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Brain Atrophy in Mild Cognitive Impairment: MRI volumetric and voxel-based method study

Doctoral dissertation

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ABSTRACT

Background: Mild cognitive impairment (MCI), a heterogeneous status, has gained increased interest from clinicians and researchers, because it has been shown to represent a transitional stage between normal ageing and very early Alzheimer's disease (AD). Neuroimaging data, genetic findings, biological markers and neuropsychological tests have been evaluated as predictors of conversion from MCI into dementia, most commonly AD. Magnetic resonance imaging (MRI) using volumetric measurements of regions of interest (ROI), as well as more advanced imaging techniques, such as voxel based morphometry (VBM), increasingly used in studying AD patients, now were engaged in studying MCI subjects to determine which of them are to convert into dementia. Apolipoprotein E (APOE) allele ε4 is the most consistently confirmed genetic risk factor for Alzheimer's disease (AD).
Objectives: By using MRI volumetric studies to compare MCI subjects with controls and patients with mild AD, we intended to determine whether the entorhinal atrophy precedes hippocampal atrophy in AD. Moreover, with the help of VBM we wanted to map the gray matter loss in the entire brains of MCI subjects. As previous studies have suggested that the APOE genotype can influence the size of various brain structures, our objective was to investigate the difference in the morphologic expression of MCI in subjects carrying the APOE allele ε4 compared to the noncarriers using VBM. Finally we intended to conduct a follow-up study and determine the predictors for conversion into AD.
Results: In the ROI-volumetric MRI study, we showed that the entorhinal volume loss predominated over the hippocampal volume loss in MCI, whereas more pronounced hippocampal volume loss appeared in mild AD. Using VBM in MCI subjects vs. controls, the greatest atrophy was found in the right hippocampus-amygdala region and in the right hippocampal tail and thalamus, while less extensive areas of atrophy were detected in the right superior temporal gyrus, the left thalamus, the left inferior temporal gyrus, and the left anterior cingulated gyrus. The extent of the atrophy was significant in the medial temporal lobe, on the right side. Between cases heterozygous for the APOE ε4 and those who were APOE ε4 noncarriers, only the right parahippocampal gyrus, with entorhinal cortex included, reached a level of statistical significance. In cases homozygous for the ε4 allele vs. noncarriers, the greatest atrophy was located in the right amygdala followed by the right parahippocampal gyrus, the left amygdala and the left medial dorsal thalamic nucleus. During the follow-up time of about 34 months, 21.7 % of the MCI subjects converted into dementia, with 15% developing AD. The right hippocampal and entorhinal volumes significantly predicted the conversion into AD.
Conclusions: In the present study, the ERC atrophy appears to be dominant over the hippocampal atrophy in MCI, whereas more pronounced hippocampal atrophy was seen in mild AD. The VBM method revealed involvement of other brain areas, in addition to the MTL, in the state of MCI. The vast majority of the brain atrophy observed in individuals with MCI appears to be due to the small group homozygous for the ε4 allele. The atrophy of the MTL seen in the baseline, predicted the conversion to AD during a follow-up of 34 months, while the severity of cognitive impairment, the white matter lesions or the APOE provided no additional contribution to the prediction of conversion of MCI to AD.

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Medical Subject Headings: Alleles; Alzheimer Disease/physiopathology; Amygdala/pathology; Apolipoproteins E/genetics; Atrophy; Brain/pathology; Cognition Disorders/genetics; Dementia/physiopathology; Entorhinal Cortex; Hippocampus/physiopathology; Magnetic Resonance Imaging/methods

To Jussi, Carmen and Robert

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Corina Pennanen

ABBREVIATIONS

AACD	Age-associated cognitive decline
AAMI	Age-associated memory impairment
AD	Alzheimer's disease
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ApoE	Apolipoprotein E protein
APOE	Apolipoprotein E gene
BBSI	Brain boundary shift integral
BSI	Boundary shift integral
CDR	Clinical Dementia Rating
CERAD	Consortium to Establish Registry for Alzheimer's Disease
CI	Confidence interval
CMRgl	Cerebral metabolic rates for glucose
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic acid
EADC	European Alzheimer's Disease Consortium
EEG	Electroencephalography
ERC	Entorhinal cortex
FLAIR	Fluid-attenuated inversion-recovery
GDR	Global Deterioration Scale
GM	Gray matter
HC	Hippocampus
HR	Hazard ratio
ICA	Intracranial area
ICD 10	International Classification of Diseases
MCI	Mild cognitive impairment
MMSE	Mini-Mental Status Examination
MRI	Magnetic resonance imaging
MTL	Medial temporal lobe
MCADRC	Mayo Clinic Alzheimer's Disease Research Center
NFT	Neurofibrillary tangle
NIA	National Institute of Aging-Reagan Institute
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association
PET	Positron emission tomography
PD	Proton density
ROI	Region of interest
SD	Standard deviation
SPSS	Statistical package for social sciences
T	Tesla
T1	Longitudinal relaxation
T2	Transverse relaxation
TE	Time of echo
TR	Time of repetition
VBM	Voxel-based morphometry
VBSI	Ventricular boundary shift integral
WAIS-R	Wechsler Adult Intelligence Scale-Revised
WM	White matter
WML	White matter lesion

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications referred to in the text by the Roman numerals **I-IV**:

I. **Pennanen C**, Kivipelto M, Tuomainen S, Hartikainen P, Hänninen T, Laakso MP, Hallikainen M, Vanhanen M, Nissinen A, Helkala E-L, Vainio P, Vanninen R, Partanen K, Soininen H. Hippocampus and entorhinal cortex in mild cognitive impairment and early AD. Neurobiol Aging. 2004;25:303-310.

II. **Pennanen C**, Testa C, Laakso MP, Hallikainen M, Helkala E-L, Hänninen T, Kivipelto M, Kononen M, Nissinen A, Tervo S, Vanhanen M, Vanninen R, Frisoni GB, Soininen H. A voxel based morphometry study on mild cognitive impairment. J Neurol Neurosurg Psychiatry. 2005;76:11-14.

III. **Pennanen C**, Testa C, Boccardi M, Laakso MP, Hallikainen M, Helkala EL, Hänninen T, Kivipelto M, Könönen M, Nissinen A, Tervo S, Vanhanen M, Vanninen R, Frisoni GB, Soininen H. The effect of apolipoprotein polymorphism on brain in mild cognitive impairment - a voxel-based morphometric study. Dement Geriatr Cogn Disord 2006;22:60-66.

IV. Tapiola T, **Pennanen C**, Tapiola M, Tervo S, Kivipelto M, Hänninen T, Pihlajamäki M, Laakso MP, Hallikainen M, Hämäläinen A, Vanhanen M, Helkala E-L, Vanninen R, Nissinen A, Rossi R, Frisoni G, Soininen H. MRI of hippocamus and entorhinal cortex in mild cognitive impairment: a follow-up study. Neurobiol Aging. *Accepted for publication.*

CONTENTS

1. INTRODUCTION

Dementia is a major cause of disability in the developed countries and places a considerable financial burden on the medical services and health care systems (Erkinjuntti et al., 1986; Hay and Ernst, 1987). An important public health concern of developed countries is aging of the population and the associated increases in the prevalence of various dementias, particularly Alzheimer's disease (AD). The concept of mild cognitive impairment (MCI) refers to a transitional stage between normal ageing and very early AD. MCI, a heterogeneous entity, has been associated with a 10-fold risk for developing dementia, most commonly AD (Petersen et al., 2001 a). AD is one of the most important causes of dementia, and one of the most important reasons for the need for long-term patient care. Therefore, an increasing interest has been focussed on MCI in research into aging-related cognitive disorders, particularly in identifying early AD both for research purposes and as a way to achieve early therapeutic intervention. An annual conversion rate of 6-25% from MCI to AD has been reported, which greatly exceeds that seen in the normal population (1-2%) (Petersen et al., 2001 a). Nevertheless, the epidemiology of MCI is not well known, and some longitudinal population based studies have cast some doubt on the concept of MCI. In the study by Larrieu et al., an annual conversion rate of 8.3% was observed during a five-year period, but again the cases had a tendency to fluctuate, and as many as 40% of MCI cases reverted to normal instead of progressing to dementia during follow up (Larrieu et al., 2002).

Subjects with MCI are characterised by memory complaints, normal general cognitive functions, impaired memory for age, preserved activities of daily living, and they are not demented, but the criteria used for diagnosing MCI have varied in different studies. For this reason, new recommendations for the general criteria for MCI, as published by the Stockholm consensus group, suggesting that the MCI construct should be expanded to include cognitive impairment in other domains such as language, attention, visuospatial skills, perceptual speed, and executive function (Winbland et al., 2004). Several approaches have been attempted to identify among MCI subjects, those who will progress to dementia and AD. The heterogeneity in the use of term has been recognised and independently from the criteria used to diagnose the MCI, predicting factors to AD, such as neuroimaging data, genetic findings, biological markers, and neuropsychological tests have been suggested. For that purpose magnetic resonance imaging (MRI) using volumetric measurements of regions-of-interest (ROI) have been used in many studies. It was shown that the neuropathological changes

occurring in AD develop gradually, first appearing in the entorhinal cortex (ERC) and later progressing to the isocortical areas. Accordingly, studies in vivo on brains such as volumetric MRI indicated that atrophy of the hippocampus and the ERC could be a sensitive indicator of early AD (Jack et al., 1999; Killiany et al., 2002). Moreover, atrophy of the ERC has been suggested to predict AD during its preclinical stage (Killiany et al., 2002). While volumetric studies focus on selected ROIs, voxel-based morphometry (VBM) is able to map the entire brain and to provide a global picture of the studied group of subjects with high reproducibility (Ashburner et al., 2003). Apolipoprotein E (APOE) ε4 allele is the most consistently confirmed genetic risk factor for AD. The APOE ε4 has been associated with the development of AD among individuals with MCI (Petersen et al., 1995) and it is also associated to amnestic-MCI (Lopez et al., 2003). The ε4 carriers show an earlier age of onset and enhanced AD related pathology compared to non-carriers (see for review Lehtovirta et al., 2000) and more pronounced atrophy in the medial temporal lobe (MTL) structures (Juottonen et al., 1998 a; Geroldi et al., 1999; Geroldi et al., 2000). Finally, to conclude what are the neuropsychological, brain imaging and genetic factors that better predict the conversion of MCI subjects to dementia, especially to AD, we need to conduct follow-up studies of MCI subjects.

In this study, brain atrophy was examined using two different imaging methods for volumetric measurements in a preclinical stage of AD so called MCI, and the predicting value of the MTL atrophy for developing AD, together with other features characteristic for AD were examined in MCI subjects.

2. REVIEW OF THE LITERATURE

2.1. Aging, Dementia and AD

2.1.1. Aging

Successful aging can be accounted for surviving and functioning. A decline in cognitive functioning that affects on the daily life will conduct towards dementia. Therefore, bringing up the subject of AD, dementia or mild cognitive impairment, one would first stop on the condition of aging, with different descriptions: usual, normal, and successful (Rowe and Kahn, 1987). Unfortunately, forgetfulness is present to some degree, from time to time in almost all of us. Memory function as measured by delayed recall of newly learned material is not clearly impaired in most elderly individuals (Geffen et al., 1990; Mitrushina et al., 1991; Petersen et al., 1992; Knopman et al., 2003). Nonetheless, variability has been proposed to be increased with age, and life style and psychosocial factors to have influence on the heterogeneity of the older population and their aging process (Rowe and Kahn, 1987). While normal aging can be associated with a deterioration in different aspects of cognitive performance, the progression of mentioned phenomena could transform normal aging into dementia.

2.1.2. Definition of dementia

Dementia is a major public health problem, with no *mercie* for gender or for different ethnic and socioeconomic groups. Just as fever is attributed to many etiologies, dementia is a non-specific term that encompasses many disease entities. The word dementia is derived from latin *de-* "apart, away" and *mens* - on genetive, *mentis* "mind", so *de mens* - "without mind" and it is used scientifically for describing a progressive decline in cognitive function due to damage or disease in the brain, beyond what might be expected from normal aging. The term cognition comes from Latin as *cogito*, "to think" and it is used in several loosely-related ways to refer to a facility for the intelligent processing of information. In other words, dementia is a syndrome of brain dysfunction, with symptoms dependent upon the etiology of the disease. The affected areas may be memory, attention, language and problem solving, although particularly in the later stages of the condition, affected persons may be disoriented in time, place and identity.

There are different sets of criteria available for the diagnosis of dementia, as many authors and associations have tried to define it (American Psychiatric Association 1980 (DSM-III), 1987 (DSM-III-R) and 1994 (DSM-IV); Small et al., 1982; Cummings and Berson, 1983; McKhann et al., 1984; Dementia. Council on Scientific Affairs**,** 1986). The Diagnostic and Statistical Manual of Mental Disorders (DSM) of the American Psychiatric Association provides the most frequently used definition of dementia in the version III (DSM-III) (American Psychiatric Association, 1980), in the revised third version (DSM-III-R) (American Psychiatric Association, 1987), and in the version IV (DSM-IV) (American Psychiatric Association, 1994). The DSM-IV defines dementia as "the development of multiple cognitive deficits that include memory impairment and at least one of the following: aphasia, apraxia, agnosia, or a disturbance in executive functioning" (American Psychiatric Association, 1994.). The executive functioning is seen as the ability to inhibit inappropriate answers and to select behaviors for action. Thus, the memory impairment is necessary for defining this condition and the disturbances should interfere with the daily life. The cognitive impairment must be more pronounced than encountered in normal aging.

2.1.3. Alois Alzheimer and Alzheimer's disease

In medicine, eponymy has been traditionally used to memorialize the first diagnostician of a syndrome or a disease. That happened also with AD. Alois Alzheimer was born in June 14, 1864 in Marktbreit near Wurzburg in Franconia, Germany. In 1888 he obtained his first post at the Mental Asylum, Irrenanstalt, in Frankfurt am Main, where later, the 51-year-old patient, Frau Auguste D was admitted, on November 25, 1901 and died on April 8, 1906. Alzheimer described her case of presenile dementia at the meeting of South-West Germany Psychiatrists in Tubingen, 1906 and published his presentation in 1907 (Alzheimer, 1907). While in life, the patient had shown a progressive decline in cognitive function, disorientation, aphasia, delusions, and psychosocial incompetence, at autopsy there were plaques and neurofibrillary tangles (NFT) and arteriosclerotic changes (Maurer et al., 1997). This was the original AD patient. As Bick KL remarked in the detailed historical description of Alzheimer's disease (Terry et al., 1999), "Alzheimer did not bestow his name on the condition he described", in fact Emil Kraepelin was the individual who introduced this disease as Alzheimer's disease in the category of presenile dementias in the 1910 edition of his textbook (Kraepelin, 1910). On the other hand, Alzheimer did not object to Kraepelin's designation, as he refers to it in his

publication (Alzheimer, 1911 - *English translation in* Förstl and Levy, 1991) on the report of a second patient with Alzheimer's disease, the case of 56-year-old male, Johann F. He states that Kraepelin has already given a summarized account on this disease and called it "Alzheimer's disease" (Möller and Graeber, 1998).

2.1.4. Diagnosis of AD

2.1.4.1. Clinically

Dementia has attracted extreme interest from public, clinicians and researchers, as the world population is aging. A major cause of dementia has been proposed to be AD, a progressive neurodegenerative disease, with a gradual onset varying between 40 and 90 of age and with a life span varying between 5 to 15 years after onset. The National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984) presented the most widely used criteria for clinical diagnosis of AD (Table1). The NINCDS-ADRDA criteria for clinical diagnosis of probable AD are equivalent with the criteria for defining the dementia of the Alzheimer type (DSM-IV) (American Psychiatric Association, 1994). Early diagnosis of AD is mainly based on clinical, neuropsychological, and brain imaging findings and exclusion of other possible causes for dementia, but no definite early tool for diagnosing the disease in point has been found until today. According to the NINCDS-ADRDA criteria, AD is divided into three categories: possible, probable and definite AD. The diagnosis of definite AD can be confirmed only by histopathological examination of the brain tissue obtained either by biopsy or at autopsy. Before that, a diagnosis of probable or possible AD has to be accepted (Table 1). Shortly, patients with probable AD are characterized by gradual onset and progression of memory and cognitive decline, and do not present any signs of other disorders that could cause dementia. The diagnosis of AD is supported by: progressive deterioration of specific cognitive functions such as language (aphasia), motor skills (apraxia), and perception (agnosia); impaired activities of daily living and altered patterns of behavior; family history of similar disorder, particularly if confirmed neuropathologically; normal cerebrospinal fluid (CSF) samples as evaluated by standard techniques; normal pattern or nonspecific changes in electroencephalography (EEG); and evidence of cerebral atrophy on brain imaging with progression documented by serial observation.

Table 1. The criteria for diagnosing AD according to the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer ’s disease and Related Disorders Association (NINCDS-ADRDA)

Criteria for clinical diagnosis of PROBABLE Alzheimer's disease: -dementia established by clinical examination and documented by objective testing; -deficits in two or more areas of cognition; -progressive worsening of memory and other cognitive functions; -no disturbance of consciousness; -onset between ages 40 and 90; -absence of systemic disorders or other brain diseases that in and of themselves could account for the progressive deficits in memory and cognition. Criteria for clinical diagnosis of POSSIBLE Alzheimer's disease: -may be made on the basis of the dementia syndrome, in the absence of other neurologic, psychiatric, or system disorders sufficient to cause dementia, and in the presence of variations in the onset, in the presentation, or in the clinical course; -may be made in the presence of a second systemic or brain disorder sufficient to produce dementia, which is not considered to be the cause of dementia; -should be used in research studies when a single, gradually progressive severe cognitive deficit is identified in the absence of other identifiable cause. Criteria for diagnosis of DEFINITE Alzheimer's disease are: -the clinical criteria for probable Alzheimer's disease; -histopathologic evidence obtained by biopsy or autopsy.

2.1.4.2. Neuropathologically

The neuropathological hallmarks of AD are amyloid plaques and neurofibrillary tangles (NFT) (Khachaturian, 1985; Braak and Braak, 1991 b; Mirra et al., 1991), but also other important signs are present: neuronal loss (Gomez-Isla et al., 1997) and synaptic loss (Hamos et al., 1989). The one neuropathological abnormality required for the definite diagnosis of AD is an adequate number of extracellular amyloid plaques (Khachaturian, 1985). This is according to the currently used neuropathological diagnostic criteria for AD, the Neuropathology Task Force of the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) (Mirra et al., 1991), a hallmark feature of the disease. The second neuropathological hallmark is the presence of neurofibrillary tangles (NFT) found inside the neurons, which form the basis of the Braak&Braak criteria with topographical staging (Braak and Braak, 1991 a; Braak and Braak, 1995). Accordingly, six stages of disease propagation can be distinguished with respect to the location of the NFTs: “transentorhinal stages I-II: clinically silent cases; limbic stages III-IV: incipient Alzheimer's disease; neocortical stages

V-VI: fully developed Alzheimer's disease" (Braak and Braak, 1995). Recently, a new set of criteria - based on both guidelines of CERAD and Braak&Braak criteria – was proposed by the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer's disease (NIA) (NIA, 1997). The fact is that there are aged people free of any symptoms, and nevertheless on autopsy they present the neuropathological changes characteristic of AD. It was concluded in a recent review that disease-related pathological changes are the signs for an incipient disease like AD, rather than the effects of aging, even though there is a lack of clinical symptoms (Thal et al., 2004).

2.2. MCI

2.2.1. The concept of MCI

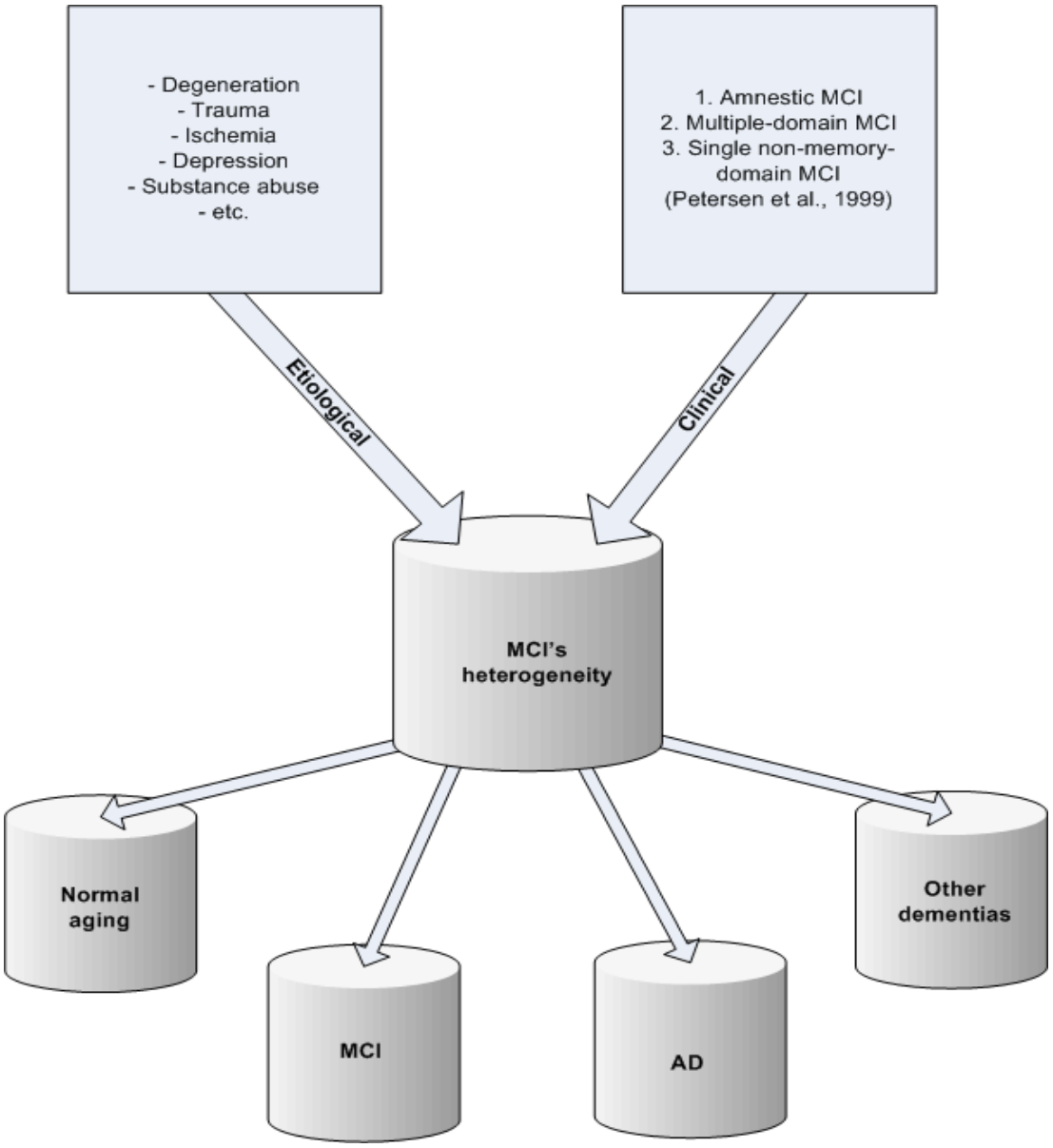
MCI is currently the most widely used concept in classifying cognitive impairment in the elderly who do not fulfil the criteria for dementia. Up to the present, a great interest was given to the changes responsible for cognitive impairment that are providing towards dementia, or to AD. For that, the boundary between normal aging and early AD has become the area of major interest for researchers and clinicians for theoretical and practical reasons (Petersen et al., 2001 a). Over the years, different concepts have been used to identify an intermediate stage of cognitive changes, for example: Benign senescent forgetfulness (Kral, 1962), Age-associated memory impairment (AAMI) (Crook et al., 1986), Age-associated cognitive decline (AACD) (Levy, 1994), Age-related cognitive decline (DSM-IV) (American Psychiatric Association, 1994), Mild cognitive disorder (ICD 10), Cognitive impairment - no dementia (Graham et al., 1997), Mild Cognitive Impairment (MCI) (Smith et al., 1996; Petersen et al., 1999). The concept of MCI has been the most recent research topic with still open questions on a large spectrum of issues involved in it (Petersen, 2003).

It should be noted that mildly impaired non-demented subjects form a heterogeneous group that includes stable subjects and subjects who will develop AD (Petersen, et al., 1995; Petersen, et al., 1999; Petersen et al., 2001 a), but also subjects that revert to normal aging (Larrieu et al., 2002). The heterogeneity (Figure 1) of the MCI group consisting of subjects declining to non-AD dementia, those progressing to AD, those that are stable and those that will revert to normal aging, may be viewed from two perspectives (Petersen, 2003): etiological or clinical presentation (Petersen et al., 1999). The most of decliners to AD are

those from the amnestic-MCI group of subjects, and they are characterized by impairment of the memory to 1.5 SD below age- and education matched normal subjects (Petersen et al., 1999). The memory impairment is a clinical judgment based on the subject's history, on the clinician's examination and on the neuropsychological profile, and it represents the main feature in the definition of MCI. Subsequently, memory impairment will remain the most important feature in diagnosing dementia, while other cognitive disturbances will interfere with the daily life (DSM-IV) (American Psychiatric Association, 1994).

In the Mayo Clinic, a progression rate of almost 12% per year was seen in declining from MCI to dementia or to probable AD, while the control cohort declined to MCI or to AD with a rate of only 2% per year (Petersen et al., 1999). The Report of the Quality Standards Subcommittee of the American Academy of Neurology reviewed in 2001 a number of studies and, even though these studies had used various criteria for MCI, they indicated an annual conversion rate of 6%–25% from MCI to AD (Petersen et al., 2001 a). Thus, investigating MCI might provide a means to study AD in its earliest phases and this might prove beneficial in terms of finding preventive or interventive measures for AD. However, some recent longitudinal population based studies have cast some doubt on the concept of MCI. Ritchie and colleagues suggested that MCI is a poor predictor of dementia, that the group is unstable, with cases changing category almost yearly, and they called for modifications to the current criteria (Ritchie et al., 2001). In the study by Larrieu et al. (2000), an annual conversion rate of 8.3% was observed during a five-year period, but again the cases had a tendency to fluctuate, and as many as 40% of MCI cases reverted to normal instead of progressing to dementia during the follow up. When considering the new recommendations for MCI criteria (Winbland et al., 2004), Alexopoulus and colleagues (2006 a) found that 17% of the MCI subjects returned to normal over a mean follow-up period of time of 3.5 years. The multiple-domain type of MCI had a less favorable prognosis than the amnestic type in progressing to dementia ($p<0.014$) (Alexopoulos et al., 2006 b). Contrary, Yaffe and colleagues (2006) found that nonmemory and multiple-domain MCI subjects were less likely to progress to dementia than amnestic MCI subjects were, while among those that converted to AD, most had prior amnestic MCI.

Figure 1. The meaning of heterogeneity in MCI subjects. Both, the etiology of MCI and the clinical presentation will affect the heterogeneity of MCI group of subjects (adapted from the book edited by Petersen RC, 2003). Accordingly, MCI subjects will revert to normal, remain stable, or progress to AD or other dementias with time. The clinical presentation after Petersen et al.: Amnestic MCI – memory impaired to 1.5 SD below age- and education-matched normal subjects, while other domains are impaired to 0.5 SD; Multiple-domain MCI – several cognitive domains impaired to 0.5 SD below age- and education- matched normal subjects; Single non-memory-domain MCI – impairment is seen in other cognitive functions, for example in: executive function, visuospatial function, language.



In different studies, different criteria for defining MCI subjects have been used and the source of study differs, yielding different results. In some studies, the subjects are those who have actually sought an evaluation from a dementia clinic for different symptoms, and in others, they derive from community-based settings. Thus, differences exist in study designs, and ultimately the investigators need to interpret the results according to the limitations of their own study settings. Many ongoing studies continue to concentrate on MCI as a transitional stage between normal aging and AD or dementia.

2.2.2. Diagnosis of MCI

The criteria for MCI have been refined with time, but those used by many studies, including the current work, were based on the adaptation of the criteria suggested by Mayo Clinic Alzheimer's Disease Research Center (MCADRC) (Petersen et al., 1995; Smith et al., 1996):

1) memory complaint by patient, family or physician;
2) normal activities of daily living;
3) normal global cognitive function;
4) objective memory impairment or impairment in one other area of cognitive function as evidenced by scores >1.5 SD below age apropiate mean;
5) Clinical Dementia Rating score of 0.5; and
6) not demented.

Later those criteria were modified (Petersen et al., 1999):

1) memory complaint;
2) normal activities of daily living;
3) normal general cognitive function;
4) abnormal memory for age;
5) not demented.

A group of experts in aging and MCI (Petersen et al., 2001 b) and the subcommittee of the American Academy of Neurology (Petersen et al., 2001 a) have summarised the criteria for MCI, and placing the emphasis on the heterogeneity of this group of subjects, they defined 3 subgroups (Petersen et al., 2001 b): amnestic-, multiple domains slightly impaired- and single nonmemory domain MCI. Thus, MCI criteria became:

1) memory complaint, preferably corroborated by an informant
2) objective memory impairment
3) normal general cognitive function
4) intact activities of daily living
5) not demented.

Moreover, the amnestic MCI, considered as being the precursor for AD, is defined as:

1) memory complaint, preferably corroborated by an informant
2) impaired memory function for age and education
3) preserved general cognitive function
4) intact activities of daily living
5) not demented.

In 2003 during the First Key Symposium in Sweden, on the topic of MCI, an international multidisciplinary group of experts discussed the current status and new perspectives on clinical, cognitive neuroimaging, biomarkers and genetics fields in MCI. They gave recommendations for the general criteria for MCI, and published them in 2004 (Winblad et al., 2004):

1) Not normal, not demented (does not meet the DSM-IV, ICD 10 criteria for a dementia syndrome)
2) Cognitive decline:
- self and /or informant report and impairment on objective cognitive tasks
and/or
-evidence of decline over time on objective cognitive tasks
3) Preserved basic activities of daily living/minimal impairment in complex instrumental functions.

This new set of criteria allows inclusion of deficits in cognitive domains other than memory, while the amnestic MCI would be then left as the precursor for AD, rather than for other dementias. Still, the clinical criteria as proposed by Petersen and colleagues, have varied from one study to another. Nevertheless, the criticisms raised against MCI criteria have not disappeared, and perhaps the criteria will be modified in the future. Recently, during a session of the MCI working group from the European Alzheimer's Disease Consortium (EADC), in 2005 a new set of criteria were presented for review (Portet et al., 2006):

1) cognitive complaint emanating from the patient and/or his/her family
2) the subject and/or informant report a decline in cognitive functioning relative to previous abilities during the past year
3) cognitive disorders evidenced by clinical evaluation: impairment in memory and/or another cognitive domain
4) cognitive impairment does not have major repercussions on daily life. However, the subject may report difficulties concerning complex day-to-day activities,
5) no dementia.

However, based on this report of the EADC, Portet and colleagues considered that in addition to those proposed criteria that would identify patients at risk to develop dementia, some more elaborate tests, like for example neuroimaging, will be still needed to determine the underlying cause of a diagnosed MCI state (Portet et al., 2006).

2.3. Imaging as an *in vivo* tool to study the brain

In studying the *normal state* of human brain and diagnosing cognitive pathology, MRI gained an important role as it provides qualitative and quantitative *in vivo* insights into brain morphology. New techniques are being developed continuously, and neuroimaging has become part of the diagnostic process of persons suspected for dementia. Quantitative structural studies are conducted both cross-sectionally and longitudinally and consist of volumetric measurements that focus on selected ROI, and computational methods that give insights into the global morphological changes in brain.

The goal for imaging studies is to achieve a consensus on applying imaging methods for early and differential correct diagnosis in dementia as a routine clinical procedure, as well as for monitoring the progress and outcome in drug trials. For that rationale, a "combination of a distinctive biochemical profile with quantitative neuroimaging, used in conjunction with clinical and neurobehavioral assessment" is urgently needed, as remarked by Black (1999) in what concerns the diagnosis of AD. According to the practice parameter on dementia (Knopman et al., 2001) the neuroimaging with MRI or computed tomographic (CT) plays a key role in the initial evaluation of a demented patient, as a means of ruling out structural lesions such as brain tumors, abcesses, strokes and hematomas. For this reason, the practice parameter supported the use of MRI or CT at the time of the initial dementia assessment. The Consensus Report of the Alzheimer's Association Neuroimaging Work Group recommended an extension of the guidelines of the American Academy of Neurology to include brain imaging as a part of the dementia evaluation in subjects when not only AD is suspected but also including amnestic MCI subjects (Neuroimaging Work Group, Alzheimer's Association. Consensus Report, 2004). The Work Group has recommended standardised analyses on the structural MRI, including the brain measurements of global loss and MTL volume, with the accent on longitudinal structural MRI study on brain atrophy rates that would provide information useful for the design of treatment studies (Neuroimaging Work Group, Alzheimer's Association. Consensus Report, 2004).

In what concerns the clinical practice, the visual rating method has high potential to be used in the clinical routine procedure for dementia investigations in the future. The visual rating method developed by Scheltens et al. (1992, 1995) has been applied to determine its value in differentiating AD patients from controls based on the MTL evaluation and has been

compared to that of a stereological volumetry method, a simplified version of manual tracing (Wahlund et al., 1999; Wahlund et al., 2000).

As for monitoring treatment effect in drug trials in AD promising results have been obtained by the boundary shift integral (BSI) method, an algorithm developed by Fox and colleagues (1996) and tested for brain (BBSI)-derived changes registered from serial MRI (Freeborough and Fox, 1997 and Fox and Freeborough, 1997). The BSI technique has been recently tested also for ventricular (VBSI)-changes and compared to BBSI changes in trials of a follow-up of 6 months and 1 year (Schott et al., 2005).

The BBSI and VBSI rates of change have been also compared to the ROI-based methods for ERC and hippocampus atrophy rates in classifying AD patients and controls (Ezekiel et al., 2004). While the rates of change were VBSI > ERC & hippocampus > BBSI in both groups, the combined rates of ERC and VBSI were the best explanatory variables for differentiation between the two groups of subjects. However, this study suggested that ERC and hippocampal atrophy rates might be more sensitive than BBSI or VBSI in monitoring the AD progression and the potential effects of disease modifying agents.

The neuroimaging methods and their efficacy differ according to the intention of use, such as clinical or research imaging tool, and the characteristics of those instruments with potential use in different clinical settings, are found in the consensus paper produced by the Neuroimaging Working Group of the European Alzheimer's Disease Consortium (EADC) (Frisoni et al., 2003).

The present work focuses on the most widely used neuroimaging tools for assessing the brain morphology, the manual tracing ROI-based MRI volumetry and the voxel-by-voxel method.

2.3.1. Imaging in normal aging brain - a view towards AD

2.3.1.1. Structural MRI and gray matter

Before one can define the *abnormal* there has to be a guideline for what is *normal*. Therefore, the imaging of the normal aging brain is an important issue, both for an *in vivo* regular anatomical presentation of the brain, and for the changes that are related to it.

Neuropathological studies have revealed changes in brain with advancing age. There is a constant hemisphere volume in individuals between the ages of 20 and 50 years (Miller et al., 1980), but thereafter there is a decrease in volume of 2% per decade (Miller et al., 1980; Jernigan et al., 1990).

Thus, after age of 50, the brain size suffers atrophy increased with age, but different studies have reported changes in brain connected with the process of maturisation of brain during its life span: both global and selective decreases in cortical volume and an increase in ventricular volume have been detected in MRI studies. While Coffey and colleagues detected an age-associated decline in the volume of cerebrum both gray and white matter included (Coffey et al., 1992), Pfefferbaum and colleagues measured only the volume of cortical gray matter and found as well a linear decrease associated with age (Pfefferbaum et al., 1994). Different brain structures are involved in the process of age-related shrinkage and this is not uniformly distributed in the brain (Raz et al., 1997). Age related changes have been described in posterior frontal lobe with a decrease of about 1% per year, but not in temporal lobe in a group of subjects aged from 19-92 (DeCarli et al., 1994). Accordingly, Coffey and colleagues found in 76 subjects aged 30 to 90 years a frontal lobe volume reduction of 0.55% per year, but they also described a small decrease in temporal lobe (0.28% per year) (Coffey et al., 1992).

Regional brain volumes, such as those of hippocampus, parahippocampal gyrus and amygdala declined with increasing age according to some studies (Jack et al., 1997; Convit et al., 1995), while other studies detected no age effect on the hippocampal volume (Sullivan et al., 1995; Jack et al., 1989) or a very weak age effect (Raz et al., 1997). The "negative" results were obtained in studies including younger subjects, aged 21 to 70 years (Sullivan et al., 1995), or even 20 to 40 years (Jack et al., 1989). Thus, the results on age effect are somewhat contradictory but much of the variation is due to the different protocols used in choosing the study subjects as concerns age and number, or in measuring brain structures. For this same reason some workers have found a decline associated with age in hippocampal volume, but not in temporal lobe or the whole-brain (a study on 29 young adults) (Bhatia et al., 1993), while others found by contrast that left and right temporal lobe gray matter volumes, exclusive of the hippocampal measures, each decreased significantly with age (a study on 72 subjects aged 21 to 70 years) (Sullivan et al., 1995).

Brain changes rely mostly on cross-sectional studies. However, a better perspective on what may be the effect of age on brain's volume should be given by longitudinal studies. In a five-year study, Raz and colleagues reported an age-related shrinkage in the medial temporal lobe with a significant hippocampal decline, but minimal entorhinal changes (Raz et al., 2004). Later the longitudinal study by Raz and colleagues did detect age related shrinkage of the hippocampus and ERC, but accelerated shrinkage only in the hippocampus (Raz et al., 2005). These results are of particular importance when studying changes in brain related to dementia or AD.

2.3.1.2. A view towards AD

There is evidence that, although the hippocampus is affected early in the course of AD, the ERC is the first region to exhibit AD-type pathology (Van Hoesen and Hyman, 1990; Van Hoesen et al., 1991; Braak and Braak, 1991 a; Arriagada et al., 1992; Huesgen et al., 1993; Gomez-Isla et al., 1996). While in cognitively normal subjects (Clinical Dementia Rating (CDR) Scale = 0) the number of neurons in the ERC has remained constant between 60 and 90 years of age, even in the mildest form of dementia (CDR = 0.5) the number of neurons decreased by 32% (Gomez-Isla et al., 1996). Neurodegenerative changes in AD are accompanied by brain atrophy with changes generally seen in both gray and white matter (Mann, 1991). In layer II of the ERC, neuronal loss together with atrophy exists prior to the onset of dementia and is correlated with MMSE (Kordower et al., 2001), and hippocampal volumetry has been shown to correlate with neuronal (Bobinski et al., 2000) and tangle counts (Huesgen et al., 1993). Moreover, while MMSE and Braak&Braak stage are correlated, hippocampal volume measured on MRI scans correlates with each of them (Jack et al., 2002).

2.3.1.3. Structural MRI and white matter

Age-related white matter changes have been reported in some studies to appear between 30 to 79 years (Jernigan et al., 1991). Other studies reported that until 20 years of age, the cortical white matter volume increased steadily (subjects aged 3 months to 30 years), while after that age it remained constant (subjects aged 21 to 70 years) (Pfefferbaum et al., 1994). Coffey and colleagues reported a 6.3% per year increase in subcortical hyperintensity in the deep white matter in a sample of young healthy adults (Coffey et al., 1992). Age-related changes in white

matter have been presented also by Raz and colleagues, with no or very weak association with age (Raz et al., 1997).

2.3.1.4. VBM - gray and white matter

Apart of structural studies that concentrate on specific brain regions, of great importance are also other quantitative MR techniques that measure functional, blood-flow, biochemical or global anatomic changes occurring in the brain (Kantarci and Jack, 2004). One such approach is the voxel-based morphometric (VBM) method for studying the brain, which allows measurements of whole brain instead of a specific brain region. The voxel-by-voxel measurements indicate that prefrontal cortex and MTL are relevant structures both in aging and age-related cognitive decline in healthy elderly subjects (Tisserand et al., 2004). In the study by Good et al. (2001 a), a total of 465 normal adults were examined for age effects on gray and white matter. Accelerated volume loss with increasing age was seen in the insula, superior parietal gyri, central sulci and cingulate sulci, while little or no age effect was seen in the amygdala, hippocampus and ERC. Generally, a linear decline in gray matter was seen in normal aging, with sparing of the temporal lobe. White matter was in global terms not affected by age; nevertheless, local areas with age-related changes were seen.

2.3.2. Imaging in AD

Quantitative MRI measurements have been used to assess the volumetric AD changes in well-defined brain regions, selected according to the pathological changes known to occur in AD (Van Hoesen et al. 1991; Braak and Braak, 1991 a; Arriagada PV et al., 1992; Huesgen et al., 1993; Gomez-Isla et al., 1996). Neuroimaging, otherwise a part of the diagnostic workup of persons suspected for dementia, is even thought to be superior to the neuropsychological tests for early diagnosis of AD (Zamrini et al., 2004). A backup for studying certain ROIs are the reports of evidence that even cognitive intact subjects or MCI subjects can carry a burden of AD pathology (Huesgen et al., 1993; Bobinski et al., 2000; Jack et al., 2002). The MTL became therefore, an attractive target for MRI measurements. Structural brain studies are assessed with both ROI based measurements and global, computational based measurements, such as the VBM approach. In ROI measurements, the most consistent finding in AD has been hippocampal atrophy accompanied by ERC atrophy, and in general an atrophy of the MTL (see for review Dickerson and Sperling, 2005; Masdeu et al., 2005). Compared to

controls, the annual rate of hippocampal volume loss is two to three times greater in mild AD patients, ranging from 4-8% per year (Laakso et al., 2000; Jack et al., 2000). In cross-sectional studies, volume losses of 38% for the hippocampus (Laakso MP et al., 1995) and 40% for the ERC (Juottonen K et al., 1998 b) were registered in patients with mild to moderate AD versus controls. Accordingly, compared to MCI subjects, a volume loss of 19% for the hippocampus and 30% for the ERC has been reported (Du et al., 2001). While structures of the MTL, especially hippocampus and ERC, are considered MRI markers for AD, there are volumetric studies, which have focussed on other than temporal regions. Callen and colleagues studied the limbic system throughly: hippocampus, amygdala, parahippocampal cortex and "beyond the hippocampus", the anterior thalamus, hypothalamus, mamillary bodies, basal forebrain, septal area, fornix, and cingulate and orbitofrontal cortices (Callen et al., 2001). All the limbic structures exhibited significant atrophy, with the exception of one region: the anterior cingulate cortex.

The volumetric measurements were also studied from the discriminant point of view, since this has implications for the early diagnosis of AD (see for review Chetelat and Baron, 2003). Reviewing a good set of cross-sectional case-control studies, in 2004 Kantarci and Jack remarked that "the entorhinal cortex and hippocampus volumes are generally considered to be the most accurate in differentiating patients clinically diagnosed with AD from normal" (Kantarci and Jack, 2004). A combined atrophy of the limbic structures was used to distinguish patients with AD from controls with over 90% accuracy (Callen et al., 2001). Lehericy and colleagues (1994) achieved 100% accuracy in discriminating AD patients from controls combining the volume of hippocampus with that of amygdala; nonetheless, with hippocampus alone the accuracy achieved was only 89% (Lehericy et al., 1994). In addition, 100% accuracy in distinguishing mild AD patients from controls was achieved by combining partial ERC volume (measured from 3 slices) with measurements of the banks of the superior temporal and anterior cingulate sulci (Killiany et al., 2000). While some investigators have found almost no differences in the power of hippocampus and that of ERC for discrimination between AD patients and controls (Juottonen et al., 1999; Du et al., 2001), some others have reported a lower accuracy for the ERC (67%) than for the hippocampus (85%) for the same purpose (Frisoni et al., 1999).

More recent studies have paid attention on computational methods for analysing the atrophy throughout the brain, and in general if, focusing on VBM studies, they have confirmed

previous ROI-based findings that the brain atrophy is concentrated in the temporal lobe in AD (Ohnishi et al., 2001; Frisoni et al., 2002; Busatto et al., 2003). Yet, some investigators have found a widespread distribution of the atrophy (Baron et al., 2001; Karas et al., 2003). This raises questions about what is happening globally in brain morphology in patients at high risk for dementia, moreover for AD.

2.3.3. Imaging in MCI

In recent years, subjects at high risk for developing dementia, or more exactly AD, have been intensively studied using *in vivo* neuroimaging methods, though the results must be interpreted with caution taking into account the set of subjects studied and the appropriate way of defining them. Even if we concentrate only on MCI subjects, we meet different categories of criteria used for collecting the study group, which can influence the results obtained in different studies. Furthermore, the neuroimaging protocols employed differ from one study to another as well. This thesis will focus rather on ROI-based measurements and VBM analyses on brain to review some basic findings in this field.

2.3.3.1. MRI volumetric studies in MCI

Due to the reduced volumes detected by MRI in AD, the ERC and hippocampus have consequently been the regions of major interest in MCI. Thus, most of the MRI studies in MCI have been dedicated to ROI measurements. An extensive review on neuroimaging in MCI stated that the decline in volumes reported in different studies ranged from 13% to 32% in the ERC and from 9% to 15% in hippocampus (Wolf et al., 2003) in MCI subjects versus controls. Nonetheless, the MCI subjects were chosen based on different criteria in the various studies as were the protocols used for measurements of ERC. Therefore, a straight comparison between studies is difficult to make. However, the ERC and hippocampal volumes are significantly reduced in those subjects determined to be at a transitional stage between normal aging and dementia, when compared with controls (Convit et al., 1997; Dickerson et al., 2001; Xu et al., 2000; Du et al., 2001). Yet, some investigators reported more atrophy in the ERC than in hippocampus (Xu et al., 2000), while others found no differences in the magnitude of the volume loss between these two regions (13% versus 11%) in MCI versus controls (Du et al., 2001). Convit and colleagues (1997) described a hippocampal volume reduction of 14% between subjects with minimal cognitive impairment,

termed as MCI and controls. In that study, the MCI subjects were defined according to Global Deterioration Scale (GDR) = 3, not using the MCADRC criteria for MCI (Petersen et al.1995; Petersen et al., 1999) and there were no reports on the ERC volume.

For defining the boundaries of the ERC, researchers have used different protocols, like that by Insausti et al. (1998) in studies by Du et al. (2001) and Xu et al. (2000), but also that by Goncharova et al. (2001). With this latter protocol, Dickerson and colleagues (2000) studied ERC and hippocampus in subjects "who presented at the clinic with cognitive complaints, but did not meet criteria for dementia (non-demented)". As this was a longitudinal study, they found that the converters were differentiated from non-converters only by the ERC volume, suggesting that the ERC atrophy appears before that of the hippocampus and thus, it is a better predictor of conversion. Moreover, the non-demented subjects were better distinguished from controls with the ERC volume (69% accuracy) than with the hippocampal volume. Xu et al. (2000) reported a more powerful overall classification with the hippocampal volume than with the ERC volume, between MCI subjects and controls, while Killiany et al. showed that only the ERC volume could discriminate normals from "questionables" (83% accuracy) and from converters (84% accuracy) (Killiany et al., 2002). "Questionables" were defined using a clinical dementia rating of 0.5 and not the MCADRC criteria for MCI (Petersen et al., 1995; Petersen et al., 1999) and the ERC volume in this study was measured from only 3 slices of 1.5mm each. "Questionables" and converters could not be discriminated with any of these two regions. In another study by Killiany et al. (2000) the best discrimination between the groups of normal subjects and "questionables" or converters was achieved with the ERC, the banks of the supperior temporal sulcus, and the anterior cingulate (accuracy 85%, respectively 93%). In that study, no data on the hippocampal atrophy has been reported.

Apart of the two above discussed MRI markers for AD, other regions started more recently to gain interest in neuroimaging. The cingulate cortex is part of the limbic system, involved in memory functions, and here a severe hypometabolism of glucose rate has been found, especially in the hippocampal complex, medial thalamus, mamillary bodies, and posterior cingulate, in patients with MCI and mild AD (Nestor et al., 2003). Yet, this is a study using a combination between the techniques of MRI with that of positron emission tomography (PET). A ROI study reporting the cingulate atrophy in preclinical AD is for instance that of Killiany et al. (2000).

Results presented by the studies searching for brain atrophy with ROI are in a way limited to the fact that, not all of them included subjects defined as MCI according to the probably most largely used criteria of MCADRC for MCI (Petersen et al., 1995; Petersen et al., 1999); furthermore, the number of subjects was small as for such a heterogenic group; the neuroimaging protocols are not constant throughout the studies; and one should beforehand plan what brain region to study, thus, significant atrophy may exist and not be detected in other regions. For the last inconvenience more recent studies on MCI have employed rather semi-, or automatic methods, for example the voxel-by-voxel measurements for detecting global brain atrophy.

2.3.3.2. VBM studies in MCI

There are only a few studies employing VBM analysis in MCI (Chetelat et al., 2002; Karas et al., 2004; Chetelat et al., 2005; Bell-McGinty et al., 2005), while before the VBM work included in this thesis was started there was only one published study on this topic (Chetelat et al., 2002). That study applied VBM on 22 patients with amnestic MCI compared to 22 controls and reported significant gray matter (GM) loss in the hippocampal region, and the cingulate gyri, with extension into the temporal neocortex. Bell-McGinty's study had an interesting design, presenting not only the GM atrophy in MCI subjects versus controls, but also morphologic changes in subgroups of MCI: amnestic group and group with more diffuse cognitive impairment (Bell-McGinty et al., 2005). Atrophy in the hippocampus and ERC, and in the amygdala and the neocortex was found in the amnestic MCI group of subjects when compared to controls.

A recent study reported significant atrophy in global GM of 12.3% in AD compared to controls, while the GM atrophy in the MCI group was not significantly different from either controls (6.5%) or AD group (6.2%) (Karas et al., 2004). Still, they found a spatial difference between the study groups. The atrophy of the MTL, thalamus and insula were significant for the MCI group versus that of controls, while the GM atrophy in the parietal association cortices and in the cingulate cortex was found in AD versus MCI. Nevertheless, while very early thalamic atrophy is not specific for Braak&Braak staging for AD, a glucose hypometabolism has been reported to appear early also in this region (Nestor et al., 2003). The thalamic involvement in MCI has been shown with VBM analysis (Chetelat et al.2005; Chen and Herskovits, 2006). As the VBM can estimate a global GM loss, annual rates ranging

from 0% to 4% have been reported during 18 months of follow-up of 18 MCI subjects, with the highest rates present in frontal temporal and cingulate cortices in converters, and in frontal areas in non-converters (Chetelat et al., 2005). The same group of researchers found an annual GM loss of ~4% in the temporal neocortex, 1-2% in the hippocampus and 3-4% in the ERC in converters. GM loss has been intensively studied in AD and MCI with ROI and VBM, but it seems that this is not the only morphological change in the brain associated with dementia.

2.3.4. WMLs and associations with MCI

There is evidence that the prevalence of white matter lesions (WMLs), defined by volume, distribution or substrate increases with age, and while the elevated blood pressure is considered to be the strongest predictor of WML, there are also other disease conditions and evermore, genetic factors that may contribute to the presence of WMLs (see for review Launer, 2003). Moreover, in 51 healthy subjects aged 19 to 91 years without cerebrovascular risk factors, the white matter hyperintensity volume was predictive of reduced cognitive scores, reduced brain volume and increased ventricular volume (DeCarli et al., 1995). The above-mentioned study considered the white matter hyperintensity volume to be abnormal if it represented more than 0.5% of the intracranial volume. In a very recent study on dementia- and stroke-free subjects, the so called large white matter hyperintensity volume was significantly associated with decreased cognitive functioning dependent rather on the frontal lobe systems, but also on the medial temporal area, though to a lesser extent (Au et al., 2006). Yet, associations between the memory impairment and WMLs remain uncertain, based on a study of 40 normal subjects (O'Brien et al., 1997). In a MCI study, apart of APOE ϵ4 and age, the white matter hyperintensity volume was also associated with increased risk for MCI, even when excluding subjects who had suffered cerebrovascular accidents (DeCarli et al, 2001). Wolf and colleagues (2000) found an inverse relationship between WMLs and the temporal lobe atrophy in the group of MCI subjects that progressed to dementia during a follow-up period of 2-3 years. It has been suggested that WMLs could accelerate the cognitive decline in MCI subjects and contribute to the dementia process (Wolf et al., 2000), though a more recent longitudinal study reported that WMLs did not predict decline (Korf et al., 2004). Thus, there is a need of including the WML burden into both cross-sectional and longitudinal studies on MCI to try to achieve a consensus on whether and to what degree those changes contribute to the development of dementia in subjects with MCI.

2.3.5. MRI volumetry - a predictor of AD in MCI

An annual conversion rate of 6-25% from MCI to AD has been reported, regardless of the MCI criteria used in different studies (see for review Petersen et al., 2001 a). For the conversion to dementia, respectively to AD in MCI studies different parameters have been used: APOE genotype, neuroimaging and neuropsychological batteries, as being *in vivo* predictive markers for AD. The visual assessment of the MTL atrophy was shown to predict dementia in MCI subjects independently of age, gender, education, MMSE, CDR sum of boxes score, APOE ϵ4, and WML burden (Korf et al., 2004). Important MCI longitudinal study designs are both finding predictors to dementia/AD and comparing groups of converters with nonconverters as concerns different features. In terms of MTL atrophy and hippocampal atrophy significant differences between MCI converters and nonconverters were reported, with increased atrophy being seen in decliners (Erten-Lyons et al., 2006). That study did not provide data about the entorhinal cortex volume. In a recent longitudinal study with a follow-up of 5 years, the most important MRI markers for AD, being ERC and hippocampus, were examined as predictors for AD, but only the ERC volume and its rate of decline was found to be an independent predictor of AD, not the hippocampal volume (Stoub et al., 2005). The MCI subjects they studied had been diagnosed according to the Mayo Clinic criteria for amnestic MCI (Petersen et al., 1999), and the volumetric MRI protocol used for the ERC volume was that developed by Goncharova and colleagues (Goncharova et al., 2001). The data reported were obtained from 58 nondemented elderly participants including both amnestic MCI subjects and healthy controls with no cognitive impairment, all studied as one group. In a review on predictors of conversion to dementia, Modrego suggested that a combination of a neuroradiological technique, APOE genotype and cognitive tests may be the best option for prediction purposes, until a 100% marker will be detected. Moreover, he considered that the ERC and hippocampal atrophy markers are problematic because of anatomic variations and artefact possibilities (Modrego, 2006).

2.4. APOE and implications in MCI and AD

2.4.1. What is APOE?

ApoE is a plasma lipoprotein involved in lipid transport and metabolism, which is synthesized and secreted by many tissues, primarily liver, but also by brain, as well as by skin and tissue

macrophages throughout the body (Saunders et al., 2000). The polymorphism of the human APOE gene, located on chromosome 19, consists on 3 different alleles: ε2, ε3 and ε4, whith the APOE ε3 being the most common in the general population (77%-78%), while APOE ε2 represents only 7%-8% and APOE ε4 14%-15% (Cedazo-Miguez and Cowburn, 2001). It is known that the human ApoE is a 299 amino acid apolipoprotein, where the three isoforms (ApoE2, E3 and E4) differ from one another due to the amino acid changes in positions 112 and 158. ApoE3 has cysteine at residue 112 and arginine at residue 158, E2 has cysteine at residue 112 and 158 and E4 has arginine at residue 112 and 158. The amino acid changes in these positions lead to different lipid binding properties and they are responsible for the association of each of the isoforms with different diseases (Mahley and Rall, 2000; Cedazo-Miguez and Cowburn, 2001).

2.4.2. APOE and implications in AD

The presence of the APOE allele ε4 has been the most consistently confirmed genetic risk factor for AD (Farrer et al., 1997). In a meta analysis, it was shown that the effect of APOE as a major risk factor for AD was seen both in men and women, in all ages between 40 and 90 years, though the risk effect diminished after the age 70 years (Farrer et al., 1997). Some studies have also suggested that AD patients carrying APOE ε4 have more pronounced neuropathological changes, exhibit more prominent atrophy of the MTL, and suffer more severe memory loss compared to AD patients with no ε4 allele (Lehtovirta et al., 1995; Petersen et al., 1995; Alberts, 1996; Lehtovirta et al., 2000; Engelborghs et al., 2003).

Regarding the atrophy of MTL regions in AD, a dose-dependent effect of the APOE ε4 allele was shown using ROI-based methods, where atrophy increased significantly from non-carriers to homozygous carriers via heterozygous carriers (Lehtovirta et al., 1996; Juottonen et al., 1998 a; Geroldi et al., 1999). In a study by Boccardi et al. (2004) using VBM analysis, some of the MTL regions (amygdala and right hippocampal tail) were atrophied in AD carriers of APOE ε4 versus AD non-carriers. Again, using volumetric measurements, Lehtovirta and colleagues (1995) showed that amygdaloid damage was greater in AD patients homozygous for the ε4 allele, than in heterozygous or noncarriers.

The presence of APOE ε4 has shown a significant role in predicting conversion from MCI to AD (Petersen et al., 1995; Alberts, 1996; Kryscio et al., 2006), though there are also data

reporting negative results on the predictive value of APOE ε4 for conversion from MCI to AD (Korf et al., 2004; Wang et al., 2006). Korf and colleagues presented data showing that visual assessment of MTL structures on MRI using a standardized rating scale is a predictor of dementia in MCI subjects independently of age, gender, education, MMSE**,** CDR sum of boxes, APOE genotype, and WML burden, while APOE did not predict conversion to dementia (Korf et al., 2004).

2.4.3. APOE and implications in MCI

The presence of APOE ε4, as mentioned above, is strongly implicated in the conversion from MCI to AD, although there are contradictory results on this topic, but even more, it increases the risk of MCI (DeCarli et al., 2001; Lopez et al., 2003; Tervo et al., 2004; Kryscio et al., 2006). Recently, Fleisher and colleagues found that MCI women carriers of one or two APOE ε4 allele have more reduced hippocampal volume than controls, and that this situation in MCI men was repeated only for carriers of APOE ε4/ε4 (Fleisher et al., 2005). Earlier, Farlow and colleagues reported that the APOE ε4 genotype in MCI subjects was associated with greater impairment in memory and functional activities, and a greater decline in the hippocampal volume, results that resemble those obtained in AD patients (Farlow et al., 2004). Contradictory, Killiany and colleagues found no significant difference in the volume of the ERC or the hippocampus in relation to APOE genotype; moreover, the APOE status did not add power on the discrimination between groups of controls, questionables, converters and AD patients (Killiany et al., 2002).

Of interest is the finding from a longitudinal study of a total of 129 subjects followed-up for 3 ± 1 years, where the proportion of individuals with APOE ε4 increased in the following order of clinical outcome: control-stable (10%) < control-decliner (20%) < MCI-stable (28%) < MCI-decliner (39%) < AD (46%) (Jack et al., 2000). However, there are conflicting results in the literature about APOE and its effect on brain morphology in MCI subjects and on the conversion from normal to MCI and to AD. Moreover, little is known about the global effect of APOE on the brain, especially on the GM.

3. AIMS OF THE STUDY

The focus of this thesis was to concentrate on MCI subjects in the Kuopio MCI study applying the hypotheses: those who later convert to AD have lower memory test scores, more hippocampal and ERC atrophy and more often have APOE ε4 allele, compared to controls and stable MCI subjects. Additionally, MCI carriers of APOE ε4 allele have more profound atrophy of the brain than noncarriers, and higher risk to convert to dementia.

The specific aims of studies **I-IV** were:

1. To determine whether the ERC atrophy precedes hippocampal atrophy in AD, and to evaluate the power of discrimination between the diagnostic groups using MRI volumetry (**study I**).
2. To map the GM loss in the entire brain of MCI patients using VBM method (**study II**).
3. To investigate the difference in the morphologic expression of MCI in subjects carriers and noncarriers of the ApoE ε4 allele using the VBM method (**study III**).
4. To conduct a follow-up study and to determine what is the predictive value of the MRI-derived volumes of the hippocampus and ERC, WML, APOE, age, gender, education, MMSE and CDR sum of boxes on conversion from MCI into AD (**study IV**).

4. SUBJECTS AND METHODS

4.1. Subjects

The study was approved by the local ethics committee, and all the participants gave informed consent for their participation in the study. The original set of the studied subjects was 172, including 59 controls, 65 MCI subjects and 48 subjects with AD. The MCI and control subjects were derived from two population-based cohorts in whom cognitive functions of the elderly had been evaluated (Hänninen et al., 2002 and Kivipelto et al., 2001). AD patients were selected from hospital series and were investigated in the Department of Neurology, Kuopio University Hospital, Kuopio, Finland. **Study I** was a volumetric study that used ROI-based measurements and included the entire set of 172 subjects: 59 controls, 65 MCI subjects and 48 subjects with AD. **Study II** and **III** used VBM method for inspecting the brain atrophy and included 32 controls and 51 MCI subjects, for whom technically adequate scanning parameters were available for the VBM method. **Study III** was designed to assess the brain atrophy in MCI subjects related to their APOE genotype. The individuals with MCI were divided into three subgroups according to their APOE status: 28 were ε4 noncarriers, 15 were heterozygous for the ε4 allele, and 8 were homozygous for the ε4 allele. **Study IV** included 60 subjects with MCI for whom clinical follow-up data and brain volumetry data was available. The baseline visit included the brain MRI scan in addition to clinical and neuropsychological evaluation. There were no reasons for excluding subjects from the study on the basis of vascular pathology based on T2 weighted images. Three follow-up visits were performed in 1999-2004, and they included neuropsychological tests and clinical neurological examination. Finally, the medical history (hospital records) was obtained for those participants who did not participate in all study visits to detect the possible conversion to dementia. The conversion to dementia was considered as the end-point of the follow-up.

4.1.1. Control subjects

Control subjects were volunteers from the population-based cohorts. They had neither dementia nor MCI and were matched by age and gender with the MCI/demented subjects. The methods used for the identification of control subjects have been published earlier in detail (Hänninen et al., 1997 and Kivipelto et al., 2001). The controls showed no impairment in the cognitive tests, and had no history of neurological or psychiatric diseases.

4.1.2. AD subjects

The subjects with AD were derived from hospital series of well characterized AD patients investigated in the Department of Neurology, Kuopio University Hospital, Kuopio, Finland. The diagnostic evaluations for AD patients included medical history, physical and neurological examinations performed by a physician, and a detailed neuropsychological evaluation administered by a neuropsychologist. The severity of cognitive decline was graded according to the CDR Scale (Berg, 1988). Furthermore, brain MRI scan, CSF analysis, ECG, chest radiography and blood tests were performed. These were not used in the diagnostic phase except for excluding other possible pathologies underlying the symptoms. The diagnosis of dementia was based on the criteria of the DSM-IV (American Psychiatric Association, 1994) and the diagnosis of AD on the NINCDS-ADRDA criteria (McKhann et al., 1984).

4.1.3. MCI subjects

The subjects with MCI were identified from two different population cohorts. In both cohorts, the evaluation consisted of a structured interview including the CDR Scale and an extensive neuropsychological assessment. The scoring of the CDR was independent of the scores obtained from neuropsychological tests. MCI was diagnosed using the criteria proposed by Mayo Clinic Alzheimer's Disease Research Center. Later, these criteria have been modified, but at the time this study was conducted the criteria required: (1) memory complaint by patient, family, or physician; (2) normal activities of daily living; (3) normal global cognitive function; (4) objective impairment in memory or in one other area of cognitive function as evident by scores >1.5 S.D. below the age-appropriate mean; (5) CDR score of 0.5; and (6) absence of dementia (Petersen et al., 1995 and Smith et al., 1996).

In the first cohort, the following test battery was used for a comprehensive neuropsychological evaluation of different cognitive domains: *Memory*: Visual Reproduction Test (immediate and delayed recall) from Wechsler Memory Scale (Russel, 1975), Logical Memory Test (immediate and delayed recall) from Wechsler Memory Scale-Revised (Wechsler, 1987), Word List Recall (immediate and delayed recall) from the CERAD Neuropsychological Assessment Battery (Morris et al., 1989), delayed recall of the Constructional Praxis from CERAD (Morris et al., 1989), New York University Paragraph

Recall (immediate and delayed recall) (Kluger et al., 1999); *Language*: Abbreviated (15 items) Boston Naming Test (Kaplan et al., 1991), vocabulary subtest from the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Wechsler, 1981); *Attention and executive function*: Verbal Fluency Test (Borkowski et al., 1967 and Butters et al., 1987), Trail Making Test (Reitan, 1958) parts A and B; *Visuospatial skills*: Constructional Praxis from CERAD, Block Design from the WAIS-R (Wechsler, 1981); *Global functioning*: Mini-Mental State Examination (Folstein et al., 1975) (MMSE), Clock Drawing Test (the CERAD version) (Morris et al., 1989). In this cohort, however, two memory test scores only were used as the objective psychometric criteria of memory impairment in MCI diagnosis: according to the normative data (Hänninen et al., 2002) in delayed recall in the Logical Memory Test from the WMS-R or in the Visual Reproduction Test from the WMS, he or she was defined as impaired. All the MCI subjects included in the present study had memory impairment.

The diagnostic procedure used in the second cohort has been previously described in detail (Kivipelto et al., 2001). The cognitive functions were screened using the MMSE. Subjects scoring < 24 in the MMSE were invited to participate in the clinical phase to assess the possibility of MCI. Ultimately, 86% subjects took part in the clinical phase, which consisted of taking of medical history, thorough neurologic and cardiovascular examinations performed by a physician, and a detailed neuropsychological evaluation conducted by a neuropsychologist that included the Buschke Selective Reminding Test (Buschke and Fuld, 1974), the Logical Memory Test from the Wechsler Memory Scale–Revised (Wechsler, 1987), the Boston Naming Test (Kaplan et al., 1983), the Vocabulary subtest of the WAIS-R (Wechsler, 1981), the Verbal Fluency Test (Borkowski et al., 1967), the Copy a Cube Test (Goodglass and Kaplan, 1972), the Clock Setting Test (Goodglass and Kaplan, 1972), the Block Design subtest of the Wechsler Adult Intelligence Scale (Wechsler, 1981 b), the Wisconsin Card Sorting Test using Nelson's version (Nelson, 1976), and the Trail Making Test (Reitan, 1958). The severity of cognitive decline was graded by the study physician according to the CDR scale. A review board consisting of the study physician, the study neuropsychologist, and a senior neurologist ascertained the preliminary diagnosis based on all available information. As defined by the MCADRC criteria, the cut-off point 1.5 SD below the norm in the neuropsychological tests was used as a guideline in the clinical assessment of cognitive performance. Thus, both psychometric and clinical aspects were taken into account in the ultimate diagnosis of MCI as suggested by the MCADRC criteria. All the MCI subjects included in the present study had memory impairment.

4.2. Imaging of the brain

4.2.1. MRI and volumetric studies

4.2.1.1. MRI technique for volumetric study

The subjects were scanned with a 1.5 T Siemens scanner (Siemens Magnetom SP or Vision, Erlangen, Germany) using a three-dimensional magnetization prepared rapid acquisition gradient echo sequence (For the 13 controls and 30 AD patients scanned before year 1998: time of repetition (TR) 10 ms, time of echo (TE) 4 ms, matrix 256×256, 1 acquisition and in plane resolution=0.98 mm; For 32 controls, 4 AD patients and 51 MCI subjects scanned in 1998/1999: TR=9.7, TE=4, matrix 256×256, 1 acquisition and in plane resolution=0.98 mm; For 14 MCI subjects scanned in 1999/2000/2001: TR=13.5, TE=7, matrix 256×256, 1 acquisition and in plane resolution=0.94 mm) resulting in contiguous T1-weighted partitions with a slice thickness of 1.5-2.0 mm oriented perpendicular to the long axis of the hippocampus. The images were then aligned to correct for the undesirable effects of head tilt and rotation. Standard neuroanatomical landmarks (such as the orbits, sulci and the commissures) were used to correct for possible deviations in any of the orthogonal planes and the scans were reconstructed into 2.0 mm thick contiguous coronal slices, oriented perpendicular to the intercommissural line. T2 weighted images were also acquired, and used to study the possibility of vascular pathology in the cases.

4.2.1.2. Determination of volumes

The hippocampi and ERCs (Figure 2) were manually traced (Figure 3) by a single tracer (C.P.), blinded to the clinical data, using custom-made software for a standard Siemens work console. The boundaries of the ROI were outlined by a trackball driven cursor and number of voxels within the region was calculated by using the in-house developed program for standard work console. Thus, once the ROI had been traced, the software calculates the volume for every structure by computing the number of voxels for each traced image. The outlining of the boundaries always proceeded from anterior to posterior. Data from a standard anatomical atlas of the human brain (Duvernoy, 1999), and from previous articles were used as guidelines

to determine the boundaries of the hippocampus (Laakso et al., 1998) and the ERC (Insausti et al., 1998).

Figure 2. Hippocampus and ERC in controls, MCI, mild AD and moderate AD

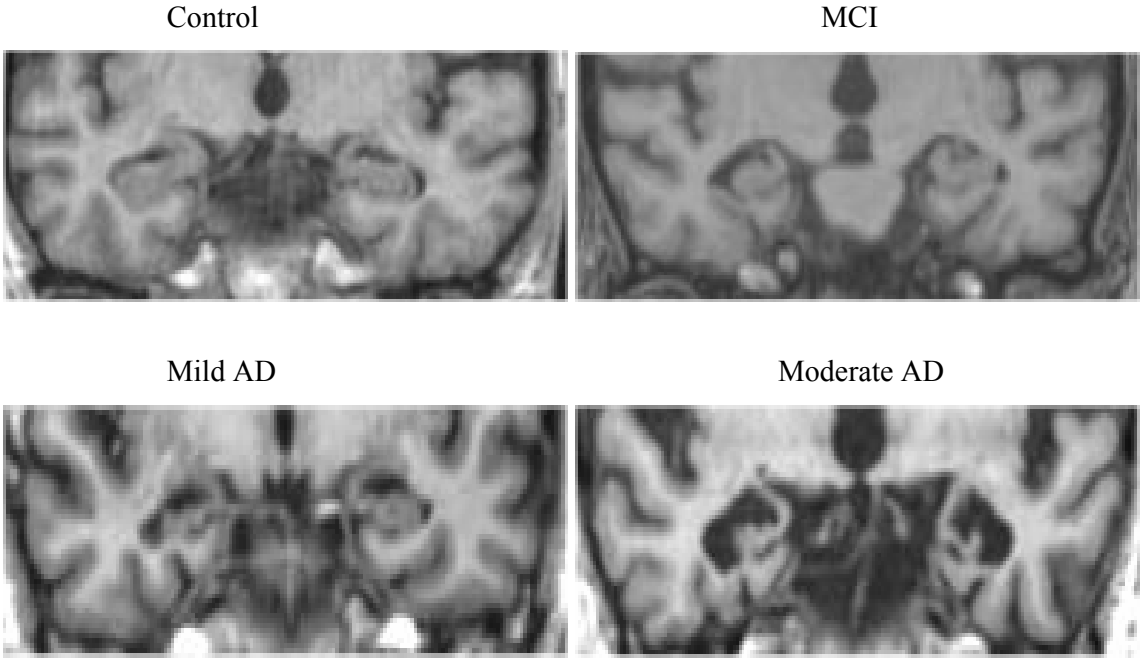
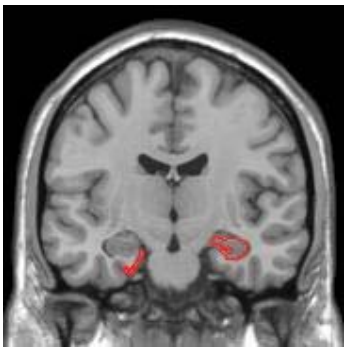


Figure 3. ROI-volumetry of the left ERC and right hippocampus



4.2.1.3. Measurement of the hippocampal volume

Tracing of the hippocampus started rostrally where the hippocampus first appears below the amygdala and ended posteriorly in the section where the crura of the fornices depart from the lateral wall of the lateral ventricles. The hippocampus included the dentate gyrus, the hippocampus proper and the subicular complex.

4.2.1.4. Measurement of the ERC

The ERC volumes were traced according to the histology-based criteria designed for MRI volumetric measurements (Insausti et al., 1998). In brief, the most anterior slice measured was the one after the appearance of the temporal stem, and the last slice was the one where the uncus and gyrus intralimbicus were no longer separable.

4.2.1.5. Measurement of the ICA

The coronal intracranial area (ICA) was measured at the level of the anterior commissure and it was used for normalization of the volumetric data. For the purpose of data presentations, the volumes were normalized to the intracranial area according to the formula:
(volume/intracranial area) × 100.

4.2.1.6. Validation studies

The intraclass correlation coefficients for intrarater reliability were 0.96 for the hippocampus and 0.95 for the ERC measured from 10 subjects.

4.2.2. VBM

In the VBM method, the brains of groups of subjects are modified in a pre-processing phase, in order to fit a reference template, such that a stereotactic point refers to the same structure in each normalized brain. Then automatic statistical analyses are carried out, that compare the concentration of gray matter in each voxel.

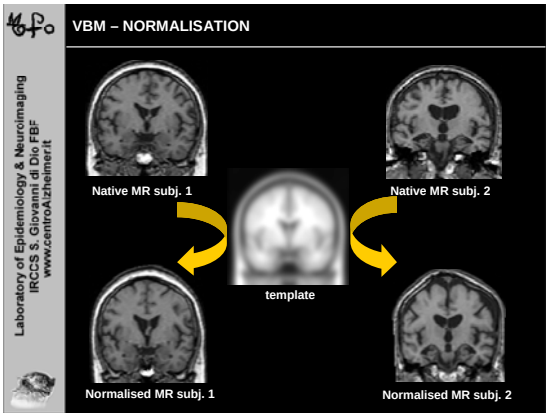
4.2.2.1. VBM pre-processing

After removing the voxels below the cerebellum with MRIcro software (www.psychology.nottingham.ac.uk/staff/cr1/mricro.html) (Rorden and Brett, 2001) the MR images were analysed with Statistical Parametric Mapping (SPM99) (www.fil.ion.ucl.ac.uk/spm) running under Matlab 5.3 (Mathworks, Sherborn, MA, USA).

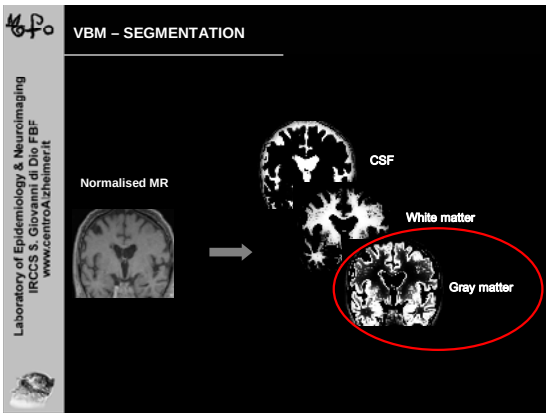
The MR images were pre-processed following a protocol, which included **(1)** generation of a ***customised template***, **(2)** generation of ***customised prior probability maps***, and **(3)** the ***main VBM steps*** (Figure 4): *normalisation* of the original MR images, *segmentation* of normalised images, cleaning of grey matter images, modulation of grey matter images, and *smoothing* of modulated images.

Figure 4. Main VBM steps, after LENITEM.

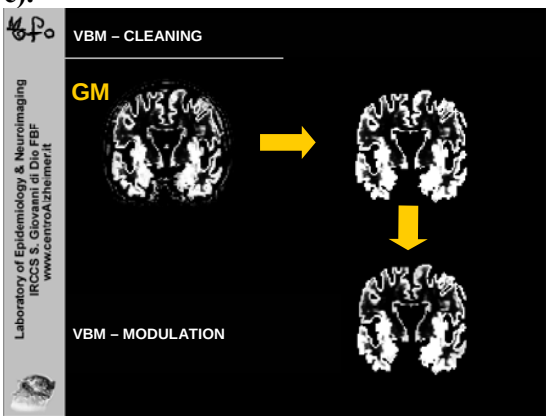
a).



b).



c).



The advantages of this procedure over the traditional simple protocol are that (1) the use of template and prior probability maps computed from the population under study reduces the error in the normalisation and segmentation steps, (2) the cleaning step permits removal of non-brain voxels erroneously classified as grey matter (Good et al., 2002), and (3) modulation permits preservation of the original volume of grey matter within each voxel (Ashburner and Friston, 2000; Ashburner and Friston, 2001; Good et al., 2001 b).

(1) *Customised template*

The customised template was obtained by normalising the images of the 51 MCI patients and 32 controls to the Montreal Neurological Institute template (Evans et al., 1994) of SPM99 using a 12 parameter affine transformation, smoothing the normalised images with an 8 mm isotropic Gaussian kernel and averaging the smoothed images. The anterior commissure was manually set as the origin of the spatial coordinates and images were reoriented coronally perpendicular to the intercommissural line. The normalisation procedure uses a bilinear interpolation algorithm to reslice images to a voxel size of 2x2x2 mm. This voxel size was used in the following processing and analysis.

(2) *Customised prior probability maps*

Customised prior probability maps were computed by segmenting the normalised images into GM, white matter (WM), and CSF, then smoothing with an 8 mm Gaussian filter, and finally averaging the segmented images, thus obtaining the customised prior probability maps specific for GM, WM, and CSF (Good et al., 2002). The voxels, which probability of being brain was greater than 0.5 were smoothed with an 8-mm isotropic Gaussian kernel in order to create the customized brain mask.

(3) *Main VBM steps*

Original images were *normalised* to the customised template through affine and non-linear transformations, medium regularisation, reslicing 2x2x2 mm, and no masking (Baron et al., 2001). The normalised images were *segmented* into GM, WM, and CSF using the customised prior probability maps. The *Xbrain* routine, based on erosions and dilatations, was used to remove voxels of non-brain tissue from the segmented images, thus obtaining a brain mask to clean the GM images by intersection with the mask. In the modulation step, voxel values of the cleaned GM images were multiplied by the measure of relative volumes of warped and unwarped structures derived from the non-linear step of spatial normalisation (Jacobian determinant) (Good et al., 2002). The modulated GM images were *smoothed* with a 12 mm isotropic Gaussian kernel. The final output is a 3D matrix where the three indices are the spatial x, y, and z coordinates of voxels in the reference space, and each value of the matrix is proportional to the volume of GM within each voxel. It should be emphasised that the output of each stage of the analysis was visually checked to ensure that the algorithms had actually carried out the expected changes.

4.2.3. Determination of WMLs

The WMLs were evaluated by a single rater (R.R.) on MRI images on a computer screen with either proton density (PD) and T2 weighted images or on T2 or FLAIR images by using the rating scale by Wahlund et al. (2001). The WMLs were defined as bright lesions ≥5 mm on T2, PD or FLAIR images. In frontal, temporal, parieto-occipital, and infratentorial regions, WMLs were scored: 0 = no lesions (including symmetrical, well-defined caps or bands), 1 = focal lesions, 2 = beginning of confluence of lesions, 3 = diffuse involvement of the entire region; and in the basal ganglia: 0 = no lesions, 1 = one focal lesion (≥5 mm), 2 = more than one focal lesion, 3 = confluent lesions. The sum score of frontal, temporal, parieto-occipital, basal ganglia, and infratentorial regions were used in the analysis.

4.3. Determination of APOE genotype

APOE genotype was determined from blood leukocytes. DNA was extracted by a standard phenol-chloroform extraction, and APOE genotypes were analyzed by polymerase chain reaction and HhaI digestion as described previously (Tsukamoto et al., 1993).

4.4. Statistical analyses

4.4.1. MRI volumetric analyses

Study I

The statistical software SPSS for Windows V10.0 (SPSS Inc., Chicago, IL) was used to analyze the data. In all statistical analyses of the volumetric data, volumes normalized for the intracranial area we used. One-way ANOVA with Bonferroni post hoc analysis was used to compare the means of age, education, and MMSE scores between the groups. The relationship of volumes with gender, diagnostic groups and age was assessed with the ANCOVA test, which had hippocampal and ERC volumes as dependent variables, gender and diagnostic groups as factors, and age as covariate. Pearson's correlation coefficients were used to analyze the correlation between the hippocampal and ERC volumes within each study group. We tested the value of the hippocampal and ERC volumes in discriminating between the groups using discriminant analyses with enter and stepwise methods, respectively. In the analyses, the normalized volumes to the intracranial area and adjusted for age were used.

First, discriminant analyses with an enter method were used to analyze the value of normalized total volumes of the hippocampus and ERC to distinguish AD patients or MCI subjects from controls and MCI subjects from patients with AD, using volumes as independent variables. Then, the value of the hippocampal and ERC total volumes for group classification were tested using stepwise discriminant function analyses (Wilks' method). Finally, stepwise discriminant analysis was used to test unilateral volumes: the right and left hippocampal volume, the right and left ERC volume in discrimination between pairwise combinations of clinical groups. The results are expressed as mean±S.D. The level of statistical significance of differences is $P<0.05$. The discrimination between groups was also represented with ROC curves.

Study IV

The statistical software SPSS for Windows V11.5 (SPSS Inc., Chicago, IL) was used to analyze the data. In all statistical analyses of the volumetric data, we used volumes normalized for the intracranial area. Student's T test was used to assess group differences. The effect of side (within group variable) and outcome (between-group variable) on the volumes of the hippocampus and ERC was assessed by ANOVA. Interaction terms were included in the model as needed. Because of the variability in the follow-up length, the predictive accuracy of volumetry for dementia was assessed by Cox's regression analysis with follow up time as the time variable and conversion to dementia or AD as the status variable. The hazard ratios (HR) with 95% CI and significance (p value) are presented. The level of statistical significance of differences was set at $p<0.05$.

4.4.2. VBM analyses

Study II

A "Single subject: Conditions and Covariates" model was used to compare the volume of GM between MCI and controls on a voxel by voxel basis. The analysis was controlled for APOE genotype including genotype (ε3/3, ε3/4, and ε4/4) as a dummy variable. Intracranial area, age, and sex were included as nuisance covariates. The resulting *t* map was thresholded at $p<0.001$, uncorrected. The demographic and clinical data were analysed using one way ANOVA or χ^2 test, the level of significance was set at $p<0.05$.

Study III

The individuals with MCI were divided into three subgroups according to their APOE status: 28 were ε4 noncarriers, 15 were heterozygous for the ε4 allele, and 8 were homozygous for the ε4 allele. The "Single subjects – Conditions and Covariates" procedure was used to compare the gray matter volume between MCI and controls and between MCI carrying and not carrying the ε4 allele. Intracranial area, age and sex were included as nuisance covariates. The regions atrophic in individuals heterozygous and homozygous for the APOE ε4 allele were detected by contrasting all MCI subjects to controls and inclusively masking atrophy of individuals heterozygous and homozygous for the APOE ε4 allele relative to noncarriers. This approach allows detecting areas of atrophy of noncarriers related to both heterozygous and homozygous carriers of the ε4 allele. The resulting t map was thresholded at $p<0.001$, uncorrected.

5. RESULTS

5.1. Descriptive characteristics

The groups from **study I** were well matched for age (F=1.5, *P*=0.24) and gender (χ^2=2.4, p=0.30). The MMSE scores declined in the following order: control>MCI>AD (p<0.001) (Table 1). The level of education differed significantly across the study groups (F[2,164]=4.3, p<0.05); the subjects with MCI had significantly a lower level of education compared to controls (Bonferroni post hoc analysis *P*<0.05).

In **study II** there was no difference in age (F = 1.96, p = 0.17), sex (χ^2 = 0.45, p = 0.50), education (F = 0.20, p = 0.66), or frequency of the APOE ε4 allele (χ^2 = 2.5, p = 0.11) between the controls and cases with MCI. As expected, the Mini-Mental State Examination scores were significantly lower in cases with MCI compared with the controls (F = 24.8, p<0.0001) (Table 2).

In **study III** the groups of MCI individuals according to the APOE polymorphism were also matched for age, gender and MMSE (Table 2).

For the subjects from **study IV**, the mean follow-up time was 34 (SD 8.7, range 10 to 54) months. During this period, 13 (21.7 %) patients had progressed to dementia (progressive MCI). Nine (69%) patients in this group had a clinical diagnosis of probable AD, three had vascular dementia and one had dementia of mixed type. In the stable MCI group, seven subjects had a neuropsychological profile of control subjects in the last follow-up visit. At baseline, there were no differences between the outcome groups in education, age, MMSE score (Table 2), follow-up time [months as mean (SD): 35.2(8.2) for the stable MCI group versus 30.7(9.9) for the progressive MCI group], or in the WML load [mean (SD): 4.2(4.5) in the stable MCI group versus 4.5(2.9) in the progressive MCI group]. Patients with stable MCI had lower (p<0.05) CDR sum of boxes [1.2(0.5), for subjects n=45] compared to patients with progressive MCI [2.1(1.1), for subjects n=12]. The progressive MCI group included eight patients with at least one APOE ε4 allele. Three patients were heterozygous for the APOE ε allele (ε3/ε4) and five were homozygous (ε4/ε4). Five patients with the progressive MCI were ε4 non-carriers (ε3/ε3). The stable MCI group consisted of 21 APOE ε4 allele carriers. Eighteen of them were heterozygous for the APOE ε4 allele (17 ε3/ε4, 1 ε2/ε4), and three

were homozygous. Twenty-six subjects in the stable MCI group were ε4 non-carriers (25 ε3/ε3, 1 ε2/ε3). The APOE groups (at least one ε4 allele, no ε4 allele) did not differ in age, education, follow-up time, baseline MMSE score, or CDR sum of boxes in progressive MCI. In subjects with stable MCI the only statistically significant difference was found in the follow-up time as subjects without the APOE ε4 allele had a longer follow-up time compared to subjects with ε4 allele (mean ± SD: 37.9 ± 5.46 versus 31.7 ± 9.79, $p < 0.05$).

Table 2. Descriptive characteristics

	CONTROLS		MCI						AD
Study	I	II&III	I	II&III			IV		I
				ϵ-/-	ϵ4/-	ϵ4/4	stable	progressive	
Number	59	32	65	28	15	8	47	13	48
Age	73(4)	74(4)	73(5)	72(5)			73(4)	72(4)	71(8)
				73(4)	71(5)	70(3)			
Sex	37(63)	19(59)	43(66)	17(61)	13(87)	4(50)	33(70)	8(62)	25(52)
Education	8(3)	7(2)	7(2)	7(2)			7(2)	7(2)	7(3)
MMSE	27(2)	27(2)	24(3)	24(2)			24(3)	23(2)	21(4)
				24(4)	24(3)	23(2)			
ApoEϵ4+/-	13/45	9/22	30/35	23/28			21/26	8/5	32/16

MCI: mild cognitive impairment; AD: Alzheimer's disease; MMSE: Mini-Mental State Examination; Sex: female (%); Age, Education and MMSE expressed as mean (SD); ApoEϵ4+/-: carriers of at least one ApoE ϵ4 allele / noncarriers. For one control subject, the ApoE genotype is not known.

5.2. MRI volumetry of hippocampus and ERC in controls, AD subjects and MCI subjects (study I)

The objective in this study was to determine the volumetric differences for the hippocampus and the ERC between the study groups of controls, MCI and AD. Moreover, we intended to determine the power in discrimination between the study groups for unilateral and total volumes of hippocampus and ERC.

5.2.1. Comparative volumetric measurements in controls, AD subjects and MCI subjects

The diagnostic groups (controls, MCI and AD) differed significantly in the volumes ($F[2,164]=88.2$, $p<0.001$ for hippocampal volume; $F[2,164]=50.1$, $p<0.001$ for ERC volume), gender had no influence on the volumes ($F[1,164]=1.1$ for hippocampal volume,

F[1,164]=0.03 for ERC volume, p>0.05), gender×group had no influence on the volumes (F[2,164]=0.2 for hippocampus, F[2,164]=2.3 for ERC volume, p>0.05), and age did not affect the hippocampal volume (F[1,164]=2.6, p>0.05), but it did affect the ERC volume (F[1,164]=8.2, p=0.01). While age was not correlated with total ERC volume in the control group (r=−0.23, p=0.09) or in the MCI group (r=−0.15, p=0.22), in the group of patients with AD there was a significant correlation between age and the total ERC volume (r=−0.30, p=0.04). Therefore, all the analyses were adjusted for age.

The total hippocampal volume and the total ERC volume, as well as the unilateral volumes of hippocampus and ERC were significantly reduced in the following order: control>MCI>AD (Table 3). The total hippocampal and total ERC volumes were correlated within each group (control: r=0.35, p=0.01; MCI: r=0.53, p<0.001; AD: r=0.58, p<0.001).

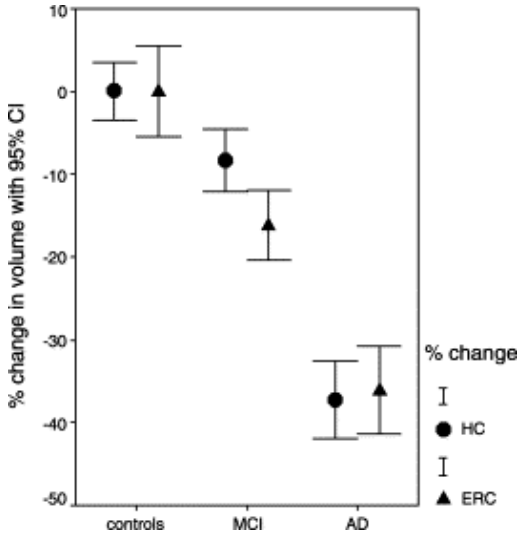
Table 3. Volumetric measurements and percentual decrease in volume

Region	Controls	MCI		AD	
	Volume	Volume	Decrease (%)	Volume	Decrease (%)
Hippocampus					
Right	16.37±2.19	15.20±2.51*	7	10.55±2.86§‡	36
Left	15.36±2.23	13.90±2.54†	10	9.34±2.48§‡	39
Total	31.73±4.19	29.10±4.77†	8	19.90±5.10§‡	37
Entorhinal cortex					
Right	9.02±1.89	7.65±1.59†	15	5.88±1.92§‡	35
Left	8.47±1.94	7.00±1.50†	17	5.29±1.62§‡	38
Total	17.49±3.63	14.66±2.96†	16	11.18±3.20§‡	36

The analyses represent normalized volumes. The results are expressed as mean±S.D. and the decrease % is compared with controls. Right: right side; Left: left side; Total: sum of the right plus left side volumes; MCI: mild cognitive impairment; AD: Alzheimer's disease.
*p < 0.05 for MCI vs. controls
§p < 0.001 for AD vs. MCI
‡p < 0.001 for AD vs. controls
†p < 0.001 for MCI vs. controls

The percentual decrease in ERC volume was higher compared to that in the hippocampal volume in the MCI group versus controls, whereas the volume losses in the AD group versus controls were of the same magnitude for the hippocampus and ERC (Figure 5).

Figure 5. The percentual decrease of total ERC and total hippocampal (HC) volumes within each study group relative to mean volumes for the control group. The 95% CI is considered.



5.2.2. Discriminant function analyses

In discriminant function analysis, we used first the enter method for the volumes of the total (right and left) hippocampus and total (right and left) ERC, adjusted for age and thereafter a stepwise method to test the accuracy of first total and thereafter unilateral volumes in classifying the groups (Table 4).

In the classification between MCI subjects and controls, the volume of the total hippocampus yielded an overall classification of 59.7% (Wilks' λ=0.92, χ^2=10.1, p=0.01), the volume of the total ERC yielded an overall classification of 66.7% (Wilks' λ=0.84, χ^2=21.3, p<0.001). In the stepwise analysis, only the total ERC volume entered the model with an overall classification of 65.9% (Wilks' λ=0.84, χ^2=21.0, p<0.001). In the stepwise discriminant function analysis including unilateral hippocampal and ERC volumes only left ERC volume entered the model (Wilks' λ=0.85, χ^2=20.3, p<0.001) (Table 4).

Distinguishing AD patients from control subjects, the volume of total hippocampus yielded an overall classification of 90.7% (Wilks' λ=0.36, χ^2=105.1, p<0.001), the total ERC volume yielded an overall classification of 82.1% (Wilks' λ=0.50, χ^2=70.8, p<0.001). In the stepwise analysis both the hippocampal volume and the ERC volume entered the model (overall classification 90.6%, sensitivity 87.6%, specificity 93.1%; Wilks' λ=0.34; χ^2=111.1; p<0.001). In the stepwise discriminant function analysis including unilateral hippocampal and ERC volumes, the left hippocampal volume and the right ERC volume entered the model (Wilks' λ=0.35, χ^2=108.1, p<0.001) (Table 4).

Table 4. Classification between the groups using discriminant function analysis with the enter method including in the model hippocampus (HC), or entorhinal cortex (ERC), as well as with the stepwise method including in the model both the HC and ERC

Groups and variables	Sensitivity %	Specificity %	Overall classification %	*P*
MCI and Controls				
Enter: HC	56.9	62.7	59.7	0.01
Enter: ERC	63.1	70.7	66.7	<0.001
Stepwise: ERC	66.2	65.5	65.9	<0.001
AD and Controls				
Enter: HC	85.4	94.9	90.7	<0.001
Enter: ERC	85.4	79.3	82.1	<0.001
Stepwise: HC + ERC	87.6	93.1	90.6	<0.001
AD and MCI				
Enter: HC	77.1	83.1	80.5	<0.001
Enter: ERC	70.8	70.8	70.8	<0.001
Stepwise: HC	81.3	83.1	82.3	<0.001

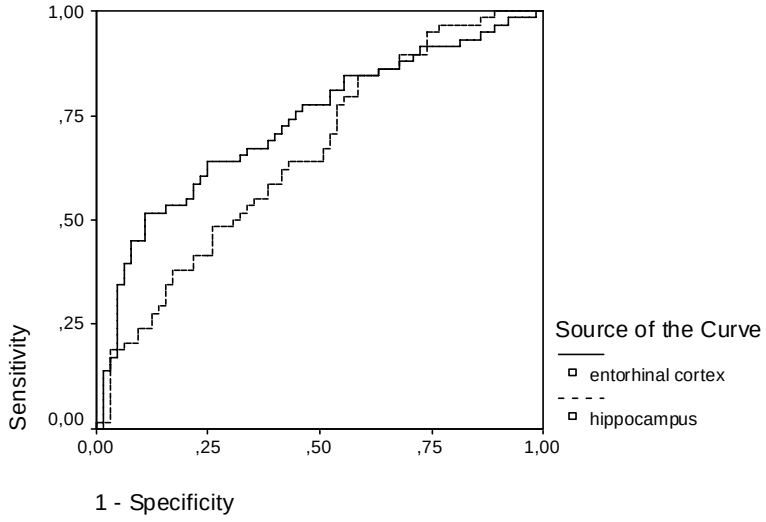
The analyses included the bilaterally summed and normalized volumes adjusted for age. MCI: mild cognitive impairment; AD: Alzheimer's disease.

Distinguishing AD patients from MCI subjects, the total hippocampal volume showed an overall classification of 80.5% (Wilks' λ=0.52, χ^2=72.0, p<0.001), the ERC volume yielded an overall classification of 70.8% (Wilks' λ=0.73, χ^2=34.1, p<0.001). In the stepwise analysis, only hippocampal volume entered the model (overall classification 82.3%, sensitivity 81.3%, specificity 83.1%; Wilks' λ=0.53, χ^2=69.3, p<0.001). In the stepwise discriminant function analysis including unilateral hippocampal and ERC volumes, only left hippocampal volume entered the model (Wilks' λ=0.55, χ^2=66.0, p<0.001) (Table 4).

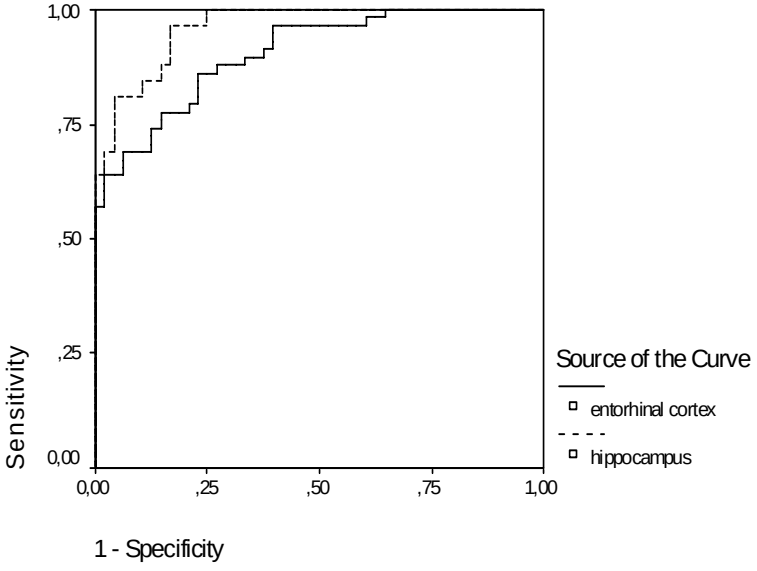
ROC curves were used to illustrate the discrimination between groups with hippocampal and ERC volumes (Figure 6).

Figure 6. ROC curves representing discriminations between groups with MRI volumetry: a). control-MCI; b). control-AD; c). MCI-AD

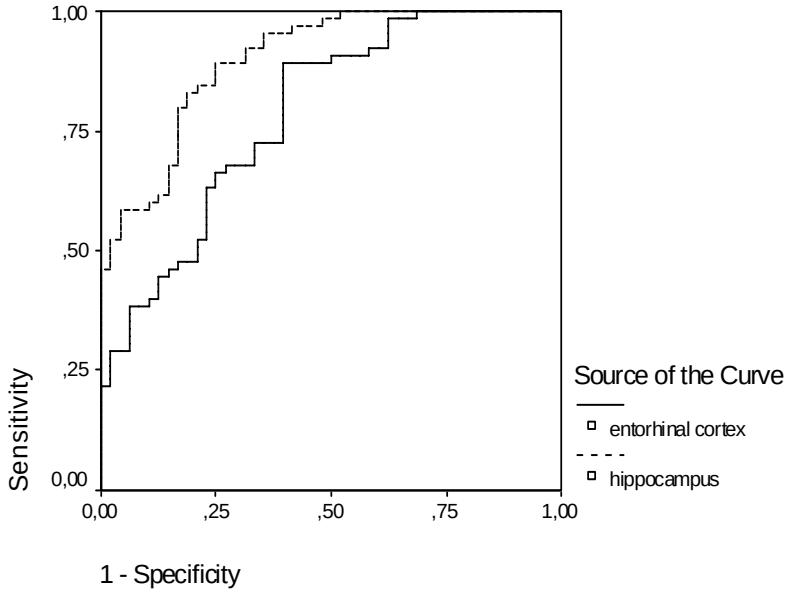
a).



b).



c).



5.3. VBM analyses in MCI subjects (study II)

The objective of this study was, using APOE genotype as a confounding factor, to determine the pattern of brain atrophy in MCI subjects.

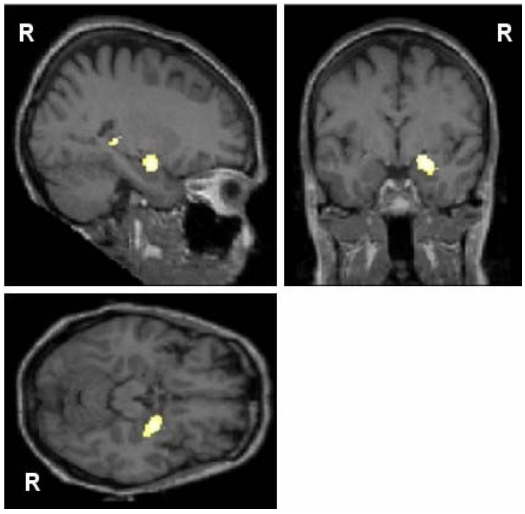
MCI subjects exhibited the greatest atrophy in the right hippocampus-amygdala region, and in the right hippocampal tail and thalamus, when compared to controls (Figure 7, Table 5). The medial temporal structures on the left did not quite achieve the level of statistical significance. Other regions with significant gray matter atrophy were detected in the right superior temporal gyrus, the left thalamus, the left inferior temporal gyrus, and the left anterior cingulate gyrus (Table 5).

Table 5. Atrophic regions of MCI cases compared with controls (p<0.001, uncorrected)

Cluster size k	Region	Stereotactic coordinates (mm)			Z score
		x	y	z	
147	Right thalamus	12	–14	12	3.74
	Right hippocampus (tail)	24	–34	4	3.42
	Right thalamus	16	–26	0	3.31
246	Right amygdala-hippocampus	24	–4	–16	3.72
14	Left superior parietal lobule	–16	–60	66	3.36
10	Right superior temporal gyrus	56	8	–6	3.31
6	Left thalamus	–8	–14	12	3.27
15	Left anterior cingulate gyrus	–10	2	42	3.24
2	Left inferior temporal gyrus	–66	–20	–28	3.16
10	Left thalamus	–16	–30	8	3.14

Region denotes the areas of maximal grey matter volume loss within each cluster. For example: the first line denotes the presence of a 3D cluster created from 147 contiguous voxels of significantly decreased (p<0.001) gray matter volume. Within the same cluster, there are two more peaks of significance more than 8 mm from the former and located at 24, –34, 4 and at 16, –26, 0. The most significant voxel of the cluster has stereotactic coordinates of 12, –14, and 12 and is located in the region of the right thalamus.

Figure 7. Medial temporal involvement in MCI patients compared with controls with p<0.001, uncorrected. Significant voxels are superimposed on MR images of a single control subject. Atrophy affects the amygdala and the head and tail of the hippocampus.

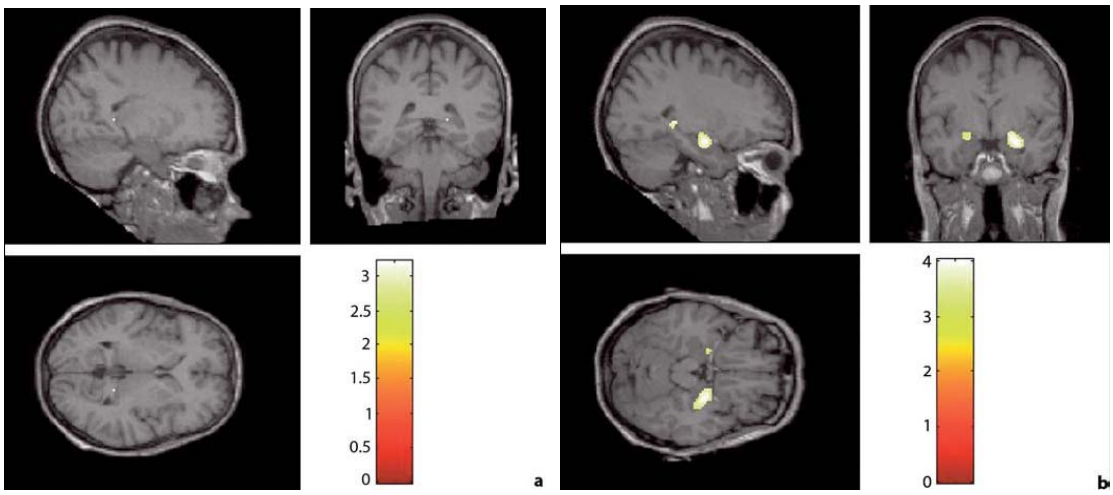


5.4. VBM analyses in MCI subjects, carriers of APOE ϵ4 (study III)

The objective of this study was to determine first the pattern of brain atrophy in MCI subjects with no APOE confounding used, thereafter to map the gray matter loss in MCI subjects carriers of APOE ε4.

MCI subjects exhibited the greatest atrophy in the right and left amygdala, right parahippocampal gyrus, left medial dorsal thalamic nucleus and right superior temporal gyrus, when compared to controls (Figure 8, Table 6).

Figure 8. Regions of atrophy in MCI. **a** Atrophy in 15 MCI with one ε4 allele compared to 28 MCI without APOE ε4. **b** Atrophy in 8 MCI with 2 ε4 alleles compared to 28 MCI without APOE ε4.



In the comparison between MCI individuals heterozygous for the APOE ε4 allele and noncarriers only the right parahippocampal gyrus, with the ERC included, reached the level of statistical significance (Figure 8 a, Table 6). The most significant atrophy between homozygous for the ε4 allele and noncarriers was located in the right amygdala followed by the right parahippocampal gyrus, the left amygdala and the left medial dorsal thalamic nucleus (Figure 8 b, Table 6).

When compared to controls, APOE ε4 noncarriers showed the widest and most significant clusters of atrophy in the parahippocampal gyrus bilaterally (on the right: cluster size 102 voxels, coordinates 24, –36, 2, z = 3.73; on the left : cluster size 34 voxels, coordinates –22, –4, 14, z = 3.31) and in the left medial dorsal thalamic nucleus (cluster size 24 voxels, coordinates –6, –14, –12, z = 3.43). Other smaller clusters were located in the temporoparietal regions bilaterally.

Table 6. Atrophic regions of MCI subjects compared to controls ($p<0.001$ uncorrected) and of MCI subjects in study groups subdivided according to the APOE genotype

Cluster size k	Region	Stereotactic coordinates (mm)			Z score
		x	y	z	
MCI versus controls					
326	Right amygdala	26	-6	18	3.81
152	Right parahippocampal gyrus	24	-36	2	3.73
24	Left medial dorsal thalamic nucleus	-6	-14	12	3.43
53	Left amygdala	-22	-4	-14	3.31
3	Right superior temporal gyrus	56	8	-6	3.17
MCI ε 4+/– versus MCI ε 4–/–					
1	Right parahippocampal gyrus	18	-40	6	3.10
MCI ε 4 +/+ versus MCI ε 4 –/–					
326	Right amygdala	26	-6	-18	3.81
133	Right parahippocampal gyrus	24	-36	2	3.73
52	Left amygdala	-22	-4	-14	3.31
2	Left medial dorsal thalamic nucleus	-6	-16	8	3.18
1	Left medial dorsal thalamic nucleus	-4	-14	10	3.14

Cluster size is given in number of voxels. Region denotes the areas of maximal grey matter volume loss within each cluster. For example, the first line denotes the presence of a 3D cluster created from 326 contiguous voxels of significantly decreased ($p<0.001$) gray matter volume, with stereotactic coordinates of 26, -6, 18 and located in the right amygdala.

5.5. Predictors of AD in MCI subjects (study IV)

The aim of this study was to determine the hippocampal and ERC volume loss in those MCI subjects that progressed to dementia, or to AD during a period of mean follow-up time of 34 months, a group so called "progressive MCI" compared to those MCI subjects that did not convert to dementia, so called "stable MCI". One additional objective of this longitudinal study was to determine the predictive value of the MRI-derived volumes of MTL structures, WML, severity of cognitive impairment, and APOE genotype on conversion of MCI to dementia and AD.

5.5.1. Volumetric measurements at baseline

At baseline, patients with progression of MCI to dementia had significantly reduced ($p<0.01$) volume of the right hippocampus and the right ERC (Table 7) versus patients with stable MCI. A similar trend was found in the analysis of the volumes of the left hippocampus and the left ERC, but this difference did not reach statistical significance. However, the total volume (sum of the right and left side) of the hippocampi and the ERCs were significantly reduced ($p<0.05$) in the progressive MCI group vs. stable MCI group (Table 7). The lowest volumes were found in patients with progression of MCI to AD (right hippocampus: 12.9 ± 2.4, left hippocampus: 12.2 ± 2.7, right ERC: 6.1 ± 1.3, left ERC: 5.8 ± 0.6), and all of the analyzed volumes were significantly reduced ($p<0.05$) compared to the stable MCI group.

In the progressive MCI group there was a tendency of decreased volumes in those patients with at least one APOE ε4 allele, and the volumes of the left hippocampus and the total volume of the hippocampi reached statistical significance ($p<0.05$) (Table 7, Figure 9). In the group of stable MCI, there were no significant differences in the volumes of hippocampi or ERCs in the APOE groups.

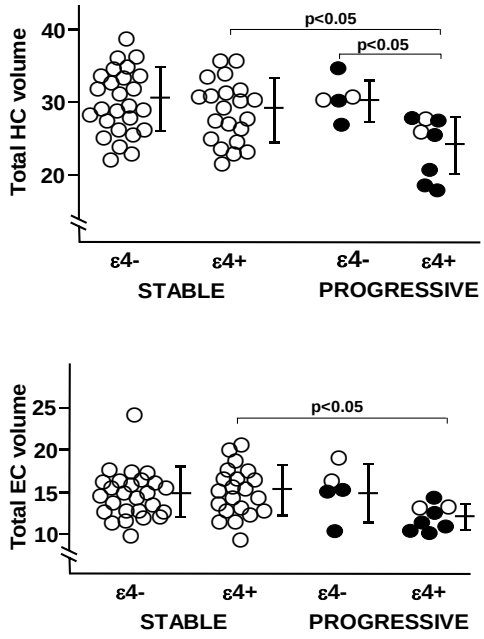
In the progressive MCI group, patients with at least one ε4 allele had significantly reduced total volumes in hippocampus and ERC as well as lowered volumes of the right and the left side separately ($p < 0.05$) compared to subjects with stable MCI with at least one ε4 allele (Table 7, Figure 9). On the other hand, there were no differences in these volumes between the stable or progressive MCI groups in the subjects without the APOE ε4 allele (Figure 9).

Table 7. Volumes of hippocampus and ERC

	Stable MCI	Progressive MCI
Right HC	15.52 (2.36)	13.53 (2.26) #
APOE ε4+	14.99 (2.48)	12.75 (2.38)
APOE ε4-	15.96 (2.20)	14.79 (1.51)
Left HC	14.19 (2.25)	12.80 (2.68)
APOE ε4+	13.96 (2.02)	11.26 (2.03)
APOE ε4-	14.38 (2.43)	15.27 (1.42)
Total HC	29.71 (4.42)	26.33 (4.54)*
APOE ε4+	28.94 (4.39)	24.01 (3.83)
APOE ε4-	30.34 (4.42)	30.06 (2.81)
Right ERC	7.91 (1.56)	6.62 (1.44) #
APOE ε4+	7.96 (1.73)	6.11 (0.95)
APOE ε4-	7.87 (1.44)	7.42 (1.82)
Left ERC	7.11 (1.53)	6.44 (1.45)
APOE ε4+	7.22 (1.37)	5.88 (0.75)
APOE ε4-	7.02 (1.68)	7.34 (1.91)
Total ERC	15.02 (2.98)	13.06 (2.74)*
APOE ε4+	15.17 (3.00)	12.00 (1.59)
APOE ε4-	14.90 (3.02)	14.76 (3.50)

Results are mean (SD). HC: normalized volume of the hippocampus; ERC: normalized volume of the entorhinal cortex; Total: sum of the right plus left side volumes; APOE ε4+/ε4-: at least one APOE ε4 allele/ no APOE ε4 allele.
* $p < 0.05$; # $p < 0.01$

Figure 9. Total volumes of the hippocampus and entorhinal cortex (EC) in stable and progressive MCI in subjects with at least one APOE ε4 allele or without any ε4 allele. Filled circles represent patients with conversion of MCI to AD during the follow-up period.



5.5.2. Predictors for conversion to dementia

In the univariate Cox's regression analysis**,** the volumes of the hippocampus and ERC on the right side, the total volume of the hippocampi, and the CDR sum of boxes significantly predicted the progression of MCI to dementia (Table 8). The baseline MMSE score, WML burden, or the APOE ε4 allele were not significant predictors of progression. When patients with non-AD dementia were excluded**,** the right, left and total hippocampal and ERC volumes significantly predicted the progression to AD (Table 8). In this analysis CDR sum of boxes, MMSE scores, WML burden or APOE ε4 allele were not significant predictors for AD.

Table 8. Cox's univariate regression analysis with follow-up (months) as time variable and outcome (Dementia, AD) as status variable

	Dementia	AD
Right HC	0.738 (0.58 - 0,94) *	0.668 (0.49 - 0.91) #
Left HC	0.866 (0.68 - 1.10)	0.739 (0.55 - 1.00) **
Total HC	0.881 (0.77 -1.00) **	0.815 (0.69 - 0.97) *
Right ERC	0.597 (0.38 - 0.93)*	0.439 (0.25 - 0.77)#
Left ERC	0.836 (0.56 - 1.24)	0.568 (0.34 - 0.95)*
Total ERC	0.824 (0.65 - 1.04)	0.651 (0.47 - 0.90)#
Age	0.991 (0.87 - 1.12)	0.972 (0.84 - 1.12)
Gender	0.625 (0.20 - 1.98)	0.543 (0.15 - 2.04)
MMSE at baseline	0.919 (0.73 - 1.16)	0.918 (0.70 - 1.20)
CDR sum of boxes	2.730 (1.55 - 4.81)##	1.948 (0.90 - 4.21)
Education	0.979 (0.68 - 1.42)	1.198 (0.78 - 1.84)
APOE ε4 allele	0.534 (0.17 - 1.69)	0.359 (0.89 - 1.44)
WML load	1.007 (0.89 - 1.14)	1.016 (0.88 - 1.17)

Results are expressed as Hazard Ratio (95 % confidence interval). HC: normalized volume of the hippocampus; ERC: normalized volume of the entorhinal cortex; total: sum of the right plus left side volumes; AD: conversion of MCI to Alzheimer's disease; MMSE: Mini-Mental State Examination; CDR: Clinical Dementia Rating Scale; WML: white matter lesions.
* $p < 0.05$
** $p = 0.05$
# $p < 0.01$
$p < 0.001$

In bivariate Cox's regression analysis, the CDR sum of boxes and the volumes of the right ERC remained significantly associated with progression of MCI to dementia, whereas progression of MCI to AD was significantly associated only with the baseline volumes of the ERCs (Table 9).

Table 9. Cox's bivariate regression analysis with follow-up (months) as the time variable and outcome (Dementia, AD) as the status variable

	Dementia	AD
Right HC	0.805 (0.61 - 1.07)	0.731 (0.52 - 1.03)
CDR sum of boxes	2.627 (1.43 - 4.84)#	1.685 (0.71 - 4.00)
Left HC	0.952 (0.73 - 1.24)	0.812 (0.59 - 1.11)
CDR sum of boxes	2.721 (1.53 - 4.83) ##	1.829 (0.80 - 4.17)
Total HC	0.932 (0.81 -1.07)	0.864 (0.72 - 1.03)
CDR sum of boxes	2.675 (1.48 - 4.84) ##	1.723 (0.73 - 4.05)
Right ERC	0.577 (0.35 - 0.94)*	0.448 (0.24 - 0.83) #
CDR sum of boxes	3.124 (1.67 - 5.86) ##	2.222 (0.93 - 5.33)
Left ERC	0.787 (0.50 - 1.24)	0.542 (0.30 - 0.97)*
CDR sum of boxes	3.001 (1.60 - 5.64) ##	2.268 (0.93 - 5.50)
Total ERC	0.799 (0.62 - 1.02)	0.658 (0.47 - 0.92)*
CDR sum of boxes	3.175 (1.67 - 6.05) ##	2.285 (0.93 - 5.60)

Results are expressed as Hazard Ratio (95 % confidence interval). HC: normalized volume of the hippocampus; ERC: normalized volume of the entorhinal cortex; total: sum of the right plus left side volumes; AD: conversion of MCI to Alzheimer's disease; CDR: Clinical Dementia Rating Scale.

* $p < 0.05$

# $p < 0.01$

$p < 0.001$

6. DISCUSSION

6.1. Study subjects

The MCI and control subjects studied in this work have been collected from two large population-based cohorts. This is of importance when interpreting the results from a brain MRI study-design that concentrates on MCI, compared to studies where subjects are collected from a memory clinic. Noteworthy is also the careful clinical evaluation and diagnosis applied in the Kuopio cohorts. The criteria set used for MCI diagnosis (Petersen et al., 1995; Smith et al., 1996) represent that most widely used at the time when MCI patients started to be collected in our research center. From the two Kuopio cohorts, a database was compiled suitable for the objectives and aims of this work, resulting in a large and representative group of subjects (n=65 MCIs and n=59 controls), for whom the brain MRI scans were evaluated. Thus, this is a relatively large MR imaging study with a well documentated MCI population.

Limitations of such a long-term imaging-study are the changes and updates in scanning parameters and protocols. This had implications on the number of subjects included in the VBM-based study (n=51 MCIs and n=32 controls). In addition, a further limitation is the small number of subjects homozygous for APOE ϵ4 allele, especially when studying the differences between and within the groups of stable MCI and progressive MCI (n=3, respectively n=5), but this is the case for our study-population in the way the APOE alleles are distributed.

6.2. MRI techniques

The ROI-based volumetry is a basic method to investigate atrophy in the brain, though limited to the region planned to be studied, and it could be easy to apply also in the clinical work, provided that the tracer would have enough experience in performing the task. Nevertheless, the time needed to accomplish such a task may still be of inconvenience for a clinical purpose. A visual rating method is certainly much quicker, with a time required of 1-2 minutes per subject (Wahlund et al., 2000), and for that reason it is more convenient for use as a routine diagnostic tool in future dementia investigations. The VBM method is a more advanced method intended to investigate atrophy taking a global view of the brain, but it is not possible to apply it as a diagnostic tool in routine clinical work. However, VBM method is

a very useful research tool. It provides an *in vivo* insight into morphological changes in the brain at the group level, but it requires a set of normative images taken with the same scanner. Nevertheless, the purpose of this study was not to determine what would be the best imaging method for detecting atrophy in brain during clinical work, but this could be one point for future study.

A strong point of this work is that with only ROI-based method this imaging study is an *in vivo* evidence supporting the Braak&Braak statement (Braak and Braak, 1991 a; Braak et al., 1993), showing that entorhinal atrophy precedes the atrophy in the hippocampus in MCI, whereas hippocampal atrophy is more prominent in AD. Additionally, the present imaging study could map the atrophy that occurs in the brain during a preclinical AD phase, the MCI stage, moreover taking into account the influence of APOE genotype, a well known risk factor for AD (Farrer et al., 1997). The novelty of this work, compared to previous research done in MCI subjects, is that we examined the effects of the APOE ϵ4 allele on brain morphometry in MCI using the VBM method.

Although there are other good predictors for AD, the ERC and hippocampal volumes were the only features to predict conversion to AD from the MCI stage, which had been diagnosed according to the Mayo Clinic criteria (Petersen et al., 1995; Smith et al., 1996). When using VBM, other regions were also shown to be implicated in MCI, but are there other and/or better predictors of dementia/AD than the ERC and hippocampal volumes, it is not shown in this work. This is an objective for a future study for the Kuopio cohorts, when applying for that purpose an automatic imaging method to map the entire brain, with VBM or other new imaging techniques. If VBM analyses are to be conducted, then newer software than the SPM99 (www.fil.ion.ucl.ac.uk/spm) could be used, and that would allow more accurate image processing. However, that would make it more difficult to compare the results even within the same research group. There are divergences already when trying to compare the findings using ROI and VBM (Good et al., 2002, Tisserand et al., 2002 and Karas et al., 2003). For example, in **study I** the percentage volume loss in MCI relative to controls was greater on the left hippocampus and ERC, while in **study II** the predominant unilateral atrophy was on the right side. This may be accounted for the different amount of subjects included in the two studies, **study II** having a reduced amount of both, MCI and control subjects, or for the differences in the applied two MRI techniques to study the brain: manual and automatic estimations, or for both of the reasons. The VBM method also entails some

limitations and possible confounders that may have importance on the results. For example, the quality of the scans given by the reduction of gray/white matter contrast in the diseased people and the greater frequency of motion effects in this group may be of importance. Moreover, the registration errors of regional specificity that may appear with the spatial normalisation, especially of small structures, such as hippocampus or ERC are, may be more variable in a diseased group, which might reduce the sensitivity of VBM to detect changes, that otherwise would have higher intensity on manual measurements. Another inconvenience when trying to compare studies is the statistical p value used for analysis when applying VBM, some using $p<0.001$ value uncorrected, while others have the p corrected for multiple comparisons. For a very heterogeneous group such as MCI, a $p<0.001$ value uncorrected may be a better choice, while from the statistical point of view the other method would be preferable. Ultimately, the choice depends on the study design. We used $p<0.001$ uncorrected, but we found quite large, anatomically coherent groups of voxels that are significant, therefore in statistical terms this design is not a major problem.

6.3. ROI- and VBM-based volumetry

The volumetric studies performed here using both ROI- and VBM- based techniques were never intended for determining the comparative value of these two techniques. They are of complementary importance, one to determine *in vivo* the value of ERC atrophy over that of hippocampal atrophy in MCI (**study I**), other to *in vivo* map the gray matter loss in MCI (**studies II** and **III**). To the best of our knowledge until **study II**, there was only one published study using VBM in MCI (Chetelat et al., 2002), but this had a slightly smaller sample and different operational criteria for MCI.

6.3.1. ROI-based volumetry (study I)

6.3.1.1. ROI-based volumetry in AD vs. controls

An imperative amount of MRI volumetric studies have been dedicated to examining MTL atrophy in AD, and our results of significantly reduced ERC and hippocampal volumes in patients with AD compared with controls are in consensus with those from the literature. Our findings revealed a 36% ERC volume loss in the AD group compared with controls, which is in agreement with an earlier study by Juottonen et al. (1998 b) where a 40% reduction of ERC

volume was found between the two studied groups. In the study by Laakso et al. (1995), the right and left hippocampal volumes decreased by 38% in patients with AD (*n*=32) compared with controls (*n*=16). Similarly, our findings revealed a 37% total hippocampal volume loss and a reduction of 36% in the right and 39% in the left hippocampus, in the AD group compared with the controls. While in our study the magnitude of the ERC changes (36%) was similar to that of the hippocampal changes (37%) in AD patients, Du et al. reported a more intense atrophy in the ERC (39%) than that in the hippocampus (27%) in the AD group (Du et al., 2001). However, the variability in measurements of ERC is greater than that of the hippocampus, and anatomical ambiguity exists when depicting the ERC boundaries on MRI, especially in the AD patients (Juottonen et al., 1999).

6.3.1.2. ROI-based volumetry in AD vs. MCI

While the interest in the MCI group of subjects increased, also the MRI volumetric studies that where dedicated to AD brought results from comparisons between the two study groups: AD and MCI. In our study, though both regions were significantly atrophied in AD patients compared with MCI subjects, significantly greater volume loss in hippocampus (32%) than that in ERC (23%) appeared in between the two groups. A study designed similarly to ours, although the criteria used for diagnosing the MCI did not exactly overlap wth those proposed by the Mayo Clinic, was that conducted by Du and colleagues (2001). Yet, our results are in contrast to their findings. They claimed that, in the AD group compared with the MCI group, the ERC volume loss (30%) was significantly greater than that of the hippocampus (19%). We attribute these discrepancies to the differences in the study populations and to the variability in depicting a badly atrophied ERC on the MRI scans. Even so, our results are in agreement with the Braak&Braak staging theory (Braak and Braak, 1991 a; Braak et al., 1993), moreover they are substantiated by the results comparing the MCI and control groups. Thus, hippocampal atrophy appears to be more representative than ERC atrophy for AD.

6.3.1.3. ROI-based volumetry in MCI vs. controls

Our findings showing that compared with controls, the volumes of the ERC and hippocampus are significantly reduced in individuals with MCI, confirmed those from some earlier studies (Convit et al., 1997; Killiany et al., 2000; Xu et al., 2000; Du et al., 2001). In addition, we found that the ERC volume loss (16%) was significantly greater than the hippocampal volume

loss (8%) in MCI versus controls. This differs from the findings of another cross-sectional study (Du et al., 2001), which detected no differences in the magnitude of the volume loss between ERC (13%) and hippocampus (11%) in MCI. Our results are of interest, given that MCI refers to a transitional stage between the cognitive changes of normal aging and those of AD, and may represent a preclinical stage of AD. Therefore, our data is well in line with the assumption that the pathology of AD starts in ERC, providing in vivo evidence for the Braak&Braak stages (Braak and Braak, 1991 a; Braak et al., 1993). The explanation for the relatively modest average ERC volume loss (16%) may be that the MCI subjects were derived from a population-based cohort, and not from a group of individuals seeking help for memory problems. It should be noted that mildly impaired non-demented subjects form a heterogeneous group that includes both stable subjects and subjects who will develop AD (Petersen et al., 1995; Petersen et al., 1999; Petersen et al., 2001 a), as well as subjects that will possibly revert to normal (Larrieu et al., 2002).

6.3.2. VBM-based volumetry in MCI vs. controls (study II & III)

The findings from this thesis when mapping the gray matter loss in MCI subjects are in accordance with the one previously published study (Chetelat et al., 2002), using VBM in MCI, but with a slightly smaller sample and different operational criteria for MCI. Their data reported bilateral thalamic atrophy and some atrophy in the temporal neocortex. Moreover, we found a small area of atrophy in the anterior cingulate cortex. This is of interest because in animal models of AD, it is claimed that the entorhinal lesions possibly the initial site of AD pathology, lead to hypometabolism of the cingulum (Meguro et al., 1999). In addition, in humans, it should be noted that hypoperfusion of the cingulate has been proposed to predict conversion from MCI to AD (Huang et al., 2002).

The thalamic involvement noted in our study is also a matter of debate in the literature. While some VBM studies on AD have not detected thalamic involvement (Ohnishi et al., 2001; Good et al., 2002), there are positive studies as well (Baron et al., 2001; Karas et al., 2003). More recently, VBM analyses have found some thalamic involvement also in MCI (Karas et al., 2004; Chetelat et al., 2005). Our finding of thalamic involvement in MCI subjects (**study II**), was proved to rather appear in carriers of the APOE ϵ4 allele (**study III**). In a study of cognitively normal subjects with a family history of probable AD, Reiman and colleagues concluded that compared to noncarriers, APOE ϵ4 carriers had lower cerebral metabolic rates

for glucose (CMRgl) in the thalamus, besides other regions already proved to be affected in AD (Reiman et al., 2001). Additionally, it has been proposed that a disconnection mechanism from the MTL could lie behind the early thalamic metabolic deficit, which may be responsible for the atrophy process (Nestor et al., 2004). Moreover, the pathology of AD has been suggested to affect also the thalamus (Braak and Braak, 1991 c), yet this issue, and the factors underlying the discrepancy, remain to be resolved in future studies. One possibility that is worth mentioning is the bias inherent in the method. As Karas et al. stated the thalamus is one of the structures, which "lie geographically in locations which are hard to evaluate with computational models" (Karas et al., 2003).

An intence atophy was present in the hippocampus-amygdala region, especially for the carriers of the APOE ϵ4 allele in MCI subjects, where amygdala atrophy was affected bilaterally. Although according to volumetric studies, the amygdala is not a primary region affected in AD, ROI-based MRI studies (Cuénod et al., 1993; Boccardi et al., 2002) and some histopathological studies have detected damage in this region in AD, with pathological changes occurring mainly in the nuclei that receive or give rise to hippocampal or entorhinal projections (Vogt et al., 1990; Scott et al., 1991; Mann, 1992). Nevertheless, when studying the results in MCI/AD with the most pronounced atrophy occurring in the amygdala, one should be aware of the fact that bias may exist when using VBM, which may be greatly affected by the reduced gray/white matter contrast in old diseased subjects and the increased motion effects on the images.

6.4. Classification with ROI-based volumetry (study I)

6.4.1. Classification of AD and controls

When classifying AD patients and controls, both the hippocampal and the ERC volumes were of statistical significance. Yet, this yielded an overall classification of 90.6%, which was similar to the 90.7% classification achieved using hippocampus alone; hence the contribution of ERC was negligible. Du et al. (2001) found no significant differences in the power of ERC and that of the hippocampus in differentiating patients with AD from controls, but they improved the classification (89%) between the two groups when using a combination of both regional volumes. For the same purpose, Lehéricy et al. (1994) reached 100% accuracy with combined volumes of the hippocampus and amygdala, but using the hippocampus alone they

achieved an accuracy of only 89% in the correct classification of 26 subjects. A similar power of classification (89%) was reported by Laakso and colleagues when using the hippocampus alone to differentiate between 55 AD patients and 42 controls (Laakso et al., 1998).

6.4.2. Classification of AD and MCI

Comparing the groups of AD and MCI, an overall classification of 80.5% was obtained when using hippocampus, and adding ERC to the model did not improve the classification. The only significant variable that entered into the model in a stepwise discriminant analysis was the total hippocampal volume, which yielded a classification of 82.3%. Our findings are similar to the results from the study by Dickerson et al. (2001) in classifying non-demented subjects and AD patients, but the volumetry protocol used was different from ours and non-demented subjects overlapped only partially wth the MCI subjects. In the study by Killiany et al., the ERC alone gave a better accuracy in discriminating mild AD from "questionables" (81%) or from converters (85%) than did the hippocampus alone (75%, respectively 76%) (Killiany et al., 2002), though again, their ERC volumetry protocol differed considerably from ours. Comparable results were reported by Du and colleagues, presenting the volume of ERC with a greater power of discrimination than that of hippocampus (Du et al., 2001). Xu and colleagues could identify no differences in the power of ERC and hippocampal volumes in classifying the MCI and AD groups (Xu et al., 2000). The differences across structural MRI studies could be partially explained by the use of the different volumetric protocols, as well as by the criteria used for diagnosing MCI and the method to recruit the subjects.

6.4.3. Classification of MCI and controls

To prove *in vivo* that MCI is a group distinct from controls adds weight to the value of such a volumetric study. When Xu et al. (2000) failed to prove the hypothesis that MRI-derived measures of the ERC would be superior to hippocampal measures in classifying controls and MCIs, de Leon et al. (2001) stated "the hypothesis that the anatomic sequence of AD affects the EC prior to the hippocampus remains to be adequately addressed by MRI". In support of this theoretical expectance (de Leon et al., 2001), we showed that the ERC volume, but not the hippocampal volume, best discriminated MCI subjects from controls. To our knowledge, this had not been demonstrated prior to our study, using the same protocol for ERC volumetric measurements (Insausti et al., 1998) applied to MCI subjects as defined by

Petersen et al. (Petersen et al., 1995; Petersen et al., 1999). For example, there are earlier studies on the same topic, but with different protocols and diagnostic criteria, with results also favouring ERC over hippocampus. Accordingly, in the longitudinal study by Dickerson et al., the authors showed that ERC volume is better than hippocampal volume at distinguishing (69%) non-demented individuals (ND) (n=28) from controls (n=34) (Dickerson et al., 2001). However, the protocol they used for the ERC volumetric measurements (Goncharova et al., 2001) was different from ours and the concepts of ND and MCI overlap only partially. In another longitudinal study, Killiany and colleagues showed that only ERC could discriminate normals from "questionables" (83% accuracy) and from converters (84% accuracy), while the comparable discriminant analysis with the hippocampus in both cases was not significant (Killiany et al., 2002). The "questionables" and converters could not be discriminated by any of the two measured regions. For the classification of "questionables" a clinical dementia rating of 0.5 was considered and not the MCADRC criteria of MCI (Petersen et al., 1995; Petersen et al., 1999). Retrospectively shown, only a part of them met the criteria for MCI. Additionally the ERC protocol was different from ours: they measured only the midregion of the ERC from three slices of 1.5 mm thickness each.

6.5. APOE and patterns of brain atrophy in MCI (study III & IV)

The exact mechanisms by which APOE influences AD are not fully understood. Strong associations for APOE genotype with AD were shown to exist, since the APOE allele ε4 is the most consistently confirmed genetic risk factor for AD (Farrer et al., 1997). It was suggested that AD patients, carriers of APOE ε4 allele have more pronounced neuropathological changes, and exhibit more prominent atrophy of the MTL, as well as more severe memory loss compared to those AD patients with no ε4 allele (Petersen et al., 1995; Alberts, 1996; Lehtovirta et al., 2000; Engelborghs et al., 2003). The genetic asset (the presence of APOE ϵ4 allele) is consistent with a theory of greater brain vulnerability in ϵ4 carriers. Our results (**study III**) show that the presence of APOE ϵ4 in MCI subjects is associated with a greater volume loss in the parahippocampal gyrus, including the ERC, as well as in amygdala and in the medial dorsal thalamic nucleus. This is in a way, an association *in crescendo* observed in our MCI cohort, as it increases with the increased frequency of ϵ4 allele. In the heterozygous group for the APOE ϵ4 allele, only atrophy of parahippocampal gyrus, with ERC included, reached the level of statistical significance. As far as we are aware, this effect of the APOE ϵ4 on brain had never been previously evaluated,

using VBM-technique in MCI subjects. However, our results are of a preliminary nature, considering the small number of carriers studied, especially in the homozygous group. Nevertheless, our results, of higher frequency of the APOE ϵ4 allele associated with atrophy in the brain regions that overlap with those found to be atrophic in the total MCI group when compared to controls, are of interest considering that the APOE ϵ4 allele was shown to be a risk factor for conversion to MCI in cognitively healthy aged subjects (DeCarli et al., 2001; Tervo et al., 2004). Yet, this is just a speculation, as long as longitudinal imaging study-results on conversion to MCI in normal elderly subjects are not available now. Our results in MCI subjects, while employing the VBM-technique, resemble the dose-dependent effect of the APOE ϵ4 allele on the extent of atrophy of the MTL in AD patients, which has been detected with ROI-based volumetry and presented significantly increased atrophy from noncarriers of APOE ϵ4 allele to homozygous carriers, via heterozygous carriers (Lehtovirta et al., 1996; Juottonen et al., 1998 a; Geroldi et al., 1999; Geroldi et al., 2000).

The exact mechanism underlying the associations of APOE ϵ4 allele with the AD process is elusive. The APOE ϵ4 allele is considered to shift the age of onset of AD (Corder et al., 1993), but this was not the case in our study. In the present study (**study IV**), in the progressive MCI group, the groups of APOE did not differ in terms of age. Moreover there was no difference at baseline in the MMSE score, or CDR sum of boxes in the progressive MCI groups of APOE genotype (progressive carriers versus progressive noncarriers). However, the number of converters and accordingly the number of carriers that progressed to dementia was low in our study and this could have influenced the results achieved.

Nevertheless, the risk of APOE ϵ4 allele for AD was evident also in our sample of MCI subjects, although the APOE genotype was not a significant predictor for conversion. Thus, while for the APOE ϵ4 allele noncarriers there were no differences in MTL volumes between the progressive and stable MCI groups, for the APOE ϵ4 allele carriers the differences in MTL volumes were significant between the two groups (progressive versus stable MCI). Moreover, in the progressive MCI group, the carriers of at least one ε4 allele had significantly reduced hippocampus compared to noncarriers.

The functions of the APOE in brain are still not fully understood. A greater accumulation of histopathological AD hallmarks in APOE ϵ4 carriers has been proposed (see for review Cedazo-Minguez and Cowburn, 2001). In addition, in the case of a neuronal damage occurred

by different injurious agents, the needed neuronal repair of synaptodentritic connections was considered to be modulated by the presence of APOE, where the ϵ4 allele was associated with impaired cell repair and synaptic remodeling (Mahley and Rall, 2000). The plastic neuronal reorganisation, as evaluated by changes in the length and arborization of dendrites, has been shown to be impaired in AD carriers of the APOE ϵ4 allele (Arendt et al., 1997). Thus, a poor compensatory mechanism to repair neuronal damage additional to more accumulation of different histopathological AD features, might account for the more prominent brain atrophy in MCI carriers of APOE ϵ4 allele versus noncarriers, with this being even more apparent in the MCI progressive group.

6.6. Prediction of AD in MCI (study IV)

During the follow-up time of ~34 months, 13 subjects (21.7%) converted to dementia, with the annual conversion rate being 7.7% emphasizing the heterogeneity of MCI. Larrieu and colleagues in their longitudinal study found an annual conversion rate of 8.3%, but more than 40% of cases reverted to normal during 5 years of follow up (Larrieu et al., 2002). In our study, seven (12%) out of 60 MCI subjects who entered the follow-up had undergone an improvement of cognition by the last evaluation, indicative of the instability inherent in the MCI category as a diagnostic entity. MRI volumetric data in combination with neuropsychological data and APOE ε4 allele have shown a significant role in predicting the risk of AD (Petersen et al., 1995; Albert, 1996). In the present study, we found that the right sided volumes of the hippocampus and ERC and the CDR sum of boxes significantly predicted the progression of MCI to dementia. In contrast, the MMSE score, WML burden, or APOE genotype were not significant predictors of progression. It is well known that the ERC occupies a key position for the communication between the hippocampus and the rest of the brain. Accordingly, the degeneration of the neuronal architecture in the ERC destroys a large functional hippocampal pathway respectively causing memory impairment and cognitive deficits associated with AD (Hyman et al., 1984). Indeed, if the patients who developed non-AD dementia were excluded from our analysis, the only volumes of hippocampi and ERCs predicted the progression to AD. These findings are well in line with data reported by Korf and colleagues, showing that the visual assessment of MTL structures on MRI using a standardized rating scale is a predictor of dementia in MCI subjects independently of e.g. age, gender, education, MMSE**,** CDR sum of boxes, APOE genotype, and WML burden (Korf et al., 2004).

It has been proposed that the WML burden is associated with cerebrovascular or vascular phenomena (Launer, 2003). Additionally, vascular risk factors have been shown to be associated with increased risk for AD and MCI (Kivipelto et al., 2001; Kivipelto et al., 2002; Tervo et al., 2004). Moreover, there are suggestions that WMLs could contribute to the dementia process by accelerating the cognitive decline in MCI subjects (Wolf et al., 2000). Nevertheless, we found no association between WMLs and progression of MCI to dementia in this population-based cohort. The extent of the WML burden was relatively modest, which may be explained by the criteria of MCI used in this study emphasizing the memory loss. However, also other studies have indicated that atrophy of MTL structures is a stronger predictor of dementia than the amount of WMLs (Korf et al., 2004)**.**

6.7. Future studies

Studies on brain with different MR techniques are important to be applied in the future on even larger database of MCI subjects formed in the limit of possibility to please both, the objectives and the technical factors implicated in the study protocols. This is of importance to further on map the brain in MCI subjects, to try to predict conversion to dementia/AD and to determine what the best techniques to apply for those reasons are, from different points of view: grade of technical difficulty, cost level and time consuming.

A large post-mortem study-design including histopathological diagnosis and *in vivo* imaging volumetry measurements, as well as *in vivo* brain mapping findings would provide reliability of neuroimaging results and add differential diagnostic value in what concerns dementia investigations with high implications in the treatment of dementia. In the coming years visualization of neuropathologiacal features such as beta-amyloid accumulation and even earlier events in the the pathogenesis of AD using either PET or MRI will be a great challenge. In addition, new techniques such as diffusion tensor imaging and arterial spin labelling are worth for further studies.

Neuroimaging should be of an imperative value when used in drug trials to investigate rates of atrophy and changes in brain during a specific treatment, with high implications in monitoring the prognosis and outcome in MCI cohorts and in AD patients. Rates of brain atrophy and ventricular dilatation are of interest in longitudinal studies and useful in drug

trials in MCI and AD cohorts. For the evaluation of the brain tissue in clinical trials importance should be given to both the global brain rates of atrophy and the MTL rates of atrophy, possibly with accent on ERC and hippocampus.

7. CONCLUSIONS

In conclusion, the strength of the present study is the large size of the MCI sample derived from population-based cohorts. Volumetric MRI analysis of the ERC and hippocampus provided *in vivo* evidence that ERC atrophy precedes hippocampal atrophy in AD. In the MCI subjects, involvement of other brain areas in addition to the MTL was also present. The novelty of this work, compared to previous research done in MCI subjects, is the examination of the effects of the APOE ϵ4 allele on brain morphology in MCI using the VBM method. An annual conversion rate of 7.7% from MCI to dementia over a follow-up of 34 months was observed. Prediction of conversion to AD was aquired with the atrophy of the MTL. The APOE ϵ4 allele, although not a predictor of progression to dementia, does seem to modulate neurodegeneration, by increasing brain susceptibility to the effects of the disease. MRI volumetry remains a useful tool in identifying the anatomical markers for incipient AD.

In summary:

1). The ERC volume loss was dominant over the hippocampal volume loss in MCI, whereas more pronounced hippocampal volume loss appeared in mild AD.
2). Volumetric measurements of the ERC were more powerful than those of the hippocampus in discriminating MCI subjects from controls.
3). Mapping the GM loss with VBM in MCI, the involvement of other brain areas in addition to the MTL was found: left superior parietal lobule, left cingulate gyrus and, notably, the thalami bilaterally.
4). The vast majority of the brain atrophy observed at the group level in MCI appears to be due to the small group homozygous for the ϵ4 allele.
5). Atrophy of the ERC and hippocampus, mostly on the right side, predicted the conversion to AD.
6). The CDR score, WML load or ApoE genotyping provided no additional value over that of the MTL atrophy in the prediction of progression of MCI to AD.

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APPENDIX: ORIGINAL PUBLICATIONS I-IV

I

HIPPOCAMPUS AND ENTORHINAL CORTEX IN MILD COGNITIVE IMPAIRMENT AND EARLY AD

Neurobiology of Aging

25: 303-310, 2004

by

Pennanen C, Kivipelto M, Tuomainen S, Hartikainen P, Hänninen T, Laakso MP, Hallikainen M, Vanhanen M, Nissinen A, Helkala E-L, Vainio P, Vanninen R, Partanen K, Soininen H

II

A VOXEL BASED MORPHOMETRY STUDY ON MILD COGNITIVE IMPAIRMENT

J Neurol Neurosurg Psychiatry

76: 11-14, 2005

by

Pennanen C, Testa C, Laakso MP, Hallikainen M, Helkala E-L, Hänninen T, Kivipelto M, Kononen M, Nissinen A, Tervo S, Vanhanen M, Vanninen R, Frisoni GB, Soininen H

III

THE EFFECT OF APOLIPOPROTEIN POLYMORPHISM ON BRAIN IN MILD COGNITIVE IMAPIRMENT: A VOXEL-BASED MORPHOMETRIC STUDY

Dement Geriatr Cogn Disord

22: 60-66, 2006

by

Pennanen C, Testa C, Boccardi M, Laakso MP, Hallikainen M, Helkala EL, Hänninen T, Kivipelto M, Könönen M, Nissinen A, Tervo S, Vanhanen M, Vanninen R, Frisoni GB, Soininen H

IV

MRI OF HIPPOCAMPUS AND ENTORHINAL CORTEX IN MILD COGNITIVE IMPAIRMENT: A FOLLOW-UP STUDY

Neurobiology of Aging

Accepted for publication

by

Tapiola T, Pennanen C, Tapiola M, Tervo S, Kivipelto M, Hänninen T, Pihlajamäki M, Laakso MP, Hallikainen M, Hämäläinen A, Vanhanen M, Helkala E-L, Vanninen R, Nissinen A, Rossi R, Frisoni G, Soininen H

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