Mild cognitive impairment

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Introduction: Mild cognitive impairment: beyond controversies, towards a consensus

WINBLAD E1, PALMER K2, KIVIPELTO M2, JELIC V1 & FRATIGLIONI L2
From the 1Division of Clinical Geriatric, Neurotec Department, Karolinska Institutet, Karolinska University Hospital-Huddinge, Stockholm, Sweden, and 2Aging Research Center, Division of Geriatric Epidemiology, Neurotec Department, Karolinska Institutet, Stockholm, Sweden

The concept of mild cognitive impairment (MCI) has received a considerable amount of interest during the last years, especially as a possible prodromal phase of Alzheimer’s disease (AD). AD has a long preclinical period, which presents an opportunity for identifying subjects in a phase when pathology has already begun but AD diagnosis is not achievable. Early diagnosis of AD is important to initiate symptomatic treatment, and will be of even greater significance if medications that might delay or halt the dementia process become available. Furthermore, better identification of elderly persons at high risk of future dementia will provide doctors with opportunities for prognostic counselling, and improve health care planning. Due to the worldwide ageing phenomenon, dementia and MCI have growing public health relevance. There is emerging evidence suggesting a high prevalence of cognitive impairment even in younger ages. For all these reasons, the concept of MCI is useful both clinically and as a research entity. However, as the literature of MCI has expanded there has been confusion concerning the specific boundaries of the condition, and controversies regarding its definition, assessment, management and intervention strategies. A greater consensus and standardization of definitions and research methodology for MCI in needed to make further studies more comparable and useful for designing intervention strategies.

Persons with MCI are known to have an increased risk of progressing to dementia compared to elderly persons with normal levels of cognitive functioning. However, the evolution is heterogeneous, as some persons have been seen to improve over time, whereas others have an increased risk of mortality. To date, much of the research on MCI has derived from clinical sources, with the focus on symptomatic patients within specialized medical settings, although there are increasing results emerging from population-based sources. The aim of the first Key Symposium was to combine the clinical perspectives with epidemiological evidence to reach a consensus concerning a broader concept of MCI and to identify future research questions. This symposium brought together both a multidisciplinary and a worldwide group of experts to focus solely on this subject.

A group of 100 international physicians and researchers from Asia, Australia, Europe and the US met in Stockholm. Five aspects of MCI were covered and presented as articles in this issue, reflecting the authors’ perspectives of the current state of the art: clinical presentation [1], cognitive assessment and markers [2], neuroimaging [3], biomarkers [4] and genetics [5].

The meeting concluded with a general discussion followed by a day of deliberations involving the speakers, discussants, chairpersons and the symposium’s Scientific Committee. This resulted in a report from this international working group, describing the state of the art of MCI, recommendations for management and treatment, and future research questions [6]. The main questions addressed by the working group included whether MCI represents a useful clinical and research entity, as well as the
challenge of further refining the criteria so that treatments can be targeted towards a specific MCI population.

Conflict of interest statement
No conflict of interest was declared.

References

Correspondence: Professor Bengt Winblad, Aging Research Center, Karolinska Institutet, Box 6401, S-11382 Stockholm, Sweden. (fax:+46 8 690 5954; e-mail: bengt.winblad@neurotec.ki.se).
Mild cognitive impairment as a diagnostic entity

R. C. PETERSEN
From the Department of Neurology, Alzheimer’s Disease Research Center, Mayo Clinic College of Medicine, Rochester, MN, USA


The concept of cognitive impairment intervening between normal ageing and very early dementia has been in the literature for many years. Recently, the construct of mild cognitive impairment (MCI) has been proposed to designate an early, but abnormal, state of cognitive impairment. MCI has generated a great deal of research from both clinical and research perspectives. Numerous epidemiological studies have documented the accelerated rate of progression to dementia and Alzheimer’s disease (AD) in MCI subjects and certain predictor variables appear valid. However, there has been controversy regarding the precise definition of the concept and its implementation in various clinical settings. Clinical subtypes of MCI have been proposed to broaden the concept and include prodromal forms of a variety of dementias. It is suggested that the diagnosis of MCI can be made in a fashion similar to the clinical diagnoses of dementia and AD. An algorithm is presented to assist the clinician in identifying subjects and subclassifying them into the various types of MCI. By refining the criteria for MCI, clinical trials can be designed with appropriate inclusion and exclusion restrictions to allow for the investigation of therapeutics tailored for specific targets and populations.

Keywords: mild cognitive impairment, Alzheimer’s disease, aging.

Background

Increasing attention is being paid in recent years to the mild end of the cognitive spectrum spanning normal ageing to Alzheimer’s disease (AD). There likely is a transitional period between normal ageing and the diagnosis of clinically probable very early AD, and this transitional zone has been described using a variety of terms such as mild cognitive impairment (MCI), dementia prodrome, incipient dementia, isolated memory impairment, amongst others [1]. We will use the term, ‘mild cognitive impairment or MCI’, in the present discussion. In particular, we will discuss this construct as a diagnostic entity.

Cognitive continuum

The diagnosis of definite AD can only be made by neuropathological confirmation of persons who had been studied in life and met criteria for dementia [2]. However, the accuracy of the clinical–pathological correlation has been quite good when standard published criteria for the clinical diagnosis of AD are employed [3]. In Fig. 1, this conceptual scheme presumes that individuals are functioning normally as they age. In a subset of persons, in particular those who are destined to develop AD, there is a decline in cognitive function, which can be very subtle at first. Currently, the criteria for clinically probable AD identify people after a substantial
The degree of cognitive decline has taken place. The construct of MCI proposes to identify these individuals at an earlier point in the cognitive decline such that if therapeutic interventions become available, clinicians can intervene at this juncture.

Another manner in which to view this continuum is shown in Fig. 2. This figure demonstrates MCI as interposed between the cognitive changes of normal ageing and what might constitute very early dementia. Note that there is overlap on both ends of the MCI bar indicating that the distinction between normal ageing and MCI can be quite subtle and, in addition, the specific transition between MCI and very early dementia can also be challenging. We will use this theoretical construct as a background against which to discuss the concept of MCI as a diagnostic entity.

The continuum outlined in Fig. 2 can apply to a variety of underlying dimensions. Most commonly, we refer to clinical criteria to distinguish between normal ageing and MCI and between MCI and dementia. One could also put measures of cognitive function such as neuropsychological testing, biomarkers or neuroimaging measures on this continuum as well. Presumably there are features of each of these measures that will help distinguish between normal ageing and MCI and MCI and dementia. It may ultimately be the case that a combination of measures, clinical features, neuropsychological testing, biomarkers and neuroimaging may be necessary to improve our diagnostic accuracy.

Terminology

Over the years, several terms have been used to describe an intermediate stage of cognitive impairment. Benign senescence forgetfulness was one of the initial descriptors of this concept, and this term was believed to be a variant of normal ageing [4]. In recent years, there has been a further study of this concept, and whilst it was originally felt to reflect a stage of normal ageing, more recent data have cast some doubt on that [5]. In 1986, an National Institute of Mental Health (NIMH) work group proposed the term, age-associated memory impairment (AAMI) and this concept was meant to characterize memory changes in ageing which were felt to be a manifestation of normal cognition [6]. These criteria referenced memory function in older individuals to the performance of younger adults and this proved to be problematical for a wider application of the term [7]. More recently, the term, ‘age-associated cognitive decline’ (AACD) has been proposed by individuals of the International Psychogeriatric Association to refer to multiple cognitive domains presumed to decline in normal ageing [8]. The Canadian Study of Health and Aging has used the term, ‘cognitive impairment no dementia’ (CIND), to characterize intermediate cognitive function of insufficient severity to constitute dementia [9]. This concept has been rather heterogeneous with regard to its inclusion of a variety of types of cognitive dysfunction, but more recently has been refined to correspond more closely to MCI [10].

Consequently, there have been a variety of terms used to discuss transitional stages between normal ageing and early dementia in the literature. MCI has come to be recognized as a pathological condition, i.e. not a manifestation of normal ageing, and has received a great deal of attention as a clinically useful entity.
Clinical characterization

There is no agreement in the field on a single set of criteria for MCI. A great deal of research is moving forward to characterize certain features of the construct and likely additional work will continue. In general, the concept of MCI refers to a group of individuals who have some cognitive impairment but of insufficient severity to constitute dementia. Usually these individuals have very slight degrees of functional impairment and most clinicians would have difficulty distinguishing these functional problems from those encountered by normal individuals as they age. The most important aspect of the criteria concerns the judgement on the part of the clinician that the person does not meet criteria for dementia. This represents a challenge in the field as there are no strict criteria as to the degree of functional impairment necessary to constitute a dementia.

Whilst there is not a consensus on set of criteria for MCI, when the studies are combined in aggregate, there does appear to be an increased risk of developing dementia relative to an age-matched normal population [11]. The most typical MCI patient is one who has a memory impairment beyond what is felt to be normal for age but is relatively intact in other cognitive domains. More recently, as will be discussed below, the concept of MCI has been expanded to include other types of cognitive impairment beyond memory. Most of the literature to date, however, pertains to those individuals with a memory impairment and, as such, it is useful to review these studies to determine the outcome of these individuals.

Outcome

As indicated above, with the increasing attention being paid to MCI, several studies have been conducted in recent years in a variety of research settings. At the Mayo Alzheimer’s Disease Research Center/Alzheimer’s Disease Patient Registry, a group of approximately 220 individuals of a mean age of 79 years has been followed for 3–6 years using the Mayo criteria [12]. The original Mayo criteria focused on a memory impairment with relative preservation of other cognitive domains. Specifically, these criteria were as follows: (i) memory complaint, preferably corroborated by an informant, (ii) objective memory impairment for age, (iii) relatively preserved general cognition for age, (iv) essentially intact activities of daily living, and (v) not demented. As will be discussed below, these criteria have since been expanded and refined but in their initial form serve as the set of data against which many other studies in the literature have been compared. Using these criteria, the subjects in the Mayo studies have progressed to dementia at a rate of approximately 12% per year [11]. This is in distinction to incidence rates from the same community which document a progression from normal to dementia at a rate of 1–2% per year. When these subjects are followed for up to 6 years, approximately 80% of them will have converted to dementia and consequently, this group represents a population at risk.

When individual measures are evaluated in the MCI group, the subjects tend to fall midway between individuals ageing normally in the community and those with very mild AD as is shown in Fig. 3. Similarly, the progression rates are intermediate between normal control subjects and those with very mild AD.

Mayo investigators have also evaluated a variety of measures which are thought to predict a more rapid progression to dementia. Amongst these apolipoprotein E4 allele carrier status has been one of the most prominent variables [13]. In addition, there is a trend towards abnormal performance on a cued memory task that predicts a more rapid progression and several neuroimaging measures such as volumetric measurements of the hippocampus have also been useful as predictors [14–16].

Tierney and colleagues in Toronto have followed a similar group of individuals who had been recruited from family doctor [17]. As these subjects were followed for 2 years, 29 individuals developed AD and 94 remained stable. They found that memory tests for delayed recall and an index of mental control were better predictors of progression and that apolipoprotein E4 carrier status was a reliable indicator only when combined with memory tests.

Investigators from the Alzheimer’s Disease Patient Registry in Seattle followed subjects for 5 years and found that slightly <50% of the subjects developed dementia over this period [18]. No individual memory test was felt to be better than any other.

The New York University research team using the Global Deterioration Scale followed subjects with a Global Deterioration Scale Rating of 3 which they felt represented a mild impairment [19]. Over a time span of 2 years, 32 of the subjects with a Global
Deterioration Scale of 3 were followed and 23 of them progressed to dementia.

In a study from Harvard, Daly and colleagues recruited a cohort of individuals through media advertisements and followed them longitudinally. These investigators used a modification of the Clinical Dementia Rating (CDR) to detect individuals who had subtle memory impairments [20]. This group demonstrated a progression rate of 6% per year which is somewhat lower than other studies. This lower rate may represent a combination of factors including the recruitment strategy using media advertising and the use of the CDR as the sole instrument for evaluation.

A study from France employed MCI criteria and criteria for AACD to evaluate a cohort of 833 subjects who had been followed longitudinally [21]. In this epidemiological study, the investigators retrospectively applied neuropsychologically based criteria to diagnose MCI and compared the outcome of those subjects with that of a group who met criteria for AACD. These investigators felt that MCI was an unstable construct and that the AACD subjects showed a higher conversion rate. However, the literal retrospective application of MCI criteria using neuropsychological cutoff scores likely contributed to the instability of these rates.

The PAQUID study from France also followed a population-based cohort 1265 subjects longitudinally [22]. These investigators found that overall MCI was a good predictor of progression to AD but also indicated that MCI may be unstable over time. These investigators used one neuropsychological test of nonverbal memory to characterize the memory impairment. As such, they found that 40% of their sample had reverted to normal and therefore concluded that MCI could be unstable. However, their use of a single nonverbal memory test may have also contributed to the instability and therefore, further refinement of the implementation of the memory criteria are needed.

The Religious Order Study is a research project concerning nuns and priests who constitute a volunteer cohort and are being followed longitudinally [23]. Investigators from Rush Alzheimer’s Disease Center followed 211 of these individuals and diagnosed them with MCI which included multiple domains of impairment in addition to memory. These subjects were followed for a mean of 4.5 years and the authors concluded that MCI subjects developed AD at a rate 3.1 times those subjects who did not meet criteria for MCI.

Investigators from the Cardiovascular Health Study applied criteria for the amnestic type of MCI (a-MCI) and multiple domain-MCI (md-MCI) to their sample and calculated prevalence figures [24]. They found that the overall MCI prevalence in the Pittsburgh site was 22% with a-MCI accounting for 6% and md-MCI representing 16%. These are the first population-based prevalence data on MCI subtypes.

The Canadian Study of Health and Aging evaluated separate features of the criteria for a-MCI from their CIND subjects to determine the relative contribution of each of the factors [10]. They concluded the subjective complaint and an impairment in instrumental activities of daily living may be
unnecessary for the definition of MCI; however, regardless of the definition, most people with MCI progress to dementia, mostly AD.

Investigators from Stockholm used the Kungsholmen Project to evaluate the outcome of subjects with their definition of CIND [based on Mini-Mental State Exam (MMSE) scores] [25]. They found an increased risk of developing dementia based on the level of severity of CIND and noted that those subjects who improved from CIND did not have an increased risk of subsequently progressing to dementia.

A decade ago, Dawe and colleagues reviewed the concept of MCI at that time and concluded that there was a wide discrepancy in rates of progression varying from 1 to 25% [26]. These investigators speculated that there were several factors contributing to this variability including the source of the subjects, specific criteria used, methods for implementing the criteria and length of follow-up. All of these issues remain relevant today.

A more recent review of MCI with recommendations for future research was recently reported by Luis and colleagues at Mt Sinai Medical Center in Miami [27]. They advocated for additional research to develop appropriate and sensitive neuropsychological and functional measures, reliable methods to assess progression, and epidemiologically oriented instruments that are sensitive to multiple cultures.

As is apparent from the literature, there is variability with regard to the characterization of subjects with MCI. Some of this variability relates to the source of the subjects, i.e. clinic based versus epidemiologically derived and also to the specific criteria employed as well as the implementation strategies. When one defines a memory impairment, there are numerous procedures that can be used to characterize these subjects and this likely contributes to differences amongst studies. Nevertheless, in spite of this apparent lack of agreement, there also is a relatively consistent pattern that indicates that subjects with MCI, however defined, are at an increased risk of developing dementia and consequently merit further study [28]. We will now turn to the issue of applying MCI criteria diagnostically.

Diagnosis of dementia and Alzheimer’s disease

To put MCI in perspective, we need to consider how we make other comparable clinical diagnoses, e.g. dementia and AD. If we consider the diagnosis of dementia or AD in the clinic, we can evaluate a typical set of criteria such as those in Diagnostic and Statistical Manual of Mental Disorders (DSM) IV [29]. The essential features of these criteria include: (i) memory impairment, (ii) aphasia and/or apraxia, agnosia or an impairment in executive function. In addition, these deficits must include a significant impairment in social or occupational functioning and constitute a change from a previous level of performance. They also need to exclude other psychiatric disorders or neurological explanations for the decline in function. Practically, the requirements for apraxia, agnosia, and executive dysfunction have been substituted with impairments in relevant cognitive domains such as language, attention/executive function, and visuospatial skills. These domains can be assessed by commonly used cognitive measures. At times other domains are also used such as problem-solving, constructional praxis or behavioural features, but, in general, any commonly accepted domain beyond memory is regarded as being sufficient for the diagnosis of AD. It is important to note that in addition to these cognitive impairments, there needs to be a concomitant impairment in functioning either socially or occupationally. These features constitute the hallmarks of AD.

Most clinicians are well familiar with these criteria and make this diagnosis on a regular basis. A recent evidence-based medicine review of the literature on the validity of the criteria for dementia and AD demonstrated that these criteria and other variations of them are quite accurate in identifying AD clinically when the diagnosis is confirmed with postmortem analysis [30]. As such, the criteria are valid. Although, as will be discussed later, it is likely that these criteria are more precise when applied in the clinic setting than in epidemiological field work.

The most relevant issue concerning these criteria for the present purposes relates to the manner in which they are operationalized. Certainly, the DSM-IV criteria are quite open with respect to the manner in which the criteria are to be met. Specifically, cognitive functions are mentioned but not specified, e.g. agnosia, and certainly instruments or cutoff scores are not designated. The National Institute of Neurologic and Communicative Disorders and Stroke (NINCDS) and Alzheimer’s Disease and Related Disorders Association (ADRDA) criteria are somewhat more specific insofar as they indicate
that the diagnosis of AD can be established by mental status testing and confirmed by neuropsychological tests [31]. This is a bit more precise but also stops short of specifying particular instruments and cutoff scores.

This approach to the diagnosis of AD is quite reasonable. It would be virtually impossible for the clinical/scientific community to agree upon domains, instruments and cutoff scores for cognition and equally problematic for social or occupational function impairments. Therefore, the assumption is that clinicians will use their experience and instruments of their choosing to make these judgements. As indicated above, these decisions have been quite accurate.

In addition, in the setting of presumed AD, the aetiology of the clinical disorder is assumed to be degeneration. This is accounted for in the criteria by statements concerning the gradual onset and progression of symptoms. In the more general situation the diagnosis of dementia using criteria such as those found in DSM-III-R or in DSM-IV, criteria are presented for the cognitive impairment in multiple domains in addition to a functional impairment component. These criteria do not include any statements about the nature of the onset or the course of the progression. Rather, in these instances once the diagnosis of a general dementia has been made, one searches for potential aetiologies by evaluating the nature of the onset, time course, variability in cognitive presentation, etc. Occasionally, ancillary tests such as laboratory studies and imaging procedures can contribute to the differential diagnosis of the dementia. These data then in combination lead to the conclusion that the dementia is based on a degenerative process, e.g. AD, frontotemporal dementia, dementia with Lewy bodies or a vascular basis such as in vascular dementia. The diagnosis is essentially made in a two-step procedure with the clinical determination of dementia emerging first, followed by the aetiology of the dementia. This would be a typical approach to the diagnosis of a dementing condition made by most clinicians. Again, this approach has been shown to be quite accurate.

As research on MCI has advanced, it has become apparent that several clinical subtypes of MCI exist [1, 32]. Most research has focused on the a-MCI but other types have been recognized as well. A second type of MCI called md-MCI involves various degrees of impairment in multiple cognitive domains such as language, executive function and visuospatial skills with or without a memory impairment. Those with a memory impairment (amnesia) are labelled md-MCI + a and those without are labelled md-MCI – a. This distinction becomes relevant when one discusses the outcomes of subjects with MCI. The third, and least common type of MCI, is single non-memory domain MCI in which a person has an impairment in a single non-memory cognitive domain such as language, executive function or visuospatial skills. These subjects likely have a different outcome from those with a memory impairment. It is also imperative that all of these clinical subtypes of MCI have minimal impairments in functional activities, i.e. do not represent a significant change in function from a prior level, and do not meet criteria for dementia.

In addition to the clinical subtypes, there can also be multiple aetiologies or causes for each subtype as depicted in Fig. 4. Therefore, if one selected the a-MCI subtype of a presumed degenerative aetiology, this would likely represent a prodromal form of AD. However, one could also add the subtype of md-MCI + a since this subtype has a high likelihood of progressing to AD also [33]. However, the other subtypes such as those emphasizing impairments in non-memory domains such as executive function and visuospatial skills, may have a higher likelihood of progressing to a non-AD dementia such as dementia with Lewy bodies [34]. Therefore, the combination of clinical subtypes and putative aetiologies can be useful in predicting the ultimate type of dementia to which these persons will evolve.

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**Fig. 4** Classification of clinical subtypes of mild cognitive impairment with presumed aetiology (with permission from Ref. [1].

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Application of amnestic MCI criteria

If we now consider the proposed approach for making the diagnosis of a-MCI, we can consider a parallel set of procedures to those used for making the diagnosis of AD. The criteria for a-MCI are shown in Table 1. In a fashion similar to that used for the DSM-IV or the NINCDS/ADRDA criteria for AD, the MCI criteria can be implemented. The first criterion refers to the subjective memory complaint. This is meant to capture the notion of a change in performance. Ideally this should be corroborated by an informant, but occasionally this can be difficult. This criterion is ‘soft’ and may be a challenge to implement, but without prior cognitive function testing, it is critical for the purpose of excluding individuals with lifelong static cognitive deficits [9].

The second criterion refers to an objective memory impairment for age. This has been a major source of contention in the literature. This criterion can be fulfilled with the assistance of neuropsychological testing, but once again, no particular test or cutoff score is specified. Rather, this was left to the judgement of the clinician in the appropriate clinical context. In the literature, the cutoff score of 1.5 SD below age norms has been suggested by some investigators. In the original description of the MCI cohort followed at the Mayo Clinic, the MCI group’s mean performance was 1.5 SD below their age-mates. However, this was not a cutoff score, and of course, nearly half of the group had memory performance score falling somewhat <1.5 SD below the mean. This criterion should be interpreted in conjunction with the first criterion. The memory complaint is meant to represent a change in function for the person. The second criterion corroborates the complaint by attesting to and an actual impairment in performance. The clinician may be challenged by persons who are of either high intellect whose performance is now in the statistically ‘normal’ range, but this level of performance represents a change for that person, and by the person with a low education whose lower cognitive performance may not represent a change. However, the preferable approach to this challenge is to allow the clinician to use judgement in combining all of these criteria. A precise history from the patient and an informant coupled with neuropsychological testing can be invaluable.

The third criterion regarding general intellectual function can be interpreted in a comparable fashion. General intellectual function refers to the other nonmemory cognitive domains, e.g. language, executive function, visuospatial skills, in a fashion similar to the constructs of apraxia and agnosia were used in the diagnosis of AD. Here again, performance in these domains should be judged relative to age-appropriate standards, but no specific instruments or cutoff scores are predetermined. Neuropsychological testing can be very useful in this context in making these determinations, but ultimately, the judgement of the clinician is required.

The essentially normal activities of daily living criterion can be fulfilled largely through a history from the subject and preferably from an informant as well. Several activities of daily living scales are available to assist in this determination, but just as is done with the dementia history, the degree of impairment is a clinical judgement [35]. Often there are minor inconveniences in daily function because of the memory deficit, but these are generally believed to be of insufficient severity to constitute a major disability. This also, however, can be a difficult judgement especially with older subjects. The criterion requires that the functional impairment be due to the cognitive reasons, and this can be difficult to determine in older subjects who may have several medical comorbidities and physical limitations. This underscores the necessity of a clinical assimilation of all of the data available.

Finally, the last criterion, ‘not demented’, is also made on the basis of the clinician’s best judgement. This results from a combination of the assessment of criteria 1–4 and hinges on the degree of functional impairment. Many of these subjects will have a slight degree of general cognitive impairment, but it will not be of sufficient magnitude to be clinically significant. Similarly, there may be some functional impairment, but in the setting of medical comorbidities, this impairment is best judged to be not due to cognitive dysfunction and is sufficiently minor. In general, these subjects appear more normal than not. The difficult

<table>
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<th>Table 1</th>
<th>Criteria for amnestic mild cognitive impairment (a-MCI)</th>
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<tr>
<td>Memory complaint usually corroborated by an informant</td>
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<tr>
<td>Objective memory impairment for age</td>
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<tr>
<td>Essentially preserved general cognitive function</td>
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<tr>
<td>Largely intact functional activities</td>
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<tr>
<td>Not demented</td>
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distinction is between normal ageing and MCI rather than between MCI and AD.

As one can see, the application of the MCI criteria is very similar to that employed for the diagnosis of dementia or AD. The fundamental change involves a shift in threshold of cognitive impairment that the clinician is willing to recognize. This presents a challenge for all of us insofar as the standards for normal ageing, as will be discussed below, can be difficult to determine. In the older subjects, a degree of memory impairment is frequently seen and felt to be associated with ageing and consequently, the demarcation between normal ageing and early MCI can be a clinical challenge. Nevertheless, the concept is reasonable and several multicentre studies have now documented that these criteria can be implemented on a reliable basis across institutions [35].

**Proposed diagnostic scheme**

To consider a procedure for generating a diagnosis of MCI in a new patient, Fig. 5 may help to guide the diagnostic process. Ultimately this scheme will lead to the classification outlined in Fig. 4. Presuming a person or another individual with knowledge about the person expresses some concern about the person’s cognitive function, the doctor must make a judgement. Based on the history and a mental status exam, the doctor makes a judgement as to normal cognition or suspected dementia. For example, if the person has a clear impairment in functional activities and scores 20 of 30 on the MMSE, this person will likely be demented. Although, if the person scores 29 of 30 on the MMSE and shows no impairments in complex activities of daily living, despite the subjective complaint, the person may be normal. Of course, other explanations for this cognitive complaint, such as depression, must also be entertained. There are many instances, however, in which a clinician is uncertain as to the precise cognitive status of the person and may entertain the diagnosis of MCI. In this instance, the diagram in Fig. 5 may help the diagnostic process [36].

Once the clinician has determined that the person is neither normal nor demented, the next decision involves assessing a decline in function. This is done through a careful history from the patient and preferably a collateral source. If there is evidence for a decline in cognition, the clinician must then determine if this change in cognition constitutes a significant impairment in functional activities such that the person might be considered for having a very mild dementia. However, if the functional impairment is not significant, the clinician may entertain the diagnosis of MCI and the next task is to identify the clinical subtype. The clinician should next assess memory more carefully, perhaps with a word list learning procedure or paragraph recall. There are no generally accepted instruments for this determination, and neuropsychological testing may be useful.

If the clinician determines that a significant memory impairment is present, the person is described as having a-MCI with a memory impairment. However, if no memory impairment is present then the person has non amnestic MCI (na MCI). The next step in the process is to determine if the person has an isolated cognitive domain impairment or not. Therefore, if the person has a-MCI, the clinician needs to assess other cognitive domains such as language, attention/executive function or visuospatial skills, to determine if the impairment is just memory or involves other domains as well and hence is a-MCI-multiple domain. If memory is the only domain impaired in a relative sense, then the classification is a-MCI-single domain. If other domains are impaired in addition to memory, the classification is a-MCI-multiple domain. Similarly, if memory is not impaired, na-MCI is the classification, and again the determination is then made of either a single nonmemory domain or multiple nonmemory domains being impaired yielding na-MCI-single domain or na-MCI-multiple domain, respectively. This classification scheme will serve of heuristic value to

![Fig. 5 Flow chart of decision process for making diagnosis of subtypes of mild cognitive impairment (with permission from Ref. [36]).](image-url)
determine the ultimate outcome of these four subtypes of MCI.

Variability in the literature

As research on MCI progresses, several areas of controversy have arisen. Some studies have used alternate criteria for MCI leading to variable results. In particular, the operationalization of the criteria have led to mixed results. Longitudinal studies have differed on their outcomes and follow-up characteristics.

Rating scales

Whilst the criteria outlined in Table 1 represent commonly accepted guidelines for a-MCI, these recommendations are by no means universally accepted. It is not uncommon for research groups to substitute various stages on rating scales as equivalent to the clinical diagnosis of MCI. This can lead to difficulties. A commonly used instrument in research on ageing dementia is the CDR [37, 38]. The CDR is a rating scale ranging from normal (CDR 0) to questionable dementia (CDR 0.5) and ultimately to varying stages of dementia, mild (CDR 1), moderate (CDR 2) and severe (CDR 3). Some research studies have equated a CDR 0.5 to MCI; however, it should be noted that the CDR is a severity rating scale and not a diagnostic instrument. Therefore, subjects with a CDR of 0.5 may meet the criteria stated above for MCI or they may represent very mild AD. This can have implications for the interpretation of progression of subjects. For example, in clinical trials, some studies have enrolled subjects with MCI and used progression to AD as a primary outcome [39]. Other studies have proposed progression along the CDR from 0.5 to 1. This is fundamentally different, however, as the clinical classification of AD may reside in the CDR of 0.5 or 1. Therefore, if one uses the clinical progression from MCI to AD as the outcome measure, the subject may still remain in a CDR 0.5 category.

Similarly, the Global Deterioration Scale (GDS) is another commonly used severity rating scale for dementia [40]. On the GDS, a score of 1 or 2 represents variations of normal function with and without a subjective complaint, respectively. Higher stages of GDS from 3 to 7 represent varying stages of increasing degree of cognitive impairment. In this scale, a GDS of 3 can represent either MCI or AD in much the similar fashion as a CDR of 0.5. Consequently, if the research group characterizes their subjects as having a GDS 3, one is not certain if they are referring to subjects of similar MCI category or mild AD [19, 41, 42].

Therefore, whilst rating scales can be useful in certain settings, they also have inherent limitations and should not be confused for the operational equivalent of clinical criteria. This can cause confusion in the literature.

Normal reference standards

A great deal of clinical judgement is involved in making a decision concerning normal versus MCI. Inherent in this decision is a discussion of what is ‘normal’ as a reference point. Normal performance can be viewed from a variety of perspectives including, amongst others, optimally normal, typically or statistically normal. Some groups have studied individuals who are ageing optimally [43]. These subjects have been selected from those elderly individuals who are relatively devoid of comorbid illnesses. This is a reasonable approach to studying optimal health and characterizing potential performance of individuals. This approach can lead to the inference that any decline in performance is due to some disease, yet studies often demonstrate that even in the absence of significant disease, some cognitive decline is inevitable.

Another approach is to study typical or statistically normal subjects [44]. In this instance, criteria for normal function are identified similar to those used in the Mayo research studies: (i) no active neurological or psychiatric disease, (ii) no psychotropic medications, and (iii) the subjects may have medical disorders but neither they nor their treatment compromise cognitive function. This group of reference subjects constitutes a typical ageing cohort but it must be recognized that these subjects may also be experiencing slight cognitive impairments. A question arises as to whether these cognitive impairments are associated with disease or the ageing process itself.

The NIMH work group in the mid-1980s suggested using young normals’ performance as a reference point [6]. This position assumed that any change in performance relative to young normals represented a change because of ageing. However, subsequent research has documented that depending
upon which instrument and measures were chosen, one could classify up to 90% of all older individuals as having AAMI [7, 45].

Finally, some investigators have argued that a change in performance is an index of abnormal function [46]. This position assumes that normal subjects will remain stable or improved over time but any decline likely represents incipient disease. The difficulty with this position is that the course of change in normal subjects is variable and may be instrument-dependent. Some research has indicated that repeated testing of normal subjects results in improved performance, at least initially [47].

None of these approaches to defining normal performance in ageing is absolutely right or wrong. Each reflects a different position for characterizing performance. Many research groups use a variation of age-adjusted normative neuropsychology data to gauge impairment. Some argue that this approach results in an underassessment of impaired performance as most normal subject groups include incipient cases of dementia who are going to develop the full manifestation of the disease process in ensuing years [48]. Counter arguments claim that this effect is minimal [45]. At present, there is no definite solution to this problem, but readers of the literature need to be aware of the set of assumptions being employed in any research study.

Source of subjects

A third component of the variability with regard to studies in the literature pertains to the sources of subjects for a particular study. A great deal of literature on MCI has been generated from clinical settings such as dementia or memory disorders clinics [12, 17–20]. This rather constrained environment has several implications for the outcomes of these studies. The referral nature of these clinics predisposes to a certain type of patient population. Depending upon the recruitment mechanism of the clinic, certain preselection criteria may be in place, which may lead to recruiting subjects who may have a degenerative aetiology of their symptoms. Cases, which are ‘messy’, such as those involving trauma or substance abuse, may be excluded. As such, the resultant cohort might be ‘cleaner’ than a community-based sample. In addition, these subjects may have a cognitive complaint or their problems may have been brought to the attention of family members who are concerned about the subject’s cognitive function. There may be a higher representation of cases with a positive family history for dementia as well.

On the contrary, cases recruited in these settings may get more thorough evaluations including extensive histories from the subject and an informant as well as detailed neuropsychological testing and a variety of neuroimaging studies. This evaluation may lead to more precise diagnoses and a possible careful classification of MCI subtypes. Presumably, this may also lead to a more pure culture of cases with greater stability in their outcome.

Alternatively, if the MCI cases are derived from an epidemiological study, other considerations apply [21, 22, 49]. For example, by definition there is no restriction on the nature of the sample. If it is a truly random sample of certain age groups, there will likely be multiple types, degrees and causes of cognitive impairment encountered. As such, this type of study must be capable of addressing clinical heterogeneity and determining which types of cognitive impairment may lead to various forms of dementing illnesses. Therefore, in certain respects, the challenge is greater for the epidemiological studies, yet the tools of evaluation may be somewhat limited. Since the study of large populations requires relatively brief assessments with screening batteries, questionnaires and assessment techniques, the breadth of the data may be somewhat compromised. This is not a criticism of the investigators performing the studies; rather, it is a result of the research environment. Out of necessity, less thorough examinations need to be done because of the large number of evaluations that must be carried out. These are generally carried out by very skilled research staffs who are trained at performing screening evaluations on large cohorts of subjects as opposed to making fine clinical discriminations as may be required in the setting of MCI.

Consequently, whilst the sources of subjects can be very important for the outcome of the study, this factor is likely confounded with the nature of the evaluation procedures. In the clinic setting, the subjects may be subtly preselected to yield a certain type of presentation, and the subjects likely undergo very thorough evaluations by expert clinicians. In the field study, there is likely to be more heterogeneity in the subject population and out of necessity, the evaluations must be somewhat more cursory.
Therefore, since the studies from the two settings are being performed for different purposes, the outcomes are likely to be different.

One example of this may manifest itself in the longitudinal outcome rates of the various studies. Clinic-based longitudinal studies of MCI cohorts report instability rates in the range of 10%; whereas population-based studies show instability rates of 25–40% in various studies [21, 22, 49]. It should be noted, however, that just because the population-based study show instability on the short-term basis, this does not mean that the construct under investigation or the criteria used are necessarily inaccurate. It may well be the case that the population-based studies with their less precise clinical diagnostic tools may show more year-to-year variation, but these subjects will eventually decline in longitudinal follow-up. Therefore, one should be cautious in concluding prematurely that the construct based on these findings is unstable. Greater longitudinal follow-up will be necessary to document the eventual fate of these subjects.

Conclusion

In summary, MCI represents a useful clinical entity. Most practitioners recognize persons in their clinical practices who meet criteria for MCI. The challenge is to help the clinicians revise their thresholds for detecting subtle cognitive impairments to enable them to identify persons with suspected MCI. It is most important to realize that MCI is a clinical diagnosis which is the same as are the diagnoses of dementia or AD. Whilst cognitive tests and functional measures are very useful, ultimately, the final determination relies on the clinician’s judgement. The procedures outlined here were designed to assist in that process.

As the field matures, we will learn more about the various subtypes of MCI and their ability to predict various forms of cognitive impairment. Hopefully, as therapeutic interventions become available, we will be able to tailor treatments for specific prodromal forms of cognitive impairment and dementia.

Conflict of interest statement

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Correspondence: Ronald C. Petersen, Department of Neurology, Alzheimer’s Disease Research Center, Mayo Clinic College of Medicine, Rochester, MN 55905, USA.

(fax: +1-507-538-6012; e-mail: peter8@mayo.edu).
Multiple cognitive deficits during the transition to Alzheimer’s disease

L. BÄCKMAN 1, S. JONES 1, A.-K. BERGER 1 E. J. LAUKKA 1 & B. J. SMALL 2
From the 1 Department of Geriatric Epidemiology, Aging Research Center, Neurotec, Karolinska Institute, Stockholm, Sweden, and 2 School of Aging Studies, University of South Florida, Tampa, FL, USA


The literature on cognitive markers in preclinical AD is reviewed. The findings demonstrate that impairment in multiple cognitive domains is typically observed several years before clinical diagnosis. Measures of executive functioning, episodic memory and perceptual speed appear to be most effective at identifying at-risk individuals. The fact that these cognitive domains are most implicated in normal cognitive aging suggests that the cognitive deficit observed preclinically is not qualitatively different from that observed in normal aging. The degree of cognitive impairment prior to the diagnosis of Alzheimer’s disease (AD) appears to generalize relatively well across major study characteristics, including sample ascertainment procedures, age and cognitive status of participants, as well as time to diagnosis of dementia. In episodic memory, there is evidence that the size of the preclinical deficit increases with increasing cognitive demands. The global cognitive impairment observed is highly consistent with observations that multiple brain structures and functions are affected long before the diagnosis of AD. However, there is substantial overlap in the distribution of cognitive scores between those who will and those who will not be diagnosed with AD, hence limiting the clinical utility of cognitive markers for early identification of cases. Future research should consider combining cognitive indicators with other types of markers (i.e. social, somatic, genetic, brain-based) in order to increase prediction accuracy.

Keywords: Alzheimer’s disease, preclinical, cognition, markers, transition, memory.

Background

This paper focuses on patterns of cognitive performance during the transition to Alzheimer’s disease (AD). Specifically, we will provide a review of recent evidence pertaining to cognitive alterations in preclinical AD across (i) different cognitive domains, and (ii) major sample and task characteristics. There is pervasive evidence for a performance decrement at a baseline assessment point amongst persons who are currently nondemented, but will receive an AD diagnosis after a follow-up interval [1]. That is, persons who will go on to be diagnosed with AD after a follow-up period exhibit poorer cognitive performance relative to persons who will not be diagnosed with dementia. Such preclinical deficits...
have been observed across multiple cognitive domains, including episodic memory [2], executive functioning [3], verbal ability [4], visuospatial skill [5] attention [6] and perceptual speed [7]. Similar deficits have been observed for global indicators of cognitive functioning such as the Mini-Mental State Examination [8] and composite measures of cognitive ability [7]. It is of note that cognitive impairment in persons who will develop AD has been observed several years [9, 10], and in some cases even many decades [11, 12], prior to dementia diagnosis.

Despite the seemingly global cognitive impairment in preclinical AD, it has oftentimes been argued and occasionally empirically demonstrated that tasks assessing episodic memory (e.g. word recall, face recognition) are particularly useful in the preclinical detection of at-risk individuals [5, 9, 13]. To be sure, impairment of episodic memory functioning is expected in preclinical AD. It is well documented from both lesion and imaging research that the hippocampus and neighbouring regions are critically involved in the encoding, storage and retrieval of episodic information [14]. It is equally well documented from histopathology [15], structural imaging [16] and functional imaging [17] that the hippocampal complex is implicated long before the diagnosis of AD.

A global cognitive deficit in preclinical AD

However, close examination of the available evidence suggests that episodic memory does not have a unique status amongst cognitive measures in differentiating those who will develop AD versus those who will not. Specifically, effect-size measures such as Cohen’s $d$ [18] or $\eta^2$ as well as epidemiological indicators of differentiation (e.g. sensitivity and specificity) show strikingly similar accuracy in discriminating preclinical AD cases from controls for measures of episodic memory [2, 5, 9, 13, 19], executive functioning [3, 19, 20] and perceptual speed [3, 7, 21]. Somewhat smaller, albeit quite sizable, differences between cases and controls are typically observed for measures of verbal ability [4–6, 22, 23], visuospatial skill [3, 19, 24, 25] and attention [13, 26, 27]. The point that multiple cognitive functions are strongly implicated in preclinical AD is further substantiated by the observation that indicators of global cognitive ability appear to be equally impaired as specific measures of episodic memory, executive functioning and perceptual speed at identifying individuals in the preclinical phase of AD [7, 8, 27–29].

The notion of a multiple cognitive systems breakdown is consistent with data that conversion rates to AD over 3 years are considerably greater for persons with deficits in episodic memory and some other cognitive domain (e.g. verbal ability, visuospatial skill) at baseline than for those who have isolated memory impairment [30, 31]. As such, multiple cognitive deficits prior to the diagnosis of AD may be the more typical presentation of preclinical AD. Of further note is that the three specific cognitive domains that seem to best discriminate between cases and controls (i.e. episodic memory, executive functioning, perceptual speed) have also been implicated as particularly sensitive to the effects of normal aging [32–34]. These two sets of observations suggest continuity rather than discontinuity from normal aging to preclinical AD with regard to the patterns of cognitive impairment [35, 36].

Biological underpinnings

How then should we reconcile the apparent multiple cognitive systems breakdown in preclinical AD with extant knowledge concerning the biological basis of cognitive deficits in this phase of the disease? As noted, in searching for biological correlates of the cognitive impairment in preclinical AD, heavy emphasis has been placed on the medial – temporal lobe (MTL), with its obvious link to episodic remembering. However, recent brain imaging and histopathological evidence suggests that multiple brain structures and functions in addition to those related to the MTL may be affected before the diagnosis of AD. These findings include volume reductions of anterior cingulate and temporal sulcus [37], posterior cingulate and neocortical temporoparietal regions [38] and frontal regions [39]; decreased blood flow in posterior cingulate and precuneus [40], reduced glucose metabolism in temporoparietal regions [41]; and deposits of amyloid plaques in temporal [42] and frontal [43] cortex. In addition, more general alterations of brain functions in preclinical AD have been observed, including an increase of white matter hyperintensities [44] as well as a reduction of whole-brain glucose metabolism [45].

Thus, given that these studies indicate a rather widespread affection of brain structures and functions
in preclinical AD, it should come as no surprise that a similar extent of impairment is observed at the behavioural level. This point is further strengthened by recent evidence indicating strong relationships of volumetric measures of the whole brain and the temporal and frontal lobes to cognitive performance (e.g. memory, verbal skill, executive functioning) in mild cognitive impairment (MCI) and early AD, although no relationships were observed between regional brain damage and impairment of specific cognitive functions [46].

**Study characteristics**

Having established that impairment of many, if not most, higher-order cognitive functions in preclinical AD is a biologically plausible outcome, it is of interest to address the extent to which the magnitude of cognitive deficits is influenced by various sample and task characteristics. Here we will consider briefly four factors related to the nature of the study sample: (i) onset age of AD; (ii) length of the follow-up period between cognitive assessment and dementia diagnosis; (iii) sampling method (i.e. population-based versus convenience sample); and (iv) participant status {i.e. whether or not the sample was classified as cognitively impaired [e.g. MCI, cognitive impairment, no dementia (CIND)] at baseline}. This discussion will focus on episodic memory and global cognitive ability, because these two domains comprise a sufficient number of studies to make comparisons meaningful. In addition, for the domain of episodic memory we will also examine whether clear performance deficits in preclinical AD are more likely to occur for some task conditions than for others. In so doing, we will contrast studies involving (i) immediate versus delayed testing of memory, (ii) recall versus recognition, and (iii) verbal versus nonverbal materials.

The impressions from the available literature can be summarized as follows. First, baseline differences between cases and controls appear to be larger for earlier-onset cases than for later-onset cases. This pattern is evident when grouping the relevant studies into those that involve persons who received the dementia diagnosis before 75 years of age versus those who were diagnosed after 75 years of age, and it applies to both episodic memory [2, 6, 7, 22, 24, 27, 50, 57–59] and global cognitive ability [19, 41, 44, 51, 60–65] alike. Notwithstanding this pattern, it is of interest to note that the degree of cognitive impairment is quite sizable also for studies employing retest intervals of 5 years or longer [2, 6, 61, 63, 64]. This observation is interesting to view in the light of recent longitudinal evidence on the trajectory of cognitive decline in preclinical AD. In two studies, stability of cognitive impairment amongst incident AD patients was observed from 6 to 3 years prior to eventual diagnosis [2, 63]. In another study, disproportionate decline was seen amongst incident AD cases from 3.5 to 1.5 years before diagnosis [19]. Thus, these studies suggest that the preclinical period in AD is characterized by an early onset followed by relative stability until a few years before diagnosis when precipitous cognitive decline occurs [8, 16, 26].

Clear preclinical deficits are observed both in studies that employ population-based sampling and those that use convenience samples recruited via memory clinics, newspaper advertisements and the like. However, for both global cognitive ability [7, 21, 25, 27, 29, 41, 66] and episodic memory [4–6, 27, 40, 58, 59, 67], the size of the impairment appears to be somewhat larger in population-based samples. Conceivably, this reflects the fact that memory and other cognitive problems are common amongst people who actively seek participation in studies on aging and cognition, irrespective of whether or not they are in a preclinical phase of dementia. Consequently, cognitive performance would be expected to be lower also for the controls in these types of studies, resulting in smaller group differences.

A key difference between studies in this area, and one that is of particular relevance to the current collection of papers, concerns whether participants have been classified as cognitively impaired already at baseline assessment, using categories such as MCI or CIND. On the one hand, it may appear reasonable...
that studies that follow a group of preclassified persons prospectively should yield larger effect sizes than those that work retrospectively from diagnosis to baseline. On the other hand, recent evidence indicates that categories like MCI are rather heterogeneous. For example, it has been shown that, although a large portion of cognitively impaired older adults go on to develop AD within a few years, a sizable portion remain stable or even improve across the same time period [68, 69]. Obviously, the latter fact speaks against prospective studies resulting in larger effect sizes.

For global cognitive ability, the evidence suggests that MCI-type studies and retrospective studies yield quite similar differences between incident AD cases and controls [8, 60–62, 64, 70–72]. By contrast, for episodic memory, the size of the impairment appears to be larger in studies that preclassify subjects on the basis of cognitive impairment at baseline assessment [2, 20, 23, 24, 26, 48, 50]. The latter finding may be expected, given that episodic memory impairment constitutes a cardinal criterion for inclusion in the MCI category [73].

Task variation in episodic memory

Within the domain of episodic memory, the size of the preclinical deficit varies in a rather orderly fashion. First, it is clear that the deficit is exacerbated in studies that employ delayed testing [3, 19, 23, 57] as opposed to those in which memory is tested immediately after study [3, 6, 19, 21, 25]. Provided that delayed testing taxes consolidation processes to a greater extent than immediate testing [74, 75], this result is consistent with the view that failure in transferring information from temporary storage to a more permanent memory representation is a characteristic feature of the episodic memory impairment in preclinical AD [35]. Secondly, it is equally clear that larger group differences are obtained when memory is tested with recall [4, 7, 26, 50, 67] compared with recognition [2, 5, 19, 25, 48]. Given that the retrieval demands are considerably greater in recall than in recognition [76, 77], these data suggest that retrieval problems, in addition to difficulties in encoding and consolidation, may be characteristic of preclinical AD. Finally, verbal episodic memory tasks [2, 5, 19, 25] appear to yield somewhat larger group differences than nonverbal episodic memory tasks [3, 6, 24, 25, 31]. This may reflect the fact that verbal materials (e.g. words, paired-associates) are typically poorer in terms of the features available at encoding compared with nonverbal materials (e.g. faces, pictures). As a result, the requirement of self-initiated elaborative encoding operations are generally greater for verbal materials [78], which may be particularly handicapping for preclinical AD cases. The patterns of data described above indicate that the episodic memory deficit in preclinical AD is exacerbated with increasing cognitive demands.

Albeit these influences of study and task characteristics, it is important to note that large group differences are manifest across the board for global cognitive ability and episodic memory. Thus, the differences observed should not conceal the fact that preclinical cognitive deficits in AD generalize across (i) several key domains of cognitive functioning; (ii) major characteristics of research studies; and (iii) multiple aspects of episodic memory.

Caveats

Although the present analysis of cognitive markers in preclinical AD provides a comprehensive account of the current state of knowledge in this domain of research, there are limitations to note. First, few of the available studies provide information on all, or even most, of the cognitive ability domains targeted. Thus, the discussion pertaining to the influence of sample characteristics was restricted to global cognitive ability and episodic memory. As a result, it is not possible to examine whether differences between preclinical AD cases and controls vary across cognitive domain depending on the time between assessment and diagnosis. For example, advocates of the view that episodic memory is the earliest cognitive marker of incipient AD could still argue their case; the present analysis is not informative regarding the onset of decline for different cognitive functions. However, for studies that employ long follow-up periods, differences between incipient AD cases and controls appear to be larger for measures of global cognitive ability [27, 41, 60, 64, 66, 72] than for episodic memory measures [2, 6, 27, 48, 50]. Note also that those studies using assessment periods spanning several decades have found clear preclinical impairment in domains other than episodic memory such as linguistic skill [11] and general intelligence [12].
Another consequence of the limited database is that it is not possible to examine the influence of different study characteristics simultaneously. In some cases, study characteristics may be confounded. For example, studies in which participants are preselected based on their cognitive performance may primarily use convenience sampling rather than population-based sampling. Disentangling the influence of these and other variables would require considerably more studies than what is available in the current literature. Thirdly, the available studies vary quite markedly with regard to the length of the follow-up interval. Future longitudinal studies should address this issue in order to provide more definite information as to the specific time at which precipitous cognitive decline normally occurs in preclinical AD.

Conclusions

With the above caveats in mind, the current overview clearly suggests that impairment in multiple cognitive domains several years before clinical diagnosis is characteristic of AD. The generality of the cognitive impairment observed is highly consistent with recent observations that numerous brain structures and functions are affected prior to the diagnosis of AD. We would also like to highlight the finding that the magnitude of the preclinical cognitive impairment seems to be only marginally affected by various study and task characteristics.

Issues to consider in future research

Although cognitive performance scores obtained several years before diagnosis can discriminate quite well between incident AD cases and controls at the group level, it is clear that the distributions of scores for the two groups overlap to a considerable degree. This fact hampers optimism regarding the utility of cognitive markers in identifying persons eligible for pharmacological intervention and other clinical purposes. The large cognitive overlap between cases and controls deserves several comments. First, it is well known that a vast number of factors, in addition to being in a prodromal phase of dementia, may affect cognitive functioning in aging. These include, but are not limited to, demographic factors (e.g. education), social and life-style factors (e.g. activity patterns), genetic factors [e.g. apolipoprotein E (APOE) status], as well as a variety of health-related factors [e.g. vitamin and thyroid deficiency, circulatory disturbance, depression; 79, 80]. Analogously, there is interindividual variability amongst persons who will develop AD both with regard to performance at a given point in time (e.g. 4 years before diagnosis) and in rate of decline during the preclinical period [26, 81]. These two facts make it unlikely that cognitive performance scores alone would yield a clear separation between incident AD cases and controls.

Extending the pool of preclinical markers

In the current overview, however, different cognitive domains were examined separately. There is evidence that the ability to identify persons at risk for developing AD increases substantially when tasks assessing different cognitive domains (e.g. episodic memory, executive functioning, verbal ability) are combined into the same prediction model [3, 5, 19]. In addition, the cognitive variables used in this research may be broadened from traditional cognitive performance scores (e.g. number of words recalled, lexical decision time) to alternate measures such as those dealing with within-person performance variability. Evidence suggests that increased variability across trials in RT tasks may be associated with several factors known to be related to brain functioning [e.g. impending death, clinical dementia, head injury, physical health; 82, 83]. Importantly, this association remains strong even after partialing out mean performance levels. Thus, including measures of within-person variability may increase prediction accuracy.

Further, indicators of other domains of functioning may be useful in identifying at-risk individuals. This includes multiple measures of brain structure and function such as volumetric measures [37, 38], glucose metabolism [41, 45], blood flow [17, 40], amyloid deposits [42, 43] and white matter hyperintensities [44]. It also includes markers for genetic predisposition such as presence of the APOE ε4 allele [84] as well as subjective memory complaints [85], family reports of cognitive impairment [20] and depressive symptoms [86]. The identification of precipitating factors may provide further help in differentiating between preclinical AD and cognitive impairment of other origins. Possible precipitating factors include medical
events such as unrecognized hypertension [87], head trauma [88] and stroke [89] as well as social factors such as isolation [90].

Given this state of affairs, an important avenue for future research would be to combine predictors from different behavioural and biological domains. Such an approach may not only increase overall prediction accuracy; it also enables examining possible interactive effects amongst various preclinical markers (e.g. poor episodic memory, unrecognized hypertension, depressive symptoms). Although it is conceivable that such a multivariate approach may decrease the overlap between preclinical cases and controls, very large samples will be required to fully utilize its potential. Moreover, improved classification will, of course, occur only to the extent that the predictor variables included contribute unique variance.

Onset of precipitous decline

Another unresolved issue of importance to the correct identification of at-risk individuals has to do with the time at which precipitous decline occurs amongst those who will develop AD. Although there is consensus that persons who will develop AD show precipitous cognitive decline during the last 2–3 years before diagnosis [8, 19, 26], evidence is mixed regarding whether accelerated decline is observed longer before diagnosis. Using data from a population-based study of individuals aged 75 years and older at the outset, we observed stability of the magnitude of preclinical impairment from 6 to 3 years before diagnosis for measures of episodic memory [2] and global cognitive ability [63]. Coupled with other evidence that preclinical impairment may be seen decades before diagnosis [10–12], this pattern could be interpreted to mean that accelerated cognitive decline may not be expected until various biological events (e.g. the accumulation of amyloid and neurofibrillary tangles, inflammation, oxidative stress, loss of synapses, death of neurones) have reached a certain threshold [91, 92]. This notion rests on the assumption that the brain is capable of counteracting AD-related neural changes to a point at which compensatory responses are no longer possible. Indeed, there is evidence from cognitive psychology [93], neuroimaging [94], histopathology [95] and neurochemistry [96] that the aging brain possesses compensatory capabilities.

However, other evidence suggests that precipitous decline may occur in persons who will be diagnosed with AD longer before diagnosis. In two studies [49, 97] accelerated decline in measures of episodic memory was demonstrated more than 5 years before diagnosis, although speeded measures of performance IQ exhibited accelerated decline around 2 years before diagnosis. Several factors are likely to contribute to the equivocal findings, including the nature of the study sample. For example, it is conceivable that accelerated cognitive decline longer before diagnosis is more likely to be observed when strict selection criteria (e.g. removal of persons with other conditions that affect cognitive functioning) are employed. Obviously, more information pertaining to this issue is vital in order to achieve a better understanding of the preclinical process in AD, and to improve differentiation between cases and controls.

Individual differences in onset and rate of change

An important factor in this context has to do with whether there are individual differences with regard to onset and rate of change in preclinical AD. Indeed, clinical observations suggest that some individuals may show accelerated decline for only a short period of time before diagnosis, whereas others show gradual decline across a much longer time period. However, delineating factors that are systematically related to rate of cognitive change in preclinical AD has proved difficult. Negative findings have been documented for a variety of factors, including age, sex, education, depression, APOE status, family history of dementia, circulatory disease, vitamin B deficiency, social network and substance use [81, 98–99]. Note that most of these factors have been implicated as risk factors for AD, or been found to influence cognitive performance in normal aging.

One possibility is that the negative findings reflect the fact that the influence of individual difference variables is overshadowed by the emerging pathogenetic process [81]. However, they could also, in part, be a function of the analytical methods employed (e.g. ANOVA or regression procedures with listwise deletion). More recently developed analytical tools such as multilevel modelling may be more sensitive in identifying factors that share systematic relationships with rate of cognitive change in preclinical AD. Specifically, these procedures allow
for differences in baseline functioning, as well as differences in longitudinal rate of change, to be incorporated into the statistical models, which is directly relevant to the longitudinal trajectories in preclinical AD seen previously [2, 19]. If systematic relationships between specific individual difference variables and rate of change were to be revealed, they would be informative in identifying people destined to develop AD at the earliest possible time.

Conflict of interest statement
No conflict of interest declared.

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Correspondence: Lars Bäckman, Department of Geriatric Epidemiology, Aging Research Center, Neurotec, Karolinska Institute, Stockholm, Sweden. (fax: +46-8-6905954; e-mail: lars.buckman@neurotec.ki.se).
MRI and CSF studies in the early diagnosis of Alzheimer’s disease

M. J. de LEON1,2, S. DESANTI1, R. ZINKOWSKI3, P. D. MEHTA4, D. PRATICO5, S. SEGAL1, C. CLARK5, D. KERKMAN3, J. DEBERNARDIS3, J. LI1, L. LAIR1, B. REISBERG1, W. TSUI1,2 & H. RUSINEK1

From the 1Center for Brain Health, New York University School of Medicine, NY; 2Nathan Kline Institute, Orangeburg, NY; 3Molecular Geriatrics, Vernon Hills, IL; 4Institute for Basic Research, Staten Island, NY; and 5University of Pennsylvania, PA, USA


The main goal of our studies has been to use MRI, FDG-PET, and CSF biomarkers to identify in cognitively normal elderly (NL) subjects and in patients with mild cognitive impairment (MCI), the earliest clinically detectable evidence for brain changes due to Alzheimer’s disease (AD). A second goal has been to describe the cross-sectional and longitudinal interrelationships amongst anatomical, CSF and cognition measures in these patient groups. It is now well known that MRI-determined hippocampal atrophy predicts the conversion from MCI to AD. In our summarized studies, we show that the conversion of NL subjects to MCI can also be predicted by reduced entorhinal cortex (EC) glucose metabolism, and by the rate of medial temporal lobe atrophy as determined by a semi-automated regional boundary shift analysis (BSA-R). However, whilst atrophy rates are predictive under research conditions, they are not specific for AD and cannot be used as primary evidence for AD. Consequently, we will also review our effort to improve the diagnostic specificity by evaluating the use of CSF biomarkers and to evaluate their performance in combination with neuroimaging. Neuropathology studies of normal ageing and MCI identify the hippocampal formation as an early locus of neuronal damage, tau protein pathology, elevated isoprostane levels, and deposition of amyloid beta 1-42 (Aβ42). Many CSF studies of MCI and AD report elevated T-tau levels (a marker of neuronal damage) and reduced Aβ42 levels (possibly due to increased plaque sequestration). However, CSF T-tau and Aβ42 level elevations may not be specific to AD. Elevated isoprostane levels are also reported in AD and MCI but these too are not specific for AD. Importantly, it has been recently observed that CSF levels of P-tau, tau hyperphosphorylated at threonine 231 (P-tau231) are uniquely elevated in AD and elevations found in MCI are useful in predicting the conversion to AD. In our current MCI studies, we are examining the hypothesis that elevations in P-tau231 are accurate and specific indicators of AD-related changes in brain and cognition. In cross-section and longitudinally, our results show that evaluations of the P-tau231 level are highly correlated with reductions in the MRI hippocampal volume and by using CSF and MRI measures together one improves the separation of NL and MCI. The data suggests that by combining MRI and CSF measures, an early (sensitive) and more specific diagnosis of AD is at hand. Numerous
studies show that neither T-tau nor P-tauX (X refers to all hyper-phosphorylation site assays) levels are sensitive to the longitudinal progression of AD. The explanation for the failure to observe longitudinal changes is not known. One possibility is that brain-derived proteins are diluted in the CSF compartment. We recently used MRI to estimate ventricular CSF volume and demonstrated that an MRI-based adjustment for CSF volume dilution enables detection of a diagnostically useful longitudinal P-tau231 elevation. Curiously, our most recent data show that the CSF isoprostane level does show significant longitudinal elevations in MCI in the absence of dilution correction. In summary, we conclude that the combined use of MRI and CSF incrementally contributes to the early diagnosis of AD and to monitor the course of AD. The interim results also suggest that a panel of CSF biomarkers can provide measures both sensitive to longitudinal change as well as measures that lend specificity to the AD diagnosis.

Keywords: MCI, Alzheimer’s disease, biomarkers, MRI, longitudinal, early diagnosis.

Introduction

The prevalence of Alzheimer’s disease (AD) is expected to double over the next 30 years [1] and there is still no currently accepted early diagnosis for AD. As reported by the biomarkers in the AD working group of the Reagan Research Institute [2], with improved understandings of the pathophysiology of AD and the promises of mechanism-based and preventative therapeutic approaches, there is an urgent need to develop biomarkers for early diagnosis.

Magnetic resonance imaging (MRI) and CSF chemistry studies have been pointed to as candidate modalities for diagnostic biomarkers because they accurately diagnose AD, predict decline in mild cognitive impairment (MCI) patients, and in the case of serial MRI track the course of AD. Nevertheless, for several reasons these modalities are not widely accepted: (i) MRI tissue volume changes are not specific for AD and require intensive skilled labour; (ii) the pioneering CSF studies measured total T-tau level and CSF amyloid beta1-42 (Aβ42) which are not specific for AD, and do not readily change with disease progression; (iii) CSF Aβ42 levels are not easily interpreted because CSF Aβ is not exclusively brain-derived and because production and clearance are not well characterized; (iv) CSF acquisition is invasive; and (v) experimental amyloid imaging protocols are not clinically available nor adequately tested [3–8].

The neuropathology of early AD

The principal hallmarks of AD include: Aβ deposition in extracellular plaques and vascular walls, the accumulation of intracellular neurofibrillary tangles (NFT), synaptic reductions, neuronal loss and volume loss (atrophy)[9–12]. The hippocampal formation includes the EC, hippocampus proper, and subiculum, and it comprises the regions most vulnerable to the early deposition of AD lesions [12–19]. A pattern of hippocampal formation NFT deposition [20–29], with relative sparing of the neocortex [21–23, 25, 30], is often found in studies of nondemented elderly (this term includes both normal and MCI patients). Braak’s neuropathology studies of 2369 cases demonstrated that in the most mildly affected brains, only the transentorhinal EC showed NFT and neuropil thread pathology [31]. These findings suggest that isolated NFT may first occur in the natural history of AD [24, 28, 32] but it does not exclude the possibility that soluble Aβ peptides are interactive [29, 33]. Of direct relevance to early diagnosis using neuroimaging, numerous pathology studies have shown that in mild AD there is damage to the hippocampal formation that includes synaptic loss (a site of active glucose metabolism), neuronal loss, volume reductions, and tau and Aβ pathology [18, 34–40]. It is well documented, that in AD hippocampal neuronal damage is reflected in volume losses that are detectable with MRI imaging [41, 42]. Of immediate importance to this application, studies by Price have shown that tau and Aβ pathology precede EC and hippocampal neuronal losses in nondemented and preclinical AD patients [43]. However, once the AD process is underway, it appears that the extent of hippocampal neuronal loss exceeds the number of NFT lesions [44]. Our pathology data [37, 38] is in
agreement with these observations and this leads to our main hypothesis: that baseline elevations of the CSF P-tau231 level and an elevated rate of MRI hippocampal formation atrophy will both within and combined across modalities optimize the prediction of longitudinal cognitive decline in normal elderly (NL) and in patients with MCI.

Compared with NFT, Aβ deposition tends to accumulate at greater ages. They too affect the hippocampal formation but have a preference for the accumulation at greater ages. They too affect the elderly (NL) and in patients with MCI.

It is very important that CSF prediction and differential diagnosis studies in MCI and AD consistently show that P-tau181 or P-tau231–235 [70, 71] or P-tau396/404 [72] are diagnostically equivalent or better than the T-tau. Predicting the conversion of MCI to AD, in the absence of controls, Arai et al. achieved equivalent accuracies from P-tau231–235 and T-tau levels [70]. However, Hampel et al. found in MCI that elevated levels of P-tau231 were superior to T-tau in the binary prediction of progressive cognitive decline to AD [73]. In a recent cross-sectional paper, Hampel’s group reported that when compared with T-tau, P-tau231 showed significantly better specificity for AD [74]. Specifically, the levels of P-tau231, but not T-tau, were consistently elevated in AD when compared with frontotemporal dementia (FTD), Lewy body dementia (LBD), and NL controls. Others have compared AD with FTD [71, 75] and with non-AD dementia controls [76, 77] and demonstrated a superior diagnostic specificity with P-tau181 relative to T-tau. Similarly, CSF P-tau396–404 but not T-tau differentiated AD (n = 52) from vascular dementia (n = 46) and NL (n = 56) (accuracy not reported) [72]. However, the ratio of P-tau396–404/T-tau differentiated the groups with a sensitivity and specificity greater than 95%. It was concluded that the major increase in X-tau detected in the CSF of AD is in the form of P-tauX.

Overall, these studies suggest that P-tau231 may provide unique and relatively specific diagnostic information for AD, whereas, T-tau may be a nonspecific marker for general brain damage [78]. To date, in vivo studies, have not examined any of the longitudinal relationships between MRI-determined EC, hippocampal, or neocortical volumes and CSF T-tau or P-tau231.

**CSF tau studies**

It is widely believed that increases in the CSF T-tau level reflect neuronal and axonal damage. Many studies demonstrate elevated CSF concentrations of T-tau in AD [53–64] and in MCI [54, 65, 66]. However, clinical studies show that elevated CSF T-tau levels are not specific to AD as they are elevated in other neurodegenerative diseases [67]. It was recently shown in acute stroke that the T-tau but not the P-tau levels were increased and later returned to normal [68]. One MCI study reported that T-tau alone was not an accurate baseline predictor of the decline to AD [65].

Moreover, considerable uncertainty exists with respect to the influence of ageing on the CSF T-tau levels. Normal ageing studies show both positive [69] and negative [53, 54] age effects. These studies are difficult to interpret due to the small numbers of subjects, narrow age distributions, diverse strategies for excluding cognitive impairment, and absent neuroimaging and autopsy validation.

The genetic mutations causing familial AD elevate the production of Aβ, particularly Aβ42 [79]. However, there is little evidence for elevated CSF or plasma Aβ42 levels in sporadic AD. One study reported that the levels of Aβ42 are elevated in MCI and AD [80] but these results are not consistent [54]. Cross-sectional CSF Aβ studies consistently show that relative to normal control, Aβ42 levels

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are reduced in AD [53, 81–85]. One longitudinal AD study showed that Aβ42 levels decreased over time [82]. Major obstacles to the interpretation of these CSF data include samples derived from multiple collaborating sites with potentially different thresholds for recognizing 'early' AD and the very limited availability of longitudinal data on normal individuals. The influences of normal ageing on CSF turnover and specifically on Aβ clearance are also poorly understood [86, 87]. It has been experimentally demonstrated that with increasing age, amyloid plaques start to accumulate in the brain and may act as a sink for soluble Aβ [88]. Based on this view, assuming production is constant, one would predict age-related plaque deposition with an associated decrease in the CSF Aβ level. However, if clearance were also reduced, then this potentially explains why cross-sectional studies show little evidence for a relationship between CSF Aβ and age [53, 54, 84]. Moreover, recent observations of reduced CSF Aβ levels in other dementias without plaque formation, alternatively, suggest that reduced neuronal production of Aβ is yet another variable. Evidence from transgenic AD-mice studies indicates that the relationships amongst brain, CSF and plasma levels change over time due to the progressive sequestration of Aβ42 in plaques. Nine-month-old AD mice with brain plaques showed a twofold higher CSF-to-plasma Aβ ratio than age and genetically matched animals without plaques [89].

The diagnostic utility of CSF Aβ40 as a biomarker is less well understood than Aβ42. A limited number of reports have shown elevated Aβ40 levels with increasing age [84, 90] and in MCI. However, several cross-sectional AD studies have failed to observe differences from NL [85, 91]. Longitudinal AD data for Aβ40 are limited and not consistent [82, 92]. It remains to be examined how well Aβ40 predicts the transitions between NL and MCI and between MCI and AD.

On the bright side, considerable agreement exists that in cross section, reduced CSF Aβ42 combined with elevated X-tau measures improves the diagnostic accuracy for AD [53, 54, 61, 62]. However, compared with non-AD dementia patients, Aβ42 reductions offer limited specificity for AD. Moreover, there is inadequate longitudinal data to make a judgement about the predictive value of Aβ4X for either future MCI or AD.

**Physiological bases for stable CSF tau concentrations**

Given the characteristic progressive clinical decline and increasing topography of brain atrophy in AD [93], it is surprising that CSF T-tau concentrations are not consistently found to be progressive. Whilst a few longitudinal AD studies report increases in T-tau levels [92, 94, 95], others do not show changes [65, 96–98]. Longitudinal AD levels of P-tau231 have not yet been reported. In a longitudinal MCI study using P-tau231–235, cross-sectional but not longitudinal level changes were found [70]. Speculative explanations for the negative longitudinal X-tau findings include the inadequacy of the follow-up interval and variability in the pathological course of AD. Our recent findings suggest that atrophy-related anatomical and physiological factors may also play a role [99]. We observed that only after controlling for the progressive ventricular enlargement in MCI and the resulting dilution of the CSF P-tau231 concentration were significant longitudinal level increases detected.

Our rationale for the ventricular volume correction is based on observations, that as a predominantly brain-derived protein, tau levels are higher in the ventricular than lumbar CSF. Reiber [100] has shown that the concentrations of brain-derived proteins are higher in ventricular than in lumbar samples (1.5 : 1 for tau and 18 : 1 for S-100B). For systemically derived proteins ratios <1 are found (e.g. albumin 1 : 205). Aβ levels are lower (1 : 2) in ventricular samples compared with LP-derived samples (K. Blennow, unpublished communication), likely reflecting the derivation of Aβ from both central and peripheral sources [101]. It is well known that AD patients show progressive enlargement of ventricular and subarachnoid compartments due to tissue loss. The increased fluid volume then dilutes the CSF concentration of brain-derived proteins (e.g. X-tau). AD patients also show reduced CSF turnover [102] which may stagnate and further increase ventricular CSF protein levels. Reiber has proposed that reduced CSF turnover would not only increase the ventricular concentration, but would also result in an increased concentration gradient for tau that would enhance transependymal clearance, leaving the lumbar CSF X-tau levels largely unchanged. However, empirical validation studies have not been carried out. Because of the diverse protein sources for Aβ, it is unknown how reduced
CSF turnover would affect the ventricular and lumbar levels. Overall, the available data suggest that in AD the X-tau concentration from an LP may underestimate the volume released from the brain. For CSF Aβ4X, ventricular levels are lower than lumbar levels and ventricular volume corrections are not warranted. In the studies below, we test the hypothesis that P-tau231 levels adjusted for ventricular volume improve the cross-sectional and longitudinal diagnostic classifications. Virtually nothing is known about cisternal and subarachnoid tau concentrations and therefore we did not correct for this CSF pool. In summary, it is generally accepted that lumbar CSF X-tau and Aβ4X concentrations are potentially useful surrogate markers of brain AD. However, the poor understanding of the physiological mechanisms governing protein production and clearance from brain [103] and accumulations in CSF and plasma may limit the clinical utility of the protein level. We propose that volume dilution studies of CSF X-tau are important first steps towards understanding the dynamics of this system.

CSF isoprostane levels

Whilst the precise cascade resulting in cell death in AD is unknown, recent studies have identified a role for oxidative stress and lipid peroxidation [104]. Isoprostane brain levels, by-products of lipid peroxidation are increased at post-mortem in AD [105, 106], and increased in cross-sectional in vivo CSF studies of both AD [106, 107] and MCI [108]. There are no longitudinal data available.

Neuroimaging and CSF biomarker studies

We could not find prior MCI or AD studies of the longitudinal relationships between neuroimaging measures and CSF measures of X-tau and/or Aβ4X levels. One encouraging prediction study of MCI and AD subjects reported that baseline CSF P-tau181 and Aβ42 levels predicted, at 16 months, ventricle volume increases [109].

Neuroimaging markers for MCI and AD

In 1989 we published the first study showing that qualitative estimates of hippocampal atrophy in MCI predicted decline to AD [110]. This finding has been replicated [111, 112] and more recently, predictions of future AD were demonstrated with hippocampal volume (see [113, 114] for review), and with hippocampal perfusion [115]. These early studies also demonstrated that the prevalence hippocampal atrophy increased with age and was very common in MCI and AD [116] (see Fig. 1).

Additional recent findings show that reduced EC size can discriminate between MCI and NL [117–122] and accurately predict future conversion of MCI subjects to AD [118, 122–124]. There is also evidence to show that size or glucose metabolism (METglu) in temporal neocortex [125–128] and posterior cingulate gyrus [115] can predict the MCI conversion to AD. However, the regional MRI brain volume or metabolism reductions determined by FDG-PET are not disease-specific. For example, both the EC and hippocampal volumes are reduced in AD and FTD when compared with control, and, these anatomical changes do not distinguish between the two disorders [129]. In addition, longitudinal whole-brain atrophy changes estimated from the boundary shift integral method fail to distinguish the abnormal rates of atrophic change characteristic of both AD and FTD [130, 131].

In 2001 using FDG-PET, we published the first evidence that EC METglu reductions in NL uniquely
predict the decline to MCI and also predict future hippocampal glucose metabolism reductions [124]. Our most recent MRI work shows that the medial temporal lobe atrophy rate during the normal stage, estimated with a boundary shift protocol, predicts the future conversion of NL to MCI [132]. Previously, Jack et al. demonstrated that NL patients who converted to MCI showed a greater rate of hippocampal volume loss than nondeclining subjects. However, baseline effects (prediction) were not observed [133]. Overall, these MRI/PET studies indicate the current potential of hippocampal formation atrophy measures to predict stage transitions related to AD as well as to describe disease progression from NL to MCI to AD levels of impairment. In our current research, we are now examining the added sensitivity and specificity that CSF biomarkers bring to the brain image in the early diagnosis of AD.

Methods

MRI image acquisition

Diagnostic evaluations. Fast spin-echo fluid-attenuated inversion-recovery (FLAIR) images (TR = 9000 ms, TE = 133 ms, 1 NEX, TI = 2200 ms, 3.3 mm slice thickness; 24 cm FOV) were obtained in 32 axial planes using a 256 × 192 acquisition matrix, with a 4-min acquisition time. The FLAIR sequence images the entire brain and is used to identify white matter lesions.

Research scan sequences. For the brain volume and the boundary shift analysis protocols, we used fast-gradient echo (FGE) images from a 3D coronal T1-weighted acquisition. Images are intensity normalized and baseline and follow-up scans are co-registered. This MRI protocol is remarkable for its high tissue contrast and good spatial resolution. The FGE sequence is defined as: TR 35 ms, TE 9 ms, 60° flip angle, 256 × 192 acquisition matrix, the section thickness is 1.7 mm which encompasses the entire brain without wrap artefact. We acquire 124 sections with a 24-cm FOV and 1 NEX for a total acquisition time of 12 min.

All quantitative work is blinded to all clinical data. File names are assigned sequential code numbers and image headers are stripped off demographic information. All images are transferred to our central image data bank and then to satellite Sun workstations for further processing. Image analysis is performed on a graphic workstation (Sun Microsystems, Santa Clara, CA, USA) using our locally developed MIDAS software running on a Unix operating system.

MRI image analysis

Qualitative assessments of hippocampal atrophy. Several years ago we demonstrated that both CT and MRI protocols could be used interchangeably to obtain sets of axial images parallel to the long axis of the hippocampus in order to evaluate the extent of hippocampal atrophy [116]. For both CT and T1-weighted MRI this consisted of contiguous 5 mm axial slices acquired at approximately 20° negative to the CM plane. For all study subjects, the hippocampus was examined for CSF accumulation in the regions of the transverse, choroidal and hippocampal fissures (see Fig. 2, arrow). The anatomical basis for this assessment is described in detail in a previous publication [111]. For each hemisphere, using previously published procedures, the extent of hippocampal atrophy was rated on a 4-point scale: (0 = none, 1 = questionable, 2 = mild/moderate and 3 = severe). A cut-off score of 2 or greater (definite CSF accumulation) on either hemisphere was considered evidence for qualitative hippocampal atrophy.

Fig. 2 Arrow highlights the body of the hippocampus. Image on right is from a patient with atrophy.

Fig. 3 Arrows mark the entorhinal cortex on MRI.
Quantitative assessments of regional brain size. EC. Measurement of the surface area of the EC requires drawings on the surface of the parahippocampal gyrus. In the coronal plane, the anterior limit of the EC is defined as 4 mm posterior to the ‘fronto-temporal junction’ (limen insulae). The posterior EC limit is demarcated by the anterior aspect of the lateral geniculate nucleus (LGN) defined by the recess of the LGN. Based on our validation study that used the FGE scan protocol, the lateral and medial EC boundaries are indicated by arrows in Fig. 3. The lateral (inferior) boundary of the EC in the more anterior sections is in the depth of the rhinal sulcus (panel C). In more posterior sections, the rhinal sulcus is often no longer recognizable, and the depth of the collateral sulcus is the lateral limit. This definition ensures the maximal inclusion of perirhinal and entorhinal cortex in the samples. For anterior levels, the medial EC boundary is in the sulcus semianularis between the convexities of the semilunar and ambient gyri. At more posterior levels with an uncal sulcus present, the medial limit is the unicus of the parahippocampal gyrus (panel B). This coincides with the grey matter found by extension of the white matter fibres of the angular bundle to the gyral surface (panel A). We measure on each coronal section the cortical ribbon from the medial to the lateral landmarks. The length is marked at the grey matter-CSF margin. The EC surface area is computed across slices.

Hippocampus.

In the human brain the hippocampus is well developed and occupies the floor of the temporal horn of the lateral ventricle. The length of the hippocampus is 4–5 cm, with a maximal width of about 2 cm and a maximal height of about 1.5 cm [134]. Our rules for the hippocampal volume have been validated at post-mortem, we have used them for many years, and they are similar to those of other investigators [135, 136]. Moreover, we have high levels of agreement between two independent raters for amygdala volume measurements (ICC = 0.93, n = 57) [137]. In anterior hippocampal levels, the superior border is defined by the alveus and temporal horn of the lateral ventricle (LV, see Fig. 4). Laterally, the hippocampus is covered by the alveus and it sits under the floor of the temporal horn. The medial border of the anterior pes (uncal [UN]) hippocampus abuts the ambient cistern. The uncal sulcus [two arrows] separates the inferior surface of the pes hippocampus from the subiculum of the parahippocampal gyrus (PG). At posterior levels, the smaller hippocampal body is bounded on its medial side by the lateral transverse fissure of Bichat (LTF) and the inferior boundary is the white matter of the parahippocampal gyrus. The superior boundary from lateral to medial is formed by the white matter of the temporal stem, tail of the caudate nucleus, lateral geniculate body (LGB) and pulvinar (PUL). As the boundary between the CA1 of Ammon’s horn and the subiculum [S] cannot be distinguished on MRI, we include subiculum in the hippocampal volume. The medial boundary of included subiculum is determined with reference to the supero-medial limit of the parahippocampal gyrus white matter. The most posterior limit of the hippocampus is the anterior crus of ascending fimbria-fornix.
Premorbid brain size correction.

To correct for head size variations across individuals we obtain an intracranial supratentorial volume using 2 mm thick sagittal images reformatted from the coronal FGE data [137, 138]. We trace the outline of the supratentorial compartment following the dural and tentorial surfaces on every third slice (mid-points every 6 mm). This estimate of premorbid brain size (prior to atrophy) is needed to statistically control for the relationship between regional volumes and brain size.

Regional boundary shift analysis.

Using this semi-automated protocol, relative atrophy in several 3D rectangular solids, size scaled by reference to x, y and z brain dimensions, are evaluated. The primary region examined was the MTL. The MTL region is centred over the hippocampus body (see Fig. 7 below) at the lateral geniculate level and extends both anteriorly to the amygdala to include pes hippocampi and posteriorly to the hippocampal tail to the level of the crus of the fornix [132].

Neuropsychological evaluations

A standard neuropsychological test battery was routinely administered to all subjects. The battery was developed to assess cognitive abilities that change with age, MCI and AD. The measures include the Guild Memory Test (Guild) [139], the Wechsler Memory Scale- Revised (WMS-R) [140], the NYU Computer Battery [141] and other supplemental tests. Multiple versions for many of these tests are routinely and systematically used over successive follow-ups.

Lumbar puncture and CSF collection

Patients arrived at the radiology suite at 9 AM after overnight fasting (12 h). A 15-cm³ volume of clear CSF is collected into three polypropylene tubes using a fine LP needle guided by fluoroscopy. Just prior to the LP, 35 cm³ of blood is collected. All CSF samples are kept on ice for a maximum of 1 h until centrifuged for 10 min at 450 g at 4 °C; 0.25 mL aliquots of the extracted plasma and CSF are stored in polypropylene tubes at −80 °C. After the LP, all patients are expected to rest for 2–3 h (to avoid headache).

CSF phosho-tau 231. CSF P-tau231 measurements were determined by Applied Neurosolutions (Vernon Hills, IL, USA) with a sandwich ELISA that detects tau phosphorylated at threonine 231 (P-tau231) in CSF. In this assay, tau is captured with two backbone-directed antibodies, tau-1 and CP-27. The captured tau is then detected by CP9, which is specific for P-tau231. The standard used in this assay is full-length recombinant human tau (441 aa) which is phosphorylated using a neuroblastoma cell extract in the presence of an ATP regeneration system. Additionally, the recombinant tau is reduced to a monomeric form to mimic the phospho-tau found in human CSF. The detection limits for these assays are 60 pg mL⁻¹ for total tau (INNOTEST hTau) and 9 pg mL⁻¹ for P-tau231 (MGC assay). The coefficients of variability for both assays ranged 5.5–11% (intra-assay) and 11.6–13.6% (inter-assay). Blind to clinical groups, we measure levels of T-tau and P-tau231 in batch mode.

CSF amyloid beta assays. Aβ 40 and Aβ42 ELISA. Plasma and CSF Aβ levels are blindly measured using monoclonal antibody 6E10 (specific to an epitope present on Aβ-16) and rabbit antisera to Aβ 40 and AB42, respectively, in a double antibody sandwich ELISA [85, 142]. The detection limit for Aβ40 and AB42 was 10 pg mL⁻¹. The coefficients of variability ranged 8–14% (intra-assay) and 10–18% (inter-assay).

CSF levels of rabbit antisera to CSF Aβ40 and Aβ42. Aβ32-40 and Aβ33-42 peptides synthesized commercially (Ana Spec, San Jose, CA, USA) were conjugated to keyhole limpet haemocyanin in PBS with 0.5% glutaraldehyde. Rabbits were immunized with the peptides and the specificity of antisera was examined in a sandwich ELISA. There was a strong response of rabbit antiserum to Aβ40 with 1 ng mL⁻¹ of Aβ40 but there was no detectable response with 10 ng mL⁻¹ of Aβ42. Similarly, antiserum to Aβ42 showed strong response with 1 ng mL⁻¹ of Aβ42 but showed no reaction with 10 ng mL⁻¹ of Aβ40. Western blot also showed that antiserum to Aβ40 was specific for Aβ40 and antiserum to Aβ42 was specific for Aβ42 [143]. Blind to clinical groups, the
levels of Aβ40 and Aβ42 in batch mode were measured.

Isoprostane (8,12-iso-iPF2α-VI). CSF samples were spiked with a fixed amount of internal standard (d4-8,12-iso-iPF2α-VI) and extracted on a C18 cartridge column. The eluate was purified by thin-layer chromatography and finally assayed by negative ion chemical ionization gas chromatography/mass spectrometry [107]. The coefficient of variation ranged 4–7% (intra-assay) and 4.5–6.5% (inter-assay). Blind to clinical groups, the isoprostane levels in batch mode were measured.

Results

Hippocampal size, a marker in MCI for future AD

For 15 years, we have studied longitudinal and post-mortem hippocampal imaging in normal (NL) ageing, MCI, AD and normal pressure hydrocephalus [110, 111, 116, 144–149]. In our early studies using qualitative techniques, we were the first to show that the hippocampal size reduction is found in MCI is a predictor of future AD [110, 111]. In more recent cross-sectional and prediction studies, logistic regression analyses showed that hippocampal volume was the only anatomical measurement to significantly classify MCI and elderly NL controls [150, 151]. When contrasting MCI and AD patients, inclusion of the fusiform gyrus volume in the model significantly improved the ability of the hippocampal volume to separate the groups [125]. These data provide strong evidence that AD-related volume losses are most readily detected in the hippocampus in MCI, and indicate that in predicting the transition to dementia, it is important to consider both hippocampal and lateral temporal lobe volume reductions.

Hippocampal size and declarative memory performance

In our cross-sectional studies of NL and MCI, when compared with a temporal lobe neocortical reference volume, the hippocampal volume showed an anatomically unique correlation to delayed verbal recall [150, 152]. In a 4-year follow-up study of 44 NL subjects, we observed that reduced delayed recall performance was predicted by a smaller baseline hippocampus [153] ($R^2 = 0.65$, $P < 0.001$). However, the diagnostic accuracy of the hippocampus to predict progressive memory decline was poor (sensitivity 63% and specificity 80%). Overall, these data suggested that the hippocampal volume was more useful in predicting decline at the MCI stage than at a stage of normal cognition. These studies led to the development of the EC work (below).

Neuropathological validation studies of the MRI hippocampal volume

We recently completed a neuropathological validation study of the MRI hippocampal volume [42]. Specifically, the hippocampal volumes from 16 AD and four NL were determined from hemispheric tissue sections and from comparably sliced post-mortem T1-weighted MRI scans. We made unbiased estimates of the number of hippocampal neurons. There was a strong correlation between the MRI and the histological-derived hippocampal volumes ($r = 0.97$, $P < 0.001$). Restricting the analysis to the AD group left the correlation unchanged ($r = 0.97$, $P < 0.001$). The difference in the hippocampal volumes between normal and AD groups was 42% for the MRI data, and 40% for the histology data after adjusting for tissue shrinkage during specimen processing. Moreover, both the histology-based and the MRI based hippocampal volume measurements were significantly associated with the number of hippocampal neurones, ($r = 0.91$, $P < 0.001$ and $r = 0.90$, $P < 0.01$, respectively, see Fig. 5).

EC glucose metabolism predicts conversion from NL to MCI

In a longitudinal FDG-PET study of NL (mean age = 72 years, range 60–80 years), the EC volume was precisely defined on MRI using our published criteria [121] and used to sample the co-registered PET scan. We reported [124] that only baseline EC METglu reductions accurately predicted decline to MCI (sensitivity 83%, $n = 12$, specificity 85%, $n = 13$), [$\chi^2 (1) = 20.8$, $P < 0.001$, odds ratio = 1.42, 95% CI = 1.08–1.88]. Those NL subjects who progressed to MCI also had, at follow-up, metabolism reductions in the EC, hippocampus and temporal neocortex. These FDG-PET data further support the value of the EC and hippocampus examinations in the characterization of the earliest brain changes associated with cognitive decline.
Neuropathological validation studies of the MRI entorhinal cortex surface area

We recently published the validation for the MRI measurement of the surface area of the EC [121]. The grey and white matter boundaries of the entorhinal and perirhinal cortices (EC) are poorly demarcated on MRI making cortical ribbon volume studies unreliable with standard MRI imaging protocols. Using post-mortem materials we validated an MRI image analysis method that avoids this problem by estimating the surface area of the EC (the sum across slices of the ribbon lengths multiplied by the slice thickness). We used serial 3 mm sections stained with cresyl violet to define three measurements: a histology-based EC volume, a histology-determined EC surface area, and EC surface area based on sulcal and gyral landmarks visible on MRI (EC-MRI). We studied 16 AD patients and four NL controls. The histology surface area was measured between the most medial boundary (pyriform cortex, or amygdala, or presubiculum, or parasubiculum) and the most lateral aspect (alternatively referred to as perirhinal, transentorhinal cortex, or Brodman’s area 35). Using the MRI landmark method, the surface area was bounded medially (superiorly) by the sulcus semianularis on anterior sections and the medial parahippocampal gyrus on posterior sections. The lateral (inferior) boundary, in the anterior sections was the depth of the rhinal sulcus and in the posterior sections, the depth of the collateral sulcus. The results showed that the volume of the EC was significantly related to both surface area measurements (histological $r = 0.94$, $P < 0.001$, and landmark $r = 0.91$, $P < 0.001$; see Fig. 6). Between the two groups, the following measures were significantly ($P < 0.01$) reduced in AD: volume 61%, histological surface area 49% and landmark surface area 45% (see appendix). In addition, an in vivo study of eight NL and eight mildly impaired AD patients was included in this publication. Significant between group differences of 27% were observed for the landmark EC method and 12% differences for the hippocampal volume. Individually, the EC correctly classified 100% of the controls and 87% of the AD group. By comparison, the hippocampus classified 88% of the controls and 75% of the AD patients. Multivariate logistic regression models showed that the EC was superior to the hippocampus in the diagnostic classification of the groups [$\chi^2 (1) = 22.2$, $P < 0.001$]. The landmark technique will be used in the proposed work.

Semi-automated medial temporal lobe atrophy predicts the conversion from NL to MCI

Forty-five NL elderly subjects were given a comprehensive battery of clinical and neuropsychological tests at baseline and at three follow-ups (2, 4 and 6 years [132] (see Table 1). Serial imaging was acquired twice with the same GE 1.5T MRI machine, at baseline and after 2 years. Brain atrophy rate was assessed using an automated procedure following the Boundary-Shift Algorithm (BSA) approach of Fox [154, 155]. Volumetric analyses were restricted to 3-D boxes that were applied to the baseline and the follow-up scans (Fig. 7). The MRI signal inten-
sity in the regions was normalized using regional (r) brain signal averages and converted to the brain volume using the partial volume decomposition method [156]. The BSA-r volume conversion assumes a fixed average contrast (3.13 : 1) between the brain parenchyma and the CSF as determined by a phantom study with our T1-weighted imaging sequence.

The atrophy at baseline and follow-up was defined as the ratio of the CSF volume to the total ROI volume. The annual percentage atrophy rate in each ROI was expressed as follow-up minus the baseline brain volume, divided by the baseline volume and by the time between the two MRI scans. In this study we examined the averaged right and left medial temporal lobe regions and a large region encompassing most of the brain. At the 2 year time point, seven of the 45 subjects showed objective evidence of cognitive decline. By the 6 year time point, a total of 13 had declined. The results in Table 2 show that both baseline and 2 year annualized rates of change for the MTL separated the declining and nondeclin-

### Table 1 Study subject (n = 45) grouping by clinical outcome

<table>
<thead>
<tr>
<th>Gender (male/female)</th>
<th>NL → NL (n = 32)</th>
<th>NL → MCI (n = 13)</th>
<th>P*</th>
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<tr>
<td></td>
<td>12/20</td>
<td>8/5</td>
<td>0.19</td>
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<td>Education (years)</td>
<td>15.7 ± 5.1</td>
<td>14.9 ± 1.9</td>
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<tr>
<td>Age (years)</td>
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<td>APOE type (E4+/E4–)</td>
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<td>3/9</td>
<td>0.33</td>
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<tr>
<td>GDS (baseline)</td>
<td>1.9 ± 0.4</td>
<td>2.0 ± 0.0</td>
<td>0.10</td>
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<tr>
<td>GDS (year 2)</td>
<td>2.1 ± 0.5</td>
<td>3.2 ± 0.9</td>
<td>0.001</td>
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<tr>
<td>MMSE (baseline)</td>
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<td>0.59</td>
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<tr>
<td>MMSE (year 2)</td>
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</tr>
<tr>
<td>Years between scans</td>
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<td>2.3 ± 1.1</td>
<td>0.49</td>
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<tr>
<td>Years to last examination</td>
<td>6.3 ± 1.0</td>
<td>5.3 ± 2.4</td>
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</table>

*Proportions were examined using Fisher’s exact test. t-Tests were used for comparison of mean values.

### Table 2 Distribution of cross-sectional and longitudinal atrophy

<table>
<thead>
<tr>
<th></th>
<th>NL → NL (n = 32)</th>
<th>NL → MCI (n = 13)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole brain % CSF (baseline)</td>
<td>20.4 ± 1.6</td>
<td>22.1 ± 1.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Whole brain % CSF (year 2)</td>
<td>21.4 ± 1.8</td>
<td>23.9 ± 1.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>MTL % CSF (baseline)</td>
<td>18.0 ± 3.5</td>
<td>21.5 ± 3.2</td>
<td>0.004</td>
</tr>
<tr>
<td>MTL % CSF (year 2)</td>
<td>18.4 ± 3.7</td>
<td>23.1 ± 3.3</td>
<td>0.0003</td>
</tr>
<tr>
<td>Whole brain annual atrophy rate</td>
<td>0.6 ± 0.4</td>
<td>1.3 ± 1.6</td>
<td>0.14</td>
</tr>
<tr>
<td>MTL annual atrophy rate</td>
<td>0.3 ± 0.4</td>
<td>0.9 ± 0.3</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*Tests for group differences were carried out using t-test.

Fig. 7 Overall head-size adjusted boxes placed over the hippocampal region to facilitate the automated calculation of atrophy change using the regional boundary shift technique.

### CSF and MRI biomarkers

In a 1-year follow-up study of NL elderly (n = 10, GDS = 1.6 ± 0.5, MMSE = 29.4 ± 0.7, age = 62.5 ± 9.2) and MCI (n = 8, GDS = 3.0 ± 0.4, MMSE = 28.5 ± 1.2, age = 69.8 ± 9.2) we examined lumbar CSF levels (pg mL⁻¹) of P-tau231, Aβ40, Aβ42 and isoprostane. At baseline, follow-up and longitudinally, we compared the MCI group and the NL control group. During the study, one MCI patient converted to AD.
Table 3 Diagnostic specificity and accuracy for significant univariate and combinations of cognitive, MRI and CSF measures (sensitivity ≥ 75%)

<table>
<thead>
<tr>
<th>Logistic regression model</th>
<th>Results at last step</th>
<th>specificity (%)</th>
<th>overall accuracy (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIP volume</td>
<td>80</td>
<td>78</td>
<td>≤ 0.01</td>
<td></td>
</tr>
<tr>
<td>P-tau 231 level</td>
<td>80</td>
<td>78</td>
<td>≤ 0.05</td>
<td></td>
</tr>
<tr>
<td>P-tau 231 load</td>
<td>70</td>
<td>78</td>
<td>≤ 0.05</td>
<td></td>
</tr>
<tr>
<td>Isoprostane</td>
<td>90</td>
<td>89</td>
<td>≤ 0.001</td>
<td></td>
</tr>
<tr>
<td>Aβ40</td>
<td>100</td>
<td>89</td>
<td>≤ 0.01</td>
<td></td>
</tr>
<tr>
<td>Paragraph immediate recall</td>
<td>90</td>
<td>89</td>
<td>≤ 0.01</td>
<td></td>
</tr>
<tr>
<td>Paragraph delayed recall</td>
<td>90</td>
<td>83</td>
<td>≤ 0.001</td>
<td></td>
</tr>
<tr>
<td>Longitudinal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-tau 231 level</td>
<td>50</td>
<td>61</td>
<td>≤ 0.05</td>
<td></td>
</tr>
<tr>
<td>P-tau 231 load</td>
<td>90</td>
<td>83</td>
<td>≤ 0.01</td>
<td></td>
</tr>
<tr>
<td>Isoprostane</td>
<td>90</td>
<td>89</td>
<td>≤ 0.05</td>
<td></td>
</tr>
</tbody>
</table>

CSF protein levels. P-tau 231. P-tau 231 levels were elevated in the MCI group at baseline (U = 14.0, P ≤ 0.05, n = 18) and at follow-up (U = 6.0, P ≤ 0.01, n = 18). This resulted in an overall classification accuracy of 78% at baseline [χ²(1) = 5.2, P ≤ 0.05, see Table 3]. In the longitudinal analysis, a small but significant change was observed.

Amyloid beta. CSF Aβ40 levels were elevated in the MCI group at baseline (U = 13.0, P ≤ 0.05, n = 18) and follow-up (U = 17.0, P ≤ 0.05, n = 18). This resulted in an overall classification accuracy of 89% at baseline [χ²(1) = 7.1, P ≤ 0.01]. After controlling for age, Aβ40 lost the baseline effect (P < 0.05) and kept the follow-up effect (U = 17, P < 0.05, n = 18). Aβ42 levels did not differ between the two groups at baseline. No longitudinal effects were observed for either Aβ40 or Aβ42.

Isoprostane. Isoprostane levels were elevated at both baseline (U = 3.0, P ≤ 0.001, n = 18) and follow-up (U = 6.0, P = 0.001, n = 18). This resulted in an overall classification accuracy of 83% at baseline [χ²(1) = 15.9, P ≤ 0.001]. After controlling for age, there was little change in the results. Moreover, a significant longitudinal change was seen in the MCI patients relative to control (U = 15.0, P ≤ 0.05, n = 18). This resulted in an overall classification accuracy of 89% for the delta [χ²(1) = 4.0, P ≤ 0.05]. The post hoc examination showed a significant isoprostane increase restricted to the MCI group (Wilcoxon signed ranks test Z = −2.38, P ≤ 0.05, n = 18).

MRI volume data. Hippocampal volume. The hippocampal volume ratio was reduced in MCI by 19% at baseline [t(16) = 3.4, P ≤ 0.01] and by 21% at follow-up [t(16) = 3.5, P ≤ 0.01]. This resulted in an overall classification accuracy of 78% at baseline [χ²(1) = 9.3, P ≤ 0.01]. After controlling for age, there was little change in the results. No longitudinal hippocampal volume change was observed.

Dilution correction-protein load. To correct for the dilution of tau in the ventricular compartment, typically enlarged in AD (see central white area of Fig. 8) we estimated ventricular CSF P-tau231 load (ng) by multiplying the P-tau231 level (pg mL⁻¹) by the ventricular volume (mL) and dividing by 1000 [99]. P-tau231 loads were elevated in the MCI group at baseline (Mann Whitney U = 11, P < 0.01, n = 18) and at follow-up (U = 6, P = 0.001, n = 18, see Table 4). In the longitudinal design, we observed a significant group by time interaction for the annualized P-tau231 load (U = 12.0, P < 0.05, n = 18). Follow-up examination showed a significant P-tau231 load increase in the MCI group (Wilcoxon signed ranks test Z = −2.1, P < 0.05, n = 8). No longitudinal load effects were observed for the controls.

We directly compared annualized longitudinal P-tau231 load and P-tau231 level changes, using two logistic regression models with reversed orders of entry, in the prediction of diagnostic group. At the first entry steps, both ΔP-tau231 level and ΔP-tau231 load significantly predicted group membership [χ²(1) = 4.45, P ≤ 0.05 and χ²(1) = 9.08, P ≤ 0.01 respectively. Comparing the second entry steps, the ΔP-tau231 load uniquely increased the variance explained by the ΔP-tau231 level [R² change = 0.23, F(1,15) = 5.8, P ≤ 0.05].

Longitudinal correlation in MCI of AD-related CSF proteins with hippocampal volume

Because of the known inverse relationships at post-mortem between the hippocampal volume and tau pathology and between brain Aβ42 load and hippocampal volume reductions, we examined the hypothesis that these relationships could be inferred in vivo using MRI and CSF. At baseline, for the entire
sample \((n = 18)\), a significant inverse relationship was found between the hippocampus volume and the P-tau\textsubscript{231} level \((r = -0.47, \ P < 0.05)\). After controlling for age, there was little change in the results. In the 2 time-point longitudinal design, the MCI group, \(n = 8\), showed a strong inverse relationship between hippocampal volume reductions and elevations in P-tau\textsubscript{231} level \((r = -0.80, \ P < 0.05, \text{Fig. 9a})\). Also for MCI, the reduction in CSF A\textsubscript{\beta}42 levels showed a strong positive relationship to the reduction in the hippocampal volume \((r = 0.75, \ P < 0.05, \text{Fig. 9b})\). Moreover, in the MCI group the longitudinal changes in A\textsubscript{\beta}42 and P-tau\textsubscript{231} showed a trend for the expected inverse relationship \((r = -0.56, \ P = 0.07, \text{one-tail})\).

### Conclusions

Both neuropathology and neuroimaging studies converge on observations that hippocampal formation pathology is an early feature of AD. Over the past 15 years, numerous studies have identified hippocampal atrophy as a predictor of the decline from MCI to AD. It also appears that the EC of the hippocampal formation has value potentially as an even earlier marker of brain AD. Specifically, EC changes may actually precede hippocampal changes in NL at risk for future MCI. Additional work is required to better understand the temporal relationships between EC and hippocampal changes as well as the optimal image acquisition and image measurement strategies to use. Nevertheless, solely imaging the structural defects or the patterns of glucose metabolism reductions are not likely to be diagnostically specific indicators for AD. Recent studies show that neither the hippocampal atrophy nor its longitudinal rate of change are useful in

### Table 4 Baseline and follow-up MRI and CSF measures (mean ± SD, group % differences and \(P\))

<table>
<thead>
<tr>
<th>Measure</th>
<th>Baseline</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NL (n = 20)</td>
<td>MCI (n = 8)</td>
</tr>
<tr>
<td>VV (cm\textsuperscript{3})</td>
<td>29.7 ± 9.8</td>
<td>42.3 ± 15.2</td>
</tr>
<tr>
<td>P-tau\textsubscript{231} level (pg mL\textsuperscript{-1})</td>
<td>160.0 ± 190.4</td>
<td>534.7 ± 451.8</td>
</tr>
<tr>
<td>P-tau\textsubscript{231} load (ng)</td>
<td>5.6 ± 9.1</td>
<td>19.7 ± 15.3</td>
</tr>
<tr>
<td>A\textsubscript{\beta}40 level (pg mL\textsuperscript{-1})</td>
<td>9596 ± 2317</td>
<td>12393 ± 2389</td>
</tr>
<tr>
<td>A\textsubscript{\beta}42 level (pg mL\textsuperscript{-1})</td>
<td>1015.1 ± 448.3</td>
<td>943.7 ± 486.7</td>
</tr>
<tr>
<td>IP level (pg mL\textsuperscript{-1})</td>
<td>28.0 ± 6.36</td>
<td>47.9 ± 11.3</td>
</tr>
</tbody>
</table>

VV, ventricle volume; load = ng, IP = isoprostane; % Diff, cross-sectional differences: (MCI–NL)/NL. Cross-sectional effects: \(^aP \leq 0.05; \ ^bP \leq 0.01\). Annualized longitudinal effects: + = \(P \leq 0.05\).

### Fig. 8 Ventricular anatomy highlighted in a control and in an AD patient.

![Ventricular anatomy highlighted in a control and in an AD patient.](image)

### Fig. 9 Relationships between longitudinal changes in hippocampal volume and changes in CSF levels of (a) P-tau\textsubscript{231} and (b) amyloid beta 1-42.

![Relationships between longitudinal changes in hippocampal volume and changes in CSF levels of (a) P-tau\textsubscript{231} and (b) amyloid beta 1-42.](image)

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separating fronto-temporal dementia from AD. CSF P-tau231 in combination with Aβ4X studies offers the promise of a specific in vivo diagnosis of AD. By combining MRI and CSF measures, the preliminary results suggest that both an early sensitive and specific diagnosis for AD is a possibility. Studies by Hampel suggest that elevated P-tau231 measurements may be specific for AD and this important observation requires replication as well as extension to MCI.

In the MCI stage of AD, both the hippocampal volume and the CSF P-tau231 concentration predict future AD. However, the magnitude of the agreement between these predictors of conversion has not been reported. Our preliminary data suggest that they are related and potential indicators of a localized process. In our 1-year longitudinal MRI and CSF data, the changes in the measures were correlated in the MCI group and the combined use of these markers contributed to improving the diagnostic accuracy for MCI and normal control groups. However, because our study did not yet yield sufficient numbers of decliners we are not able to report on conversion questions.

Another promising avenue for clinical research concerns the dilution or clearance of brain-derived proteins from the CSF. Numerous studies point to abnormal CSF clearance as part of the late-onset AD syndrome. Although there is minimal information regarding age-related and/or AD related changes in CSF turnover, there is ample speculation that reduced clearance could affect the accuracy of the LP measured concentrations of P-tau231 and Aβ4X (which are presumed to reflect the rate of delivery and or egress from the CSF pool) and possibly even influence the course of the neural degeneration. Our recently published results suggest that correction for the dilution of the biomarker in the enlarged CSF pool typically found in AD contributes to detecting longitudinal concentration changes in MCI patients. Clearly additional studies on CSF flow and clearance dynamics are needed.

In conclusion, the combined use of MRI and CSF diagnostic measures for AD have the promise to improve the early and specific diagnosis of AD as well as to improve our understanding of the course of AD on both brain and behaviour. Such information will contribute to improved selection of study subjects in clinical trials and for improved monitoring of treatment effects.

Conflict of interest statement

No conflict of interest was declared.

Acknowledgements

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References

10 Braak H. On areas of transition between entorhinal allocortex and temporal isocortex in the human brain. Normal


BIOMARKERS IN THE EARLY DIAGNOSIS OF AD


Correspondence: Dr Mony de Leon, Department of Psychiatry, Center for Brain Health, NYU School of Medicine, 560 First Avenue, New York, NY 10016, USA.
(fax: +1 212 263 3270; e-mail: mony.deleon@med.nyu.edu).
CSF biomarkers for mild cognitive impairment

K. BLENNOW
From the Department of Clinical Neuroscience, Section of Experimental Neuroscience, The Sahlgrenska Academy at Göteborg University, Göteborg, Sweden


A correct clinical diagnosis of Alzheimer’s disease (AD) early in the course of the disease is of importance to initiate symptomatic treatment with acetylcholine esterase inhibitors, and will be even more important when disease-arresting drugs, such as β-sheet breakers or γ-secretase inhibitors, will reach the clinic. However, there is no clinical method to determine if a patient with mild cognitive impairment (MCI) has incipient AD, i.e. will progress to AD with dementia, or have a benign form of MCI without progression. Thus, there is a great clinical need for diagnostic biomarkers to identify incipient AD in MCI cases. Three cerebrospinal fluid (CSF) biomarkers: total-tau (T-τ), phospho-tau (P-τ) and the 42 amino acid form of β-amyloid (Aβ42) have been evaluated in numerous scientific papers. These CSF markers have high sensitivity to differentiate early and incipient AD from normal ageing, depression, alcohol dementia and Parkinson’s disease, but lower specificity against other dementias, such as frontotemporal and Lewy body dementia. However, if the CSF biomarkers are used in the right clinical context, i.e. together with the cumulative information from the clinical examination, standard laboratory tests and brain-imaging techniques [single photon emission tomography (SPECT) and magnetic resonance tomography (MRT) scans], they may have a role in the clinical evaluation of MCI cases.

Keywords: Alzheimer’s disease, β-amyloid, biomarkers, cerebrospinal fluid, tau proteins, diagnosis.

Alzheimer’s disease (AD) is the most common form of dementia. There are exceptional cases with familial (autosomal dominant) forms of AD, but the large majority of patients have a sporadic form of the disease [1]. Microscopically, AD is characterized by degeneration of the neurones and their synapses, and the presence of extensive amounts of senile plaques and neurofibrillary tangles [2]. The degenerative process probably starts 20–30 years before the clinical onset of AD [3]. During this preclinical period, the number of plaques and tangles increase, and at a certain threshold the first symptoms, most often impairment of episodic memory, appear.

According to the clinical tradition, AD cannot be diagnosed until dementia is present. However, in recent years, the clinical phase of AD with mild memory impairment, but without overt dementia, has attained increased attention in the medical community. Patients with mild memory impairment, but without dementia, are often diagnosed as having mild cognitive impairment (MCI). To make a diagnosis of MCI, memory disturbances should be ‘verified’ by objective measures adjusted for age and education [4]. However, although many patients with MCI have incipient AD, i.e. have early AD pathology and will progress to AD with dementia,
others probably have a benign form of MCI as part of the normal ageing process [5]. In other patients, cerebrovascular pathology (e.g. infarcts, white-matter lesions) may contribute to the symptoms. Thus, MCI is an aetiologically heterogeneous disorder.

The conversion rate from MCI to AD with dementia is probably around 15% per year [5]. Research efforts have been directed to find methods to identify which patients with MCI that will progress to AD and which will not. In this paper, the potential of cerebrospinal fluid (CSF) analyses to identify incipient AD in MCI cases is reviewed.

The need of diagnostic markers for AD and MCI

The introduction of effective symptomatic treatment of AD with acetylcholine esterase (AChE) inhibitors has highlighted the importance of early and accurate diagnosis of AD. The increased general knowledge on AD in the population, and the awareness of the possibilities for drug treatment, has also made patients seek medical advice early in the disease, in the MCI phase, when the characteristic clinical picture of dementia with parietal lobe symptoms [6] has not yet developed.

In the MCI stage, there is no clinical method to determine which patients that will progress to AD with dementia, except for a very long clinical follow-up. Thus, there is a great clinical need for diagnostic instruments to identify incipient AD in MCI cases. This need will be even larger when new disease-arresting drugs, such as β-sheet breakers or β- and γ-secretase inhibitors, reach the clinic. Such compounds will probably be most effective in the earlier stages of the disease, before neurodegeneration is too severe and widespread. Further, although the NINCDS-ADRDA criteria [7] have a relatively high accuracy rate, about 80–90% [8, 9], these figures come from specialized expert research academic centres, and are based on patients in later stages of the disease which were followed longitudinally for several years before autopsy. There are few data on the diagnostic accuracy of incipient AD in MCI cases, but it is reasonable to that it will probably be considerably lower.

The basis for CSF markers for AD and MCI

The CSF is in direct contact with the extracellular space of the brain, and thus biochemical changes in the brain are reflected in the CSF. Since AD pathology is restricted to the brain, CSF is an obvious source of biomarkers for AD. Candidate biomarkers for AD should be a protein, or molecule, reflect the central pathogenic processes in the brain, i.e. the neuronal degeneration, the aggregation of β-amyloid (Aβ) with subsequent deposition in plaques, and the hyperphosphorylation of tau with subsequent formation of tangles. Hitherto, three CSF biomarkers, total-tau (T-τ), Aβ isoforms, in particular the 42 amino acid variant (Aβ42), and different phospho-tau (P-τ) epitopes, have been found to have the highest diagnostic potential.

Tau protein

τ- Protein is a microtubule-associated protein located in the neuronal axons. Because of alternative splicing of τ-mRNA, there are six isoforms ranging in size from 352 to 441 amino acids, with molecular weights of approximately 50–65 kDa [10]. A schematic drawing of the τ isoforms is shown in Fig. 1. Tau binds to tubulin in the microtubules in the axons, thereby promoting microtubule assembly and stability [10]. In AD, an abnormally hyperphosphorylated form of tau is the principal component of the paired helical filaments (PHFs), which make up the neurofibrillary tangles, neuropil threads and senile plaque neurites [11]. Because of the hyperphosphorylation, tau also loses its ability to bind to the microtubules and to stimulate their assembly [12]. Using different techniques, more than 30 phosphorylation sites have been described on tau in the brain [10]. A schematic drawing of tau with phosphorylation sites is shown in Fig. 2.

CSF total tau

The first report on CSF T-τ as a biomarker for AD was published in 1993. In this paper, an enzyme-linked immunosorbent assay (ELISA) with a polyclonal reporter antibody was used [13]. Subsequent studies used ELISA methods based on monoclonal antibodies that detect all isoforms of τ-independent of phosphorylation state of tau [14, 15]. An increase in CSF T-τ in AD has consistently been found in numerous studies, with a mean of 3.2 times higher levels in AD than in controls [16].

In acute conditions such as stroke, there is a marked transient increase in CSF T-τ that shows a
correlation with computerized tomography measurements of infarct size [17]. Further, the level of increase in CSF T-τ is highest in disorders with the most intense neuronal degeneration, such as Creutzfeldt-Jakob disease [18], whilst a moderate to marked increase is found in AD, with less intense degeneration [19], and normal levels are found in patients with depression, with no or limited degeneration [14]. Thus, the CSF level of T-τ probably reflects the intensity of the neuronal damage and degeneration.

**CSF phospho-tau**

The ELISA methods have been developed for several different phosphorylated epitopes of tau, including threonine 181 + 231 [14], threonine 181 [20], threonine 231 + serine 235 [21], serine 199 [21], threonine 231 [22], and serine 396 + 404 [23]. A marked increased level of P-τ in CSF in AD has been found using all these different ELISA methods.

In contrast to T-τ, there is no change in CSF P-τ after acute stroke [24]. Further, CSF P-τ levels are normal in Creutzfeldt-Jakob disease, despite a very marked increase in T-τ [25]. These indirect evidence suggest that CSF P-τ is not simply a marker for neuronal damage, like CSF T-τ, but that it specifically reflects the phosphorylation state of tau.

**β-Amyloid isoforms**

β-Amyloid (Aβ or β/A4 protein) is the main protein constituent of plaques [26]. Aβ is constitutively generated by proteolytic cleavage of its precursor, the amyloid precursor protein (APP) [27]. APP is a single membrane-spanning protein with a large ectodomain and a smaller cytoplasmic tail [28]; a schematic drawing of APP is given in Fig. 3.

Aβ is metabolized along two pathways (Fig. 3). In the nonamyloidogenic pathway, APP is cleaved within the Aβ domain (between Aβ16 and Aβ17) by a protease referred to as z-secretase. This cleavage results in the release of a large N-terminal derivative called z-secretase-cleaved soluble APP (z-sAPP). Recent studies have shown that two enzymes of the ADAM family of disintegrin metalloproteases, tumour necrosis factor-α converting enzyme (TACE) [29] and ADAM10 [30] have z-secretase activity.

Importantly, cleavage of APP by z-secretase within the Aβ domain precludes generation of Aβ. Instead, the 83 amino acid C-terminal fragment (CTF) of APP (C83) is cleaved by γ-secretase releasing a shorter peptide called p3 (Fig. 3). Recent
studies have shown that the presenilins constitute the catalytic subunit of the γ-secretase [31, 32], whilst other membrane proteins, including nicas-trin, APH1a, APH1b, and PEN2, regulates γ-secretase cleavage [33–35], and together with presenilin forms a functional complex, the γ-secretase complex, responsible for cleavage of the APP-CTF.

In the second, amyloidogenic pathway, APP is cleaved at the N-terminus after position 671 by a protease referred to as β-secretase, later identified as β-site APP-cleaving enzyme (BACE) [36]. This cleavage results in the release of a large N-terminal derivative called β-secretase-cleaved soluble APP (β-sAPP) (Fig. 3). In the second step, the 99 amino acid CTF of APP is cleaved by the γ-secretase complex releasing free β-amyloid. In the next step, the 83 amino acid C-terminal fragment (CTF) of APP (C83) is cleaved by the γ-secretase complex releasing a shorter peptide called p3. In the amyloidogenic pathway, APP is first cleaved by β-secretase, resulting in the release of β-secretase-cleaved soluble APP (β-sAPP). In the second step, the 99 amino acid CTF of APP is cleaved by the γ-secretase complex releasing free β-amyloid.

CSF total Aβ

Initial reports on Aβ in CSF used ELISA methods that did not discriminate between different Aβ isoforms, i.e. the CSF level of total Aβ was measured. Although some studies found a slight decrease in the CSF level of total Aβ in AD [37–39], there was a large overlap between AD patients and controls, and other researchers found no change in CSF total Aβ in AD [40–42].

Aβ42 and Aβ40

An important change came after the discovery that there are several C-terminal forms of Aβ (Fig. 4). Focus was set on the longer form ending at Ala-42 (Aβ42). The rationale for this was that Aβ42 was found to aggregate more rapidly than Aβ40 [43], and to be the initial form of Aβ deposited in diffuse plaques [44, 45], and also the predominating form of Aβ in senile plaques [44, 45]. These data made it logical to develop ELISA methods specific for Aβ42 [41, 46].

A decrease in CSF-Aβ42 to about 40–50% of control levels has been found in AD in several papers [16]. The reduced CSF level of Aβ42 in AD was initially hypothesized to be caused by the deposition of Aβ42 in plaques, with lower levels diffusing to CSF [41]. However, subsequent studies found a marked reduction in CSF-Aβ42 also in disorders without Aβ plaques, such as Creutzfeldt-Jakob disease [47], amyotrophic lateral sclerosis [48], and multiple system atrophy [49]. These findings make the relation between low CSF-Aβ42 and deposition of Aβ in plaque questionable. On the contrary, a recent autopsy study found strong correlations between low Aβ42 in ventricular CSF and high number of plaques in the neocortex and
hippocampus [50]. Thus, the reduction in CSF-\(\beta\)42 in AD may at least partly be due to a deposition of \(\beta\) in plaques.

The importance of assaying specific \(\beta\)-isoforms is shown by the finding that, in contrast to the marked reduction of CSF-\(\beta\)42 in AD, there is no change in CSF-\(\beta\)40 [51–55]. As a consequence, a marked decrease in the ratio of \(\beta\)42/\(\beta\)40 (or increase in the ratio of \(\beta\)40/\(\beta\)42) in CSF has been found in AD in several papers [52–54]. The reduction in the CSF-\(\beta\)42/\(\beta\)40 ratio was more marked than the reduction in CSF-\(\beta\)42 [52–54]. Future studies are needed to determine if the CSF-\(\beta\)42/\(\beta\)40 ratio has a larger diagnostic potential than CSF-\(\beta\)42 alone. Preliminary data suggest that the CSF-\(\beta\)42/\(\beta\)40 ratio may be of special use in early AD and MCI cases.

**Other \(\beta\) species**

Studies using mass spectrometry have found that there is a heterogeneous set of \(\beta\) peptides in CSF [56]. Also using urea-based sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western immunoblot [57], it is possible to separate several C-terminally truncated \(\beta\) peptides, including \(\beta\)1–37, \(\beta\)1–38, \(\beta\)1–39, \(\beta\)1–40 and \(\beta\)1–42 in human CSF. In contrast to what was previously believed, the most abundant of these peptides is \(\beta\)1–40, followed by \(\beta\)1–38 and \(\beta\)1–42 [57]. In AD, increased CSF levels of both \(\beta\)1–40 and \(\beta\)1–38 were found, together with a decrease in \(\beta\)1–42 [57]. Similar data has been found using surface-enhanced laser desorption/ionization (SELDI) time-of-flight (TOF) mass spectrometry [58]. Further studies are needed to examine to diagnostic potential of these \(\beta\) species.

**Diagnostic performance of current CSF markers for AD**

The diagnostic performance of the three most extensively studied biomarkers (T-\(\tau\), \(\beta\)42 and P-\(\tau\)) is reviewed below.

**CSF T-\(\tau\)**

For the most commonly used ELISA method [14], sensitivity and specificity figures are available from more than 35 studies, including more than 2500 AD patients and 1400 controls [for review see 59]. At a specificity level of 90%, the mean sensitivity to discriminate AD from nondemented aged individuals is above 80% [for review see 59].

Besides in aged nondemented individuals, normal CSF T-\(\tau\) is found in depression, alcoholic dementia, and in chronic neurological disorders such as Parkinson’s disease and progressive supranuclear palsy [14, 19, 53, 60–63]. Thus, CSF T-\(\tau\) has a clinical diagnostic value in the differentiation between AD and these important, and often difficult, differential diagnoses.

As the CSF level of T-\(\tau\) reflects neuronal and axonal degeneration, high levels will be found in all disorders with neuronal degeneration or damage, such as stroke [17] and Creutzfeldt-Jakob disease [18]. Of other dementias, high CSF T-\(\tau\) has been found in vascular dementia (VAD) in some studies [14, 19, 63], whilst only in occasional VAD cases in other studies [15, 53, 64]. These discrepant findings may depend on the patient characteristic and diagnostic criteria used. High T-\(\tau\) in clinically diagnosed VAD cases may be caused by the presence of concomitant AD pathology, which is a frequent finding at autopsy [9, 65], which also was suggested in a CSF study with

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**Fig. 4** Schematic drawing of different C-terminally truncated \(\beta\)-amyloid (\(\beta\)) isoforms present in cerebrospinal fluid.
longitudinal MRT scans in VAD cases [19]. Similarly, a mild-to-moderate increase in CSF T-τ has been found in frontotemporal dementia (FTD) in some [14, 66], but not in all [20, 67] studies.

**CSF P-τ**

An increased level of P-τ in CSF in AD has been found using all the different ELISA methods [for review see 16]. Today, 11 different studies presenting sensitivity and specificity figures have been published, including approximately 700 AD patients and 350 controls [for review see 59]. At a specificity level of 92%, the mean sensitivity of CSF P-τ to discriminate between AD and nondemented aged individuals is around 80% [59].

Interestingly, the specificity of CSF P-τ to differentiate AD from other dementias seems to be higher than for T-τ and Aβ42; increased P-τ has only been found in AD, whilst normal P-τ levels is not only found in psychiatric disorders such as depression [14, 68] and in chronic neurological disorders such as amyotrophic lateral sclerosis and Parkinson’s disease [14, 48, 67], but also in other dementia disorders such as VAD, FTD, and Lewy body dementia [20, 48, 67, 69–72]. Thus, addition of P-τ will increase the specificity of CSF biomarkers in the discrimination between AD and other dementias.

There is a relatively large variation in sensitivity and specificity figures of P-τ between different studies. Thus, further large studies are needed to determine if there is a difference in sensitivity and specificity figures for the different ELISA methods for P-τ. In the first study comparing the diagnostic performance of P-τ<sub>181</sub>, P-τ<sub>199</sub> and P-τ<sub>231</sub> in the same patient material, including cases with AD, DLB, FTD, VAD, and other neurological disorders, all three P-T-τ assays performed equally well in the discrimination of AD from nondemented controls [72]. However, group separation was maximized between AD and FTD using P-τ<sub>231</sub> and between AD and DLB using P-τ<sub>181</sub> [72]. Thus, differences in the phosphorylation of specific τ-epitopes between dementia disorders may be reflected in the CSF level of the corresponding P-τ variant.

**Aβ42**

For the most commonly used method, the ‘Innogetnetics ELISA’ [46, 73], sensitivity and specificity figures are available from 13 different studies, including approximately 600 AD patients and 450 controls [for review see 59]. At a specificity level of 90%, the mean sensitivity of CSF-Aβ42 to discriminate between AD and normal ageing is above 85% [59].

Besides in nondemented aged individuals, normal CSF-Aβ42 is found in psychiatric disorders-like depression, and in chronic neurological disorders such as Parkinson’s disease, and progressive supranuclear palsy [48, 49, 63, 74]. Therefore, CSF-Aβ42 helps in the clinical differentiation between AD and these important, and often difficult, differential diagnoses. However, data on the performance of CSF-Aβ42 in the discrimination between AD and other dementias and neurological disorders are relatively limited. Moderately low levels are found in Lewy body dementia [73, 74], a disorder also characterized by the presence of senile plaques, and a mild-to-moderate decrease in CSF-Aβ42 is found in a percentage of patients with FTD and VAD [48, 60, 63].

**Use of CSF markers to identify incipient AD**

Several studies have examined the performance of CSF markers in AD cases with mild dementia, i.e. with Mini-Mental State Examination (MMSE) scores above 23–25. Also is this phase of the disease, high CSF T-τ and P-τ, and low CSF-Aβ42 are present, with sensitivity figures similar to those found in later stages of the disease [19, 60, 73, 75–78].

In recent years, research efforts have aimed at evaluating the performance of CSF markers in MCI cases that during a clinical follow-up period of 1–2 years developed AD. All studies have found high CSF T-τ and P-τ, and low CSF-Aβ42 already in this early phase of AD, with sensitivity figures similar to, or slightly lower than, those found in AD cases with dementia [79–86].

Some studies have evaluated the diagnostic value of CSF biomarkers to identify MCI cases that later will progress to AD with dementia, i.e. have ‘preclinical’ or incipient AD. A pitfall with these studies is that since only around 15% of MCI cases progress to AD each year [5], a very extensive follow-up period (>5 years) would be needed to be certain that MCI patients will not develop dementia, i.e. have stable MCI. In the first study using this approach, high CSF T-τ was found to discriminate MCI patients that later progressed to AD from those...
that did not progress with 90% sensitivity and 100% specificity [87]. A recent study also found high CSF T-\(\tau\) and low CSF-A\(\beta\)42 in 90% of MCI cases that later progressed to AD with dementia when compared with 10% of stable MCI cases [83]. Similarly, a marked increase in CSF P-\(\tau\) was found in MCI cases that at follow-up had progressed to AD compared with stable MCI cases [88]. Although further longitudinal studies are needed, these data suggest that CSF markers are positive very early in the disease process in AD, and may be of clinical value to differentiate MCI cases with incipient AD, which will progress to AD, from benign MCI cases. Indeed, a recent population-based study also found that reduced CSF-A\(\beta\)42 is present in asymptomatic elderly that during a 3-year follow-up period developed dementia [89].

CSF markers in clinical practice

Diagnostic value of CSF markers for AD

Numerous studies have evaluated the diagnostic value of CSF markers for AD cases, finding high CSF T-\(\tau\) and P-\(\tau\), and low CSF-A\(\beta\)42, with sensitivity figures around 85–90%, but with lower specificity against other dementia disorders. A limitation with these studies is that almost all data are based on clinically diagnosed patients. This brings in a risk of circular evidence, i.e. the diagnostic performance of CSF markers will not be higher than the accuracy of the clinical diagnostic criteria used.

It is not possible on clinical grounds to exclude that patients with other dementias have concomitant AD pathology, or are mis-diagnosed AD cases [8, 9]. It is also known that asymptomatic aged control subjects may harbour presymptomatic AD lesions in their brains [90]. This makes it difficult to judge whether suboptimal sensitivity and specificity figures are due to shortcomings of the CSF markers or of the clinical patient and control materials. Indeed, even after exclusion of five clinically mis-diagnosed cases, AD cases confirmed by autopsy showed a tendency for lower A\(\beta\)42 and higher T-\(\tau\): 170 vs. 187 pg mL\(^{-1}\) and 677 vs. 559 pg mL\(^{-1}\), respectively [91].

Lumbar puncture in patients with suspected dementia

Lumbar puncture (LP) is often avoided because of fear of postlumbar puncture headache (PLPH). However, the incidence of PLPH is clearly age-related and much more uncommon in individuals over 60 years of age than in younger individuals [92, 93], and even lower in patients admitted for cognitive symptoms, below 2% [80, 93]. Thus, LP can safely be introduced in clinical routine at the dementia clinic.

Confounding factors and laboratory procedures

Potential preanalytical and biological confounding factors for CSF analyses include, for example, possible concentration gradients along the spinal cord, which is present for neurotransmitters [94], but also for some proteins [95], presence of the protein in plasma and passage over the blood–brain barrier, as for neuronal thread protein [96], influence of CSF haemorrhage [97], and degradation or loss of the protein in vitro after the CSF tap [98]. For CSF T-\(\tau\), P-\(\tau\) and A\(\beta\)42, the only preanalytical confounding factor is that these proteins tend to stick to the walls of test tubes made of glass and hard plastic, resulting in falsely low levels [73]. Therefore, it is important to tap CSF into nonabsorbing test tubes made of polypropylene. The CSF sample can be sent to the laboratory at room temperature, since storage for up to 3 days does not influence levels of these proteins. Reproducibility of ELISA results are confirmed by analysing two internal controls (stored in aliquots at \(-80\) ºC), on each ELISA plate. Using this procedure, the analytical variation for these ELISA methods is adequate, around 10–15% [80].

Performance of CSF markers in clinical routine

The diagnostic potential of the CSF markers have been evaluated in numerous scientific paper, but almost all have been performed in research settings, with selected patient samples and CSF analyses run on one occasion, i.e. under conditions providing figures on the optimal performance of the markers. In two studies, the CSF markers have been evaluated on prospective patient samples, with ELISA assays run each week in clinical neurochemical routine, which may give figures closer to their true performance as diagnostic markers [19, 80]. The ability of CSF T-\(\tau\) [19] and the combination of CSF T-\(\tau\) and A\(\beta\)42 [80] to differentiate AD from normal ageing, depression
and Parkinson’s disease was in the same range as in the other studies, but again the specificity against other dementias (VAD and Lewy body dementia) was suboptimal.

Therefore, like in other areas of medicine, diagnostic biomarkers should not be used as isolated tests. For example, the clinical diagnosis of myocardial infarction is based on the combined information from the clinical examination, electrocardiogram, and biomarkers (e.g. troponine T). Similarly, we propose that the clinical diagnosis of AD should be based on cumulative information from the clinical examination, brain-imaging techniques (e.g. SPECT and MRT scans), and CSF biochemical markers.

Today, patients with cognitive disturbances seek medical advice at a very early stage, often when mild memory impairment is the only objective symptom. In these MCI cases, there is no clinical method to determine which patients have incipient AD and which have benign MCI. After the clinical examination and standard auxiliary investigations, secondary dementias (e.g. hypothyreosis and subdural haematoma) and often also dementias with differing history, symptoms and findings on brain imaging (e.g. FTD, VAD) can be identified. Functional brain imaging (SPECT) may be of value in the differentiation of AD from FTD and VAD [99, 100]. A common ‘final question’ is often whether a patient with MCI has incipient AD or benign MCI as part of the normal ageing process. Other possible differential diagnoses that may be hard to differentiate from incipient AD are depression and variants thereof and alcohol-related cognitive dysfunction. Although more studies are needed to determine the diagnostic performance of CSF markers to identify incipient and early AD, we suggest that CSF markers have a clinical potential help to resolve this diagnostic challenge. Early diagnosis of AD is not only of importance to be able to initiate symptomatic treatment with AChE inhibitors, but will be the basis for initiation of treatment with drugs aimed at slowing down or arresting the degenerative process, such as γ-secretase inhibitors, if these prove to affect AD pathology and to have a clinical effect.

Conflict of interest statement

No conflict of interest was declared.

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Correspondence: K. Blennow, Department of Clinical Neuroscience, Section of Experimental Neuroscience, The Sahlgrenska Academy at Göteborg University, Göteborg, Sweden. (fax: +46 31 3432426; e-mail: kaj.blennow@neuro.gu.se).
Prospects of genetic research of mild cognitive impairment

C. M. VAN DUIJN
Genetic Epidemiology Unit, Department of Epidemiology and Biostatistics, Erasmus MC, Rotterdam, The Netherlands


Mild cognitive impairment (MCI) is a common problem in the elderly. Genetic research may yield valuable clues to improve the diagnostic and prognostic tools for MCI. As the majority of patients progress into Alzheimer’s disease (AD) and other forms of dementia, the genetics of MCI cannot be separated from the genetics of AD and dementia in general. Follow-up studies of carriers of mutations underlying AD may yield valuable clues for the development of new diagnostic tools for MCI. In particular, large-scale studies of carriers of the apolipoprotein E 4 allele may still be of interest. Our knowledge of the genes involved in MCI is still very limited. In addition, we need powerful and carefully designed candidate gene studies aiming to discover new genes involved in the risk and progression of MCI. Although the genetics of MCI will be difficult to disentangle, there is ample opportunity to improve research of the genes involved.

Keywords: genetics and dementia, mild cognitive impairment.

Introduction

Mild cognitive impairment (MCI) is a trait characterized by memory complaints in subjects with normal global cognitive function (see earlier this issue). In a large number of patients, MCI progresses into Alzheimer’s disease (AD) [1]. There is increasing interest in the genetics of MCI for various reasons. MCI is still difficult to diagnose and its prognosis is extremely unpredictable. If we can identify genes involved in the aetiology of MCI, this opens the opportunities for improvements in the diagnosis of MCI and the prediction of the course of disease patients. However, the clinical heterogeneity of MCI limits the possibilities of genetic research. It is unlikely that MCI is a single pathological entity by itself. Given the progression of a substantial number of MCI patients into AD and dementia, one may argue that one cannot separate the genetics of MCI from the genetics of AD and dementia in general [1–4].

In this paper, the prospects of genetic research of MCI are reviewed. First, the role of genes in the diagnosis of MCI is discussed. Next, I will review the prospects of genetic research into the aetiology and prognosis of MCI. Finally, the general conclusions of the paper are summarized.

Genetics in the diagnosis of MCI

The diagnosis of MCI is far from straightforward (see earlier this issue). In the majority of patients MCI may be caused by early AD pathology, as the majority of patients progress into AD. Based on this clinical observation, it has been argued that asymptomatic carriers of AD mutations may be studied in order to
improve diagnostic criteria for MCI. The four genes for which the relation to AD is established are the amyloid precursor protein (APP) [5–8], presenilin-1 (PSEN1) [7–13] and presenilin-2 (PSEN2) genes [14, 15] which are involved in rare autosomal dominant forms of AD, and the apolipoprotein E (APOE) gene, which is a common genetic risk factor for AD.

There have been a number of studies of asymptomatic carriers of APP mutations which have been studied with the view to characterize MCI and improve its diagnosis [16, 17]. Although these studies have been valuable, there are a number of problems encountered. First, these studies have been hampered by small numbers of carriers. Mutations in APP are rare [16, 18, 19]. Also PSEN-1 mutations, the most common cause of autosomal dominant forms of AD [9–13] are relatively rare occurring in about 0.065% of all patients with AD. This makes it difficult to study these mutations with sufficient statistical power, even in multi centre studies [16, 18, 19]. Another problem is that in each gene there are different mutations (http://molgen-www.uia.ac.be/ADMutations/), which may deviate in clinical phenotype. Further, each of these dominant mutations is usually involved in early onset forms of disease, which makes it difficult to translate the findings to the general population.

Given its role in early as well as late-onset forms of AD, the APOE gene may be a more useful model for studies aiming to improve diagnostic criteria for MCI. The problem when studying MCI in carriers of the APOE*4 allele is that the risk of MCI (and AD) is relatively low. Therefore, a large number of carriers must be studied in order to identify sufficient numbers of carriers. The studies of the role of APOE in MCI conducted to date have yielded inconsistent findings. However, this may be attributed in part to the small number of subjects studied. We need larger, perhaps multi-centred studies, in which carriers and noncarriers are studied in detail on cognitive function, potential biomarkers, pathology at cerebral MRI and the presence of (co-)morbidity.

Research of genes involved in MCI is of interest not only to characterize the clinical phenotype, but may also be used directly in the diagnosis of MCI. Again, we will have to lean on our knowledge of AD, when evaluating at this time the potential of genetic testing in the diagnosis of MCI. If one considers the AD genes known to date and extrapolates these findings to how useful these genes are expected to be for the diagnosis of MCI, the contribution of the individual genes in the diagnostic process may be of limited value. Together, dominant mutations in APP, PSEN-1 and PSEN-2 occur in only 0.075% of AD patients [18, 20]. Assuming the impact of APP, PSEN1, PSEN2 on MCI will be lower than their impact on AD, screening for each of these mutations will have no value in diagnosing MCI in the general population. The situation may be more favourable in subsets based on onset age and family history. In these subgroups, also other genes may be considered candidates for diagnostic research, i.e. genes involved in prion disease or frontal lobe dementia [2].

Although the APOE E4 allele (APOE*4) is consistently associated with late-onset AD [17, 21], risks are moderately increased for APOE*4 carriers. For this reason, the APOE gene has been shown to unsuitable for diagnosis of AD [2]. As MCI is more heterogeneous than AD, APOE or genes with a comparable effect are predicted to be not suitable for MCI diagnosis [22, 23]. However, there may be alternative causes of MCI than AD including vascular pathology, diabetes, depression and other psychiatric conditions. As each of these disorders is in part of genetic origin, a very large number of different genes may underlie the pathology of MCI. Given the heterogeneity of the disease, one may argue that most likely a large number of low-risk genes are involved. Most of these genes are yet to be discovered, as discussed in the next paragraph. Ultimately, the combination of the outcomes of multiple genes may be helpful in the diagnosis [24–26].

Finding genes involved in the aetiology of MCI

As has been the case with other studies of complex traits, findings of studies aiming to discover new genes that are involved in MCI have been inconsistent. Most studies found that the APOE is associated with cognitive decline and MCI [27–33]. Other candidate genes that have been studied in relation to MCI include genes involved in hypertension, lipid metabolism, haemostasis, homocystein metabolism, diabetes and inflammation. But also these studies failed to yield reproducible results.

There may be several reasons for the non-replication of candidate gene studies of MCI and other complex traits. First of all there may be false
positive or false negative findings due to laboratories errors and multiple testing. Secondly, there may be bias due to serious mismatch of patients and controls for population of origin (population stratification). Third, most studies have been relatively small, in particular as genes are studied that convey only a small effect on the risk of MCI. There is increasing awareness that a considerable number of studies of the genetics of complex disorders have been underpowered and that this problem may be a very important source of inconsistencies of studies. Fourth, we need consistency in the diagnosis of MCI, which has differed substantially between studies. The chances of success of candidate gene studies will increase considerably if one was able to define a more homogeneous trait, based on clinical criteria, imaging, clinical subgroups or biomarkers. One cannot escape the conclusion that there is ample opportunity to improve the validity as well as the power of candidate gene studies.

An alternative approach to find new genes involved in MCI will be to screen the full genome. As MCI is such a complex trait consisting of a large putative number of pathways in which multiple genes may be involved, genome screens are not likely to be powerful. However, one has to raise the question whether searching the genome for genes involved in MCI is the most rational approach. It is very questionable whether MCI is an entity by itself. More likely MCI may be an extremely heterogeneous trait comprising groups of patients with (early forms of) various forms of dementia. Taking this into consideration, it is more powerful to first find genes involved in the subjects affected with subpathology independent of the presence of MCI, i.e. find new genes using AD or other MCI-related pathology such as hypertension. Next, one can study these genes as candidate genes for MCI.

**Genetics and the prognosis of MCI**

It is clear that a substantial number of patients with MCI develop into AD or dementia, while others do not. Perhaps one of the most important challenges from the view point of MCI patients will be to identify genes which can be used to predict which individuals progress into dementia. When treatment becomes available in the future, it will be crucial to halt the disease in an early stage in which neurodegeneration has not progressed into the major personality and behavioural changes that characterize AD and other types of dementia. In particular, subjects who are genetically susceptible to these disorders may benefit from early treatment. If only the disease can be postponed a limited number of years, this may improve the quality of life of these subjects and their relatives tremendously.

Studies of candidate genes influencing the progression of MCI have also been hampered by the same problems as genetic studies on the aetiology of MCI. Again, candidate gene studies are probably the approach with the highest statistical power [27–32]. Candidate genes may be sought primarily in genes that play a role in the aetiology and prognosis of dementia. For prognostic research, the question arises whether genetic tests will be useful in predicting the outcome of patients with MCI. Despite the promises in the literature of individualized medicine using simple DNA-based tests, there is an ongoing debate on this issue [24–26]. Rare mutations with major effects may indeed be useful in clinical practice for predictions [24, 25]. However, it is important to realize that for low risk mutations there is increasing evidence that their usefulness for genetic testing in clinical medicine and public health may be limited due to the low positive predictive value [24]. Only the combination of tests of large numbers of genes will be informative [24–26].

What to expect in MCI? As research on the heritability of the progression of MCI is extremely difficult, it is impossible to estimate how many genes are involved and how strong their relation to the prognosis of MCI is. Given the heterogeneity of MCI, most likely we will be dealing with a high number of low risk genes. This makes prediction using a single gene test impossible. Further, this implies that single gene test are not feasible and that we will have to combine the information of tests of various low-risk genes in order to develop a test that can reliably used in individual patients. This brings us to the conclusion that the identification of these low-risk genes will be the first challenge for the near future.

**Conclusions**

There is no doubt that studies of the genetics of MCI will be extremely relevant in light of early detection, prevention and prognosis of MCI. Finding genes for MCI may also increase our understanding of the neuropathological mechanisms underlying MCI.
The piece the resistance is to find genes involved in MCI, which most likely involve frequent mutations, which increase the risk of MCI only modestly. These genes have not been identified due to the complexity of MCI and the difficulties we face in the diagnosis of patients.

Although findings of studies on the genetics of MCI conducted to date have been disappointing, research may be improved relatively easily. Large studies are simply needed for gene discovery. Most likely, data will have to be pooled from various centres in order to improve the power. The technical developments in molecular genetics have made it possible to genotype large numbers of genes in a time- and cost-effective way. The candidate gene approach is the most powerful approach to discover new genes involved in MCI. For these candidate genes, research of the genetics of MCI will have to rely on the developments in research of AD and other dementias. It will be a matter of time before new candidate genes can be studied.

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*Correspondence: C. M. van Duijn PhD, Department of Epidemiology and Biostatistics, Erasmus MC Rotterdam, PO Box 1738 Rotterdam, The Netherlands.*

(fax: +31 10 4089406; e-mail: c.vanduijn@erasmusmc.nl)


1 Division of Geriatric Medicine, Neurotec Department, Karolinska Institutet, Stockholm, Sweden; 2 Aging Research Center, Division of Geriatric Epidemiology, Neurotec Department, Karolinska Institutet, Stockholm, Sweden; 3 Division of Molecular Neuropharmacology, Neurotec Department, Karolinska Institutet, Stockholm, Sweden; 4 Department of Neurology, John Hopkins University School of Medicine, Baltimore, MD, USA; 5 Department of Geriatric and Complementary Medicine, Tohoku University Graduate School of Medicine Sendai, Miyagi, Japan; 6 Department of Public Health/Geriatrics, Uppsala University, Sweden; 7 Department of Clinical Neuroscience, Sahlgrenska Academy, Gothenburg University, Sweden; 8 Center for Brain Health, New York University School of Medicine, New York, NY, USA; 9 Department of Neurology, Alzheimer’s Disease Center, University of California at Davis, Sacramento, CA, USA; 10 Department of Clinical Neurosciences, Helsinki University Hospital, Helsinki, Finland; 11 Department of Geriatrics, University of Geneva Medical School, Switzerland; 12 Division of Experimental Geriatrics, Neurotec Department, Karolinska Institutet, Stockholm, Sweden; 13 Laboratory of Neurogenetics, National Institute on Aging / National Institute of Health, Bethesda, MD, USA; 14 Department of Diagnostic Radiology and MR Research Laboratory, Mayo Clinic, Rochester, MN, USA; 15 Centre for Mental Health Research, Australian National University, Canberra, Australia; 16 Department of Nervous System Pathologies, French National Institute of Medical Research (INSERM), Montpellier, France; 17 Departments of Epidemiology and Biostatistics and Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands; 18 Department of Psychiatry and Neuropsychology, University of Maastricht, The Netherlands; 19 Department of Neurology, Mayo Clinic, Rochester, MN, USA

Abstract. Winblad B, Palmer K, Kivipelto M, Jelic V, Fratiglioni L, Wahlund L-O, Nordberg A, Bäckman L, Albert M, Almkvist O, Arai H, Basun H, Blennow K, de Leon M, DeCarli C, Erkinjuntti T, Giacobini E, Graff C, Hardy J, Jack C, Jorm A, Ritchie K, van Duijn C, Visser P, Petersen RC (Karolinska Institutet, Stockholm, Sweden; John Hopkins University School of Medicine, Baltimore, MD, USA; Tohoku University Graduate School of Medicine Sendai, Miyagi, Japan; Uppsala University, Sweden; Gothenburg University, Sweden; New York University School of Medicine, New York, NY, USA; University of California at Davis, Sacramento, CA, USA; Helsinki University Hospital, Helsinki, Finland; University of Geneva Medical School, Switzerland; National Institute on Aging/National Institute of Health, Bethesda, MD, USA; Mayo Clinic, Rochester, MN, USA; Australian National University, Canberra, Australia; French National Institute of Medical Research (INSERM), Montpellier, France; Erasmus Medical Center, Rotterdam, The Netherlands; University of Maastricht, The Netherlands). Mild cognitive impairment – beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment (Key Symposium). J Intern Med 2004; 256: 240–246.

The First Key Symposium was held in Stockholm, Sweden, 2–5 September 2003. The aim of the symposium was to integrate clinical and epidemiological perspectives on the topic of Mild Cognitive Impairment (MCI). A multidisciplinary, international group of experts discussed the current status and future directions of MCI, with regard to clinical presenta-
Mild cognitive impairment (MCI) was the topic of the First Key Symposium held in Stockholm, Sweden, 2–5 September 2003, and supported by The Royal Swedish Academy of Sciences and the Journal of Internal Medicine. The aim of the symposium was to integrate clinical and epidemiological perspectives in discussing current concepts in MCI and to identify pertinent questions for future research.

A multidisciplinary and a worldwide group of experts from Asia, Australia, Europe and North America participated in the meeting. During the first day and a half, five topics were debated by three experts: one main speaker, and a clinical and epidemiological discussant. The topics covered clinical presentation, cognitive profiles, genetics, neuroimaging and biomarkers. Following this, a day of discussion on the topics was conducted with the speakers, discussants, chairpersons and symposium’s Scientific Committee. The current status of MCI and new perspective discussed at the meeting are summarized here, in addition to recommendations for management, treatment and future research.

Clinical presentation

Current status

The term MCI is generally used to refer to a transitional zone between normal cognitive function and clinically probable Alzheimer’s disease (AD). Although many researchers have suggested and utilized a variety of criteria for defining cognitive impairment, they are essentially common with regard to their aim and theoretical framework in that they (i) refer to non-demented persons with cognitive deficits measurable in some form or another, and (ii) represent a clinical syndrome that can be utilized to classify persons who do not fulfill a diagnosis of dementia, but who have a high risk of progressing to a dementia disorder. As the literature on MCI has expanded, there has been some confusion concerning the specific boundaries of the condition.

Agreement of new perspectives

Mild cognitive impairment is useful both clinically and as a research entity, and is a concept encompassing much more than a preclinical state of AD. The heterogeneous aetiology of MCI is reflected in the literature. When persons with MCI are followed over time, some progress to AD and other dementia types, but some are stable or even recover. Moreover, epidemiological studies on elderly persons have shown that the risk of mortality is high amongst persons with MCI. MCI is also heterogeneous in its clinical presentation and should be considered in a

![Fig. 1 Heterogeneity of the clinical presentation of mild cognitive impairment (MCI) and potential multiple aetiologies.](image-url)
broad clinical context. The principal cognitive impairment can be amnestic, single nonmemory domain or involving multiple cognitive domains. Each of these clinical presentations could have multiple aetiologies (see Fig. 1). For example, although a neurodegenerative process could be the aetiology of a patient with amnestic MCI, memory impairment could also evolve as a result of other conditions such as ischaemia, trauma, metabolic disturbance, etc. Within this theoretical framework, numerous additional aetiologies may potentially be involved, such as psychiatric illness (burn out or depression) or other somatic conditions such as cardiovascular disease. For an alternative clinical interpretation of this figure, please refer to Petersen in the current issue (Fig. 4).

Cognitive and functional assessment

Current status

A wide range of cognitive functions appear to decline in persons who will be later diagnosed with AD compared with persons who remain dementia-free, including memory, attention, language, visuospatial skill, perceptual speed and executive functioning. However, controversy exists as to how MCI can be best assessed and defined, as there is insufficient evidence to recommend specific tests or cut-off scores. In a clinical setting, the degree of impairment can be assessed neuropsychologically, but fulfilment of MCI criteria is ultimately determined through clinical judgement using information from these tests within a framework including other tools.

Agreement of new perspectives

Both cognitive and functional abilities need to be considered in the evaluation of MCI. Individual slopes of decline in both functional and cognitive performance may be better measures than deficits assessed according to age-specific norms. However, consensus can only be achieved after longitudinal studies establish the age-specific levels of cognitive functioning, as well as normal rates of cognitive decline over specific time periods. The same issues apply to the assessment of complex instrumental activities of daily living. Specific domains of instrumental activities that might be impaired in MCI need to be determined.

Neuroimaging

Current status

The few MCI studies on neuroimaging have used magnetic resonance imaging (MRI) evaluations of atrophy of the hippocampus or entorhinal cortex, where relationships with transition of MCI to clinical AD and from normal ageing to MCI have been found. There is also evidence that deficits in regional cerebral blood flow as measured by SPECT and regional cerebral glucose metabolism as measured by FDG-PET could predict future development of AD in individuals with MCI.

Agreement of new perspectives

Neuroimaging techniques (such as MRI, CBF-SPECT and FDG-PET) are an essential part of the general evaluation of MCI subjects. Neuroimaging can be used from two essential perspectives. First, brain imaging has an important role in identifying specific and treatable causes of cognitive decline (e.g. subdural haematoma, brain tumour and normal pressure hydrocephalus), and thus, in establishing differential diagnoses. Secondly, neuroimaging can be used for predicting probability of developing dementia and measuring progression of neurodegenerative disease. Thus, brain imaging may provide supplementary diagnostic information on the pathological processes responsible for cognitive decline.

Biomarkers

Current status

There are limited studies investigating biomarkers in MCI. To date, most work has focused on tau and/or Aβ42 and the relationship to neuroimaging and clinical symptoms in persons at risk for AD. Some investigations have indicated that CSF markers [e.g. total tau (t-tau), phospho tau (p-tau) and 42 amino acid form of β-amyloid (Aβ42) etc.] may differentiate early and incipient AD from normal ageing and certain other dementia types. Focus on these biomarkers in the CSF raises a number of other issues, for example, access to CSF requires an invasive procedure (risks/benefits and patient acceptance of lumbar puncture), and there is lack...
of normative data on changes of these CSF markers with age. Furthermore, the effect of medications on changes in CSF markers is not established.

**Agreement of new perspectives**

Currently, biomarkers, particularly CSF markers, can be used mainly as a research tool and optionally by specialists with the purpose of identifying persons at risk of progressing to AD in conjunction with other instruments. The findings from a small number of studies conducted in selected clinical samples cannot yet be generalized to the general population.

**Genetics**

**Current status**

Mild cognitive impairment is a genetically complex condition and currently there are no major genes known to be involved in MCI. Each of the disorders possibly underlying MCI (such as AD, vascular pathology and depression) may partly have a genetic origin, and thus, different genes could underlie the aetiologies of MCI. Furthermore, various factors (both genetic and environmental) may interact, which creates an even more complex picture.

**Agreement of new perspectives**

Identification of mutations in amyloid precursor protein (APP), presenilin 1 (PSEN1) and 2 (PSEN2), tau, PRNP and α-synuclein may be useful in determining the aetiology of cognitive impairment in younger patients where there is a family history of AD or other neurodegenerative diseases. Prospective phenotypic studies of mutation carriers (APP, PSEN and α-synuclein) and apolipoprotein E (APOE) ε4 carriers may be useful for understanding the early clinical features of AD.

There may be several prognostic genes that may help to identify persons with a higher risk for progression from MCI to dementia. A few studies have suggested that the APOE ε4 allele is associated with a greater likelihood of progressing from MCI to AD. However, more studies are needed to determine the value of APOE and other genes in this context taking into account age, gender and gene–environment interactions.

**Recommendations**

**General criteria for MCI**

- Not normal, not demented (Does not meet criteria (DSM IV, ICD 10) for a dementia syndrome)
- Cognitive decline
  - Self and/or informant report and impairment on objective cognitive tasks and/or
  - Evidence of decline over time on objective cognitive tasks
- Preserved basic activities of daily living / minimal impairment in complex instrumental functions

**Fig. 2** Recommendations for the general criteria for mild cognitive impairment (MCI).

**Fig. 3** MCI classification process (adapted with permission from Lippincott-Raven Publishers, Williams & Wilkins.)

**Recommendations**

**General criteria for MCI**

The recommendations for general MCI criteria are shown in Fig. 2. The classification of MCI can be carried out in a stepwise fashion, taking into account each criterion. First, persons should be judged as not normal besides not fulfilling diagnostic criteria for dementia. Secondly, functional activities of the person are mainly preserved, or at least that impairment is minimal. Furthermore, the person should have evidence of cognitive decline, measured either by self and/or informant report in
conjunction with deficits on objective cognitive tasks, and/or evidence of decline over time on objective neuropsychological tests.

Figure 3 provides a flow chart that could guide the classification process in a diagnostic setting. An alternative depiction is also shown in Fig. 5 of Petersen’s manuscript in this issue. First, the patient or another individual with knowledge about the person expresses some concern about the person’s cognitive functioning. Based on the history and a mental status examination, the doctor would judge whether the person has normal cognition or suspected dementia. For example, if the person has a clear impairment in functional activities and scores low on the Mini-Mental State Exam, it is likely that this person has dementia.

Once the clinician has determined that the person is neither normal nor demented, assessing decline in cognitive functioning would be the next step. This could be achieved via taking a structured history from the patient and, where possible, a close relative or friend. If there is evidence for decline in cognition, the clinician must then determine whether this change causes impairment in functional activities to an extent that the person would be considered as having very mild dementia. If the functional impairment is not significant, MCI would be the appropriate classification. The clinical presentations of MCI can then be classified according to three subtypes: amnestic, multiple domain and single nonmemory domain (e.g. language and visuospatial). In order to determine the specific subtype of MCI, comprehensive cognitive testing is necessary, using neuropsychological testing, although there are currently no generally accepted instruments recommended. Specific domains of episodic memory might be assessed with, for example, a word list learning procedure or paragraph recall. If the subject’s memory is significantly lower than would be expected for their age, the clinician must determine whether other cognitive domains are also impaired, e.g. language, executive function or visuospatial skills. If the nonmemory domains are intact, the person would be classified as having amnestic MCI. If there are mild deficits in a number of different domains, the person would be considered as having multidomain MCI (with or without a memory component). Alternatively, if there appears to be a cognitive impairment in a single nonmemory domain, such as an isolated deficit in visuospatial skill, then single nonmemory domain MCI would be the appropriate classification. Please see Petersen’s Fig. 5 in this volume for another characterization of these concepts.

Once the clinical subclassification has been made, the proposed cause or aetiology of the clinical syndrome should be determined, similar to the evaluation that most clinicians do to determine subtypes of dementia. For example, if the clinician suspects that a person with amnestic MCI has a degenerative disorder, then this would likely be prodromal AD. Other explanations for cognitive complaint, such as depression, should also be considered.

Management

The recommendations for management follow two perspectives, clinical and epidemiological, with suggestions at three levels: general population, primary care and specialized secondary care.

At the population level, evidence-based information on established risk factors could be disseminated for broad public use (increase knowledge on modifiable risk factors for cognitive impairment, dementia, vascular problems, etc). Screening at the population level for either MCI or prodromal AD cannot currently be recommended. There is insufficient evidence for sensitive and specific tools (such as cognitive tests, imaging techniques, or biomarkers) that have both high positive and negative predictive values for use in the general population.

At the primary care level, general practitioners should pay attention to subjective cognitive complaints and verify cognitive deterioration by structured history taking. This, in addition to routine clinical examinations, can identify possible treatable causes of cognitive impairment such as somatic illness (e.g. hypothyroidism and anaemia), medication side-effects, modifiable cerebrovascular risk factors (e.g. diabetes, hypercholesterolaemia and high blood pressure), psychiatric illness (e.g. depression) and vitamin deficiency (e.g. B₁₂ and folate). As many of these conditions (such as depression) could also be risk factors for dementia development or possible precursors of AD and other dementias, periodical follow-ups are necessary with emphasis both on the primary disorder and cognitive deficits. In case of persistent or deteriorating cognitive impairment, patients should be referred to secondary care.
At the specialist level, patients with memory complaints should be clinically examined (including somatic and neurological status) to determine cognitive status, with detailed neuropsychological investigation to determine cognitive subtypes of MCI and laboratory investigations such as neuroimaging (MRI, SPECT and CT) and possible CSF biomarkers and PET. The physician can utilize these tools to make a clinical judgement and then follow the patient to assess progression.

Treatment: pharmacological and lifestyle interventions

There is no evidence for long-term efficacy of currently approved pharmacological treatments in MCI, and only modest evidence for symptomatic treatment efficacy in AD. Epidemiological studies have indicated a reduced risk of dementia in persons taking antihypertensive medications, cholesterol-lowering drugs, antioxidants, anti-inflammatories and oestrogen therapy; however, data from randomized clinical trials are needed to verify these associations. Currently, population-based intervention strategies relevant to MCI can only be limited to information on maintaining a healthy lifestyle. At the primary care level, intervention is restricted to primary prevention and management of known modifiable risk factors for cognitive impairment and dementia. Specialized medical care should focus on exclusion of treatable causes of cognitive impairment, treatment of behavioural/psychiatric symptoms and longitudinal assessment and re-evaluation of persistent or deteriorating cognitive impairment.

Pharmacological treatment for primary degenerative dementia and particularly AD currently show only moderate effects on cognition, behaviour and function. Acetylcholinesterase inhibitors (AChEI) approved for the symptomatic treatment of mild-to-moderate AD stabilize disease symptoms up to 1 year. Another antidementia drug, memantine, has been approved for treatment in severe AD. There are no randomized controlled clinical trials providing evidence that currently approved drugs for dementia could have efficacy in MCI and risk-benefit ratio is questionable. Answers may be provided in future by currently ongoing clinical trials in MCI with several AChEIs, their combination with vitamin E and a piracetam trial. In the light of the current recommendations for MCI, clinical trials could begin focusing on specific subtypes of MCI such as amnestic MCI with presumed degenerative aetiology.

Future research

An imperative element of reaching a consensus on the current and future directions of MCI is to establish clear research goals and provide evidence for the unanswered questions described above. Here we provide a summary of important areas for future research at the population level, as well as in primary and specialized medical settings.

Future research should focus on identifying the prevalence of the three clinical presentations of MCI as well as to establish the aetiology behind the impairment, both with clinical data and especially population-based studies. Specific questions should determine the prevalence and incidence of the MCI subtypes in different populations and age groups. Comparisons between the general population and clinical settings are of particular importance. For example, is amnestic MCI more common than multidomain MCI in memory clinics, but less frequent in the general population? Which aetiologies do the subtypes commonly relate to? Is the most common aetiology for amnestic MCI degenerative in nature? Can other aetiologies (such as psychiatric and somatic) be added to the current conceptual framework? What factors can help to determine the aetiology and future outcome?

Verifying and validating screening instruments and neuropsychological scales both for assessing MCI and detecting preclinical dementia is needed. Focus is required to establish normative rates of cognitive decline in specific domains in ageing. Of special interest will be comparisons between defining cognitive impairment based on age- and education-specific norms and the individual decline on cognitive tasks. Assessment of complex activities of daily living in MCI is potentially of great interest, as there is little information on this topic so far. Which activities are impaired in MCI and are there tasks of complex activities that can help predict outcome of persons with MCI? Establishing tools, norms and normative rates of decline on such rests are needed before conclusions can be made.

The possibility of neuropsychological testing laboratories at the primary care level has been discussed. This could involve general practitioners
referring patients to a setting in which cognitive functioning can be assessed using computerized tests. Much in the same way as other laboratory tests are analysed (e.g. blood test analysis, X-rays, etc.), a neuropsychological test profile could then enable the clinician to determine the cognitive status of the patient and thus assess the need for further examinations. The efficacy and cost of such a proposal will be of interest in future investigations.

Developments in brain imaging techniques include the potential for neurochemical imaging such as neurotransmitters, enzymes or receptors and possible imaging of specific pathological aggregates such as beta amyloid or neurofibrillary tangles. The use of standardized neuroimaging protocols would permit greater use of results from individual centres and pooling of such data could provide important insights into the value of neuroimaging in MCI. Relevance of white matter lesions to the diagnosis of MCI and prediction of progression to AD/dementia also needs to be further evaluated. Another future focus should be on developing guidelines for the use of neurophysiological methods (EEG and quantitative EEG in particular) as widely available, cheap and noninvasive diagnostic tools in the assessment of MCI and its subtypes. For example, there is evidence that EEG is normal in pseudodementia (depression) and that MCI subjects who progress to AD differ at baseline from those who remain stable. Consensus on recording and analysing standards as well as multicentre replication studies with population-based subjects are needed.

Biomarker investigations should aim at standardizing methodology and establish validation in larger cohorts and more heterogeneous populations. Furthermore, efforts should be made to assess changes over time and to investigate markers of other aspects of pathology, including inflammation, trophic factors and synaptic loss. It is also essential to investigate the relationship of current markers with genetic factors and quantify the added value of clinical markers to, for example, neuropsychological testing and neuroimaging. Another interesting question is whether biomarkers for identifying early stage disease (i.e. for AD) are the same as those that should be used to monitor progression within MCI.

Genetic studies will hopefully identify more susceptibility genes not only for AD, but also for other dementia subtypes. Current evidence suggests that genes involved in lipid metabolism, hypertension, haemostasis and homocysteine might also be candidates for involvement in susceptibility for various dementia syndromes. Research may also find prognostic genes that could help to predict persons with a higher risk for transition from MCI to dementia. The value of APOE genotype in this context should be further evaluated taking into account age, gender and gene–environment interactions.

Additionally, there is potential for the future implementation of a multivariate approach that combines demographic and genetic variables, cognitive measures, brain-imaging data and laboratory test results into a common prediction model. Of specific interest will be whether the various markers contribute unique variance and thus increase overall prediction accuracy for dementia. For example, can neuroimaging identify persons who will develop AD who are not detected with neuropsychological measures?

Better definition and earlier recognition of MCI could lead to revision of the current diagnostic criteria of AD or possibly other dementia subtypes. Research efforts regarding treatment should focus on designing drug trials for MCI and incorporate knowledge on natural history (time needed to reach next clinical milestone–transition to dementia) and surrogate markers of progression suitable for primary and secondary outcome measures (cognitive, neuroimaging, and CSF biomarkers). These methodological aspects are crucial for future preventive trials with neuroprotective and disease-modifying drugs currently under development.

Conflict of interest statement

No conflict of interest was declared.