindl, et al., 2010; Shehzad, et al., 2009). The findings above indicate, in line with previous studies, that RSNs can be reliably reproduced over time.

Relevance for early memory impairment

As known from the literature, posterior parietal regions are important nodes for memory encoding and retrieval and show strong FC [35]. The vulnerability of these nodes for AD pathology is related to their extensive connections with other AD signature regions (e.g. hippocampus) and high metabolic activity compared to other regions of the DMN [36].

A number of studies found a reduction in connectivity between both the hippocampus [37, 38] and precuneus [6, 7, 39-41] and several other brain regions in (amnesic) MCI or AD. The present study found that in HO subjects, instability of FC between the precuneus and the rest of the DMN was linked with subtle alterations in global cognitive impairment and immediate recall, even after correction for multiple comparisons. This finding is of particular relevance since structural and functional alterations in precuneal regions are a consistent finding in various diseases such as AD. The precuneus, acting a hub, has extensive connections with other brain regions, increasing its sensitivity for metabolic and pathological influences. Abnormal connectivity patterns were also found within PCC regions in patients suffering from early AD [42, 43]. In this study, however, no associations were found between instability of FC in hippocampal/posterior cingulate regions and neuropsychological test performance. Besides the fact that our study population comprises of healthy subjects, this finding could also be due to the fact that no spatial memory task was administered which would have possibly been more sensitive to subtle hippocampal or PCC dysfunction. Recent evidence pointed out that stable RSNs may represent healthy aging, whereas decreased RSN reliability may indicate progressive neuro-functional alterations before the actual manifestation of clinical symptoms [23]. In order to strengthen our conclusion that instability in precuneal connectivity is a precursor of cognitive problems, this hypothesis requires testing in subjects with subjective and/or mild cognitive impairment. Associations between other nodes of the DMN and neuropsychology may possibly become apparent in later stages of the disease process.

Limitations and future perspectives

The present study has several limitations. First, a predefined template was used instead of a data-driven independent component analysis (ICA) approach. It may be argued that predefined networks limit the scope of observations by restricting the criterion of connectivity to temporal similarity within a given network. These networks were however chosen on the basis of previous studies that had reproduced them in entirely different populations [14, 44]. An advantage is that typical ambiguities associated with ICA are avoided (e.g. specificity of the investigated group, model order). Second, older subjects with memory problems were not included in this study. Future studies could additionally include subjects with memory problems for discriminant analysis or longitudinally follow groups of healthy subjects in order to re-evaluate their cognitive status after a number of years. Third, it is known that certain mechanisms related to BOLD-variability might have an influence on BOLD-reactivity differences between old and young subjects [45]. One study found that there is substantial variability in responses collected across subjects and that differences between multiple scans sessions within a single subject seem to be less variable [46]. Another study suggests including an estimate of hemodynamic response function in order to solve some of the variability issues across subjects [47]. It is also known that other factors such as caffeine intake [48], physiological factors [49] and sleep during scanning might have an influence on the BOLD-response [50]. In the present study, we did not control for caffeine intake nor for respiratory factors by means of external recordings of physiology. We however corrected for the influence of noise by using methods inherent to the ICA-approach, e.g. correction for head motion and low frequency artefacts. Regarding sleep and vigilance, we did not measure EEG-activity during fMRI, which would have been the most sensitive technique. However, a questionnaire was administered in which subjects, after scanning, reported their sleepiness during scanning and whether they had fallen asleep. From the total group of subjects, over all scanning sessions, one subject fell asleep during one of the three scanning sessions. Fourth, stability was assessed at one-month interval. Neuronal changes due to the process of aging possibly require longer intervals. On the other hand, short intervals form the optimal paradigm for evaluation of the resting state technique and to validate FC measurements as such. Fifth, previous studies showed that resting-state FC changes overlap with structural connectivity alterations and that combining these modalities

can enrich our understanding of underlying brain networks [51]. Future studies could include techniques such as diffusion tensor imaging to extensively investigate the process of healthy aging or disease, both on a functional and structural level. Finally, recent evidence points out that healthy subjects with amyloid plaque deposition in the brain (investigated by means of Positron Emission Tomography (PET)) show patterns of decreased FC at rest [52]. A recent study was unable to replicate these findings in diagnostic groups [53]. To investigate whether amyloid depositions precedes aberrant FC in the DMN, and to explore the diagnostic value of each of these techniques, future studies could combine resting state modalities together with PET. A major strength of this study is the correction for gray matter atrophy and education. A large fraction of previous rs-fMRI studies did not correct for gray matter atrophy nor for cognitive reserve, which both play a prominent role in the process of aging. Furthermore, the present study used dual regression techniques to evaluate differences in FC between groups and time points. Hence, rater time is limited with the majority of the analysis being automated.

Clinical implications

The exploration of FC in RSNs by means of rs-fMRI has the advantage of being noninvasive, readily available, and does not require complex subject interaction [54]. This is essential in older patients and especially when working with patients suffering from AD. From a research perspective, RSNs can be used for the investigation of disease progression over time, and for monitoring of healthy older subjects. From a clinical point of view, RSNs can be used to select patients for clinical trials or to aid the early diagnostic process. A recent study found high diagnostic accuracy rates for the investigation of the DMN by means of rs-fMRI, ranging from 64-97%, depending on the method of analysis used [55].

CONCLUSION

The present study provides important insights into the reproducibility of RSNs in healthy aging. Measurements of FC in RSNs, investigated by means of rs-fMRI were stable over different time points, showing good to excellent reproducibility, both in healthy old and healthy young subjects. Age-effects were identified, indicating a decrease in FC in the DMN and in the auditory system, associated with aging. Loss of stability of FC in vulnerable regions of the DMN (e.g. precuneus) could become a predictor for future memory problems on an individual level.

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Chapter 9

General Discussion

Future directions

Rationale

The main objective of this thesis was to expand insight into the use of novel magnetic resonance imaging (MRI) techniques for the early detection, diagnosis and prognosis of Alzheimer's disease (AD). The clinical value of both conventional and novel imaging markers was evaluated for their possible contribution to patient management.

Added value of MRI in clinical practice

Current clinical practice in most Memory Clinics consists of a cognitive examination of patients by means of a neuropsychological test battery, together with a physical and neurological investigation, (hetero)anamnesis, a blood test and a brain scan. Based on the results of all these tests a diagnosis is made, treatment is initiated, and a follow-up trajectory is defined. Given the limited resources in health care, an efficient use of diagnostic tests with a minimum of costs per diagnosis together with a maximum accuracy is important. A first question that automatically arises from this clinical approach formed the starting point of the thesis: *'What is the added value of MRI to general diagnostics, and more specific: to neuropsychological test performance?'*

First of all, by means of MRI one can exclude other underlying diseases or possible causes of cognitive impairment [1, 2], and assess the presence of vascular (i.e. white matter lesions, lacunes, infarcts) and treatable diseases (i.e. subdural hematoma, tumors). Second, structural MR images allow an evaluation of atrophy in medial temporal lobe (MTL) regions, a key marker for AD, by means of a qualitative visual rating scale [3]. In most memory clinics, MRI investigation is actually limited to these assessments.

In *Chapter 2* we found that MRI hippocampal volume was the best predictor of conversion from mild cognitive impairment (MCI) to AD. Measuring hippocampal volume furthermore improved the accuracy over memory tests alone. In line with others was found that the delayed recall test is the best neuropsychological predictor for AD in subjects with MCI. We pointed out that when MRI-assessment is available, that adding a measurement of hippocampal volume to neuropsychological test performance is preferred. We also found that the added value of performing

a visual rating scale on top of neuropsychological testing is limited in terms of sensitivity and specificity. Assessing disease progression by means of episodic memory tests (delayed recall) and MMSE-score is an alternative at the cost of a lower sensitivity and specificity. In line with the results in *Chapter 3*, we concluded that the likelihood to identify MCI patients at risk for AD-conversion is highest for volumetric measurements. Assessment of hippocampal volume together with a delayed recall test may be sufficient for short-term prognostic purposes (e.g. follow-up of MCI-patients). Recent evidence pointed out that MTL atrophy is not completely specific for AD since it can also be found in other forms of dementia [4]. It is furthermore known that AD pathology is not restricted to the MTL. *Chapter 4* therefore explored differences between healthy controls, MCI and AD-patients in and outside the MTL. We evaluated if four techniques assessing gray matter (GM) atrophy differences in the brain show the same pattern of abnormalities throughout the same dataset. We found that the sensitivity of each method to detect group differences was region-specific. Manual volumetric measurements together with voxel based morphometry are preferred techniques for assessing GM differences showing abnormalities in most of the investigated regions, with a predominance of the MTL in the early phase. Automated FreeSurfer volumetric measurements show similar performances in the early phase, displaying group differences in the posterior parietal cortex (PPC) but not in MTL regions. Measurements of cortical thickness are less sensitive in the MCI stage and its sensitivity is restricted to the MTL and PPC regions in later stages of the disease (AD).

Taken together, the results of the studies described in *chapters 2 to 4* allow us to draw three major conclusions. First, MRI has indeed an added value on top of neuropsychological assessment, both in the field of diagnosis and prognosis (disease tracking). Second, we confirmed the early involvement of the MTL (*Chapter 2 to 4*) and its clinical importance in predicting conversion to AD in subjects with MCI (*Chapter 2 and 3*). We also showed that atrophy of the MTL is related to cognitive decline (*Chapter 3*). Third, our findings add to the existing body of knowledge that the diagnostic value of MR imaging in AD is not limited to qualitative assessments in MTL regions (*Chapter 4*). Quantitative, volumetric measurements show higher predictive accuracy rates than visual rating scales (*Chapter 3*) and the sensitivity of volumetric markers for (early) AD is not restricted to MTL regions since also posterior parietal and prefrontal regions show macroscopic alterations throughout

the disease process (Chapter 4). However, a major pitfall for implementing volumetric measurements in clinical practice is access to an infrastructure which allows quantitative assessments (see discussion below).

Gray matter atrophy or white matter connectivity?

Besides GM atrophy as a key hallmark of AD, white matter (WM) integrity is decreased in MCI and AD suggesting a potential diagnostic value that may possibly help to improve the diagnostic accuracy of GM atrophy measurements [5, 6]. In *Chapter 5* we evaluated the potential of DTI as an early marker for AD, in relation to MTL atrophy measurements, by using a meta-analysis approach. We concluded that in general, effect sizes of volumetric MTL atrophy measurements were equal or larger than effect sizes of DTI. However, in the early stages (controls vs. MCI), hippocampal mean diffusivity showed larger effect sizes than hippocampal volume. Despite studies stating that DTI measurements seem to be more sensitive than volumetric measurements [7] our meta-analysis did not clearly support the notion that DTI is superior to structural MTL assessment in detecting early stage AD. In chapter 6, we tried to replicate the findings of chapter 5 by means of a case-control study. Cross-sectionally, we found a constrained pattern of GM differences in left parahippocampal gyrus (healthy controls versus subjects with MCI and AD) and left posterior cingulate regions (controls versus AD). This in contrast with previous findings that show a widespread pattern of GM atrophy in AD and a more restricted GM atrophy pattern in MCI patients [8]. In addition, diffusivity metrics were more sensitive for group differences than atrophy measurements, and were less influenced by the effects of aging, gender and scan parameters in a priori selected ROIs. Diffusivity differences were found in all the investigated structures, whereas changes in absolute diffusivity (mean diffusivity (MD), radial diffusivity (DR) and axial diffusivity (DA)) were more pronounced than changes in anisotropy (FA).

Since longitudinal cognitive and behavioral data were available, we additionally investigated the value of these markers for predicting decline at 2 years follow-up. We found that diffusivity differences in the genu of the corpus callosum but not volumetric measures predicted decline in a group of MCI-subjects. Important to note is that, in contrast to our meta-analysis in chapter 5, automated atrophy measurements (FreeSurfer) were evaluated here, both in and outside the MTL. The novelty of this study was that the pattern of gray and white matter alterations in both MCI and AD-patients was investigated in a multicenter setting, and that

diagnosis was based on expert panel judgements.

From the results in chapter 5 and 6 we can conclude that DTI is a sensitive method to detect white matter changes in subjects with MCI and AD. In widespread brain regions diffusivity is already impaired early in the disease process, with more severe white matter disruptions with increasing disease severity. Diffusivity measurements show higher effect sizes than volumetric measurements in discriminating healthy controls from MCI and AD and are less influenced by the effects of aging, gender and center in apriori selected ROIs. DTI metrics in medial temporal, posterior parietal and callosal regions may therefore serve as an early marker for degeneration, even before atrophy is detectable. Diffusivity metrics in the genu of the corpus callosum can predict decline in a group of MCI-subjects.

Robustness of structural atrophy and functional connectivity measurements

In the search for new imaging markers for disease, we must be well aware that techniques may be influenced by internal (e.g. patients age) and external (e.g. software, hardware) factors. In order to explore some of these influences, we carried out a number of methodological evaluations in the third and last part of the thesis.

Automated segmentation techniques become more and more popular nowadays due to the time-intensive nature of manual volumetry. The question therefore arises if processing conditions such as workstation type, operating system and software version affect automated segmentation results and thus influence group differences. *Chapter 7* investigated the robustness of FreeSurfer to capture morphological changes in the brain against varying processing variables and in comparison to manual measurements. We concluded that later versions of FreeSurfer were more accurate than earlier versions, especially in medial temporal and posterior parietal regions. A lot of research in the last decade has pointed out resting state functional connectivity as a marker for AD [9]. The first step toward a biomarker is assuring that age-effects do not influence the reliability of RSNs. *Chapter 8* investigated whether functional connectivity patterns can be reliably reproduced over time in healthy old and young subjects. It was found that measurements of functional connectivity, investigated by means of resting state functional MRI were stable over different time points, showing good to excellent reproducibility, both in healthy old

and healthy young subjects. Age-effects were furthermore identified, indicating a decrease in FC in the DMN and in the auditory system, associated with aging.

From the findings in chapter 7 and 8 we can conclude that both FreeSurfer automated volumetry and resting state functional connectivity are reliable measurements to study the process of aging and disease. Prior work of our group has shown that mixing up versions, workstations or operating systems might lead to significant differences in cortical thickness or volumetry in schizophrenic patients [10]. The findings in *chapter 7* were much more positive, but we however agree with the software developers that it is advised to keep the research environment stable. The necessity to control for age-effects in dementia research is furthermore quite evident as age might cover or lead to an overestimation of group-specific differences [11].

Underlying mechanisms of gray and white matter pathology in AD

Alterations in gray and white matter are independent pathological mechanisms in AD [12-14]. GM atrophy essentially results from neurodegeneration by the accumulation of aggregated hyperphosphorylated tau protein ('tangles'), deposition of amyloid-beta ('plaques'), oligodendrocyte death and reactive gliosis [15, 16]. The exact neurobiological substrate of diffusion changes still remains unresolved. Alterations of anisotropy (FA) have been suggested to correlate primarily with the integrity of the axonal membrane [17]. An increase in mean diffusivity (MD) is thought to reflect enlargement in the extracellular space due to altered cytoarchitecture (i.e. loss of neurons, axons, and dendrites), suggesting immaturity or degeneration of the tissue leading to elevated water diffusivity within these regions [18, 19]. Intra-axonal structures are thought to determine the diffusion parallel to the axonal fibers (DA) [20], whereas the changes in myelin sheaths and axonal membranes underline diffusion perpendicular to the axonal fibers (DR) [21]. In *Chapter 6* was found that the discriminative value of diffusivity metrics is higher than that of GM atrophy suggesting that in most regions microstructural changes in WM precede macrostructural atrophy. The pattern of decreased WM integrity in *Chapter 6* was consistent with the retrogenesis model of WM degeneration in AD, which shows that myelin breakdown follows a pattern that is the reverse of myelogenesis, thus first affecting late-myelinating fibers [22-24]. This retrogenesis model of WM change is furthermore consistent

with the pattern of functional changes in early AD where memory, language and executive problems are present in the absence of sensory and motor problems [25]. Apart from retrogenesis, also Wallerian degeneration plays a role in WM pathology in AD. In the Wallerian degeneration model, degradation of microstructure occurs secondary to GM pathology [26, 27]. The genu, splenium and cingulum fiber pathways investigated in *Chapter 6* all share connections to MTL structures (e.g. hippocampus) known to be affected early in the course of AD [28] and changes in these structures (e.g. atrophy) could lead to Wallerian degeneration [29]. The results of *Chapter 6* did not change when analyses were corrected for GM volume, suggesting that WM diffusivity differences were independent of GM atrophy patterns and that Wallerian degeneration is not the primary mechanism of WM change in AD. The lack of a significant correlation between diffusivity and volumetric measurements in regions outside the hippocampus strengthens the idea that DTI and MR-based volumetry measure different aspects of underlying pathology [30]. Since the data in *chapter 6* was mainly cross-sectional we cannot adjudicate about causality. However, our findings suggest that WM changes in AD are primary related to alterations in the integrity of myelin[21] rather than to Wallerian degeneration secondary to distal GM atrophy. These findings are in line with previous work [24, 31] and neuropathological evidence indicating that WM changes in AD are not solely due to Wallerian degeneration [32].

Scientific implications

The results described in this thesis have several implications for future studies into the pathogenesis of early AD. In line with other studies, we showed that both GM atrophy and WM diffusivity play an important role in discriminating between healthy controls and (early) AD, predicting AD-conversion in subjects with MCI or predicting decline after 2 years [13, 33-37]. Associations with genetic, CSF and neuropsychological markers were investigated and techniques were validated both in single center and multi-center settings.

Relation with other (non-imaging) AD-biomarkers

In *chapter 3* we found, in line with the literature, that hippocampal volume significantly correlated with CSF t-tau and p-tau but not with A β 1-42 [38-40]. These findings may be explained by the observation that antemortem hippocampal volume significantly correlates with the density of neurofibrillary tangles at autopsy [41, 42]

but not with amyloid beta plaque load [41]. The qualitative MTA-score and lateral ventricle volume correlated with CSF A β 1-42. This correlation may indicate that these MTL measures in part reflect generalised brain atrophy as previous studies showed that lower A β 1-42 levels but not t-tau levels were associated with total brain atrophy and ventricular volume [43]. Automated hippocampal volume at baseline was furthermore associated with APOE-e4 allele status in *chapter 3*.

Multi-center effects

Besides the above mentioned differences in software and hardware during pre-processing and analysis, also scanners (e.g. manufacturer, type, field strength) or scanparameters often vary in multi-center studies and may influence imaging results or group differences. Since the data used in *chapter 3* consisted of scanners with different field strengths (1T and 1.5T), we investigated the possible effects of these differences on hippocampal volume measurements. First, we identified a study from one of our co-authors comparing hippocampal volumes of subjects scanned at both 1.5T and 3T scanners (Wolz 2013, in press). Hippocampal volume was measured by means of the LEAP method using data from the ADNI study. The correlation between the measurements on each type of scanner was very high ($r=0.98$). The volumes measured on 3T were on average 24.4 mm³ or 1.17% larger than on 1.5T. Important to note is that the volume was also about 1.5% larger if the hippocampus was measured the second time on a scanner with the same magnetic field strength (either 1.5T or 3T; Wolz 2013, in press). This indicates that for the LEAP method, even a difference of 1.5T in field strength has no significant impact on hippocampal volume. Secondly, we tested the effect of field strength on the volumetric measures. We compared the volumes of 127 subjects scanned on a 1T scanner with that of 201 subjects scanned on a 1.5T scanner with correction for age, gender, educational level, baseline MMSE score and follow-up diagnosis. The difference in volume between 1T and 1.5T scanners was only 0.2% for the LEAP method (p -value = 0.9 [44]), 0.9% for manual hippocampal volume (p -value = 0.6), and 1.5% for the lateral ventricle volume (p -value = 0.8). Third, we tested whether field strength modulated the effect of the volumetric measures on conversion to AD-type dementia. The interaction between field strength and volumetric measure was not statistically significant for any of the volumetric measures ($p>0.15$). These data indicate that the difference in field strength had no effect on hippocampal or ventricular volume.

In *chapter 7*, we used multi-center DTI-data consisting of different scanners (manufacturers) and scan parameters with equal field strength (3T). One healthy volunteer (25 years old, male) was scanned twice at all centers to determine intra and inter center differences in DTI-values. In the hippocampal region we found variability values between 0.79-3.88% (depending on the DTI parameter) compared to 0.22-9.58% in the posterior cingulum. As expected, inter center variability was higher than intra center variability, and also region-dependent (hippocampus 2.07-6.85% vs. posterior cingulum 7.12-16.45%). Our results are in line with recent evidence which reported a variation in FA across multiple scanners/field strengths both using a physical phantom and human brain scans [45].

The findings above indicate the importance of harmonization in the field of scanners, scan protocols, software versions, pre-processing steps and methods of analysis. These premonitions are not limited to the field of imaging since other markers (e.g. CSF, neuropsychology) also require standardisation of methods and validation of cut-offs. It is virtually impossible to correct for all sources and types of errors in research practice. It is however essential that we are aware of potential sources of error, that research protocols and analyses account for these factors and that corrections are performed, if possible.

MRI in clinical practice: necessity or option?

At this point, we are back to where we started in the beginning of this chapter: ‘What is the role of MRI in clinical practice?’ and ‘Is MRI investigation necessary in clinical practice or does it only lead to additional burden from a patient’s point of view?’. Taken together all three parts of the thesis, and building upon the existing body of knowledge, we can conclude that MRI plays an important role both for diagnostic and prognostic purposes. However, despite the clinical relevance of certain imaging modalities (e.g. hippocampal volumetry, connectivity), many of these findings are not readily transferrable to clinical practice. Translation to clinical settings remains to be demonstrated due to restrictions inherent to the technique (e.g. no standardization, no longitudinal validation, no availability of cut-offs, no expertise), or due to the unavailability of software or hardware amenities.

For implementation in clinical practice, a marker requires that its dynamics are known at different disease stages and that its relationship with other imaging and biological markers is understood [46]. In chapter 3 was found that the predictive accuracy of both volumetric hippocampal measurements for AD-type dementia (manual and LEAP) was higher than that of the qualitative rating and lateral ventricle measure. Both measures of hippocampal volume significantly correlated with CSF t-tau and p-tau but not with A β 1-42. LEAP hippocampal volume at baseline was furthermore associated with APOE-e4 allele status (lower LEAP volumes in APOE-e4 carriers compared to APOE-e4 non-carriers). For the limited rater time (four minutes control time on a standard computer), LEAP automated hippocampal measurement may be preferred for clinical implementation.

A lot of effort has been made to point out the best biomarker (or combination of markers) for diagnosing early AD [47]. To date, indirect evidence has indicated that amyloid markers (assessed through PET-imaging or CSF) show more substantial abnormalities than structural MRI in the asymptomatic to MCI stage, and that CSF-levels are overall more sensitive as diagnostic tool [48]. Atrophy markers on the other hand are more sensitive to change (disease tracking) than CSF-markers [49, 50]. However, a lot of inconsistencies are found regarding the initiator and accelerator roles of certain pathological events and thus markers for different disease stages [48, 51, 52]. According to recent findings one might consider all CSF and neuroimaging biomarkers (together with the presence of cognitive impairment) to the same degree whereas the presence of each factor increases the risk of underlying AD pathology [53]. Despite the high diagnostic accuracy rates of CSF biomarkers, lumbar puncture is feared by many patients due to the risk of severe complications and post-LP headaches in 2-4 percent of cases [54]. From a patients perspective, an MRI scan is a more feasible and less intrusive diagnostic alternative in clinical practice.

Future research directions

The restrictions mentioned above should be thoroughly investigated and resolved before quantitative measurements (structural atrophy or connectivity) can be successfully implemented in everyday clinical practice. In order to implement 'MRI-beyond-MTA' as a biomarker for AD, future studies need to standardise automated techniques for use in clinical practice, and establish and validate cut-offs in studies with large sample sizes. Such cut-offs are needed to achieve the ultimate goal of

an MRI-biomarker, namely a reliable diagnosis on a subject-level based on a single cross-sectional MRI scan.

Future studies need to assess the clinical relevance of the above mentioned techniques in large-scale clinical trials which aim to examine the diagnostic and prognostic value of certain techniques. Furthermore, treatment effects need to be mapped with different multimodal imaging modalities. The latter is even more important if disease modifying treatments become available that require easy accessible, cost-effective and non-invasive techniques to diagnose AD in its earliest stages.

A necessary stopover... to know or not to know?

The research area of early biomarkers for AD is highly motivated by the on-going development of disease-modifying treatment. However, to date there is no cure available for AD yet, so there is no direct benefit from an early diagnosis. The question therefore arises if preclinical persons need to be informed about their status in clinical routine?

In short, early diagnosis may also negatively affect health and daily life of an early-stage AD patient. A few of the possible implications are feelings of hopelessness and despair which may lead to depression, loss of job and/or driver's license, and insurance issues. Besides an impact on the patient level, also the social system (relatives, employer) is concerned. On the contrary, even without treatment options, early diagnosis might be clarifying for the patient, facilitate access to tools which can help to cope with the progressive decline and enhance assistance from the health care system.

Extended use of early diagnostic tools in research environments is furthermore essential since these developments may lead to increased enrollment of patients in clinical trials which can in turn lead to novel treatment approaches.

Conclusion

In conclusion, this thesis expands insights into the added value of imaging markers as diagnostic tool for the early detection of AD. Atrophy of MTL structures is a candidate marker to assess change from MCI to moderate AD stages (prognosis).

We showed that relatively novel and thus less validated MRI markers such as DTI and resting state functional MRI are promising for diagnosing AD in an early stage but need to be evaluated empirically. Standardization of operating procedures (for e.g. volumetric and connectivity approaches) will further consolidate our understanding of AD-pathology, facilitate comparison and integration of results, enable the establishment of cut-offs and consequently pave the way to clinical implementation.

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Appendix

Summary

Nederlandse samenvatting

Curriculum vitae

List of publications

Dankwoord

SUMMARY

Worldwide, 115 million people are at the forefront of an Alzheimer's tsunami. The baby boom generation is now entering their mid-sixties and many of them will suffer from Alzheimer's disease (AD), which now already affects 1 in every 8 people over age 65. Early diagnosis and treatment may help to reduce both patient and health-cost burden. Before clinical or cognitive deficits become apparent, a distinct set of underlying brain abnormalities such as a decrease in cerebral connectivity are present. Early detection of these (often subtle) abnormalities is therefore an important public health goal. The research described in this thesis aimed to expand insight into the use of both conventional (e.g. hippocampal volumetry) and novel (e.g. diffusion tensor imaging) magnetic resonance imaging (MRI) markers for early detection, diagnosis and prognosis of AD. MRI provides more information than macrostructural atrophy and vascular abnormalities. However, the added value and exact position of novel imaging techniques in the diagnostic field of AD was still unclear. The principle question of the thesis was whether MRI investigation is necessary in the diagnostic process of AD and which MRI measurements can incrementally contribute to the early and specific diagnosis of Alzheimer disease.

The clinical value of novel imaging markers for AD was evaluated in relation to current (gold standard) methods (i.e. hippocampal volumetry, visual rating scale). In the first part of the thesis (Chapter 2 to 4) we evaluated well-known imaging techniques which assess gray matter atrophy in the brain. In the second part (Chapter 5-6) we examined the discriminative and predictive value of a novel imaging marker for AD: Diffusion Tensor Imaging (DTI). In the third part (Chapter 7-8) we investigated the robustness of structural and functional abnormalities in AD.

In *chapter 2* we investigated in a multicenter setting (n=184) if MR imaging markers increased predictive accuracy for AD relative to a model which only included demographical and neuropsychological information. We found that hippocampal volume is the best predictor of conversion from mild cognitive impairment (MCI) to AD. Measuring hippocampal volume furthermore improved the accuracy over memory tests alone. In line with others was found that the delayed recall test is the best neuropsychological predictor for AD in subjects with MCI. We concluded that when MRI-assessment is available, that adding a measurement of hippocampal

volume to neuropsychological test performance is preferred. Additionally, it was found that the added value of performing a visual rating scale on top of neuropsychological testing is limited in terms of sensitivity and specificity. Since the MTL still receives a lot of attention in diagnosing AD, discriminating converters from non-converters and AD from non-AD, *chapter 3* compared the predictive value of 4 different MTL measurements in a multicenter (n=328) and single-center setting (n=172). Results showed that volumetric hippocampal measurements are the best predictors of conversion to AD-type dementia in subjects with MCI after 2 years follow-up (manual and LEAP hippocampal volume). Both volumetric measurements strongly correlate with cerebrospinal fluid (CSF) markers of neuronal injury (CSF t-tau and p-tau), are able to predict cognitive decline, and show consistent cut-off values between different cohorts. LEAP hippocampal volume has the advantage over manual volumetry that it needs much less rater time and shows no interrater variability effects. In addition, LEAP has a low technical failure rate.

Recent evidence pointed out that MTL atrophy is not completely specific for AD since it can also be found in other forms of dementia. It is furthermore known that AD pathology is not restricted to the MTL. Since the contribution of brain areas outside the MTL has become more evident, in *chapter 4* we evaluated the discriminative value of several techniques measuring GM atrophy in the brain, both in and outside MTL regions. It was investigated if four techniques assessing gray matter (GM) atrophy differences in the brain show the same pattern of abnormalities throughout the same dataset. We found that the sensitivity of each method to detect group differences was region-specific. Results showed that manual volumetric measurements together with voxel based morphometry are preferred techniques for assessing GM differences showing abnormalities in most of the investigated regions, with a predominance of the MTL in the early phase. Automated FreeSurfer volumetric measurements showed similar performances in the early phase, displaying group differences in the posterior parietal cortex but not in MTL regions. Measurements of cortical thickness are less sensitive in the MCI stage and its sensitivity is restricted to the MTL and PPC regions in later stages of the disease (AD).

Chapter 5 describes a meta-analysis which was conducted to evaluate the discriminative value of DTI in a group of controls subjects, patients with MCI and AD-pa-

tients. We included seventy-six studies on MTL atrophy including 8122 subjects and fifty-five DTI studies including 2791 subjects. Effect sizes (ES) were calculated for several regions of interest known to be altered by pathophysiological mechanisms of AD, and were compared with ES of medial temporal lobe measurements. Results showed that DTI is a sensitive method to detect white matter changes in subjects with MCI and AD. It seems that in widespread brain regions diffusivity is already impaired early in the disease process, with more severe white matter disruptions with increasing disease severity. DTI abnormalities were found in many different regions. FA and MD changes seem to follow a slightly different anatomical pattern whereas highest ES (relative to controls) for DTI-FA were typically seen in cingulate and callosal areas (total cingulum, posterior cingulum, parahippocampal cingulum, splenium CC and uncinate fasciculus). For DTI-MD the largest differences were seen in the hippocampus, followed by the parietal lobe, splenium CC and temporal lobe. It was furthermore found that the discriminative power of DTI-MD is slightly higher than for DTI-FA.

Chapter 6 aimed to validate the findings of chapter 5 by means of a case control study. Cross-sectionally, we found a constrained pattern of GM differences in left parahippocampal gyrus (healthy controls versus subjects with MCI and AD) and left posterior cingulate regions (controls versus AD). This in contrast with previous findings that show a widespread pattern of GM atrophy in AD and a more restricted GM atrophy pattern in MCI patients. In addition, diffusivity metrics were more sensitive for group differences than atrophy measurements, and were less influenced by the effects of aging, gender and scan parameters in a priori selected ROIs. Diffusivity differences were found in all the investigated structures, whereas changes in absolute diffusivity (mean diffusivity (MD), radial diffusivity (DR) and axial diffusivity (DA)) were more pronounced than changes in anisotropy (FA). We furthermore found that diffusivity differences in the genu of the corpus callosum but not volumetric measures predicted decline in a group of MCI-subjects. Due to the time-intensive nature of manual volumetry, automated segmentation techniques become more and more popular nowadays. As a result, the question arises if processing conditions such as workstation type, operating system and software version affect segmentation results and thus influence group differences. In *chapter 7* we investigated the robustness of FreeSurfer to capture morphological changes in the brain against varying processing variables and in comparison to manual

measurements. We concluded that later versions of FreeSurfer were more accurate than earlier versions, especially in medial temporal and posterior parietal regions. A lot of research in the last decade has pointed out resting state functional connectivity as a marker for AD. The first step toward a biomarker is assuring that age-effects do not influence the reliability of RSNs.

In *chapter 8* we investigated whether functional connectivity patterns can be reliably reproduced over time in healthy old and young subjects. It was found that measurements of functional connectivity, investigated by means of resting state functional MRI were stable over different time points, showing good to excellent reproducibility, both in healthy old and healthy young subjects. Age-effects were furthermore identified, indicating a decrease in functional connectivity in the DMN and in the auditory system, associated with aging.

Chapter 9 provides a general overview of the findings described in this thesis. Clinical and research implications resulting from the presented studies are discussed.

In conclusion, this thesis expands insights into the added value of imaging markers as diagnostic tool for the early detection of AD. Atrophy of MTL structures is a candidate marker to assess change from MCI to moderate AD stages (prognosis). We showed that relatively novel and thus less validated MRI markers such as DTI and resting state functional MRI are promising for diagnosing AD in an early stage but need to be evaluated empirically. Standardization of operating procedures (for e.g. volumetric and connectivity approaches) will further consolidate our understanding of AD-pathology, facilitate comparison and integration of results, enable the establishment of cut-offs and consequently pave the way to clinical implementation.

NEDERLANDSE SAMENVATTING

De ziekte van Alzheimer (ZvA) is de meest voorkomende vorm van dementie die wereldwijd ongeveer 34 miljoen mensen treft. Onderzoekers voorspellen een tsunami van dementie, waarbij de prevalentie van de ziekte zal verdriedubbelen als gevolg van de vergrijzing van de bevolking. Dit leidt tot een toenemende nood aan vroegdiagnostiek van deze ziekte om behandeling in een vroege fase mogelijk te kunnen maken. Een vroege diagnose en behandeling kan immers naast een toename van levenskwaliteit ook een kostenbesparing teweegbrengen, zowel op het niveau van de patiënt als van de gezondheidszorg. Vooraleer klinische of cognitieve afwijkingen daadwerkelijk objectiveerbaar worden, is er reeds sprake van onderliggende abnormaliteiten op hersenniveau, waarbij volumeverlies in bepaalde regio's en een afname van cerebrale connectiviteit een voorbeeld zijn. Beeldvorming met magnetische resonantie (NMR of MRI) biedt een kader om de structurele en functionele veranderingen geassocieerd met het proces van neurodegeneratie te visualiseren.

Het onderzoek beschreven in dit proefschrift kadert binnen het eerder aangehaalde onderzoeksveld van vroegdiagnostiek en heeft primair als doel de inzichten in het gebruik van zowel de traditionele (bijv. volumebepaling van de hippocampus) als de meer recente (bijv. diffusiviteit in de witte stof) beeldvormingstechnieken te vergroten. Deze technieken werden geëvalueerd zowel op het vlak van vroegdetectie, diagnose, als prognose van de ziekte van Alzheimer. De huidige aanpak in de meeste Nederlandse geheugenpoli's vertrekt van een interview met patiënt en zijn naaste, gevolgd door een neurologisch onderzoek. Hierna wordt een neuropsychologische test batterij afgenomen en vindt er een bloedtest en een hersenscan plaats. Aangezien MRI beelden ons meer informatie kunnen verschaffen dan zuiver macroscopische (bv. tumor) of vasculaire afwijkingen, is het gebruik van hersenscans in de kliniek van exclusie van andere ziekten (bv. tumoren, infarcten) uitgebreid naar vroegdetectie (bv. ZvA versus gezonde veroudering) en differentiaaldiagnostiek (bv. ZvA versus Fronto-temporale dementie). Echter, de toegevoegde waarde en exacte rol van de nieuwe beeldvormingstechnieken voor de vroegdiagnostiek van de ZvA is nog steeds onduidelijk. De vraag of MRI onderzoek überhaupt noodzakelijk is in het diagnostisch proces van de ZvA en welke MRI technieken potentieel zinvol zijn vormde het startpunt van dit proefschrift.

De klinische waarde van nieuwe beeldvormingstechnieken voor de ZvA werd geëvalueerd in relatie tot de meer traditionele ('gouden standaard') methoden (bv. volumemeting van de hippocampus en een visuele beoordelingsschaal voor volumeverlies van de mediale temporaalkwab).

In het eerste deel van dit proefschrift (Hoofdstuk 2-4) werden technieken die volumeverlies in de grijze stof van het brein meten geëvalueerd. Het tweede deel (Hoofdstuk 5-6) beschrijft het onderscheidend en voorspellend vermogen van een nieuwe beeldvormingstechniek: Diffusie-gewogen beeldvorming ('diffusion tensor imaging' of DTI). Het derde en laatste deel van dit proefschrift (Hoofdstuk 7-8) heeft zich gericht op het evalueren van de robuustheid van structurele en functionele afwijkingen bij de ZvA.

In *hoofdstuk 2* hebben we in een multicenter setting onderzocht (n=184) of het toevoegen van MRI-beelden de voorspellende waarde voor de ZvA verhoogd ten opzichte van een model dat enkel demografische en neuropsychologische informatie bevat. Hippocampaal volume (gemeten op MRI-beelden) bleek de beste voorspeller te zijn voor conversie van milde cognitieve klachten (MCI) naar de ZvA. Het toevoegen van het volume van de hippocampus verbeterde bovendien de voorspellende waarde van een episodische geheugentaak. In lijn met eerder onderzoek werd gevonden dat een test voor uitgestelde reproductie van een woordenlijst de beste voorspeller is voor de ZvA op neuropsychologisch vlak. We concludeerden dat wanneer MRI-onderzoek mogelijk is (oa. infrastructuur en expertise aanwezig, patiënt MRI-compatibel), dat een volumemeting van de hippocampus naast neuropsychologisch onderzoek wenselijk is. Verder bleek dat de toegevoegde waarde van een visuele beoordelingsschaal (MTA-score) beperkt was in termen van sensitiviteit en specificiteit.

Het evalueren van volumeverlies in de mediale temporaalkwab is tot op heden nog steeds een belangrijk onderdeel van de diagnostiek bij de ZvA. Volumeverlies in deze regio kan converters van non-converters onderscheiden en mensen die lijden aan de ZvA onderscheiden van mensen met een ander type dementie. In *hoofdstuk 3* hebben we de voorspellende waarde van 4 verschillende maten die volumeverlies van de mediale temporaalkwab meten onderzocht in een multicenter (n=328) en single center (n=172) setting. Resultaten hebben aangetoond dat een volumemeting van de hippocampus de beste voorspeller is voor conversie naar

de ZvA bij mensen met MCI (zowel de manuele als automatische meting van hippocampaal volume (LEAP methode)). Beide maten toonden bovendien een sterke samenhang met markers in hersenvocht (CSF) die schade aan de hersencellen meten (CSF t-tau en p-tau). Er werden consistente afkapwaarden gevonden tussen verschillende cohorten en beide maten voorspelden cognitieve achteruitgang na 2 jaar. De LEAP volume meting heeft als voordeel ten opzichte van de manuele methode dat deze minder tijdsintensief is en geen variabiliteit tussen beoordelaars laat zien. Bovendien heeft LEAP een erg lage technische foutenmarge.

Recent onderzoek heeft aangetoond dat volumeverlies in de mediale temporaalkwab niet geheel specifiek is voor de ZvA en dat we dit ook terugvinden bij andere vormen van dementie. Uit eerder onderzoek is bovendien gebleken dat Alzheimer pathologie zich niet beperkt tot deze gebieden. Aangezien ook andere regio's aangetast zijn bij de ZvA hebben we in *hoofdstuk 4* onderzocht welke de onderscheidende waarde is van verschillende technieken die volumeverlies in de grijze stof van de hersenen meten, zowel in de mediale temporale gebieden als daarbuiten. We hebben onderzocht of 4 verschillende technieken die grijze stof atrofie in het brein meten dezelfde resultaten laten zien binnen eenzelfde dataset. De sensitiviteit of gevoeligheid van elke methode bleek afhankelijk van de onderzochte regio. Resultaten toonden aan dat een manuele volumemeting en VBM (voxel based morphometry) de voorkeur genieten voor het in kaart brengen van volumeverlies in de grijze stof. De meeste regio's lieten groepsverschillen zien met een duidelijk overwicht in mediaal temporale gebieden. Automatische volumetrische methodes (FreeSurfer) toonden gelijkaardige prestaties waarbij verschillen in de posterieure delen van de parietaalkwab (en dus niet in de mediaal temporaalkwab) het meest uitgesproken waren in de vroege fase van de ziekte. Het meten van de dikte van de hersenschors ('cortical thickness') bleek minder gevoelig in het vroege stadium van de ziekte.

In *hoofdstuk 5* hebben we een meta-analyse gepresenteerd die primair als doel had de diagnostische waarde van een nieuwe beeldvormingstechniek (DTI of diffusie tensor beeldvorming) te evalueren in een groep van gezonde controles, patiënten met MCI en patiënten met de ZvA. Naast 76 studies die volumeverlies in de mediale temporaalkwab onderzochten (8122 subjecten) werden er ook 55 DTI studies (2791 subjecten) geïnccludeerd. Voor verschillende regio's in het brein werden ef-

fectgroottes berekend die op hun beurt vergeleken werden met effectgroottes van technieken die atrofie van de mediale temporaalkwab meten. Resultaten toonden aan dat in wijdverspreide regio's van het brein de beweging van watermoleculen (diffusie) reeds in een vroege fase van de ziekte verstoord is, waarbij we meer ingrijpende witte stof veranderingen zien bij progressie van de ziekte. Abnormaliteit en op het vlak van fractionele anisotropie en gemiddelde diffusiviteit toonden een verschillend anatomisch patroon. De maat van anisotropie was het meest gevoelig voor verschillen in cingulate en callosale regio's. De gemiddelde diffusiviteit daarentegen was het meest sensitief voor verschillen in de hippocampus, gevolgd door de parietaalkwab, het splenium van het corpus callosum en de temporaalkwab. Over het algemeen genomen was de fractionele anisotropie index even gevoelig als de gemiddelde diffusiviteit. Een lagere gevoeligheid van fractionele anisotropie ten opzichte van gemiddelde diffusiviteit was het meest opmerkelijk in de corticale gebieden.

In *hoofdstuk 6* hebben we gepoogd de bevindingen van hoofdstuk 5 te valideren door middel van een case control studie. Onze resultaten toonden aan dat het patroon van grijze stof afwijkingen in deze studie beperkt was tot de linker parahippocampale gyrus (controles versus subjecten met MCI en ZvA) en de linker posterior cingulate (controles versus subjecten met ZvA). Dit is deels in tegenstelling met eerdere bevindingen die een wijdverspreid patroon van afwijkingen laten zien bij patiënten met de ZvA en een meer beperkt patroon van grijze stof afwijkingen bij MCI-patiënten. Er werd daarenboven gevonden dat diffusiviteitsmaten meer gevoelig zijn voor groepsverschillen dan metingen van volumeverlies en dat deze minder beïnvloed worden door leeftijdseffecten, effecten van geslacht en scanner instellingen. Verschillen qua diffusiviteit werden gevonden in alle onderzochte structuren waarbij veranderingen in absolute diffusiviteit (gemiddelde diffusiviteit (MD), radiale diffusiviteit (DR) en axiale diffusiviteit (DA)) meer uitgesproken waren dan veranderingen in anisotropie (FA). Verder werd gevonden dat niet het volumeverlies maar de verschillen in diffusiviteit in de genu van het corpus callosum achteruitgang voorspelden in een groep van MCI-patiënten. Door het tijdsintensieve karakter van manuele volumemetingen hebben automatische metingen meer en meer aan belang gewonnen. De potentiële invloed van hardware en software instellingen op de resultaten van dergelijke technieken en dus ook het indirecte effect op groepsverschillen is tot nog toe vrij onderbelicht gebleven. In *hoofdstuk 7* onderzochten we of FreeSurfer resistent is tegen deze verschillen. Om hierbij een

zuivere vergelijkingsbasis te hebben werd deze techniek rechtstreeks vergeleken met de manuele segmentatie. Onze bevindingen toonden aan dat het effect van hardware (workstation) kleiner is dan van software (FreeSurfer versie) en dat latere versies van FreeSurfer meer accuraat zijn dan eerdere versies, vooral in posterieur pariëtale gebieden.

Recent onderzoek heeft aangetoond dat ‘resting state’ functionele MRI (rsfMRI), een techniek die de hersenactiviteit in een taakvrije omgeving meet, een belangrijk marker is voor de ZvA. De eerste stap richting ontwikkeling en validatie van een zogeheten ‘biomarker’ is onderzoeken of leeftijdseffecten de betrouwbaarheid van deze ‘resting state’ netwerken niet beïnvloeden. In *hoofdstuk 8* werd onderzocht of patronen van functionele connectiviteit stabiel zijn over de tijd bij gezonde ouderen en jongeren. Resultaten toonden aan dat metingen van functionele connectiviteit, onderzocht door middel van rsfMRI, stabiel waren over verschillende tijdsmomenten, en een goede tot uitstekende reproduceerbaarheid laten zien. Leeftijdseffecten werden geïdentificeerd en er werd een afname in functionele connectiviteit gevonden in het ‘default mode netwerk’ (DMN) en het auditieve systeem, welke in verband staan met veroudering.

In *hoofdstuk 9* wordt een algemene discussie van onze bevindingen beschreven. Klinische en wetenschappelijke implicaties worden hier uitgebreid toegelicht en suggesties voor verder onderzoek worden gegeven.

We kunnen besluiten dat volumeverlies in de mediale temporaalkwab een kandidaat-marker is voor het meten van progressie van een milde cognitieve stoornis naar de ZvA (prognostische waarde). Het onderzoek hier beschreven toont bovendien aan dat relatief nieuwe en dus minder gevalideerde MRI technieken (DTI en resting state fMRI) veelbelovend zijn voor de toekomstige vroegdiagnostiek maar dat deze nog uitgebreid empirisch getest en gevalideerd moeten worden. Standardisatie van de procedures (voor bijv. volumetrische maten en maten van structurele en functionele connectiviteit) zullen onze kennis wat betreft onderliggende Alzheimer pathologie verder uitbreiden en ervoor zorgen dat we resultaten kunnen vergelijken en integreren. Bovendien kunnen we op die manier stabiele afkappunten gaan ontwikkelen dewelke de weg naar klinische implementatie verder kunnen faciliteren. Het ultieme einddoel van een MRI-biomarker is immers een betrouwbare diagnose op subjectniveau, gebaseerd op een enkele MRI-scan.

CURRICULUM VITAE

Lies Clerx was born on October, 25th 1982 in Diest, Belgium. After completing secondary school at the Voorzienigheid Diest, she obtained a bachelor degree in Information Technology at the PHL in Hasselt. In 2004 she started studying Clinical Psychology at the University of Leuven. As a result of her growing interest in neuropsychology, the subject of her master thesis at the lab of Biological Psychology in Leuven was the impact of hippocampal lesions on different

forms of spatial and non-spatial learning (supervision: Prof. Dr. R. D’Hooge) (Summa Cum Laude). During her master she also completed a clinical internship at the Psychiatric Hospital/ University Center in Kortenberg (Leuven) where she gained a lot of diagnostic experience with patients suffering from Alzheimer’s disease and other dementias. This again stimulated her interest in ageing and neurodegenerative diseases. After obtaining her master’s degree in Psychology (Cum Laude) in June 2009, she started as a PhD student at the Department of Psychiatry and Neuropsychology at Maastricht University in August 2009. She investigated the value of novel markers for the early diagnosis of Alzheimer’s disease under the supervision of Prof. Dr. F. Verhey, Dr. P. Aalten and Dr. P.J. Visser. During her PhD, Lies’ work was rewarded with a poster prize on the Euron PhD days and several presentations at international conferences. From June to September 2012 she worked as an intern at the Forschungszentrum in Juelich, Germany on a research project which focused on task-free brain activity in healthy aging. During her PhD trajectory, Lies also enrolled in a 2-year Master Specialization ‘Clinical Neuropsychology’ at the Free University of Brussels of which she graduated in July 2013 (Summa Cum Laude). Assuming that there was no life after obtaining your PhD degree, she grip her Phd-time to build a house, to give birth twice and to marry the man of her life. In January 2014, Lies started as a post-doctoral fellow at the Forschungszentrum in Juelich, Germany.

PUBLICATIONS

International Publications

- ❖ **Clerx L.**, Visser P.J., Verhey F.R.J., Aalten P. (2012). New MRI markers for Alzheimer's disease: a meta-analysis of diffusion tensor imaging and a comparison with medial temporal lobe measurements, *Journal of Alzheimer's Disease*, 29(2), 405-29.
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- ❖ Lies Clerx, Pauline Aalten. (2012). De meerwaarde van diffusie gewogen beeldvorming bij de vroegdiagnostiek van de ziekte van Alzheimer, *Neuropraxis*, 2012, 5(5), 143-148.

DANKWOORD

Beste (ex)collega, vriend, familie, verdwaalde lezer,

Meer dan vier jaar geleden, op een regenachtige lentedag (uiteraard in België) kreeg ik onderweg van Leuven naar huis telefoon. Een nummer uit NL... Zou het? Ik durfde amper te antwoorden: "Hallo Lies, Pauline Aalten uit Maastricht, stoor ik?" "Euh nee hoor" *slik* "Wij hebben besloten dat jij de ideale kandidaat bent voor de MRI onderzoeksplek binnen het LeARN project..." *say whaaaaat* "...dus als jij het ook ziet zitten verwelkomen we jou graag binnenkort in Maastricht". Hoe graag ik ook "Jaaaaa natuuuurrrllliijjk" wou schreeuwen... toch probeerde ik de rustige Lies te zijn (bestaat-die-dan?) en antwoordde ik "Ja hoor, heel graag" ...

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Gisela, mama Gis.. onze kamers lagen de afgelopen jaren 'ver' uit elkaar maar toch vonden we ieder om beurt de tijd om lekker even bij te kletsen (en voor nu: leve whatsapp!). Fijn om samen met jou dikke buik en andere kinderderelateerde pret te kunnen delen.

Sanne, Peggy en Ieke, mijn ex-buurvrouwen: leuk dat jullie de witte en andere chocotoffs met mij wilden delen en tegelijk ook steeds into een leuke babbel waren! Ieke: jouw gouden tip van M&Ms eten in de auto en dan het pakje naar achter gooien – ik pas hem regelmatig toe! Dank je wel daarvoor ;) S&P: veel succes nog met het afronden van jullie boekje!

Nico en Ron M., wat mij betreft zijn jullie de levende versie van de Rescue Rangers: geen karwei te groot, geen klus te zwaar, die 2 staan altijd klaar! De verdeling van wie knabbel en wie babbel is laat ik aan de lezer over. Bedankt voor jullie IT-hulp-in-nood!

Elsa, "la mama" van de afdeling! Voor alles kunnen we bij jou terecht – en we worden bovendien nog ontvangen met een brede glimlach ook. Bedankt voor alle hulp tijdens mijn promotie en voor het extra-tandje-erbij bij de afronding!

Astrid, bedankt voor jouw logistieke en andere hulp bij het organiseren van NPO's, huisbezoeken, scansessies enz.!

Tanja en Carla: bedankt voor jullie ondersteuning en harde werk tijdens de vele patiëntencontacten!

Claire, bedankt voor de leuke samenwerking binnen PSI/LeARN!

Marjolein H., we hebben elkaar niet zo vaak 'in person' gezien de afgelopen jaren maar we hebben samen wel een erg leuke tijd gehad in Riga (oa. in die Amerikaanse eettent haha) en gelukkig bestaat er social media die veel mogelijk maakt! Veel succes met de opleiding verder!

Alle andere collega's van divisie 1 wil ik eveneens bedanken voor de leuke tijd samen!

I would also like to thank my German colleagues: Eckart, we had some nice low-level conversations im Seecasino, thanks for your daily help in choosing the right sausage for lunch. Kim and Niels (and later: Boris), we had a great time together in the summer of 2012! I enjoyed our FSL-discussions and the little course we organized. Ozgur, our contact is limited but however valuable. It's nice to have little boys who share the same age. Juraj, thank you for giving me the opportunity to work on the RIMCAD data.

Uiteraard en niet in het minst zijn er ook een heleboel mensen buiten Maastricht die het verdienen om hier vernoemd te worden:

Lieve Anneke, Moorsie, my BFF! Zo leuk dat we ineens terug in elkaars leven zijn gekomen - it was meant to be! Heerlijk om samen ADA te zijn en uren met jou te messagen, bellen of chatten. YOLO! Elkaar in het echt zien is natuurlijk nog veel beter maar dan lukt het ongeneerd praten niet zo goed door die 4 kleine varkentjes van ons. Ik hoop dat ik nog vaak spreuken mag sturen omdat je overtijd gaat.. en dat jij nog vaak (kuch) als eerste op de materniteit kan staan. Voor de geur alleen al, toch? :) (ps: wa IS dees?!).

Lieve Ellen S. aka foefie, we kennen elkaar nu zo'n 13 jaar (say whaaaaat?) en ik herinner me nog heel goed wat een leuke tijd we hebben gehad op de PHL. Fully open, samen met Mie. Ik ben blij dat we nog steeds contact hebben en dat we

elkaar bij leuke en minder leuke dingen altijd weten te vinden. 2014 kan niet meer stuk, jullie wens mag eindelijk uitkomen... en die van Fre en mijzelf dus ook een beetje! Muchos besos voor jou en Steve-man!

Lieve Ellebelle, hoe krijgen we het toch voor elkaar om telkens weer dezelfde keuzes te maken in het leven? Zo fijn om een blond kopietje van mezelf te hebben! Tha blond and tha brunette! Foreva! Ik kan heel wat wist-je-datjes gaan bedenken maar eentje blijft essentieel: groene fluostift + tranen = gele fluostift (ik WIST van bij het ontstaan van die spreuk dat deze ooit gepubliceerd ging worden: bij deze!) ;) Keep on shining Miss van Crieke! En jij ook nog veel succes met afrondingsmanoeuvres – ik zal er zijn he! ;)

Lieve Stephanie T., Celine, Ann. R., Nathalie, Sylvie, Katrien V., Vroni - bedankt voor jullie oprechte interesse in mijn doen en laten van de afgelopen 4 jaar. Een mens kan zich geen betere vriendinnen wensen!

Graag wil ik ook mijn bredere familie en schoonfamilie bedanken voor hun interesse in de ongetwijfeld vaak abstracte inhoud van mijn job. Fijn dat jullie vanuit de kantlijn zo enthousiast wilden mee supporteren!

Steven, dank je wel voor het prachtige ontwerp van de kaft! Een half uurtje hebben we hiervoor samen gezeten – ik probeerde mijn vage ideeën zo goed mogelijk over te brengen en jij noteerde HELEMAAL NIETS. Ik wachtte rustig (en toch ook wat sceptisch) af tot ik enkele weken later een knaller van een cover toegestuurd kreeg - ongeloofelijk! PAL EROP! Exact wat ik wou en dat zonder enige voorkennis en met een schamele uitleg – dat kan alleen een echte professional.

Kristof, lieve schoonbroer (of is het schoonboer???), fijn dat we op tijd en stond eens nuchter (?) kunnen kletsen over het reilen en zeilen binnen onze beide jobs. Hoewel erg uiteenlopend weten we allebei wat het is om gebeten te zijn door ons vak, hard te werken en een heel klein beetje veel work-a-holic te zijn. Ik vind het heerlijk om zo een leuke schoonboer te hebben! Bedankt om bovendien zo'n leuke peter/nonkel te zijn voor onze kids!

Nico, je suis heureuse que tu fasses partie de la famille. Tu es l'homme grâce à qui

ma petite soeur n'a pas mal tourné! :) Et n'oublie jamais: kleine t**** and dikke p***! :)

Elisabeth, lieve schoonzus, wat heerlijk dat jij er plots bijkwam. Eindelijk iemand waarmee ik ongegeneerd kon kletsen, shoppen en chocola eten. Een lieve tante Bee voor ML, een uitmuntende metie voor Maurice en een geweldige vriendin voor mij. Ik hoop dat we samen nog veel stukken chocola kunnen kraken, voor-, tijdens en na onze shoppinguitjes!

Sonia, lieve zus, we kennen elkaar ondertussen iets minder dan 30 jaar en hoewel we 400km uit elkaar wonen, toch slagen we er telkens in om bij belangrijke gebeurtenissen bij elkaar op de 1e rij te staan. Mijn jeugd had er heel anders uit gezien zonder jou erbij, dank je voor alle leuke momenten en ook om ze allemaal te onthouden zodat we ze nu elke keer weer opnieuw kunnen beleven. A demain, portemonnaie vous bien.. En dan? :) Ma das de wiiinnnddd! :p ML kan zich geen betere meter wensen en ik geen betere zus. Sokke, you rock BIG time!

Marc en Monique: schoonouders krijg je er gratis bij - Wel, voor jullie had ik gerust heel wat willen bijbetalen! Ongelofelijk hoe goed het tussen ons allemaal klikt en wat voor een prettig gestoorde bende we samen zijn. Ik hoop dat we nog vaak samen kunnen 'zakdoek leggen' op Italiaanse (of andere) bodem. Marc, bedankt om ons telkens op culinair en ander vlak in de watten te leggen, geen zalm is veilig in jouw buurt! Monique, mémé, mijn respect voor jou is eindeloos. Ondanks het ziek zijn, de pijn en de moeilijke periodes sta je toch altijd klaar om ons te helpen als dat nodig is. 100km rijden om mijn achterstallige berg strijk te komen doen geschiedt met de glimlach, ML en M opvangen, een luisterend oor bieden en een flinke dosis amusement voorzien; niets is teveel gevraagd. Mede dankzij jou (jullie) is dit doctoraat geworden wat het vandaag is en daar kan ik je niet genoeg voor bedanken!

Mijn rotsen in de branding: mama en papa! Eerst en vooral sorry voor het bloed, het zweet en de tranen die ik jullie gekost heb tijdens mijn kleuter-, tiener- en puber-tijd. Vechten om niet naar het oudercontact te moeten gaan, moedeloos thuiskomen en ontvangen worden door een kind dat blijft volhouden dat 'alles goed komt'. Maar hey, had ik gelijk of had ik gelijk? Duizend maal dank om me bij te sturen

waar nodig, maar toch ook vrij te laten in de keuzes die ik wou maken en uiteraard om mij alle kansen te geven! Bedankt om er altijd te zijn voor ML en M, om bij te springen waar nodig en om urenlang mijn klaagzangen te aanhoren (vooral tijdens mijn Leuven-tijd: 'Ik ga effe wenen he, maar eet gerust verder ge moet ni naar mij kijken'). Papa, bedankt voor je bescheiden maar steeds übernuchtere kijk. Ik ben er zeker van dat er niemand is die met nog minder woorden zoveel duidelijk kan maken. Het is er gewoon altijd 'boenk op'! Ookal zijn het vaak niet de zaken die ik op dat moment wil horen. Ik heb enorm veel bewondering voor alles wat je bereikt hebt in je leven en ook voor alles waar je tot op de dag van vandaag nog voor gaat. Hoezo maar ene keer per verlof de Ventoux doen?! Mijn superpapa! Mama, er zijn maar weinig dochters die het geluk hebben dat hun moeder EN een moeder EN een beste vriendin is. Er is niets waarmee ik bij jou niet terecht kan. Wellicht kunnen we vaak met veel minder woorden iets aan elkaar duidelijk maken maar hey wat hindert dat, happytime nemen wij gewoon heel letterlijk. Ik kan nog pagina's vullen maar het boekje is uiteindelijk al dik genoeg dus het volstaat om hier af te ronden met een welgemeende dankjulliewel en een 'ikziejullieongelofelijk graag'!

Fre, schattie, hubbie, ondertussen zijn wij al meer dan 6 jaar samen en ik kan me niet herinneren dat ik mij ooit 1 seconde verveeld heb. Sinds april 2008 staat de funmode in mijn leven permanent aan. Het dagelijks leven met jou en de kids is gewoon DE MAX! Ik hoop dat wij, 2 prettig gestoorde psychologen, nog veel crazy avonturen samen kunnen beleven. Soms (heel soms dan wel) zijn woorden overbodig: Ik hou zielsveel van je!

En dan nu de aller aller aller allerbelangrijkste persoontjes in mijn leven: DE KIDS!

Lieve Marie-Lou en Maurice,

Zonder jullie ... was het zéééér zéker OOK gelukt... haha! Grapje!

Marie-Lou, Louke, Louisie, pippi langkous, mijn mooie lieve prinses: jij bezorgt papa en mij al meer dan 3 jaar topentertainment van de bovenste plank. Mijn liefde voor jou en broer is grenzeloos. Ik hoop dat we nog vaak samen kunnen shaken op K3 en dat ik nog vaak voor jou slaapwel verhaaltjes mag verzinnen. Lieve meid, ik kijk uit naar alles wat nog komt en ben superfier elke dag opnieuw jouw mama te mogen zijn. Love you van hier tot aan de maan en terug!

Maurice, Mr. Mau, Maurizio, broeder Tuc, stoere man van me: vanaf de eerste minuut was ik totaal aan jou verhangen. Jouw grote donkere deugenieten-kijkers doen me elke dag opnieuw beseffen wat écht belangrijk is. Ik hoop dat jij en zus nog vaak apenkuren gaan uithalen en ons huis overhoop gaan zetten - gooi die dvd's maar allemaal door de kamer, haal die kasten maar leeg! Ik kijk er naar uit om ook dan weer samen met papa de uitdaging aan te gaan in het heerlijke opvoedingsavontuur. Want sinds jij in onze leven bent is die uitdaging wel minimaal verdriedubbeld!!! Love you!