Hippocampal volumetry in mild cognitive impairment

Doctoral thesis by

Carl Eckerström

UNIVERSITY OF GOTHENBURG

Section of Psychiatry and Neurochemistry

Institute of Neuroscience and Physiology

Sahlgrenska Academy at University of Gothenburg

Cover: Detail from *De humani corporis fabrica* by Andreas Vesalius (1514-1564)

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“Worse by far than all bodily hurt is dementia: for he who has it no longer knows the names of his slaves or recognized the friend with whom he has dined the night before, or those whom he has begotten and brought up. Yes, by a cruel will he cuts off his own flesh and blood and leaves all his estate to Phiale — so potent was the breath of that alluring mouth which had plied its trade for so many years in her narrow archway.”

Juvenal (60-140 AD)

Abstract

Dementia is a syndrome with cognitive decline as a prominent feature. MCI is similarly a syndrome of cognitive decline, albeit with much subtler symptoms, and has been identified as a condition at risk for progression to dementia. A considerable clinical challenge lies in identifying MCI patients with an underlying dementia disorder. The overall aim of this thesis is to examine hippocampal volume in MCI with regard to prognostics.

Currently, Alzheimer's disease (AD) and vascular dementia are the two most important causes of dementia. Subcortical ischemic vascular dementia (SIVD), characterized by white matter lesions (WMLs), is considered the most important cause of vascular dementia in the elderly. Hippocampal atrophy has been identified as a common feature of AD and increasing evidence suggests that hippocampal atrophy is present in SIVD as well.

In Paper I, baseline hippocampal volume was studied in MCI patients who either converted or remained stable and in a control group. In Paper II, the predictive value of hippocampal volume was examined alone and in combination with CSF biomarkers Aβ42 and T-tau, respectively. In Paper III, the amount of WML was measured and compared with hippocampal volume in a group of MCI patients with different clinical outcomes. Finally, the possible association between hippocampal volume and performance on psychometric test was evaluated.

It was found that MCI patients subsequently converting to dementia have smaller hippocampi than stable MCI patients. Hippocampal volume seems to be a useful marker in MCI patients with different underlying disorders. It can therefore be argued that hippocampal volume may be viewed as a broad cognitive marker. Hippocampal volume was also found to supplement the prognostic ability of CSF Aβ42 and T-tau in MCI. Furthermore, measurement of WML shows that WML volume is related to hippocampal volume in patients with high WML burden, suggesting that WMLs may be involved in the development of hippocampal atrophy in SIVD. Left hippocampal volume was consistently a better prognostic marker than right hippocampal volume. When evaluating their respective association with psychometric test performance, the left hippocampus was found to be more closely related to test performance.

Populärvetenskaplig sammanfattning

Demens är ett syndrom - en kombination av symtom. Ett av de mest framträdande symtomen är en gradvis försämring av de kognitiva funktionerna (hjärnans tankefunktioner, exempelvis minne). Lindrig kognitiv störning (mild cognitive impairment; MCI) är ett tillstånd där de kognitiva funktionerna är nedsatta, dock inte lika uttalat som vid demens. Många MCI-patienter försämras inte, men MCI har visats vara ett risktillstånd för kognitiv försämring och tillståndet utgör ett förstadie till flera demenssjukdomar.

En utmaning för sjukvården består i att identifiera de MCI-patienter som har en underliggande demenssjukdom. För att utreda om en person har en begynnande demenssjukdom används en kombination av metoder. Hjärnavbildning ger information om eventuella skador i hjärnans strukturer, exempelvis i nervcellskärnan hippocampus som är nödvändig för att lagra minnen. Analys av ryggvätska visar om proteinnivåerna i hjärnans skyddande vätska – cerebrospinalvätskan – är onormala. Dessa refereras ofta till som ”biomarkörer”. Neuropsykologiska tester används för att undersöka minnet och andra av hjärnans funktioner.

Alzheimers sjukdom och vaskulär (kärlrelaterad) demens är de två vanligaste orsakerna till demens. Småkärlssjuka, som karaktäriseras av skador i hjärnans vita substans, bedöms vara den viktigaste typen av vaskulär demens hos äldre. Förtvining av hippocampus har visats vara ett vanligt fynd vid Alzheimers demens och det finns tecken på att hippocampus minskar även vid småkärlssjuka. Huvudsyftet för denna avhandling är att undersöka storleken på hippocampus vid lindrig kognitiv störning för att se om denna information kan ge vägledning avseende framtida sjukdomsutveckling.

(i) I det första delarbetet studerades hippocampus storlek hos MCI-patienter som antingen hade utvecklat demens två år efter mätningen eller inte hade försämrats avseende kognitiv funktion. En grupp friska kontrollpersoner studerades också. Resultaten visade att MCI-patienter som senare utvecklade demens hade mindre hippocampus än oförändrade MCI-patienter och kontroller. Detta gällde oavsett vilken typ av demens som patienterna utvecklade. Slutsatsen är att storleken på hippocampus har ett samband med försämrad kognitiv funktion och demens, för flera olika demenstyper.

(ii) I det andra delarbetet jämfördes hippocampusvolymerna med de mest använda demensmarkörerna i cerebrospinalvätska. Studien visade att förmågan att förutsäga sjukdom förbättrades hos biomarkörerna av att kombineras med mått på hippocampusstorlek.

(iii) I det tredje delarbetet mättes graden av vitsubstansförändringar i en blandad grupp MCI-patienter och jämfördes med storleken på hippocampus. Resultaten visade att det fanns ett samband mellan uttalad vitsubstansskada och minskad hippocampus,

vilket tyder på att vitsubstansskada kan vara delaktig i en process som leder till minskad hippocampus.

(iv) Slutligen utvärderades sambandet mellan hippocampusvolym och prestation på neuropsykologiska test. Vänster hippocampus visades genomgående vara en bättre markör för kognitiv försämring än höger hippocampus. Vänster hippocampus hade en starkare koppling till neuropsykologiska testresultat än höger hippocampus.

Sammanfattningsvis visar resultaten från avhandlingen att minskad hippocampus har ett samband med framtida demens, och att hippocampusmätning är ett kliniskt användbart instrument.

Table of contents

List of original papers

This thesis is based on the following papers, referred to in the text by their roman numerals:

I. Eckerström C, Olsson E, Borga M, Ekholm S, Ribbelin S, Rolstad S, Starck G, Edman A, Wallin A, Malmgren H. **Small baseline volume of left hippocampus is associated with subsequent conversion of MCI into dementia: the Göteborg MCI study.** *J Neurol Sci.* 2008 Sep 15;272(1-2):48-59.

II. Eckerström C, Andreasson U, Olsson E, Rolstad S, Blennow K, Zetterberg H, Malmgren H. Edman H, Wallin A. **Combining hippocampal volume and CSF biomarkers improves predictive value in MCI.** *Dement Geriatr Cogn Disord* 2010;29:294-300

III. Eckerström C, Olsson E, Klasson N, Bjerke M, Göthlin M, Jonsson M, Rolstad S, Malmgren H, Wallin A, Edman Å. **High white matter lesion load is associated with hippocampal atrophy in MCI.** Manuscript.

Abbreviations

Aβ42	The 42 amino acid long amyloid beta peptide
AD	Alzheimer's disease
ApoE	Apolipoprotein E
CDR	Clinical Dementia Rating
CSF	CerebroSpinal Fluid
CT	Computer Tomography
EEG	ElectroEncephaloGraphy
GDS	Global Deterioration Scale
MCI	Mild Cognitive Impairment
MCI-MCI	Mild Cognitive Impairment remaining stable
MCI-c	MCI Cognitive Impairment converting to dementia
MCI to AD	Mild Cognitive Impairment converting to Alzheimer's disease
MCI to non AD	Mild Cognitive Impairment converting to dementia other than AD
MCI-s	Mild Cognitive Impairment remaining stable
MCI-Vasc	Mild Cognitive Impairment converting to Vascular Dementia or mixed dementia
MMSE	Mini Mental State Examination
MRI	Magnetic Resonance Imaging
NFT	NeuroFibrillary Tangles
SIVD	Subcortical Ischemic Vascular Dementia
SP	Senile Plaques
SPECT	Single Photon Emission Computer Tomography
T-tau	Total levels of the protein tau
VaD	Vascular Dementia
WMLs	White Matter Lesions

Introduction

Dementia, mild cognitive impairment and aging

Dementia and mild cognitive impairment are syndromes or clusters of symptoms that are believed to be linked. The most important risk factor for dementia and mild cognitive impairment is high age.

Dementia

Dementia is defined as cognitive impairment and a decreased ability to perform the tasks of everyday life [1]. Dementia is not an illness in itself, but rather a syndrome of cognitive and functional impairment secondary to a disease of, or damage to, the central nervous system. The incidence of dementia is highly age-related and it is estimated that the prevalence of dementia doubles every five years from 1.5% in 60-69 years old to 40% in persons over 90 years old [2]. As life expectancy increases worldwide it is estimated that the prevalence of dementia will increase from 24 million people today to 81 million people in 2040 [3]. Dementia does not only lead to suffering for the patient and next of kin, but also poses a great challenge to the healthcare system. In Sweden, the estimated yearly cost for dementia is SEK 40 billion [4]. In the United Kingdom the cost for cognitively impaired patients living in institutions is estimated to £4.6 billion [3].

Historically, the view on dementia has differed. The first written description of age-dependent cognitive decline dates back to Greek and Roman philosophers and physicians in the 7th century BC [5]. Aging in itself was seen as an illness and Hippocrates observed that "the entire person is ill from the moment of his birth" [6].Dementia was then regarded as an inevitable process of mental decline caused by aging. Pythagoras noted that the development of new skills early in life reverses later on and that this natural regression begins at the age of 60 [5]. This view persisted until studies by Giovanni Battista Morgagni (1682-1771), showing that distinct morphological changes to the bodily organs could lead to illness, paved the way for the organic view of disease [6]. Under the influence of materialism and humanism,

dementia was gradually recognised as a condition separated from normal aging [5]. In 1907, Alois Alzheimer published a case report on Auguste Deter, a 50-year old woman suffering from progressive confusion and hallucinations. The report included a neuropathological examination, describing senile plaques and neurofibrillary tangles. Although Alzheimer's report was not the first description of plaques and tangles, and despite the fact that a clinicopathological study with 12 patients describing the same changes was published the same year [7], Emil Kraepelin named the disease after Alzheimer in his influential textbook published in 1910 [7]. In the first part of the 20th century, dementia was considered to be caused by stiffening of the blood vessels in the brain and Alzheimer's disease (AD) was considered an unusual presenile condition [8]. In the later part of the 20th century, the finding that elderly dementia patients had senile plaques [9] shifted the focus from vascular causes towards a more AD oriented view. Further evidence for the importance of senile plaques came with the discovery that familiar forms of AD had mutations in genes encoding amyloid precursor protein (APP) or APP cleaving enzymes [10,11], which lead to the proposal of the amyloid cascade hypothesis [12]. The amyloid cascade hypothesis had a profound influence and senile dementia was successively recognised as Alzheimer's disease. Recently, however, a number of studies have been published that challenged the amyloid cascade hypothesis. An article published in 1997 showed that the amount of AD neuropathology (senile plaques and neurofibrillary tangles) alone could not explain the variation of cognitive functioning in patients who fulfilled the neuropathological criteria for AD [13]. Findings like these have increased the interest in mechanisms that affect cognitive functioning; and the idea that cerebrovascular function is important for cognitive health has been revitalized. Another field of research that has attracted interest lately is the effect of aging on cognitive health and the question where the line between dementia and healthy aging should be drawn. In order to address these issues two clinical rating scales were introduced in 1982; the clinical dementia rating (CDR) and the global deterioration scale [14,15]. Both the CDR and GDS assess intermediary stages between normal cognitive function and dementia. The term MCI was first used in 1988, describing a group of patients classified as GDS 3 [16]. The concept of MCI has since been refined and different classifications have been proposed.

Mild cognitive impairment

Mild cognitive impairment is a state with mildly impaired cognitive functions but intact ability to perform basic daily activities. The MCI population consists of mainly three categories: patients subsequently recovering from the cognitive impairment, patients with stable cognitive impairment during follow-ups, and patients eventually converting to dementia. An early MCI classification, describing MCI as a transitional state between normal aging and AD was introduced in 1999 and received much interest in the field [17]. Subsequent research on MCI populations have identified MCI as an early stage of several dementia disorders [18]. As research in the field increased, the original MCI classification was widened to better correspond to the new results. In 2004, the international working group on mild cognitive impairment presented a consensus report proposing the following criteria for MCI: (i) the patient is neither normal nor demented; (ii) there is evidence of cognitive deterioration shown by either objectively measured decline over time or subjective report of decline by self and/or informant in conjunction with objective cognitive deficits; and (iii) activities of daily living are preserved and complex instrumental functions are either intact or minimally impaired[19]. The incidence rate is estimated to 12 – 15 per 1000 person years in people aged over 65 [20], which is roughly ten times the global incidence rate of dementia [2]. Clinical MCI studies generally show higher rates of conversion to dementia than population based studies. The annual conversion rate is 5 – 10 % and less than half of the MCI patients convert to dementia, even with a long follow-up time [21]. The etiology is multi-factorial, including primary degenerative, vascular, psychiatric, metabolic, toxic, traumatic, and pharmacologically induced deterioration [19]. Neuropathological studies in MCI have shown presence of AD and cerebrovascular pathologies suggesting that MCI may be an early stage of both AD and vascular dementia [22].

Aging

As the organism ages it undergoes irreversible changes. These changes are progressive and lead to reduced functionality. The process is not fully understood but there is increasing evidence for a number of key elements. Currently, the oxidative stress theory is the most influential. It states that as a metabolically active cell ages, the continual production of free radicals leads to an accumulation of oxidatively damaged biomolecules, such as DNA,

proteins and lipids [23]. The combined effect of these changes is thought to represent functional aging.

Structural changes can be seen in the aging brain. Typically, there is a reduction in cortical grey matter volume and an increase of lateral ventricle volume [24-26]. White matter volume is more resistant to aging, as is cerebellar volume [24-26]. Studies using diffusion tensor imaging techniques suggest, however, that white matter changes in aging may appear earlier than was previously believed [27]. The effect of aging on the hippocampus and related structures such as the entorhinal cortex is not clear. Several studies have found hippocampal and entorhinal cortical volume loss in aging [24,26,28], but others have found no or only a small aging effect [29-31]. In conclusion, it seems that the hippocampi and the entorhinal cortices are more resistant to aging compared to other grey matter regions.

Most cognitive functions are affected by aging. Mental speed and processing intensive tasks such as attention and working memory are affected first in the aging process as opposed to language functions which are more resistant to aging [32]. Since the aging process is highly individual, the chronological age does not reflect how many more years the subject will live. In acknowledgement of this researchers have found the need to measure age not from birth but from death. The findings that elderly individuals will undergo a faster deterioration in the time prior to death lead to the development of the concept of terminal decline [33]. It has been shown that an accelerated global cognitive loss precedes death by several years [33,34]. Questions regarding when the terminal decline begins and how the effect is related to the direct cause of death remain to be answered.

Dementia disorders

There are numerous causes of dementia. The material used in this thesis consists of patients seeking care at a memory clinic. Typically, patients with cognitive deficits caused by large cerebral infarcts, malignant tumors, major depression or one of many severe somatic disorders that can cause dementia, will not be referred to the memory clinic as the cause of the cognitive impairment is obvious. The dementia disorders most commonly found in the material are Alzheimer's disease, subcortical vascular dementia, frontotemporal dementia, and Lewy-body dementia. As the cases of frontotemporal dementia and Lewy-body dementia were too few for a specific analysis, they will not be described further.

Alzheimer´s dementia

Alzheimer´s dementia (AD) is regarded as the most common form of dementia [2]. Clinically, AD is characterized by progressive impairment of memory, visuospatial abilities and language. As the disease progresses it results in global cognitive impairment. The most commonly used diagnostic criteria is the NINCDS-ADRDA [35], according to which the diagnosis is classified as definite, probable or possible AD based on clinical findings with or without histological confirmation. Clinical symptoms include progressive impairment of memory, language, and visuospatial functions, and functional and behavioral disturbances [36]. As the disease progresses it leads to global cognitive impairment [36]. Risk factors for AD include high age, hypertension, diabetes mellitus, and smoking [37].

The neuropathological hallmarks of the disease are senile plaques (SP) and neurofibrillary tangles (NFT). The distribution pattern of the neuropathological changes in AD was carefully described by neuropathologists Heiko and Eva Braak. According to the Braak staging, the distribution of SP begins in stage A in the basal portions of the frontal, temporal and occipital lobe, from where it spreads to other parts of the neocortex in stages B and C [38]. The distribution of NFT begins in the entorhinal region of the medial temporal lobe. From there, it spreads first to the hippocampus and the limbic system and later to the neocortex [38].

The most influential theory regarding the pathophysiology of AD is the amyloid cascade hypothesis [12]. It states that the driving mechanism of AD is the accumulation of insoluble β amyloid peptides (Aβ), by either increased production or by decreased elimination [12] . The β amyloid aggregates and forms plaques which elicit inflammatory activity, damaging the nearby tissue [39]. Aside from activating inflammatory response, dimers and trimers of Aβ seem to be neurotoxic on their own [40,41]. NFT consist of hyperphosphorylated forms of the cytoskeleton-associated protein tau. The role of NFT in the pathophysiology of AD is not clear but it has been suggested that accumulation of hyperphosphorylated tau is driven by accumulation of Aβ [42]. Levels of Total-tau (T-tau) and phosphorylated Tau in CSF have also been shown to correlate with cognitive function [43].

There are alternative theories regarding the pathophysiology of AD. These theories consider early-onset AD as a genetic disease and a primary Aβ amyloidosis, while maintaining that amyloidosis in late-onset AD is a consequence rather than a cause of the disease.

The vascular hypothesis states that cerebrovascular pathology is the cause of AD. Briefly, the main argument for this theory is the finding that reduced cerebral bloodflow precedes neurodegeneration in AD [44]. According to the theory, the reduced blood flow affects the hippocampus and the posterior parietal cortex first as they are the regions most sensitive to hypoxia [45]. The following metabolic energy crisis leads to oxidative stress and increased inflammatory activity resulting in neuronal damage and cognitive decline. Further argument comes from the facts that not all patients with even widespread deposits of SP and NFT display AD symptoms [13], and that the known risk factors for AD are shared with VaD [37,46].

The retrogenesis model of AD hypothesizes that white matter myelin degeneration is the driving cause in AD, and that this degeneration is reverse to that of myelogenesis [47]. Thus, the late developing language and executive functions should be affected early in the disease process, while the early developing sensorimotor functions should be affected late in the disease process. Clinical studies have shown that this is indeed the case in AD [47]. A number of neuroimaging studies have examined the assertions of the hypothesis. As predicted by the model, the late developed neocortical association and allocortical fibers are the first to be affected in AD [48]. These findings have since been confirmed in studies using diffusion tensor imaging [49,50].

Vascular dementia

Vascular dementia (VaD) is regarded as the second most common cause of dementia [2]. It is caused by ischemic, hypoperfusive or haemorrhagic damage to the brain. VaD is classified as large-vessel disease, small-vessel disease, ischemic-hypoperfusive vascular dementia, or haemorrhagic vascular dementia [51]. Subcortical ischemic vascular dementia (SIVD), a subgroup of the small-vessel diseases, is a major cause of dementia in the elderly and probably the most common subtype of VaD [52,53]. The clinical symptoms of SIVD are cognitive and motor slowing, impairment of executive functions, personality change, and short-stepped gait [51,54]. Risk factors for SIVD include (in common with AD, see above)

high age, hypertension, and diabetes mellitus [54,55]. Pathologically, SIVD is characterized by occlusion or stenosis of the lacunar arteries, leading to ischemia and hypoperfusion of the deep cortical white matter [51]. A number of pathological features have been identified in the development of SIVD. The most prominent seem to be arteriolosclerosis and lipohyalinosis [55]. Arteriolosclerosis is characterized by concentric stenosis of the vessel lumen of arterioles [55]. Lipohyalinosis is characterized by initial damage to the vessel wall leading to leakage of plasma proteins into the vessel wall and the perivascular space [55]. On CT and MRI examinations, these changes appear as white matter lesions in the deep white matter.

Mixed dementia

The term mixed dementia describes a state where both AD and cerebrovascular pathologies are present. Since the finding of the genetic mutations causing familial AD and the development of the amyloid cascade hypothesis, much of the focus in the field has been on amyloid pathology and its role in cognitive impairment. A paper published in 1997 did, however, reawaken the interest in the role of vascular pathology. The finding from that study was that the progression of cognitive impairment is more rapid when both pathologies are present [13]. Subsequent studies have found that AD patients with cerebrovascular pathology will be more cognitively impaired than AD patients with similar levels of AD pathology but without vascular pathology [56,57]. It has been suggested that mixed pathology accounts for the majority of cases of dementia in the elderly [58,59]. There are large discrepancies, however, between these neuropathological findings and reported prevalence levels of dementia from epidemiological and clinical studies, reporting that AD accounts for 50 % - 70 % of all cases of dementia [2,4]. Further research is needed to elucidate the interaction between AD and cerebrovascular pathology.

The hippocampus

The hippocampus is a 3-4 cm^3 paired structure located in the medial temporal lobe. The name “hippocampus” is Greek and comes from the resemblance of the structure to a sea-horse. It was first used by anatomist Arantius in his 1587 work “De Humano Foetu” [60]. Ramon y

Cajal, using new histological staining methods, gave the hippocampal formation the first comprehensive description in 1910 [60].

Figure 1. Two hippocampi.

Preparation by Prof Laszlo Seress. Published under CC-BY-SA.

The hippocampus receives the majority of its incoming projections via the entorhinal cortex from frontal, temporal, and parietal association areas, and projects to the same regions [61-63]. Bilateral hippocampal lesions lead to extensive memory impairment with three distinct characteristics [62]. First, the impairment is multimodal, meaning that the memory impairment encompasses information received by all sensory modalities. Second, delayed memory is impaired but immediate memory is intact. The consequence of this is that very simple information may be rehearsed and remembered for a short period of time but more complex information is quickly lost. Third, the impairment is limited to memory; perceptual functions are intact and the patient may have a normal score on intelligence tests and tests for semantic memory.

The hippocampus is regarded as a neurogenic region of the brain, with the presence of both precursor cells and a microenvironment suitable for production of new neurons [64]. There is increasing evidence, mainly from animal studies, that adult neurogenesis plays an important role in learning and memory [65]. The most important regulator of hippocampal neurogenesis is hippocampal dependent learning [66]. Learning increases the survival of differentiated cells which were born recently before training, but induces apoptosis in cells born at the start of training [67]. Animal studies have shown that living in an enriched environment have similar effects on hippocampal neurogenesis as learning [68]. Physical training has also been shown to increase hippocampal neurogenesis, but it is by increased proliferation of progenitor cells rather than by increased survival in differentiated cells [69]. The structure of cortical gray matter is highly determined by genes. In studies comparing monozygotic twins to unrelated subjects it was found that the structure of frontal, sensorimotor, and language cortices were genetically determined by 70 % to 90% [70]. The hippocampus, however, seems to be an exception. The structure of the hippocampus is determined equally by genes and environment, meaning that environmental factors shape the hippocampus much more than the rest of the brain [71].

The hippocampi are well vascularized. The majority of the blood supply comes from the posterior cerebral artery, but blood is also supplied from the anterior choroidal artery [72]. These vessels have anastomotic sites in the hippocampus. Studies have shown that the hippocampus is one of the regions in the brain most sensitive to hypoxia [73,74].

Asymmetry

It is well known that, as opposed to what is the case in lower primates, the human cerebral hemispheres are not functionally equivalent. Briefly, the left hemisphere is considered dominant, handling verbal information while the right hemisphere handles non-verbal information. Damage to the left hippocampus impairs verbal learning, regardless if the information is read or heard, while damage to the right hippocampus leaves verbal learning intact but impairs the ability to recognize visual and auditory patterns [75]. Recent studies have, however, questioned the clear left - right, verbal – non-verbal dichotomization [76,77]. Furthermore, temporal lobe surgery studies have reported that unilateral hippocampal lesions

can be tolerated surprisingly well [78], supporting the view that many important functions of the hippocampus are bilaterally represented.

Structurally, the right hippocampus seems to be larger than the left hippocampus in the cognitively healthy [79], and this pattern remains in MCI and AD [80]. The asymmetry does not seem to be affected by handedness [81], although left-handed women may have overall larger hippocampal volume [82]. The cause of asymmetry may be hormonal [83], but the functional consequences of the size differences remain unknown.

White matter lesions

White matter lesions are often seen in the elderly undergoing CT or MRI investigation. The lesions appear on MRI images as patchy or confluent hyperintensities most commonly seen in the deep white matter surrounding the lateral ventricles. Based on their localization they are often classified as periventricular or subcortical white matter lesions. A number of terms are used synonymously in the scientific literature to describe white matter lesions, including age-related white matter changes, white matter changes, white matter hyperintensities, and leukoariosis. The prevalence of subcortical white matter lesions increases with age [55].

WMLs can be focal or diffusive in appearance. The focal WMLs are believed to be fluid filled cavities resulting from small infarcts and the diffuse WMLs are believed to be areas of incomplete infarction [84]. WMLs are the pathological hallmark of SIVD and have been found to be associated with progression of MCI to dementia [85]. It has also been found that progressive WMLs are related to a parallel decline in cognitive function [86]. Furthermore, in a community based study, WML volume was shown to be inversely correlated with hippocampal volume in healthy elderly, independently of vascular risk factors [87]. Smaller clinical studies have found similar results in SIVD and AD [88,89]. Others have found no association between WML volume and hippocampal volume [90]. Although there seems to be some support for a relationship between WMLs and hippocampal atrophy, the underlying mechanism is not understood. There remains a possibility that the relationship is caused by a confounder such as age or vascular risk factors.

Diagnostic tools for the investigation of cognitive decline

Beside the clinical interview and medical history, there are a number of examinations to assist the clinician in finding the cause of cognitive decline.

Cerebrospinal fluid biomarkers

The cerebrospinal fluid (CSF) surrounds the brain and spinal cord. It provides physical protection of the central nervous system (CNS), absorption and removal of selected compounds and regulation of CNS activity by circulating neurotransmitters and hormones. It is separated from the blood by the blood-brain barrier which restricts the flow of proteins to the brain [91]. The CSF is in direct contact with the extracellular space of the CNS and thus reflects the chemistry of the brain, allowing analyses of the CSF to detect pathologic processes in the brain [91]. CSF samples are collected by lumbar puncture. A number of cerebrospinal fluid biomarkers are well established for the prediction of AD. The level of Aβ42 is believed to reflect the non-soluble Aβ-peptides present in senile plaques [91-94]. Neurofibrillary tangles, consisting of phosphorylated forms of the microtubule-associated protein tau, are displayed by the biomarkers total-tau and phosphorylated tau [91-94]. These biomarkers show abnormal values before the onset of dementia but appear to remain stable as the disease progresses [95,96]. They are therefore of limited value as markers of progression. The CSF biomarker profile for the prediction of VaD is not as well studied as that for AD. There is, however, some evidence that levels of the myelinated axon related protein Neurofilament light may be increased early in VaD [97-100].

Psychometric markers

A neuropsychological test battery is designed to assess performance across the cognitive domains: speed and attention, learning and episodic memory, visuospatial functions, language functions, and executive functions. Studies examining the neuropsychological performance of MCI patients compared to healthy elderly have revealed that MCI patients are impaired across several cognitive domains [101,102]. These findings suggest that MCI is a heterogeneous condition. Several studies have attempted to identify different cognitive profiles as markers of the underlying disorders. Thus, incipient AD is characterized by broad cognitive impairment,

most pronounced in attention, verbal learning and memory functions [103-105]. Incipient SIVD is not as well studied but the disorder seems to involve an early decline in executive functions, attention functions, and mental speed [106,107]. There are, however, considerable overlap in the cognitive profiles of incipient AD and SIVD [104,108]. As AD and SIVD progress they result in global cognitive impairment.

Neuroimaging markers

Neuroimaging in cognitive disorders include structural and functional imaging. Functional imaging visualizes functional and metabolic aspects of the brain using functional magnetic resonance imaging (fMRI), positron emission tomography (PET), and single photon emission computer tomography (SPECT). Structural imaging gives morphometric information using magnetic resonance imaging (MRI) and computer tomography (CT). Since structural MRI is the topic of this thesis, the other modalities will not be presented further.

As the hippocampus is necessary for formation of new memories and neuropathological studies have shown that the hippocampal region is an early site for AD pathology, the question was raised more than two decades ago if measurement of the hippocampus in vivo could provide information on the presence of pathology. The first study showing hippocampal atrophy in AD using magnetic resonance imaging was published in 1989 [109]. Several studies have since linked hippocampal atrophy to AD and incipient AD [18,110-115]. Surrounding structures such as the entorhinal cortex and amygdala also seem to be affected [116-118]. These alternative markers have, however, not been proved superior to the hippocampus which remains the most used structural marker ofAD and incipient AD. Studies have shown that grey matter volume decreases with increasing age in the normal population [26,28,29], but hippocampal volume seem to decrease less than cortical grey matter [26,29]. The variance of hippocampal volume seems to be the same in young and old healthy adults [28], implying that there is a group of healthy people with small hippocampal volumes. This raised the question if small hippocampal volume should be regarded not as a disease marker but rather as a risk factor for dementia. Twin-studies allow for studying the relationship between genetic and neuropathological influence on brain structure. One such study reported significantly smaller hippocampus in the twin with AD compared to both the non-demented twin and to healthy controls [119]. Other studies have found increased rate of hippocampal

atrophy in patients with incipient AD compared to controls, strengthening the belief that hippocampal atrophy is a disease marker in AD [120,121].

Less is known about hippocampal structure in vascular cognitive impairment and SIVD. There is, however, some evidence that cognitive impairment in SIVD is associated with hippocampal atrophy [122,123].

Objectives

The objective of the thesis is to examine hippocampal volume and other putative markers of cognitive decline in a clinical setting at a memory clinic. The specific objectives are to:

1. Examine hippocampal volume in two groups of MCI patients with and without cognitive deterioration and in a matching control group (Paper I)
2. Examine longitudinal changes in hippocampal volume in these groups and determine if it is related to cognitive decline (Paper I)
3. Examine whether hippocampal volume can distinguish between MCI patients converting to AD or SIVD (Papers I and III)
4. Examine how hippocampal volume performs as a marker of cognitive decline, alone and in combination with CSF biomarkers (Paper II)
5. Examine the presence of white matter lesions in MCI and their relation to hippocampal volume (Paper III)
6. Examine the relationship between hippocampal volume and performance on a comprehensive neuropsychological test battery (Unpublished data)

Material

Göteborg MCI study

The Göteborg MCI study started in 1999 and is ongoing. It is based at the memory clinic in Mölndal which is part of the Sahlgrenska University Hospital. The aims of the study are to identify neurodegenerative, vascular and stress-related disorders prior to the development of overt dementia [101]. The study has a longitudinal design with biannual assessments. The baseline investigation includes neurologic, psychiatric, and cognitive screening, neuropsychological testing, MRI examination, SPECT examination, EEG examination, and sampling of blood and CSF. The Göteborg MCI study is approved by the local ethics committee (diary number: L091-99, date: 15-March-1999).

MCI classification procedure

Study participants were classified using the global deterioration scale (GDS) [15]. The classifications were based on medical history and the following checklists and instruments for cognitive function: Stepwise Comparative Status Analysis (STEP) [124], IFlex, which is a short form of the Executive Interview (EXIT) [125], Mini Mental State Examination (MMSE) [126], and Clinical Dementia Rating (CDR) [127]. The CDR assessment was based on information from both the participant and an informant. For inclusion, subjective and objective (by an informant) verifications of a progressive cognitive impairment for more than 6 months was required. In addition, a positive outcome on STEP, I-Flex, MMSE, or CDR was required. Based on the results, patients were at each examination classified according to the Global Deterioration Scale (GDS) where 2 equals subjective cognitive impairment, 3 equals MCI, and 4 equals mild dementia [15].

Dementia diagnostic criteria

Dementia diagnoses for MCI patients converting to dementia were founded on somatic examination, anamnesis, neuropsychiatric evaluation, and MRI. AD was diagnosed according to the NINCDS-ADRDA criteria [35], SIVD according to the Erkinjuntti criteria [128], frontotemporal dementia according to the Lund and Manchester criteria [129], Lewy body dementia according to the consensus guidelines by McKeith et al. [130], and dementia non

ultra descriptum (Dementia NUD) according to the International Classification of Diseases, tenth revision (ICD-10) [131].

Healthy controls

Healthy controls were recruited mainly from senior citizens' organisations, while a few were spouses of study patients. They were invited if they were physically and mentally healthy. Controls were not included if they had subjective or objective signs of cognitive disorder as assessed from above procedure.

Study participants

The total patient group used in Papers I and II (N=42) included 21 patients with MCI (GDS 3) at baseline who had converted to mild dementia (GDS 4) by the time of the two year follow-up (converting MCI; MCI-c), and 21 patients with MCI both at baseline and follow-up (stable MCI; MCI-s). Thirteen of the converting MCI patients converted to AD, 4 to SIVD, 2 to mixed Alzheimer/vascular dementia and 2 to frontotemporal dementia. The AD converters were grouped into the MCI to AD group and the other converters were grouped into the MCI to non-AD group. Twenty-six healthy controls were also included. All converting MCI patients with two clinical assessments and a baseline MRI investigation with the 0.5 T scanner were included. Stable MCI patients were classified as GDS 3 at baseline and follow-up, and were included consecutively to achieve matching group sizes.

In the total patient group used in Paper III (N=122), all MCI patients with at least two clinical examinations and at least one MRI investigation with the 1.5 T scanner were included. The earliest MRI examination was used if a patient underwent more than one examination. The patient group included 30 patients who were classified as GDS 2 or 3 at baseline and had deteriorated to GDS 4 at subsequent examinations (converting MCI; MCI-Con), and 92 patients who were classified as GDS 2 or 3 at baseline and GDS 2 or 3 at subsequent examinations (stable MCI; MCI-MCI). Forty-four healthy controls were also included. Sixteen of the converting MCI patients converted to AD, 6 converted to SIVD, 5 to mixed dementia, 1 to Lewy body dementia, and 2 to dementia NUD. The AD converters were grouped into the MCI-AD group, the SIVD and mixed dementia converters were grouped into the MCI-Vasc group.

Table 1. Characteristics of study participants

Paper	Group	N	Men/Women	Age	Education	MMSE
I & II	Converting MCI	21	6/15	69.3 ± 6.3	10.4 ± 3.4	27.2 ± 2.0
	Stable MCI	21	12/9	66.6 ± 7.0	12.5 ± 3.5	28.3 ± 1.8
	Controls	26	8/18	67.7 ± 5.3	11.7 ± 3.4	29.4 ± 0.9
III	Converting MCI	30	16/14	66.9 ± 8.5	11.5 ± 3.6	25.4 ± 4.5
	Stable MCI	92	38/54	64.3 ± 7.4	12.4 ± 3.6	29.0 ± 1.1
	Controls	44	15/29	66.2 ± 7.2	11.8 ± 3.6	29.4 ± 0.4

Method

Two different MR scanners have been used since the start of the Göteborg MCI study. A 0.5 T magnet (Philips NT5) was used until 2004 and a 1.5 T magnet (Siemens Symphony) was used thereafter. Data from the 0.5 T scanner were used for Papers I and II. Data from the 1.5 T scanner were used for Paper III.

Imaging in Papers I and II

Manual hippocampal volumetry was performed by means of a custom method on an interactive Cintiq LCD tablet screen. The method has been validated on two other datasets (unpublished data). The segmentation was done on T1 weighted coronal slices which were scanned perpendicularly to the hippocampal principal axis. Hippocampal volumes were normalized using intracranial volume.

The segmentation process consisted of two steps: 1. Landmark setting was done by point-wise cross setting in the sagittal view of the reformatted coronal image where the demarcation in the original coronal image is indiscernible or difficult to interpret. 2. Segmentation of the

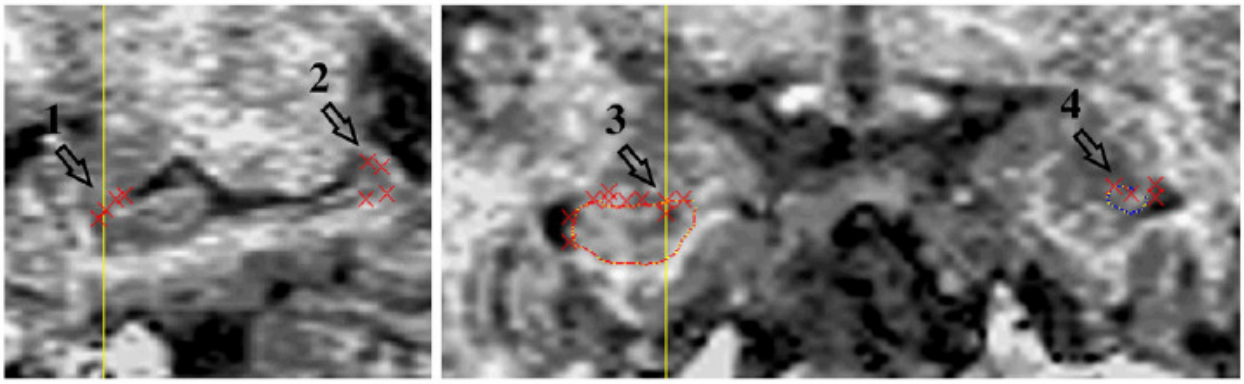
hippocampus on coronal images was done by continuous pen drawing and point-wise sampling. By means of the landmark setting, and intensity and noise preprocessing, the whole hippocampus including the tail [79] could be segmented without ad hoc determination of the most anterior and the most posterior slice [132]. Interpretation of the image in relation to the sectional anatomy of the hippocampus [133] and segmentation guidelines [134,135] is necessary as there often are parts of the image where no distinct border can be found between adjacent anatomical regions of similar tissue types.

A 3D visualization was done after the preliminary segmentation to check for apparent deviations from the expected anatomical figure.

Intrarater reliability for the two hippocampal segmentations performed by Carl Eckerström with a 3 month interval: single measure ICC=0.712. Interrater reliability for CE's first measurement and Erik Olsson's measurement: single measure ICC=0.663.

Paper II also included CSF samples collected by lumbar puncture (LP). Both baseline and follow-up LPs were performed in the morning to exclude influence on the results from possible diurnal fluctuations in biomarker levels. All CSF analyses from the same patient were performed on one occasion. CSF T-tau and Aβ42 levels were determined using a sandwich enzyme-linked immunosorbent assay (ELISA) constructed to measure T-tau and Aβ42, respectively.

Figure 2. Segmentation process using sagittal reformation



Left: sagittally reformatted view of the right hippocampus of a control subject.

Right: Coronal view of the same subject at the level of the front of the hippocampal head.

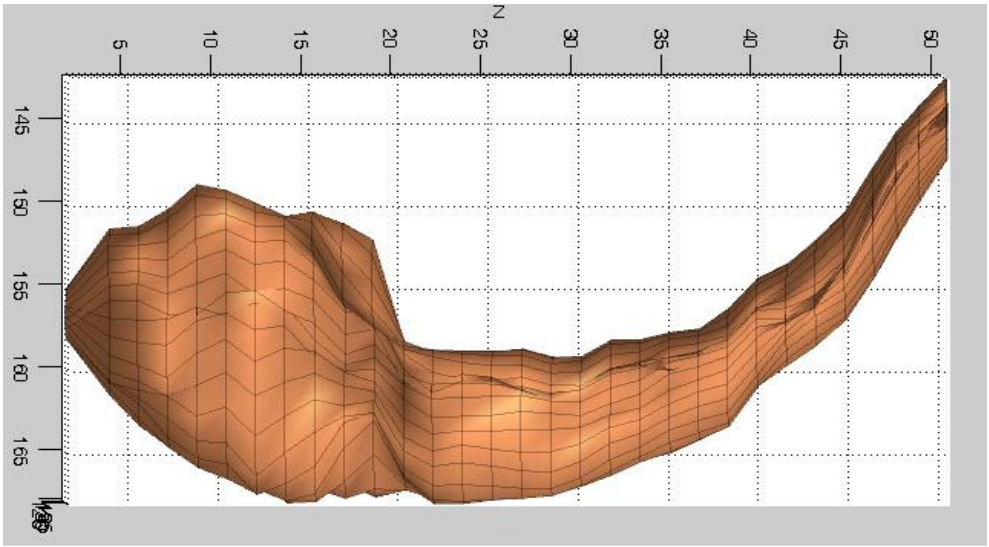
1: Marking the transition between hippocampal head and amygdala.

2: The tip of the hippocampal tail.

3: Same cross as 1, now appearing in the coronal view of the right hippocampal head;

4: Left hippocampal head.

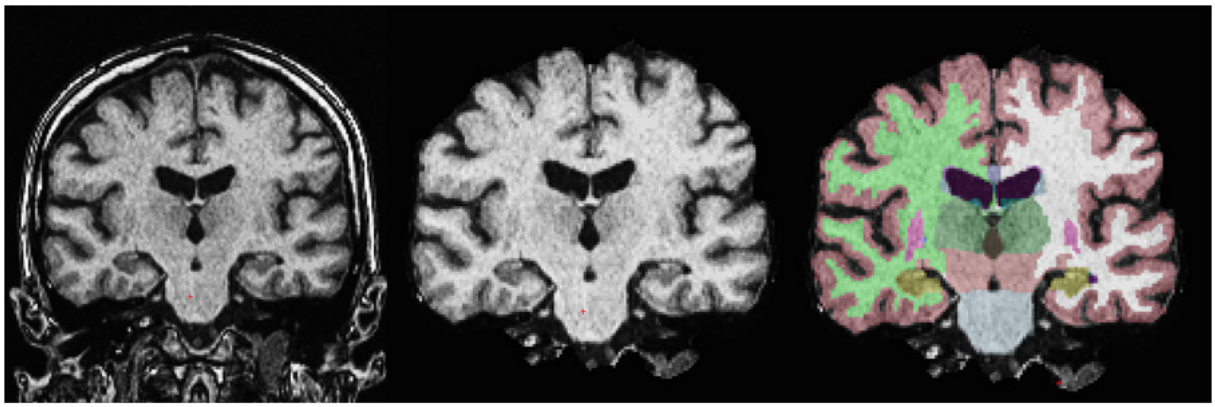
Figure 3. 3D visualization of the left hippocampus from a participant in the Göteborg MCI study.



Imaging in Paper III

Grey and white matter volumetry in Paper III was performed using FreeSurfer. FreeSurfer is one of the most widely used software packages for automated segmentation of the brain. The segmentation process can briefly be described in 3 steps; step 1 is intensity normalization, step 2 is computer assisted stripping of the skull, step 3 involves assigning each voxel a tissue class using a probabilistic atlas [136,137].

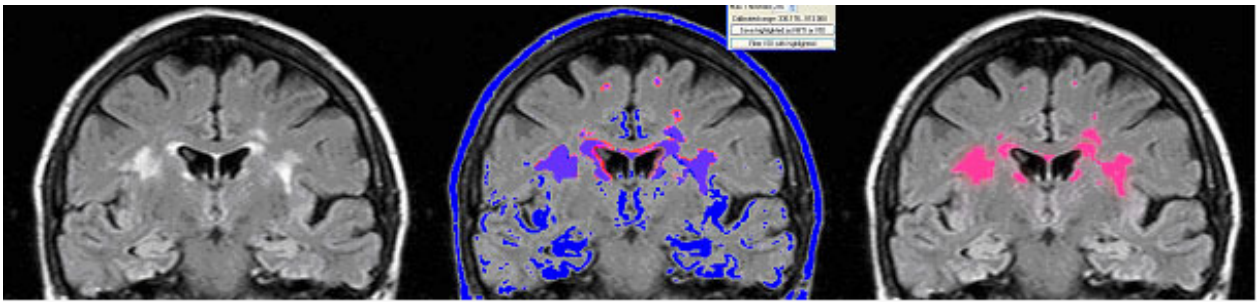
Figure 4. FreeSurfer images from the Göteborg MCI study



Left image shows original image, middle image after skull stripping, right image shows complete segmentation

Manual volumetry of white matter lesions was performed using MRicron. The segmentation process consists of three steps; step 1 is manual outlining of supratentorial white matter lesions, step 2 involves applying an intensity threshold to correspond with the intensity of the white matter lesions in the image, and step 3 is automatic measurement of the area of overlap of the outlined lesions and the applied intensity threshold.

Figure 5. MRicron images from the Göteborg MCI study.



Left image shows original image, middle image shows manually outline white matter lesions (pink) and intensity threshold (blue), right image shows complete segmentation

Statistical analyses

Hippocampal volume tends to follow a normal distribution both in healthy controls and cognitively impaired which allowed the use of parametric tests when hippocampal volumes were analyzed. White matter lesions are not normally distributed and were consequently analyzed with non-parametric tests.

In all Papers, demographic data were analyzed using Chi-square for sex and ApoEε, and ANOVA for comparisons of age, education and MMSE scores.

In Paper I, unpaired t-tests (two-tailed) were used for comparing hippocampal volume between the groups. Intraindividual comparisons were done with the paired t-test. In evaluating the reliability of the hippocampal measurements, raw correlations and intraclass correlations were used.

In Paper II, unpaired t-tests (two-tailed) were used for group comparisons regarding hippocampal volumes and CSF biomarkers. Receiver operating characteristic (ROC) analyses were performed to assess the diagnostic value of the variables separately and in combination using the orthogonal projection to latent structures discriminant analysis (OPLS-DA) algorithm. Cutoff values were determined by finding the maximum for the sum of sensitivity and specificity.

In Paper III, Mann-Whitney's U test was used for group comparisons with regard to white matter lesion volumes. Unpaired t-tests were used for group comparisons in the evaluation of hippocampal volumes and total white matter volumes. Correlation analyses were performed with Spearman's ρ for correlations with WMLs, while Pearson's r were used for correlations with hippocampal volumes and total white matter volumes.

Pearson's r were also used for comparisons between hippocampal volumes and psychometric test performance in the unpublished data.

Results

Hippocampal volume in converting and stable MCI patients

The main finding in Paper I was that the converting MCI patients had significantly smaller total hippocampal volume than both the stable MCI group and the controls. There was no significant difference between the stable MCI group and the controls. When looking at left and right hippocampus respectively, only the left hippocampus was significantly smaller in the converting MCI group than in both the stable MCI group and the control group. It was therefore concluded that the left hippocampus was the best marker for conversion.

Table 2. Total, right and left normalized hippocampal volumes at baseline (Paper I).

	Converting MCI	Stable MCI	Controls
N	21	21	26
Total	4.0 ± 1.1 **, ##	4.5 ±1.1	4.5 ± 1.2
Right	2.1 ± 0.6 #	2.3 ± 0.6	2.4 ± 0.6
Left	1.9 ± 0.5 **, ##	2.2 ± 0.5	2.2 ± 0.6
Difference (R,L)	0.2 ± 0.3	0.2 ± 0.3	0.2 ± 0.3

Volumes are given as cm^3, mean ± 2SD.

* and ** indicate significant results compared to MCI-s.*: $p < 0.05$, **: $p < 0.01$

and ## indicate significant results compared to Controls. #: $p < 0.05$, ##: $p < 0.01$

Longitudinal change in hippocampal volume and cognitive function

Although not significant, mean hippocampal volume decreased in the converting MCI group and in the control group between baseline and the two year follow up. The stable MCI group had an increase in volume over the same time period. The unexpected large volume loss in the control group required further investigation. From examination of the medical records of the control subjects, it was found that two of the three subjects with the largest reductions in hippocampal volume had recently received cancer diagnoses. Apart from this, psychiatric or

somatic disorders of possible relevance was found in additionally four control subjects. When these six subjects were excluded from the analysis, the remaining controls showed a much smaller hippocampal volume loss of 0.09 cm³ over two years.

For the converting MCI group, the hippocampal volume loss amounted to 5.8 % of baseline volume or a yearly loss of 2.9 %. The yearly loss for the purged control group was 1.0 % while the stable MCI group had a yearly volume increase of 1.8 %.

Table 3. Volume change over two years (Paper I).

	Converting MCI	Stable MCI	Controls	Purged controls
N	14	15	19	13
Volume change (cm³)	– 0.23	0.16	– 0.32	– 0.09
p-value	0.2	0.07	0.008	0.3

Hippocampal volume in MCI patients converting to AD or SIVD

In Paper I, there was an overall trend for smaller hippocampal volumes in the patients converting from MCI to AD than in the MCI to non-AD group, both with regards to left, right and total hippocampal volumes. The subgroups were, however, small and none of the differences reaches significance. The MCI to AD demonstrated significantly smaller total, right, and left hippocampal volumes than the non-converters and controls. In the MCI to non-AD group, only the left hippocampal volume was significantly smaller compared to the stable MCI group.

Table 4. Total, right and left normalized hippocampal volumes at baseline (Paper I).

	MCI to AD	MCI to non-AD	Stable MCI	Controls
N	13	8	21	26
Total	3.9 **,##	4.2	4.5	4.5
Right	2.1 *, ##	2.3	2.3	2.4
Left	1.9 **, ##	2.0 *	2.2	2.2

Volumes are given as cm^3, mean value.

* and ** indicate significant results compared to stable MCI.*: $p < 0.05$, **: $p < 0.01$

and ## indicate significant results compared to Controls. #: $p < 0.05$, ##: $p < 0.01$

As not all scans analysed in Paper III were performed prior to conversion, the more pronounced differences between the converting and stable patients seen in Paper III may, therefore, be due to some patients in the converting MCI group having progressed further in the disease process. The findings were, however, similar to those of Paper I. Note the overall higher volumes in Paper III compared to those in Paper I and II, reflecting the different methods used.

Table 5. Total, right and left normalized hippocampal volumes (Paper III).

	MCI-AD	MCI-Vasc	MCI-MCI	Controls
N	16	11	92	44
Right HC	3.2 ± 0.7 * #	3.2 ± 0.6 * #	4.0 ± 0.4	3.9 ± 0.5
Left HC	3.2 ± 0.6 * #	3.1 ± 0.6 * #	3.9 ± 0.4	3.8 ± 0.4

Volumes are given as cm^3, mean ± 2SD.

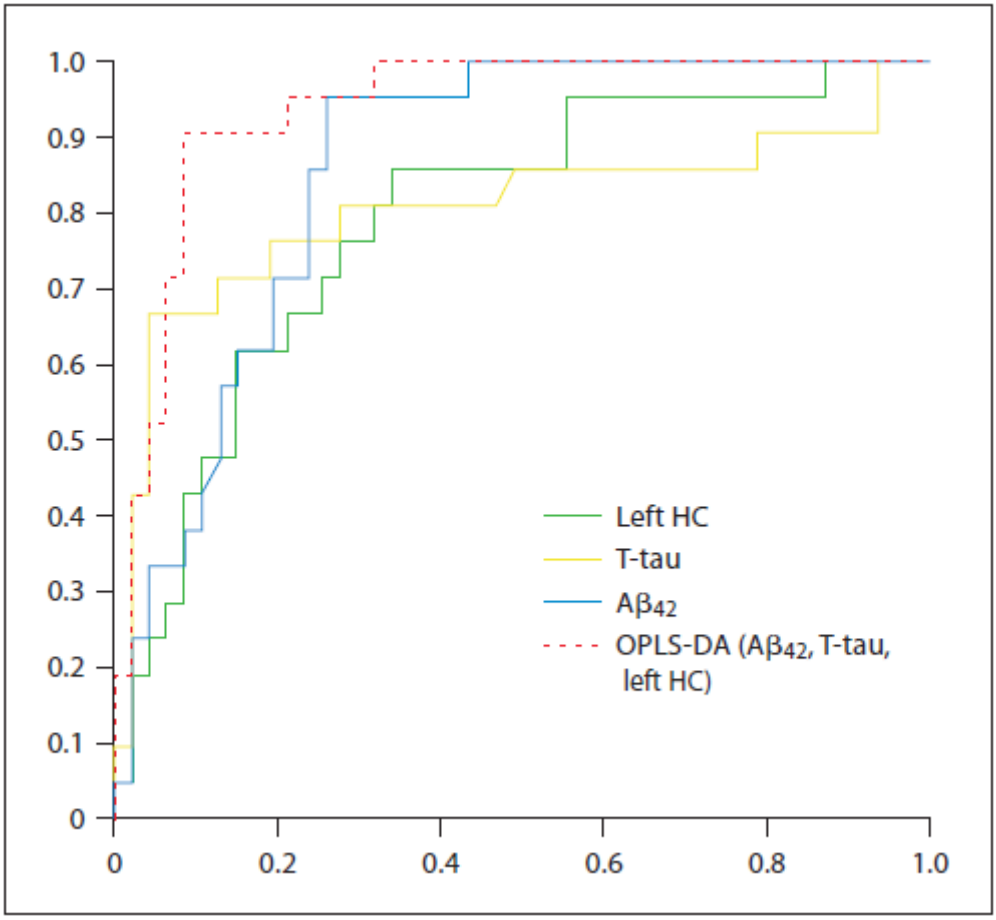
* and ** indicate significant results compared to stable MCI. *: $p < 0.05$, **: $p < 0.01$

and ## indicate significant results compared to Controls. #: $p < 0.05$, ##: $p < 0.01$

Combination of hippocampal volume and CSF biomarkers in MCI

Paper II examined the prognostic accuracy of left hippocampal volume in combination with CSF biomarkers T-tau and Aβ42. Group comparisons showed highly significant differences for all three markers between the converting MCI group and both the stable MCI group and the controls. None of the markers showed a significant difference between the stable MCI group and the control group. When examining predictive values, the combination of all three markers was found to be the best predictor of conversion to dementia, with a sensitivity of 90 % and a specificity of 91 %.

Figure 6. ROC analysis (Paper II).



Prognostic accuracy for different variables.

Table 6. Summary of ROC analysis (Paper II).

	Sensitivity %	Specificity %	AUC
Left HC	86	66	0.79
T-tau	67	96	0.81
Aβ42	95	74	0.86
OPLS-DA (Aβ42, T-tau)	95	79	0.91
OPLS-DA (Aβ42, Left HC)	86	91	0.92
OPLS-DA (T-tau, L HC)	71	87	0.82
OPLS-DA (Aβ42, T-tau, Left HC)	90	91	0.94

OPLS-DA: orthogonal projections to latent structures discriminant analysis. AUC: area under the curve.

White matter lesions in MCI and their relation to hippocampal volume

The stable MCI group (MCI-MCI) had lower WML volume compared to both the converting MCI group (MCI-Con) and the control group. The MCI patients converting to SIVD or mixed dementia (MCI-Vasc) stand out with high WML volumes, which was expected as MRI verified WMLs are included in the diagnostic criteria for SIVD. As noted above, the MCI-Con group had smaller hippocampal volume compared to both the MCI-MCI group and the control group, while there was no difference between the MCI-MCI group and the control group. Furthermore, there was no difference in hippocampal volumes between MCI-AD and MCI-Vasc and both groups had smaller hippocampal volume compared to the MCI-MCI group and the control group. There was no significant difference in total white matter (Total WM) volume between the groups.

Table 7. White matter lesion volume, hippocampal volume and total white matter volume. (Paper III)

	MCI-Con (N=30)	MCI-AD (N=16)	MCI-Vasc (N=11)	MCI-MCI (N=92)	Controls (N=44)
WMLs	21.1 ± 26.0 [a]	14.6 ± 21.8	34.5 ± 30.2 [b x]	8.0 ± 7.3 [y]	11.6 ± 8.9
Right HC	3.3 ± 0.7 [b x]	3.2 ± 0.7 [a x]	3.2 ± 0.6 [a x]	4.0 ± 0.4	3.9 ± 0.5
Left HC	3.2 ± 0.6 [b x]	3.2 ± 0.6 [a x]	3.1 ± 0.6 [a x]	3.9 ± 0.4	3.8 ± 0.4
Tot WM	433 ± 48	428 ± 51	428 ± 46	443 ± 52	435 ± 54

Volumes (cm^3) are presented as Mean ± SD

No statistical analysis between the MCI-Con group and its subgroups (MCI-AD, MCI-Vasc) were performed.

[a] and [b] indicate significant results compared to MCI-MCI. [a]: $p < 0.05$, [b]: $p < 0.001$

[x] and [y] indicate significant results compared to Controls. [x]: $p < 0.05$, [y]: $p < 0.001$

Table 8 shows that age is negatively correlated to Left hippocampal (Left HC) volume and total WM volume, but positively correlated to WML volume. Total WM volume is positively correlated to left and right hippocampal (Right HC) volume but not to WML volume. Left and Right HC volumes are highly correlated. The negative correlations between WMLs and Right/Left HC volume are not significant.

Table 8. Correlations for the whole cohort. (Paper III)

	Left HC	Right HC	Total WM	Age	Education
WMLs	-0.14	-0.16	0.04	0.39 **	-0.12
TotWM	0.50 **	0.47 **	1	-0.27 **	0.07
Left HC	1	0.88 **	0.50 **	-0.44 **	0.13
Right HC	0.88 **	1	0.48 **	-0.48 **	0.18

**Indicate significant results. **: $p < 0.01$

In order to examine the effect of high WML load, the subjects were divided into quartiles. Correlations for the quartile with the highest WML volume (> 11 cm³, N = 42) are displayed in Table 9. It shows that WML volume is negatively correlated to both Left and Right HC volume but not correlated to age, education or total WM volume. WML volume was not correlated to Left or Right hippocampal volume in any of the other quartiles.

Table 9. Correlations for the top WML quartile. (Paper III)

	Left HC	Right HC	Total WM	Age	Education
WMLs	-0.58**	-0.44 *	-0.33	0.28	-0.16
TotWM	0.57 **	0.51 **	1	-0.32	0.14
Left HC	1	0.85 **	0.57 **	-0.23	0.1
Right HC	0.85 **	1	0.51 **	-0.18	-0.09

* Indicate significant results. *: $p < 0.05$

** Indicate significant results. **: $p < 0.01$

Relationship between hippocampal volume and psychometric performance

A correlation analysis was performed to evaluate the association between neuropsychological test performance and hippocampal volume in MCI. The hippocampal volumes from the MCI patients from Papers I and II were used for the analysis. The cognitive domains most strongly associated with hippocampal volume were learning and memory, and visuospatial function. Across the battery, left hippocampal volume was more closely related to test performance than right hippocampal volume. This was true for both verbal and non-verbal learning and memory tests.

Table 10. Correlation coefficients for hippocampal volume (HC) and test scores (Unpublished data).

		MCI (N=42)	
Cognitive domain	*Test*	*Right HC*	*Left HC*
Speed and attention	Trail Making Test A	-0.02	-0.10
	Trail Making Test B	-0.15	-0.14
	WAIS-R Digit Span	-0.08	-0.07
	WAIS Digit Symbol Coding	-0.09	0.05
Learning and memory	RAVLT Immediate recall	0.21	0.33*
	RAVLT Delayed recall	0.29	0.42**
	RAVLT Recognition	0.35*	0.42*
	Rey Complex Figure recall	0.37*	0.47**
	Rey Complex Figure delayed recall	0.42**	0.49**
	Face Recognition	0.30	0.40**
Visuospatial	Rey Complex Figure copy	0.17	0.18
	WAIS-R Block Design	0.14	0.29
	VOSP Silhouettes	0.30	0.48**
Language	Controlled Oral Word Association	0.21	0.25
	WAIS-R Similarities	-0.04	0.09
	Boston Naming test	0.10	0.32
	Token test	-0.01	-0.04
Executive funtions	PASMO	0.10	0.10

* Indicate $p < 0.05$.

** Indicate $p < 0.01$

Discussion

To be able to track the first changes in dementia, perhaps many years before the first symptoms are present, would be of great importance. An early diagnosis would enable the possibility of early symptom alleviating therapy and ensure that when any disease modifying treatment is made available, this will be administered at an early stage of the disease.

Hippocampal volume as a broad cognitive marker in MCI

The findings in Paper I that MCI patients subsequently converting to dementia have smaller hippocampi than stable MCI patients support the notion that hippocampal volume can be used as a marker for cognitive deterioration in an MCI group with varying underlying diseases. The view that hippocampal atrophy is a strict AD marker is likely not entirely correct. In the present material, the MCI patients converting to AD have pronounced hippocampal atrophy but patients converting to SIVD and mixed dementia also have a significant hippocampal volume loss compared to stable MCI patients and controls. It would seem that the pathophysiological process in AD affects the hippocampus at an early stage. This assumption is supported by both pathological and neuropsychological data [38,103-105]. In SIVD and mixed dementia the hippocampus seems to be involved in the pathophysiological process, as well, but more indirectly. While hippocampal atrophy in AD has been shown in numerous studies, hippocampal atrophy in SIVD is not as well established in the literature. Much of the research performed in the field of MCI and dementia is focused on predicting specific diseases e. g. AD or less commonly, vascular dementia. Although this is valuable research, the focus is inevitably moved from MCI to the studied disease. By selecting MCI patients with prodromal AD or other isolated diseases, the information conferred applies only to a subgroup of the MCI population. Instead of a disease-oriented view, the aim of the present research has been to focus on the MCI group as a whole to attempt to determine which patients will subsequently convert to dementia and which patients will remain stable or even improve. It should be noted that the approach in the Göteborg MCI study relies on clinical diagnostics and not on neuropathologically verified diagnosis. The participants in Papers I and II seem representative of the general dementia population, however. Among the converting

patients, 62% converted to AD with VaD as the second most common diagnosis. These proportions of dementia disorders are in fair concordance with what has been reported in larger studies [2]. Taken together, the results from Papers 1-3 support the assumption that hippocampal atrophy may have predictive value not only in incipient AD, but also in incipient SIVD and mixed dementia. This is of interest in the clinical setting as the design of the Göteborg MCI study reflects the flow of patients to a memory clinic.

Longitudinal change in hippocampal volume and cognitive function

There is much interest in finding a reliable marker of progression in dementia disorders. Not only would a progression marker confer information regarding the pathologic process, but it would also provide a way of evaluating interventions such as disease modifying drugs. The cerebrospinal fluid markers T-tau and Aβ42 are valuable in providing diagnostic information, but studies have shown that these markers are not sensitive to disease progression [95,96,138]. Similarly, psychometric markers provide excellent information about cognitive functioning but suffer from a floor-effect and remain static as the patient deteriorates. There are findings that suggest that hippocampal volume may be used as a progression marker in AD [120,121,138]. Although the material in Papers I and II is small, the 2.9 % yearly rate of decline for converting MCI patients is similar to the 3.3 % yearly decline previously reported in MCI patients converting to AD [120]. Surprisingly, the stable MCI patients showed an increase in hippocampal volume of 1.8 %. A possible explanation for this is that some patients with stable MCI sought help for cognitive impairment caused by something else than dementia disorders. These causes may include high levels of circulating cortisol/chronic stress, post-traumatic stress disorder, or depression. While these conditions have all been shown to be associated with reductions in hippocampal volume [18], there are some findings suggesting that clinical improvement is linked to an increase in hippocampal volume [139,140].

Combination of hippocampal volume and CSF biomarkers in MCI

Paper II compares the predictive ability in MCI of hippocampal volume alone and in combination with CSF biomarkers T-tau and Aβ42. It was found that while Aβ42 was the best single predictor, by combining Aβ42 with hippocampal volume the sensitivity was increased from 74 % to 91 %. Aβ42 and hippocampal volume seem to reflect different disease processes

or different aspects of the same disease process. The results provide evidence for the increased value gained by combining markers in MCI. Judging from the high prognostic accuracy of the three markers studied, one may speculate that inclusion of further markers would enable the construction of a formula with still better prognostic ability. Paper III (see below) suggests that hippocampal atrophy can be seen in SIVD and that it may be secondary to WMLs. The increased sensitivity provided by hippocampal volume in combination with Aβ42 in Paper II may be due to the capture of patients with WMLs but without changes in Aβ42 levels.

White matter lesions in MCI and their relation to hippocampal volume

It remains unclear to what extent the presence of WMLs is linked to hippocampal atrophy. The question is fundamental for the division between VaD and AD. According to the traditional view, AD begins by accumulation of SP and NFT in the medial temporal lobe and progresses through the brain with predictable clinical manifestations, beginning with disturbances of memory, visuospatial and language functions. Vascular dementia and SIVD in particular, on the other hand, begins by hypoperfusion of deep white matter by ways of reduced permeatibility of lacunar arteries and arterioles. The symptoms of this process should be frontosubcortical reflecting the damage done to the axons in this region. There are, however, reasons to question such a clear distinction. Studies have shown hippocampal atrophy in SIVD [123,141], and there is increasing evidence for a correlation between hippocampal volume loss and WMLs [87-89]. The underlying process is not known but several mechanisms have been proposed. WMLs and AD share the same risk factors and it has therefore been suggested that WMLs and hippocampal atrophy are not directly related. There is evidence that the correlation remains after adjustment for known risk factor [87], but more studies are needed to settle the issue [142]. It has also been suggested that hippocampal atrophy in SIVD is a result of ischemia leading to apoptosis of hippocampal neurons [141]. Although the hippocampi have high metabolic activity and are sensitive to hypoxia, they are also well vascularized with anastomoses and hippocampal infarcts are rarely seen [143], favoring a view that the hippocampi are more indirectly affected in SIVD. Another proposed hypothesis is that not only WMLs but also hippocampal atrophy is caused by hypoxia or ischemia due to vascular factors. A third proposed mechanism is that WMLs involves damage

to afferent and efferent axons of the hippocampus, slowly disconnecting it and thereby causing atrophy. The finding in Paper III that a correlation between WMLs and hippocampal volume is only seen in subjects with large WMLs may be interpreted as support for a causal link between WMLs and hippocampal atrophy. Clinical and neuropsychological studies have found that subcortical symptoms are the earliest and most pronounced symptoms in SIVD, whereas memory impairment may be present early but is typically more pronounced in later stages of the disorder [54]. Furthermore, a recent study using an animal model for SIVD showed that hippocampal atrophy developed as a consequence of WMLs [144].These observations provide support for the view that the development and progression of WMLs is the driving cause of SIVD and that WMLs are involved in the development of hippocampal atrophy in SIVD.

Hippocampal asymmetry and psychometric performance

One of the findings in Paper I was that the left hippocampus was a better marker of cognitive deterioration than the right hippocampus. Although other researchers have found similar results in MCI, there are also several studies reporting decreased asymmetry (i.e. the larger right hippocampus is more atrophied) in AD [145,146]. In order to investigate whether there is a difference in how left and right hippocampus are associated with psychometric test performance a correlation analysis was performed. In the MCI material from Papers I and II hippocampal volumes were associated with visuospatial function and learning and memory function. The results showed that left hippocampal volume was more strongly correlated with test performance and this applied to both verbal and non-verbal functions. Although there may be reason to question a strong dichotomization in function of the hippocampi [76,77], the results are nonetheless unexpected. One interpretation is that the hippocampal volumes and the psychometric test should be regarded as markers of an underlying disorder, and the patterns discerned should be viewed in that perspective. However, other studies support the notion that the left hippocampus is more strongly associated with a decline in psychometric performance. A recent meta-analysis reported that the left hippocampus may be more affected in MCI prior to the bilateral atrophy seen in AD [80]. In a large longitudinal study investigating hippocampal volume in MCI and AD it was found that volume loss of the right hippocampus seemed to lag volume loss of the left hippocampus [147]. Interestingly, it was

also reported that the ApoEε4 allele was associated with an accelerated rate of volume loss in the left but not in the right hippocampus [147]. It may be that the increased asymmetry found in Paper I is due to ApoE status. Further analysis is warranted.

Limitations

A problem with volumetric estimation of the hippocampus is the lack of a standardized measure protocol. A large number of manual and automated techniques are being used to measure hippocampal volume in vivo [132], making it difficult to compare hippocampal volumes between different study centres. As manual methods are both time-consuming and subjective, automated methods are preferable if hippocampal volumetry is introduced in everyday clinical practice. The present automated methods can detect hippocampal atrophy but studies have reported that they systematically over- or underestimates hippocampal volume [148,149]. Manual hippocampal volumetry is still regarded as the golden standard.

The Göteborg MCI study is designed to reflect the flow of patients to a memory clinic. In order to examine early dementia, it is necessary to capture the patient prior to conversion from MCI to dementia within the timeframe of the study. Furthermore, for a proper longitudinal examination, it is desirable that the patient is examined with the same MRI scanner both before and after conversion to dementia. A consequence of scanner upgrades is that it is difficult to achieve large group sizes, especially with regard to groups of patients converting to specific types of dementia. The group sizes in the present study are smaller than what would be preferable and it was not possible to analyze less frequent types of dementia such as Lewy body dementia or frontotemporal dementia.

A difficulty when interpreting the data is that the statistical differences do not necessarily represent real differences. Extra caution is warranted with regard to correlation analyses. The significant correlations reported need not reflect a causal relationship but may instead be associated through a variable not included in the analysis.

Conlusions

One of the conclusions of the present study is that hippocampal volume may be viewed as a broad cognitive marker in MCI. Rather than a marker for a specific disease, reduced hippocampal volume should be viewed as a marker for neurodegeneration and impaired cognitive functioning. It would seem that AD neuropathology affects the hippocampal region early in AD and at a later stage in SIVD. While hippocampal volume loss in AD is probably caused by local neurotoxicity, SIVD may be caused by the disconnection of hippocampal projections by WMLs.

Furthermore, the results of the present study suggest that hippocampal volume may function as a marker of disease progression in MCI. Finding a good marker of progression would be of great value in the evaluation of therapeutic interventions.

Several CSF biomarkers are being used in the evaluation of MCI. By combining hippocampal volume with two of the most widely used CSF biomarkers, Aβ42 and T-tau, the predictive ability in MCI is enhanced. Measurement of hippocampal volume may capture MCI patients with progressive cognitive decline in the absence of a typical AD biomarker profile.

Examinations of WMLs in MCI showed that patients converting to SIVD or mixed dementia have larger WMLs than other MCI patients and controls. The finding that WMLs are negatively associated with hippocampal volume in patients with high WML burden suggests that such a burden may be an alternate cause of hippocampal atrophy in MCI and dementia.

In both the 0.5 T and 1.5 T materials, left hippocampal volume was found to be the best marker of cognitive deterioration. When comparing left and right hippocampal volume with psychometric test performance, left hippocampal volume was found to be more closely related to test performance. Left hippocampal atrophy seems to be more strongly associated with a decline in psychometric performance but it remains unclear if the link is causal.

Future directions

Trajectories of hippocampal volume loss

Hippocampal atrophy is seen in AD and SIVD. There is information in the literature suggesting that hippocampal volume loss in AD is a process that continues even at later stages of the disease [138]. Less is known regarding the dynamics of hippocampal volume in the development of SIVD, and also in MCI with different outcomes. It would, therefore, be interesting to study trajectories of hippocampal volume and relate these to cognitive status and biomarkers.

WML subtypes

Not all white matter lesions progress over time and not all white matter lesions seem to be linked to cognitive decline [150]. Possibly, some white matter lesions are more malignant than others. Currently there is insufficient knowledge regarding subtypes of white matter lesions. Furthermore, it would be interesting to examine the localization of white matter lesions in relation to a careful assessment of cognitive functions both cross-sectionally and longitudinally.

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