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Μελέτη των αλλαγών του σήματος του νερού σε ασθενείς με Ηπια Γνωστική Διαταραχή και νόσο Alzheimer

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Myelin content changes in probable Alzheimer's disease and mild cognitive impairment

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Περίληψη

Αντικείμενο της Μελέτης. Μεταβολές στην λευκή ουσία έχουν αναφερθεί τόσο σε ασθενείς με πιθανή ν. Alzheimer (ΝΑ), όσο και σε άτομα με Ηπια Γνωστική Διαταραχή (ΗΓΔ) και σχετίζονται με αυξημένη συννοσηρότητα με κατάθλιψη. Η φύση των αλλαγών στην λευκή ουσία, που σχετίζονται με φυσιολογική γήρανση, άνοια, κατάθλιψη και ήπια γνωστική διαταραχή, δεν εχει μελετηθεί με την χρήση μεθόδων ευαίσθητων στο βαθμό ακεραιότητας της μυελίνης. Η νεότερη πολυεκθετική τεχνική Τ2 χρόνου μαγνητικής αποκαταστάσης επιτρέπει τη διάκριση των σημάτων από μόρια νερού που βρίσκονται σε διάφορετικά περιβάλλοντα στον εγκέφαλο. Ιδιαιτέρως το κλάσμα νερού/μυελίνης (MWF) σχετίζεται στενά με την περιεκτικότητα του ιστού σε μυελίνη. Στην παρούσα μελέτη χρησιμοποιήσαμε την πολυεκθετική ακολουθία MESE για να εξετάσουμε την περιεκτικότητα σε μυελίνη και την συσχέτιση της με ηλικία και με νευροψυχιατρικές παραμέτρους σε ασθενείς με ν. Alzheimer, ΗΓΝ και υγιείς μάρτυρες.

Μέθοδοι. Μετρήθηκαν οι συνιστώσες του σήματος Τ2 και ο δείκτης MWF σε 12 περιοχές ενδιαφέροντος (περικοιλιακή λευκή ουσία και την εν τω βάθη λευκή ουσία των τεσσάρων λοβών, και το μεσολόβιο) σε 25 ασθενείς με ΝΑ, 43 ασθενείς με ΗΝΔ, και 33 φυσιολογικούς ηλικιωμένους. Ολοι οι συμμετέχοντες υποβλήθηκαν σε νευροψυχολογική και νευροψυχιατρική αξιολόγηση.

Αποτελέσματα. Οι προσαρμοσμένες για την ηλικία τιμές τόσο της βραχείας όσο και της μακράς συνιστώσας του σήματος Τ2 βρέθηκαν σημαντικά αυξημένες (και αντιστοίχως μειωμένες οι τιμές του κλάσματος νερού/μυελίνης) στους ασθενείς με ΝΑ στη λευκή ουσία του αριστερού κροταφικού και βρεγματικού λοβού και στην περικοιλιακή λευκή ουσία αμφοτερόπλευρα σε σύγκριση με τους φυσιολογικούς μάρτυρες και τους ασθενείς με ήπια γνωστική διαταραχή. Παρατηρήθηκε επίσης αύξηση των τιμών Τ2 και αντίστοιχη μείωση των τιμών του κλάσματος νερού/μυελίνης με την ηλικία στην πλειοψηφία των περιοχών που μετρήθηκαν. Σημειώθηκαν επίσης σημαντικές συσχετίσεις μεταξύ των τιμών της βραχείας συνιστώσας του σήματος Τ2 και του κλάσματος νερού/μυελίνης στην κροταφική και μετωπιαία λευκή ουσία καθώς και στην περικοιλιακή λευκή ουσία με νευροψυχιατρικές μετρήσεις (επεισοδιακή και σημασιολογική μνήμη και συμπτώματα κατάθλιψης) στους φυσιολογικούς μάρτυρες και στους ασθενείς με ΗΝΔ.

Συζήτηση. Η παρούσα μελέτη εκμεταλεύεται την αυξημένη ειδικότητα της πολυεκθετικής τεχνικής Τ2 χρόνου μαγνητικής αποκαταστάσης για να εντοπίσει το βαθμό αλλοίωσης της μυελίνης ακόμα και σε ήπιες μορφές ΝΑ, ιδιάιτερα στον αριστερό κροταφικό και βρεγματικό λοβό καθώς και στην περικοιλιακή φυσιολογικά απεικονιζόμενη λευκή ουσία. Αλλαγές στην λευκή ουσία, και κατα συνέπεια και στην μυελίνη, έχουν καταγραφεί σε σχετιζόμενες με την ηλικία νευροεκφυλιστικές ασθένειες και ενδεχομένως να προηγούνται της παθολογικής εναπόθεσης β αμυλοειδούς και πρωτείνης tau. Αξιοσημείωτο είναι ότι ο αυξημένος ρυθμός απομυελίνωσης με την ηλικία στους ασθενείς με ΗΝΔ σχετίζονταν με τη βαρύτητα των ατομικών δυσκολιών στη μνήμη επεισοδίων καθώς και αυξημένα συμπτώματα κατάθλιψης.Το γεγονός ότι οι παραπάνω συσχετίσεις ανιχνεύτηκαν σε περιοχές με σημαίνοντα ρόλο στα παραπάνω νευροψυχιατρικά προβλήματα υποστηρίζουν την εγκυρότητα της μεθόδου στην κλινική αξιολόγηση νευροεκφυλιστικών νόσων.

Abstract

Background. White matter (WM) changes, including myelin breakdown, have been reported in Alzheimer's disease (AD) and mild cognitive impairment (MCI) and both conditions are associated with increased comorbidity with late-life depression. The nature of NAWM changes associated with normal aging, dementia, depression and/or MCI has not been systematically investigated using measures particularly sensitive to regional myelin integrity.

Multiexponential T2 (MET2) imaging calculates short T2 and myelin water fraction (MWF) values, strongly correlated with histological measures of myelin. In the present study a multi-echo, spin-echo (MESE) sequence was utilized to examine myelin content as a function of age and severity of neuropsychiatric impairment in patients diagnosed with AD or MCI and cognitively intact elders.

Methods. Measurements of Short T2, Long T2 and MWF values were obtained within 12 NAWM areas in patients and controls, involving periventricular and deep frontal, parietal, temporal and occipital WM, separately in each hemisphere, as well as the genu and splenium of the Corpus Callosum (CC) in patients diagnosed with probable AD (n=25) or MCI (n=43) and cognitively intact elderly controls (n=33). All participants received a comprehensive neuropsychological and neuropsychiatric assessment.

Results. AD patients displayed higher age-adjusted long and short T2 values and reduced MWF values in left temporal/parietal and bilateral periventricular NAWM than controls and MCI patients. Preliminary analyses revealed significantly increased Long and Short T2 values with age in the majority of ROIs in the NI and MCI groups. With few exceptions, corresponding reductions in MWF values were also found. Age-related effects in the Dementia group were restricted to the left temporal (Long T2) and periventricular NAWM (Short T2).

Short T2/MWF values in temporal, frontal, and periventricular NAWM of controls and/or MCI patients were significantly associated with episodic and semantic memory performance and

depressive symptomatology. In addition, the impact of age on memory performance was significantly mediated by age-related changes in short T2 and MWF values in these regions.

Discussion. To our knowledge, this is the first study utilizing the Multi-echo T2 relaxation time technique to improve specificity in detecting myelin degradation, predominantly in left hemisphere temporal, parietal and periventricular NAWM, even in mild dementia. WM and myelin changes have been documented in age-related neurodegenerative disorders, such as AD and MCI, and potentially precede amyloid and tau pathology. Moreover, age-related demyelination was associated with reduced memory function and increased depression symptom severity in an anatomically specific manner. This effect was more pronounced for episodic memory function in prodromal dementia states (MCI). These changes suggest an important role of WM integrity in clinical and neuropsychiatric manifestations of these patients.

1. Introduction

1.1 Alzheimer's Disease (AD)

The World Health Organization (WHO) proposes that dementia should be regarded as a global health priority as 47 million people may be currently affected by dementia and the number is projected to reach 75 million in 2030 and 131 million in 2050 with the majority of patients living in low and middle income countries (Prince et al 2015). Dementia has a great health, social, and economic impact. For instance, the annual cost incurred by dementia in the US in 2015 alone was estimated at $818 billion. Only 16% of this cost is for direct treatment and prevention of the disease and the rest includes care and societal costs.

AD is the most common form of dementia. Familial AD is a rare form of the disease characterized by early onset (<age 65), and is caused by mutations in the amyloid precursor protein (APP) gene and the presenilin 1 (PSEN1), or PSEN2 genes. Non familial (sporadic) AD has an older age of onset and is the most common form of AD accounts for up 95% of all cases. Polymorphism of the APOE ε4 allele is consistently found to be more frequent among persons who either have developed AD or exhibit greater than expected cognitive decline (see Section 1.2).

The median survival range for people aged 65 years or older diagnosed with AD is 3-9 years, with some living for as long as 20 years (Ganguli et al. 2005, Todd et al. 2013). In the early stage of AD the main symptoms are forgetful, language difficulties and mood changes. In the middle stage of AD patients may have increased difficulty with speech, may be very forgetful and they need help for self-care activities. In the late or severe stage, 5-9 years, symptoms get worsen with serious memory disturbances and nearly total dependence and inactivity. According

to the US Alzheimer's Association, AD was the sixth leading cause of death across all ages and the fifth leading cause of death for people aged 65 years or older in the USA. Women with AD live longer than men because they tend to survive longer in severe stage. So it is crucial to develop new methods for treatment and diagnose AD which should include all costs of the disease so that could improve quality of life for patients with AD. Epidemiological studies are crucial to understanding and address the challenges of AD, provide us knowledge about the distribution, genetic or non genetic risk factors, cost of health care and treatment and intervention strategies such as therapeutic and preventive interventions.

1.1.1 Pathophysiology of AD

In 1906 Alois Alzheimer described the pathological changes present in the brain of a patient exhibiting dementia symptoms that are currently recognized as AD. The pathology of AD involves the intracellular accumulation of amyloid plaque: small spherical structures, formed by the protein fragment Aβ and tau protein, forming fibrillary tangles (see Fig. 1.1). These observations led to the amyloid cascade hypothesis, which propose that Aβ initiates a molecular cascade of toxic effects leading to neurodegeneration and to clinical manifestation of AD. Aβ is an antioxidant, has antimicrobial activity, activates other signaling proteins and modulates cholesterol transport. The main efforts of treatment targeting Aβ is through inhibitors of β-secretase and γ-secretase, the enzymatic proteins that result in Aβ generation (for a schematic illustration of the hypothetical flow of pathophysiological changes sees Fig, 1.2). This hypothesis guided efforts to find treatments but because of biological role of Aβ is largely unknown there is a potential risk of serious side effects in targeting Aβ production for the treatment of AD.

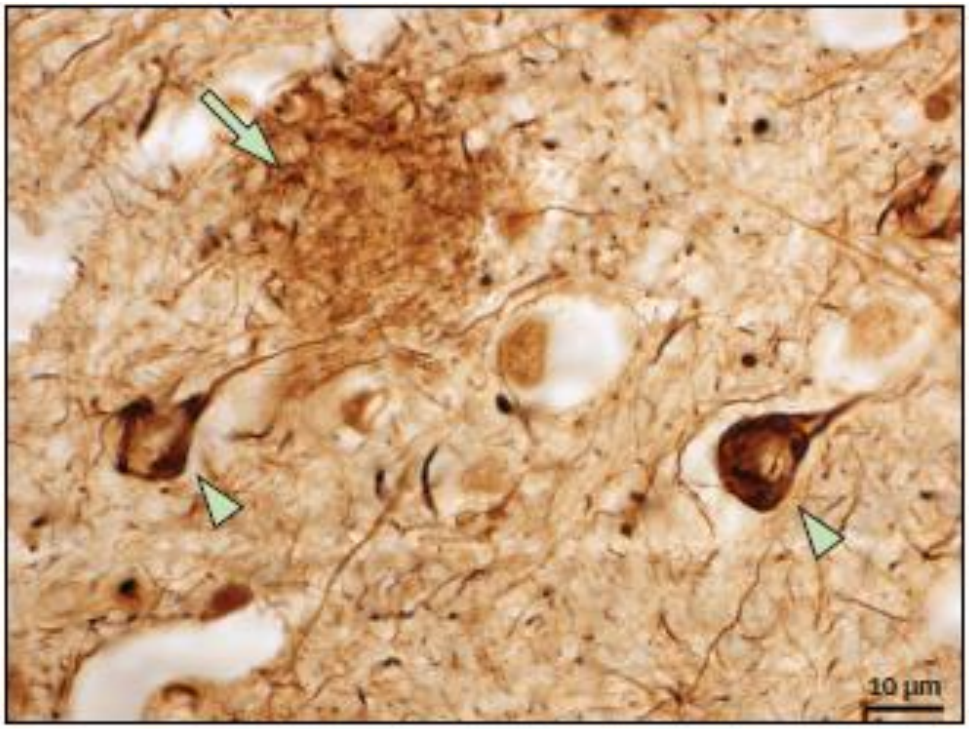


Fig 1.1. Neuropathology of Alzheimer's disease. Postmortem Bielschowsky silver staining of frontal cortex from a patient with Alzheimer's disease, showing the presence of a neuritic amyloid plaque (arrow), consisting of aggregated extracellular amyloid β fibrils, and intraneuronal neurofibrillary tangles (arrowheads), and consisting of hyperphosphorylated tau protein. (From Pater, C. Current Alzheimer 2011; 8: 798-807).

Formation of tau protein tangles in brain neurons is considered to play a secondary role in AD pathology despite its direct correlation with neuronal death and disease progression. Tau regulates microtubule assembly, dynamics and spatial organization and participates in the axonal transport of organelles and vesicles. Tau in neurofibrillary tangles is abnormally hyperphosphorylated. Hyperphosphorylation causes aggregation of tau into paired helical filaments, leading to the formation of tangles inside neurons and to impairments of cytoskeletal organization and of the transport of proteins and organelles and as a result converts tau into a microtubule-disrupting protein.

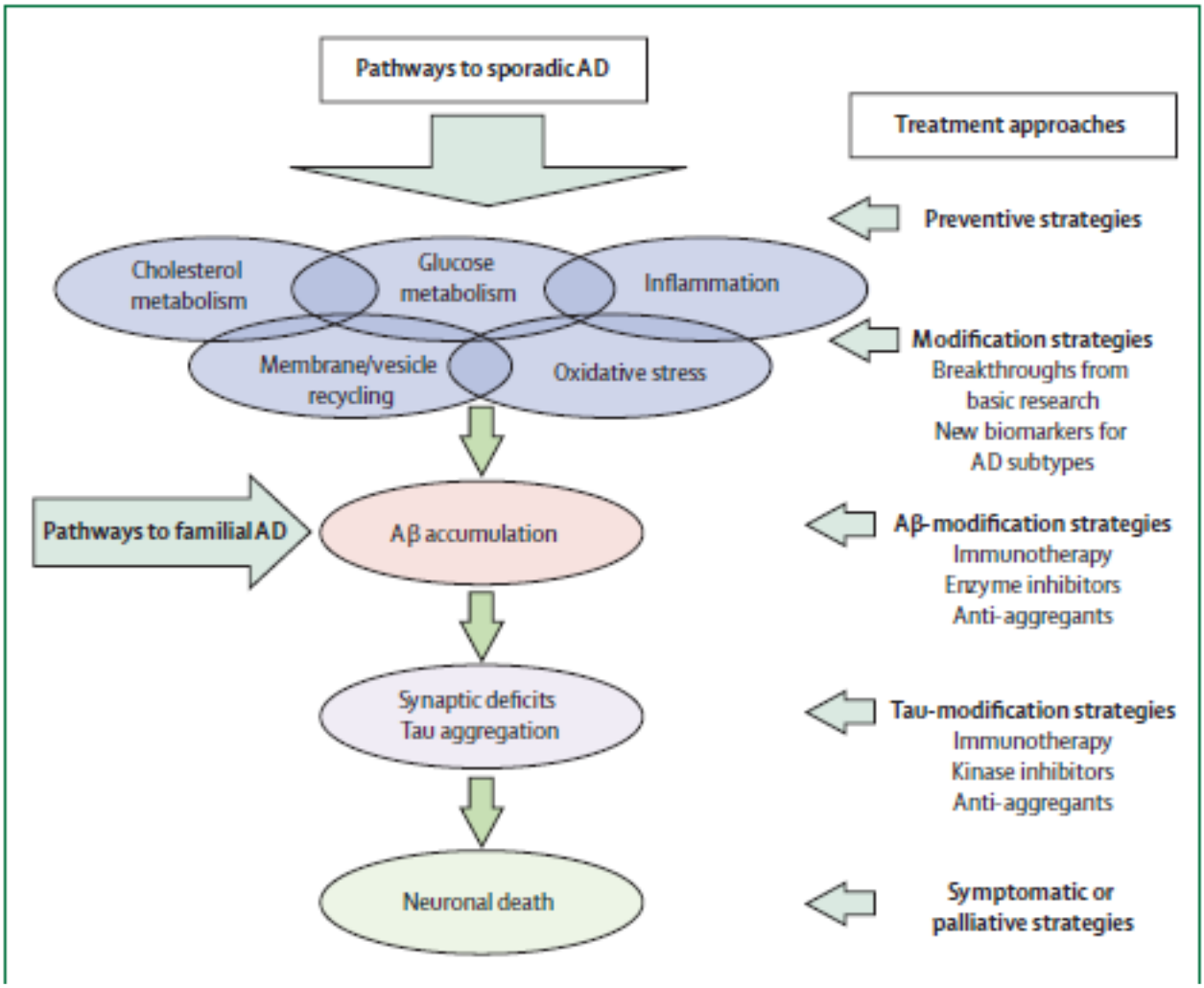


Fig 1.2. Pathways to Alzheimer's disease (from Winblad et al. Lancet Neurol 2016; 15: 455-532).

1.1.2 Risk and protective factors for dementia and AD

The major risk factor for Alzheimer is increasing age and is expected that the number of people with AD and other dementias will increase as life expectancy increases. Cardiovascular and metabolic risk factors are also believed to increase the overall dementia risk, including smoking, diabetes, hypercholesterolemia, hyperlipidemia, hypertension, and obesity in young or middle age, but not necessarily in late life (Winblad et al. 2016). Several studies have shown that having multiple cardiovascular risk factors in middle age or several years before dementia onset,

increases the risk of AD and dementia (see Table 1.1). Life-style and psychosocial characteristics have also been recognized as potential contributors to the onset of dementia and AD, such as chronic stress, depression, and loneliness (Winblad et al. 2016).

Except of risk factors which increased the rate for developing AD there are a lot of late-life protective factors which could reduce the risk of developing AD, including maintenance of a significant cognitive engagement, such as reading, playing games, doing crossword puzzles, an active social life, as well as known cardiovascular disease prevention factors such as Mediterranean diet and mild physical activity. Unfortunately, the results of these largely epidemiological studies have yet to be translated into knowledge of specific interactions among hypothetical predictor variables and etiological pathways that identify direct and indirect effects of each factor on AD emergence and progression. Large scale randomized controlled trials (RCTs) are needed to determine if prevention strategies that target potential risk and protective factors, from daily-life factors to drugs for prevention, can actually decrease the occurrence of AD. Effective approaches of prevention strategies depend on appropriate timing of the intervention. For example, starting before the onset of AD is likely to be more effective than starting when AD is established.

Risk factors	Protective factors
Older age	Genetic factors
Genetic factors	• Some genes proposed (eg, *APP*, *APOE* ε2 allele)
• Familial aggregation (two or more family members with the disease)	Psychosocial factors
• *APOE* ε4 allele	• High education and socioeconomic status
• Other susceptibility genes (eg, *CR1*, *PICALM*, *CLU*, *TREM2*, *TOMM40*)	• High work complexity
Vascular risk and metabolic factors	• Rich social network and social engagement
• Atherosclerosis	• Mentally stimulating activity
• Cerebral macrovascular and microvascular lesions	Lifestyle factors
• Cardiovascular diseases	• Physical activity
• Diabetes mellitus and pre-diabetes	• Light-to-moderate alcohol intake
• Midlife hypertension	Diet and nutritional factors
• Midlife overweight and obesity	• Mediterranean diet
• Midlife high serum cholesterol	• Polyunsaturated fatty acid and fish-related fats
Lifestyle factors	• Vitamin B6, vitamin B12, and folate
• Sedentary lifestyle	• Antioxidant vitamins (A, C, E)
• Smoking	• Vitamin D
• Heavy alcohol consumption	Drugs
Diet and nutritional factors	• Antihypertensive drugs
• Saturated fats	• Statins
• Hyperhomocysteinaemia	• Hormone replacement therapy
• Deficiencies in vitamin B6, B12, and folate	• Non-steroidal anti-inflammatory drugs
Other factors	Many risk and protective factors for dementia and Alzheimer's disease have been proposed and investigated; however, the evidence to support the factors listed here is variable, and the relevance of several proposed factors is open to debate. The most pronounced risk factors are advancing age and carrying one or two *APOE* ε4 alleles.
• Depression	
• Traumatic brain injury	
• Occupational exposure (eg, heavy metals, extremely-low-frequency electromagnetic fields)	
• Infectious agents (eg, herpes simplex virus type I, *Chlamydophila pneumoniae*, spirochetes)	

APOE=apolipoprotein E. *CR1*=complement component receptor 1. *PICALM*=phosphatidylinositol-binding clathrin assembly protein. *CLU*=clusterin. *TREM2*=triggering receptor expressed on myeloid cells 2. *TOMM40*=translocase of outer mitochondrial membrane 40 homologue. *APP*=amyloid precursor protein.

Table 1.1. Putative risk and protective factors for late-onset dementia and Alzheimer's disease. (From Winblad et al. Lancet Neurol 2016; 15: 455-532).

1.1.3 Clinical assessment and diagnosis

Although definite diagnosis of AD can be made only through neuropathological examination, a diagnosis of probable AD can be made on the basis of clinical examination, aided by laboratory tests, conventional neuroimaging, and neuropsychological evaluation. Despite the use of biomarkers and cognitive tests in clinical diagnose of AD post mortem examination is necessary

to confirm the presence of extracellular Aβ deposits and intraneuronal aggregates of neurofibrillary tangles in brain tissue. Unfortunately the numbers of autopsies in the USA and in many European countries have decreased by at least half since 1970s. This could cover diagnostic errors and reduce the power of research studies. Also it could leads to less reliable records of cause of deaths which is greater in ageing population which the cause of death could be due to multiple factors. The International Classification of Diseases have set specific criteria for clinical diagnosis of probable AD, such as memory deficit at initial presentation and slowly progressive onset and disease course and also language variant (logopenic aphasia), visuospatial (posterior cortical atrophy) and executive function deficits.

	NIA-AA criteria[325-327]	IWG criteria[328-330]	Comments
Diagnosis in the absence of symptoms	Preclinical AD: Stage 1: asymptomatic cerebral amyloidosis (CSF Aβ or amyloid imaging) Stage 2: asymptomatic cerebral amyloidosis with evidence of neuronal injury (volumetric MRI, CSF tau, or ^{18}F-FDG PET) Stage 3: cerebral amyloidosis with evidence of neuronal injury and subtle cognitive decline	Asymptomatic at risk: Normal cognition with one pathophysiological marker of AD (CSF Aβ and tau or P-tau, or amyloid imaging) Pre-symptomatic AD: Normal cognition with an autosomal dominant AD-causing mutation	The disease process, including accumulation of amyloid and tau pathology, can begin years or decades before symptoms emerge. The NIA-AA criteria specify three stages of preclinical AD, whereas the IWG criteria specify two different conditions in cognitively healthy individuals
Diagnosis of cognitive impairment due to AD (prodromal stage)	MCI due to AD—high likelihood: Biomarkers of amyloidosis (CSF Aβ or amyloid imaging) and neuronal injury (volumetric MRI, CSF tau, or ^{18}F-FDG PET) present MCI due to AD—intermediate likelihood: Biomarker of amyloidosis or neuronal injury present MCI—possibly due to AD: Biomarkers gave conflicting results MCI—unlikely due to AD: Biomarkers of amyloidosis and neuronal injury absent	Prodromal AD: Amnestic syndrome of the hippocampal type or a specific phenotype compatible with atypical AD, with one pathophysiological marker of AD (CSF Aβ and tau or P-tau, or amyloid imaging)	The IWG-2 criteria propose a specfic type of memory impairment for AD and a confirmation of the diagnosis by biomarkers. The NIA-AA criteria do not propose a specfic type of cognitive impairment in MCI and discuss different biomarker patterns in terms of different likelihoods of the presence of AD.
Diagnosis of dementia due to AD	Probable AD dementia: AD dementia with documented clinical decline AD dementia with an autosomal dominant AD-causing mutation Possible AD dementia: AD dementia with an atypical course AD dementia with evidence of mixed aetiology Probable AD dementia with evidence of the AD pathophysiological process: High likelihood of AD aetiology (biomarkers of amyloid abnormalities and neurodegeneration present) Intermediate likelihood of AD aetiology (biomarker of amyloid abnormalities or neurodegeneration present) Possible AD dementia with evidence of the AD pathophysiological process: High likelihood of AD aetiology (biomarkers of amyloid abnormalities and neurodegeneration present) Intermediate likelihood of AD aetiology (biomarker of amyloid abnormalities or neurodegeneration present) Pathophysiologically proved AD dementia: Clinical phenotype of probable AD with neuropathology findings indicative of AD	AD dementia: Episodic memory impairment or atypical AD phenotype with impaired activities of daily living and a pathophysiological marker of AD (CSF Aβ and tau or P-tau, or amyloid imaging)	The IWG criteria view the disease as a clinicobiological entity, so a diagnosis of AD dementia can be made in patients with typical or atypical clinical features only if a pathophysiological marker of AD is present

Table 1.2. Classification of Alzheimer's disease subtypes across NIA-AA and IWG criteria. (From Winblad et al. Lancet Neurol 2016; 15: 455-532).

It is currently recognized that the progression of AD typically includes a long preclinical phase, followed by a prodromal phase characterized by mild signs and symptoms (see Fig. 1.3). Although such symptoms can be masked by emotional manifestations (such as late-life depression which is highly prevalent in this age group) and changes in life conditions (i.e., reduced daily function demands associated with retirement) reliable identification is possible through comprehensive clinical and neuropsychiatric evaluation. The challenge in clinical diagnosis of AD is the transfer of concepts and methods that were developed for AD to earlier stages of the disease. This is very important for future treatment to starts in early stages, so being more effective. As top priorities that we can set are the identification and the effect of treatment of very early symptoms of disease, and predictors of treatment outcomes at the mild symptomatic stage.

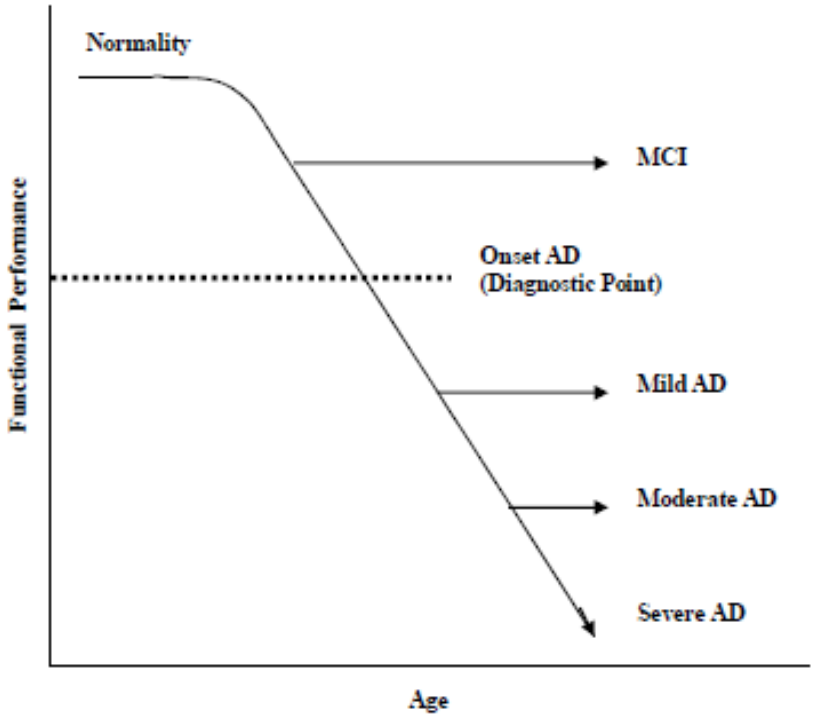


Fig. 1.3. Schematic representation of cognitive decline with progressive aging. (From Pater, C. Current Alzheimer 2011; 8: 798-807).

At present, the tools used in clinical care and diagnosis of AD include cognitive screening tools, psychometric (neuropsychological) tests, rating scales of functional impairment, assessments of neuropsychiatric symptoms, quality of life and disease-related burden. The disadvantages of these methods are that address to people to mild and severe stage of AD and display reduced sensitivity for persons with very high premorbid cognitive capacity (who have typically achieved high educational levels).

The most widely used cognitive screening tool in both primary and secondary care facilities is the Mini-Mental State Examination (MMSE) a brief assessment of several domains of cognitive function (semantic and episodic memory, orientation, attention, language, visuoconstructive function) with possible scores ranging between 0 and 30 points. The test was designed for use with elderly patients who are able to cooperate for few minutes and can be used for detection of decline in cognition, for monitoring response to treatment and disease progression. Although, MMSE tests vary considerably with age and education level, scores of 23 or lower for persons with elementary education (and <24 or even 25 points for persons with higher education) are generally considered as indicative od significant cognitive impairment. Using these cut-off levels MMSE has shown very high sensitivity (i.e., > 90%) for detecting the presence of depentia, with somewhat lower overall specificity (between 75-85%). An important limitation of MMSE is the very low sensitivity and specificity it possesses for detecting milder forms of cognitive decline which are considered as prodromal to dementia (see Fig. 1.4). Other brief cognitive screening tools, such as the Montreal Cognitive Assessment (MOCA) and the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-Cog), may ensure somewhat greater sensitivity and specificity for detection of non-dementing cognitive impairment. The presence of quantifiable early symptoms of cognitive decline (subjective cognitive decline) is also taken into account in diagnosis, as it correlates with both objective cognitive measures and biological markers, such as decreased concentration of Aβ42 or increased concentration of tau in CSF.

Unfortunately, the degree of awareness and ability to rate the severity of cognitive decline varies significantly among persons leading to low sensitivity and specificity as the sole indicator of dementia-related cognitive decline.

Major problems associated with the use of comprehensive psychometric assessments of cognitive function among elders include, in addition to significant cost and limited availability for wide-scale application, their modest reproducibility (especially for tests depending more heavily upon—potentially fluctuating—attention levels), and functional specificity (i.e., performance on a given test requires more than one cognitive function), difficulty in estimating premorbid performance levels for each patient, and strong dependence upon education level and prior experience on formal testing.

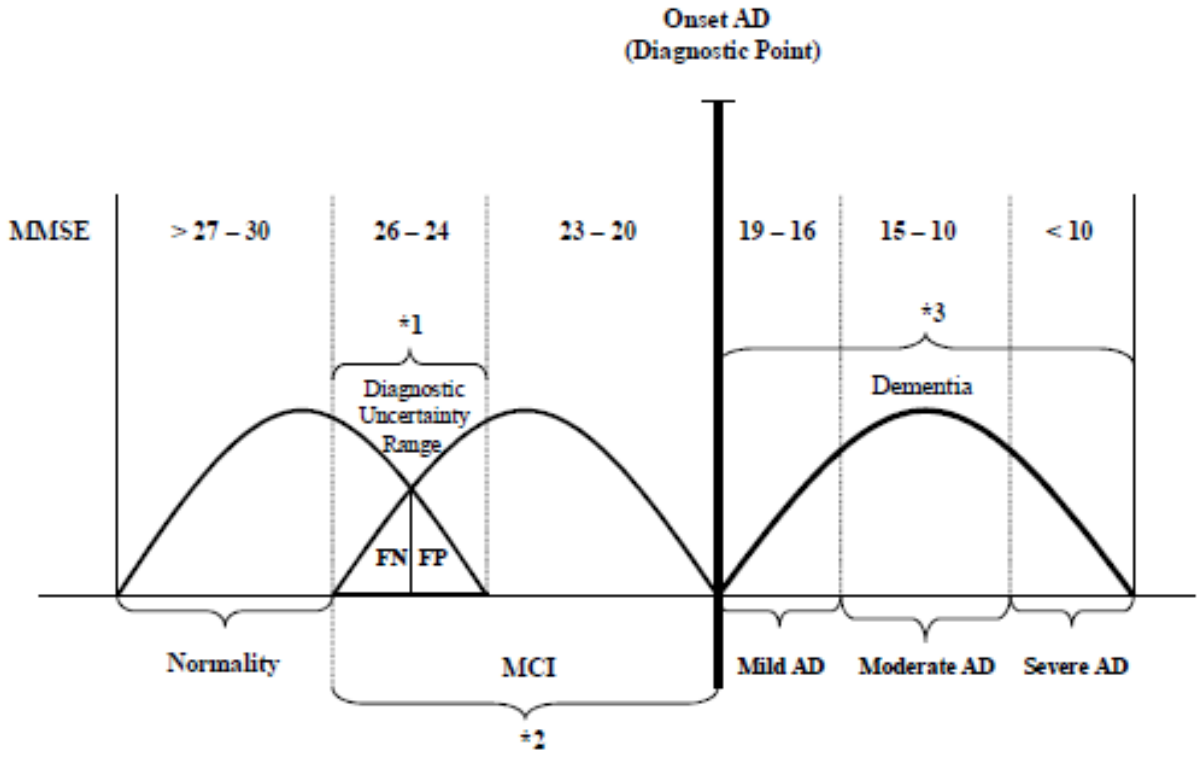


Fig 1.4. Conventional diagnostic entities crudely discriminated by MMSE. Notes; FN: false negative diagnoses, FP: false positive diagnoses. (From Pater, C. Current Alzheimer 2011; 8: 798-807)

1.1.4 Biomarkers in AD diagnosis

Disease- modifying approaches will probably work better to prevent the progression of the clinical syndrome in individuals with very mild or no clinical symptoms, with a genetic background to AD or positive CSF (see Fig. 1.5) or radiological AD biomarkers. There are studies suggesting that it might be possible to discriminate older adults with AD from cognitively healthy individuals by the use of a blood test. Methods by using MRI, PET or CSF biomarkers for detection of other processes, such as inflammation or cerebrovascular pathology might also be useful. Finally, the use of novel markers of neurodegeneration such as markers of synaptic dysfunction would be helpful to assess the effects of disease-modifying treatments intended to decelerate neurodegeneration.

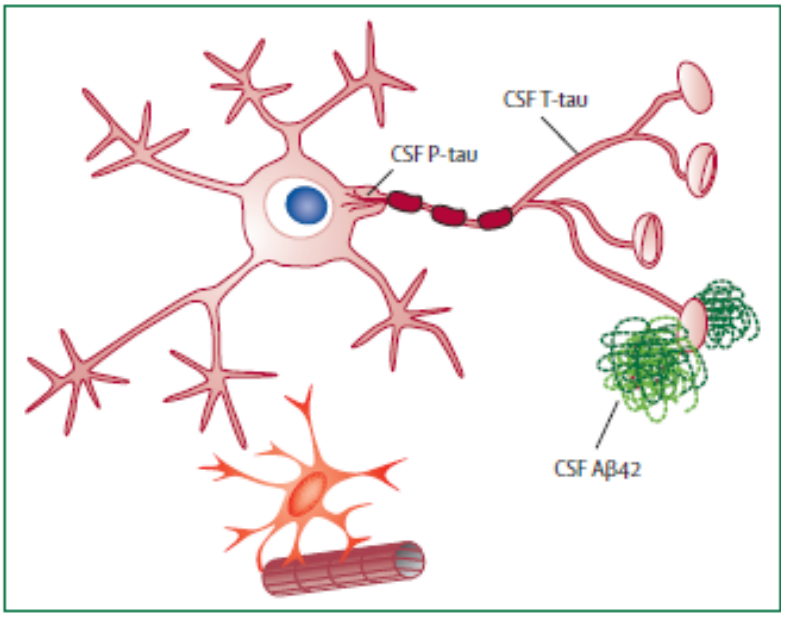


Fig 1.5. Schematic representation of a neuron shown the pathological changes associated with three core CSF biomarkers of Alzheimer's disease. While increased CSF concentration of T-tau is a marker of axonal degeneration, increased CSF of P-tau suggests the presence of neurofibrillary tangles, and decreased CSF concentration of the 42- amino acid form of Aβ (Aβ42) relates to senile plaque pathology. (From Winblad et al. Lancet Neurol 2016; 15: 455-532).

1.1.5 Cognitive impairment in AD

Comprehensive neuropsychological evaluation aiming to determine chief weakness and residual cognitive abilities is of value mainly in the early stages of the disease, when impairment of daily living is not pronounced and cognitive decline not yet global. In these cases, a detailed cognitive profile may contribute significantly to the differential diagnosis of AD from multidomain-amnestic MCI and/or cognitive decline due to other frequent comorbid disorders such as depression.

Progression brain pathology in AD displays extensive individual differences although the most consistent finding in the early stages of the disease involves medial temporal (typically asymmetric) atrophy. It is thus not surprising the earliest sign in the majority of AD patient's is impaired episodic memory (Salmon et al 2000). Episodic memory tests use a variety of cognitive procedures (free recall, recognition and paired-associate learning) across virtually all modalities (auditory, visual). These tests include word list learning tasks such as those from Consortium to Establish a Registry for Alzheimer Disease (CERAD; Welsh et al. 1991) and the California Verbal Learning Test (CVLT; Delis et al. 1991). A rather universal finding in these studies is that AD patients forget information over time at significantly higher rates than age- and education-level matched healthy participants and they are impaired on both recognition and free recall tasks of memory tests, even at the early stages of the disease. This performance profile is consistent with impaired consolidation rather than merely ineffective retrieval of new information.

Studies of episodic memory measures for early detection of AD have identified different characteristics for differentiating AD patients and normal elders. Measures of delayed recall with absolute delayed recall scores (amount recalled after the delay divided by the amount recalled on

the immediate learning trial) can differentiate mildly demented AD patients from Healthy elders with 85-90% accuracy (Flicker et al. 1984; Butters et al. 1988; Morris et al. 1991). To be-remembered information is not accessible after a delay even if retrieval demands are reduced by assessing recognition instead of free recall (Delis et al. 1991). This may at least in part due to difficulty in transferring information from primary to secondary memory (Bayley et al. 2000), and/or in difficulty in engaging semantic organization strategies during memory encoding.

Another domain of cognitive function where deficits are apparent even in early stages of the disease concerns semantic knowledge (semantic memory) as assessed by tasks of verbal fluency, semantic categorization, and confrontation naming. Several studies have shown that these deficits reflect deterioration in the structure and content of semantic memory (meanings of words, general knowledge of facts, concepts), they miss across different semantic memory tests that employ unique modes of access and output (fluency versus confrontation naming), that support language. As a result AD patient exhibit a loss of semantic knowledge rather that only an impaired ability to retrieve information from intact semantic memory stores (Salmon et al., 2000). The ability of patients with AD to efficiently generate words from a small and highly related set of exemplars during tests of verbal fluency is reduced due to loss of knowledge of the attributes and associations that define a particular semantic category. Rohrer et al. (1995, 1999) showed that AD patients experience deterioration of the structure and organization of semantic memory rather than a general inability to retrieve or access semantic knowledge.

Visuospatial deficits may also appear in during early AD stages or even in preclinical stages and are apparent on visuoconstructive and tasks that require visuoperceptual abilities and visual orientation. Treisman et al. (1996) and Foster et al. (1999) have shown that when AD patients perform a visual search task to quickly identify targets on the basis of the conjunction of two or more features that are processed in different brain areas, they have greater response times compared to healthy elders when required to identify targets solely on the basis of a single

feature. AD patients react more slowly to peripheral stimuli as measured with the Usefull Field of View (UFOV) paradigm, in which reaction time to peripheral visual targets is measured in the presence of various levels of distracting stimuli (Ball et al.1988), compared to healthy elders and younger controls. Although direct anatomo-clinical correlations generally modest associations between deficit severity and extent of regional atrophy, such impairments are thought to reflect primarily pathological changes in the temporal and parietal lobes.

Executive deficits may also be present in the early stages of AD progression, including sustained attention and control, mental manipulation of information, and problem solving (Perry and Hodges 1999; Chen et al. 2001). Such deficits may serve as additional negative prognostic signs in addition to episodic memory deficits. Lefleche and Albert et al. 1995 showed that mildly demented AD patients were significantly impaired relative to healthy elders on tests that required self- monitoring, and set shifting. Mental manipulation deficits in AD patients have impact in working memory tests and studies have shown that deficits in working memory are initially mild and primarily involve disruption of the central executive with relative sparing of immediate memory, whereas working memory is severely compromised only in later stages of the disease (Baddeley et al. 1991; Collete et al 1999). Albeit less consistently, patients in the early stages of AD may also show deficits in problem solving tests that require mental manipulation, such as the Tower of London puzzle (Lange et al 1995), the modified Wisconsin Card Sorting Task (Bondi et al. 1993), Porteus Maze Task, Part B of the Trail-Making Test, and the Raven Progressive Matrices (Grady et al. 1988). The extent to which such deficits depend upon the severity of neuropathological changes in prefrontal cortices is debatable.

1.1.6 Neuropsychiatric (NP) symptoms in Alzheimer's disease

Behavioral and psychoemotional symptoms of dementia are mixed with respect to type, severity, and course, affecting more than 80% of AD patients during course of the disease. The most common NPs associated with brain changes in people with AD include delusions, apathy and depression. *Delusions* are present in 30-40% of patients with AD, and are a sign of a worse prognosis. There are different subtypes of delusions such as delusions of theft, abandonment, persecution, jealousy, phantom boarder symptoms, misidentification of people, delusional misidentification of a mirror image and the belief that a deceased family member is still alive (Forstl et al. 1994; Hirono et al. 1998; Cook et al. 2003; Nakano et al. 2006). Memory, attentional and visuoperceptual deficits may contribute to delusions with paranoid features (Staff et al. 1999), a notion supported by an association between hippocampal atrophy and delusions. Another study implied a link between the right brain dysfunction and the development of delusion phenomena (Shanks et al. 2004).

The most common behavioral symptoms in AD are apathy and aboulia with a prevalence as high as 40-60% in some series (Lyketsos et al. 2002).Whereas apathy is sometimes used to refer to emotional bluntness aboulia is defined as the loss of the ability to initiate a motivated behavioral response and includes several aspects such as behavior, emotion and cognition. Also, could be considered as an early clinical marker of developing dementia because it is detectable in a high proportion of MCI patients. One study of 31 autopsied AD cases showed a strong association between apathy and the amount of neurofibrillary tangles in the left anterior cingulate gyrus (ACC; Tekin et al. 2001).

Depression, either supported by clinical diagnosis or evidenced by clinically significant depressive symptomatology is also very prevalent among AD patients ranging from 20% to

45%. The most common late-life depressive symptoms include restlessness, loss of interest for previously pleasurable activities, sense of hopelessness, and sadness (Fountoulakis et al. 2003). Despite the significant comorbidity of late-life depression and AD, it is not clear whether depression is a risk factor for development of AD or whether is a prodromal manifestation of dementing illness. Some studies have shown that a history of depression at any point in life approximately doubles one's risk of developing AD. Depression can precede cognitive decline in AD (Jicha et al. 2010) and may accelerate the rate of this cognitive decline (Rapp et al. 2011). Other studies have shown that depression increases conversion rates from MCI to AD (Lee et al., 2012) and may shorten the asymptomatic phase of the disease (Raskind et al. 2008). Such findings are consistent with a common pathophysiological mechanism involved in both AD and depression.

Late-life depression is itself associated with both structural and physiological changes in the brain. Structural imaging studies, such as diffusion tensor imaging, have shown reduced white matter structural integrity in late life depression involving a frontostriatal disconnection syndrome. Volumetric studies have linked depression with volume declines in left frontal WM and hippocampus between elderly non-demented with and without depression. One study addressing depressive symptoms in AD showed decreased cerebral blood flow in right dorsolateral and superior prefrontal regions (Levy-Cooperman et al. 2008) and other studies showed right ACC changes related to depression (Minnix et al. 2004).

There is evidence that pathophysiological changes in late life depression accelerate age-related cognitive decline. There is a high association between depression and hypercortisolemia, some studies have proposed a depression- as –stress model-related glucocorticoid neurotoxicity, particularly for hippocampus. So depression-associated hippocampal neuronal loss may negatively and synergistically affect the underlying neurodegenerative processes in AD, leading to earlier clinical expression of disease. Dysfunction of frontal lobe is an area of intersection in

apathy, depression and AD. But there is available evidence linking frontal lobe dysfunction to a variety of geriatric NPs making difficult to argue that frontal lobe dysfunction is a unique brain circuit for AD-associated depression.

Only recently, however, have neuroimaging studies began to systematically explore the neural basis of the association between depression and AD. For instance, Lee et al. (2012) showed that depressed MCI patients who also exhibited significant depressive symptomatology had greater frontal, parietal and temporal WM atrophy compared with patients without depression. Other researchers have proposed vascular pathology as the common link between cognitive decline and depression. According to the vascular depression hypothesis, cerebrovascular disease may predispose to both cognitive decline and depression. Once accelerated pathophysiological chances take place a downward spiral phenomenon may be initiated whereby depression-related cognitive changes (such as aboulia and attention disturbances) have detrimental effects on the performance of tasks in other cognitive domains (such as episodic memory, visuoconstructive ability, and other executive functions). Awareness of reduced functional capacity may, at least in MCI and in the early stages of dementia, further enhance feelings of helplessness, trigger symptoms of anxiety, and result in a disproportionate reduction in daily functional capacity (through reduced sense of self-efficacy).

The incidence of neuropsychiatric symptoms increases with disease progression. Delusions are rarely observed at terminal stages of dementia and hallucinations occur at a moderate stage of AD (Ropacki et al 2005). Apathy appears early in AD pathology and even earlier in MCI patients (Palmer et al. 2007). Some pharmacological and non-pharmacological treatment approaches for NPs have been shown to modify the natural characteristics of some NPs (Devanand et al. 1998). As an example, memantine and donezepil may be effective in the improvement of NPSs in patients with mild to moderate AD (Howard et al. 2012). The presence, frequency and severity of neuropsychiatric symptoms are considered as key determinants of

caregiver distress and burden (Germain et al. 2009), worsened quality of life (Missotten et al. 2008), hospitalization (Voisin et al., 2010) and institutionalization rates (Rocca et al. 2010).

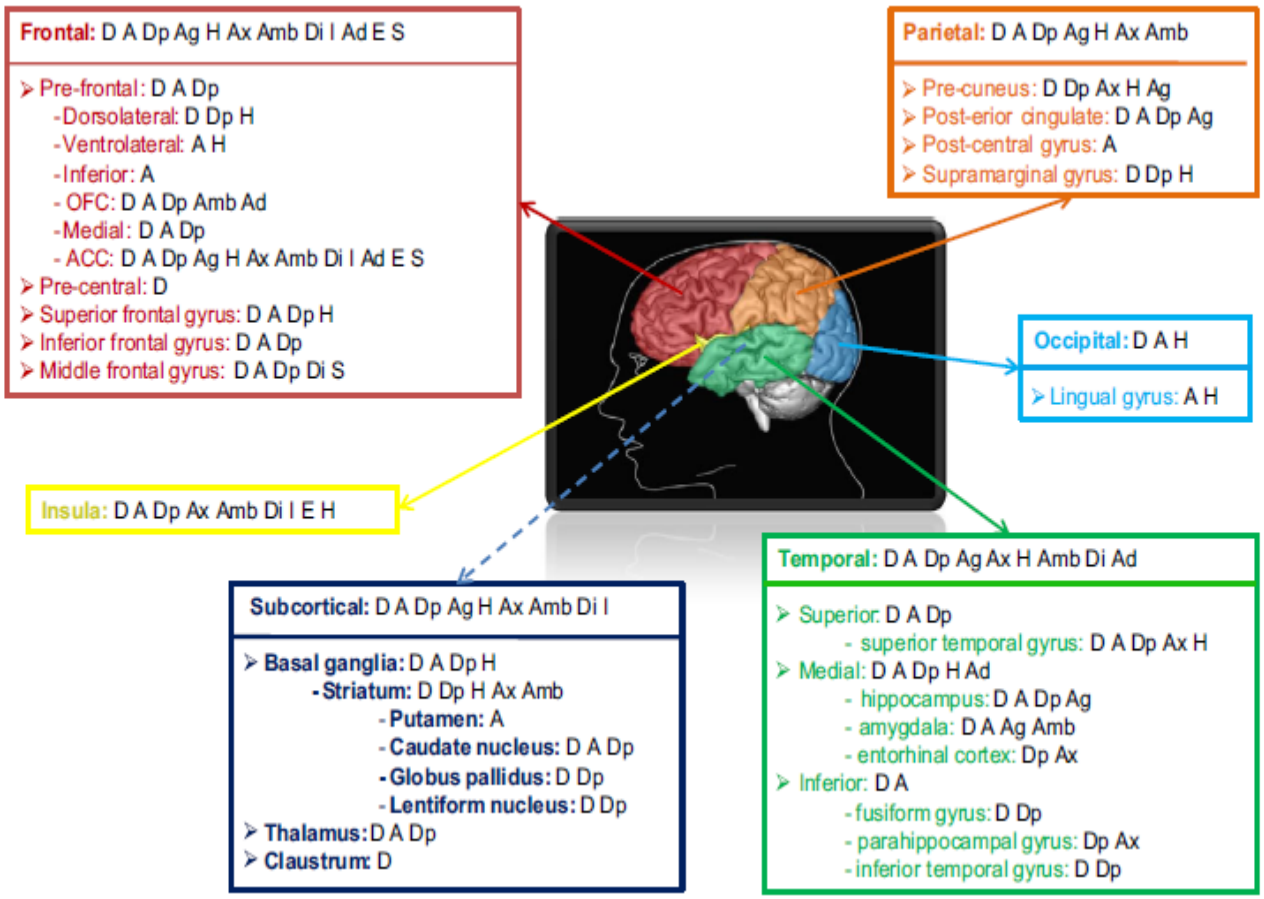


Fig 1.6. Associations between neuropsychiatric symptoms and affected brain regions. D, delusions; A: apathy; Dp: depression; Ag: Agitation; H: hallucinations; Ax: anxiety; Amb: aberrant motor behavior; Di: disinhibition; I: irritability; Ad: appetite disorders; E: euphoria; S: sleep disorder. (From Boublay et al. European Journal of Neurology 2016; 0: 1-10).

1.1.7 Pharmacological treatment of AD

Acetylcholinesterase inhibitors, donepezil, galantamine and rivastigmine and NMDA receptor antagonist memantine are approved drugs for AD in Europe which are indicated for mild- to-severe AD. In Europe, no drugs are available to patients in prodromal AD or MCI.

Although AD influences many functional domains the main focus in most AD trials is instrumental and cognitive function. Efficacy based on cognitive tests of daily activities, can be assessed in clinical trials of drugs for AD. However the effects of the marketed acetycholinesterase inhibitors have been statistically small, and remain controversial of how effective and cost effective they are. A drug that could ensure 1-2 years of stable function with good quality of life would be useful and cost effective, irrespective of whether the underlying pathology of AD is targeted. Finally, combined drug treatment in various settings is perhaps the best approach.

Drugs in various testing phases that target Aβ through disruption of the Aβ production or clearance include vaccines and antibodies to Aβ and inhibitors and modulators of β-secretase and γ-secretase. The first vaccine which tested in patients with AD removed amyloid plaques, but caused brain toxicity and had no clinically significant benefits. γ-secretase is an enzyme that cleaves the precursor protein APP intracellularly to produce Aβ fragments activity. Drugs targeting γ-secretase activity in AD patients, like γ-secretase inhibitors failed with an unexpected degree of toxicity and worsening of cognition. Drugs targeting β-secretase activity and specifically the form known as BACE1, which cleaves APP extracellularly to produce Aβ peptides, like BASE1 inhibitors have been used in clinical trials in people with prodromal AD or AD and some of them in phase 1 reduced CSF concentrations of total and soluble Aβ by up to 84% and 88% respectively.

Other treatments focus on the down-regulation of tau-related toxicity through reducing the pathological hyperphosphorylation of tau protein, or the fibrillation or deposition of tau. Activation of phosphatases has been proposed as a strategy to reduce tau phosphorylation. One phosphatase which involved in the desphosphorylation of tau is protein phosphatase 2A (PP2A) and treatment of PP2A activator sodium selenate in transgenic mice reduced tau

hyperphosphorylation and tangle formation, improved memory and prevented neurodegeneration.

To date there is very limited success by any of the (several) drugs that completed phase 2 and phase 3 trials. This is might due to the pursuit of the amyloid cascade hypothesis, and clinical research efforts are now being directed more broadly. The main issue of these proposed treatments is that there is no successful translation from preclinical to clinical studies in treating AD. For both Aβ-based and tau-based approaches the lack of good predictive animal models, efficient biomarkers for disease progression and well-defined target populations in clinical trials have been crucial in revealing future benefits in AD. So, preclinical research and early-phase clinical trials need to be replicated before drug development moves to later phases.

1.1.8 Non-pharmacological interventions for AD

Several non-pharmacological interventions have been proposed and tested as means to prevent or delay the onset of AD. For instance, FINGER, was tested on persons aged 60-77 years and showed significant positive effects of diet, cognitive training, exercise and management of vascular risk factors on cognitive function, with the largest benefits seen for attention and executive functions. These multidomain trials are feasible and can provide cognitive benefits.

Exercise is believed to have the greater impact of these non pharmacological interventions. One study included 170 adults with subjective memory complaints, 92 of whom had mild cognitive impairment, showed that a 6 month program of physical activity improved significantly cognitive function of these patients. These positive effects maintained for 18 months and were more pronounced in people with mild cognitive impairment. There are several studies that have shown that aerobic exercise in people with mild cognitive impairment has significantly improved motor performance, cognitive function, brain plasticity, cardiovascular fitness and AD

biomarker concentrations. Similarly, other studies have shown that aerobic exercise provides cognitive benefits in people with preclinical or prodromal AD. Lot of studies point out, that exercise has better effects than any other non-pharmacological interventions for people with preclinical AD and we should focus in exercise interventions as a basic part of the clinical management of people which is in high risk developing dementia. Summarizing, exercise, nutritional support, lifestyle changes and all non-pharmacological interventions might have significant effects at all phases of AD.

More research needs to be done, to clear which of these non-pharmacological interventions contribute to the reported benefits, to improve the cost-effectiveness of these interventions and to guide the implementation of these interventions.

1.2 Mild Cognitive Impairment (MCI)

MCI is characterized by significant decline in one or more cognitive functions (e.g., memory, attention, language, executive abilities) which, in contrast with dementia, are not sufficiently severe and generalized to cause dementia and significantly impair daily function (Eshkoor, Hamid, Mun, & Ng, 2015; Gauthier et al., 2006; Petersen, 2008). Although MCI is distinct from dementia, it is often regarded as a precursor of or as a high risk condition for various non-reversible dementia syndromes. Both conditions are associated with increased comorbidity with major depression and/or elevated depressive symptomatology (for a review see Panza et al., 2010). The original Mayo Clinic criteria for amnestic MCI are: 1) a memory complaint; 2) impaired memory for age on psychometric testing; 3) normal general cognitive function; 4) intact activities of daily living; 5) not demented. MCI diagnosis is universally recognized as incurring a significant risk factor for developing dementia of any type, with reported annual conversion rates ranging between 6-15% (Daly E et al 2000; Petersen RC et al. 2005) as

compared to the general population of 1-2%. According to their neuropsychological profiles, MCI patients can be classified to the following subtypes: 1) MCI-single domain, amnestic; 2) MCI-single domain, non-amnestic; 3) multi-domain, amnestic; and 4) multi-domain non-amnestic (see Fig. 1.7).

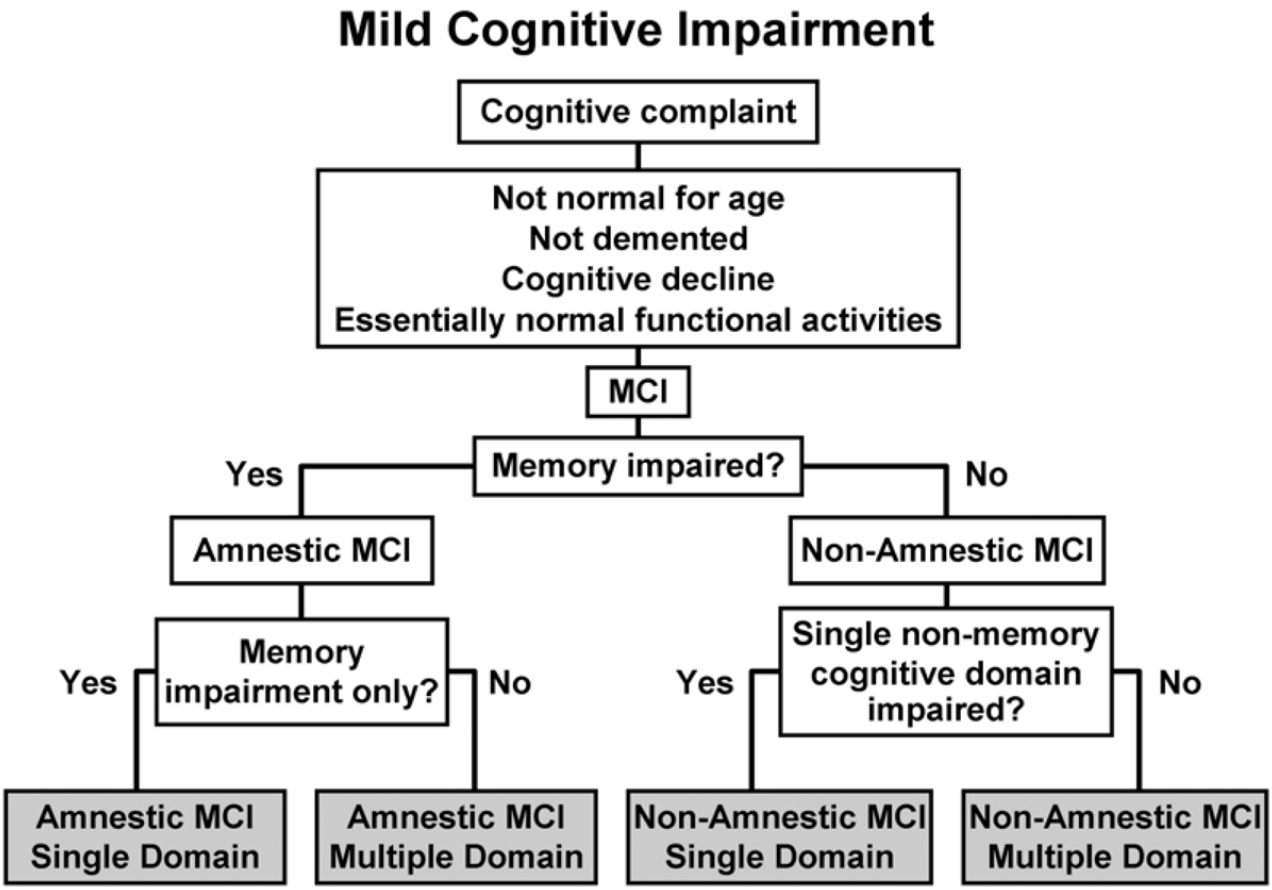


Fig 1.7. Diagnostic algorithm that can be pursued to arrive at a diagnosis of a particular subtype of MCI.

The overall prevalence of MCI in persons older than 60 years is 6.36% in western countries (Petersen RC et al .2010), with a slight preponderance of men (7.24% vs. 5.73%%). However, given that MCI prevalence varies notably with education level (according to some estimates the prevalence is twice as high among persons with less than 12 years of education), estimates of MCI prevalence are known to vary greatly among study cohorts. Clinical, neuropsychological

and neuropsychiatric evaluation of persons suspected for MCI largely involves the same procedures and tests as those outlined in Section 1.1.7 for dementia evaluation.

1.2.1 Brain Pathology in MCI

Studies on the presence and extent of typical neuropathological markers in brain specimens from patients diagnosed with MCI are somewhat limited and results highlight the significant intraindividual variability that characterized the disease. Thus, the density of brain amyloid beta (Aβ) deposition appears to be only slightly higher among MCI patients as compared to cognitively intact controls (Priceet al., 2009), limiting the use of Aβ plaques as a significant pathologic marker for the distinction between normal aging and MCI. Other studies demonstrate intermediate Aβ plaque load in MCI as compared to both cognitively intact controls and AD patients (Markesbery et al. 2010). Another characteristic neuropathological finding in AD, neurofibrillary tangles (NTFs) were found increased in the amygdala, entorhinal cortex (ERC), subiculum and the inferior parietal cortex (IPC) in MCI compared to controls (Braak et al. 1991; Guillozet et al. 2003; Markesbery et al. 2006).

Clinico-pathologic investigations have shown a significant increase in tau positive NFTs and neuropil treads in the entorhinal and perirhinal cortex in MCI as compared to healthy elders (Mitchell et al. 2002), coincident with significant reduction in the density of ERC layer II stellate neurons, which display prominent NFTs. These findings suggest a continuum of tau-induced NFT pathology underlying the transition between normal aging, MCI and AD.

Several studies have shown changes in neural density in MCI, focusing on medial temporal structures. There have been reports of reduced cell density in the entorhinal cortex even in the very early stages of AD and in MCI (Gomez-Isla et al. 1996) suggesting that accelerated neuronal death is already taking place at symptom onset. Furthermore, entorhinal atrophy correlated with impairment on tests of episodic memory.

The neuropathologic substrate of MCI is complex and involves cellular dysfunctions and the neuroplastic responses in addition to senile plaque formation and NFT pathology. MCI pathology is initiated via a trans-synaptic neuron to neuron disconnection syndrome affecting multiple levels within the central nervous system and is clear that the pathologic mechanism underlying MCI begins years before the onset of cognitive decline.

1.2.2 Available treatments for MCI

Several clinical trials have examined the relative effectiveness of pharmacological interventions in MCI patients. These trials are promising because possibly will uncover new information in the detection and intervention of the disease while it is still in a transitional clinical stage. The same therapeutic agents that are used for the treatment of AD have been tested for treatment of MCI such as cholinesterase inhibitors, antioxidants, anti-inflammatories, nootropics and glutamate receptor modulators but as with AD there is no specific treatment for MCI. For instance, according to data pooled from several review studies, cholinesterase inhibitors show very limited benefit toward reducing the risk of progressing from MCI to AD (Russ & Morling, 2012).

1.3 Brain Imaging

Several diagnostic tools are available to contribute to the diagnosis of AD by assessing pathophysiological markers of the disease. For instance, although the accumulation of neuritic plaques composed of aggregated extracellular AB fibrils and neurofibrillary tangles are not amenable to brain imaging, their impact on macroscopic anatomy and brain physiology and function can be inferred through several neuroimaging techniques. Magnetic Resonance Imaging (MRI) is the method of choice to detect and quantify changes in brain volume and structure, such as the presence of asymmetric hippocampal atrophy.

Baseline brain physiology in the form of cerebral metabolism and blood flow can be studied using positron emission tomography (PET) and perfusion MRI, respectively. PET with fluro-deoxy-D-glucose (FDG) and amyloid tracers such as Pittsburgh Compound-B (PiB) have shown characteristic changes in brains of patients with AD, even in early stages of the disease. Another diagnostic marker for AD is a CSF test which shows concentration of total tau (T-tau) and phosphorylated tau (P-tau) and concentration of Aβ42. Increased concentration of T-tau and P-tau and decreased concentration of Aβ42 suggests AD-like neurodegeneration.

PET for amyloid plaques and CSF biomarkers may assist in the early detection of AD and perhaps also in the identification of MCI patients who are at higher risk to progress to dementia and AD. The use of these biomarkers in cognitively healthy individuals, which are at risk of developing AD, has shown that pathophysiological process of AD begins a decade or more before the appearance of symptoms and Aβ deposition could take around 20 years before the first clinical symptoms appears. Each of these imaging modalities has unique strengths and weaknesses. The combination of them to most efficiently facilitate diagnosis, disease staging and development of effective disease-modifying therapies is the future challenge.

1.3.1 PET Imaging

In the early stages of the disease, MRI studies may not show any abnormality, whereas a decreased blood flow pattern on SPECT or decreased glucose utilization on PET could be noted in areas of the brain reported as normal on MRI. In addition, molecular PET imaging can reveal the accumulation of proteins such as Aβ and hyperphosphorylated tau.

1.3.1.1 Flurodeoxyglucose PET

Brain fluorodeoxyglucose (FDG) PET enables quantification of regional metabolic rate at rest by assessing the degree of uptake of a glucose analog (FDG) containing a positron-emitting fluorine isotope (Fluorine-18) by brain tissue (Schwartz et al. 1979; Magistretti et al. 2006). FDG-PET scans in AD patients typically demonstrate regions that display reduced basic metabolic rate in limbic and association regions (Foster et al. 1983; Reiman et al. 1996; De Santi et al. 2001), including the precuneus, posterior cingulate gyrus, inferior parietal lobule, posterolateral portions of the temporal lobe, hippocampus and medial temporal cortices. Such findings are much less consistent in MCI patients as compared to healthy elders. The limitations of FDG-PET are that is quite expensive and has limited availability. It requires intravenous access and involves exposure to radioactivity. Also brain FDG can be deranged for a variety of reasons (ischemia or inflammation) and may be irrelevant or not related to any AD-related process.

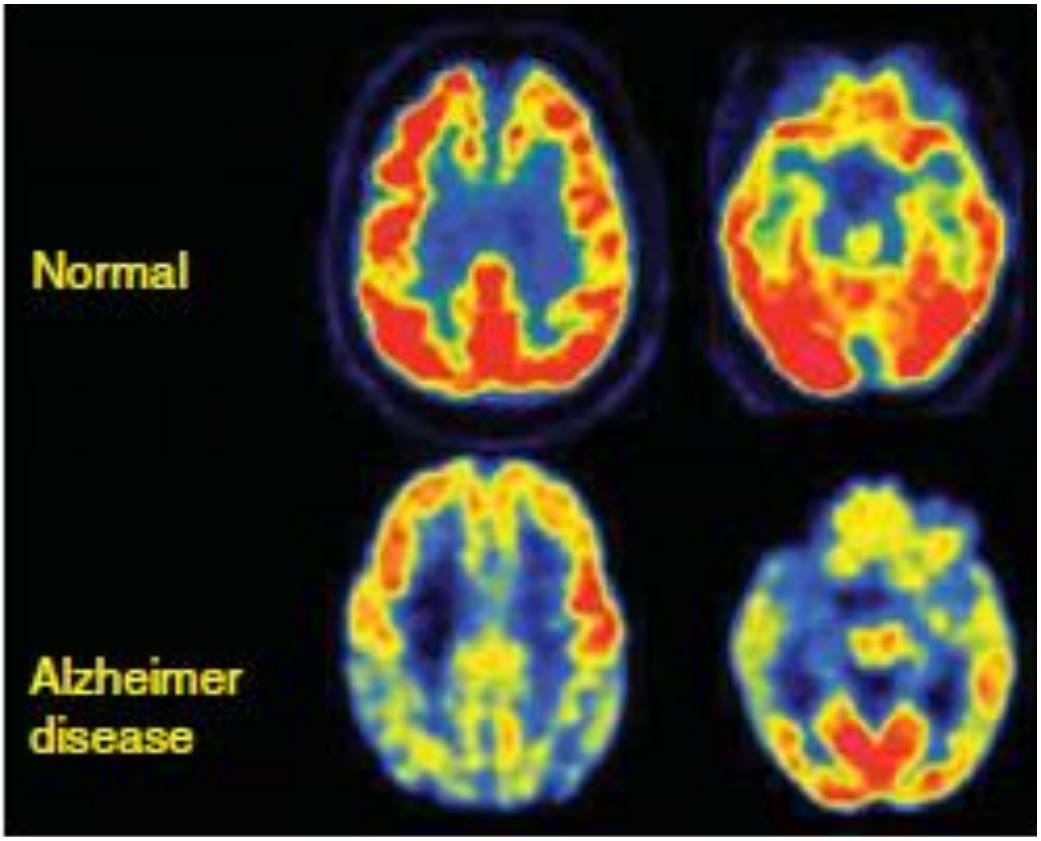


Fig 1.8. Axial FDG-PET images of a normal control and an AD patient with mild dementia demonstrate severe hypometabolism (blue and yellow cortical regions) in association and limbic cortex. (From Johnson et al. Cold Spring Harb Perspect Med 2012; 2:a006213)

1.3.1.2 Amyloid Positron Emission Tomography (PET)

Given that Aβ amyloid is considered as one of the neuropathological hallmarks of AD in vivo methods that reliably describe the amount of this protein in the brain is highly desirable and can be provided by PET (Amyloid PET). The earliest and perhaps more widely used amyloid ligand is the Pittsburgh Compound B (PiB: thioflavin T) whereas the only FDA-approved compound is 18F-florbetapir. Results from relatively small samples suggest that as many as 96% of AD patients are amyloid positive (Kemppainen et al. 2006; Aizenstein et al. 2008; Edison et al .2008; Shin et al. 2008; Devanand et al. 2010). Amyloid PET studies further suggest that as many as many as 60% of MCI patients may be amyloid positive as compared to only 20-30% of cognitively intact elders (Forsberg et al. 2008; Koivunen et al. 2008; Lowe et al. 2009; Johnson et al. 2008). The advantages of amyloid PET relate primarily to its modest anatomical resolution and associated regional specificity of results and its suitability for serial measurements assessing the progress of brain pathology. There is an early, steep rise of CSF Aβ42 levels, roughly paralleling amyloid PET results. However, CSF Aβ42 levels reach a plateau very early during the source of the disease are not therefore suitable as indicators of further disease progression (Blennow and Hampel 2003; Hansson et al. 2006; Fagan et al. 2007, 2009). Therefore, amyloid PET is preferable when the goal is to detect changes in brain Aβ load given that amyloid tracer retention correlates directly with Aβ load (Ikonomovic et al. 2008).

The main limitations of amyloid PET is the cost and availability. Also, it is not a good surrogate marker of progression during the clinical stage of the disease (Engler et al. 2006; Kadir et al. 2010). Moreover, the threshold of sensitivity of amyloid PET has to precisely determine in

each laboratory, whereas a negative result is not informative regarding the probability of any other form of dementia. In contrast MRI and FDG PET may give information of a frontotemporal or vascular pathology when amyloid PET would be ambiguously negative in both cases.

It should be noted that FDG-PET metabolic rate correlates with PiB accumulation, suggesting that cerebral metabolism appears to changing as amyloid is accumulating and it has been suggested that hypometabolism may signify an intermediate process between the initiating pathologic event and the subsequent development of synaptic failure and neurodegeneration (Cohen et al. 2009). In this context, hypometabolism may be viewed as a biomarker of neurodegeneration that precedes the appearance of cognitive symptoms and predicts the rate of progressive cognitive decline in individuals who will progress to AD. Notably, however, the intraindividual trajectories of amyloid deposition and metabolism are not parallel at least after the onset of AD: whereas amyloid deposition in most regions reaches plateau relatively early in the course of the disease, FDG continues to decline along with cognitive function (Engler et al. 2006).

In sum, the diagnostic value of any imaging technology will be determined by its contribution to finding and evaluating effective therapies. The major goal is to find biomarkers that could identify disease-slowing effects earlier and with fewer subjects exposed to treatment. Imaging is incorporated into trials designs to measure the effects of a therapy on fibrillary amyloid (amyloid imaging), on atrophy (MRI) and on metabolism (PET and fMRI).

1.3.2 MRI Imaging

MRI is a noninvasive imaging technique that offers a spatial resolution of tens of microns, can be used longitudinally within the same subject and does not rely on ionizing radiation.

Images can be acquired in a relatively short amount of time with high anatomic resolution providing anatomic and functional insights into pathologic processes. MRI is a very important tool to diagnose, monitoring progression and responses to therapeutic strategies in AD and MCI patients. Also is safe, does not involve ionizing radiation and offers a range of different sequences that can probe different tissue characteristics providing multiple clinical and research measures in the same session.

1.3.2.1 Volumetric approaches

Progressive cerebral atrophy is the main characteristic of neurodegeneration that can be visualized with MRI. Dendritic and neuronal loss are thought to be the major contributors to atrophy. AD is characterized by insidious onset and an unavoidable progression of atrophy that is first appears in the medial temporal lobe (Scahill et al. 2002). Entorhinal cortex is the first site of atrophy, followed by the hippocampus, amygdala, parahippocampal gyrus, and posterior cingulate. Typically, atrophy then spreads to neocortex in the temporal and parietal lobes.

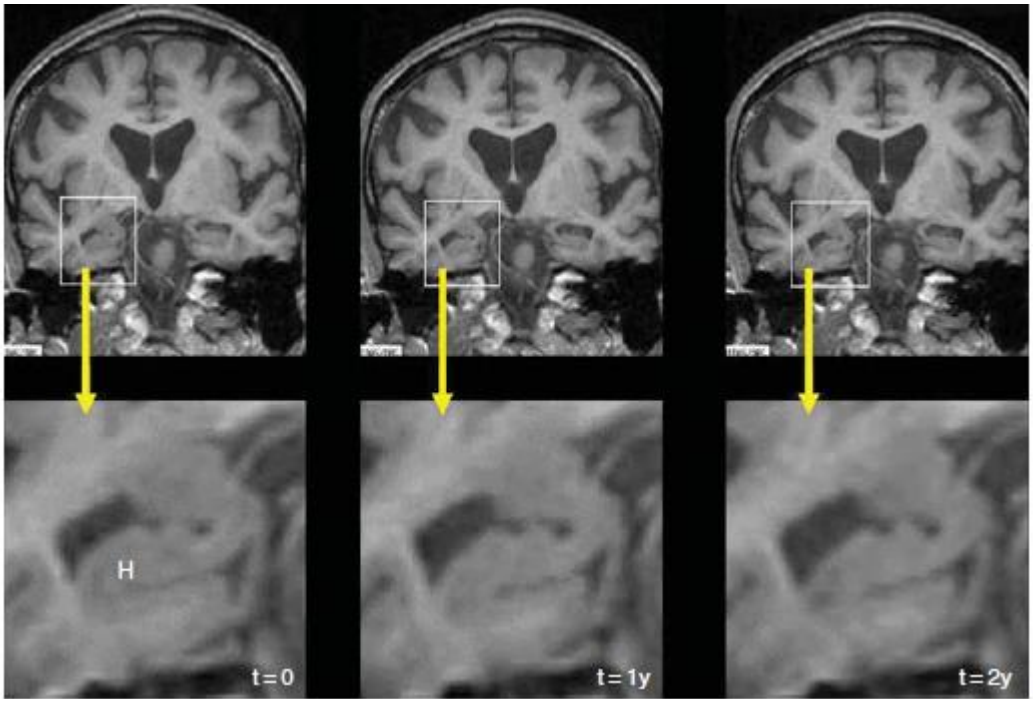


Fig 1.9. Three coronal T1-weighted images from an individual with autopsy-proven AD, were each acquired 1 year apart and show progressive hippocampal (H) atrophy as the individual progressed from memory complaints (left column, t=0) to MCI (center, t=1) and on to fulfill criteria for AD (right column, t=2). Johnson et al. Cold Spring Harb Perspect Med 2012; 2:a006213

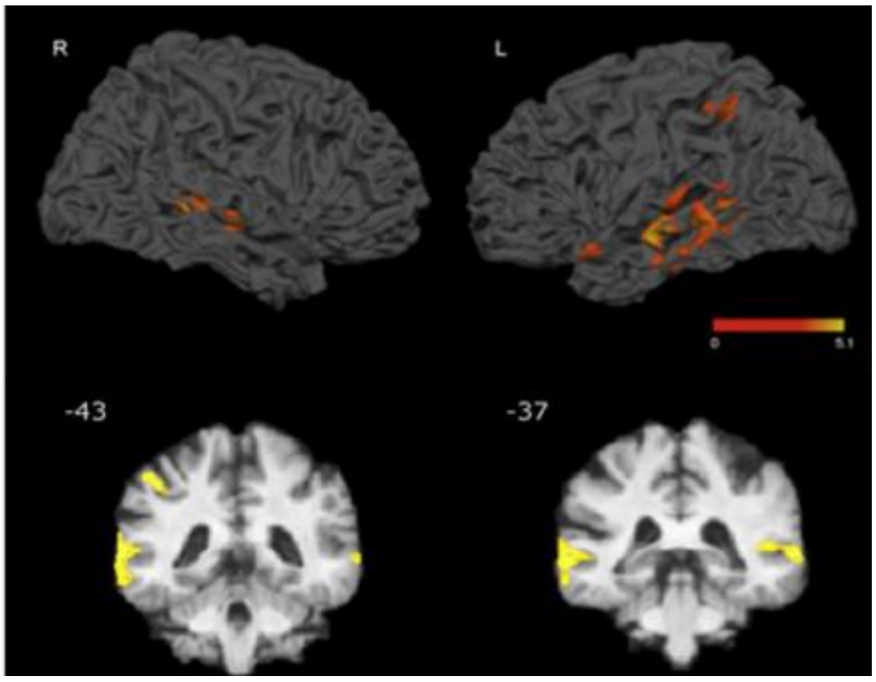


Fig 1.10. Higher rate of atrophy in the temporoparietal cortex among incident AD cases. The yellow voxels indicate brain regions in which gray matter volume loss from baseline to follow-up was significantly higher in incident AD patients compared to healthy elders (Beinard et al. 2014)

The average reduction of hippocampal volume is estimated at 3-5% per year in mild AD implying the presence of a period of several years before diagnosis, where medial temporal atrophy was already under way. According to MRI studies hippocampal volumes are already reduced by 10% three years before diagnosis for AD in asymptomatic individuals who

subsequently develop AD and the rate of this atrophy increases gradually within 5 years before diagnosis. Visual assessment of medial temporal lobe atrophy can differentiate mild AD from normal aging with a sensitivity and specificity of 80-85% (Sheltens et al .1992; Duara et al. 2008; Burton et al. 2009). Voxel based morphometry studies have shown a significant decrease in the volume of the parahippocampal white matter in amnestic MCI compared to healthy elders. Other studies showed a relationship between MTL atrophy and the severity of declarative memory dysfunction (Johnson KA et al. 2012). Differentiation between MCI who will progress to AD and those who will not according to medial temporal atrophy in MRI is more difficult with a sensitivity and specificity rate to 50-70% (DeCarli et al. 2007). For these reasons one of the biomarkers included in proposed criteria for diagnosing prodromal AD at a pre-dementia stage is medial temporal lobe atrophy (Dubois et al. 2007). In a similar vein, serial measures of medial temporal atrophy could be used as indices of AD progression and a potential outcome measure in trials.

White matter integrity has been extensively studied in age-related neurodegenerative disorders, such as Alzheimer's disease, in persons experiencing age-related cognitive decline without dementia (i.e., Mild Cognitive Impairment; MCI). To date the methods of choice have been diffusion-weighted imaging (DTI) and magnetization transfer imaging (MTI) that will be described in more detail in the following sections.

1.3.2.2 Diffusion weighted Imaging (FWI/DTI)

Except of volumetric studies in AD and MCI patients and studies focused on the extent of areas of white matter (WM) hyperintensity which is believed to reflect ischemic demyelination, loss of neurons and subsequent gliosis (Fazekas et al., 1993; Maillard et al., 2012), a complementary type of structural MRI is DTI. DTI allows the mapping of water diffusion in biological tissues and can be used to locate changes in white matter integrity. DTI is based on

the principles of diffusion imaging in which each voxel represents the rate and direction of diffusion in 3D space and can estimate more subtle perturbations in neuronal circuitry. The structural integrity of normal-appearing white matter (NAWM) has been examined systematically in AD and MCI using metrics obtained through DTI, such as functional anisotropy (FA) and mean diffusivity (MD).

One of the key indices in DTI imaging is fractional anisotropy (FA) taking up values between 0 and 1 with an FA value close to 0 representing a state of unrestricted diffusion of water, whereas an FA value close to 1 indicates restricted diffusion of water which is characteristic of a very ordered system. Several studies have shown promising results in using DTI to differentiate dementia with Lewy bodies from AD. Firbank et al. (2016) found greater mean diffusivity in AD than Dementia with Lewy body in the left parietal and temporal lobe. Although the extent and location of NAWM alterations varies widely across studies in both disorders, in general, reduced FA and increased MD has been found less consistently in MCI

Some studies reported that very specific regions of white matter are affected in the early stages of AD including the parietal sector of the corpus callosum and the parahippocampal cingulum. For instance, Zhuang et al. (2010) found reduced FA in the corpus callosum, fornix, lateral temporal and superior frontal WM and Alves et al. (2012) identified reductions in FA in the genu and body of the corpus callosum, anterior corona radiata and cingulate gyrus, whereas Nir et al. (2013) failed to find significant FA changes in MCI patients. In the majority of studies involving AD patients widespread reductions in FA are reported as compared to healthy controls.

Moreover, in one of the few neuroimaging studies examining the independent impact of cognitive decline and depressive symptomatology a further reduction in FA was noted in corpus callosum, superior longitudinal fasciculus (SLF), corona radiate and posterior thalamic radiation in MCI without depression as compared to controls and furthermore in internal and external

capsule, sagittal striatum, fornix, uncinate capsule and right cingulum between MCI with depression and healthy elders. No significant differences in FA found between MCI with and without depression point out that there is a common mechanism of structural white matter changes which leads to cognitive impairment to both MCI groups (Duffy et al., 2014).

Also, there are studies that have demonstrated atrophy of the entorhinal cortex and hippocampus in aMCI compared to cognitively healthy elders. These MRI results are in line with postmortem studies suggesting that AD-related pathology affects the entorhinal area before the hippocampus and that white matter volume changes reflect not only loss of afferent and efferent fibers in the parahippocampal region but may also be due to demyelination in remaining fibers in MCI

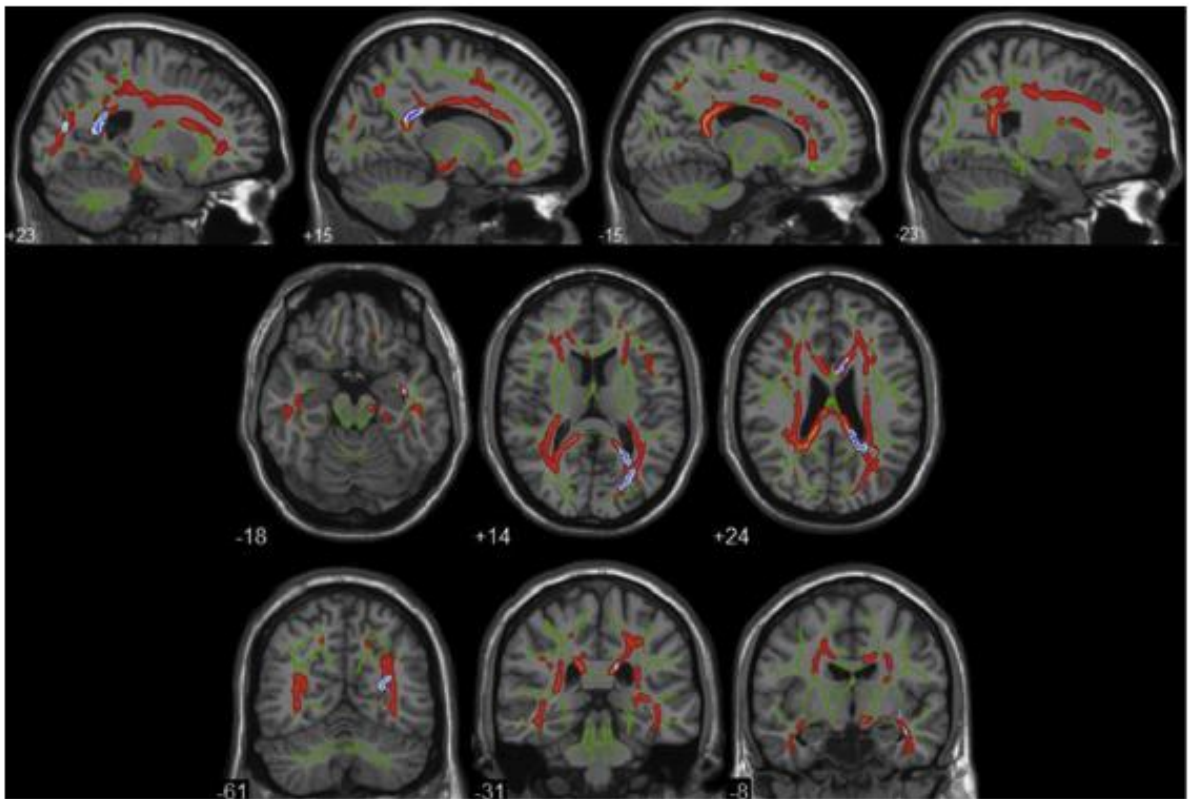


Fig 1.11. Group differences in Mean Diffusivity (MD) in AD patients versus Controls. Significant voxels in the white matter skeleton corrected for multiple comparison where increase

in MD in AD> control (red-yellow) and AD>Lewy Body Dementia (blue-pink). The white matter skeleton is shown in green (Firbank et al. 2016).

An advantage of structural MRI is its availability and ease of use, with a typical high-resolution volumetric sequence can be acquired in 5-10 minutes and more basic sequences in less time. Atrophy as a measure has advantages over conventional clinical ratings because it is not subject to practice or to ceiling effects and it has greater ability to detect disease slowing and when compared with other imaging markers has as strength, the correlation with cognitive decline. The main disadvantage of structural MRI is that lacks molecular specificity, so it cannot detect the amyloid plaques or the neurofibrillary tangles and such it is downstream from the molecular pathology. Another disadvantage is that atrophy patterns overlap with other diseases and unusual forms of AD have atypical patterns of atrophy too. Also, people with claustrophobia or more severe symptoms of the disease cannot be examined with MRI and finally, structural MRI cannot assess brain function.

1.3.2.3 Magnetization Transfer Imaging (MTI)

The integrity of lipid macromolecules in NAWM can be measured more directly through magnetization transfer imaging (MTI). For instance, Carmeli et al. (2013) reported reduced Magnetization transfer effect (as indexed by the Magnetization Transfer Ratio; MTR) in the splenium of the corpus callosum, right posterior corona radiata and in medial temporal lobe in 42 patients diagnosed with amnestic MCI as compared to an equal-sized group of healthy elders. In contrast, Granziera et al. (2015) failed to find MTR differences in WM between 42 patients diagnosed with various subtypes of MCI and 77 non-impaired elders as Mascalchi et al. (2013) in 27 patients with aMCI and 30 healthy controls. Late-life depression without MCI may also be associated with reduced MTR values in the corpus callosum, and frontal WM regions in the left

hemisphere (Dalby et al., 2010). Although AD and MCI are not considered as natural consequences of aging, their respective incidence rates rise steeply with advancing age.

1.3.2.4 Task-related and resting-state functional MRI

Another MRI technique, affording an indirect index of neuronal activity by measuring changes in blood oxygen level-dependent (BOLD) MR signal is functional MRI (fMRI; Ogawa et al. 1990; Kwong et al. 1992). FMRI is considered to reflect the integrated synaptic activity of neurons via MRI signal changes because of changes in blood flow, volume and oxyhemoglobin/deoxyhemoglobin ratio (Logothetis et al. 2001). FMRI can be used during cognitive task comparing an active condition (e.g., encoding new information) to a control condition (baseline information-cross fixation), or during resting state condition to investigate the functional connectivity within brain networks. Resting state and task-related fMRI could detect early brain dysfunction related to AD and to monitor therapeutic response over short time periods.

There are several fMRI studies in MCI and AD using episodic memory tasks, focusing on the pattern of fMRI activation in the hippocampus and medial temporal lobe. Most of these studies showed decreased hippocampal activity during the encoding of new information in AD patients (Small et al. 1999; Rombouts et al. 2000; Kato et al. 2001; Gron et al. 2002). Other studies have suggested the existence of a compensatory mechanism through increased activity in other networks in response to hippocampal failure in AD patients, featuring for instance increased prefrontal activity (Grady et al. 2003; Sperling et al. 2003; Sole-Padulles et sl. 2009). fMRI studies in MCI are more limited, with some and some reporting decreased mesial temporal lobe activation during the performance of memory tasks (Small et al. 1999; Machulda et al. 2003; Johnson et al. 2006). There have been contradicting results, however, with some studies

reporting increased MTL activation in very mild MCI cases (Dickerson et al. 2004, 2005; Celone et al. 2006; Hamalainen et al. 2006) and cognitively intact individuals with genetic risk for AD (Bookheimer et al. 2000; Smith et al. 2002; Wishart et al. 2004; Bondi et al. 2005). These discrepant results may be related to specific paradigm demands, behavioral performance and stage of impairment. Studies which found increased fMRI activity was perform to subjects that were able to perform the fMRI tasks quite well. Cross-sectional studies suggest that the hyperactivity may be present only at early stages of MCI, followed by a loss of activation in late stages of MCI, similar to the pattern seen in AD (Celone et al. 2006). Furthermore, other studies suggest that the presence of hyperactivity as baseline is a predictor of rapid cognitive decline (Bookheimer et al. 2000; Dickerson e t al. 2004; Miller et al. 2008), and loss of hippocampal function. This evidence suggest that hyperactivity may be a marker of neuronal failure and may reflect cholinergic or other neurotransmitter up- regulation (DeKosky et al. 2002), inefficiency in synaptic transmission (Stern et al. 2004), increased calcium influx and evidence of excitotoxicity (Palop et al. 2007; Busche et al. 2008).

Resting state fMRI is a novel technique and lot of studies has focused studying spontaneous brain activity and the interregional correlations. According of these studies, the brain is organized into multiple large-scale brain networks (Damoiseaux et al. 2006; Vincent et al. 2007) such as Default mode network (DMN) which includes the precuneus, posterior cingulate, lateral parietal, temporal and medial prefrontal regions. Multiple studies have shown impaired intrinsic functional connectivity in the DMN during the resting state in MCI and AD (Greicius et al. 2004; Rombouts et al. 2005; 2009; Sorg et al. 2007; Bai et al. 2008; Koch et al. 2010). Other studies highlight that DMN dysconnectivity in MCI and AD overlap the anatomy of regions with the highest amyloid burden in AD patients (Klunk et al. 2004; Buckner et al. 2005). Also, recent studies have showed evidence of disrupted DMN activity in cognitively normal older individuals with evidence of amyloid deposition on PET imaging (Hedden et al.

2009; Sheline et al. 2009; Sperling et al. 2009), suggesting that these markers could be used to track response to antiamyloid therapies in preclinical trials.

fMRI, either task related or resting state, could be very useful for the evaluation of novel pharmacological strategies to treat AD. Lot of studies suggested that fMRI can detect acute pharmacological effects on memory networks (Thiel et al. 2001; Sperling et al. 2002; Kukolja et al. 2009), but only a few number of these studies have shown enhanced brain activation after acute or prolonged treatment with cholinesterase inhibitors in MCI and AD (Rombouts et al. 2002; Goekoop et al. 2004; Saykin et al. 2004). Task related fMRI is quite problematic in examining patients with more severe cognitive impairment, as it is very sensitive to head motion. Resting state may be more feasible in more severe impaired patients as it easier to apply to AD and MCI patients than task fMRI, as no special equipment is required, subjects do not have to be able to perform a cognitive task, and a resting state run could be added to the end of a safety or volumetric MRI protocol. Also, BOLD fMRI response varies across subjects and very few studies examining the reproducibility of fMRI activation in older and cognitively impaired subjects. Finally, more studies are needed to track the evolution of alterations in the fMRI activation over the course of the cognitive decline from preclinical to prodromal to clinical AD.

1.3.2.5 Multi-echo T2 relaxation technique

In the studies reviewed above, WM integrity was assessed through DTI and MT techniques, which are characterized by adequate sensitivity for quantifying WM pathology but limited specificity. Thus, FA values are affected by fiber tract orientation order and packing properties of large fiber bundles and may not provide accurate measurements of myelin content. MTR serves as a direct index of the relative tissue composition in myelin and water, yet reduced values may be attributed to either a decrease in myelin content and axonal loss *or* an increase in water content.

There are different MRI approaches to measure signals originating from hydrogen protons in water molecules. These protons can be free or part of macromolecules such as proteins and lipids constituting myelin. The first display moderate to long T2 times (>10 ms) and the latter, which are less more mobile, have shorter T2 times (10 μs< T2< 1ms). Conventional imaging techniques can not detect the fast decay signals which are attributed to these molecules. There are direct and indirect methods for myelin imaging. Ultrashort TE imaging is a direct approach of measuring myelin. Indirect approaches include diffusion tensor imaging, magnetization transfer imaging and multiexponential T2 imaging. Multi-exponential T2 technique has been used to measure water trapped within the myelin layers and considered both indirect but and direct measure of myelin as water is a significant and critical component of the total myelin composition.

Quantitative T2 relaxation studies require images to be collected as multiple TEs, resulting in a T2 decay curve. Vasilescu et al. (1978) were the first to describe three distinct components from T2 relaxation curves, attributed to water associated with proteins and phospholipids, axoplasmic and extracellular water, respectively, using nuclear magnetic resonance in frog sciatic nerve. Another study by Menon and Allen et al. (1991) showed four T2 components in the WM of excised cat brain tissue and crayfish nerve cord. These four components were linked to extra-axonal water protons (T2=600±200 ms), axonal water protons (T2=200±30 ms), intramyelinic water protons (T2=50±20 ms), and lipid protons (T2=7±4 ms). MacKay et al. (1994) obtained by T2 relaxation data in CNS two different T2 components, a shorter T2 (10-40ms) and a longer T2 (70-100ms), with the first attributed to water trapped between the myelin bilayers and the other to the intra and extracellular water. A third (longer) component was found which was attributed to signal produced by water in the CSF. Finally they defined the term MWF as the ration of myelin water to the total water. Short T2 values strongly correlate with histological measures of myelin in guinea pig (Gareau et al., 1999), rat (Pun et al., 2005; Webb et al., 2003) and formalin-

fixed human brains (Laule et al., 2006; Laule et al., 2008). Expanding the data acquisition window of the Multi-echo T2 relaxation sequence to 300 ms (Laule et al., 2004; Oh et al., 2006; Tozer et al., 2005) and higher (320-1120 ms; Laule et al., 2007) permits identification of water reservoirs in brain tissue that may indicate the presence of edema or inflammation by mapping the anatomic distribution of intermediate and long T2 components. These findings have helped validate the use of myelin water imaging as a biomarker for myelin content in tissue.

FA and Short-T2 values from normal WM can be considered as complementary, with the former reflecting primarily integrity of axonal membranes and the latter reflecting the integrity of myelin sheaths (Beaulieu, 2002). Whereas Long-T2 and MTR values are highly correlated (Vavasour, Whittall, Li, & MacKay, 2000), the Short-T2 component appears to complement MTR as a more direct measure of myelin integrity. Despite several applications of the multicomponent T2 relaxation technique to probe pathological changes in WM in multiple sclerosis, its potential utility in identifying the pathophysiological processes involved in WM alterations in age-related neurodegenerative diseases remains unexplored. Myelin water fraction (MWF) is the ratio of the myelin water amplitude (short T2 component) to the total signal (Whittall et al., 1997) and corresponds to the anatomical distribution of myelin (Moore et al., 2000).

1.4 Age-related decline in WM integrity

While raw neuropsychiatric scores (especially those reflecting performance on neuropsychological tests) are expected to deteriorate with age, WM integrity is also known to show age-related decline. For instance, Draganski et al. (2011) reported significant reduction in FA and MT with age (range: 18-85 years) in frontostriatal, prefrontal, and temporoparietal

portions of WM, and Callaghan et al. (2014) identified higher age-related reductions in MTR in the genu of the corpus callosum than the splenium in 138 healthy controls (range 19-75years). Similar age-related reductions in FA in posterior and anterior periventricular WM, which were generally paralleled by corresponding increases in radial diffusivity, have also been reported (Westlye et al., 2010). These findings suggest a complex pattern of WM microstructural damage, involving axonal degeneration and myelin breakdown among other processes.

More recently, Wu et al (2016) reported widespread age-related reduction s in superficial white matter MTR extending across all brain lobes: inferior frontal, bilateral rostral middle frontal, lateral orbitofrontal and medial superior frontal, bilateral superior and middle temporal, fusiform and left inferior temporal, bilateral angular gyrus, bilateral cuneus, lingual and lateral occipital cortices. It should be noted however that regionally specific increases in FA (e.g., Inano et al., 2011) and MTR have been reported as well Armstrong et al. (2004). There is also some evidence that pathological aging may be associated with distinct temporal and spatial patterns of WM changes (FA changes) as compared to cognitively healthy aging. Head et al. (2004) showed that age effects in older adults without dementia were greater in anterior as opposed to posterior brain areas. In contrast, in the early stages of dementia WM deficits were minimal in anterior regions and more notable in posterior regions. In contrary, Damoiseaux et al. (2009) found age related effects on FA in healthy orders in frontal, temporal and parietal lobes, corpus callosum and internal capsule and in AD patients as compared to older healthy subjects decreased FA found in anterior left temporal.

In the majority of neuroimaging studies to date, age was typically used as a covariate in assessing the changes in WM integrity as a function of cognitive impairment. Only recently the complex paths between age, WM integrity indices and cognitive performance have been examined within the same study. In one of the first studies, Charlton et al. (2008) used path analysis to model potentially causal relationships between age, mean diffusivity aggregated

across periventricular and centrum semiovale WM, and four composite indices of cognitive ability in 118 healthy adults aged 50-90 years. Although mediated effects were not assessed explicitly, results were consistent with an age-related decline in working memory that was at least in part due to increasing mean WM diffusivity as a function of age. In a similar vein Voineskos et al. (2012) modeled associations between age, FA in several major WM tracts and three major cognitive domains (memory/executive function, visuospatial construction ability, and visuomotor dexterity) in 48 healthy participants aged 18-85 years. Results were, again, consistent with a negative impact of age on each cognitive domain that is mediated by age-related reductions in FA in the corpus callosum, inferior occipitofrontal fasciculus, and inferior longitudinal fasciculus, respectively. Mediated effects of age on cognitive performance through MTR-based measures of NAWM integrity were only recently examined in a large community sample (N=355) of adults aged 38-86 years Seiler et al. (2014). In addition to significant direct (i.e., age-adjusted) positive effects of NAWM on memory and executive measures in all but the temporal lobe, results revealed a significant mediated effect of age on executive measures through whole-brain MTR.

To summarize, it appears that although the effect of aging on myelination is robust on a variety of indices (e.g.,), the effect of clinical diagnosis of cognitive decline (in the form of dementia or MCI) is not well-established on measures that are primarily sensitive to regional myelin integrity (e.g., MTR). Moreover, there is little information on the extent to which the well-established impact of aging on cognitive performance may be mediated by age-related deterioration in WM, and especially in NAWM. It is further not known if the effect of age (direct and/or indirect through WM integrity) varies with clinical diagnosis of dementia or MCI.

1.5 Aim of the study

The present study utilizes the multicomponent T2 relaxation technique to examine NAWM integrity in patients diagnosed with probable AD or MCI and cognitively intact elders, as a function of age and severity of neuropsychiatric impairment (performance on neuropsychological tests and depression symptomatology). The study had the following specific aims concerning short T2, long T2 and MWF values obtained from lobar and periventricular NAWM:

(i) Average short/long T2 are increased and MWF values are *reduced* in patients diagnosed with MCI as compared to neurologically intact elders;

(ii) Average short/long T2 are increased and MWF values are *reduced* in patients diagnosed with AD as compared to both patients with MCI and neurologically intact elders;

(iii) The *overall positive* association between age and T2 values (and corresponding negative association with MWF) is stronger among patients with MCI and/or AD as compared to neurologically intact elders; moreover, there is a significant anterior-posterior gradient in the effect of age on T2/MWF values;

(iv) The *direct negative* association between T2 values (and corresponding positive association of MWF) with cognitive function measures is moderated by clinical diagnosis (presence of MCI and/or AD vs. neurologically intact elder controls);

(v) The *direct positive* association between T2 values (and corresponding negative association of MWF) with depressive symptomatology is moderated by clinical diagnosis (presence of MCI and/or AD vs. neurologically intact elder controls);

(vi) The *negative* effect of age on cognitive function measures is mediated by age-related increase in T2 values (and corresponding reduction in MWF values);

(vii) The *positive* effect of age on depressive symptomatology is mediated by age-related increase in T2 values (and corresponding reduction in MWF values). It is hypothesized that

mediated effects postulated in Specific Aims (vi-vii) will be specific (or at least stronger) among patients with MCI.

2 Methods

2.1 Participants

Data were derived from two cohorts of community dwelling elders residing in the district of Heraklion, Crete, Greece. The Cretan Aging Cohort is a sample of 3160 community dwelling adults, aged 60-100 years, who were recruited from a representative set of Primary Health Care (PHC) facilities. The recruitment pool included anyone over 60 years old who visited the selected facilities for any medical reason. All participants were interviewed by specially trained nursing staff during a face-to–face interview using a structured questionnaire, which included the MMSE scale. Based on the cutoff of 23/24 points on MMSE, 344 persons, who were deemed to be at risk for cognitive impairment, and 161 controls received a comprehensive neuropsychiatric evaluation. The second cohort was established through the "ΣΚΕΨΗ" research program and consisted of 338 self-referred elders who responded to ads in local media inviting persons aged 50 years or older to be tested for "memory and other cognitive difficulties they may be experiencing" and received comprehensive neuropsychiatric evaluation. Informed written consent as approved by the Clinical Research Ethics Board was obtained for all groups.

For both cohorts, diagnosis was based on consensus decision taking into account results from the comprehensive neuropsychiatric and neuropsychological evaluation. Diagnosis of any type of MCI was based on modified Petersen criteria (IWG-1; Winblad et al., 2004), requiring cognitive impairment that is insufficient to be dementia and generally intact daily function. Diagnosis of MCI further required that cognitive deficits could not be accounted for by clinically

significant or subclinical mood or anxiety disorder. The final data set used in the present study consisted of 43 persons diagnosed with amnesic MCI (15 presented deficits in additional cognitive domains; amnesic-multidomain type), 25 patients with possible or probable Alzheimer's disease, and 35 persons who did not present evidence of impairment in any cognitive domain.

As shown in Table 2.1, non-impaired participants as a group were younger and had completed more years of formal education than MCI patients who were, in turn, younger and more highly educated than the group of dementia patients. As expected the dementia patients had, on average, longer estimated disease duration than MCI patients. The majority of dementia patients (25.9%) experienced mild cognitive and functional impairment as indicated by CDR scores of 0.5-1, and only a quarter experienced moderate dementia severity (CDR = 2; Morris, 1997). The three groups were, however, matched on gender, depressive symptomatology (CESD score) and diagnosis of depression. Further the NI and MCI groups were matched on general cognitive ability (MMSE score).

Table 2.1. Demographic and clinical information by diagnostic group.

	Non-Impaired (n=35)	MCI (n=43)	Dementia (n=25)
Age (years)	67.9 (7.1)*$	71.0 (7.6)*¥	75.4 (5.9)$¥
Education (years)	11.4 (4.5)*$	8.0 (4.6)*¥	7.4 (2.8)$¥
Gender (%)			
Men	31.4	53.5	56.0
Women	68.6	46.5	44.0
CDR impairment class (%)			
Unimpaired (0)	94.0*	53.4*	--
Mild/Questionable (0.5-1)	6.0*$	46.6*¥	76.0$¥
Moderate (2)	--$	--¥	24.0$¥

CESD	34.3 (7.6)	35.5 (9.5)	37.8 (8.4)
Depression (%)	18.2	34.8	33.0
MMSE	26.6 (3.4)[$]	24.6 (3.3)[¥]	20.0 (4.5)[$ ¥]
Disease duration (months)	--	35.1 (23.8) [¥]	47.7 (30.4) [¥]

Symbols indicate significant pairwise differences at $p < .01$. Abbreviations; CDR: Clinical Dementia Rating; CESD: Center for Epidemiological Studies Depression scale

2.2 MR Image acquisition

All subjects underwent an MRI examination on a 1.5T superconducting MR imager (Vision/Sonata hybrid System, Siemens, Erlangen, Gradient Strength: 45 mT m^{-1}, Slew Rate: 200 mT m^{-1} s^{-1}). A standard quadrature RF body coil was used in all examinations for signal excitation and a standard 4 channel phased array head coil was used for signal detection. All subjects were placed in supine position and entered the magnet cradle using the head-first configuration. A conventional GRadient Echo (GRE) 2D multi slice multi plane turbo Fast Low Angle Shot (turboFLASH) T1-weighted imaging sequence was initially applied in the axial, sagittal and coronal planes for the localization of the head anatomy. Basic Imaging protocol comprised the following sequences: T1-weighted spin echo (TR/TE: 600ms/15ms) in the axial plane, T2 weighted turbo spin echo (TR/TE: 5000ms/98ms) in the axial plane, and turbo FLAIR (TR/TE/TI: 9000ms/120ms/2600ms) in the sagittal plane. The field of view was 250x250 mm and the scan matrix was 205x256 in all previous sequences. The total imaging time of the basic protocol did not exceed 15 min.)

Once localized, a series of a 2D, multi-slice, Multi Echo Spin Echo (MESE), PD- to T2-weighted sequences were obtained with no interslice delay time. The MESE sequence was applied using 32 symmetrically repeated spin echoes (TEs) obtained at 6.7 sec intervals (i.e. at 6.7, 13.4, 20.1, 26.8…207.7, and 214.4 ms). With the above chosen parameters a sensitive multi-

echo sequence for T2 measurements ranging from 10 ms to 300 ms was obtained. Repetition time (TR) was set at 2300 ms, flip angle = 90^o acquiring 5 oblique axial slices of 8 mm slice thickness and 8 mm interslice gap. A Field Of View (FOV) image area of 280x210 mm^2 was covered by each slice.

The image reconstruction matrix was 256x160 pixels respectively to the FOV dimensions, with in-plane spatial resolution (pixel size) of 1.1 x 1.1 mm^2. The cross-plane spatial resolution was equal to the slice thickness (8 mm). The overall spatial resolution expressed in raw data voxel dimension was 1.1 X 1.1 X 8 mm^3. The total imaging dimension on the cross-plane direction was 65 mm covering

The longer anatomical axis (Anterior to Posterior direction on the oblique axial slices) was always chosen as the frequency encoding axis. The highest possible receiver bandwidth (501 Hz/pixel) was used in order to eliminate geometric distortions due to susceptibility artifacts and also minimize chemical shift artifacts due to fatty components. Geometric distortion filtering was also applied in order to eliminate geometric distortions due to inherent gradient field imperfections. The overall SNR measured on the first echo proton density image was 160. One signal average was used and the total examination time was approximately 4 min major WM regions in the temporal, frontal, parietal and occipital lobes.

2.3 MR data and quantitative image analysis

All quantitative T2 MRI data were transferred to and analyzed in a separate workstation. Numerical fitting and all relevant quantitative MR image voxel by voxel based analysis for the construction of the parametric T2 maps was performed using in-house software (QMRI, Kalaitzakis et al. under review). T2 parameter values were obtained for each voxel by fitting a triple exponential decay curve to the signal intensity of the corresponding voxels in the image

stack vs. TE time (voxel T2 relaxation decay curve). The assumption of the three compartment water model present in brain tissues was followed (MacKay et al. 1994; Raj et al. 2014). Under this assumption three distinct water T2 pools exist in brain tissues. These consist of a fast relaxing myelin water pool, a slower-relaxing intra/extra cellular water pool and a very long relaxing cerebrospinal fluid (CSF) pool. These three water pools are characterized by three distinct T2 relaxation times, $T2_S$ (Short or fast relaxing), $T2_L$ (Long or slow relaxing) and $T2_{CSF}$ (Very long or very slow relaxing) components, respectively. $T2_S$, $T2_L$, and $T2_{CSF}$ parameter maps were obtained utilizing a Multi Exponential Non Linear fitting regression method (MENL) which was based on a standard nonlinear fitting least squares minimization algorithm (Fig 2.1) (Marquardt et al. 1963). The relative T2 relaxation decay curves were analyzed, by assuming a three compartment multi-exponential decay behavior in the presence of a homogeneous image stack background (Bg) which was subtracted from the actual signal data (S).

Parametric imaging voxel signals denoted $PD_{S(x,y)}$, $PD_{L(x,y)}$, $PD_{CSF(x,y)}$, $T2_{S(x,y)}$, $T2_{L(x,y)}$ and $T2_{CSF(x,y)}$ were determined by fitting the following equation on a voxel by voxel basis (x,y):

$$S_{(x,y)}(TE) - Bg = \boldsymbol{PD_{S(x,y)}}\, e^{\frac{-TE}{T2_{S(x,y)}}} + \boldsymbol{PD_{L(x,y)}}\, e^{\frac{-TE}{T2_{L(x,y)}}} + \boldsymbol{PD_{CSF(x,y)}}\, e^{\frac{-TE}{T2_{CSF(x,y)}}} \quad (1)$$

Where, (TE) represents the time set of 32 symmetrically repeatable echoes that were applied, $[S_{(xy)}\ (TE)]$ is the corresponding voxel signals and (Bg) is the offset background signal present in all images throughout the whole image stack. (Bg) value was obtained from a non-signal producing rectangular ROI outside the main anatomical image depicting area, avoiding any prominent artifact. PD and T2 values were concurrently estimated as a result of numerical fitting on equation (1). More specifically, $T2_{S(x,y)}$, $T2_{L(x,y)}$ and $T2_{CSF(x,y)}$ correspond to the three compartment relaxation times measured for each voxel as a result of the fit. $PD_{S(x,y)}$, $PD_{L(x,y)}$, $PD_{CSF(x,y)}$,correspond to the three compartment proton density uncorrected voxel signals obtained from the fit. The short T2 relaxation times ($T2_{S(x,y)}$) were calculated from the fit under

the constrain of ranging their values from 10 up to 50 ms. The long T2 relaxation times ($T2_{L(x,y)}$) were calculated from the fit with their values ranging from 50 up to 200 ms and the very long T2 relaxation times ($T2_{CSF(x,y)}$) were calculated from the fit with their values being greater than 200 ms.

For the final measurement of Myelin Water Fraction (MWF) some valuable pre-processing is necessary. The net parametric $PD_{(x,y)}$ voxel signal for the three compartments (PD_S, PD_L and PD_{CSF}) was calculated from the following equations :

$$Net\ \boldsymbol{PD}_{\boldsymbol{CSF}(x,y)} = \boldsymbol{PD}_{\boldsymbol{CSF}(x,y)}$$

$$Net\ \boldsymbol{PD}_{\boldsymbol{L}(x,y)} = \boldsymbol{PD}_{\boldsymbol{L}(x,y)} - Net\ \boldsymbol{PD}_{\boldsymbol{CSF}(x,y)}$$

$$Net\ \boldsymbol{PD}_{\boldsymbol{S}(x,y)} = \boldsymbol{PD}_{\boldsymbol{S}(x,y)} - (Net\ \boldsymbol{PD}_{\boldsymbol{L}(x,y)} + Net\ \boldsymbol{PD}_{\boldsymbol{CSF}(x,y)})$$

T1 corrections on the voxel net signals will outcome an expression for the voxel signals that should be obtained at infinite TR. The method for T1 signal corrections on the T2 decay relaxation curves was firstly introduced by Whittall et al. (1997). For the purposes of this study, the necessary normal T1 values for the application of T1 correction factors for the three myelin water compartments were obtained from the literature Deoni et al. (2003). The final infinite TR voxel signals for the three compartments are given by the equations.

$$Net\ \boldsymbol{PD}_{\boldsymbol{S}(x,y)}(\infty) = Net\ \boldsymbol{PD}_{\boldsymbol{S}(x,y)} \,/\, \mathbf{(1\text{-}e^{-TR\,/\,T1_S})}$$

$$Net\ \boldsymbol{PD}_{\boldsymbol{L}(x,y)}(\infty) = Net\ \boldsymbol{PD}_{\boldsymbol{L}(x,y)} \,/\, \mathbf{(1\text{-}e^{-TR\,/\,T1_L})}$$

$$Net\ \boldsymbol{PD}_{\boldsymbol{CSF}(x,y)}(\infty) = Net\ \boldsymbol{PD}_{\boldsymbol{CSF}(x,y)} \,/\, \mathbf{(1\text{-}e^{-TR\,/\,T1_{CSF}})}$$

$T1_S$ was set to 465 ms, $T1_L$ was set to 1070 ms (Deoni et al. 2003) and $T1_{CSF}$ was set to 4500 ms for the operating static magnetic field of 1.5T. These values were used in all subsequent

data analysis. At a final step Myelin Water Fraction (MWF) was computed as the ratio of the T1 corrected net myelin water signal amplitude $Net\ PD_{S(x,y)}(\infty)$ to that of total T1 corrected net water signal amplitude on a voxel by voxel (x,y) basis according to the equation:

$$MWF = \frac{NetPD_{S(x,y)}(\infty)}{NetPD_{S(x,y)}(\infty) + NetPD_{L(x,y)}(\infty) + NetPD_{CSF(x,y)}(\infty)}$$

The method of measurement of MWF as presented in this study is based on the initial assumption of three basic compartment model (MacKay et al., 1994; Raj et al. 2014). Its mathematical formulation has its origin on a technique initially proposed by Vasilescu et al (1978). Relevant methods are presented in the literature by Du et al. (2007) on a post-mortem study and completed by Hwang et al. (2010) on a standard clinical study. In this mathematical rationale no distribution fitting through the regularized Non Negative Least Squares (NNLS) algorithm is necessary. Data were simply fitted on a triple compartment T2 decay scheme utilizing MESE methods with prolonged TE ranges.

Measurements of short T2, long T2 and MWF values were obtained within 12 NAWM areas in patients and healthy controls, involving periventricular and deep frontal, parietal, temporal and occipital WM, separately in each hemisphere, as well as the genu and splenium of the CC. Regions of interest (ROIs) were drawn at the MESE sequence, to ensure detection and exclusion of even minute WMH, and then automatically transferred to the short T2, long T2 and MWF maps (Figure 2.2 & 2.3). In order to reduce measurement error, three short T2, long T2, and MWF measurements were obtained from each of the different WM areas, which were then averaged. ROIs were placed at the same cross-sectional positions and the same locations in patients and healthy controls.

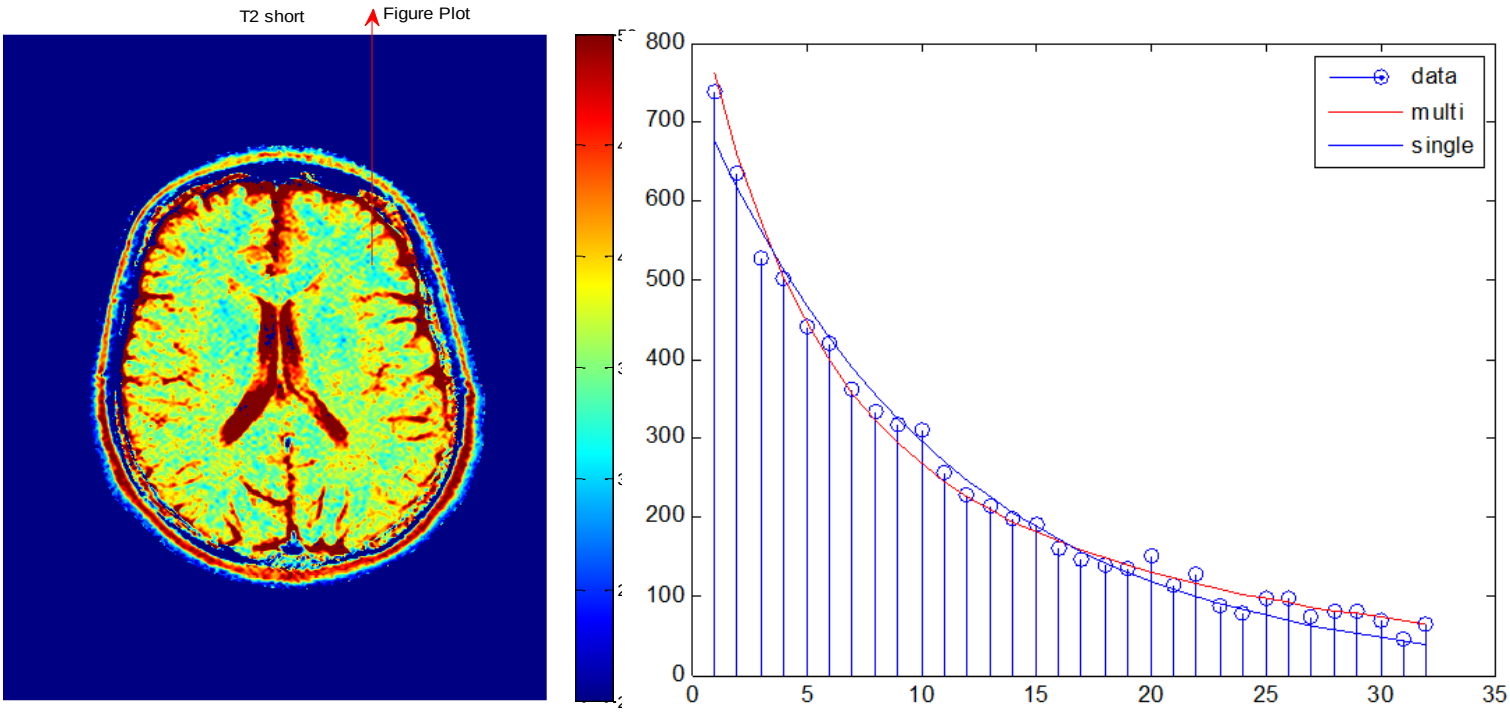
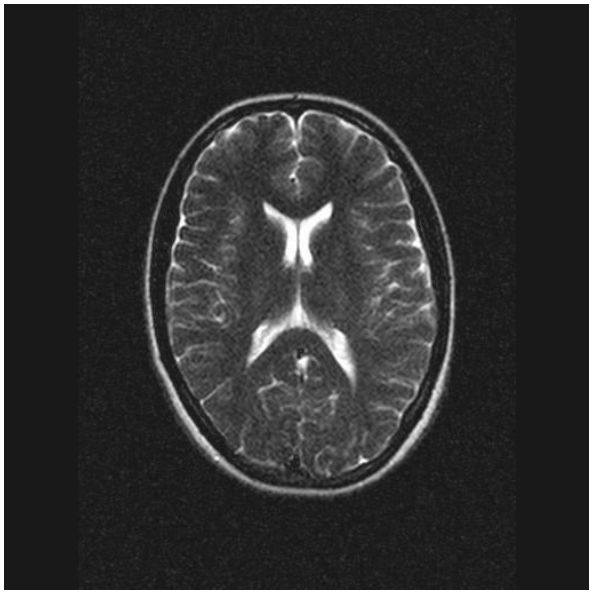
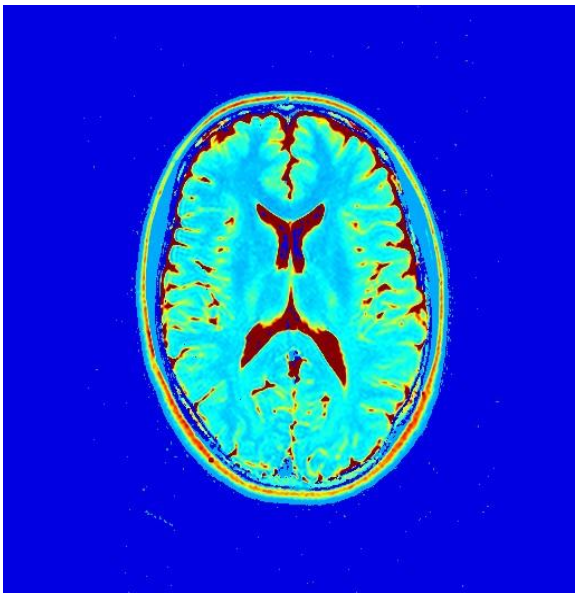


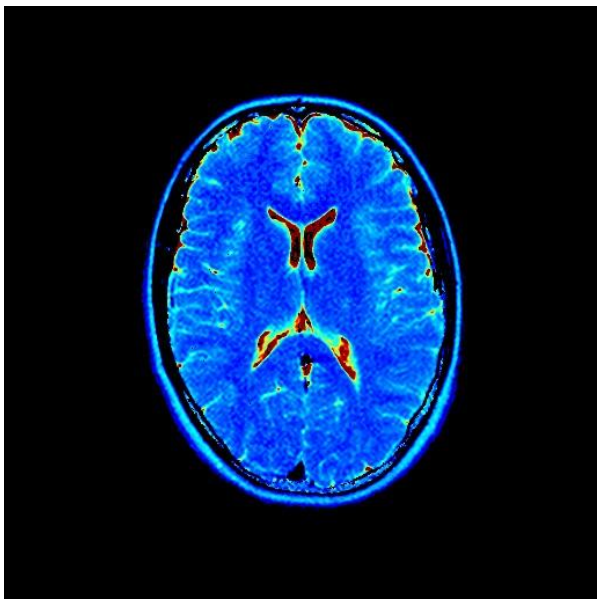
Fig 2.1. The Multi Exponential Non Linear model (MENL) includes at least two molecular environments represented by a multi exponential T2 relaxation decay curve-parameter estimation. Left-hand panel: The base of the red arrow indicates a specific sampling point on a single-subject MWF map. Right-hand panel: Blue circles represent vales obtained at 32 symmetrically repeated spin echoes. The data were fitted using a single-compartment model (blue line) and a multi-compartment model (red line) using the non-linear Levenberg-Marquant algorithm. The observed data are best-fitted by the latter model, especially at the lowest and highest values ranges.



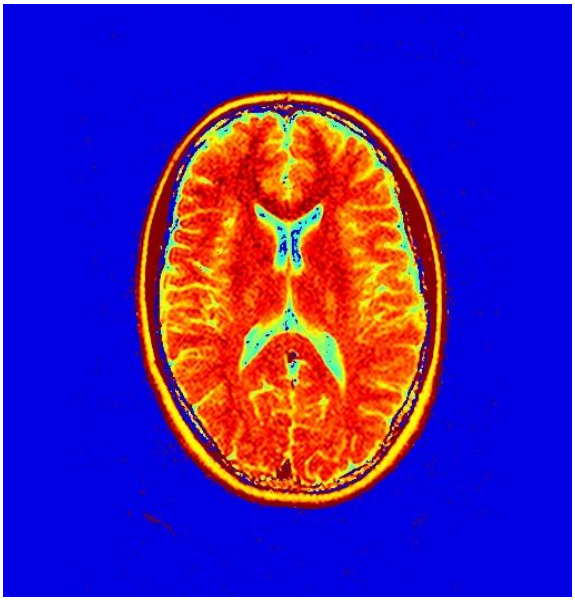
Multi-echo spin echo (MESE) sequence



Long T2 Map



Short T2 Map



MWF Map

Fig 2.2. Construction of the parametric T2 maps using in house software

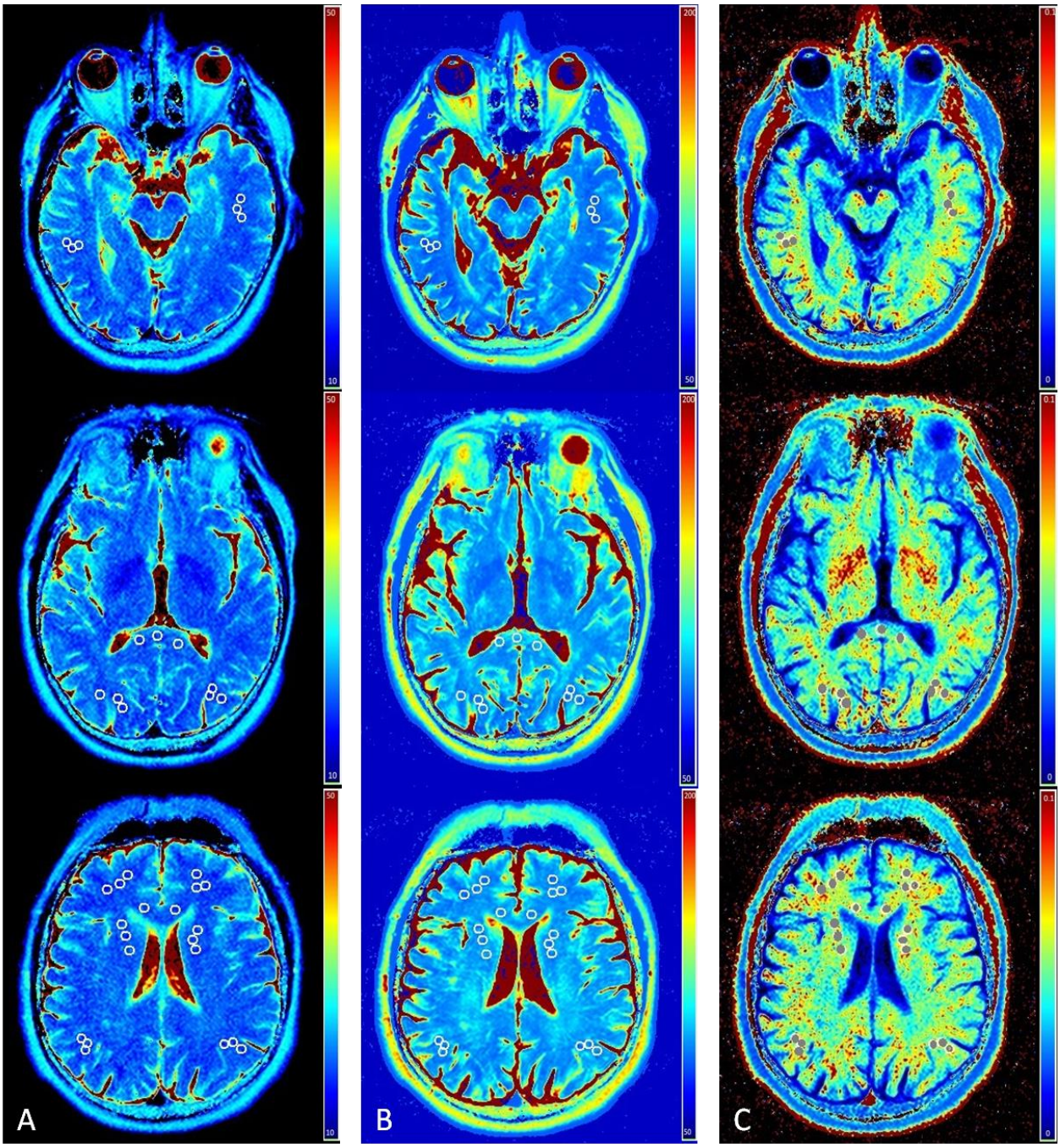


Fig 2.3. Examples of sampling locations displayed on axial sections of Long T2 (A), Short T2 (B) and MWF (C) maps from a single participant.

Measuring MWF could be achieved with other techniques such as mcDESPOT, which does not rely on measurements of the T_2 decay curve, and is based in multicomponent driven equilibrium single-pulse observation of T_1 and T_2. This technique is based on an extension of the driven equilibrium single-pulse observation of T_1 (DESPOT1) and driven-equilibrium single-pulse observation of T_2 (DESPOT2) techniques which are based on spoiled gradient recalled echo (SPGR) and balanced steady-state free precession (bSSFP). SPGR-based T_1 method involves the combination of SPGR images to generate a T_1 –dependent signal curve as a function of flip angle. In bSSFP sequence both longitudinal and transverse magnetization are bought into dynamic equilibrium through the application of "a" pulses and fully refocusing the transverse magnetization prior to each excitation pulse. mcDespot involves fitting the data obtained from $DESPOT_1$ and $DESPOT_2$ sequences with a two-pool model of longitudinal and transerve relaxation that includes intercompartemental water exchange (fig 2.4).

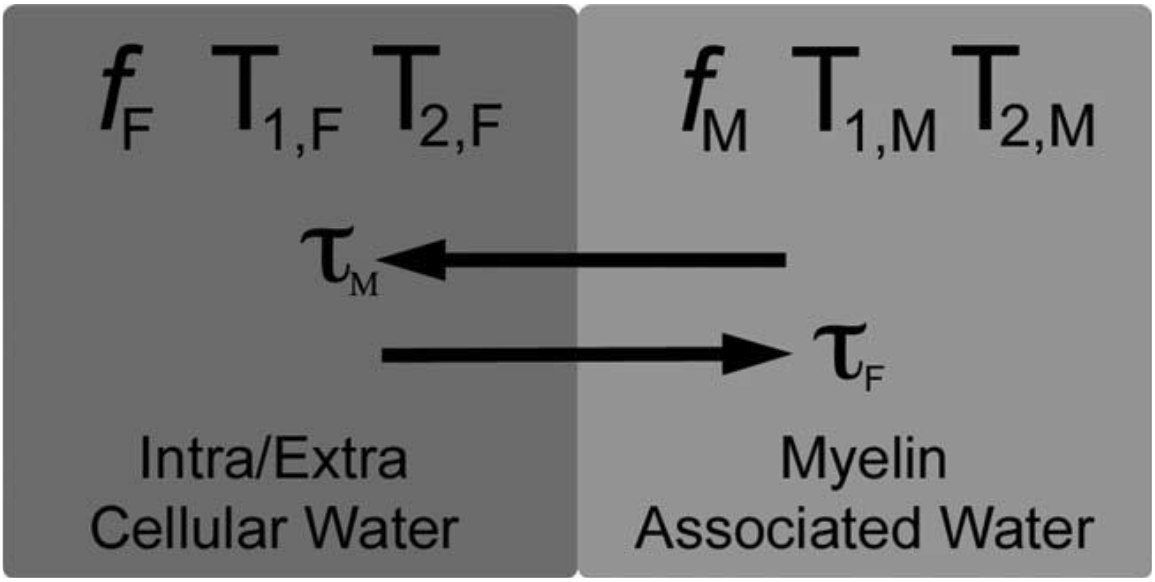


Fig 2.4. Two-pool model: f_F and f_M are the free and myelin water fractions. T_{1F} and T_{1M} are the T_1 times of the free and myelin water pools, T_{2F} and T_{2M} are the T_2 times of the free and myelin water pools and τ is the mean residence time of the free and myelin water pools.

In this model, brain tissue is composed of two water compartments, free IE water and water trapped between the lipid bilayers of the myelin sheath, in exchange with one another. The main advantage of mcDESPOT is the whole brain ME T_1 and T_2 quantification in 16 to 30 min with high SNR efficiency. Also the inclusion of intercompartmental water exchange between two pools through this technique could be helpful for two reasons, firstly, the mean residence time of myelin-associated water has been found to be a potential measure of myelin thickness (Dula e t al. 2003; Harkins et al. 2012) and secondly, the presence of intercompartmental water exchange may impact MWF quantification. In addition, Lankford et al. (2013), showed that mcDESPOT signals are not able to provide parameter estimates with useful levels of precision. This can be improved by constraining certain parameters, such as the transverse relaxation rates and water exchange. Another limitation of mcDESPOT is the acquisition time which is estimated to 16 to 30 min for each patient only for this sequence and as anyone can assume for AD patients this is a significant pitfall because the time for all the MRI examination increases too much and as a result people with severe or mild cognitive complaints won't be cooperative.

2.4 Neuropsychological assessment

Neuropsychological assessment was performed by two trained neuropsychologists affiliated with the study. Specifically, verbal short-term and working memory were assessed through the Digits Forward and Digits Reverse subtests from the Greek Memory Scale, respectively (Simos, Papastefanakis, Panou, & Kasselimis, 2011). Verbal episodic memory was assessed using the Passage Memory test from the Greek Memory Scale and the Greek adaptation of the Rey Auditory Verbal Learning Test (AVLT, Constantinidou, Zaganas, Papastefanakis, Kasselimis, Nidos, Simos, 2014; Geffen & Geffen, 2000). Visuospatial episodic memory through the 5-min delayed recall of the Taylor Complex Figure (TCF; Anita Hubley & Tremblay, 2002). Memory for autobiographic events was evaluated through the 16-item

Autobiographic Memory Scale (Simos, Papastefanakis, Panou, & Kasselimis, 2011). Orientation to place and time and memory for public facts was index by performance on the Orientation subscale from the Mattis Dementia Rating Scale (DRS; Coblent et al., 1973; Katsarou et al., 2010). Visuoconstructive ability was evaluated via performance on the TCF Copy subtest. The short forms of the Greek adaptations of the Boston Naming and Peabody Picture Vocabulary tests (BNT & PPVT-R; Simos, Kasselimis, & Mouzaki, 2011) provided measures of crystallized lexical knowledge and naming capacity, whereas the Semantic Verbal Fluency test (SVFT; Kosmidis, Vlahou, Panagiotaki, & Kiosseoglou, 2004), provided additional information on strategic retrieval capacity from semantic memory. Visuomotor processing speed and sustained attention were assessed through the Trail Making Test Part A (Zalonis, Kararizou, Triantafyllou, Kapaki, Papageorgiou, Sgouropoulos, & Vassilopoulos, 2008), and the Symbol Digit Modality Test (SDMT; Constantinidou, Christodoulou, & Prokopiou, 2012). Executive functions were evaluated using the Trail Making Test Part B (set-shifting ability), the Initiation/Perseveration subtests from DRS, and the General Ability Measure for Adults (GAMA; Naglieri & Bardos, 1997; problem solving ability). Depressive symptomatology and trait anxiety were assessed through the Greek adaptations of the Center of Epidemiological Studies Depression Scale (CESD; Fountoulakis et al., 2001; Radloff, 1977) and the Spielberger State-Trait Anxiety Inventory (Fountoulakis et al., 2006; Spielberger, 1983), respectively.

Finally, daily functional capacity, behavioral disturbances, and fluctuations in attention/alertness were assessed using the 13-item Greek IADL scale (Simos, Papastefanakis, Panou, & Kasselimis, 2011), the Cambridge Behavioral Inventory-Revised (Wedderburn et al., 2008), and the Mayo Fluctuations Scale (Ferman et al., 2004) administered in the form of structured interview to the participants' closest relative or caregiver.

For diagnostic purposes individual performance on all neuropsychological tests was converted to age- and education-adjusted standard scores using published norms developed for

the Greek population. In particular, conversion to age- and education-adjusted standard scores for all cognitive measures were computed using a regression method based on data from a semi-random community sample of 450 persons aged 16-90 years.

2.5 Statistical analyses

Specific Aims (i-ii): Diagnostic group differences on average T2 values. Overall diagnostic group differences were examined through two-way ANCOVAs, with Diagnostic Group (NI, MCI, Dementia) and Gender as between-subjects factors, on Long T2, Short T2 and MWF values in each of the 10 ROIs with age and education as covariates. The Bonferroni method was used to control for family-wise Type I error setting the alpha level to .05/10 = .005. Supplementary analyses assessed regional differences (frontal vs. temporal, frontal vs. parietal, and frontal vs. occipital) as a function of diagnostic group (controlling for age and education).

Specific Aim (iii): Associations between age and T2/MWF values. The type of association between participant age (linear or quadratic) on Long T2, Short T2 and MWF values in each ROI was assessed through linear regression analyses with gender and education level serving as covariates.

Diagnostic group differences in the magnitude of the age effect in each region were assessed through moderated regression models with age as the independent variable, each Long T2, Short T2, or MWF value as the dependent variable and Diagnostic Group as a categorical moderator (i.e., with a value of 0 indicating the NI group, 1 for the MCI group, and 2 for the dementia group) with gender and education level as covariates.

Differences between anterior (i.e., frontal NAWM) and posterior NAWM regions (temporal, parietal, or occipital) in the magnitude of the effect of age on T2/MWF values were assessed in the context of moderated regression analyses with age as the independent variable,

each Long T2, Short T2, or MWF values as the dependent variable and region as a categorical moderator (i.e., with a value of 0 indicating a posterior region and a values of 1 indicating frontal NWAM). These analyses were performed separately for each diagnostic group with gender and education level as covariates.

Specific Aims (iv-v): The degree of the association between T2/MWF values and neuropsychological test scores varies significantly across diagnostic groups. In preliminary analyses both bivariate and partial correlations (controlling for age, education and gender) were computed between T2/MWF values and neuropsychological test scores to identify brain regions where T2/MWF values may serve as predictors of cognitive performance. These aims were addressed in the context of moderated regression analyses with Short T2, Long T2, or MWF as the independent variable, test performance ((raw values; Specific Aim iv) or depression symptomatology (Specific Aim v) as the dependent variable and Diagnostic Group as a categorical moderator (i.e., with a value of 0 indicating the NI group, 1 for the MCI group, and 2 for the dementia group) with gender and education level as covariates.

Specific Aims (vi-vii): Indirect effects of age on neuropsychiatric function through changes in T2/MWF values. The main hypotheses were explored in the context of mediated regression models where age served as the independent variable, neuropsychological test scores (raw values) as the dependent variable, and T2/MWF values in each of the 10 ROIs as the mediating variable. Education level in years and gender were entered in each model as covariates. Evidence of partial mediation would be found if the unstandardized regression coefficients of age on test scores controlling for T2/MWF values (direct effect) and the corresponding coefficients for the indirect (mediated) effect were significantly different from zero as indicated by bootstrapped 95% confidence intervals that did not include zero. Evidence of complete mediation would be found if the coefficients for the indirect effect were found to be significant in the absence of corresponding significant direct effects.

Mediation analyses were performed using SPSS macros developed by Hayes (2013; model 4). All statistical analyses were performed using IBM SPSS v. 20.

3 Results

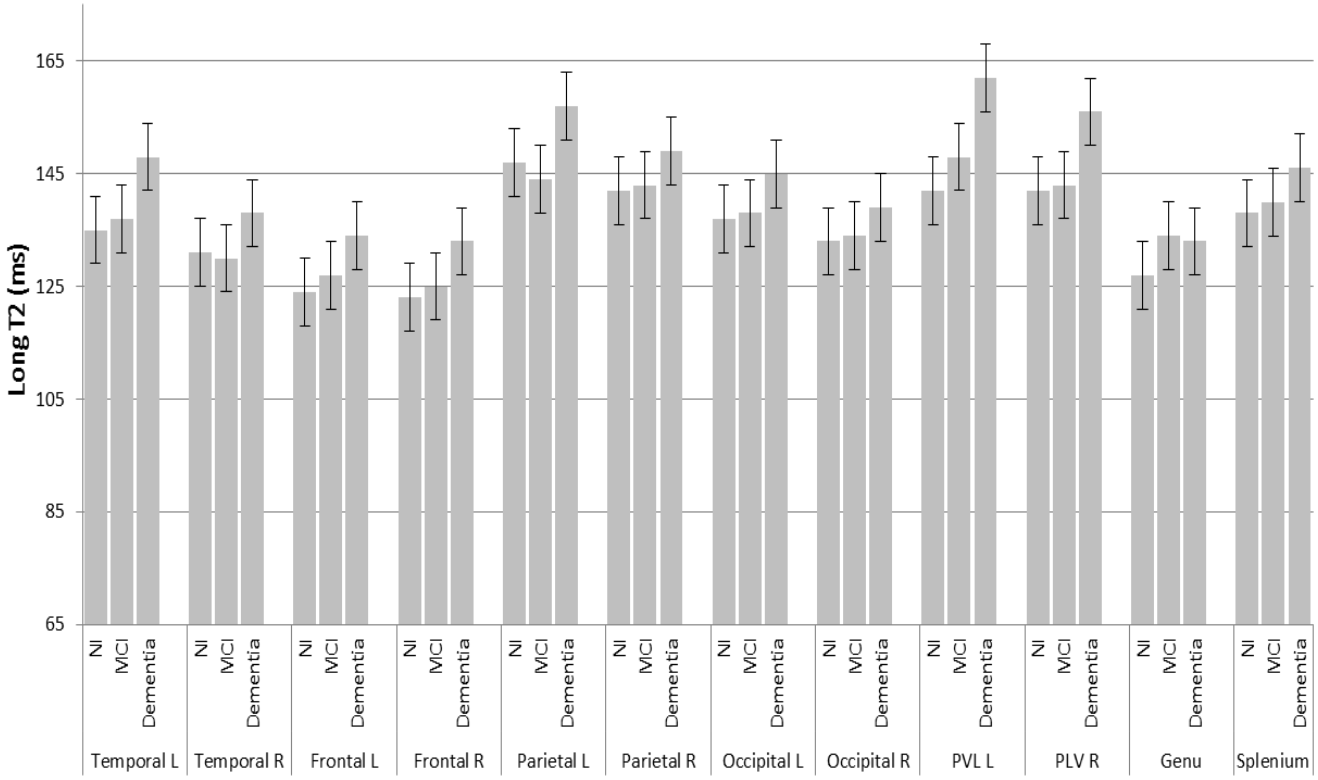
3.1. Specific Aims (i-ii): Diagnostic Group differences on average T2/MWF values

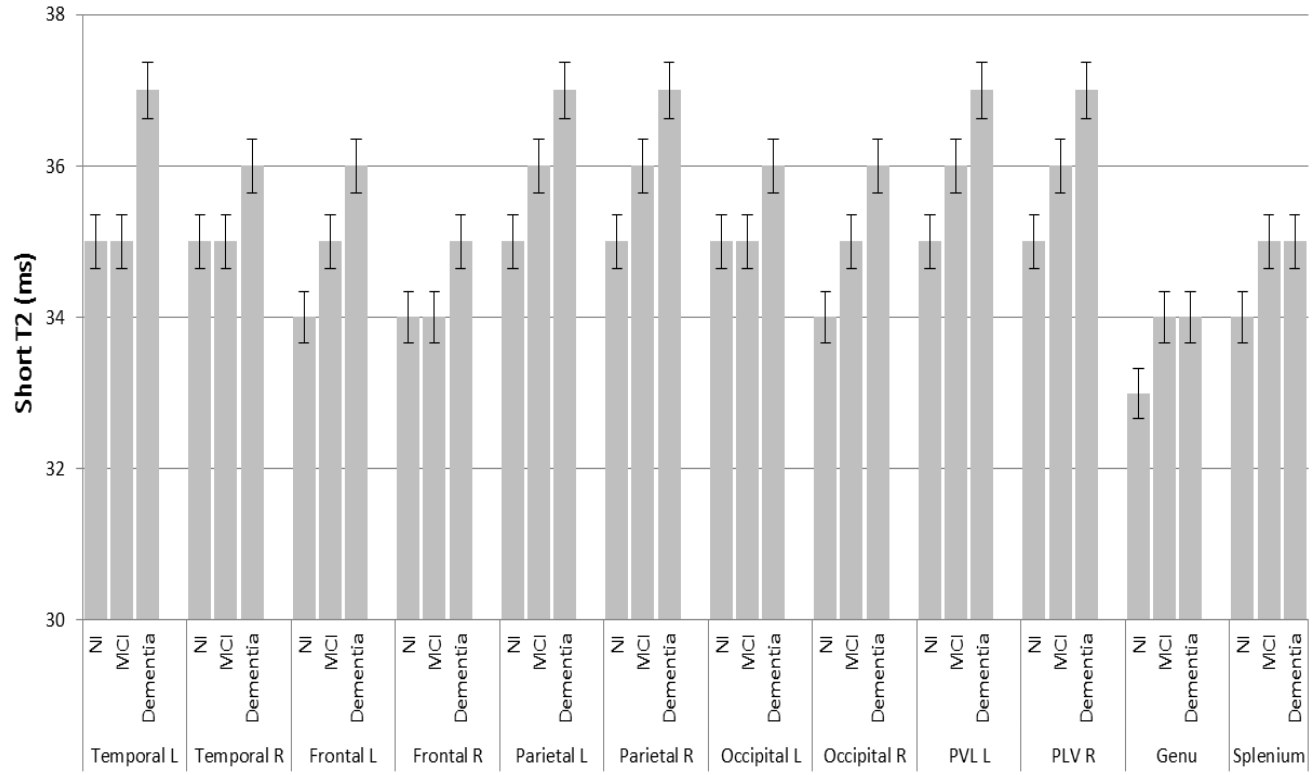
With respect to T2 values, main effects of Diagnostic Group (controlling for age and education) were found in the left temporal, $F(2,98) = 6.10$, $p = .003$, left parietal, $F(2,98) = 7.06$, $p = .001$, and in the periventricular NAWM bilaterally; left: $F(2,98) = 5.58$, $p = .005$, right: $F(2,98) = 6.71$, $p = .002$, revealing higher long T2 values in the Dementia as compared to both NI and MCI groups. Average T2 and MWF values by ROI and diagnostic group are displayed in Figure 3.1. Results of pairwise comparisons computed to explore these effects are displayed in Table 3.1. Main effects of Diagnostic Group were found on Short T2 values measured in the left temporal, $F(2,98) = 4.98$, $p = .005$, parietal, $F(2,98) = 6.68$, $p = .002$, and periventricular NAWM, $F(2,98) = 5.24$, $p = .006$. Finally, significant main effects of Diagnostic Group indicating reduced values in the Dementia group were found in the left temporal, $F(2,98) = 6.57$, $p = .002$, left periventricular, $F(2,98) = 6.52$, $p = .002$, and right periventricular NAWM, $F(2,98) = 6.14$, $p = .003$.

Table 3.1. Results of pairwise comparisons associated with main effects of Diagnostic Group.

		Non-Impaired vs. MCI		Non-Impaired vs. Dementia		MCI vs. Dementia	
		Contrast	*p*	Contrast	*p*	Contrast	*p*
Long T2	Temporal L	1.08	.5	**5.62**	**.01**	**6.16**	**.002**
	Parietal L	4.91	.1	6.03	.1	**8.49**	**.005**
	PVL L	0.24	.9	**8.59**	**.005**	**8.71**	**.002**
	PVL R	3.52	.2	6.27	.05	**8.03**	**.003**
Short T2	Temporal L	0.02	.9	**0.83**	**.01**	**0.67**	**.005**
	Parietal L	0.03	.9	**0.88**	**.01**	**0.95**	**.001**
	PVL L	0.10	.7	**0.85**	**.01**	**0.88**	**.004**
MWF	Temporal L	0.002	.4	**0.007**	**.01**	**0.008**	**.001**
	PVL L	0.001	.7	**0.010**	**.009**	**0.011**	**.001**
	PVL R	0.004	.2	0.007	.07	**0.009**	**.005**

Abbreviations; PVL: Periventricular, R: Right, L: Left. Significant associations at Bonferroni-corrected alpha = .017 are shown in bold.


Long T2 (ms)
165
145
125
105
85
65
NI
MCI
Dementia
Temporal L
Temporal R
Frontal L
Frontal R
Parietal L
Parietal R
Occipital L
Occipital R
PVL L
PLV R
Genu
Splenium


Short T2 (ms)
38
36
34
32
30
NI
MCI
Dementia
Temporal L
Temporal R
Frontal L
Frontal R
Parietal L
Parietal R
Occipital L
Occipital R
PVL L
PLV R
Genu
Splenium

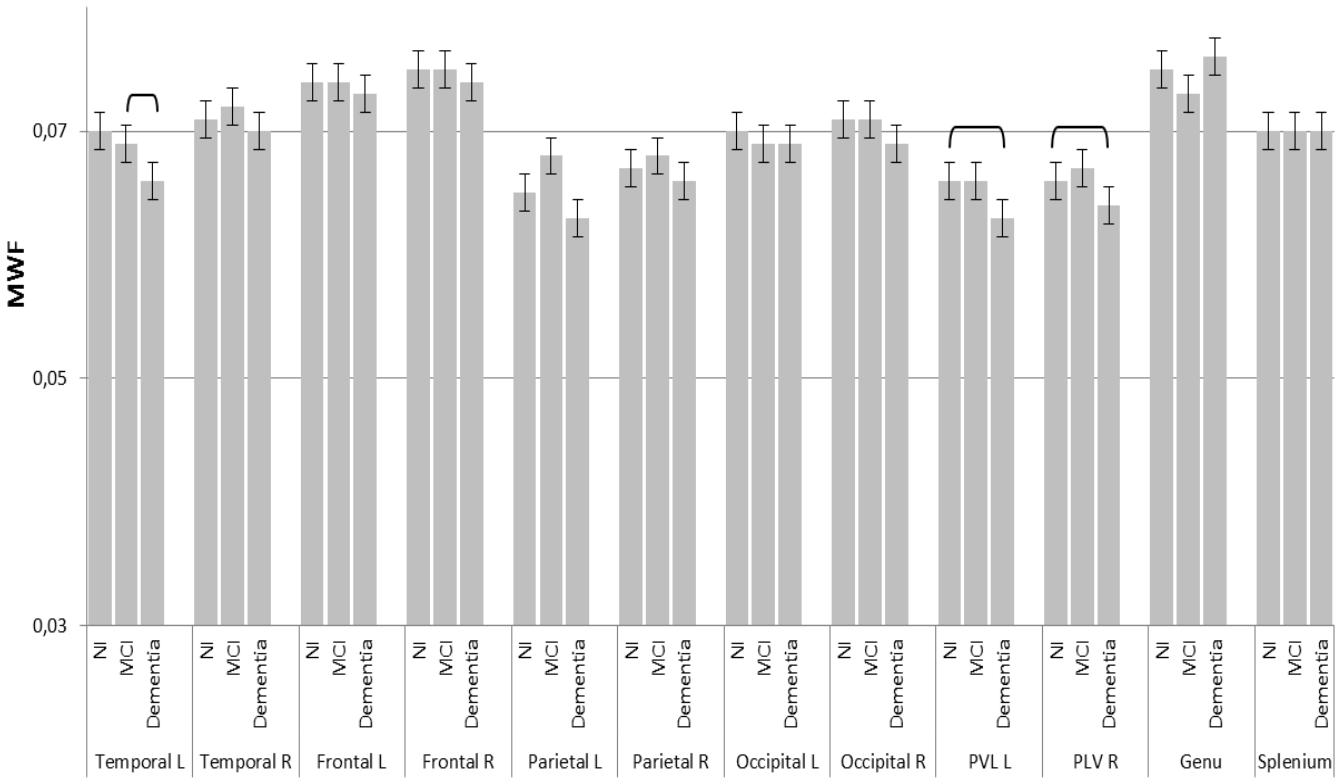

MWF
0,07
0,05
0,03
NI
MCI
Dementia
Temporal L
Temporal R
Frontal L
Frontal R
Parietal L
Parietal R
Occipital L
Occipital R
PVL L
PLV R
Genu
Splenium

Figure 3.1. Age- and education-adjusted average Long T2 (upper panel), Short T2 values (middle panel) and MWF (lower panel) in 10 NAWM ROIs for cognitively non-impaired (NI), MCI and Dementia patients. Significant differences between NI/MCI and dementia groups are shown by brackets. Bars indicate standard error.

Supplementary analyses assessing regional differences revealed higher Long T2 values in left temporal: $F(1,100) = 8.58$, $p = .004$, right temporal: $F(1,100) = 7.42$, $p = .008$, left parietal: $F(1,100) = 12.01$, $p = .001$, right parietal: $F(1,100) = 15.53$, $p = .0001$, left occipital: $F(1,100) = 6.72$, $p = .011$, and right occipital NWAM: $F(1,100) = 6.86$, $p = .01$, as compared to the corresponding frontal lobe. Similar trends were found for short T2 in left temporal: $F(1,100) = 7.71$, $p = .007$, right temporal: $F(1,100) = 6.98$, $p = .01$, left parietal: $F(1,100) = 6.62$, $p = .012$, and right parietal: $F(1,100) = 7.49$, $p = .007$. Lower MWF values compared to corresponding frontal NWAM sites were found in left temporal: $F(1,100) = 10.51$, $p = .002$, left parietal: $F(1,100) = 14.64$, $p = .0001$, right parietal: $F(1,100) = 17.64$, $p = .0001$, left occipital: $F(1,100) = 11.27$, $p = .001$, and right occipital NWAM: $F(1,100) = 14.31$, $p = .0001$. The magnitude of the anterior-posterior gradient however did not vary significantly across groups as indicated by non-significant Region by Group interactions ($p > .05$).

3.2 Specific Aim (iii): Overall association between age and T2/MWF values

In all cases quadratic trends of Long T2, Short T2 and MWF values with age did not approach significance ($p > .3$). Significant linear increases in *Long T2* values with age (controlling for gender and education level) were found in the majority of areas examined in the MCI group and in left temporal, left parietal and bilateral frontal and occipital regions in the NI group (see Table 3.2). Among patients with dementia a significant age-related increase in Long T2 values was restricted to the left temporal lobe. Education level did not exert an independent

effect on Long T2 values. Significant (positive) age effects on *Short T2* values were found in virtually all ROIs in the NI and MCI groups, and in the left periventricular NAWM in the dementia group (see Table 3.3). Finally, advancing age was associated with reduced *MWF* in all ROIs, although this tendency reached significant in bilateral temporal and frontal and in left occipital NWAM among NI and MCI patients (see Table 3.4). The effect of age was also significant in right parietal NWAM in the NI group, and in right occipital and bilateral periventricular NWAM among MCI patients. Age effects on MWF failed to reach significance in the dementia group.

Table 3.2. Unstandardized regression coefficients reflecting the contribution of age to Long T2 values, controlling for gender and education level, by clinical group.

	Non-Impaired (n=35)			MCI (n=43)			Dementia (n=25)		
	B	*SE*	*p*	*B*	*SE*	*p*	*B*	*SE*	*p*
Temporal L	**.625**	**.179**	**.002**	**.640**	**.165**	**.0001**	**1.077**	**.273**	**.001**
Temporal R	.568	.193	.006	.434	.146	.010	.510	.272	.08
Frontal L	**.782**	**.151**	**.0001**	**.974**	**.189**	**.0001**	.483	.366	.2
Frontal R	**.703**	**.150**	**.0001**	**.905**	**.185**	**.0001**	.661	.384	.1
Occipital L	**.820**	**.199**	**.0001**	**.676**	**.142**	**.0001**	.720	.286	.02
Occipital R	**.593**	**.185**	**.003**	**.479**	**.149**	**.003**	.503	.330	.1
Parietal L	.665	.339	.06	**.944**	**.276**	**.002**	.223	.330	.4
Parietal R	.699	.267	.014	.696	.268	.02	.150	.553	.7
PVL L	.595	.232	.016	**1.265**	**.206**	**.0001**	.926	.495	.08
PVL R	.478	.232	.05	**1.141**	**.213**	**.0001**	.473	.433	.3

Abbreviations; PVL: Periventricular, R: Right, L: Left. Significant coefficients at Bonferroni-corrected alpha = .005 are shown in bold.

Table 3.3. Unstandardized regression coefficients reflecting the contribution of age to Short T2 values, controlling for gender and education level, by clinical group.

	Non-Impaired (n=35)			MCI (n=43)			Dementia (n=25)		
	B	*SE*	*p*	*B*	*SE*	*p*	*B*	*SE*	*p*
Temporal L	**.089**	**.022**	**.0001**	**.088**	**.018**	**.0001**	.106	.036	.008
Temporal R	**.098**	**.023**	**.0001**	**.076**	**.019**	**.0001**	.078	.035	.037
Frontal L	**.134**	**.030**	**.0001**	**.115**	**.026**	**.0001**	.050	.043	.3
Frontal R	**.110**	**.029**	**.001**	**.131**	**.026**	**.0001**	.059	.054	.3
Occipital L	**.101**	**.023**	**.0001**	**.077**	**.017**	**.0001**	.067	.031	.04
Occipital R	**.105**	**.022**	**.0001**	**.087**	**.021**	**.0001**	.080	.033	.02
Parietal L	.084	.030	.01	**.097**	**.023**	**.0001**	.040	.047	.4
Parietal R	**.126**	**.025**	**.0001**	**.085**	**.020**	**.0001**	.039	.057	.5
PVL L	**.105**	**..025**	**.0001**	**.090**	**.026**	**.001**	**.149**	**.046**	**.004**
PVL R	**.121**	**.028**	**.0001**	**.089**	**.025**	**.001**	.086	.046	.07

Abbreviations; PVL: Periventricular, R: Right, L: Left. Significant coefficients at Bonferroni-corrected alpha = .005 are shown in bold.

Table 3.4. Unstandardized regression coefficients reflecting the contribution of age to MWF values, controlling for gender and education level, by clinical group.

	Non-Impaired (n=35)			MCI (n=43)			Dementia (n=25)		
	B	*SE*	*p*	*B*	*SE*	*p*	*B*	*SE*	*p*
Temporal L	**-.001**	**.0001**	**.0001**	**-.001**	**.0001**	**.0001**	-.002	.0001	.008
Temporal R	**-.001**	**.0001**	**.002**	**-.001**	**.0001**	**.0001**	-.001	.0001	.027
Frontal L	**-.001**	**.0001**	**.001**	**-.001**	**.0001**	**.0001**	-.001	.001	.2
Frontal R	**-.001**	**.0001**	**.0001**	**-.001**	**.0001**	**.0001**	-.001	.001	.2
Occipital L	**-.001**	**.0001**	**.0001**	**-.001**	**.0001**	**.0001**	-.0001	.0001	.05
Occipital R	-.001	.0001	.025	**-.001**	**.0001**	**.0001**	-.001	.0001	.1
Parietal L	-.001	.0001	.021	-.001	.0001	.006	-.001	.001	.8
Parietal R	**-.001**	**.0001**	**.002**	-.001	.0001	.01	-.001	.001	.8
PVL L	-.001	.0001	.008	**-.001**	**.0001**	**.0001**	-.001	.001	.02
PVL R	-.001	.0001	.009	**-.001**	**.0001**	**.0001**	-.001	.001	.2

Abbreviations; PVL: Periventricular, R: Right, L: Left. Significant coefficients at Bonferroni-corrected alpha = .005 are shown in bold.

Effect of diagnostic group on the degree of age-related increase in T2/MWF values. Although the magnitude of the effect of age on T2 and MWF values varied somewhat between diagnostic groups, the Age x Diagnostic Group interaction term failed to reach significance in all cases ($p > .11$).

Anterior-posterior gradient in the age effects on T2/MWF values. As shown in Table 3.5, significant Age by Region (Anterior vs. Posterior) interactions were found in several occasions,

especially among NI and MCI participants. Inspection of unstandardized regression coefficients indicates that the effect of age was restricted to either posterior or anterior NWAM depending on the index considered each time. With respect to the NI group, significant increases in Long T2 were restricted to frontal NWAM bilaterally, whereas increased Short values with age were restricted to posterior regions (left temporal, right parietal, and bilateral occipital NAWM). In this group, MWF decreased significantly with age only in frontal NAWM, bilaterally.

In the MCI group, significant age-related increases in T2 values were found in largely the same regions as in the NI group (anterior [right frontal] for Long T2 and posterior NAWM [left parietal and temporal bilaterally] for Short T2). In this group, the rate of age-related reduction in MWF was similar (and significant) in anterior (frontal) as well as in posterior (temporal and parietal) NAWM regions. Finally, significant age-related changes were restricted to the left temporal NAWM (increasing Long T2 and decreasing MWF).

Table 3.5. Unstandardized regression coefficients reflecting the contribution of age to Long T2 values in NAWM regions displaying anterior-posterior gradient, by clinical group.

			Non-Impaired (n=35)			MCI (n=43)			Dementia (n=25)		
Hemisphere	Gradient	Region	*B*	*SE*	*p*	*B*	*SE*	*p*	*B*	*SE*	*p*
Left	T-F	T	0.358	0.187	0.06		--[1]		**0.774**	**0.193**	**0.0002**
		F	**0.870**	**0.191**	**0.0001**		--[1]		0.428	0.304	0.2
	P-F	P	0.409	0.289	0.2		--[1]			--[2]	
		F	**0.870**	**0.191**	**0.0001**		--[1]			--[2]	
Right	T-F	T	0.567	0.241	0.02	0.468	0.195	0.02		--[2]	
		F	**0.827**	**0.213**	**0.0002**	**0.719**	**0.156**	**0.0001**		--[2]	
	P-F	P	0.338	0.245	0.2		--[1]			--[2]	
		F	**0.827**	**0.213**	**0.0002**		--[1]			--[2]	

Significant coefficients at Bonferroni-corrected alpha = .0083 are shown in bold. Gender and education level served as covariates in all analyses. Abbreviations; T: Temporal, F: Frontal, O: Occipital, P: Parietal.

[1]The effect of age was significant at both anterior and posterior sites without significant Region by Age interaction ($p > .2$).

[2]The age effect did not reach significance.

Table 3.6. Unstandardized regression coefficients reflecting the contribution of age to Short T2 values in NAWM regions displaying anterior-posterior gradient, by clinical group.

			Non-Impaired (n=35)			MCI (n=43)			Dementia (n=25)		
Hemisphere	Gradient	Region	*B*	*SE*	*p*	*B*	*SE*	*p*	*B*	*SE*	*p*
Left	T-F	T	**0.070**	**0.021**	**0.001**	**0.074**	**0.022**	**0.002**		--[2]	
		F	-0.048	0.049	0.3	-0.009	0.047	0.8		--[2]	
	P-F	P		--[2]		**0.092**	**0.032**	**0.005**		--[2]	
		F		--[2]		-0.009	0.047	0.8		--[2]	
	O-F	O	**0.099**	**0.021**	**0.0001**		--[2]			--[2]	
		F	-0.048	0.049	0.3		--[2]			--[2]	
Right	T-F	T		--[2]		**0.074**	**0.026**	**0.005**		--[2]	
		F		--[2]		-0.008	0.046	0.9		--[2]	
	P-F	P	**0.083**	**0.027**	**0.004**		--[2]			--[2]	
		F	-0.039	0.055	0.4		--[2]			--[2]	
	O-F	O	**0.088**	**0.028**	**0.002**		--[2]			--[2]	
		F	-0.039	0.055	0.4		--[2]			--[2]	

Significant coefficients at Bonferroni-corrected alpha = .0083 are shown in bold. Gender and education level served as covariates in all analyses. Abbreviations; T: Temporal, F: Frontal, O: Occipital, P: Parietal.

[1]The effect of age was significant at both anterior and posterior sites without significant Region by Age interaction ($p > .2$).

[2]The age effect did not reach significance.

Table 3.7. Unstandardized regression coefficients reflecting the contribution of age to MWF values in NAWM regions displaying anterior-posterior gradient, by clinical group.

			Non-Impaired (n=35)			MCI (n=43)			Dementia (n=25)		
Hemisphere	Gradient	Region	*B*	*SE*	*p*	*B*	*SE*	*p*	*B*	*SE*	*p*
Left	T-F	T	-.0005	.0003	0.08		--[1]		**-.001**	**.0003**	**0.0001**
		F	**-.0015**	**.0004**	**0.0001**		--[1]		-.0009	.0005	0.06
	P-F	P	-.0008	.0004	0.05		--[1]			--[2]	
		F	**-.0015**	**.0004**	**0.0001**		--[1]			--[2]	
Right	T-F	P	-.0005	.0004	0.1		--[1]			--[2]	
		F	**-.0016**	**.0004**	**0.0001**		--[1]			--[2]	
	O-F	O	-.0006	.0003	0.07		--[1]			--[2]	
		F	**-.0016**	**.0004**	**0.0001**		--[1]			--[2]	

Significant coefficients at Bonferroni-corrected alpha = .0083 are shown in bold. Gender and education level served as covariates in all analyses. Abbreviations; T: Temporal, F: Frontal, O: Occipital, P: Parietal.

[1]The effect of age was significant at both anterior and posterior sites without significant Region by Age interaction ($p > .2$).

[2]The age effect did not reach significance.

3.3 Specific Aims (iv-v): The degree of the association between T2/MWF values and neuropsychological test scores varies significantly across diagnostic groups

Significant moderating effects of diagnostic group on the association between T2 values and neuropsychiatric test scores were found on several instances as shown in Tables 3.8-3.10. In all cases a significant Group by T2/MWF value interaction indicated that the effect of T2 on test scores (controlling for age, education, and gender) was significant only among patients with MCI. Specifically, increased Short T2, long, T2, and reduced MWF in the right temporal NWAM was associated with reduced spatial episodic memory (TCF delayed recall). The same effect was noted for Short T2 and MWF in the right periventricular NAWM. Increased Short and Long T2 and reduced MWF in the left periventricular NWAM was further associated with poor memory for orientation in place and time and memory for public facts (DRS Memory Scale). Depressed DRS scores were also related with increased Short T2 and reduced MWF in the right frontal NAWM and increased Short T2 in the left temporal NAWM. Increased short T2 signal and reduced MWF in the right frontal NWAM was associated with more severe symptoms of depression (the latter were also related to increased left temporal Short T2). In addition, higher MWF in the left frontal (B = 190.4, SE = 64.6, 95% CI: 61.3 to 319.4), and periventricular NAWM (B = 257.9, SE = 74.3, 95% CI: 109.4 to 406.4) was associated with higher semantic verbal fluency (Semantic Verbal Fluency Test scores) across groups. The reverse trend was found for Long and Short T2 in the left periventricular ROI with higher values associated with poorer performance across groups (Long T2: B = -0.26, SE = 0.08, 95% CI: -0.44 to -0.09; Short T2: B = -2.19, SE = 0.77, 95% CI: -3.74 to -0.64).

Table 3.8. Significant moderation of the association between *short T2* and neuropsychiatric test scores by diagnostic group (MCI vs. controls).

		Left Temporal		Right Temporal		Right Frontal		Left PVL		Right PVL	
		B	*95% CI*	*B*	*95% CI*	*B*	*95% CI*	*B*	*95% CI*	*B*	*95% CI*
TCF recall	Group x T2	ns		-1.88	[-2.83 / -0.19]	ns		ns		-0.99	[-2.12 / -0.35]
	T2→score (MCI)	-		-1.57	[-2.99 / -0.16]	-		-		-1.26	[-2.47 / -0.46]
DRS Memory	Group x T2	-0.65	[-1.17 / -0.15]	ns		-0.49	[-0.90 / -0.09]	-0.58	[-1.03 / -0.15]	ns	
	T2→score (MCI)	-0.55	[-0.88 / -0.10]	-		-0.33	[-0.61 / -0.08]	-0.38	[-0.70 / -0.11]	-	
CESD	Group x T2	3.88	[1.70 / 7.12]	ns		2.22	[0.05 / 4.74]	ns		ns	
	T2→score (MCI)	4.48	[0.69 / 7.60]	-		2.66	[0.19 / 5.17]	-		-	

Abbreviations; CI: 95% confidence interval, PVL: Periventricular, TCF: Taylor Complex Figure, DRS: Dementia Rating Scale, CESD: Center for Epidemiological Studies Depression scale. Notes: Age, education, and gender served as covariates in all models. The effect of T2 on neuropsychiatric scores failed to reach significance in all cases in the cognitively non-impaired group ($p>.5$). Only effects significant at $p = .01$ are shown.

Table 3.9. Significant moderation of the association between *long T2* and neuropsychological test scores by diagnostic group (MCI vs. controls).

		Right Temporal		Left PVL	
		B	*95% CI*	*B*	*95% CI*
TCF recall	Group x T2	-0.27	[-0.46 / -0.07]	ns	
	T2→score (MCI)	-0.19	[-0.26 / -0.01]	-	
DRS Memory	Group x T2	ns		-.04	[-.08 / -.002]
	T2→score (MCI)	-		-.03	[-.07 / -.001]

Abbreviations; CI: 95% confidence interval, PVL: Periventricular, TCF: Taylor Complex Figure, DRS: Dementia Rating Scale. Notes: Age, education, and gender served as covariates in all models. The effect of T2 on neuropsychiatric scores failed to reach significance in all cases in the cognitive non-impaired group ($p>.5$). Only effects significant at $p = .01$ are shown.

Table 3.10. Significant moderation of the association between *regional MWF* and neuropsychiatric test scores by diagnostic group (MCI vs. controls).

		Right Temporal	Right Frontal	Left PVL	Right PVL
		B *95% CI*	*B* *95% CI*	*B* *95% CI*	*B* *95% CI*
TCF recall	Group x MWF	161.1 [20.6 / 301.4]	ns	ns	79.4 [14.2 / 217.1]
	MWF →score (MCI)	126.6 [22.9 / 230.3]	-	-	100.27 [5.2 / 203.7]
DRS Memory	Group x MWF	ns	32.9 [2.9 / 62.7]	38.6 [4.6 / 72.7]	ns
	MWF →score (MCI)	-	29.1 [1.5 / 60.0]	33.3 [1.8 / 67.6]	-
CESD	Group x MWF	ns	-220.4 [-.373.5 / -50.2]	ns	ns
	MWF →score (MCI)	-	-220.2 [-413.8 / -26.6]	-	-

Abbreviations; CI: 95% confidence interval, PVL: Periventricular, TCF: Taylor Complex Figure, DRS: Dementia Rating Scale, CESD: Center for Epidemiological Studies Depression scale. Notes: Age, education, and gender served as covariates in all models. The effect of T2 on neuropsychiatric scores failed to reach significance in all cases in the cognitive non-impaired group (p>.5). Only effects significant at p = .01 are shown.

3.4 Specific Aims (vi-vii): Indirect effects of age on neuropsychiatric function through changes in T2/MWF values.

In this set of analyses we examined the hypothesis that age-related cognitive decline was *mediated* by the effect of age on T2/MWF values, according to the model displayed in Figure 3.2. Education level in years and gender served as covariates in these analyses. Prerequisites for assessing mediation effects, namely a significant association between the independent variable (age) and the mediator (T2/MWF values) and a significant partial correlation between the mediator and the dependent variable controlling for age (MacKinnon, Lockwood, et al., 2002) were met in the combined group of cognitively non-impaired elders and MCI patients for category verbal fluency ability (Semantic Verbal Fluency Test scores) in relation to long/short T2/MWF in the bilateral frontal and left periventricular NWAM. Mediation prerequisites were met only in the MCI group for several measures of long-term memory (AVLT retention, PM Delayed Recall, DRS Orientation subscale) in relation to T2/MWF values in bilateral frontal, left temporal and periventricular NAWM and also for spatial episodic memory (TCF Recall) and T2/MWF values in right temporal and periventricular NAWM.

Results of the mediated regression analyses presented in Table 3.11 reveal significant indirect effects of age on delayed verbal episodic memory measures through Short T2 and MWF values measured in left frontal and periventricular NAWM. The effect of age on DRS scores was similarly mediated by age-related increases in left temporal, left periventricular, and right frontal Short T2. Moreover, age-related reductions in MWF in the right temporal NAWM accounted for the decline in spatial episodic memory with age (TCF Delayed Recall score).

Whereas the aforementioned effects were significant only among MCI patients, mediated regression models accounting for age-related decline in semantic verbal fluency (Semantic Verbal Fluency Test scores) fitted well data from both cognitively non-impaired elders and MCI

patients. Specifically, the effect of age on SVFT scores was mediated by age-related increases in bilateral frontal and left periventricular Short T2 and corresponding reductions in MWF in the same regions (see Table 3.12). Notably the direct effect of age on cognitive measures failed to reach significance in all cases, suggesting that the impact of age on these scores was *fully mediated* by age-related increases in Short T2 and MWF values.

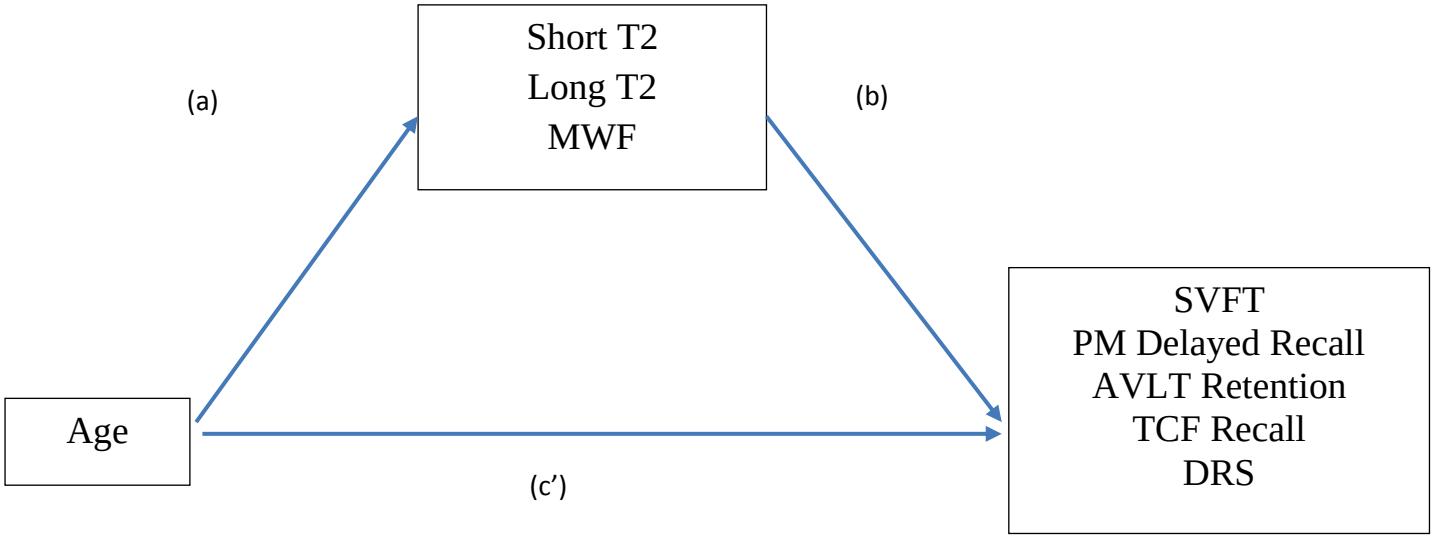


Figure 3.2. Schematic illustration of the mediated regression model accounting for age-related decline on cognitive measures associated with changes in T2 measurements. Gender and education served as covariates.

Table 3.11. Results (R^2 and unstandardized regression coefficients) of mediated regression analyses of the effects of age on memory scores in the MCI group (n=43).

				a		*b*		*c'*		*a x b*	
Age→			R^2	*B*	*95% CI*	*B*	*95% CI*	*B*	*95% CI*	*B*	*95% CI*
Short T2	L Frontal→	AVLT Retention	.305	**.11**	.05 / .16	**-.08**	-.16 / -.003	-.004	-.02 / .01	**-.01**	-.02 / -.002
MWF	L Frontal→	AVLT Retention	.261	**-.001**	-.002 / -.0008	**6.11**	.01 / 14.15	-.006	-.02 / .008	**-.008**	-.02 / -.002
Short T2	L PVL→	PM Delayed Recall	.266	**.08**	.009 / .15	**-1.65**	-3.55 / -.11	-.15	-.54 / .23	**-.13**	-.38 / -.01
MWF	L PVL→	PM Delayed Recall	.289	**-.001**	-.002 / -.0003	**178.0**	5.11 / 369.3	-.11	-.53 / .32	**-.17**	-.42 / -.03
MWF	R Temporal→	TCF Recall	.420	**-.001**	-.002 / -.0002	**102.6**	1.46 / 206.7	-.08	-.26 / .11	**-.09**	-.24 / -.001
Short T2	L Temporal→	DRS	.254	**.09**	.05 / .12	**-.55**	-.18 / -.01	-.02	-.09 / .06	**-.05**	-.12 / -.002
Short T2	R Frontal→	DRS	.253	**.12**	.06 / .18	**-.37**	-.82 / -.02	-.02	-.09 / .06	**-.05**	-.11 / -.001
Short T2	L PVL→	DRS	.270	**.08**	.02 / .14	**-.40**	-.84 / -.03	-.03	-.11 / .04	**-.03**	-.07 / -.007

a: effect of age on T2 values; b: effect of T2 measurements on test scores; c': direct effect of age on test scores controlling for T2 measurements; a x b: indirect (mediated) effect of age on test scores through T2 measurements. CI: 95% confidence interval, L/R: Left/Right, PVL: Periventricular NAWM. Coefficients significant at $p < .05$ are shown in bold.

Table 3.12. Results (R^2 and unstandardized regression coefficients) of mediated regression analyses on the effects of age on T2 values and category verbal fluency among non-impaired elders and MCI patients (n=78).

			a		*b*		*c'*		*a x b*	
Age→			R^2	*B*	*95% CI*	*B*	*95% CI*	*B*	*95% CI*	*B*	*95% CI*
Short T2	Left Frontal→	Semantic Verbal Fluency Test	.627	**.12**	.08 / .16	**-1.90**	-3.67 / -.13	-.24	-.54 / .06	**-.23**	-.48 / -.02
MWF			.642	**-.001**	-.002 / -.0009	**195.7**	58.8 / 332.7	-.21	-.48 / .06	**-.26**	-.49 / -.09
Short T2	Right Frontal→		.639	**.12**	.08 / .16	**-2.38**	-4.19 / -.54	-.19	-.47 / .10	**-.29**	-.55 / -.08
MWF			.636	**-.001**	-.002 / -.0009	**188.5**	32.2 / 344.7	-.22	-.51 / .08	**-.26**	-.50 / -.06
Short T2	Left PVL→		.643	**.10**	.05 / .15	**-2.50**	-4.14 / -.85	-.24	-.51 / .04	**-.24**	-.48 / -.09
MWF			.651	**.001**	-.001 / -.0005	**252.8**	103.8 / 401.9	-.22	-.52 / .07	**-.25**	-.51 / -.09

a: effect of age on T2 values; b: effect of T2 measurements on test scores; c': direct effect of age on test scores controlling for T2 measurements; a x b: indirect (mediated) effect of age on test scores through T2 measurements. CI: 95% confidence interval, PVL: Periventricular NAWM. Coefficients significant at $p < .05$ are shown in bold.

4 Discussion

In this work, we investigated NAWM microstructural alterations and particularly changes in myelin content using the multi-echo T2 relaxation technique, as a function of age and severity of neuropsychiatric impairment in MCI and, mainly, mild, probable AD. The results of the study can be summarized as follows: (1) Significant reductions in myelin integrity /myelin content/ (as indexed by increased Short T2 and reduced MWF values) and increased intra/extracellular water content, probably due to WM volume loss and gliosis (as indexed by increased Long T2 values) are present even in mild AD dementia; (2) In non-demented elders (i.e., NI and MCI groups), regional measures of myelin content correlate with behavioral outcomes (episodic memory and symptoms of depression) in a domain-specific manner; (3) In non-demented elders advanced age is associated with reduced myelin content, which is in turn related to reduced episodic memory capacity.

4.1 Diagnostic Group differences in average T2/MWF values

Although differences between MCI patients and healthy age-matched controls did not reach significance in the present study, previous reports present conflicting results. Some studies failed to find significant FA (Nir et al. 2013) or MTR changes in MCI patients (Granziera et al. 2015) in temporal, frontal, occipital and parietal WM. Negative results were also reported by Mascalchi et al. (2013) in a voxel based MTR study which failed to find differences in left amygdala, hippocampus and fusiform gyrus between aMCI and healthy controls. Other studies were more successful, however. Zhuang et al. (2010) found reduced FA in the corpus callosum, fornix, lateral temporal and superior frontal WM in aMCI and naMCI as compared to normal controls, Bosch et al. (2012) reported reduced FA primarily in posterior periventricular regions and Alves et al. (2012) identified reductions in FA in the

corpus callosum, cingulate and uncinate fasciculus. Furthermore, Carmeli et al. (2013) showed reduced MTR in the splenium of the corpus callosum in mMCI and sMCI patients as compared to a group of healthy elders. Finally, Kabani et al. (2002) showed decreased MTR in temporal lobes bilaterally in MCI and AD patients as compared to healthy elders.

Reductions in white matter integrity, as measured by DTI, are more evident in individuals with AD, as suggested by several studies reporting reduced FA in AD, but not in MCI patients, as compared to healthy age-matched controls. These regions include the posterior cingulate, hippocampus and temporal lobes (for review see Stebbins et al. 2009). These findings are corroborated by studies using the Magnetization Transfer Imaging technique. Thus, in accordance with our findings Fornari et al. (2012) demonstrated bilaterally reduced MTR in temporal, cingulate, parietal and prefrontal regions in their AD group as compared to healthy controls. Moreover, Kabani et al. (2002) found reduced MTR among AD, but not among MCI, patients compared to healthy controls in whole WM (segmented WM). In a similar vein, voxelwise analyses showed decreased MTR in the left mesial temporal cortex and fusiform gyrus in mild AD patients as compared to healthy elders, but no significant difference between patients diagnosed with aMCI and AD patients or controls (Mascalchi et al. 2013). These results are consistent with the notion of progressive WM deterioration in age-related neurodegenerative disorders, whereby demyelination typically progresses from the left temporal lobe in the early stages of AD to additional lobes bilaterally.

4.2 Age-related changes in white matter

Given the purported specificity of MWF as an index of myelin integrity, age effects on this measure are likely to have greater functional significance than T2 values. In the

present study, multivariate analyses (controlling for education level and gender) revealed that in the age range covered by the sample MWF decreased linearly in temporal, frontal and occipital NAWM in participants without dementia (MCI and NI groups). A similar trend in bilateral periventricular NAWM reached significance only among MCI patients. While the present study is the first to examine MWF in older adults, there have been several reports of reduced white matter volume and/or fractional anisotropy in elders without dementia. For instance, significant reductions in FA in inferior fronto-occipital fasciculus, the superior longitudinal fasciculus and the uncinate fasciculus and decreased white matter volume in parietal, temporal and prefrontal areas were detected during a 13 to 16 month period in healthy elders and patients with MCI (Teipel et al. 2010). DTI studies suggest that FA is also reduced in the course of normal aging in a broad range of regions, including the corpus callosum, posterior periventricular regions and deep frontal regions (Westlye et al., 2010; Head et al., 2004), ventromedial prefrontal and deep frontal white matter (Salat et al., 2005). Moreover, lower MTR values in NAWM, with greater age-dependence in frontal and parieto-occipital regions (Fazekas et al., 2005) and in mean NAWM (whole brain NAWM) (Split et al., 2005) have been found in elderly subjects related to younger subjects.

In addition, the present findings corroborate previous reports of greater age-related reduction in myelin integrity in anterior vs. posterior regions. Specifically, cross-sectional analyses suggested that the most prominent reductions in MWF along the course of normal aging occur in frontal NWAM. This is consistent with several DTI studies highlighting an anterior-posterior gradient of age-associated decreases in FA (Ardekani et al., 2007; Salat et al., 2004; Abe et al., 2002) and in MTR studies which MTR values were significantly higher in the prefrontal versus the posterior part of frontal lobes and in the genu of corpus callosum versus the body and splenium (Armstrong et al.2004). Inspection of age-related trends in the very mild and mild DAT group revealed a more widespread pattern of DTI reduction,

consistent with the notion that when pathological changes in cognition are present age-related demyelination may take place with greater deterioration of WM in posterior (temporal and parietal lobes) regions versus anterior ones.(frontal lobe; Head et al., 2004).

We also documented decreasing trends in MWF among patients with dementia which, however, failed to reach significance. The relatively small sample size (n=25) and increased within-group variability may have been responsible for this negative finding. Thus although the majority of patients in the dementia group met clinical and neuroimaging criteria for probable AD, available data were not conclusive for some patients for whom a tentative diagnosis of possible AD was provided. In addition, significant heterogeneity in both cognitive and neuropathological presentations is known to exist even among patients with a similar dementia diagnosis.

To summarize, we have found different anatomical patterns of age-related NAWM deterioration in MCI patients and cognitively intact age-matched controls. Thus although the two groups may not differ significantly on average Long/Short T2 or MWF values, patients presenting with cognitive difficulties consistent with MCI display age-related NAWM deterioration in posterior as well as anterior regions. These results may have clinical significance if the degree of age-related NAWM deterioration correlated significantly with neuropsychiatric test scores. This issue was addressed through mediated regression analyses which allow assessment of both direct effects of T2/MWF on neuropsychiatric test scores (by statistically maintaining age as constant) as well as the indirect effects of age on neuropsychiatric test scores which can be accounted for by age-related decline in T2/MWF values. These topics are revisited in the next section.

4.3 Associations between T2 values/MWF and neuropsychiatric test scores

Direct associations between T2 values/MWF and several cognitive measures were found, controlling for age (summarized in Table 4.1). In the control group these effects were restricted to semantic verbal fluency: increased Short T2/reduced MWF values in left periventricular and bilateral frontal NAWM was associated with reduced verbal fluency scores (b path in Table 3.10). A similar effect was found among MCI patients. This finding is consistent with the purported role of dorsolateral prefrontal regions in the retrieval of semantic memory traces and in executive functions and the large body of neuroimaging literature revealing predominant activation foci in dorsal prefrontal cortices during performance of fluency tasks (such as category fluency) (Gurd et al. 2002)

However in the latter group additional direct associations between Short T2/MWF measures and neuropsychological test scores: left frontal and periventricular NAWM integrity was associated with verbal episodic memory (retention of word list and passage items, respectively), whereas right temporal NAWM status was associated with visuospatial episodic memory (delayed reproduction of a meaningless complex geometric pattern). The domain specificity of correlations between short T2 and/or MWF values and memory found in the present study is in agreement with the extensive literature linking verbal episodic memory with an extensive network of left hemisphere frontal and temporal lobe regions and visuospatial memory with primarily temporal lobe regions in the right hemisphere (Staff et al. 1999). Finally, orientation in time and place—probably reflecting both episodic and semantic memory abilities—was found to correlate significantly with Short T2 in left temporal/periventricular and right frontal NAWM. Finally, increased depressive symptomatology was associated with reduced myelin content in both frontal and temporal NAWM bilaterally.

In general, associations between T2/MWF measures and cognitive performance displayed the expected anatomic distribution, consistent with the notion that reduced myelin content in NAWM is an important component of neuronal degeneration processes in MCI. Previous imaging studies utilizing FA and MTR have reported largely similar findings, albeit with limited anatomic specificity. For instance, Huang et al. (2007) reported that reduced FA in the temporal lobe in patients with AD and MCI correlated with poorer performance in episodic memory, whereas reduced FA in the frontal lobe in patients with AD and cognitively intact elders correlated with poorer performance on executive function tests. Goldstein et al. 2009 documented significant correlations between WM integrity in medial temporal regions and episodic verbal memory in MCI patients. Significant correlations between immediate visual performance was significantly correlated with loss of WM integrity in the left temporal lobe.

Few studies have explored associations between white mater indices and cognitive performance in healthy elders. For instance, significant correlations between lower MTR values in NAWM (parietal, frontal, occipital and temporal lobes) and in deep gray matter (amygdala, thalamus, putamen, caudate nucleus) were found with worse executive functioning (Seiler et al., 2014).

The majority of studies have found significant associations between white matter indices and cognitive test scores in AD patients. Specifically, correlations were found between lower FA in the fornix and anterior cingulum and worse performance on several neuropsychological tests (MMSE, CVLT long-delay free recall, and Wechsler Memory Scale Immediate and Delayed Recall; Mielke et al. 2009). Correlations between MTR values in the left hippocampus, putamen and thalamus and MMSE scores were reported by Duzel et al. (2010) who also found reduced MTR in the frontal lobe in healthy elders versus younger with memory impairment (Duzel et al., 2010) . Another study by Fornari et al. (2012)

revealed significant correlations between the severity of demyelination, in brain areas such as Broca's, bilateral insular region, left superior temporal extending to temporoparietal region, cuneus, cingulated sulcus and primary visual areas, with MMSE. Also, they found correlations between MTR values and episodic visual memory scores in the left hemisphere, and also between scores on a language competence test and MTR in the left superior and middle temporal regions. Finally, Van de Flier et al. (2002) showed that the peak heights of the MTR histograms of MCI and AD patients were lower than those of controls for the whole brain and particularly in the temporal and frontal lobes, reflecting structural brain damage.

Table 4.1. Summary of direct associations between NAWM integrity measures and cognitive abilities in MCI patients (unless otherwise noted).

Measure	Region	Cognitive domain
↑ Short/Long T2, ↓MWF ↑ Short T2, ↓MWF	R Temporal R Periventricular	↓ Spatial memory
↑ Short/Long T2, ↓MWF ↑ Short T2, ↓MWF ↑ Short T2	L Periventricular R Frontal L Temporal	↓ Orientation in place/time & memory for public facts
↓MWF ↑ Short/Long T2	[a]LFrontal, [a]LPeriventricular L Periventricular	↓ Semantic verbal fluency
↑ Short T2, ↓MWF ↑ Short T2	R Frontal L Temporal	↑ Depressive symptomatology

[a]The effects were significant in both NI and MCI groups.

4.4 Age-related neuropsychiatric deterioration is mediated by decreasing white matter integrity

An important finding of the present study was that in non-demented elders advanced age was associated with reduced myelin content, which is in turn related to reduced episodic and semantic memory capacity (summarized in Table 4.2). The indirect (mediated) effects of age: (a) were restricted to memory indices (semantic and episodic), (b) involved both anterior (frontal) and posterior regions (temporal lobes), and (c) were restricted to MCI patients when episodic memory (verbal and visuospatial was concerned). With respect to episodic memory, regions serving a mediating role in the age-cognition association were the right temporal (visuospatial memory), left temporal and right frontal lobes (orientation and semantic memory for public facts), and the left frontal lobe (verbal episodic memory). White matter integrity (as indicated by short T2 and/or MWF) in these regions was found to be negatively related with age in this group. With respect to semantic memory (verbal fluency) regions emerging as mediators of of the age-cognition association in both groups of participants without dementia (NI and MCI) were the frontal lobes bilaterally. Notably, significant age-related decline in MWF was restricted to these regions among cognitively intact elders.

Very few studies have explored associations between aging, changes in white matter integrity and cognitive deficits have not been studied concurrently (i.e., in the same conceptual and statistical model). For instance, Seiler et al. (2014) reported mediating effects of MTR aggregated over the entire NAWM in the association between age and performance on executive tests (Wisconsin Card Sorting test, Trail Maiking Test Part B, and Digit Span Backwards) in a large sample of adults aged 38-86 years without history of

neurologic or psychiatric disorder. Unfortunately, associations with MTR in specific NAWM regions were not assessed. A tractography study on a sample of cognitively intact volunteers aged 18-85 years found indirect effects of age on episodic memory through FA in the corpus callosum and two major white matter tracts connecting posterior and anterior brain regions (inferior longitudinal fasciculus and inferior occipitofrontal) fasciculus (Voineskos et al., 2012).

Table 4.2. Age-related decline in cognitive ability mediated by WM integrity in MCI patients (unless otherwise noted).

	Measure	Region		Cognitive domain
Age→	↓MWF	R Temporal	→	↓ Spatial memory
Age→	↑ Short T2	L Periventricular R Frontal L Temporal	→	↓ Orientation in place/time & memory for public facts
Age→	↑ Short, ↓MWF	[a]L/R Frontal L Periventricular	→	↓ Semantic verbal fluency
Age→	↑ Short T2, ↓MWF	L Frontal L Periventricular	→	↓ Delayed verbal episodic memory

[a]The effect was significant effect in both NI and MCI groups.

4.4.1 The nature of WM alterations in AD & MCI

WM changes, including demyelination, have been documented in age-related neurodegenerative disorders, such as AD and MCI, and are potentially related to amyloid and tau pathology (Fagan et al. 2009; Fagan et al. 2007; Hansson et al. 2006; Dean et al. 2017). These changes suggest an important role of WM integrity in clinical and neuropsychiatric manifestations of these patients. It is well known that WMH, revealed by conventional T2-sequences, are frequently found in AD, and may have an additive effect on cognitive decline (Gouw et al. 2008; Fazekas et al. 2005). Non-conventional MRI techniques, such as DTI and MTI, can quantify NAWM microstructural changes, but may not be appropriate to accurately measure myelin content (Bozzali et al. 2001; van der Flier et al. 2002).

Specifically widespread changes in FA were typically documented among AD patients as compared to healthy controls in several areas of the brain including cingulated gyrus, corpus callosum, parahippocampal gyrus as well as frontal, temporal, parietal and occipital white matter. Agosta et al. (2011) showed reduced FA in inferior parietal regions, parahippocampal tract and in fornix in AD patients as compared to control subjects. Huang et al. (2007) revealed decreased FA in AD patients in frontal, temporal and parietal NAWM as compared to healthy elders and in MCI found decreased FA in temporal and parietal NAWM as compared to normal cognition subjects. Another diffusion tensor imaging study have been reported significantly reduced FA values in the genu of corpus callosum (Stahl et al. 2003). Moreover Ropele et al. (2012) showed significantly lower global MTR values in AD than controls and regional MTR decrease in areas such as putamen, hippocampus and thalamus. In another study based on MTR and *D* histogram analysis, found reduced MTR and increased *D* in a large brain portion from patients with AD as compared to controls (Bozzali et al. 2001).

There are many studies with conflict results of FA in AD, MCI and healthy controls. Some of them found decreased FA in the frontal lobes (Bozzali et al.2002; Huang et al.2007; Medina et al.2006; Rose et al. 2006) but not by others (Fellgiebel et al. 2004; Stahl et al. 2007; Head et al. 2004; Takahashi et al. 2002). Most studies failed to document significant differences in FA in the occipital lobes in AD compared to MCI or healthy elders (Fellgiebel et al. 2004; Head et al. 2004; Huang et al. 2007; Stahl et al. 2007). With respect to the parietal lobes conflicting results can be found in the literature (Bozzali et al. 2002; Fellgiebel et al. 2004; Huang et al. 2007; Medina et al. 2006; Salat et al. 2008; Stahl et al. 2007). The most consistent findings of reduced FA in AD as compared to healthy elders and MCI were obtained in the medial temporal lobe (hippocampus, entorhinal cortex, parahippocampal white matter; Choo et al. 2010; Fellgiebel et al. 2004; Rose et al. 2006; Salat et al. 2008; Zhou et al. 2008), lateral temporal regions (Bozzali et al. 2002; Huang et al.2007; Fellgiebel et al. 2004; Medina et al. 2006; Salat et al. 2008; Takahashi et al. 2002; Xie et al. 2006) and posterior cingulum (Choo et al. 2010; Fellgiebel et al. 2005; Medina et al. 2006; Rose et al. 2006; Takahashi et al. 2002; Zhang et al. 2007; Zhou et al. 2008).

In the studies reviewed above WM integrity was assessed through DTI and MT techniques, which are characterized by adequate sensitivity for quantifying WM pathology but limited specificity. Thus, FA values are affected by fiber tract orientation order and packing properties of large fiber bundles and may not provide accurate measurements of myelin content. Previous studies, mostly in MS, showed that MTR measurements have been used for improve the specificity and the sensitivity of conventional MRI techniques through characterizing diffused or focal abnormalities in pathological brain and differentiating lesions such as demyelination, axonal loss, inflammation and edema. Gareau et al. (2000) showed that MTR measurements of a plaque in MS patients may give conflicts results in lesions where both inflammation and demyelination coexist. Papanikolaou et al. (2004)

showed that a linear correlation between MTR and T2 values exists in regions where either inflammation or demyelination preponderates but this correlation is lost when both demyelination and inflammation coexist.

Summarizing, MTR measurements includes contributions from both demyelination and inflammation and could serve as a direct index of the relative tissue composition in myelin and water. Also, is altered by the amount of myelin but also by inflammation or edema, thus reduced values may be attributed to either a decrease in myelin content and axonal loss *or* an increase in water content due to inflammation, edema, etc. In contrary, MWF as the ratio of the myelin water amplitude (short T2 component) to the total signal (Whittall et al., 1997) corresponding to the anatomical distribution of myelin-qualitative correlation between short T2 map and gluxol fast blue staining for myelin in a formalin fixed brain-short T2 component originates from water related to myelin (Moore et al., 2000) and studies from animal models have shown that MWF is indicating myelin content in tissue (Gareau et al 1999,2000) and could serve as a direct index for quantification of the degree of demyelination.

4.5. Advantages of the T2 multi echo technique

Several multi-echo-spin-echo multi-compartment quantitative sequence schemes exist in the literature. These include: (a): 2D single/multi slice multi-echo schemes with spectral T2 analysis post-processing algorithms based on laplacian transformations (Makcay et al. 1994), (b): 2D and 3D multi slice simultaneous GRAdient and Spin Echo (GRASE) methods (Oshio et al. 1991), (c): T2 preparation multi slice methods (Nguyen et al. 2012), (d): Multi-Gradient echo (MGRE) methods (Deoni et al. 2003) and (e): whole brain 3D multicomponent driven-equilibrium single-pulse observation time (mcDESPOT) methods

for simultaneous multi-compartment T1 and T2 measurements (Deoni et al. 2003; 2008) All sequences have specific advantages and disadvantages in relation to the accuracy and the sensitivity of T2 measurements for which they were designed.

The number of echoes, echo spacing, and the time range covered by the sequence of echoes are always crucial factors for the sensitivity of the final T2 measurement. Amongst all multi-echo spin echo sequences the method proposed by MacKay et al 1994 and its variants are those which are still considered as reference methodologies in T2 relaxometry. They all use 32 echoes as their basic characteristic for brain tissue T2 measurements. The main drawbacks are the single slice acquisitions, the elevated echo spacing (10 ms) and the long examination times (25 min) in most of the sequences. Echo spacing is considered a crucial factor, especially in the case where short T2 measurement (< 20 ms) sensitivity is a prerequisite. The choice of a short echo spacing (< 10 ms) is certainly a significant benefit to any T2 relaxometry sequence.

3D GRASE sequences (Oshio et al. 1991) offer the advantage of total brain anatomical coverage. However, they are still using elevated echo spacing, long examination times for clinical protocols and extremely low TR (1000 ms) for accurate brain tissue T2 relaxometry. The inherent drawback on these sequences is the strong signal T2* dependence on the peripheral K-space lines.

T2-prep sequences (Nguyen et al. 2012) offer the advantage of 2D multi-slice and in some cases total brain anatomical coverage. They possess the ultimate advantage of being insensitive to cumulative errors of stimulated echoes due to the T2-prep rationale. They are also using short and variable echo spacing. However, they possess the disadvantages of quite long examination times (15 min) and a limited number of echoes (12 echoes).

MGRE methods (Deoni et al. 2003) have the absolute advantage of minimal echo spacing (1.1 ms) and whole brain coverage but in this case we are measuring T2* rather than absolute T2. mcDESPOT sequences (Deoni et al. 2003; 2008) do offer an alternative indirect way to measure T1 and T2 simultaneously. These sequences possess the advantage of total brain anatomical coverage and short variable echo spacing. However, they still use long examination times (30 min) and an extremely small number of the echoes, only 3 echoes, for measuring T2. These last two types of methodologies (MGRE and mcDESPOT) are indeed promising alternatives in indirect T2 relaxometry measurements, but a clinical study comparing these sequences with conventional MESE sequences on the same human subjects is certainly missing from the current literature.

In order to optimize the clinical applicability of the MRI protocol used in the present study an optimized MESE sequence was developed. This sequence has the ultimate advantage of being fast enough (3.5 min) to be incorporated to any standard clinical examination protocol. 32 symmetrically repeatable echoes with echo spacing of 6.7 ms (starting echo) covering a the range of 208 ms was considered adequate mathematically for measurements of T2's ranging from 10 ms up to 300 ms. The 5 slices chosen were meticulously positioned at the center of cranial anatomy as the best alternative solution for not being able to cover the whole brain anatomy at the chosen scan time. In special cases the sequence could be repeated (extra 3.5 min) for extending the coverage of brain anatomical range. These five slices were positioned far apart from each other, their slice gap was one slice thickness (8 mm), in order to avoid cross talking and cumulative errors from stimulated echoes driven from the imperfect adjacent slices RF excitations. The double exponential fitting model proved to be a fast and accurate means for two compartments simultaneous T2 measurements ranging from 10 up to 300 ms. The optimized 2D MESE sequence described

above was chosen as the best alternative, for the fulfillment of the purposes of the clinical part of this study, as compared to the sequences existed in the literature.

4.6 Limitations of the study

The aforementioned findings should be interpreted with caution in view of certain study limitations. Thus, the relatively small size of the control group may have reduced the sensitivity for detecting additional indirect age-related effects on cognitive measures. Moreover, regional measures of myelin content were obtained from a small number of axial sections (n=5) employed in ROI analyses.

Age-related indirect effects on cognitive measures were established using cross-sectional data. Longitudinal measurements are required in order to (a) determine factors (clinical, life-style, demographic) that may predict the rate of white matter deterioration in older adults without dementia, and (b) if white matter integrity in specific brain regions has a prognostic value for cognitive and/or neuropsychiatric outcomes.

Finally, the relatively small size of the MCI group did not permit stratification of correlational analyses by MCI subtype in order to enhance the specificity of associations between white matter integrity in specific brain regions and performance on particular cognitive domains.

4.7. Conclusion

To our knowledge, this is the first study utilizing the Multi-echo T2 relaxation time technique to improve specificity in detecting myelin degradation, predominantly in left hemisphere temporal, parietal and periventricular NAWM, even in mild dementia. We also

provided evidence that age-related decline in memory capacity, even in the absence of dementia, is largely mediated by white matter changes. Future studies should attempt to examine age-related effects in prospective, longitudinal studies.

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