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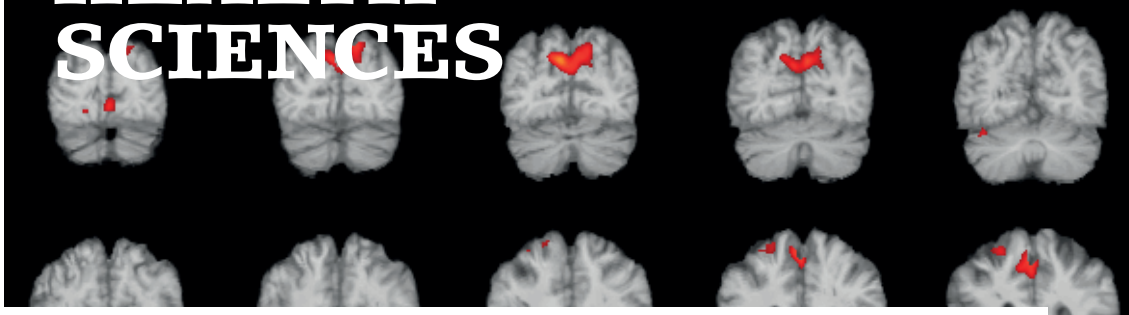
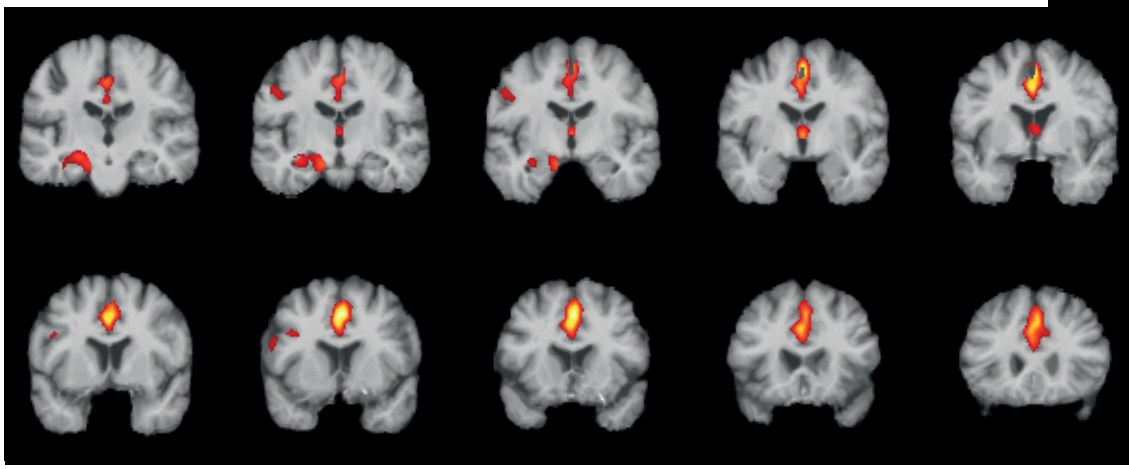


Gabriela Spulber

Imaging the progression from Mild Cognitive Impairment to Alzheimer's disease



Publications of the University of Eastern Finland
Dissertations in Health Sciences

UNIVERSITY OF
EASTERN FINLAND

GABRIELA SPULBER

Imaging the progression from Mild Cognitive Impairment to Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is a common neurodegenerative disorder currently diagnosed in the stage of dementia. There is a pressing need to identify AD at an earlier stage, so that treatment, when available, can begin early.
This thesis focuses on identifying neuroimaging differences between stable and progressive mild cognitive impairment (MCI) subjects prior to clinical conversion to AD. Various MR image analysis techniques were used to achieve suitable parameters for clinical daily practice or to provide additional information for research.
Whole brain atrophy rates were measured from two serial MRI scans. We found that an increase of 1% per additional year post-MCI identification was associated with an increased risk of progressing to AD.
The susceptibility to progressive pathological processes varies between cortical regions, and global atrophy rates overlook region specific changes. Therefore, voxel based morphometry (VBM) was used to investigate regional dynamic of grey matter atrophy. Progressive MCI subjects displayed accelerated regional atrophy as compared to stable MCI already 2 years before clinical conversion, particularly in the frontal, limbic and occipital lobes.
Based on the VBM findings we addressed the issue of possible individual risk classification based on multivariate scores derived from multiple measures (volume and cortical thickness measurements of subjects scanned with aligned MR acquisition protocols) in a single MR scan. Using orthogonal projection to latent structures (OPLS), an individual MR severity index – *the OPLS FreeSurfer based index* - was generated for MCI subjects, which were then classified as AD-, or control-like. The major challenge is to determine optimal cutoff points for such parameters and to compare their reliability, alone or in combination with other biomarkers.
Studies of MCI reported associated pathology in the white matter. Using tract based spatial statistics on diffusion tensor images (DTI) we found decreased fractional anisotropy (FA) in tracts connecting AD affected structures.
Results from this thesis point to the conclusion that structural MRI (T1 weighted and DTI sequences) are integral components for MCI/predementia evaluation. These markers support earlier and more precise diagnosis and assessment of progression. Standardized MR acquisition protocols and efficient quality control will empower the role of MRI for the benefit of the patient.

National Library of Medical Classification: WT 155, WL 141.5.M2, QZ 180
Medical Subject Headings: Alzheimer's Disease; Neuroimaging; Mild Cognitive Impairment; Magnetic Resonance Imaging; Atrophy

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TIIVISTELMÄ

Alzheimerin tauti (AT) on yleinen hermostorappeumasairaus, joka nykyisin tunnistetaan usein vasta dementiavaiheessa. Varhaisempi diagnoosi mahdollistaisi hoidon aiemman aloittamisen.
Tässä väitöskirjatyössä tarkasteltiin aivojen rakenteellisia muutoksia stabiilia ja etenevää lievää kognitiivisen tason heikkenemistä sairastavilla henkilöillä ennen heidän sairastumistaan Alzheimerin tautiin. Tutkimuksessa käytettiin aivojen magneettikuvien (MRI) automaattisia analyysimenetelmiä ja pyrittiin kehittämään mittareita, joita voidaan hyödyntää kliinisessä työssä tai tutkimuksessa.
Koko aivojen atrofian, aivokudoksen surkastumisen, etenemistä mitattiin toistetuilla MRI-tutkimuksilla. Henkilöt, joilla aivojen atrofia lisääntyi yhden prosentin jokaisena kognitiivisen tason alentuman toteamisen jälkeisenä vuonna, sairastuivat todennäköisemmin AT:iin.
Alttius patologisiin muutoksiin vaihtelee aivokuoren eri alueilla, mutta koko aivojen atrofiaa arvioidessa nämä alueelliset muutokset jäävät usein huomioimatta. Alzheimerin tautia edeltäviä harmaan aineen alueellisia muutoksia tarkasteltiin magneettikuvien vokselipohjaisella analyysillä. Etenevää kognitiivista heikkenemistä sairastavilla atrofia oli erityisen voimakasta limbisessä lohkossa sekä otsa- ja takaraivolohkoissa. Nämä muutokset olivat havaittavissa jopa kaksi vuotta ennen AT-diagnoosia.
Vokselianalyysi kykenee kuitenkin tunnistamaan erot ainoastaan ryhmätasolla. Yksilötason riskin arvioimiseksi MRI:lla saatujen tilavuus- ja aivokuoren paksuusmittausten pohjalta kehitettiin muutosten vakavuutta arvioiva monimuuttujamittari. Useiden alueiden samanaikainen tarkastelun avulla etenevää ja stabiilia kognitiivista alenemaa sairastavat henkilöt pystyttiin tunnistamaan luotettavasti. Optimaalisten luokittelurajojen määrittäminen on kuitenkin haasteellista ja menetelmän luotettavuuden arviointi tärkeää.
Valkean aineen rakennetta tarkasteltiin diffuusiotensorikuvien tilastollisella analyysilla. Todettiin, että terveisiin henkilöihin verrattuna lievää kognitiivisen tason heikkenemistä ja Alzheimerin tautia sairastavilla esiintyy muutoksia myös ratayhteyksissä niiden aivoalueiden välillä, joiden tiedetään vaurioituvan AT:ssä.
Tutkimustulosten perusteella rakenteellisen MRI:n automaattiset analyysimenetelmät soveltuvat dementiaa edeltävän kognitiivisen tason aleneman arviointiin. Nämä menetelmät mahdollistavat AT:n aiemman ja tarkemman diagnosoinnin. Kuvantamis- ja analyysiprotokollien standardisointi on kuitenkin tärkeää, jotta menetelmiä voidaan hyödyntää potilastyössä.

Luokitus: WT 155, WL 141.5.M2, QZ 180
Yleinen Suomalainen asiasanasto: Alzheimerin tauti; magneettitutkimus; atrofia; kuvantaminen – lääketiede

To my grandparents

You will never be forgotten

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Kuopio, April 2012

Gabriela Spulber

List of the original publications

This dissertation is based on the following original publications:

I Spulber G, Niskanen E, Macdonald S, Smilovici O, Chen K, Reiman EM, Jauihianen AM, Hallikainen M, Tervo S, Wahlund LO, Vanninen R, Kivipelto M, Soininen H. Whole brain atrophy rate predicts progression from MCI to Alzheimer's disease. *Neurobiology of ageing, 31:1601-5, 2010.*

II Spulber G, Niskanen E, Macdonald S, Kivipelto M, Padilla D, Julkunen V, Hallikainen M, Vanninen R, Wahlund LO, Soininen H. Evolution of global and local grey matter atrophy on serial MRI scans during the progression from MCI to AD. *Current Alzheimer Research 2012 [Epub ahead of print]*

III Spulber G, Muehlboeck JS, Mecocci P, Vellas B, Tsolaki M, Kłoszewska I, Soininen H, Spenger C, Lovestone S, Simmons A, Wahlund LO and Westman E. An MRI-based index measuring the severity of Alzheimer's disease-like pattern of structural brain changes in MCI subjects. Submitted for publication.

IV Liu Y, Spulber G, Lehtimäki KK, Könönen M, Hallikainen I, Gröhn H, Kivipelto M, Hallikainen M, Vanninen R, Soininen H. Diffusion tensor imaging and Tract-Based Spatial Statistics in Alzheimer's disease and mild cognitive impairment. *Neurobiology of ageing, 32:1558-71, 2011.*

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Contents

Abbreviations

ACC	anterior cingulate cortex
AChEI	acetylcholinesterase inhibitors
AD	Alzheimer's disease
aMCI	amnestic mild cognitive impairment
APP	amyloid precursor protein
Aβ42	Aβ peptide
BPF	brain parenchymal fraction
CC	corpus callosum
CSF	cerebrospinal fluid
CTL	healthy controls
DMN	default mode network
DTI	diffusion tensor imaging
DW	diffusion weighted
FA	fractional anisotropy
FAD	familial Alzheimer's disease
FLAIR	fluid attenuation inversion recovery
^{1}H MRS	Proton magnetic resonance spectroscopy
IPCA	iterative principal component analysis
MCI	mild cognitive impairment
MD	mean diffusivity
mPFC	medial prefrontal cortex
MRI	magnetic resonance imaging
MTL	medial temporal lobe
NFT	neurofibrillary tangles
NMDA	N-methyl-D-aspartate
NMR	nuclear magnetic resonance
OPLS	orthogonal projection to latent structures
PC	principal component
PCC	posterior cingulate cortex
PiB	Pittsburgh compound B
P-Tau	phosphorylated Tau
QC	quality control
ROI	region of interest
TBSS	tract based spatial statistics
TE	echo time
TIV	total intracranial volume
TR	repetition time
T-Tau	total Tau
VBM	voxel based morphometry
WM	white matter
WMH	white matter hyperintensities

1 Introduction

When my 79-year-old grandmother didn't remember my mom's second name, I got worried; but many around her didn't. She was just old.
The single most important risk factor for cognitive decline and AD is age itself. When should cognitive changes be of concern in old people? Can we visualise anatomical alterations behind memory complains?

1.1 BECOMING OLD

Ageing is "a fundamental biological process that can be defined, measured, described and manipulated" (Arking 2006). It is one of the most complex biological processes, whose definition is intrinsically related to its phenotype. It is familiar to us all, but defining it is not straightforward.
Although it is a universal human experience, ageing was formally studied for the first time by Muhammad ibn Yusuf al-Harawi in his book "Ainul Hayat", in 1532. In the book, one can find, amazingly, how the author discussed 500 years ago all types of behavioural and lifestyle factors (including diet, environment and housing conditions) in relation to ageing.
Ageing has been defined as *"a progressive, generalized impairment of function resulting in a loss of adaptive response to stress and in growing risk of age associated disease"* (Kirkwood 1996), and ultimately renders human beings progressively more likely to die. This process is strongly influenced by environment, lifestyle, and diseases.
In conclusion, ageing is a progressive deterioration of physiological functions, an intrinsic age-related process of loss of viability and increase in vulnerability, which implies:

- physiological changes that typically lead to a functional decline
- increasing susceptibility to diseases
- an exponential increase in mortality

1.2 THE FRAMEWORK AND FOCUS OF THE THESIS

Cognitive frailty is emerging as one of the greatest health threats of the twenty-first century, and ageing of populations is no longer an isolated concern of economically developed countries. The prevalence of dementia is constantly growing as the longevity of the population increases. Rapid demographic ageing and its accompanying set of chronic illnesses has come to characterize many developing countries, too. Of an estimated 25 million people with Alzheimer's disease (AD) worldwide in 2000, 13 million lived in less developed regions (Wimo *et al.* 2003).
The current AD treatment protocols are restricted to symptomatic relief, and hardly have an effect on the course of the disease (Mangialasche *et al.* 2010). In addition, the current diagnostic criteria (DSM-IV, ICD-10 and NINCDS-ADRDA) identify patients with AD who have overt dementia and correspond to neuropathlogically advanced disease, leaving little room for therapeutic interventions. Therefore, the research focus has shifted towards increasing the accuracy of detecting earlier phases of the disease in order to allow earlier

therapeutic intervention. Indeed, it is estimated that interventions that could delay the clinical onset of dementia by 1 year would reduce the prevalence in 2050 by 9 million cases (Brookmeyer *et al.* 2007).

Distinguishing preclinical AD from the changes attributable to normal ageing is still a challenge. The concept of mild cognitive impairment (MCI) introduced by the Mayo Clinic (Petersen *et al.* 1999) has therefore rightfully received significant attention. Although the definition varies across studies, and the variable clinical course for MCI subjects (Palmer *et al.* 2002) likely reflects different underlying causes, MCI subjects progress to AD with consistent annual rates of 10-15% (Mariani *et al.* 2007). Basic and clinical research advances over the past decades have provided detailed knowledge of the molecular mechanisms and clinical course of AD, and the diagnostic criteria should be modified accordingly. A recent proposal for updating the criteria for AD diagnosis included a number of biomarkers (autosomal dominant genes, imaging, cerebrospinal fluid (CSF)) to be used as supportive features in the diagnostic workflow (Dubois *et al.* 2007). Moreover, there have been proposed special diagnosis criteria for preclinical stages of AD and for clinical dementia due to AD (Albert *et al.* 2011, McKhann *et al.* 2011).

An increasing number of studies show that structural MRI estimates of alterations in brain regions vulnerable to AD pathology are predictive of progression from MCI to AD (Liu *et al.* 2010, Risacher *et al.* 2009), and structural MRI has become an integral part of the clinical assessment of patients with dementia.

The studies included in this thesis investigate the imaging correlates of progression from MCI to AD. To this end we used structural MRI (T1-weghted images) and DTI in both clinical, and population based settings.

2 Review of the literature

2.1 MEASURING CHANGES CAUSED BY DISEASES

"When you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of meager and unsatisfactory kind: it may be the beginning of knowledge, but you have scarcely, in your thoughts, advanced to the stage of science, whatever the matter may be" (Lord Kelvin, 1883).

The benefits of quantification are that fundamental research into biological changes caused by disease and their response to treatments can proceed in a more satisfactory way. A wide range of features can be assessed whether they lie in a normal range, and whether they have changed from the time of previous examination (Parker and Chard 2003). On the other hand, good quantification requires attention to both *data collection* and *analysis* techniques. "*The biological question to be answered and thus the bio-physical feature to be measured need very careful choice after discussion by all concerned*" (Tofts and du Boulay 1990).

Developing measurement techniques for MR images constitutes a perfect application of traditional scientific skills to a modern problem. MRI is now widespread, and accepted as the method of choice for imaging the brain. MRI is used as a scientific instrument to make measurements of clinically relevant quantities.

2.1.1 MRI principles

MRI is an imaging technique used primarily in the clinical setting in order to produce high quality images of the inside of the human body (Figure 2.1). The main roles of imaging in clinical neurological practice are firstly to identify (or exclude) an abnormality in a given patient relative to normal subjects; secondly to monitor an abnormality in a single patient over time; and thirdly to characterize disease structural phenotypes across groups of patients. The advances in medical imaging have demanded innovative image processing and analysis tools in order to assimilate large data sets and ultimately generate clinically relevant information.

MRI is based on nuclear magnetic resonance (NMR), the physical phenomenon of absorption (excitation) and emission (relaxation) of electromagnetic radiation from nuclei placed in a strong external magnetic field. Felix Bloch and Edward Purcell were awarded the Nobel Prize in 1952 for discovering the magnetic resonance phenomenon, and Paul C. Lauterbur and Sir Peter Mansfield were awarded the Nobel Prize for Medicine or Physiology *"for their discoveries concerning magnetic resonance imaging"* in 2003 (www.nobelprize.org).

MRI is based on the NMR signals from hydrogen nuclei (protons). The human body is primarily composed of fat and water, which have many hydrogen atoms (*e.g.* body fluids contain over 95% water). When the human body is placed in the magnetic field inside the scanner, the magnetic fields of the spinning protons align parallel to the external magnetic field (*magnetization* in Figure 2.1). Because the static longitudinal magnetization cannot be measured, the protons are excited using a radio frequency (RF) electromagnetic pulse

which pushes the magnetic fields of individual protons out of alignment (*excitation* in Figure 2.1). After the RF pulse, the magnetization gradually returns to equilibrium (*relaxation* in Figure 2.1). During the relaxation process, the protons emit electromagnetic waves in the RF spectrum. The signal detected by the receiver coil is called Free Induction Decay (FID) and is the summation of waves of different frequencies, amplitudes and phases. The spectral features (frequency and amplitude) depend on the proton density of the tissue and on the strength of the external magnetic field. Using a Fourier transform, the signal (in the time domain) is converted to spectrum (in the frequency domain). The Fourier transform resembles a musician hearing a tone (time domain signal) and determining what note (frequency) is being played.

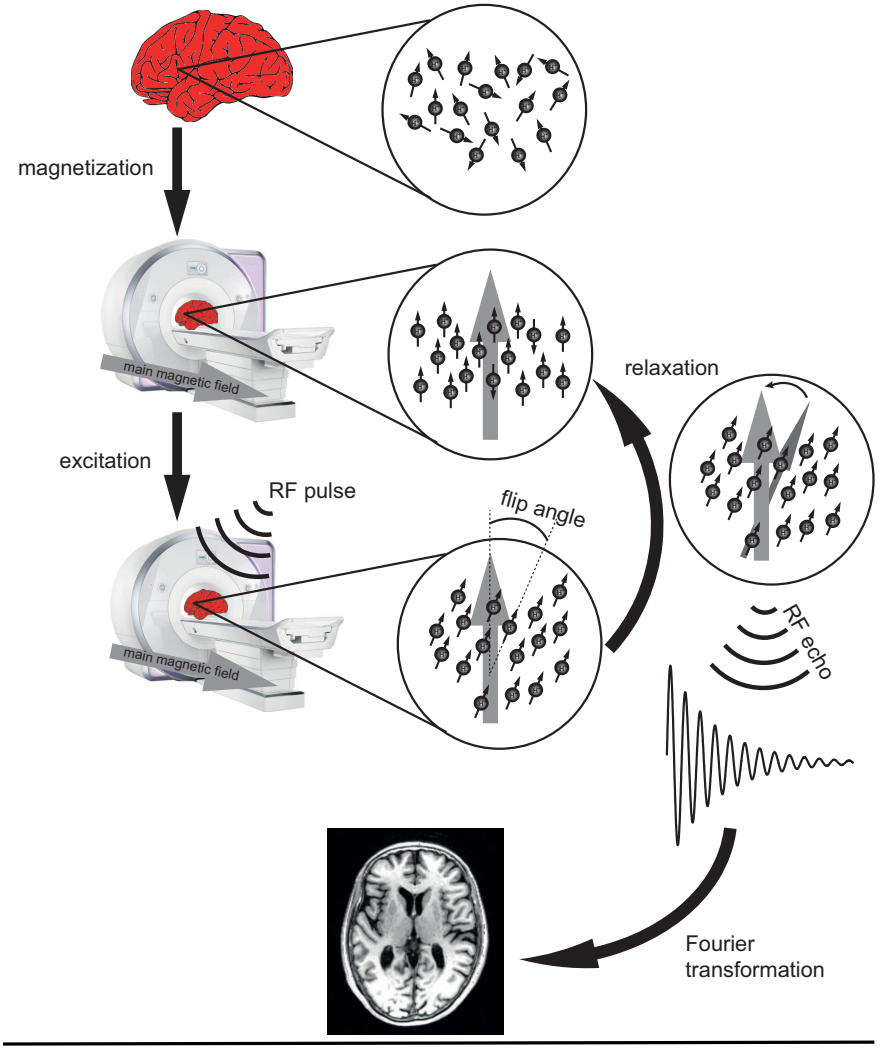


Figure 2.1 Principles of magnetic resonance imaging (MRI)

The magnetization returns to equilibrium *via* two relaxation processes measured by the T1 and T2 relaxation times, which are characteristic for each tissue. The RF signal that comes back from the patient is collected as an echo by the receiver coil. Relaxation times can be measured using special pulse sequences created by varying the repetition (TR) and echo

(TE) times, where TR is the time between two RF excitation pulses and TE is the time between the RF pulse and the recorded echo.

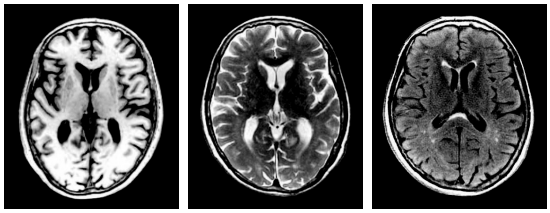
Table 2.1 T1 and T2 values for common brain tissues at 1.5T (Sled and Pike 2001, Stanisz *et al.* 2005)

Tissue	T_1 (ms)	T_2 (ms)
CSF	2400	160
White	780	90
Gray	920	100
Fat	250	80

2.1.2 Basic MRI sequences

The intrinsic properties of human tissues (proton density, T1, and T2 relaxation times), provide ways of creating contrast in the acquired images.

T1-weighted images are often known as "anatomy scans". They require short TR and short TE, and the contrast is excellent: fluids are very dark, fat-rich tissues are very bright, while water-rich tissues are mid-grey.



T1-weighted T2-weighted FLAIR

Figure 2.2 Basic MR contrasts. Note the difference in appearance between liquid and brain parenchyma.

T2-weighted images are often known as "pathology" scans. These scans require short TR and long TE; fluids are very bright, and water- and fat-based tissues are mid-grey

T2-weighted images with suppressed CSF signal are known as FLAIR sequences (Fluid Attenuated Inversion Recovery) and are used for detecting lesions in the white matter (Figure 2.2; see also Figure 2.4).

2.1.3 Diffusion tensor imaging

Diffusion tensor imaging (DTI) permits the interrogation of the brain's microstructure, allowing for a more refined characterization of white matter (WM) and the complex network of nerve fibers connecting different brain areas. DTI measures the translational displacement of water molecules. Water molecules' motion or diffusion was found to be much faster along the WM fibers than perpendicular to them (Basser and Pierpaoli 1996, Basser and Pierpaoli 1998) and the difference between these two motions (parallel and perpendicular to the fibers, also termed diffusion anisotropy) is the basis of DTI. The most frequently used DTI summary measures are mean diffusivity (MD) and fractional anisotropy (FA). FA is near 0 in ventricular CSF but can approach 1 in callosal regions where fibers are arranged parallel. WM with high structural integrity will typically put a greater degree of directional restriction on diffusion. Therefore, higher FA is conventionally interpreted as preserved integrity of WM (Pierpaoli *et al.* 1996).

Diffusion weighted (DW) contrast behaves like an inverse T2-weighted: very watery tissues have very mobile molecules that give little signal while more solid and static tissues give a stronger signal. Fractional anisotropy and mean diffusivity are frequently employed parameters from DTI acquisitions as measures of fiber tract integrity. FA describes the directionality of fiber tracts while MD determines the overall diffusivity (see Figure 2.3).

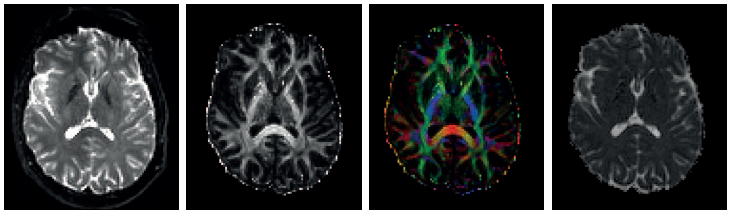


Figure 2.3 Diffusion-weighted MR images and main DTI metrics (fractional anisotropy and mean diffusivity). Courtesy of Amirhossein Manzouri, SMILE, Karolinska Institutet.

2.1.4 Image analysis principles

For research purposes, the analysis of MR images is a complex process involving a number of specific, sequential steps.

1) Aligned acquisition MRI protocols.
2) Comprehensive quality control (QC), by both scanning of phantoms and by clear QC protocols for subject scanning. Image QC should be done immediately after the images are acquired to avoiding recalling the patients for MRI scanning if there were problems on the first attempt.
3) On-site inspection of the images by a radiologist in order to exclude potential serious pathology.
4) Image registration (intramodality for the same sequence or intermodality for different sequences) allows the alignment of two or more images into the same geometric space. In order to make within-subject or between-subject comparisons in a meaningful way, images need to be in the same anatomical framework.
5) Skull stripping using different brain extraction procedures.
6) Intensity non-uniformity correction.
7) Segmentation (manual, semi-automated, automated procedures).
8) Postprocessing QC.

Brain tissues may broadly be divided into white matter (WM) and grey matter (GM) volumes. The spatial relationship between WM, GM and CSF are complex and the relative signal intensities vary across the brain.

- GM/WM contrast is less sharp than brain/CSF contrast and is strongly dependent upon acquisition parameters.
- The border between GM and WM is not necessarily truly distinct, and may be blurred due to partial volume effect.
- Spatially varying imaging hardware characteristics (non-uniformity) cause artefactual fluctuations in signal intensity and contrast.

These factors make the task of GM and WM segmentation rather complex and it is therefore not surprising that different methods output different results (Parker and Chard 2003). This

variability highlights the difficulty in measuring brain volumes. The use of probabilistic atlases can improve the confidence of segmentation into specific tissue classes.
The brain may be divided into anatomically defined regions rather than tissue specific regions. Localized measures have the potential to offer more direct insight into the relationship between tissue damage and clinical impairment. Specific measures may be more sensitive to evolving pathology throughout the course of a given disease process.
Regional estimates can be done using manual, semiautomatic, or automated segmentation procedures. All methods are prone to different type of errors. Manual outlining procedures are time consuming and prone to rater subjectivity. Automated methods are often rapid and reproducible, but suffer from the fact that standard templates cope poorly in the presence of significant abnormalities.
Brain MRI measurements vary across methods, meaning that there is no gold standard against which to validate measurement accuracy. The lack of compatibility between methods results in a lack of compatibility between studies performed with different methodologies. Care should be used when interpreting results of any study with respect to literature values of atrophy rates or absolute volume differences between groups.

2.2 THE AGEING BRAIN

The advent of MRI provided the opportunity to observe and monitor age-related changes in the human brain. However, recognition of profound gross anatomical differences between younger and older brains was accompanied by the realization of the extent of individual differences within narrow age ranges. Thus, normal ageing is accompanied by profound global and regional changes in the brain, structurally as well as functionally.

2.2.1 Structural brain changes

The ageing brain decreases in volume, though not uniformly across regions. Cumulative records of post-mortem studies of the ageing brain indicate a moderate linear reduction in the gross weight and volume of about 2% per decade (Kemper 1994). Early computer tomography (CT) studies concluded that normal ageing is associated with ventricular expansion and enlargement of the cerebral sulci (Stafford *et al.* 1988) and more recently MRI volumetry confirmed the age-related reduction of cerebral volume (Blatter *et al.* 1995).
GM consists of neuronal cell bodies in the cortex and in the subcortical nuclei, while WM is made of myelinated axonal tracts. Therefore, GM and WM exhibit different vulnerability to alterations due to *e.g.* ageing (Pantoni and Garcia 1997).
The question of differential effects of age on GM *vs.* WM was raised by early post-mortem investigations (Miller *et al.* 1980). Miller *et al.* concluded that WM is more vulnerable to ageing than GM. This results were revised in several *in vivo* studies that found a linear decline for GM, while WM is following a more sinuous trajectory (Courchesne *et al.* 2000, Pfefferbaum *et al.* 1994, Sullivan *et al.* 2004).
GM volume peaks at about 4 years and thereafter declines steadily through the life span with approximately 5% per decade. In contrast, WM volume shows an initial increase until early 20s, followed by a plateau stretching into the 60s. A consistent decline was reported only in the oldest old (Courchesne *et al.* 2000).
However, because of the greater number of anatomically diverse components involved, the landscape of age-related differences is far more complicated than a contrast between global

GM and WM volumes. The CSF space, for instance, is expanding continuously, and this is possible on the expense of shrinking brain parenchyma during ageing.

Selective brain structures and regions display different patterns of changes with ageing. Thus, converging data indicate an excessive vulnerability of the prefrontal cortex (Haug and Eggers 1991, Raz *et al.* 2004, Resnick *et al.* 2003, Tang *et al.* 2001).

The hippocampus and the entorhinal cortex, structures located in the medial temporal lobe, are involved in episodic memory, a faculty that declines with age (Verhaeghen *et al.* 1993). Although the hippocampus is a focal point of multiple pathological events associated with age and age-related diseases, its involvement in normal ageing is uncertain. In contrast, the grey matter of the temporal lobe does decrease with age (Sullivan *et al.* 1995), and this has been mistakenly interpreted as hippocampal volume decline. The relative resilience of the hippocampal volume to ageing makes hippocampal size deviations sensitive indicators for pathology (Jack *et al.* 2005). Thus, cross-sectional and longitudinal studies (Morrison and Hof 1997, Persson *et al.* 2006, Raz *et al.* 1998) linked hippocampal atrophy with memory loss in normal ageing, but the magnitude of these effects are mild to moderate in range (Sullivan *et al.* 2005).

All studies vary in methodological make-up (*e.g.* MR acquisition protocol, image analysis method, region of interest (ROI) definition, correction for head size, sample characteristics), and these are important aspects. For example, the definition of the hippocampal ROI may be related to the age effect size. When the anterior hippocampus is not measured, the results point to an age-related decline in the hippocampal volume (Laakso *et al.* 2000). Given that the anterior and the posterior parts of the hippocampus are dealing with different mnemonic operations, the border definition rules need to be taken into account (Gabrieli *et al.* 1997). This heterogeneity prevents a direct comparison of the outcome of different studies, and slows down the transfer of the marker from the research laboratory to the clinical setting, and efforts are made to standardize the manual hippocampal volumetry (Boccardi *et al.* 2011).

Similarly to the hippocampus, the surrounding entorhinal cortex remains relatively unaffected by the normal ageing process (Insausti *et al.* 1998).

Age effects on the basal ganglia and the diencephalon are moderate (Raz *et al.* 2003). The caudate is the only structure where all the relevant studies are in coherence, showing a linear negative relationship with age (Walhovd *et al.* 2005).

Walhovd *et al.* showed age effects at a global level, but with substantial differences in the amount of variance explained by age (Walhovd *et al.* 2011). For this study, all structures were significantly affected by age when the whole sample (883 subjects) was used, indicating that when statistical power is sufficiently high, age effects are observed throughout the human brain.

The integrity of cerebral WM is critical for efficient cognitive functioning, and Norman Geschwind revolutionized neuroscience research by proposing the disconnection's theory (Geschwind 1965, Geschwind 1965). He asserted that disconnection syndromes resulting from WM lesions could underlie deficits in higher-order functions, thereby advancing the idea that disconnection of GM regions, by interrupting the communication between them, could be as disruptive as trauma to those regions per se.

While the presence and degree of age-related volume loss in cerebral WM remains somewhat inconsistent, the frequent presence of foci of increased signal on MRI scans of older adults is a well-established phenomenon. WM hyperintensities (WMH) are areas of

increased intensity appearing on T2 and FLAIR images and are interpreted as WM damage. WMH can be located in different brain regions, may be discrete, punctuate, confluent and may reflect multiple pathological causes, vascular and neural (Pantoni and Garcia 1997). The presence of WMH is common among normal elderly adults and age appears to be the strongest predictor of severity (Brickman *et al.* 2008). Additional risk factors include hypertension, hypercholesterolemia, and cardiovascular disease (Liao *et al.* 1996, Liao *et al.* 1997, Manolio *et al.* 1994).

Research over the past decades has associated WMH with cognitive deficits (Gunning-Dixon and Raz 2000), physical disability (Sakakibara *et al.* 1999), gait abnormality (Starr *et al.* 2003) and psychiatric disorders such as depression (Steffens *et al.* 2002). Regarding specifically the cognitive deficits, a number of studies (Gunning-Dixon and Raz 2000, Gunning-Dixon and Raz 2003, Raz and Rodrigue 2006) have established that WMH burden (*i.e.* volume or severity) is negatively associated with performance across a range of cognitive tests, particularly those involving executive functioning, processing speed, and attention.

Despite fairly consistent findings, the associations between age and WMH volume and between WMH and cognitive function are imperfect. The severity of WMH varies in older adults as does the amount of cognitive dysfunction associated with the degree of WMH. Post-mortem studies of WMH histology suggest that WM abnormalities reflect a number of pathological processes, but the existing volumetric methodology prevents reliable discrimination among mechanisms. Recent advances in segmentation methods using support vector machines will improve the automated identification of WMH and will make results replicable between studies (see Figure 2.4).

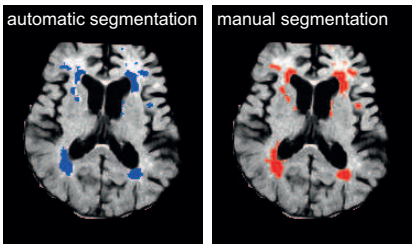


Figure 2.4 FLAIR-overlaid WMH automatic segmentation using a cascade of reduced support vector machine algorithm. Note the accuracy of segmentation (comparable with manual segmentation). Courtesy of Soheil Damangir, Karolinska Institutet

Age-related differences as measured by DTI occur both in the overall measures of WM integrity (*e.g.* FA and MD) and in component measures (*e.g.* axial and radial diffusivity). The anterior-posterior gradient of the decline in WM integrity is characteristic with increasing age.

One of the most robust findings describing age-related differences in regional FA has been a distribution of low FA selective to frontal WM (Salat *et al.* 2005, Sullivan *et al.* 2001). With ageing FA declines and MD increases (Head *et al.* 2004, Pfefferbaum and Sullivan 2003, Pfefferbaum *et al.* 2005). The magnitude of the FA-MD relationship varies across brain regions and is greater in older than in younger healthy individuals (Pfefferbaum and Sullivan 2003).

The pattern of age-related DTI changes may be related to age-related changes in WM demonstrated in post-mortem studies of ageing: change in the axon's cytoskeleton, reduction in axon density, decline in number and length of myelinated fibers, breakdown in the myelin sheets (Aboitiz *et al.* 1996, Bartzokis *et al.* 2004, Marner *et al.* 2003, Meier-Ruge *et al.* 1992).

DTI studies reviewed to this point confirm the role of disconnection among distributed neural systems as a fundamental mechanism of age-related variability in cognitive performance.

2.2.2 Pathological findings in normal ageing

The accumulation of pathology during normal ageing may play a role in mediating the cognitive impairment observed in the elderly.

Until recently the general belief was that age-related cognitive impairment was an inevitable result of age-related neuron loss (Brody 1955, Coleman and Flood 1987, Colon 1972). However, due to developments in counting procedures, and stereological techniques, a different picture has emerged. Specifically, it now appears that while there is limited neuron loss within restricted regions of the hippocampal gyri, there is no evidence for widespread neuron loss during normal brain ageing (Morrison and Hof 1997, Pakkenberg and Gundersen 1997, Rapp and Gallagher 1996).

If studies about age related changes in the number of neurons have yielded controversial findings, there is more agreement about neuronal size. This has been found to decrease modestly with age, particularly in the cerebral cortex (Anderson *et al.* 1983, Meier-Ruge *et al.* 1980, Terry *et al.* 1987). Studies of synapses have shown an overall decrease with age, although the branching patterns of dendrites suggest that there may be compensatory increase in some dendrites to make up for loss of others (Buell and Coleman 1981).

Normal ageing harbours a number of pathological features, including corpora amylacea (Mrak *et al.* 1995), argyrophilic grains (Tolnay and Clavaguera 2004), neuromelanin (Zecca *et al.* 2003), lipofuscin (Keller *et al.* 2004), as well as significant accumulation of iron (Zecca *et al.* 2004).

The AD-like pathology is found in a large number of healthy elderly. The AD brains possess two main pathological features: the extracellular deposition of amyloid β -protein (Aβ) and the intraneuronal generation of neurofibrillary tangles, both required for a definite AD diagnosis.

Extracellular Aβ deposits consist of aggregated Aβ and are located in the GM and, to a lesser extent, in adjoining portions of the WM. The main components of plaques are the 40 to 42 amino acid Aβ-peptides, (Mattson 1997, Mattson 2004). Aβ-peptides are generated through proteolytic cleavage from the larger amyloid precursor protein, with γ- and β-secretase as key enzymes in the cleavage process.

Neurofibrillary tangles are abnormal intracellular structures that contain paired helical filaments and straight filaments, both consisting of abnormally phosphorylated and aggregated tau protein.

The difference between healthy and AD individuals with AD-related pathology is reflected by the distribution pattern of neurofibrillary tangles and Aβ plaques. In healthy elderly, neurofibrillary tangles and Aβ deposits are restricted to distinct predilection sites (plaques: neocortex, allocortex, basal ganglia and diencephalic nuclei; neurofibrillary tangles: entorhinal and limbic areas) (Arriagada *et al.* 1992, Braak and Braak 1991, Price *et al.* 1991), whereas in AD patients, these lesions are much more widespread and occur in many areas of the brain (Braak and Braak 1991).

Depending on which neuropathological criteria are used for the postmortem diagnosis of AD (Braak and Braak 1997, Khachaturian 1985, Mirra *et al.* 1991), dementia free subjects can have levels of pathology sufficient for a diagnosis of likely or probable AD. AD-related

pathology occur in 81.5% of all individuals aged over 55 (Braak and Braak 1997), and the frequency of higher neurofibrillary tangles and plaque stages increases with age (Braak and Braak 1997). These studies highlight the potential importance of AD-related pathology to age-related declines in cognitive performance observed in the elderly.

2.3 ALZHEIMER'S DISEASE

2.3.1 Dementia as a syndrome

The term dementia (from the Latin *demens, dementis,* "out of one's mind") describes a serious loss of cognitive abilities, beyond what might be expected from normal ageing. Symptoms of dementia may include loss of memory, mood changes and communication problems. From its beginnings the concept of dementia has been synonymous with madness; these two words are deeply rooted in the common language of many cultures where the word *dementia* continues to carry the connotation of insanity.

According to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV-TR) (American Psychiatric Association, 2000) the essential feature of dementia is the development of multiple cognitive deficits that include memory impairment and at least one of the following cognitive disturbances: aphasia, apraxia, agnosia, or a disturbance in executive functioning. The cognitive deficits must be sufficiently severe to cause impairment in occupational or social functioning and must represent a decline from a previously higher level of functioning. The dementia diagnosis should not be done if cognitive impairment is present only when the subject experiences delirium. The etiology of dementia is diverse, originating from either damage or disease in the brain. It must develop during adulthood, and should be differentiated from psychiatric illnesses and mental disability.

Dementia mainly affects older people, although there is a growing awareness of cases that start before the age of 65. After age 65, the likelihood of developing dementia roughly doubles every five years (Qiu *et al.* 2007). In the 2010 World Alzheimer Report, Alzheimer's Disease International estimated that there were 35.6 million people living with dementia worldwide in 2010, increasing to 65.7 million by 2030 and to 115.4 million by 2050 (http://www.alz.co.uk/research/worldreport/).

The proportion of people aged over 60 years is growing faster than any other age group in most countries as a result of longer life expectancy and declining birth rates. This population ageing can be regarded as a success story for public health policies and for socioeconomic development, but it also challenges society to adapt, in order to maximize the health and functional capacity of older people as well as their social participation and security. Along with the positive trend, however, come special health challenges for the 21st century. Preparing health providers and societies to meet the needs of elderly people is essential: training for health professionals on old age care; preventing and managing age-associated chronic diseases; designing sustainable policies on long-term care; and developing age-friendly services and settings.

People with dementia, their families and friends are affected on personal, emotional, financial and social levels. Lack of awareness is a global problem. A proper understanding of the societal costs of dementia, the impact on families, health and social care services may help to address this problem.

AD is the most common form of dementia. Age is the most important risk factor for sporadic AD, but genetic and lifestyle risk factors may influence the development of AD. Familial AD (FAD) account for only about 5% of the AD cases and often have an early onset, generally before the age of 65. Late-onset (after 65 years of age) is typical for sporadic cases, but is also found among FAD cases.

2.3.2 Diagnostic criteria

Currently, AD is clinically diagnosed as probable or possible AD based on the presence of progressive cognitive impairment of sufficient severity to interfere with activities of daily living, and by the absence of other neurological conditions that could account for the observed impairment. A definitive diagnosis is available only through brain biopsy or postmortem examination, based on the histopathological verification of the presence of Aβ plaques and neurofibrillary tangles (NFTs).

For research purposes, the diagnosis of AD is based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV-TR) and the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) working group (McKhann *et al.* 1984). These accepted criteria are fulfilled in a two-step diagnostic process where there is an initial identification of a dementia syndrome and then the application of criteria based on the clinical features of the AD phenotype. The currently accepted criteria support a probabilistic diagnosis of AD within a clinical context where there is no definitive diagnostic biomarker. According to the NINCDS-ADRDA criteria, a definite diagnosis of AD is only possible when there is histopathological confirmation of the clinical diagnosis.

Since the publication of the NINCDS-ADRDA criteria in 1984 there was an enormous, unprecedented growth in the scientific knowledge regarding the biological basis of AD. Rapidly accumulating evidence indicates that AD is associated with specific patterns of cognitive impairment, abnormal levels of CSF biomarkers, increased cortical Aβ binding, and structural and metabolic changes in the brain. One or more of these biomarkers could be used to help earlier detection of AD.

The National Institute on Ageing and the Alzheimer's Association charged a workgroup with the task of revising the 1984 criteria for AD and the revised criteria have been published in 2011 (McKhann *et al.* 2011). The new recommendations still have the clinical criteria as the core of the diagnosis in the clinical practice, but biomarker evidence is expected to enhance the specificity of the diagnosis of AD dementia.

2.3.3 Neuropathological diagnosis

The pathology underlying AD is by no means uniform and diffuse. The distribution pattern of changes is specific to the area, lamina and even cell type.

The most conspicuous change is the progressive deposition of abnormal proteins, between and within the neurons. The extracellular deposits consist of Aβ protein, whereas abnormal tau protein contributes to the formation of the intraneuronal neurofibrillary changes (Figure 2.5).

Pathological phosphorylation of tau proteins leads to a sequence of events that result in *neurofibrillary changes*. Neurofibrillary tangles, neuropil threads, and components of neuritic plaques are all forms of neurofibrillary changes. The first neurofibrillary changes develop in the transentorhinal region of the medial temporal lobe. The destructive process follows a

predictable pattern as it spreads into the limbic area before appearing in widespread areas of association cortex, with sparing of primary sensory and motor areas until later disease stages (Braak and Braak 1991, Braak *et al.* 2006). This pattern is subject to only minor inter-individual variation and thus provides a basis for distinguishing six stages in the evolution of the lesions (the clinically silent transentorhinal stages I and II, the limbic stages III and IV, (onset of clinical symptoms), and the neocortical stages V and VI, corresponding to fully developed AD)) (Braak and Braak 1991, Braak and Braak 1991). The tau pathology in AD correlates with disease stages and appears necessary for the clinical expression of the disease. Currently, postmortem diagnosis of AD rests on establishing proof of the presence of neurofibrillary changes.

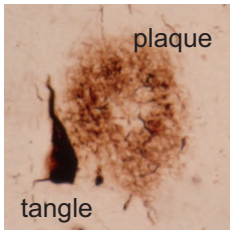


Figure 2.5 Characteristic AD pathology. Bielschowsky silver staining. Courtesy of Dr. Nenad Bogdanovic, Karolinska Institutet

Extracellular amyloid deposits occur at a few predictable sites with three stages in the development of deposits. Typically, the Aβ plaques first appear in the basal neocortex (stage A) as patches with ill-defined borders. The patches spread into the adjoining neocortical areas and the hippocampal formation in stage B and to the primary neocortex in stage C. The evolution and distribution patterns of Aβ plaques do not correspond to disease stage and are not specific for AD.

According to some studies tau pathology precedes amyloid deposition and recent findings showed that tau-positive material is visible in a high proportion of children and young adults in the absence of Aβ accumulation (Braak *et al.* 2011). According to Braak and Tredici, the pathologic process leading to abnormal tau pathology does not begin in the transentorhinal region, but in select subcortical nuclei (*e.g.* locus coeruleus). These new findings, if confirmed, could modify the current view on AD. Sporadic AD may be the result of two separate assaults: first, a tauopathy, possibly beginning in childhood; and second, negative influences of β-amyloid after a given threshold is reached. Aβ might be capable of exacerbating the underlying tauopathy so it develops into clinical AD. Thus, AD might not be a disease of the aged, but an uncommonly slowly progressive disorder that frequently extends into old age (Del Tredici and Braak 2008).

If tau hyperphosphorylation and accumulation are a cause or a consequence of AD is still a matter of debate. According to the amyloid cascade hypothesis (Hardy and Selkoe 2002), Aβ formation is the critical step and tau pathology is driven by Aβ formation. Support for the amyloid hypothesis stems from the identifications of pathogenic mutations in FAD patients that are linked to Aβ formation, as well as increased Aβ levels and a higher frequency of AD in people with trisomy 21, who carry an additional APP allele (Ittner and Götz 2011). A crucial question is where to place tau in the amyloid cascade. Is it a prime target, a mediator or a bystander of Aβ toxicity? Evidence from both *in vitro* and *in vivo* models suggest three possible models of interaction: 1) Aβ drives tau pathology, 2) Tau mediates Aβ toxicity, 3) Aβ and tau synergistically exert toxic effects (Ittner and Götz 2011).

2.3.4 Genetic risk factors

In a substantial proportion of individuals with AD there is evidence for a significant genetic component. FAD only covers only about 5% of AD cases, but linkage studies on FAD have

shed light on many aspects of AD. The literature reports disease causing variants in three genes for early onset autosomal dominant AD; amyloid precursor protein (Goate *et al.* 1991) (32 mutations to date), presenilin 1 (Sherrington *et al.* 1995) (177 mutations to date), and presenilin 2 (Rogaev *et al.* 1995) (14 mutations to date) on chromosomes 21, 14 and 1 respectively.

The remaining 95% of AD cases, however, do not exhibit familial inheritance and are referred to as sporadic, late onset AD. First-degree relatives of AD patients have a higher lifetime incidence of AD than the general population and 15%-35% of patients with AD have affected first-degree relatives (Breitner and Folstein 1984). Thus far, the only confirmed genetic risk factor for sporadic AD is the ε4 allele of the gene encoding Apolipoprotein E (*APOE* ε4) (Corder *et al.* 1993, Saunders *et al.* 1993).

In the past decade, the plethora of candidates genes and regions emerging from genetic linkage and smaller-scale association studies yielded intriguing 'hits' that have often proven difficult to replicate consistently. Genome-wide association studies confirmed the universally accepted role of *APOE* as a genetic risk factor for late-onset AD as well as additional candidate genes that require confirmation. At the time this thesis was written, 695 different genes had been evaluated for association with late-onset sporadic AD (Bertram *et al.* 2007).

2.3.5 Neuroimaging findings

Four imaging modalities have been used as secondary end points in clinical trials on AD: structural MRI, functional MRI (fMRI), MRI spectroscopy (MRS) and positron emission tomography (PET). The imaging techniques provide information on the spatial distribution and temporal dynamic changes of the AD brains.

The clinical symptoms of AD arise from progressive neuron and synapse loss, with the resulting tissue atrophy visible on high resolution structural MRI. As expected from the pathology and clinical expression of AD, significant atrophy is observed in early disease stages in the memory-related structures of the medial temporal lobe, particularly the hippocampus and entorhinal cortex (Fennema-Notestine *et al.* 2009, Morra *et al.* 2009); moreover, the degree of atrophy correlates well with memory impairment (Kovacevic *et al.* 2009, Walhovd *et al.* 2010).

The initial use of MRI for the detection of atrophy in AD used manual tracing methods, necessitating focus on a few selected regions, such as the hippocampus or entorhinal cortex, or on global measures of atrophy, such as reduced global brain volumes and increased ventricular volumes. The advent of computer-based methods for quantitative MRI has allowed for efficient quantification of AD-related atrophy across the brain (Ashburner and Friston 2000, Fischl *et al.* 2002). This revealed that mild AD is associated with widespread atrophy of cortical association areas, with significant involvement of medial and lateral temporal, inferior parietal, posterior cingulate and prefrontal cortices (Whitwell *et al.* 2008).

Structural MRI measures can discriminate AD from healthy controls with high sensitivity and specificity (Westman *et al.* 2011). Importantly, it has been demonstrated that the degree of atrophy correlates well with disease stage determined from histopathology (Vemuri *et al.* 2008), and that the topographical pattern of atrophy is relatively consistent with the distribution of NFTs in later disease stage (Whitwell *et al.* 2008). Thus, quantitative measures of atrophy from structural MRI are sensitive to neurodegeneration occurring in

AD, and although atrophy itself is nonspecific to AD, the topographical pattern of atrophy may be a sensitive and specific marker for AD.

DTI studies in AD are difficult to compare because there has not yet emerged a consensus on the best acquisition protocols, tensor metrics, and statistical analytical techniques. The DTI-detected regional WM alterations in AD seem to parallel the pattern of changes followed by the GM in the disease process, that is, greater abnormalities in posterior brain regions relative to anterior regions at the early stages of AD. Thus AD is associated with reduced FA and increased MD compared with controls, in widespread brain regions, most notably in frontal and temporal lobes, the posterior cingulum, corpus callosum, superior longitudinal fasciculus and uncinate fasciculus (Bozzali and Cherubini 2007). A recent meta-analysis of DTI studies of AD subjects confirmed a large effect size of WM damage in the posterior cingulum, but also within major WM bundles connecting the prefrontal cortex (Sexton *et al.* 2011), suggesting that WM damage affects large-scale networks in AD.

There is still much debate concerning the pathophysiology underlying WM abnormalities in AD. In particular, it is unclear whether WM pathology is related to, or independent of GM pathology. One theory proposes that microstructural WM changes occur as a result of Wallerian degeneration. In this case, it follows that WM abnormalities parallel the pattern of GM pathology (Coleman 2005). Alternatively, the retrogenesis hypothesis proposes that decreased WM integrity is the result of myelin breakdown that occurs in the reverse order to myelogenesis (Bartzokis 2004).

Proton magnetic resonance spectroscopy (^{1}H MRS) allows noninvasive assessment of brain biochemistry. ^{1}H MRS metabolite abnormalities (*e.g.* increased levels of the glial metabolite myoinositol, decreased levels of the neuronal metabolite *N*-acetylaspartate) are associated with the severity of AD pathology (Kantarci *et al.* 2008).

Task-activation fMRI studies in AD generally have involved less-impaired subjects because good cooperation is required to hold still and to follow task paradigm. Mild AD subjects have demonstrated three basic trends: 1) decreased hippocampal activation (Diamond *et al.* 2007, Sperling *et al.* 2003); 2) increased activation in neocortical brain regions normally recruited by a given task (Saykin *et al.* 1999),(*e.g.* frontal regions during semantic tasks) and 3) recruitment of new regions not seen during the task in healthy elderly subjects (Celone *et al.* 2006, Saykin *et al.* 1999). A heuristic interpretation of these findings is that increased activation or alternate processing within other brain regions in early AD try to compensate for declining function in the medial temporal lobes.

Resting state fMRI has the ability to record spontaneous brain activity fluctuations when subjects lie still in the scanner, at rest. The default mode network (DMN), which includes the posterior cingulate cortex, anterior cingulate cortex, precuneus, inferior parietal and medial prefrontal cortex has been identified as a neuronal network showing a coherent pattern of spontaneous activation (functional connectivity) while at rest (Hafkemeijer *et al.* 2012). These regions, which show correlation in their spontaneous fluctuations at rest, are believed to be similarly modulated by cognitive tasks. In this perspective, changes in strength of correlation within regions of the DMN at rest are expected to express an indirect measure of regional brain disconnection. The DMN is a particularly relevant network in AD research, since DMN structures are vulnerable to atrophy, amyloid deposition, and show reduced metabolism in AD. Decreased functional connectivity was observed in the medial prefrontal cortex, posterior cingulate cortex and the parietal cortex (Zhang *et al.* 2009, Zhou *et al.* 2010). Taken together, most of these studies showed AD-related decrease in

functional connectivity at rest and deactivation in the DMN. It is currently not clear to what extent these changes may be explained by atrophy, since the majority of the studies did not include this potential confound in the analysis (Hafkemeijer *et al.* 2012).

FDG-PET measures glucose metabolism as a proxy for neuronal activity at a resting state. In AD, neuronal activity is impaired, as FDG uptake is reduced, predominantly in temporoparietal association areas, including the precuneus and posterior cingulate cortex and frontal association regions are increasingly affected as the disease progresses (Alexander *et al.* 2002, Hirono *et al.* 2004). Virtually all FDG-PET studies report that, compared with age-matched healthy normal controls, AD patients show regional metabolic reduction involving the parieto-temporal and posterior cingulate cortices and the frontal area in advanced disease (Mosconi *et al.* 2008).

Amyloid-PET studies have shown that selective binding of a ^{11}C-labelled thioflavin analogue known as Pittsburgh compound B (^{11}C-PiB) to Aβ can be visualized in AD patients (Klunk *et al.* 2004). *In vivo* PiB studies demonstrate a roughly two-fold increase in tracer retention in AD patients compared to most cognitively normal elderly subjects (Klunk *et al.* 2004). Greatest retention values are seen in prefrontal and lateral temporoparietal cortex, posterior cingulate/precuneus, and striatum (Edison *et al.* 2007, Engler *et al.* 2006). The topographic distribution of PiB retention corresponds to Braak and Braak plaque stage C in most cases of clinically established AD. Nearly all clinically diagnosed AD subjects reported to date have PiB retention while the majority of healthy controls do not. However, approximately 30% of healthy controls display increased amyloid retention levels in cortex which are in the typical range for AD, with as yet unknown prognostic implication (Jack *et al.* 2008, Villemagne *et al.* 2008).

2.3.6 CSF biomarkers

CSF is regarded as an ideal source for viable biomarkers in AD due to its intimate contact with the cerebral tissue; therefore pathological changes in the brain are often reflected in the CSF. The most consistent finding have been obtained with the measurement of CSF concentrations of Aβ peptide ($A\beta_{42}$), total Tau (T-Tau) and phosphorylated Tau (P-Tau). AD patients characteristically display low concentrations of $A\beta_{42}$ and high concentrations of T-Tau and P-Tau. This pattern of CSF biomarkers is commonly referred to as the 'AD signature' in the CSF. $A\beta_{42}$ is a byproduct of the abnormal processing of the amyloid precursor protein (APP) leading to amyloidogenesis and formation of neuritic plaques (Wiltfang *et al.* 2005). In addition, decreased concentrations of $A\beta_{42}$ likely reflect its deposition in plaques, preventing its clearance through the CSF. P-Tau illustrates the cytoskeletal changes that arise from the deregulation of microtubule homeostasis and ultimately cause axonal dysfunction and neuronal death.

Recently, the neuropathology of AD has been linked to the accumulation of non-fibrillar forms of neurotoxic Aβ oligomers. There is evidence that soluble Aβ oligomers, more than amyloid *per se*, play a critical role triggering early pathological events of the amyloid cascade (Haass and Selkoe 2007). High levels of Aβ oligomers are observed in the brain and in the CSF of AD patients (Fukumoto *et al.* 2010).

To date, over 100 studies have been published to support the notion that this AD-positive CSF pattern has good diagnostic accuracy to distinguish between normal ageing and AD (>85%) (Hansson *et al.* 2006).

2.3.7 Treatment

The first drugs developed for AD, acetylcholinesterase inhibitors (AChEI), were designed to increase acetylcholine levels, previously demonstrated to be reduced in AD. To date, four AChEI have been approved for the treatment of mild-to-moderate AD: tacrine (First Horizon Pharmaceuticals), donepezil (Pfizer), rivastigmine (Novartis) and galantamine (Janssen). Donepezil is now also approved for severe AD. The other three AChEI are effective for mild-to-moderate AD, although it is not possible to identify patients who will respond to treatment (Birks 2006). A further therapeutic option for moderate-to severe AD is memantine. This drug is a non-competitive, N-methyl-D-aspartate (NMDA) antagonist believed to protect neurons from excitotoxicity. AChEI and memantine treatments can result in statistically significant but clinically marginal improvement (Raina *et al.* 2008).

In 2008 the America College of Physicians and the American Academy of Family Physicians developed clinical guidelines to present the available evidence on current pharmacological treatment in dementia by analyzing the targeted literature of the Food and Drug Administration approved pharmacological therapies for dementia (Qaseem *et al.* 2008). They concluded with the recommendation that clinicians should base the decision to initiate therapy with AChEI or memantine on individual assessment.

On the basis of recent additional findings on AD pathogenesis, novel treatments are under development in an attempt to interfere the course of the disease in its early stages. Drugs interfering with Aβ deposition and with Tau deposition are in different phase of testing (Galimberti and Scarpini 2011).

Despite promising premises, large phase III trials with potentially disease-modifying properties have failed to demonstrate any effect on cognition (Mangialasche *et al.* 2010). Neuropathological analysis of brains coming from patients, who received immunization, proved that, although the Aβ load was lower than in the placebo group, there was no evidence of improved survival or improvement in time to severe dementia. Another important issue is that treatments for AD appear effective only in certain phases of the disease. Some disease-modifying compounds showed some benefits in mild but not moderate AD. Therapeutic trials should be carried out as early as possible during the course of the disease, which requires the identification of more accurate tools for early diagnosis.

2.4 MCI AS A PRODROMAL STAGE FOR AD

2.4.1 Historical development

Families, caregivers, and physicians of persons with AD generally find it difficult to pinpoint, even in retrospect, the precise onset of a patient's cognitive impairment. The development of dementia due to a degenerative neurological illness typically proceeds insidiously over several years from a state of cognitive normalcy to progressively severe stages of global intellectual dysfunction.

Clinical investigators have grappled with the problem of defining the boundaries of normal cognitive ageing for over 40 years. In 1962, Kral coined the term "benign senescent forgetfulness" (Kral 1962) to describe a population of nursing-home residents with mild memory deficits. This concept has undergone many refinements resulting in a proliferation of proposed entities: age-associated memory impairment, age-consistent memory

impairment, late-life forgetfulness, and age-related cognitive decline. These constructs were intended to identify subjects whose performance had deteriorated below values established for young adults, but were not expected to undergo significant further decline and were not believed to harbor neuropathological changes. In contrast to these proposed definitions of "normal ageing", Levy's "ageing-associated cognitive decline" included subjects who performed below normative levels for their own age-group making a pathological basis more likely (Levy 1994). Other constructs such as "isolated memory loss", "mild cognitive disorder", "mild neurocognitive disorder", "cognitive impairment-no dementia", were intended to capture similar levels of overall intellectual performance.

It was in this historical context that the expression "mild cognitive impairment" (MCI) gradually entered the lexicon of the ageing dementia literature. In 1988, Reisberg *et al.* used it as a descriptive term describing subjects with stage 3 on the Global Deterioration Scale (Reisberg *et al.* 1988). Three years later, the term appeared again in the title of an article by Flicker *et al.* 1995 (Flicker *et al.* 1991), and Petersen *et al.* used MCI as an independent diagnostic category not linked to a previously defined rating scale (Petersen *et al.* 1995). In this case, the diagnosis was applied to nondemented research subjects who retained normal global cognitive function without impairment on tasks of daily living, but had subjective memory complaints and scored below age-adjusted norms on memory tests. Subsequent studies have rediscovered the verities described in the preceding critique of the 2001 (Ritchie *et al.* 2001) practice parameter. The new working criteria for MCI were proposed in Stockholm in 2003 (Winblad *et al.* 2004) and the stepwise algorithm was based around the following three diagnostic features:

- Not normal, not demented (does not meet Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition [DSM-IV] or International Classification of Mental and Behavioral Disorders [ICD-10] criteria for a dementia syndrome).
- Cognitive decline indicated by subject and/or informant report and objective cognitive tests.
- Preserved basic activities of daily living with some minimal impairment in complex instrumental functions.

2.4.2 Epidemiological considerations

Population-based studies in older adults (age ≥ 60 or ≥ 65 years) reported a prevalence of MCI ranging from 11% to 17% (Ganguli *et al.* 2004, Graham *et al.* 1997, Lopez *et al.* 2003, Ritchie *et al.* 2001).

Regarding the incidence rates, the few studies published to date considered only the amnestic MCI (aMCI) subtype, with a range from 9.9 to 21.5/1000 person/year in people older than 65 years (Larrieu *et al.* 2002, Solfrizzi *et al.* 2004).

Several longitudinal studies have shown that most persons with MCI are at increased risk for the development of dementia. As compared with the incidence of dementia in the general U.S population, which is 1 to 2% per year, the incidence among MCI subjects is significantly higher. The conversion to dementia annual rate of 5 to 10% in community-based populations (Farias *et al.* 2009, Ganguli *et al.* 2010) and 10 to 15% among those in memory clinics, the latter rates reflect the fact that cognitive impairment is typically more advanced by the time a person seeks medical attention (Ganguli *et al.* 2010).

2.4.3 Diagnostic criteria

While the notion of MCI as transitional stage between a normal cognitive state and dementia is easy enough to grasp, it is presently unclear whether an operational definition can be made sufficiently precise to define a unique and useful diagnostic entity. Should MCI be constructed as a syndrome with multiple etiological explanations or should the concept be constrained to denote only patients with prodromal AD?

The original criteria of MCI (Petersen *et al.* 1999) were related to the amnestic form, lately defined as aMCI, characterized by the presence of isolated memory impairment, memory complaint, relatively intact activities of daily leaving, normal general cognitive function and absence of dementia. Later studies showed that aMCI construct represented a relatively small group, compared with all individuals with a much broader form of mild cognitive deficits in other cognitive functions. Thus, Petersen revised the original criteria (Petersen 2004) and four different MCI subtypes have been proposed: 1) aMCI; 2) single non memory MCI with isolated impairment of a cognitive domain other than memory; 3) multiple-domain amnestic MCI; 4) multiple-domain non amnestic MCI.

In an evidenced-based review published in 2001, the American Academy of Neurology recommended that clinicians should monitor and follow MCI subjects, since they are at increased risk for dementia, particularly AD (Petersen *et al.* 2001).

The National Institute on Ageing and the Alzheimer's Association recently published new diagnostic guidelines for assessing the likelihood that MCI is caused by the underlying AD pathophysiology (Albert *et al.* 2011). This report has a *Core Clinical Criteria* designed to be used in all clinical settings and a *Clinical Research Criteria* intended to be used only in research settings. The concept of "MCI due to AD" is introduced in the clinical practice and reflects the fact that the ultimate focus of these criteria is to identify those symptomatic but nondemented individuals whose primary underlying pathophysiology is AD.

2.4.4 Neuropathology

Relatively few data on the neuropathological features of MCI are present in the literature. Results from the Religious Order Study showed that more than one half of persons with MCI met the National Institute on Ageing-Reagan criteria for AD. One third of them had also cerebral infarctions (Bennett *et al.* 2005). Petersen *et al.* reported that although most aMCI subjects did not meet the neuropathologic criteria for AD, their pathological findings suggest a transitional state of evolving AD (Petersen *et al.* 2006). All patients had pathological findings involving medial temporal lobe structures, likely accounting for their memory impairment. In addition, there were many concomitant pathologic abnormalities, including argyrophilic grain disease, hippocampal sclerosis, and vascular lesions. Presence of neurofibrillary tangles rather than amyloid deposition seems to be more prominent in MCI compared to cognitively healthy controls, with the highest density in early AD. The numbers of neurofibrillary tangles in entorhinal cortex and hippocampus are related to the progressive memory loss (Markesbery *et al.* 2006). This suggests a continuum of neurofibrillary pathologies underlying transition between normal ageing and early AD.

Because MCI is considered an intermediate state between normal ageing and dementia, those with dementia due to other neurodegenerative etiologies are also likely to pass through an MCI state. For example, subjects with non-amnestic MCI are more prone to progress to Lewy body dementia (Molano *et al.* 2010). Therefore, though it has been suggested that all cases of aMCI will progress to AD, these recent findings suggest that the

neuropathological substrate for MCI (amnestic or non-amnestic) is more heterogeneous and has to be taken into account when the main substrate of research are the MCI subjects.

2.4.5 Neuroimaging findings

Consistent with the finding that histopathological changes occur prior to the clinical diagnosis of AD, significant brain atrophy is visible prior to the onset of dementia. Amnestic MCI, with its hallmark memory impairment, is associated with entorhinal and hippocampal atrophy, and cortical thickness or volume measures intermediate between those of healthy controls and mild AD patients (Bell-McGinty *et al.* 2005, Fennema-Notestine *et al.* 2009, Morra *et al.* 2009, Singh *et al.* 2006). However, even at this pre-dementia stage of the disorder, atrophy is not restricted to the MTL areas, but extends to widespread association areas. Brain volume differences in structures outside MTL are more striking and consistent when comparing MCI and AD groups. Mild AD subjects show less brain volume in medial parietal (i.e., retrosplenial cortex, posterior cingulate and precuneus) and parietal association cortex (Chételat *et al.* 2002, Karas *et al.* 2004) . MCI subjects also have lower global cortical volume than AD patients (Wolf *et al.* 2004). MCI individuals with isolated memory impairment, which may represent an even earlier stage of the disorder, show significant atrophy in areas beyond the MTL, (*e.g.* thinning of lateral temporal cortex, posterior cingulate, inferior parietal, precuneus and caudal middle frontal cortex (Fennema-Notestine *et al.* 2009)).

Apart from GM changes, WM abnormalities have been observed in MCI. DTI indices of cerebral damage (increased MD and decreased FA) are commonly found in MCI individuals. The severity of alterations in MD and FA appears to be greater in AD compared to MCI, with MCI individuals typically displaying fewer regions of altered DTI values. The majority of DTI changes appear to be in more posterior regions (MTL including the hippocampus, entorhinal cortex and parahippocampal WM, temporal lobes proper and the posterior cingulate) (Fellgiebel *et al.* 2004, Fellgiebel *et al.* 2005, Rose *et al.* 2006) compared to frontal regional changes that are more common in healthy ageing. There is evidence that changes in MD are more common in MCI, whereas changes in MD and FA are more common in AD. There is little evidence that changes in radial diffusivity (potential marker for myelin damage), is more common in AD and MCI than changes in axial diffusivity (indicator of axonal damage) (Huang *et al.* 2007). Although studies of axial and radial diffusivity may be more pathology oriented, shortcomings of the measurements methods need to be taken into account. The interpretation of these indices might be flawed since alignment of the eigenvectors with underlying tissue could be affected by disease pathology (Wheeler-Kingshott and Cercignani 2009).

An important consideration in the utility of DTI in the study of AD and MCI is not only its sensitivity to group differences, but also its sensitivity to associations between DTI metrics and measures of cognitive performance (Fellgiebel *et al.* 2008, Kalus *et al.* 2006). MD and FA show a significant association with cognitive functions typically impaired in AD and MCI, suggesting external validity of these measures because of their relationship to cognitive performance and group differences.

Spectroscopic findings in MCI subjects are similar to those found in AD subjects, but to a lesser extent. The *N*-acetylaspartate/ creatine ratio is significantly lower in AD patients when compared to both MCI and healthy controls in the left superior temporal and the posterior cingulate volume of interest. Also, the choline/creatine ratio measured form the

posterior cingulate volume of interest is higher in AD patients when compared to both MCI and healthy controls (Kantarci *et al.* 2000). One of the circulated hypothesis is that, while the disease progresses, the initial change is an increase of the myoinositol/creatine ratio and a decrease of the *N*-acetylaspartate/ creatine ratio while the increase of choline/creatine ratio develop later in the disease course (Kantarci *et al.* 2000).

Although *f*MRI studies show group differences, similar studies yielded variable results with respect to degree, directionality, and location of functional differences (*e.g.* is MCI associated with *hypo* or *hyper*activation relative to older controls during memory encoding, and where are these changes localized?). The variability in task design, definitions of MCI, and imaging parameters across studies makes the comparison of *f*MRI findings difficult.

Task-activation fMRI studies in MCI, compared to healthy ageing and AD, have demonstrated both increased and decreased MTL activity during encoding novel visually presented material. Differences in the MTL activation patterns in MCI subjects may relate to differences in the severity of cognitive decline. There is replicated evidence to support the hypothesis that there may be an initial phase of increased MTL activation in MCI. Some MCI individuals, with smaller hippocampal volume perform similarly on memory tasks to MCI individuals with larger hippocampal volume but have relatively greater MTL activation (Diamond *et al.* 2007, Dickerson *et al.* 2005). This increase may represent an attempted compensatory response to AD neuropathology. Investigations of the MTL generally demonstrate that less impaired subjects show increased BOLD response in the hippocampus compared to control groups, whereas more impaired MCI subjects demonstrate decreased BOLD response similar to the levels observed in mild AD patients (Celone *et al.* 2006).

*Deactivation and resting state f*MRI studies focusing on the DMN showed altered functional connectivity and deactivations within the DMN for the MCI subjects (Hafkemeijer *et al.* 2012). The decreased connectivity was observed in the PCC, mPFC, ACC and hippocampus, even when controlled for GM atrophy (Lustig *et al.* 2003, Rombouts *et al.* 2005). Decreased task-induced deactivation in MCI subjects was found in the PCC, precuneus, frontal and parietal regions (Celone *et al.* 2006, Rombouts *et al.* 2005). These decreased task-induced deactivations are progressively decreased along the continuum from normal ageing to clinical AD, with one exception: the group of less impaired MCI subjects, who showed increased deactivation compared to controls (Celone *et al.* 2006).

Memory-related hyperactivation of specific brain regions becomes abnormal early in the course of the disease. At clinically more advanced stages of MCI and AD, this memory task-induced hyperactivation of the temporo-parietal memory network turns into an activation deficit (Celone *et al.* 2006). Specifically what this initial hyperactivation is due to and how that turns into an activation deficit remains unknown at this time; however it could represent a transient phase of impending breakdown of neuronal networks (Ewers *et al.* 2011). Alternatively, it could be more directly related to Aβ pathology, either as a consequence of Aβ-induced hyperexcitability of neurons (Palop *et al.* 2006), or by increasing vulnerability to Aβ toxicity (Buckner *et al.* 2009). Whatever the underlying reason, initial evidence suggest that hyperactivity might reflect impending neuronal dysfunction and clinical decline (Miller *et al.* 2008).

Approximately 33-61% of MCI subjects show AD pathophysiology by virtue of being *PiB-PET* positive (Koivunen *et al.* 2011). Studies with [^{11}C] PIB have shown that MCI subjects have significantly higher uptake *vs.* healthy controls in several brain areas (frontal cortex,

posterior cingulate, parietal and lateral temporal cortices, putamen and caudate) (Forsberg *et al.* 2008, Kemppainen *et al.* 2007). In AD, during the progression of disease there is no or relatively little increase in [^{11}C] PiB uptake (Forsberg *et al.* 2008). It has been suggested that amyloid accumulation takes place early in the AD process and increases relatively little at the stage of clinical diagnosis. Thus, it might be possible that MCI subjects would show increased [^{11}C] PiB uptake with time.

Although *FDG-PET* studies in AD demonstrate consistent and progressive reductions in cerebral metabolic rate for glucose (Alexander *et al.* 2002), MCI subjects show a more variable pattern of this reduction. Currently no specific pattern of hypometabolism is considered to be a hallmark for MCI. FDG-PET studies showed that hippocampal metabolism is evident in MCI subjects regardless of the neuropsychological profile, whereas the cortical involvement is more diversified (Mosconi *et al.* 2008).

Whether or not FDG-PET hypometabolism in MCI is linked to deposition of Aβ is not clear at this stage. Only a few studies have examined this association and weak negative associations have been reported (Reiman and Jagust 2011).

2.4.6 CSF biomarkers

CSF biomarkers for MCI include T-Tau, P-Tau and $A\beta_{42}$. Several studies have found high CSF T-Tau and P-Tau, and low CSF $A\beta_{42}$ in subjects with MCI, with sensitivity figures similar to, or slightly lower than those found in AD cases. It was suggested that also plasma $A\beta_{42}$/ $A\beta_{40}$ ratio may be a useful marker for identifying cognitively healthy elderly subjects who are at increasing risk for developing MCI and AD, reflecting the extent to which there has been selective aggregation and depositions of $A\beta_{42}$ in the brain. Graff-Radford *et. al* postulated that CSF $A\beta_{42}$, as well as plasma $A\beta_{42}$ levels, decline in parallel as **A**β_{42} deposits in the brain, and because $A\beta_{42}$ aggregation and deposition precedes MCI and AD, most subjects who develop MCI/AD have a low ratio several years before being diagnosed (Graff-Radford *et al.* 2007).

2.4.7 Predictors of conversion to AD

A question commonly raised by MCI subjects and their family members concerns the likelihood and time course of progression to dementia.

Clinical and epidemiological investigations have demonstrated that MCI subjects progress more often to AD or to other dementias than older adults without objective evidence of cognitive impairment. However, a substantial variation in the annual progression rates from MCI to AD is observed across studies, ranging from low estimates of 3% to very high estimates of 40% to 50% in samples defined according to Petersen criteria (Petersen *et al.* 1999).

The concept of MCI is definitely sensitive to identify subjects that may develop dementia/AD, since most individuals with predementia AD will present, at some point of the progression curve, with a long period of mild cognitive deficits prior to the onset of dementia. Nevertheless, as currently conceived, the clinically oriented diagnostic criteria for MCI yields a heterogeneous group of subjects with distinct short-term and long-term outcomes. In other words, the specificity and the predictive value of the MCI diagnosis are low, and the cross-sectional identification of cases of prodromal AD may not reach adequate diagnostic accuracy if based solely on clinical tools. Rather, it could benefit substantially from the combination of clinical and biological information.

Clinical, imaging, genetic and CSF aspects have been widely examined as possible markers in MCI in order to detect subjects at greater risk of conversion to dementia.

Increased GM loss is found in converters as compared to stable MCI subjects; these subjects display volumetric reductions in hippocampal and parahippocampal structures and, to a lesser extent, in the posterior cingulate cortex, middle and inferior temporal gyri, fusiform gyrus, posterior cingulate gyrus, precuneus, temporoparietal junction, and frontal cortex (Liu *et al.* 2010). A voxel-based morphometry meta-analysis indicated smaller hippocampal volumes in converters *vs.* stable MCI subjects (Ferreira *et al.* 2011).

The atrophy of the MTL structures correlates with clinical decline and conversion to AD in individuals with MCI. Retrospective comparisons between converters and stable MCI, as well as prospective studies of MCI subjects, have highlighted the importance of atrophy beyond medial temporal areas in predicting conversion to AD.

Multivariate techniques are increasingly applied for detecting patterns of regional atrophy to improve the discrimination. These studies indicate that the degree to which MCI subjects express the characteristic AD atrophy pattern is predictive of a decline in cognitive function, progressive structural brain loss and eventual conversion to AD. Discriminant analysis applied to healthy control and AD revealed that atrophy in medial temporal (hippocampus and entorhinal cortex), lateral temporal, orbitofrontal and isthmus of the cingulate cortex best differentiated AD from controls. Application of this model to MCI data showed that the degree to which an MCI individual expressed the regional AD atrophy pattern was predictive of clinical decline and progressive structural brain loss (McEvoy *et al.* 2011).

The patient-specific estimates of the risk of conversion from MCI to AD can be derived from quantitative measures of brain atrophy obtained from both single-time-point and serial MR imaging examinations. Studies comparing atrophy rates or ventricular expansion from serial MRIs have demonstrated differences between converters and stable MCI. Use of these measures substantially improves the prediction risk based on the clinical MCI diagnosis alone. An individual who receives a diagnosis of MCI based on the commonly accepted criteria has a 15%-20% risk of developing AD within 1 year (Petersen *et al.* 2010). However, use of structural MRI data from a baseline examination considerably improves risk prediction, enabling the identification of individuals who show only slightly elevated risk relative to elderly subjects without cognitive impairment (3% per year) and individuals with a substantially higher risk (up to 40% per year). Information regarding the rate of atrophy over 1-year period enables even greater risk discrimination compared with information derived from only one single MRI scan, enabling the identification of individuals who are at very high risk (69%) of developing AD within the next year (McEvoy *et al.* 2011).

Amyloid biomarkers are very sensitive in discriminating individuals with MCI who will progress to AD from those who remain stable. To date, over 100 studies have been published to support the notion that the "AD signature" in the CSF is a strong predictor of dementia outcome (Diniz *et al.* 2008). MCI converters have a CSF biomarker pattern undistinguishable from AD patients ($A\beta_{42}$ aggregation and deposition precedes MCI and AD and most MCI converters have a low $A\beta_{42}/A\beta_{40}$ ratio several years before the diagnosis of the disease). MCI subjects with progressive deficits (albeit not severe enough to characterize conversion) have a similar pattern to AD patients (Mariani *et al.* 2007). Conversely, MCI subjects with unstable (transient) MCI and those who display non-progressive deficits over time have a CSF biomarker pattern very similar to older, healthy

adults. However, the specificity of amyloid pathology is uncertain, as it is not accompanied by dementia in approximately 30% of cases and may not inevitably lead to dementia in these individuals.

Studies that have directly compared the predictive ability of amyloid biomarkers with structural measures have found that structural measure are better predictors in MCI over the near term, but that amyloid biomarkers provide important complementary information (Jack *et al.* 2009). Taken together, these findings suggest that positive evidence of amyloid pathology in the presence of AD-like atrophy pattern would strongly suggest that an individual is in a prodromal stage of AD.

With respect to functional neuroimaging, MCI converters show a pattern of cerebral hypometabolism that is largely similar to that found in patients with mild AD, in particular in the posterior cingulate cortex and the hippocampal regions (Mosconi *et al.* 2008). PiB-positive MCI subjects have a higher conversion rate than PiB-negative subjects and the amyloid load is negatively associated with time to conversion (Okello *et al.* 2009).

3 Aims of the study

The general aim of this thesis was to investigate structural correlates for the progression from MCI to AD using different MR image analysis techniques

1. To examine whether the rate of whole brain atrophy is associated with increased risk of progression to AD
2. To investigate the changes in brain tissue fractions' volumes (GM, WM, CSF) across serial MRI scans within 2 years preceding the clinical conversion to AD
3. To map the brain atrophy and its differential dynamics within 2 years preceding the clinical conversion to AD
4. To investigate the predictive power of MRI-derived severity indices for the progression to AD
5. To explore the potential of WM integrity analysis in differentiating normal from pathological ageing

4 Materials and methods

4.1 SUBJECTS

4.1.1 Studies I and II

MCI subjects were selected via the MR database at the Kuopio University Hospital. The MRI database was created to centralize all MRI data existing at the University of Eastern Finland, Department of Neurology. These two studies included MCI subjects that were taking part in two population-based studies (Kuopio MCI study (Pennanen *et al.* 2004) and the Cardiovascular Risk Factors, Ageing and Dementia, CAIDE study (Kivipelto *et al.* 2001)) using similar diagnostic procedures.

Study I consisted of 102 subjects, aged ≥55, and who had two MRI scans with a minimum follow-up of 6 months.

Study II consisted of 60 subjects, aged ≥55 and had at least 3 serial MRI scans spanning 2 years.

The MCI participants were further divided into two groups: a group of subjects that did not progress to AD (stable MCI); and a group that progressed to AD (progressive MCI). The group definition was based solely on the clinical diagnosis and was blind to all quantitative MRI.

In study II for the progressive MCI subjects, a prerequisite was to have 2 serial scans available after being diagnosed with MCI, and one scan at the point of the diagnosis of AD (*i.e.* a scan series of MCI-MCI-AD). The follow-up scans were performed during a scheduled visit, or as soon as possible after the subject was clinically diagnosed with dementia.

These studies were approved by the Ethics Committee of Kuopio University Hospital and all subjects gave written consent prior to enrolment.

4.1.2 Studies III and IV

These two studies included subjects originated in the AddNeuroMed project, part of the European Union FP6 program, InnoMed (Innovative Medicines in Europe) and the Alzheimer ’s Disease Neuroimaging Initiative (ADNI).

The AddNeuroMed project was designed to develop and validate novel surrogate markers for AD and includes a human neuroimaging strand which combines MRI data with other biomarkers and clinical data. Data were collected from six different sites across Europe; University of Kuopio, Finland, University of Perugia, Italy, Aristotle University of Thessaloniki, Greece, King’s College London, United Kingdom, University of Łodz, Poland and University of Toulouse, France.

The overall goal of the ADNI study is to define the rate of progress of MCI and AD, to develop improved methods for clinical trials in this area, and to provide a large database which will improve the design of treatment trials (http://www.adni-info.org).

Study III consisted of 1064 subjects, (329 AddNeuroMed and 726 ADNI subjects): 335 CTL, 434 MCI subjects and 295 AD patients.

Study IV consisted of 63 AddNeuroMed subjects recruited at the University of Kuopio; 19 CTL, 27 MCI subjects and 17 AD patients.

A synoptic presentation of subjects and methodologies used throughout the thesis is presented in Table 4.1. Baseline clinical characteristics of the subjects included in all the studies are presented in Table 4.2.
Written consent was obtained where the research participant had capacity, and in those cases where dementia compromised capacity then assent from the patient and written consent from a relative, according to local law and process, was obtained. This study was approved by ethical review boards in each participating country.

Table 4.1 Synoptic presentation of subjects and methodologies used throughout the thesis

STUDY	No of Subjects	Label	MRI	Target	Method
I	102	MCI	2	whole brain atrophy rates	IPCA under SPM5
II	60	MCI	3	regional dynamics of atrophy	VBM under SPM8
III	1064	CTL/MCI/AD	1	MR derived index of severity	FreeSurfer, OPLS
IV	63	CTL/MCI/AD	1	WM integrity	TBSS under FSL

Table 4.2 Subjects baseline characteristics

STUDY	Groups	Age at 1st scan	Gender (male/female)	Education years	MMSE at 1st scan
I	Stable MCI	72.4 ±5.6	37/50	6.8 ±2.2	24.7±2.8
	Progressive MCI	71.6±5.8	4/11	8.2±3.5	23.8±3.1
II	Stable MCI	73.03±5.04	21/27	6.8±2.3	26.1±2.5
	Progressive MCI	73.03 ±6.9	4/8	6.5 ±3.1	23.1±2.1
III	CTL	75±5.7	167/168	14.3±4.4	29.12±1.05
	Stable MCI	74.7±6.5	152/109	13.2±4.9	27.3±1.7
	Progressive MCI	74.3±6.9	102/71	14.8±3.7	26.6±1.7
	AD	75.2±6.8	129/165	12±4.7	22.3±3.6
IV	CTL	75±6	11/8	9±3	28±1
	MCI	75±6	15/12	8±3	26±2
	AD	76±7	6/11	9±5	22±5

4.2 MRI METHODS

4.2.1 Acquisition protocols

Studies I and II
T1-weighted images were collected using a three-dimensional magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence. All measurements are from the same scanner (Siemens, Magnetom Vision, 1.5 T) without any upgrades between visits.
The imaging parameters were as follows: TR = 13.5 ms, TE = 7ms, flip angle = 12°, FOV = 25 cm, matrix 256x256, slice thickness = 1.5 mm, 128 contiguous slices, in plane voxel dimension = 0.98 mm x 0.98 mm.
In study I, a subgroup of 33 subjects had slightly different acquisition parameters: TR = 9.7 ms, TE = 4ms, flip angle = 12°, FOV = 25 cm, matrix 256x256, slice thickness = 2 mm, 128 contiguous slices, in plane voxel dimension = 0.98 mm x 0.98 mm.

Studies III and IV

Data acquisition for the AddNeuroMed study was designed to be compatible with the Alzheimer Disease Neuroimaging Initiative (ADNI) (Jack *et al.* 2008). The imaging protocol for both studies include: high resolution sagittal 3D T1-weighted MPRAGE sequence, (voxel size 1.1 x 1.1 x 1.2 mm3) axial proton density and a T2-weighted fast spin echo images. The MPRAGE volume was acquired using a custom pulse sequence specifically designed for the ADNI study to ensure compatibility across scanners (Jack *et al.* 2008). Full brain and skull coverage was required and a detailed quality control was carried out on all MR images according to the AddNeuroMed quality control procedure (Simmons *et al.* 2009, Simmons *et al.* 2011).

DTI data was collected using gradient echo single shot EPI sequence (TR = 5000 ms, TE = 98 ms, matrix 256x256, FOV =220 mm x 220mm, 29 axial slices, slice thickness = 5 mm) with diffusion gradients applied in 30 directions (*b* values 0 and 1000s/mm^2).

4.2.2 Iterative principal component analysis (IPCA) under SPM99

IPCA was used to characterize annualized whole brain atrophy rates using two sequential MRI scans (Chen *et al.* 2004). Image pre-processing was performed under SPM99 and the IPCA software package was incorporated into SPM99 (Figure 4.1).

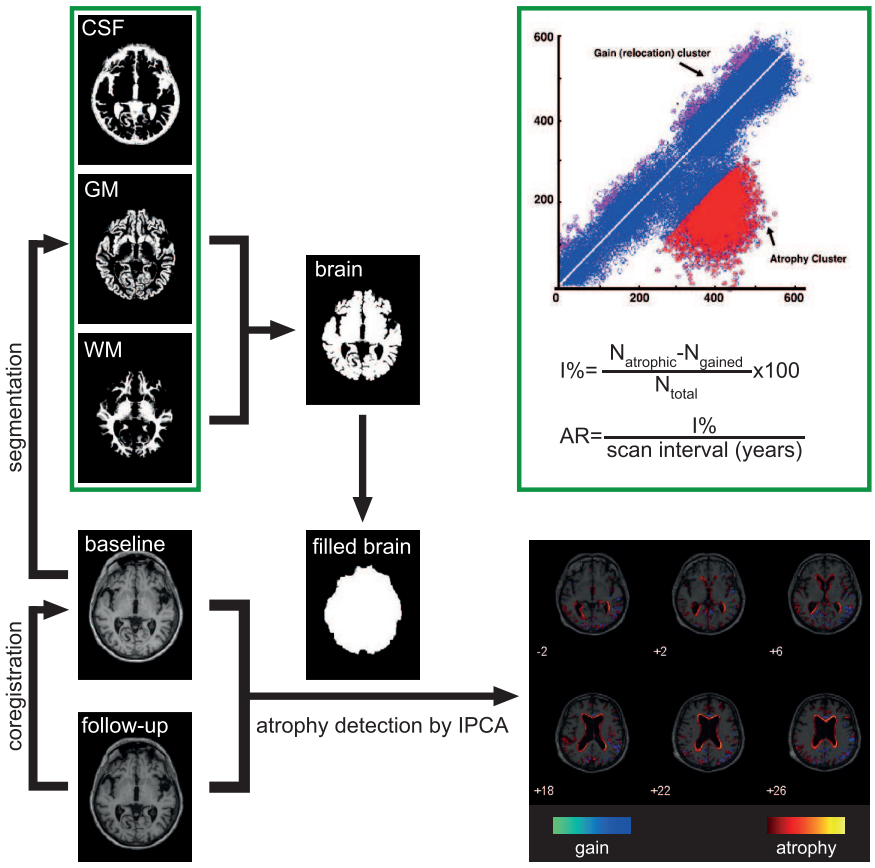


Figure 4.1 Atrophy detection using IPCA. The baseline image is used as reference

Image processing included AC-PC alignment, coregistration of the follow-up image to the baseline image, brain segmentation into three tissue types, brain extraction and filling of the brain mask. Based on the filled brain mask, baseline measurements of total intracranial volumes were calculated.

IPCA was used to compute the change in brain volumes. IPCA considers the voxel intensity pairs from coregistered images and identifies those pairs of voxels that are a sufficiently large distance away from the PCA major axis. The IPCA automatically characterize the major axis, identifies significant outliers, and computes between-scan changes in brain volume. By applying the PCA to the (x_i, y_i) points in an iterative manner, the IPCA successively removes outliers in determining the major axis direction. Once the ray is determined, outliers can be identified as those that are a sufficiently large projected distance away from the ray.

The rates of whole brain atrophy were expressed as percentage of volume change divided by the interval between the scans in years, yielding an annualized measure of brain atrophy.

4.2.3 Voxel based morphometry (VBM) under SPM8

VBM is a fully automated MR image analysis technique based on voxel-wise statistical comparisons over the entire brain providing information about regional brain concentration or volume differences between different groups.

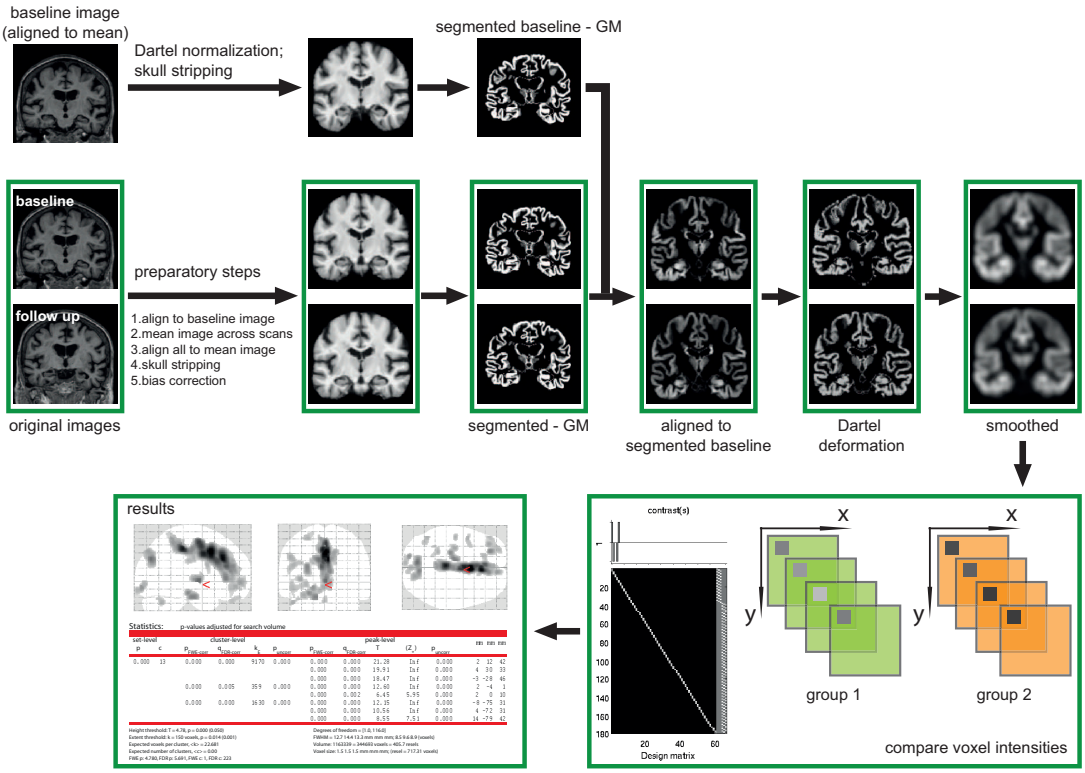


Figure 4.2 Longitudinal VBM workflow

Data pre-processing and analysis was performed using the VBM8 toolbox of SPM8 running under the Matlab R2010b environment (Mathworks, Natick, MA, USA). The VBM8 toolbox supplies a batch for longitudinal designs that was employed in study II. Longitudinal VBM workflow is presented in Figure 4.2 Finally, the smoothed, modulated, grey matter segments were used as input for the statistical model.

4.2.4 Brain volumes and cortical thickness measurements using FreeSurfer

Cortical reconstruction and volumetric segmentation was performed with the FreeSurfer image analysis suite software, version 4.5.0, which is documented and freely available to download (http://surfer.nmr.mgh.harvard.edu/).

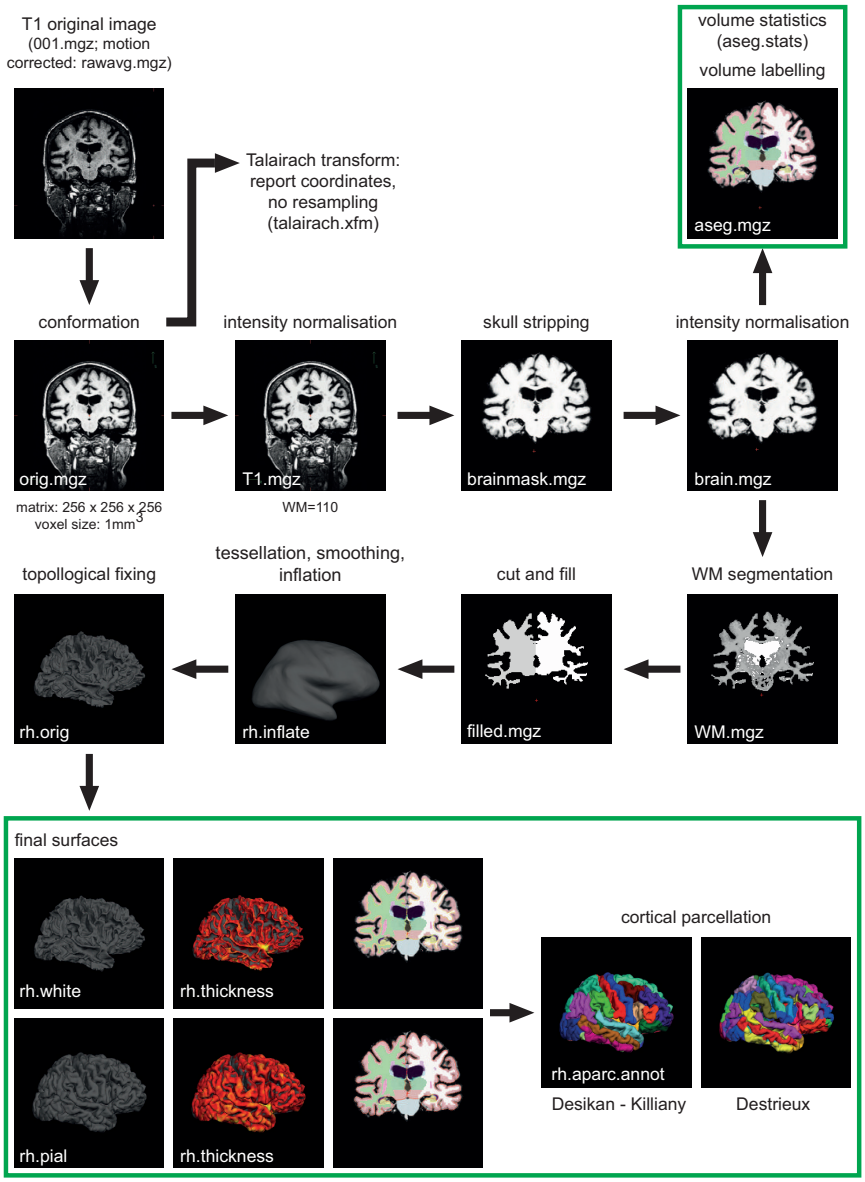


Figure 4.3 Schematic depiction of the algorithms included in the FreeSurfer pipeline

FreeSurfer has two main streams (see Figure 4.3):

- The volume based stream (Fischl *et al.* 2002, Fischl *et al.* 2004) is designed to preprocess MRI volumes and label subcortical tissue classes (white matter and deep grey matter structures including the hippocampus, amygdala, caudate, putamen, etc.). This pipeline only depends upon the skull stripping to create a mask of the brain in which the labelling is performed. For surface-based labelling, the measured value is the curvature in each of the principal directions at that vortex. For the volume-based labelling, the measured value is the intensity at that voxel. The atlas was built from a training set (prior manually labelling) and the labels are mapped into a common space to achieve point-to-point correspondence.
- The surface based stream (Dale *et al.* 1999, Fischl *et al.* 1999) consists of several stages that have as main output the representation of cortical thicknesses, calculated as the closest distance from the grey/white boundary to the grey/CSF boundary at each vertex on the tessellated surface.

The regional cortical thickness was measured from 34 areas and the regional volumes were measured from 23 areas. Left and right sided thicknesses were averaged. Volumetric measures were corrected for differences in head size by dividing each measurement by the estimated total intracranial volume.

4.2.5 Tract based spatial statistics (TBSS)

DTI data analysis was performed using the TBSS tool (Smith *et al.* 2006) under FSL (http://www.fmrib.ox.ac.uk/fsl/tbss).

Initially, the diffusion weighted images were corrected for the distortions induced by the eddy currents. The FA images were created by fitting a tensor model to the raw diffusion data using the FSL diffusion toolbox, and then brain-extracted using the FSL BET tool (Smith 2002). All subjects 'FA data were aligned into a common space using the nonlinear registration FSL tool called FLIRT and then a mean FA image was created and thinned to create a mean FA skeleton which aims to represent all tracts which are "common" to all subjects. Each subject's aligned FA data is then projected onto this skeleton in order to account for residual misalignments between subjects after the initial nonlinear registrations. The resulting data was fed into voxelwise cross-subject statistical analysis.

4.3 STATISTICS

4.3.1 Outside imaging space

Sociodemographic data analysis was performed using the two-tailed student's t-test or chi-squared tests as appropriate.

In paper I hierarchical generalized linear models under HLM 6.03 were used to model the likelihood of progression to AD as a function of time post-MCI identification, as well as the interaction between this time variable and annualized whole brain atrophy rates. Our choice was based on the 3 main characteristics of our data: longitudinal data, binary event and repeated measures over time.

Advantages of HLM modelling include:

- non-normally distributed binary outcome
- ability to model all available data

- no restriction regarding the number and the ability of measurement occasions
- dependencies among observations *between* and *within* individuals

In paper II repeated measure analysis of variance (ANOVA) was used for the analysis of brain tissue fractions (GM, WM, CFS, BPF), with time (3 time points scans) and groups (stable *vs.* progressive MCI) as independent predictors.

For paper IV an index of severity was generated using multivariate statistical analysis. Fifty-seven FreeSurfer measures were analysed using orthogonal projection to latent structures (OPLS) with no feature selection. The OPLS classifiers were trained on the data from all subjects in the combined group of CTL and AD and then applied to data from MCI subjects. This produced a discriminant score – the OPLS FreeSurfer based score – for each MCI subject, reflecting the degree to which the individual's MR pattern resemblance the pattern of AD or CTL subjects. The OPLS score tends to be closer to 1 for AD patients and 0 for CTL subjects. Scores above 0.5 indicate more AD-like characteristic and score below 0.5 a more CTL-like characteristic. Finally the AD *vs*. CTL model was used as classifier to investigate how well they could predict conversion from MCI to AD.

4.3.2 Inside imaging space

*Voxel Based Morphometry statistics (*see also Figure 4.2*)* of the smooth modulated GM volumes was performed by fitting a general linear model at each voxel using the theory of Gaussian random fields. The output of the method is a statistical parametric map showing regions where GM density differs significantly among the study groups.

Cross-sectional group differences (1 scan per subject, 2 groups) were assessed using full-factorial design. Longitudinal group differences (2 scans per subject, 2 groups) were assessed using flexible factorial design. The statistical criterion to accept or reject clusters was $p<0.05$ corrected for multiple comparison (family wise error technique) with a voxel level threshold of 150 voxels.

Tract Based Spatial Statistics

After preprocessing, the DTI data is in the form of a sparse (skeletonised) 4D image, with the fourth dimension being the subject ID. Comparisons were performed for control *vs.* AD, control *vs*. MCI and MCI *vs*. AD. Permutation-based approach was used for inference on statistic maps when the null distribution is not known. This approach tests an appropriate statistic (*e.g.* voxel *t* value) against the null distribution of maximum values of the test statistic. The null distribution was generated via 5000 random permutations of subject ID ordering with respect to the model. The random permutation is performed using a simple permutation program named *randomise* (http://www.fmrib.ox.ac.uk/fsl/randomise/) available through the FSL software library). This gives strong control for multiple comparisons, while searching over the entire skeleton for regions of significant effect. This approach does not require a Gaussian distribution for the cross-subject FA distribution. Significance was tested at $p < 0.05$ ((false discovery rate (FDR) or threshold-free cluster-enhancement correction techniques for multiple comparisons)) and $p < 0.001$ uncorrected for multiple comparisons.

5 Results and discussion

AD is a progressive neurodegenerative disease and significant irreversible brain damage is already present at the time of clinical manifestation of dementia. Therefore, the diagnosis of AD at early stages of the disease becomes a prerequisite. Earlier detection is needed in order to evaluate "predementia treatments" in the most rapid and rigorous way. There is a growing interest in the role that brain imaging and other biomarker measurements could play in the rapid evaluation of promising presymptomatic AD treatments.
This thesis focuses on identifying differences between stable and progressive MCI subjects at least one year prior to the clinical conversion to AD. To this end various MR image analysis techniques were used with the purpose to achieve suitable parameters to be used in a daily clinical practice or to provide additional information for research.
Various neuroimaging measures are potential biomarkers for early detection and prediction of AD pathology. We first investigated the whole brain atrophy and found that accelerated global atrophy is associated with an increased risk of progressing to AD. Then we investigated the patterns of regional grey matter changes between stable and progressive MCI subjects over a period of 2 years before the clinical conversion to probable AD. We found that progressive MCI subjects display accelerated regional atrophy already 2 years before the conversion. Based on these findings, we addressed the issue of possible individual risk classification based on multivariate scores derived from multiple measures in a single MRI scan (the OPLS score). We found that joint evaluation of several brain regions provide accurate separation of different disease stages. The latter two studies are based almost exclusively on grey matter changes. Therefore, we investigated the integrity of white matter in MCI and AD subjects in comparison with healthy elderly controls, and found differences that might explain certain features of AD pathology.

5.1 WHOLE BRAIN ATROPHY RATE

The question: Do the rate of brain atrophy and the number of years since MCI identification predict the risk for progression to AD?

Progressive cerebral atrophy is a characteristic feature of AD (Fox and Schott 2004) (see table 5.1). Atrophy correlates with neuronal loss at autopsy and with cognitive decline in life (Fox *et al.* 1999).

Table 5.1 Age related global brain atrophy rates

Age	Normal ageing	AD
30-50	0.2%	2-3%
70-80	0.3-0.5%	

We measured the rate of global brain atrophy on two serial MRI scans for a large sample of MCI subjects using a completely automated procedure based on iterative principle

component analysis (IPCA). We modelled the probability of progressing to AD as a function of time since enrolment in the study (diagnosis of MCI) and the amount of brain atrophy per year. Age, gender and TIV were included as covariates.

An increase of 1% in whole brain atrophy per additional year post-MCI identification was associated with an increased risk of progressing to AD. For the highest atrophy group the conversion rate was approximately 65% (*i.e.* two out of three MCI subjects with an atrophy rate of 4% per year will convert to AD during the following 3 years (see also Fig 5.1).

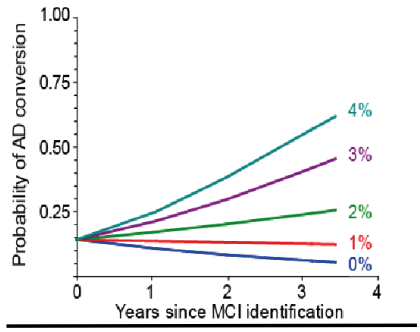


Figure 5.1 Probability of conversion to AD based on IPCA whole brain atrophy rates

Relatively few studies addressed the issue of global brain atrophy as a predictor of subsequent progression to AD and even fewer have used time-to-event statistical methods in the evaluation of MCI progression to AD. Jack *et al.* (Jack *et al.* 2005) concluded that whole brain atrophy rates provide predictive information about the hazard ratio of subsequent conversion from MCI to AD, but this ratio is complimentary to that provided by a cross-sectional hippocampus measure (Jack *et al.* 2005). According to their results, the key MRI predictor of conversion is how much the hippocampus had atrophied at baseline, as well as how fast the brain has been undergoing atrophy for 1 to 2 years before an AD diagnosis has been reached. Whole brain atrophy rates have become user friendly thanks to the newly developed techniques and could be easily implemented in the clinical practice. However, the question of how relevant is this biomarker and what is the real value added to the whole neuroimaging pattern for one single individual remains open.

5.2 DYNAMIC OF ATROPHY

The measures of whole brain atrophy do not differentiate between regions, but are certainly dominated by tissue loss that is occurring widespread throughout the brain. In this context, longitudinal assessment of regional atrophy is expected to bring additional information and even increase the predictive power of structural MRI.

5.2.1 The dynamic of brain tissue fractions

The question: which tissue fraction changes most with disease progression?

There is consensus that GM volume is smaller with higher age and that this effect is seen early in life. Less consistent results have been reported for the relationship between age and WM volume.

Global brain changes, GM or WM, are usually not pronounced in MCI. For this thesis, brain tissue fractions (GM, WM and CSF) and brain parenchymal fraction ($BPF = (GM + WM)/TIV$) were longitudinally investigated in relation with the transition from MCI to AD. As expected, the CSF fraction increased and the BPF decreased constantly both for the stable and the progressive MCI groups. These results indicate that the brain

parenchyma is shrinking in both groups during the 2-year observation period. We found significant progressive GM atrophy only in the progressive MCI group, and the difference became significant 1 year prior to the clinical conversion to AD. The WM volume changed significantly across scans in the stable MCI group, but not in the progressive MCI group. The decrease in WM volume is an indicator of WM integrity loss that together with DTI results of decreased FA indicate that connection loss underlies the cognitive impairment. Given the annual conversion rate of 10-15% for the MCI subjects, it is anticipated that many of the stable MCI subjects will convert to AD in the near future. Therefore, future studies with longer follow-up periods and larger samples will refine the estimates.

5.2.2 Dynamic of regional atrophy

The question: are there any differences in the dynamic of the GM atrophy between stable and progressive MCI?

VBM is an adaptation of the statistical parametric mapping (SPM) technique allowing a comprehensive examination of brain structural changes in a variety of conditions. SPM is a technique that evaluates the whole brain volume independent of distinct neuroanatomical regions and produces a parametric map containing an average value for each voxel. VBM objectively maps GM differences between groups on a voxel-by-voxel basis. The advantage of VBM over analysis based on region of interest (ROI) is that VBM is completely automated and insensitive to anatomical variations. It outputs an unbiased view and is capable of investigating the presence of abnormalities across the whole brain.

The first VBM study on MCI subjects showed marked GM differences predominantly affecting the hippocampal region and the cingulate gyri, extending into the temporal neocortex (Chételat *et al.* 2002). Later on many reports on MCI subjects found GM differences located in several brain regions (Ferreira *et al.* 2011).

The majority of longitudinal VBM studies available in the literature actually use independent cross-sectional VBM analysis for each timepoint. Therefore, although the design of the study is essentially longitudinal, the real dynamic of the atrophy cannot be assessed because of cross-sectional image analysis procedure.

For the present set of studies, the dynamic of GM atrophy between stable and progressive MCI was assessed using a longitudinal VBM approach implemented under SPM8. The design of the study is depicted in Figure 5.2.

Two to one years before conversion, when all subjects were still classified as MCI (Figure 5.2) we found widespread GM loss located in the frontal, limbic and occipital lobes. During 1 year immediately preceding the conversion to AD, GM loss was located mainly in the frontal lobe, cingulate and parahippocampal gyrus.

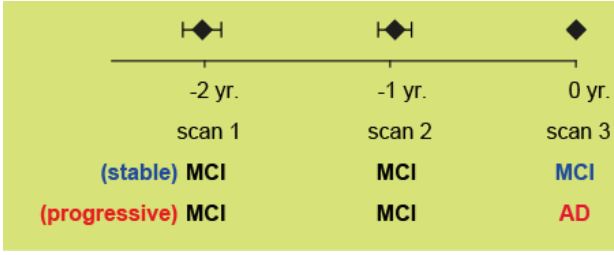


Figure 5.2 Alignment of MR scans relative to the time of conversion to AD

These results point to a severe impairment of the limbic loop components in the progressive MCI group and highlight the importance of atrophy beyond medial temporal areas in predicting conversion to AD.
VBM is a technique that has enormous popularity in the ageing research community, largely because it is relatively easy to use and has provided biologically plausible results. A simple PubMed search will provide around 1000 articles that employed VBM. Despite the relatively large number of "longitudinal" VBM investigations, results are generally difficult to compare and to validate. The specific brain regions related to conversion from MCI to AD are not completely clear.
The variability of the results is mainly due to methodological differences. Although the majority of the processing and analysis is automated in SPM, several methodological decisions remain in the hands of user (*e.g.* the size of the smoothing kernel, corrected or uncorrected results).
VBM is a two-sample approach that dichotomizes subjects (*e.g.* stable and progressive MCI) and because of this, available potentially valuable information is discarded due to methodological limitations. This limitation could be addressed by using time-to-event voxel-based techniques for the assessment of the association between regional atrophy and the risk of progression from MCI to AD (Vemuri *et al.* 2011).
VBM under SPM is for the moment only a research tool because of it outputs results exclusively at group level and the methodology is not yet standardized. The longitudinal VBM algorithm implemented in SPM8 became available very recently, and being able to assess the dynamic of atrophy brings a new dimension in research, which later may become translatable at individual level.
Our data prove that the dynamic of cortical atrophy in areas other than medial temporal lobe is different between progressive and stable MCI subjects. The methods we used identified differences at group level, but these reflect differences in the progression of atrophy at individual level (implicit consideration of consecutive measurements of the same individual).

5.3 MRI DERIVED INDEXES OF SEVERITY

The question: Can MRI measures be integrated under the form of a severity score that will differentiate between different diagnostic entities?

It is well established now that structural MRI is sensitive to neurodegeneration and that volumetric analysis of structural data can be used to quantify the risk of converting from MCI to AD. Brain changes in prodromal AD involves a pattern of widespread atrophy (volumes and thicknesses), with the involvement of different structures across the brain (*e.g.* hippocampus, entorhinal cortex, cingulate gyrus, frontal cortices, etc.). Using a limited set of predefined regions may not reflect the spatial–temporal pattern of structural and physiological abnormalities in their entirety. Therefore, as proved by previous studies, the use of singular, specific structure, as indicators of conversion is most probably not the suited approach.
Computational neuroanatomy is a continuous expanding field of research. In recent years, a number of automated, unbiased, objective techniques have been developed to

characterize structural changes in the brain *in vivo* using structural MRI. These techniques can examine the entire brain, rather than a particular structure, in an unbiased and objective manner, and have the potential to detect changes that are difficult to detect by visual assessment or manual ROI tracing. VBM is one of the most used automated techniques to examine the pattern of brain changes at group level. At individual level, FreeSurfer is one of the most widely used tools for the reconstruction of the cortical surface (surface-based analysis) and for subcortical segmentation (volume-based analysis). These techniques are powerful and can produce big amount of data.

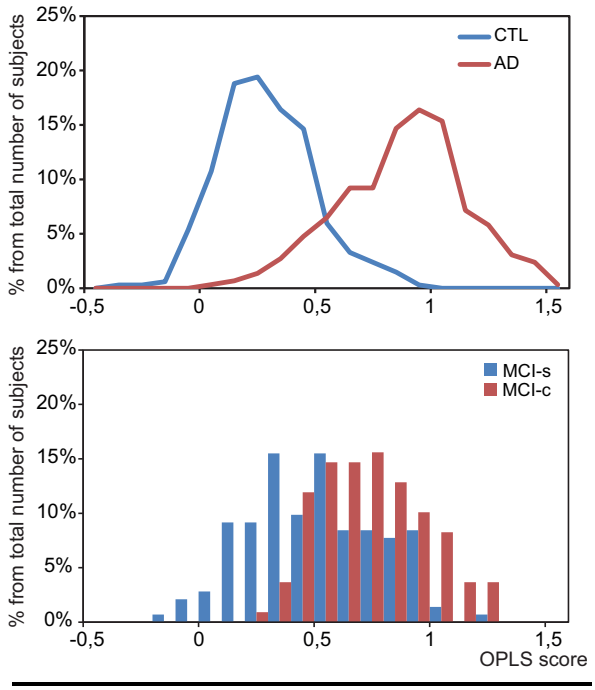


Figure 5.3 The distribution of the OPLS FreeSurfer based index

The question that still remains is how researchers can integrate all these results into more meaningful clinical interventions.

In this study we investigated the possibility of combining MR derived measures (volumes and thickness), under the form of a severity index, using multivariate analysis.

We applied the classification algorithms that distinguished between CTL and AD to generate an atrophy score. The MCI subjects were then tested on the two respective models to investigate if the pattern of atrophy is AD- or CTL-like.

The *OPLS FreeSurfer based index* of the AD and CTL individuals was found to be around 1 for the AD cases and around 0 for the CTL subjects. For the differentiation of CTL *vs*. AD the OPLS score achieved high sensitivity (86.1%) and high specificity (90.5%).

MCI converters were significantly more likely to have the AD-like phenotype (N=112) than the CTL-like phenotype (N=61). 69.6 % of the MCI subjects who converted to AD were predicted as AD-like (See Figure 5.3).

The main reason for low accuracy levels when predicting MCI converters is certainly that MCI stable are a very heterogeneous group: some subjects would convert shortly after the end of the follow-up and are thus in fact prodromal AD patients while others would remain stable for a long period of time. This may indicate that classification methods should be focused on the detection of prodromal AD (*e.g.* MCI converters), which is a much better defined entity. Results point to the fact that considering the whole brain is advantageous mostly at the most advanced stages when the atrophy is much more widespread.

While investigating causes for misclassification, we found that the youngest MCI converters and oldest MCI stable subjects were more often misclassified. So, does age influence the classification accuracy? One explanation could be that ageing is associated with disrupted WM and with alterations of brain tissue properties (*e.g.* intensity and

contrast (Salat *et al.* 2009)), which in turn can disrupt the segmentation step and thus artificially inducing increased atrophy.
Most of the classification algorithms classify AD and CTL subjects with high accuracy, while at prodromal stage, their sensitivity is substantially lower (Cuingnet *et al.* 2011). Results from our study, using multivariate analysis as classification technique, points to the same direction. Combinations with other markers and/or more sophisticated prior knowledge seem necessary to be able to detect prodromal AD with high accuracy.

5.4 WHITE MATTER INTEGRITY ASSESSED BY DTI

The question: To investigate fractional anisotropy as marker of WM integrity in CTL, MCI and AD cases using tract-based spatial statistics.

Although most imaging studies of MCI and mild AD focused GM alterations, post-mortem studies documented WM pathology associated with AD (Bronge *et al.* 2002). Also, the neurofibrillary changes and the Aβ deposition interfere with the neuronal function, producing a series of ultrastructural changes in the axons, the main components of the WM. The resulting damage affects axonal transport, structure of microtubules and neurofilaments and the integrity of the myelin sheath (Bartzokis 2004). All these ultrastructural changes in the WM affect in turn the water diffusivity and this can be measured using DTI parameters (*e.g.* FA, MD, axial diffusivity). Most of the published studies on MCI and AD have inferred impaired WM integrity from findings of both decreased FA and increased MD. The most consistent findings across studies are an impairment of WM integrity in the corpus callosum, temporal lobe, parietal lobe, and the cingulum, with greater posterior than anterior involvement (Head *et al.* 2004).
For the present set of studies, TBSS was used to assess FA differences for three groups: CTL, MCI and AD. Comparisons were performed between: AD *vs.* CTL, MCI *vs.* CTL and AD *vs.* MCI.
A decrease in FA was found for both MCI and AD *vs*. CTL subjects, with a much more extensive pattern for the AD patients (see Table 5.2). Results for AD *vs.* MCI are also presented in Table 5.2.
Because the multiple comparison correction failed in revealing significant clusters, ROI analysis with an *a priori* hypothesis was performed. The ROI analysis was based on the original uncorrected TBSS results. The results of the ROI analysis replicated most of the TBSS results (see Table 5.2).
For MCI subjects, DTI findings are to some extent discrepant, from no change in FA to significant differences in multiple regions. Most probably, DTI analysis methodological issues account for the majority of the discrepancies (*e.g.* acquisition parameters, the ROI selection, corrected or uncorrected results, subtle registration errors, cerebrovascular pathology). Although uncorrected results were reported, these were in agreement with previously published results (Stricker *et al.* 2009). We found low WM integrity for MCI and AD subjects in pathways that are among the latest-myelinating (*e.g.* corticocortical association pathways like the uncinate fasciculus, inferior longitudinal fasciculus, and superior longitudinal fasciculus, commissural pathways like the splenium of corpus calosum and limbic pathways like the fornix). These findings are consistent with the

retrogenesis model in which late myelinated pathways are particularly vulnerable to early degeneration in AD. However, many of the late myelinated pathways also connect MTL structures, so the Wallerian degeneration due to cortical atrophy cannot be ruled out (Coleman 2005). Controlling for GM volume could be one solution to differentiate between the two hypotheses. The FA evaluation combined with other DTI parameters would be helpful as well.

A very interesting finding was reduced FA in the cerebellar tracts. Although previous DTI studies did not report changes in these tracts, we could confirm with these results the GM loss found in the cerebellum for MCI subjects that progressed to AD from the longitudinal VBM study. The presence of Aβ plaques in the molecular and granular layer of the cerebellum (Yamaguchi *et al.* 1988), as well as other concomitant pathologies (*e.g.* ischemic infractions, Lewy bodies) could explain the loss in GM and WM integrity.

Findings from this study have implications for discrepancies in the literature that relate to the issue of greater anterior versus posterior WM changes. Our results indicate the WM is affected both in anterior and posterior regions and maybe is more important the distinction between early and late myelination. This is consistent with the progression of the cognitive changes in AD; relative preservation of motor and sensory functions with memory, language and executive function deficits.

Table 5.2 Anatomic locations of FA differences

Location	**TBSS**			**ROI**
	MCI *vs.* CTL	AD *vs.* CTL	AD *vs.* MCI	AD *vs.* MCI
Parahippocamppus	R	R	L	
Uncinate fasciculus	RL	RL	RL	RL
Inferior longitudinal fasciculus		RL	RL	
Superior longitudinal fasciculus	RL	RL	RL	RL
Cingulum		RL	RL	RL
Corpus callosum-genu		+	+	
Corpus callosum-splenium		+	+	
Body of fornix		+	-	
Anterior arch of fornix		RL	RL	RL
Cerebellum	RL	RL	RL	

R = right, L = left

6 Conclusions

Alzheimer's disease (AD) is the most common cause of cognitive impairment in older people. When considering the impact of AD on patients and families and the growing number of people living to older ages, there is a need to understand the progressive brain changes associated with the development of AD. There is also an urgent need to find treatments for slowing down, stopping and reducing the risk to develop AD symptoms as soon as possible.

Recent hypotheses suggest that AD biomarkers become abnormal in a temporally ordered manner as the disease progresses. Although structural MRI is the last to become abnormal, it does retain a closer relationship with the cognitive performance later into the disease course (Jack *et al.* 2005). It should be stressed that neuropathological data does not fit this hypothetical representation. Braak *et al.* have shown, as early as in 1997, that tau pathology in the entorhinal-hippocampal regions precedes Aβ accumulation by decades.

Therefore, MRI provides crucial information, particularly since the patients typically get in contact with the medical system when the symptoms are becoming evident and pathology has presumably already reached the critical threshold. Moreover, the MRI-based estimates of progression might be used to assess potential disease-modifying drugs.

Mild cognitive impairment (MCI) represents an intermediate state of cognitive function between the changes seen in ageing and those fulfilling the criteria for dementia and often AD (Petersen *et al.* 1999). Although MCI is a very heterogeneous condition and not all MCI subjects will progress to AD, this is nevertheless an intermediate state that is susceptible to MRI changes.

The aim of this thesis was to investigate structural correlates for the progression from MCI to AD using different image analysis techniques for structural MRI.

Whole brain atrophy rates, measured from two serial MRI scans, were associated with an increased risk of subsequent conversion to AD. Rates of whole brain atrophy in AD diverge from normal approximately 4 years before the dementia threshold is crossed and it has been estimated at 1.4-2.2% per year, whereas rates of atrophy during normal ageing usually do not exceed 0.7% per year (Frisoni *et al.* 2010, Ridha *et al.* 2006). Whole brain atrophy accelerates in sporadic AD during the transition from normality to cognitive impairment and as MCI individuals progress to AD (Jack *et al.* 2005). Measures of whole brain atrophy rates are indicators of widespread tissue loss throughout the brain and they could be valuable clinical markers when assessing advanced disease stages.

Structural MRI gains predictive power when atrophy is estimated at regional level. Therefore, we used longitudinal voxel based morphometry (VBM) to assess the differences in the dynamic of regional grey matter atrophy between stable and progressive MCI. To this end we used three MRI scans aligned to the time of AD diagnosis and we detected a significant difference in the dynamic of the atrophic changes between stable and progressive MCI. Results from this study point to severe impairment of the frontal, limbic and occipital lobes and highlight the importance of atrophy beyond the medial temporal lobe.

VBM outputs results exclusively at group level. Therefore, in study III we investigated whether the combination of multiple MRI-based features can improve classification accuracy for those MCI subjects that will progress to AD at individual level. Based on volumetric and cortical thickness measures and using multivariate analysis (orthogonal projection to latent structures method) a MRI atrophy index was generated. The index was estimated for the MCI subjects, which were classified as AD- or CTL-like. Most MCI converters had AD-like index, while the stable MCI subjects presented a more heterogeneous pattern.

The major challenge is to determine optimal cut-off points for such parameters and to compare their relative reliability (alone and in combination). It still needs to be clarified to what extent this type of scores can be used alone or in combination with other measurements in order to predict subsequent cognitive decline in MCI subjects. Relatively few studies use multivariate approaches to predict decline in MCI subjects. Markers of neural dysfunction such as FDG-PET and structural MRI atrophy are better indicators of more progressed individuals and, as such, might be more effective in predicting imminent decline (Chen *et al.* 2011, Landau *et al.* 2010). There is a need to continue to develop, test and compare algorithms that can capitalize on the wealth of data and that can summarize the pattern of measurements or time-dependent changes in single measurements (*e.g.* MR derived index of severity) that could later on be translated in the clinical settings.

Post-mortem and MRI studies of ageing have provided knowledge about white matter (WM) abnormalities in AD. DTI captures unique variance associated with neurodegenerative brain changes. Knowledge of the pattern of WM microstructural changes in AD and its underlying mechanisms may contribute to earlier detection and intervention in groups at risk for AD, especially if WM abnormalities are not entirely due to changes in cortical volume. Using tract based spatial statistics, we investigated fractional anisotropy (FA) as a marker of WM integrity in healthy controls, MCI and AD cases. Decreased FA was found for both MCI and AD when compared to CTL subjects, with a much more extensive pattern for the AD subjects.

In conclusion, T1-weighted structural MRI and DTI are integral components of the predementia and MCI assessment. Markers of both structural MRI and DTI support earlier and more precise diagnosis and measurement of progression. The future use of imaging in the clinical evaluation of MCI is also related to the development of effective medication treatments. Nonetheless, standardized MR acquisition protocols and an efficient quality control, both before and after image analysis, are fundamental for empowering the future role of MRI for the benefit of the patient. MRI, through structural and diffusion tensor imaging, is well placed to contribute to early diagnosis of AD and to monitoring treatments under evaluation for this devastating disease.

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Gabriela Spulber

Imaging the progression from Mild Cognitive Impairment to Alzheimer's disease

Alzheimer's disease (AD) is the most common cause of cognitive impairment in older people and can be diagnosed only at dementia stage. There is a pressing need for timely identification of AD so that interventions can begin early. This thesis focuses on identifying structural MRI parameters that differentiate between stable and progressive mild cognitive impairment (MCI) subjects prior to clinical conversion to AD. Results from this work indicate that structural MRI biomarkers support the assessment of progression to AD, and are therefore integral components in the evaluation of MCI patients.

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