Neuroimaging markers in clinical trials for pre-dementia stages of Alzheimer's disease

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“A person who never made a mistake
never tried anything new”
Albert Einstein

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Summary

The development of new drugs and the validation and standardization of neuroimaging and biological markers for Alzheimer's Disease (AD) clinical treatment trials is expected to be one of the major goals of AD research in the upcoming years. The present thesis aims to address these critical issues. The first part of the thesis is focused on the proper application of Standard Operating Procedures (SOPs) for the structural neuroimaging protocols of acquisition and the implementation of neuroimaging markers in 10 Italian Memory Clinics. The second part of the thesis deals with the application of several structural and functional neuroimaging markers in the context of clinical trials investigation in mild cognitive impairment individuals. Results revealed that the implementation of SOPs at multicentre level reduces the variance of neuroimaging markers measurement detected by different scanners. Moreover, results from the employment of neuroimaging markers in pre-dementia trials in mild cognitive impairment individuals showed a significant impact of anticholinesterase therapies in reducing the hippocampal rate of atrophy, the cortical thinning as well as in increasing the activation of brain areas related to functional Magnetic Resonance Imaging (fMRI) face and location matching tasks. These promising results support the hypothesis that structural and functional neuroimaging markers applied in a standardized manner might be utilized as candidate surrogate outcomes in future pre-dementia trials for AD.

Keywords : Alzheimer's Disease, mild cognitive impairment, neuroimaging markers, MRI, hippocampus, cortical thickness, basal forebrain, fMRI, face and location matching tasks, standard operating procedures, clinical trials, surrogate outcomes.

Résumé

Dans les années à venir, les principaux objectifs de la recherche pour maladie d'Alzheimer (MA) sont le développement de nouveaux médicaments ainsi que la validation et la standardisation des marqueurs de neuroimagerie et de biochimie. La présente thèse vise à aborder ces questions cruciales. La première partie de la thèse fait le point sur l'application correcte des Procédures Opérationnelles Standards (SOPs) pour l'acquisition de protocoles de neuroimagerie structurelle et l'application des marqueurs de neuroimagerie cérébrale dans 10 cliniques italiennes spécialisées dans les troubles de la mémoire. La deuxième partie traite de l'application de plusieurs marqueurs de neuroimagerie structurelle et fonctionnelle dans le cadre des études cliniques sur des patients ayant des troubles précoces de la MA. Les résultats ont révélé que la mise en œuvre des SOPs pour l'acquisition des séquences d'imagerie par résonance magnétique (IRM), au niveau multicentrique, réduit la variance des mesures des marqueurs de neuroimagerie détectées par différents scanners. En outre, les résultats de la deuxième partie de la thèse ont montré que les thérapies anticholinestérasiques ont un impact significatif : réduction de l'atrophie de l'hippocampe, de l'amincissement cortical ainsi que l'augmentation de l'activation de la voie dorsale visuelle des patients ayant des troubles précoces de la MA. Ces résultats prometteurs confirment l'hypothèse que les marqueurs de neuroimagerie structurelle et fonctionnelle appliqués avec SOPs pourraient être utilisés comme critère d'évaluation substitutif dans les études cliniques pour les patients ayant des troubles précoces de la MA.

Mots clés : Maladie d'Alzheimer, IRM, marqueurs de neuroimagerie, IRM fonctionnelle, hippocampe, épaisseur corticale, cerveau antérieur basal, études cliniques, Procédures Opérationnelles Standards.

List of Abbreviations

^{18}F-FDG-PET	^{18}F-fluorodeoxyglucose-PET
AD	Alzheimer's Disease
ADNI	Alzheimer's Disease Neuroimaging Initiative
AIBL	Australian Imaging, Biomarker, and Lifestyle Flagship Study of Ageing
APC	Annualized Percent Change
APOE	Apolipoprotein E
APP	amyloid precursor protein
$A\beta_{1-42}$	42 amino acid-long form of the amyloid beta peptide
BF	Basal Forebrain
CSF	Cerebrospinal fluid
DIAN	Dominantly Inherited Alzheimer's Network
E-ADNI	European-ADNI
EADC	European Alzheimer's Disease Consortium
EMA	European Medicines Agency
FCSRT	Free and Cued Selective Reminding Test
FDA	Food and Drug Administration
fMRI	functional Magnetic Resonance Imaging
IWG	International Working Group
MCI	Mild Cognitive Impairment
MPRAGE	Magnetization-Prepared Rapid Gradient-Echo
MRI	Magnetic Resonance Imaging
MTL	Medial Temporal Lobe
NbM	Nucleus basalis of Meynert
NIA-AA	National Institute on Aging-Alzheimer's Association
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
PET	Positron Emission Tomography
PSEN1	Presenilin-1
PSEN2	Presenilin-2

sMRI	Structural Magnetic Resonance Imaging
SNR	Signal-to-Noise Ratio
SOPs	Standard Operating Procedures
TOMM40	Translocase of Outer Mitochondria Membrane

I. INTRODUCTION

1. WHAT IS ALZHEIMER'S DISEASE? A HISTORICAL OVERVIEW

Since 4,000 years ago Egyptians recognized the loss of cognitive abilities in elderly individuals (Boller and Forbes, 1998). However, the clinical description of what we now consider dementia was not established until the 19th century. The French physician Philippe Pinel, considered the father of modern psychiatry, is generally credited for introducing the term dementia (démence), although the term may have been in use prior to that time. However, it was Pinel's student, Jean Etienne Esquirol, in his book *Des Maladies Mentales* (1838), who described the distinction between amentia and dementia, the former was the term used to denote an individual who developed deficits in mental functioning early in life (*amentia* meant someone who was born with mental deficiencies), the latter referring to loss of mental faculties leading to the concept of senile dementia (individuals who develop mental deficiencies as adults) (Berchtold and Cotman, 1998). In the 1860s, clinical-pathologic studies of senile dementia demonstrated a reduction of brain weight and atrophy. The late 19th century witnessed revolutionary progress in neuropathological methods including brain tissue fixation, staining, and improved microscope optics. This permitted the characterization of individual neurons in their entirety by Golgi (Pannese, 1999) in 1873. These technological developments set the stage for the identification of the lesions that now characterize Alzheimer's disease. Blocq and Marinesco using a carmine stain on brain tissue from patients with chronic epilepsy described for the first time senile plaques in 1892 (Buda, et al., 2009) without recognize any link to dementia.

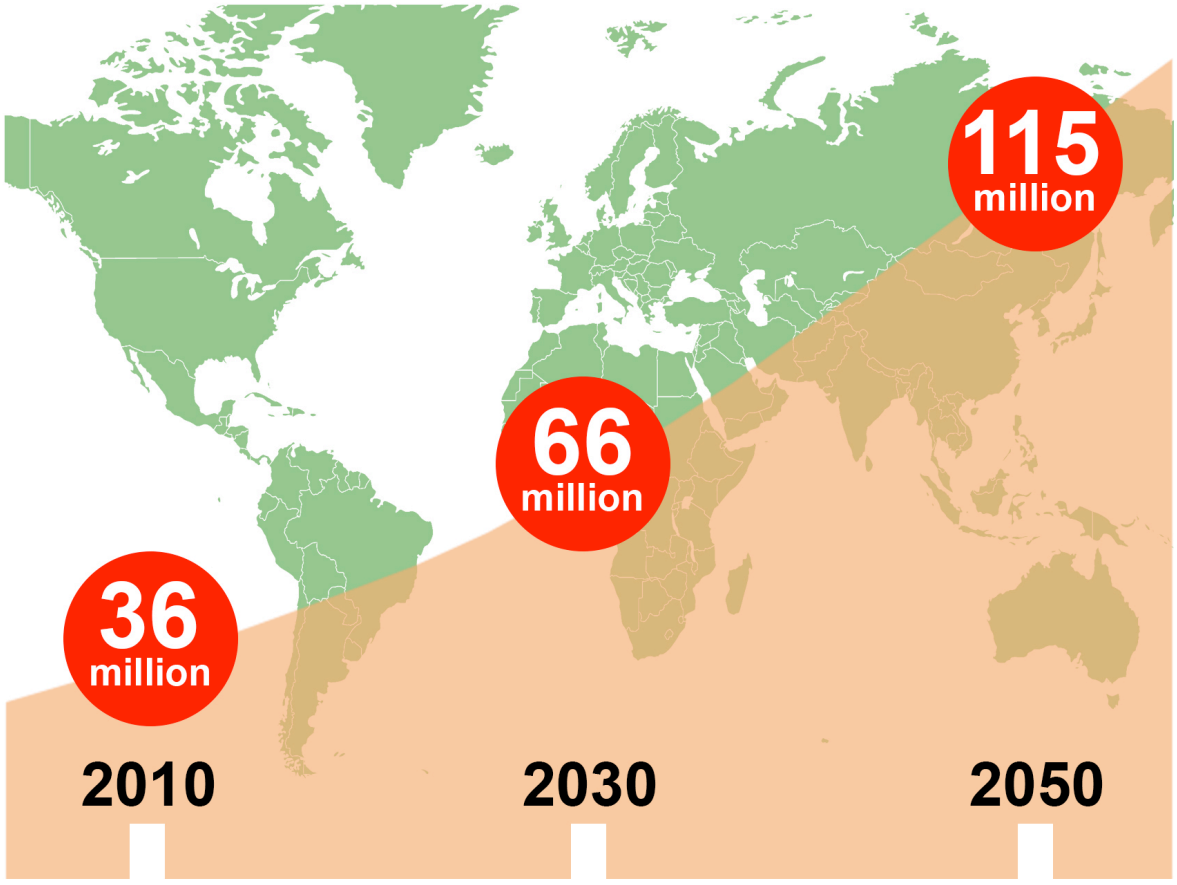
The term Alzheimer's Disease (AD) derives from the name of the German neurologist who first described this disorder, in 1906 (Goedert and Ghetti, 2007). He followed a patient in her early 50s who had suffered from memory loss and difficulty both speaking and understanding what was said. After her death, Dr. Alzheimer performed a detailed investigation of her brain, noting dramatic brain atrophy as well as unusual protein deposits called amyloid plaques and neurofibrillary tangles. That same year, Oskar Fischer separately reported a link between dementia and plaques in 12 cases of senile dementia (Goedert, 2009). In his textbook of psychiatry published in 1910, Emil Kraepelin used the term *Alzheimer's Disease* to refer to the early onset dementia and distinguished it from the later onset senile dementia (Amaducci, et al., 1986). More than a century later, these neuropathological characteristics are still recognized as the hallmarks of AD. In order to address the issues related to diagnose patients with AD at its earliest stages, the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA), and the National Institute of Mental Health published a consensus recommendations for the clinical diagnosis of AD in 1984 (Khachaturian, 1985,McKhann, et al., 1984). These recommendations and the two following iterations (Mirra, et al., 1991) provided protocols aimed to harmonize the definition of AD using terms as "definite Alzheimer's disease" (AD), "probable AD," "possible AD," and "normal brain" in order to indicate levels of diagnostic certainty. These consensus recommendations have reduced subjective interpretation, and assured common language in the diagnosis of AD. In addition, findings on the relationship between cholinergic deficits in persons with AD leading to neuronal loss in the basal forebrain, measures of AD pathologic changes, and cognitive function were found (Perry, et al., 1977, Perry, et al., 1978, Whitehouse, et al., 1981, Whitehouse, et al., 1982, Wilcock, et al., 1982). These findings

motivated, in part, the conduction of clinical trials with cholinesterase inhibitors (Davis, et al., 1992, Rogers and Friedhoff, 1996) that have been subsequently approved by the Food and Drug Administration in 1993.

2. THE GLOBAL IMPACT OF ALZHEIMER'S DISEASE

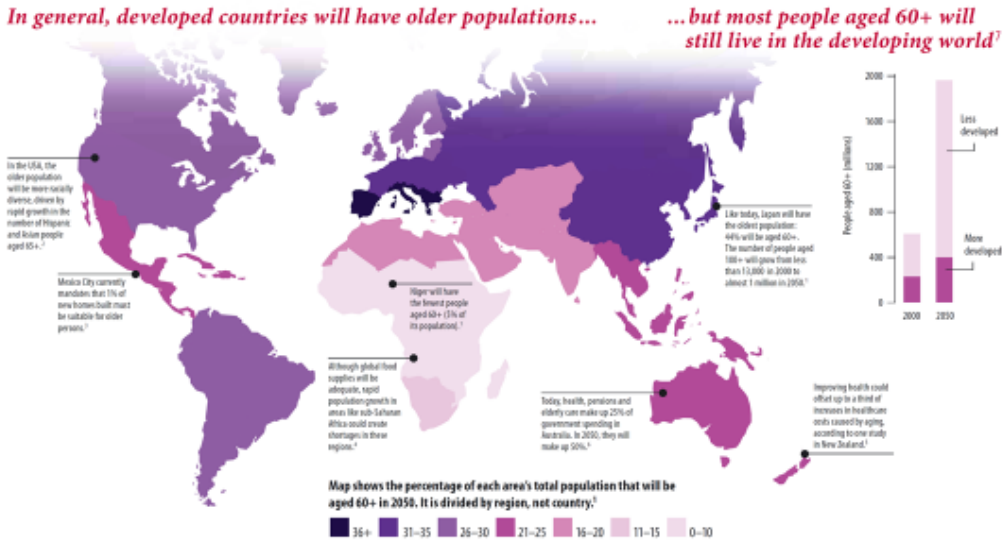
To date, the Alzheimer's Disease (AD) is the most common cause of dementia. Dementia was estimated to affect 35.6 million people worldwide in 2010, and without any preventive plans aimed to slow the disease onset, the prevalence of dementia is expected to increase to 65.7 million by 2030 and 115.4 million by 2050 (Alzheimer's Disease International: World Alzheimer's Report, 2009) (Figure 1). The astronomical numbers of people with dementia will put a huge stress on governments and public health systems around the world. In 2010, total estimated worldwide costs of dementia were USD 604 billion, about 1% of the world's gross domestic product.

Figure 1. The worldwide dementia epidemic.



If it were a country in and of itself, dementia care would rank 18th in the size of its economy, nearly the same as the economy of Turkey (Alzheimer's Disease International: World Alzheimer Report, 2010). Brookmeyer and colleagues (Brookmeyer, et al., 1998) estimated that a delay of 5 years in the onset of AD could decrease the prevalence of the disease by almost 50%. Even delaying onset by as little as 1 year would have enormous public health implications, reducing the worldwide prevalence by nearly 9.2 million cases by 2050, with most of the decline among those who need the highest level of care (Brookmeyer, et al., 2007).

Figure 2. Prevalence of aging of population among countries (Hampel H, Lista S. Silent Alarm – The Quiet Epidemic of Alzheimer's Disease. Karger Gazette. The Aging Issue. 2012;72:8-10).



Sources: Population, Ageing and Development Wallchart. UN Population Division, 2009. Older Americans 2010: Key Indicators of Well-Being. Federal Interagency Forum on Aging Related Statistics, 2010. Global Age-Friendly Cities: A Guide. World Health Organization. 2007. World Agriculture: Towards 2030/2050. Food and Agriculture Organization of the UN, 2006. Bryant J, Sonerson A: Gauging the cost of aging. Finance & Development 2006, vol. 43, No. 3. Australia to 2050: Future Challenges. Australian Government:, 2010. World Population and Ageing: 1950–2050. UN Population Division, 2001. Falls factsheet. World Health Organization, 2010.

The main reason for this worldwide explosion of dementia prevalence is the aging of the population. Indeed, people around the world are living longer and healthier owing to the medical advances that have reduced the incidence of communicable diseases (Figure 2).

It is clear that AD represents a worldwide public health crisis of unimaginable proportions, demanding a massive, integrated, multidisciplinary, and sustained worldwide response. Over the past decades, there has been significant progress in understanding the molecular and biochemical basis of the disease and in applying that knowledge to the development of new technologies and treatments. In spite of these progresses in the tentative of slowing the prevalence of the disease more attention from governments, academic institutions, and the private sector, combined with a broadened perspective, is needed. Only through such collaboration better treatments – and eventually a cure – will be found.

3. DEVELOPMENT OF DIAGNOSTIC CRITERIA FOR ALZHEIMER'S DISEASE

The first criteria for diagnosing AD were established in 1984 (McKhann, et al., 1984) when little was known about the genetic and molecular events that result in disease, and before the availability of imaging technologies revealing the dysfunction or death of brain cells. Developed by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS, now NINDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA, now the Alzheimer's Association), these criteria required a physical evaluation as well as neuropsychological testing to classify patients as having *probable* or *possible* AD. Although, according to these criteria, a definite diagnosis was made only after confirmation by a detailed study of the brain tissue at autopsy, the clinical diagnosis of probable AD could be made when a progressive impairment in at least two cognitive domains was present. Memory was considered the most common cognitive domain affected during AD; others cognitive domains affected are: concentration, problem-solving and language. In the next 20 years, these criteria have evolved in parallel to the development and discover of biological and imaging markers for AD thanks to several worldwide initiatives such as the Alzheimer's Disease Neuroimaging Initiative (ADNI), the Australian Imaging, Biomarker, and Lifestyle Flagship Study of Ageing (AIBL) and global efforts known as World Wide ADNI. On account of these initiatives, in the recent years, biomarkers for making not only an earlier diagnosis but also for providing more precise information about the pathological basis of AD have been identified. As a result of this improved understanding of the disease, six sets of research criteria reflecting the full range of the disease, from its earliest effects to its eventual impact on mental and physical function, have been proposed. The International Working Group (IWG) criteria in 2007 and the National Institute on Aging-Alzheimer's Association (NIA-AA)

workgroups in 2011 developed two independent set of criteria for AD. The proposed criteria and guidelines identify three main different phases of the disease: (1) the asymptomatic at risk, pre-symptomatic, or preclinical phase occurring many years before symptoms become evident (Sperling, et al., 2011); (2) the symptomatic phase characterized by mild problems in cognition, learning, and memory, enough to be noticed and measured but not enough to impair the ability to live independently or carry out everyday activities (Albert, et al., 2011a, Dubois, et al., 2010, Dubois, et al., 2007, Dubois, et al., 2014); (3) the probable and possible Alzheimer's dementia, characterized by a marked impairment of memory and cognition and functional dependence.

The criteria for the asymptomatic at risk, pre-symptomatic or preclinical phase identify cognitive intact people who might develop AD in their future. According to the update version of the IWG criteria (IWG-2) there are two type of preclinical phase: (i) asymptomatic at risk and (ii) presymptomatic individuals. The asymptomatic at risk individuals are cognitive intact persons with evidence of in vivo pathological biomarkers, while the presymptomatic individuals are persons without cognitive impairment or evidence of in vivo pathophysiological biomarkers but mutation carriers (Dubois, et al., 2014) (Table 1). According to the NIA-AA criteria, the pathologic process of AD is not always clinically expressed and AD is recognized as a continuum in which the initially asymptomatic AD pathophysiological cascade results in symptoms. Therefore, the NIA-AA criteria consider as Preclinical AD the pathophysiological stage when *in vivo* molecular biomarkers of AD are presents in absent of symptoms. In particular, according to these criteria, the entity of preclinical AD can be divided into three different stages. Stage 1 represents asymptomatic individuals with in vivo markers of brain amyloidosis such as high Positron Emission Tomography (PET) amyloid tracer retention, low cerebrospinal fluid (CSF) of the 42 amino

acid-long form of the amyloid beta peptide (Aβ1-42); Stage 2 is characterised by the presence of both in vivo markers of amyloidosis and neurodegeneration such as neuronal dysfunction on ^{18}F-fluorodeoxyglucose (^{18}F-FDG)-PET/fMRI, High CSF tau/p-tau, cortical thinning and hippocampal atrophy on structural MRI; Stage 3 is represented by both the above in vivo markers of amyloidosis and neurodegeneration plus subtle cognitive decline such as subtle cognitive changes such as poor performances on more challenging cognitive tests. These individuals do not meet yet the criteria for MCI (Sperling, et al., 2011) (Table1).

Regarding the symptomatic phase of AD, three sets of criteria have been established to characterize mild problems in cognition. In particular, they include biomarkers of Alzheimer's disease pathology to increase the confidence that subjects with Mild Cognitive Impairment (MCI) have Alzheimer's disease as underlying cause. However, they differ in the definition of MCI and biomarker abnormality (Visser, et al., 2012) (Table 1). The IWG-1 criteria have introduced the term prodromal AD for the pre-dementia diagnosis of AD and were designed to serve as research criteria. These criteria require episodic memory impairment and at least one abnormal AD biomarker. The biomarker can be a topographical marker, for instance the medial temporal lobe atrophy on MRI or the parieto-temporal hypoperfusion on ^{18}F-FDG-PET, or it can be a pathophysiological marker such as the decreased CSF $A\beta_{1\text{-}42}$, or the increased CSF tau, or the increased amyloid PET uptake (Dubois, et al., 2007). In 2010, the IWG-1 criteria and the lexicon used to define different stages of AD were further elucidated by the group (Dubois, et al., 2010). The major advantage of the IWG-1 criteria has been to integrate the use of biomarkers into diagnosis to allow a biologically based approach to diagnosis independent of disease severity. Recently, the updated IWG criteria (IWG2) distinguish between the pathophysiological markers (diagnostic markers) and the topographical markers (markers of progressions) that are necessary for an early diagnosis of

AD. They described the type of cognitive impairment in different cognitive domains and which biomarker can be considered for the diagnosis or for the progression. The pathological biomarkers are: decreased CSF $A\beta_{1-42}$ and increased tau, or increased amyloid PET uptake (Dubois, et al., 2014) (Table 1). These criteria specify two subtypes: typical prodromal AD if the impairment on a memory test is present; and atypical prodromal AD if only impairment on a non-memory test is present. Along similar lines, one more set of diagnostic criteria has been proposed recently by NIA-AA criteria (Aisen, et al., 2011a,Albert, et al., 2011a,Sperling, et al., 2011).

The NIA-AA criteria have used the term 'mild cognitive impairment due to AD' and were designed for both clinical and research purposes. They require cognitive impairment in any cognitive domain and abnormal amyloid markers (i.e. decreased CSF Aβ1-42 or increased amyloid PET uptake) or neuronal injury markers (i.e. medial temporal lobe atrophy on MRI, increased CSF tau, or parietotemporal hypoperfusion on ^{18}F-FDG-PET. They associated the number of abnormal biomarkers to the likelihood that MCI condition is due to AD (Albert, et al., 2011a) (Table 1).

Both criteria (IWG-1 and NIA-AA) have showed a good predictivity to detect the progression to Alzheimer's disease-type dementia in subjects with MCI (Bouwman, et al., 2010,Oksengard, et al., 2010,Petersen, et al., 2013,Prestia, et al., 2013). A recent study compared the sensibility and specificity of IWG-1, IWG-2 and NIA-AA criteria in detecting the prevalence and incidence of AD at the MCI stage by means of a large multicentre study. The results supported the use of all proposed research criteria to identify AD at the mild cognitive impairment stage. In clinical setting the use of both amyloid and neuronal injury markers, as proposed by the NIA-AA criteria, offers the most accurate diagnosis. For clinical

trial selection, the MCI high likelihood due to AD, as defined by the NIA-AA criteria, or the prodromal AD group, according to the IWG-2 criteria, could be considered (Vos, et al., 2015).

Both approaches showed important similarities and differences and clearly need further validation and standardization before being considered for pivotal studies in AD. However, as Vos and colleagues affirmed, from a regulatory point of view, the Dubois/IWG criteria seem to be less complicated and as such more readily applicable in clinical practice and more easily generalizable to everyday clinical practice (Vos, et al., 2015).

Referring to the diagnosis of full-blown AD, the criteria proposed by Mckhan on 1984 were updated by himself in 2011 (McKhann, et al., 2011). These criteria commonly referred to as the NINCDS-ADRDA criteria, have been quite successful, surviving for over 27 years. These criteria have been reliable for the diagnosis of probable AD, and across more than a dozen clinical pathological studies have had a sensitivity of 81% and specificity of 70% (Knopman, et al., 2001). They have been widely used in clinical trials and clinical research. Knowledge of the clinical manifestations and biology of AD has increased vastly. In the update criteria they proposed the following terminology for classifying individuals with dementia caused by AD: (1) Probable AD dementia, (2) Possible AD dementia, and (3) Probable or possible AD dementia with evidence of the AD pathophysiological process. The first two were intended for use in all clinical settings. The third was intended for research purposes. The updated criteria for probable dementia did not change from the 1984 NINCDS–ADRD.

Table 1. Classification of asymptomatic phase of AD, cognitive impairment phase according to the IWG-I, IWG-2, NIA-AA criteria.

		Clinical profile	Biomarkers
Asymptomatic			
NIA-AA	Stage 1	Cognitively Intact	-High PET amyloid Tracer Retention -Low CSF $A\beta_{1-42}$
	Stage 2	Cognitively Intact	-High PET amyloid Tracer Retention -Low CSF $A\beta_{1-42}$ -Cortical Thinning/Hippocampal Atrophy on sMRI -Neuronal dysfunction on ^{18}F-FDG-PET/fMRI -High CSF tau/p-tau
	Stage 3	Subtle Cognitive Decline/ Poor performance on more challenging cognitive test	-High PET amyloid Tracer Retention -Low CSF $A\beta_{1-42}$ -Cortical Thinning/Hippocampal Atrophy on sMRI -Neuronal dysfunction on ^{18}F-FDG-PET/fMRI -High CSF tau/p-tau
IWG-2	Asymptomatic at risk	Absence of amnestic syndrome of the hippocampal type/Absence of any clinical phenotype of atypical AD	-Low CSF $A\beta_{1-42}$ and High CSF tau/p-tau -High PET amyloid Tracer Retention
	Pre-symptomatic	Absence of amnestic syndrome of the hippocampal type/Absence of any clinical phenotype of atypical AD	-AD autosomal dominant mutation present (in PSEN1, PSEN2, or APP)

Table 1. Classification of asymptomatic phase of AD, cognitive impairment phase according to the IWG-I, IWG-2, NIA-AA criteria.

		Clinical profile	Biomarkers
Cognitive Decline			
IWG-I	Prodromal AD	Objective evidence of an amnestic syndrome of the hippocampal type	-High PET amyloid Tracer Retention -Low CSF $A\beta_{1-42}$ -Cortical Thinning/Hippocampal Atrophy on sMRI -Neuronal dysfunction on ^{18}F-FDG-PET/fMRI -High CSF tau/p-tau
IWG-2	Prodromal AD	- Gradual and progressive change in memory function reported by patient or informant over more than 6 months - Objective evidence of an amnestic syndrome of the hippocampal type	-Low CSF $A\beta_{1-42}$ and High CSF tau/p-tau -High PET amyloid Tracer Retention -AD autosomal dominant mutation present (in PSEN1, PSEN2, or APP)
NIA-AA	MCI due to AD high likelihood	Mild Cognitive Impairment	-Low CSF $A\beta_{1-42}$ and High PET amyloid Tracer Retention -High CSF tau/p-tau -Cortical Thinning/Hippocampal Atrophy on sMRI -Neuronal dysfunction on ^{18}F-FDG-PET/fMRI
	MCI unlikely due to AD	Mild Cognitive Impairment	-High CSF $A\beta_{1-42}$ and Low PET amyloid Tracer Retention -Low CSF tau/p-tau -No Cortical Thinning/Hippocampal Atrophy on sMRI - No Neuronal dysfunction on ^{18}F-FDG-PET/fMRI

The diagnosis of possible AD dementia is currently made whether the following circumstances are present (McKhann, et al., 2011):

Atypical course with cognitive deficits for AD dementia, but either has a sudden onset of cognitive impairment or demonstrates insufficient historical detail or objective cognitive documentation of progressive decline; aetiologically mixed presentation including: (a) concomitant cerebrovascular disease, history of stroke or presence of multiple or extensive infarcts or severe white matter hyperintensity burden; or (b) features of Dementia with Lewy bodies other than the dementia itself; or (c) evidence for another neurological disease or a non-neurological medical comorbidity or medication use that could have a substantial effect on cognition;

In persons who meet the core clinical Criteria for probable AD dementia in vivo biomarkers evidence of brain amyloidosis as well as brain injury, may increase the certainty that the basis of the clinical dementia syndrome is the AD pathophysiological process.

4. STRUCTURAL AND FUNCTIONAL NEUROIMAGING MARKERS OF ALZHEIMER'S DISEASE

4.1 Structural Neuroimaging Markers of AD

The non-invasive, non-radioactive, quantitative nature of magnetic resonance techniques has propelled them to the forefront of neuroscience and neuropsychiatric research. In particular, recent advances have confirmed their enormous potential in patients with Alzheimer disease (AD). Structural and functional magnetic resonance imaging (MRI) have demonstrated significant correlation with clinical outcomes and underlying pathology that allow their implementation in the clinic of AD (Anderson, et al., 2005).

Progressive cerebral atrophy is a characteristic feature of neurodegeneration that can be visualized in vivo with MRI (best with T1-weighted volumetric sequences). The major contributors to atrophy are thought to be dendritic and neuronal losses. Studies of regional MRI volumes (e.g., hippocampus) have shown the association of these volumes with neuronal counts at autopsy (Bobinski, et al., 2000, Gosche, et al., 2002, Jack, et al., 2002).

Structural MRI (sMRI) is the modality most used for the diagnosis of AD. In AD, cerebral atrophy – detected by sMRI – occurs in a characteristic topographic distribution (Baron, et al., 2001) which mirrors the Braak (Braak and Braak, 1991) and Delacourte (Delacourte, et al., 1999, Thompson, et al., 2003) neurofibrillary tangles (NFT) staging. The earliest sites of tau deposition and MRI-based atrophic changes typically lie along the perforant (polysynaptic) hippocampal pathway (entorhinal cortex, hippocampus and posterior cingulate cortex), consistent with early memory deficits (Thompson, et al., 2003) (Scahill, et al., 2002). Later, atrophy in temporal, parietal and frontal neocortices is associated with

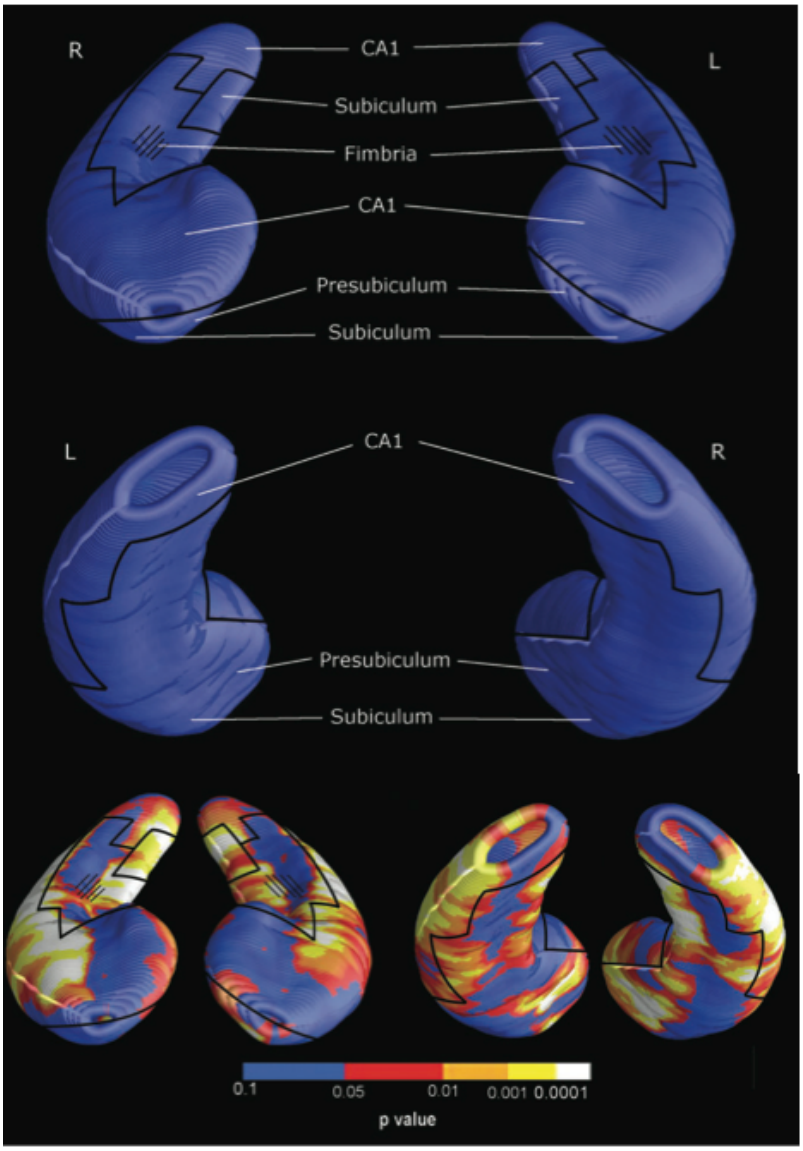
neuronal loss, as well as language, praxic, visuospatial and behavioural impairments (Frisoni, et al., 2008, McDonald, et al., 2009). Although the process of atrophy in AD correlates with the primary proteinopathies such as tau (Josephs, et al., 2008b, Whitwell, et al., 2008a, Whitwell, et al., 2007), the atrophy, however, does not follow the topography of beta-amyloid deposition nor it is particularly well correlated with plaque counts or immunostaining in imaging-autopsy (Jack, et al., 2008b, Josephs, et al., 2008a). Thus, sMRI is correctly viewed as a direct measure of neurodegeneration. The location and severity of atrophy can be extracted from grey scale images by qualitative visual grading (Scheltens, et al., 1992), by quantification of the volume of specific structures, or by measuring volume/thickness from multiple regions of interest to form AD-signature composite measures (Sapolsky, et al., 2010, Vemuri, et al., 2008). The most common sMRI measures employed in AD are at subcortical level the atrophy of the hippocampus, entorhinal cortex, temporal lobe, basal forebrain, and at cortical level the thinning of cortex. In addition, ventricle enlargements and whole brain volume represent more indirect markers of brain atrophy. Rates of change in the whole brain (Fox, et al., 1999, Josephs, et al., 2008a, Schott, et al., 2008, Sluimer, et al., 2008), the entorhinal cortex (Cardenas, et al., 2011), the hippocampus (Jack, et al., 2004, Morra, et al., 2009, Ridha, et al., 2008, Thompson, et al., 2004) and the temporal lobe volumes (Ho, et al., 2010, Hua, et al., 2009), as well as ventricular enlargement (Jack, et al., 2004, Jack, et al., 2003, Ridha, et al., 2008, Thompson, et al., 2004), closely correlate with changes in cognitive performances. These results support the validity of these markers as indexes of disease progression. In order to implement the use of marker of atrophy in the clinic it is necessary that its dynamics is known at each different stages of the disease. In addition, it is important that also the association of the marker of atrophy with the dynamics of other imaging and biological markers is understood

(Frisoni, et al., 2010). Progression of atrophy in the whole brain and in areas targeted by AD (medial temporal lobe, temporoparietal, and restrosplenial cortex) represents a reliable marker of the neurodegenerative process underlying clinical symptoms, and it seems more robust than markers of amyloidosis alone. From MCI to moderate dementia stage of AD, structural markers are more sensitive to change than markers of beta-amyloid deposition (as assessed through brain imaging or CSF analysis)(Jack, et al., 2009). In asymptomatic to MCI stages, however, indirect evidence indicates that amyloid markers show more substantial abnormalities than structural markers (Engler, et al., 2006, Fox, et al., 2001, Josephs, et al., 2008a, Minoshima, et al., 1997, Pike, et al., 2007, Ridha, et al., 2006) (Jack, et al., 2010).

Among all available MRI markers of AD, the hippocampal atrophy, assessed on high-resolution T1-weighted MRI images, is the best established and validated (Figure 3). The simplest way to assess atrophy of the medial temporal lobes is by visual inspection of coronal T1-weighted MRI. Several rating scales to quantify the degree of atrophy have been developed and are widely used (Scheltens, et al., 1992). Visual rating scales provide ≈80–85% of sensitivity and specificity to distinguish patients with AD from those with no cognitive impairment, and only slightly lower sensitivity and specificity levels for diagnosing amnestic MCI and for anticipating their decline (DeCarli, et al., 2007, Duara, et al., 2008, Korf, et al., 2004). These scales also have good predictive power to anticipate decline in MCI (DeCarli, et al., 2007, Duara, et al., 2008, Korf, et al., 2004, Scheltens, et al., 1992). Visual rating also correlates well with underlying pathology and has high diagnostic accuracy against a pathologically verified diagnosis of AD (Burton, et al., 2009). Despite its convoluted structure, the boundaries of the hippocampus (and adjacent CSF spaces) are easier to be recognized from human operators or automated algorithms than other structures such as the amygdala, the entorhinal cortex or the parahippocampal gyrus. This is because the

anatomical boundaries of the hippocampus are distinct on high-resolution T1-weighted MRI scans around most of the surface of this structure. Hippocampal volume measured *in vivo* by MRI correlates with Braak stage and neuronal counts (Bobinski, et al., 2000, Jack, et al., 2002) (Gosche, et al., 2002).

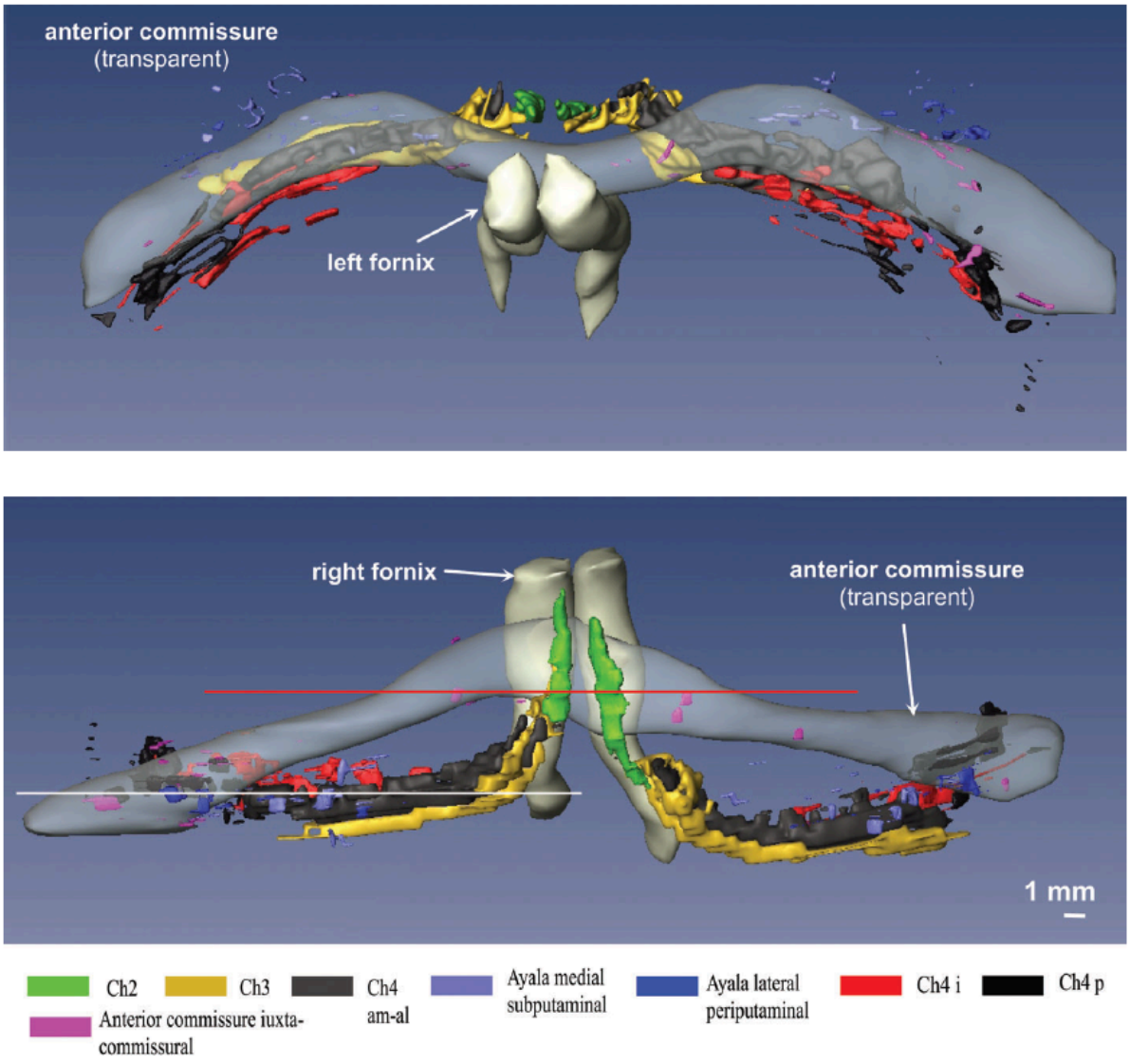
Figure 3. 3D-reconstraction of cytoarchitectonic hippocampal subregions. At the top, cytoarchitectonic subregions mapped on blank MR based models at 3Tesla of the hippocampal formation of a healthy subject (Frisoni, et al., 2006). At the bottom, effect of AD on local hippocampal volume: maps of the difference of regional volume between 19 AD patients and 19 healthy elderly controls (Frisoni, et al., 2008).



At the mild dementia stage of AD, hippocampal volume is already reduced by 15–30% relative to controls (van der Flier, et al., 2005), and in the amnestic variant of MCI the volume is reduced by 10–15% (Shi, et al., 2009) (a meta-analysis of hippocampal MRI studies is provided elsewhere (Barnes, et al., 2008)). A meta-analysis estimated that medial temporal atrophy has ≈73% sensitivity and ≈81% specificity for predicting whether patients with amnestic MCI will convert to dementia (Yuan, et al., 2009). When the medial temporal lobe atrophy is measured with a continuous metric such as hippocampal volume, specificity might be increased, but at the cost of reduced sensitivity. Recently, this marker was recommended by the revised criteria for AD as one of core biomarkers for AD (Albert, et al., 2011b, Dubois, et al., 2010, Dubois, et al., 2007, C.R. Jack, Jr., et al., 2011a, McKhann, et al., 2011). Thanks to its reliability as diagnostic marker for AD, international efforts to harmonize the definition of the hippocampus were carried out (Frisoni and Jack, 2011, Frisoni, et al., 2014, C.R. Jack, Jr., et al., 2011b). Fully automated MR-based hippocampal volumetry seems to fulfil the requirements for a relevant core feasible biomarker for detection of AD associated neurodegeneration in everyday patient care, such as in a secondary care memory clinic for outpatients.

Recently growing in vivo evidences have also confirmed the presence of the basal forebrain (BF) atrophy in AD patients and in clinical prodromal stages of AD (Grothe, et al., 2013, Grothe, et al., 2012, Teipel, et al., 2011). The BF could be considered as uninterrupted band connected to basotemporal areas such as the entorhinal cortex, hippocampal formation (Mesulam, 2013) (Figure 4). Degeneration of BF cholinergic nuclei is associated with cognitive decline, and this effect it might be mediated by neuronal dysfunction in the denervated cortical areas. MRI-based measurements of BF atrophy are increasingly being used as in vivo surrogate markers for cholinergic degeneration.

Figure 4. Computer-assisted 3D reconstruction of the human BF complex, seen from the dorsal view (at top) and from the fronto-occipital perspective (at the bottom) (Grinberg, 2007).



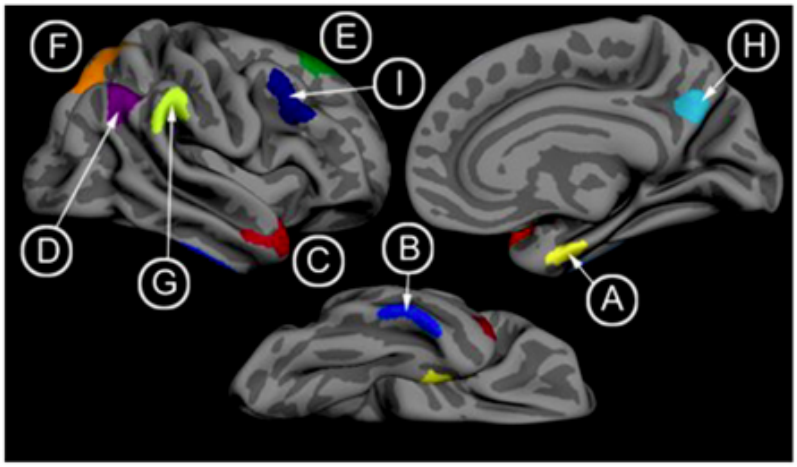
Unlike hippocampal volumes, BF volume has shown to be correlated to brain amyloid status suggesting that the atrophy in the former is closely related to cortical amyloid burden (Kerbler, et al., 2015, Teipel, et al., 2014). A recent study conducted by Kilimann and colleagues revealed significant volume reductions of all subregions of the BF in AD, in particular in the posterior nucleus basalis of Meynert (NbM). In this study, the mild cognitive

impairment group who converted to AD showed pronounced volume reductions in the Ch4p and in the nucleus subputaminalis, but preserved volumes of anterior-medial regions (Ch4am). The diagnostic accuracy of posterior NbM volume was superior to hippocampus volume in both groups, despite higher multicentre variability of the BF measurements. The data of this study suggested that the morphometry of BF may provide an emerging biomarker in AD (Kilimann, et al., 2014). Moreover, the BF atrophy was also associated with widespread cortical hypometabolism, and path analytic models indicated that hypometabolism in domain-specific cortical networks mediated the association between BF volume and cognitive dysfunction (Grothe, et al., 2015).

Cortical atrophy is reflected in a loss of grey matter that resulted in a reduction of cortical thickness. Cortical thickness measurement across the entire cerebrum was recognised as a marker for AD (Dickerson, et al., 2009). Normative data describing the cross-sectional and longitudinal brain volume decline in aging and AD showed an acceleration of whole brain volume atrophy rates associated to AD (Cardenas, et al., 2003, Fotenos, et al., 2005, Fox, et al., 1999) confirming that extensive regional changes are taking place in AD, not only in the medial temporal lobe regions. Cortical thinning in AD was first found in distributed association areas pointing out that regional atrophy can be detected across widespread cortical areas (Du, et al., 2007, Lerch, et al., 2008). Cortical thickness measurements in comparison to other biomarkers provide quantitative values describing physical property of the brain detectable at single individual level (Rosas, et al., 2002). Dickerson and colleagues identified a spatial topography of regional cortical thinning in AD patients (Dickerson, et al., 2009) (Figure 5). The cortical AD signature identified by Dickerson and colleagues was consistent with previous findings with voxel-based morphometry in mild AD (Baron, et al., 2001, Bozzali, et al., 2006, Karas, et al., 2004, Whitwell, et al., 2008b) and

similar techniques (Apostolova and Thompson, 2008, Scahill, et al., 2002, Thompson, et al., 2003). A larger degree of thinning was found in the medial temporal, inferior temporal, temporal pole, inferior parietal, and posterior cingulate/precuneus regions.

Figure 5. Regions of cortical thickness affected in Alzheimer's disease patients (image from Dickerson et al., 2009). The cortical signature of AD is composed of a priori regions of interest in which consistent atrophy has been previously observed in multiple samples of patients with mild AD dementia. Key: A: medial temporal, B: inferior temporal, C: temporal pole, D: Angular, E: superior frontal, F: superior parietal, G: supramarginal, H: precuneus, I: middle frontal, J: calcarine, K: caudal insula, L: cuneus.



These regions are those earliest affected during AD, due to the burden of pathologic accumulation (Arnold, et al., 1991, Hyman, et al., 1984, Van Hoesen, et al., 1986), neuronal loss, and gliosis (Brun and Gustafson, 1976).

Structural MRI is a widely established method to measure regional and global brain volumes in vivo. Nevertheless, volumetric MRI measurements are sensitive to variations in image contrast and resolution caused by differences in scanner settings across multiple sites. However, the multicentre variability can be effectively reduced by the use of phantom-based

standardization of acquisition protocols across sites and correction of most commonly encountered image artefacts using uniform post-acquisition image processing tools (Cavedo, et al., 2014, Jack, et al., 2008a). Methodologically, accurate volumetric assessment requires Standard Operating Procedures (SOPs) that include the know-how specific for the modality, acquisition parameters, and suitable training of the staff. For this reason, strict standardization of acquisition and measurement is being undertaken for manual hippocampal volumetry by a European Alzheimer's Disease Consortium (EADC)-ADNI consortium under the auspices of the Alzheimer's Association (Frisoni, et al., 2015, C.R. Jack, et al., 2011). The EADC-ADNI SOPs, already implemented for the manual segmentation of the hippocampus, will be used to validate automated algorithms, such as Free Surfer and Learning Embeddings Atlas Propagation. Other brain regions, and their corresponding standardized operating procedures, are under active investigation. For example, the entorhinal cortex showed performance similar to hippocampus using the Quarc analysis software (Quarc, Wedemark, Germany) (Holland, et al., 2012). The implementation of standardized operating procedures in term of MRI acquisition protocols and imaging markers detection represents one of the most important challenges in this field in order to obtain surrogate markers of progression as much repeatable and reliable as possible.

4.2 Functional Neuroimaging Markers of AD

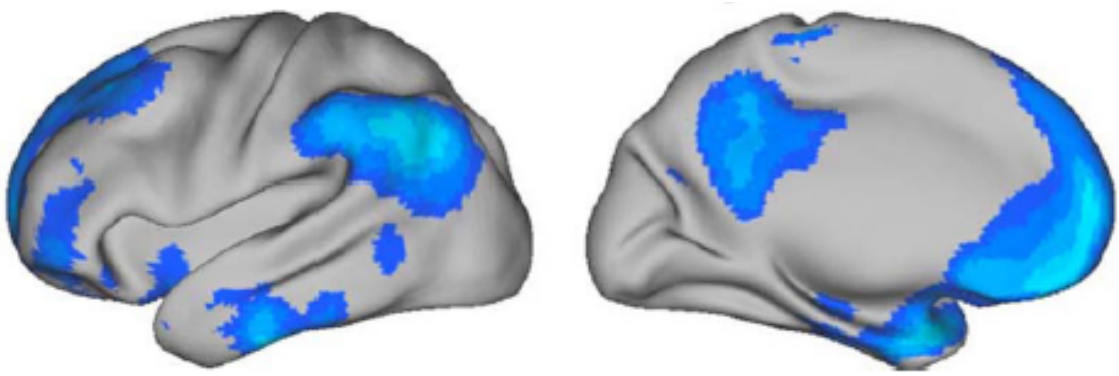
Functional MRI (fMRI) has been employed to explore the functional integrity of brain networks related to memory and other cognitive domains in aging and AD patients. Functional MRI is a non-invasive imaging technique providing an indirect measure of

neuronal activity due to increase in blood flow, which usually result in changes in blood oxygenation (Kwong, et al., 1992, Ogawa, et al., 1990). Thus, changes of image intensity can be observed, given rise to the so-called Blood Oxygenation Level Dependent signal reflecting integrated synaptic activity of neurons via MRI (Logothetis, et al., 2001). fMRI can be acquired during cognitive tasks, comparing two different conditions, for instance: encoding new information (experimental condition) and viewing familiar information or visual fixation on a cross-hair (control condition). Moreover, fMRI can be acquired during the resting state to investigate the functional connectivity within specific brain networks. Both task-related and resting fMRI techniques are able to identify early brain functional dysfunction associated to AD, and they might be implemented as surrogate markers to monitor therapeutic response in relatively short-term follow-up. Due to the small number of research groups investigating functional brain alterations using fMRI in aging, MCI, and AD patients, this field of research has been so far less investigated. The early fMRI experiments conducted in MCI and AD patients have used episodic memory tasks, and were focused on the pattern of fMRI activation in the hippocampus and related structures in the medial temporal lobe. In AD patients a reduction of hippocampal activity during the encoding of new information (Golby, et al., 2005, Gron, et al., 2002, Hamalainen, et al., 2007, Kato, et al., 2001, Machulda, et al., 2003, Rombouts and Scheltens, 2005, Small, et al., 1999, Sperling, et al., 2003) and an increment of prefrontal cortical activity (Grady, et al., 2003, Sole-Padulles, et al., 2009, Sperling, et al., 2003) was detected, suggesting that mechanisms of compensation throughout the increase activation of other networks might be present. Furthermore, decreased medial temporal lobe (MTL) activation was found in MCI (Johnson, et al., 2006, Machulda, et al., 2003, Petrella, et al., 2009, Small, et al., 1999) and in genetic at-risk individuals (Borghesani, et al., 2008, Lind, et al., 2006, Mondadori, et al., 2007, Ringman, et

al., 2011, Trivedi, et al., 2006). In the study of Gordon and colleagues two attentional control tasks were used to investigate alterations in task-evoked fMRI data related to biomarkers of AD pathology in preclinical AD population. Results showed a significant association between higher levels of tau and phosphorylated tau pathologies and block-level over activations of attentional control areas, suggesting early alteration in attentional control with increasing levels of AD pathology (Gordon, et al., 2015). By contrast, other fMRI studies have reported evidence of increased MTL activity in very mild MCI subjects (Celone, et al., 2006, Dickerson, et al., 2004, Dickerson, et al., 2005, Hamalainen, et al., 2007), and genetic at risk of AD cognitively intact individuals (Bondi, et al., 2005, Bookheimer, et al., 2000, Filippini, et al., 2009, Fleisher, et al., 2005, Wishart, et al., 2006). These discrepant results can be justified by the use of different specific paradigms, clinical stage of subjects, and behavioural performances. A common feature of the studies reporting evidences of increased fMRI activity was due to the fact that the at-risk AD individuals performed the fMRI memory tasks reasonably well. This consideration support the hypothesis that hyperactivity may represents a compensatory mechanism at early stage of AD (Dickerson and Sperling 2008; Sperling et al. 2009)(Celone et al. 2006) and predict a rapid cognitive decline (Bookheimer, et al., 2000, Dickerson, et al., 2004), and loss of hippocampal function on serial fMRI (O'Brien et al. 2010).

The default mode network represents the presence of brain activity in specific regions during passive control state compared to the processing of external stimuli (Buckner et al. 2008). The core brain areas associated with the brain's default network are: the ventral medial prefrontal cortex, the posterior cingulate cortex, the inferior parietal lobule, the lateral temporal cortex, the dorsal medial prefrontal cortex, the hippocampus and the entorhinal and parahippocampal cortices (Figure 6) (Buckner et al. 2008).

Figure 6. Brain Default mode Network. The brain's default network was originally identified in a meta-analysis that mapped brain regions more active in passive as compared to active tasks (of- ten referred to as task-induced deactivation). The displayed positron emission tomography (PET) data include nine studies (132 participants) from Shulman et al. 1997; reanalysed in Buckner et al., 2005). The images show the medial and lateral surface of the left hemisphere using a population-averaged surface representation to take into account between-subject variability in sulci anatomy (Van Essen et al., 2005). Blue represents regions most active in passive task settings.



An abnormal response during memory tasks in the default network was recently observed in clinical AD patients and individuals at risk of AD (Lustig and Buckner 2004; Celone et al. 2006; Petrella et al. 2007; Pihlajamaki et al. 2008). Notably, the same default network regions usually showing beneficial deactivations in the posterior cingulate/precuneus in cognitive intact individuals (Daselaar, et al., 2004,Miller, et al., 2008), tends to manifest an increase fMRI activity (or loss of normal default network deactivation) in AD patients and individuals at-risk of AD (Fleisher, et al., 2013, Petrella, et al., 2007, Pihlajamaki, et al., 2008, Sperling, et al., 2010). Moreover, studies in MCI and AD patients showed an impaired at rest functional connectivity between the posterior cingulate/precuneus and the hippocampus (Bai, et al., 2008, Greicius, et al., 2004, Koch, et al., 2010, Rombouts and Scheltens, 2005, Sorg, et al., 2007). In addition, disrupted default mode network activity both during task and at rest condition was found associated to in vivo

brain amyloidosis load in cognitive intact elderly (Hedden, et al., 2009, Sheline, et al., 2010, Sperling, et al., 2009), suggesting that the disruption of the default mode network may be an imaging markers particularly useful to track response to antiamyloid therapies in AD clinical trials in pre-dementia stages (Johnson, et al., 2012).

No SOPs are currently available for the implementation of fMRI markers. Moreover, several limitations are present for both fMRI application (at rest or using activation tasks). Functional MRI activation needs: (i) audio/visual support for stimuli presentation (for example, goggles) and performance recording (for example, joysticks), (ii) expert personnel, (iii) calibration procedures for each task, (iv) and proven invariance to repeated exposure (often not studied). All of these needed limit their implementation in multicentre trials or in diagnostic routine in the community. The main limitation of fMRI at rest is the impossibility to control what people are thinking during the MRI acquisition. It indeed depends on many state-dependent factors, such as the noise during the acquisition that can influence the acquisition at rest and the related interpretation of data acquired. Although the above limitations, either cognitive paradigms or during resting state fMRI, may hold the greatest potential as markers of early diagnosis or for the evaluation of novel pharmacological strategies to treat AD.

5. BIOMARKERS IN ALZHEIMER'S DISEASE DRUG DEVELOPMENT

In the mid-1980s, researchers succeeded in identifying amyloid-beta and tau proteins as the main components of plaques and tangles, which are the main hallmarks of AD. This led to the research of imaging and biological markers of AD as indirect *in vivo* measures of neuropathological processes of AD. According to the National Institute of Aging a biomarker is defined as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention' (Biomarkers Definitions Working Group., 2001). Together, these discoveries led to the development of a large amount of drug candidates that are currently tested in clinical trials for AD and may have disease-modifying effects. The most widely used drugs for treating AD are the cholinesterase inhibitors. Cholinesterase inhibitors are the only drugs currently approved by the United States Food and Drug Administration (FDA) and include: Aricept® (donepezil), Razadyne® (galantamine), and Exelon® (rivastigmine).

Designing secondary prevention trials for pre-dementia populations presents many challenges, including how to identify appropriate subjects for such trials and how to assess treatment effectiveness. The FDA published a draft guidance outlining their thinking about drug development of early AD stages (Blennow, 2010). In this guidance, they report the difficulty of using time-to-dementia as an endpoint in clinical trials since it fails to take into account the gradually progressive nature of the disease, suggesting that their might consider the possibility "that a claim of disease modification could be supported by evidence of a meaningful effect on a biomarker in combination with a clinical benefit" (Food and Drug Administration. Draft Guidance for Industry. Alzheimer's disease: Developing drugs for the treatment of early stage disease). Similarly, the European Medicines Agency (EMA) has

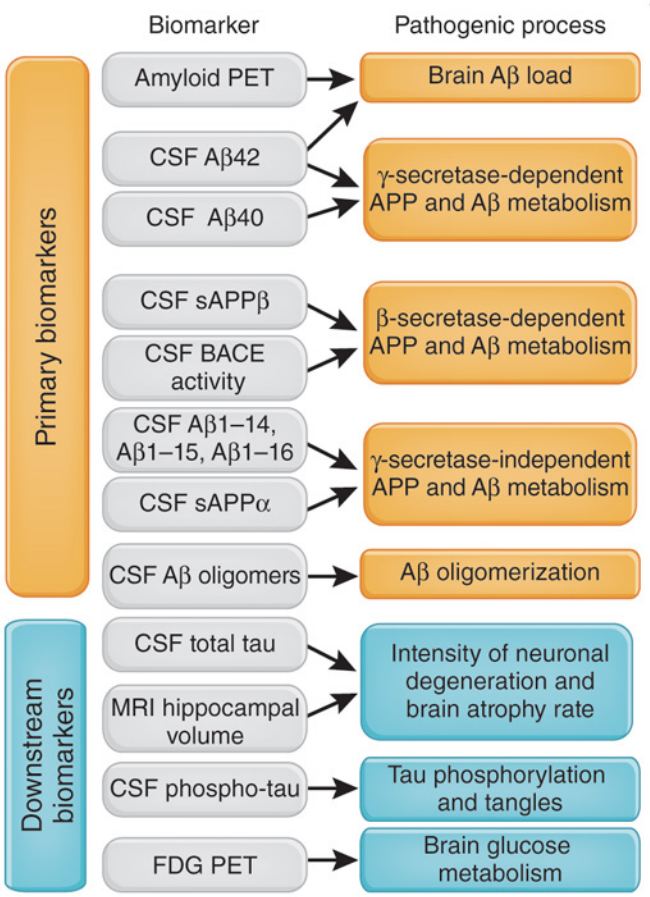
suggested that biomarkers could provide supportive evidence for disease modification recognizing that the development of such biomarkers represents a high priority particularly in dementia (European Medicines Agency Committee for Medicinal Products for Human Use (CHMP). Guideline on medicinal products for the treatment of Alzheimer's disease and other dementias. 2008). Mani (Mani, 2004) underlined that according to regulatory bodies such as FDA and EMA a therapeutic intervention has an effect of disease modification, when it shows both clinical improvement and disease modification. Similarly, according to the European regulators a disease-modifying effect is considered when the pharmacologic treatment delays the underlying pathological or pathophysiological disease processes and when this is accompanied by an improvement of clinical signs and symptoms of the dementing condition. To foster the innovation in this field, the collaboration among consortia such as 'The Biomarker Consortium' (Woodcock and Woosley, 2008), 'The Innovative Medicine Initiative' (Council of the European Union: Council regulation (EC) No. 73/2008 of 20 December 2007 setting up the joint undertaking for the implementation of the joint technology initiative on innovative medicines. Official Journal of the European Union 2008: L30/38–L30/51), the ADNI and different stakeholders are necessary (Cummings, 2010).

Biomarkers can be used in all stages of drug development in order to: (i) understand the biology and pathophysiology of a disease process, (ii) clarify the mode of action of medicinal products, and (iii) provide information on relevant subpopulations of patients who might respond to treatment or who might be susceptible to side effects.

Conceptually it might be useful to divide biomarkers into primary and secondary biomarkers. A primary biomarker can be used to identify and monitor the specific

biochemical effect, or mode of action of a drug. The main primary biomarkers in AD are: the brain amyloid load measure using imaging PET or CSF levels of $A\beta_{1-42}$ (Figure 7). Secondary biomarkers or biomarkers of progression are used to identify and monitor effects on pathogenic processes downstream of the drug, for example, CSF tau and MRI measurements of brain atrophy able to identify and monitor an effect on the rate of neuronal degeneration in an anti-abeta trial (Figure 7). Therefore, AD biological and imaging markers are needed not only for the early AD diagnosis, but also to select subjects for clinical studies and to monitor the treatment-response. Recently, all the stakeholders involved in drug development and biomarkers harmonization are encouraging the use of biomarkers as surrogate endpoints (Blennow, 2010, Hampel, et al., 2010a, Hampel, et al., 2011, Trojanowski, et al., 2010, Vellas, et al., 2008). A surrogate endpoint is a marker that is intended to substitute a clinical endpoint, which means that a change in the surrogate endpoint adequately reflects a clinical change. Even the use of a biological or imaging marker as surrogate endpoint measure has been used in many drug development programs (Frank and Hargreaves, 2003, Hampel, et al., 2010b); its role is still controversial. Before their application in clinical trials, the specific biomarker must be validated in the population and clinical setting for which the biomarker is intended – the biomarker can be considered as a 'surrogate endpoint' when it allows substitution for a clinically relevant endpoint that is a direct measure of how a patient feels, functions, or survives and can be expected to predict the effect of the therapy (Bucher, et al., 1999, Fleming and Powers, 2012, Katz, 2004).

Figure 7. Flowchart for Primary and Downstream (Secondary) biological and imaging markers for AD (figure adapted from Blennow et al., 2010).



However, a perfect correlate of the biomarker with disease progression in the untreated state is not sufficient to accept a biomarker as a surrogate for a clinical endpoint (Baker and Kramer, 2003). Surrogate endpoint can be used in ways that allow decision-making on further drug development. Ultimately, if any of these biomarkers are found to be acceptable as surrogate endpoints, definitive efficacy trials may also be considerably shorter and/or smaller than studies using more traditional clinical outcomes as primary endpoint. In those settings in which clinical outcomes cannot be assessed practically, such as in studies

examining prevention therapies of AD in which clinical outcomes (e.g. time of onset of diagnosis of clinical AD) may not occur for many years after treatment initiation, not validated surrogates biomarkers of outcome are likely to be considered. For these reasons, the standardization and validation regarding the technical aspects of acquisition, measurement, analysis and interpretation of biological and imaging marker is urgently needed. This is supported by the ongoing dialogue on harmonization of regulatory practices between EMA and FDA, both of which take into consideration the experience with biomarkers in other fields like oncology or cardiovascular medicine for the field of dementia and AD. To foster these developments, EMA has established a qualification procedure of biological and imaging markers of AD for a specific intended use in drug development through the involvement of consortia, networks, public/private partnerships. In this framework, they publish a second qualification opinion for use of low hippocampal volume (atrophy) by MRI as primary imaging marker in clinical trials in patients with pre-dementia stage of AD (EMA-CHMP, 2011). Throughout the collaboration among all stakeholders and the biomarkers validation as surrogate endpoints in clinical trials might bring to efficacious and safe medicinal products for AD patients (Broich, 2012).

6. STRUCTURAL AND FUNCTIONAL NEUROIMAGING MARKERS AS SURROGATE OUTCOMES IN ALZHEIMER'S DISEASE CLINICAL TRIALS

In the context of drugs research, disease-modifying treatments for AD have so far failed to demonstrate their efficacy (Mangialasche, et al., 2010). Despite this, a large number of candidate disease-modifiers are advancing into phase II and III testing. Part of the reason of clinical trial failure might be due to heterogeneous patient populations and clinical end-points based on insufficiently sensitive clinical features (Aisen, et al., 2011a). Recently, the EU/US Task Force discussed progress in developing three types of biomarkers that have shown promise as outcome measures in clinical trials: CSF biochemical markers, sMRI markers, and markers of brain amyloid and tau using PET (Vellas, et al., 2013). Moreover, functional connectivity measures, as assessed by means of functional MRI, are emerging as potential intermediate biomarkers for AD. A summary of previous studies describing the potential use of sMRI and fMRI in clinical trials of AD therapeutic agents, as well as the limitations of these promising imaging techniques is reported.

6.1 Structural Neuroimaging Markers in Clinical Trials for AD

The most commonly used imaging modality in the study of AD has been volumetric T1-weighted sMRI. These images provide high-resolution (~1 mm) structural images with good tissue contrast. Longitudinal natural history cohort studies have demonstrated changes in global measures based on T1 images, such as whole brain or ventricular volumes, as well as regional measures. In particular longitudinal volume reduction was found in the hippocampal volume of AD patients compared to age-matched cognitively intact individuals. Considering the all known outcome measures used in clinical trials, such as clinical

progression measurements; performances at psychometric tests; neuroimaging (functional structural and molecular) and biofluid markers, the sMRI seems to have the highest measurement precision (Cavedo E, 2014, Jack, et al., 2008c). Remarkably, sMRI techniques are becoming of great interest since they not require radioactivity exposure; they are relatively inexpensive and can be performed on machines already available in almost hospital (Frisoni, et al., 2010, Johnson, et al., 2012, Merlo Pich, et al., 2014). In clinical trials, sMRI was first of all used as a radiologic diagnostic aid to exclude individuals with incidental brain pathologies. Currently, sMRI markers (Ciumas, et al., 2008) are used as effective outcome measures in clinical trials. Questions regarding which sMRI markers are best to use, and how, are far from be resolved, and the choice must be taken considering: (i) the type of therapeutic intervention, (ii) the clinical stage of AD, (iii) the time dependence of the imaging marker change during disease progression, (iv) as well as its costs and availability (Vellas, et al., 2012). Numerous clinical trials on a wide range of compounds have considered imaging endpoints based on sMRI in AD and MCI population. A summary of findings currently published in the literature is presented in Table 2.

The most commonly used atrophy measured in clinical trials is the global measure of whole brain atrophy. Numerous drugs showed no significant treatment effects on whole brain atrophy in AD patients, such as the atorvastatin and donepezil (Feldman, et al., 2010), the CAD106 (Winblad, et al., 2012) the decosahexaeonic acid (Quinn, et al., 2010), the intravenous immuglobin (Dodel, et al., 2013), the memantine (Weiner, et al., 2011), rosiglitazone (Tzimopoulou, et al., 2010), the Scyllo-inositol (Salloway, et al., 2011), the Semagacestat (Doody, et al., 2013) and the Solaneuzumab (Doody, et al., 2014). Unexpected effects were found in the AN-1792 study, in which a strong association between antibody titre and brain volume loss was found in the antibody responders: subjects who generated

the higher titres of antibodies had greater volume loss (Fox, et al., 2005). These evidences were not confirmed in a subsequent study conducted in a small subset of study participants who underwent longer term follow-up scans (4.5 years after baseline) (Vellas, et al., 2009). Significant effect of drugs on whole brain atrophy was found in a phase II study of Bapineuzumab showing a substantial treatment effect, a less annual reduction of 10.7 ml over 71 week follow-up in the whole brain in the APOE ε 4 non-carriers treated group (Salloway, et al., 2009). This finding was not subsequently replicated in the larger phase III study of APOE ε 4 carrier and no carrier population (Salloway, et al., 2014). Three out of four studies investigating the effect of donepezil, Vitamin E and Galantamine on whole brain atrophy in MCI patients revealed a significant reduction of atrophy in the treatment group (Dubois, et al., 2015, Prins, et al., 2014, Schuff, et al., 2011).

Ventricular enlargement can be a sensitive (although nonspecific) volumetric measure in dementia. Increased ventricular expansion was seen in the AN-1792 study with some suggestion of greater ventricular expansion relative to global brain loss. A similar finding was also observed in a phase II study of scyllo-inositol and in the Bapineuzumab phase II trials exclusively in APOE ε 4 carriers individuals (Salloway, et al., 2009). This finding has been confirmed in the two phase III studies, although the increase was much smaller than the previous studies.

Table 2. Summary of studies using sMRI markers as imaging endpoints in Clinical Trials in mild to moderate AD and MCI patients

Diagnosis	Compound	Subjects Number		Follow-up (months)	Marker & Treatment effect
		Placebo	Treatment		
AD	AN-1792 (Fox, et al., 2005)	57	231[a]	11	⇑Ventricles, whole brain atrophy (⇑, antibody responders only), ⇔ hippocampus
AD	Atorvastatin and donepezil (Feldman, et al., 2010)	64[b]		18	Whole brain (⇔), hippocampus (⇓)
AD	Bapineuzumab (phase II) (Salloway, et al., 2009)	122	107	18	Whole brain (⇓, in ε4 no carriers), ventricles (⇑, in ε4 carriers)
AD	Bapineuzumab (phase III) ε 4 carriers (Salloway, et al., 2014)	238	352	18	Whole brain (⇔)
AD	CAD106 (Winblad, et al., 2012)	7/5	24/21[c]	6,12	Whole brain (⇔)[d], ventricles (⇔), hippocampus (⇓)[e]
AD	Docosahexaenoic acid (Quinn, et al., 2010)	49	53	18	Whole brain (⇔), ventricles (⇔), hippocampus (⇔)
AD	Intravenous immunoglobulin (Dodel, et al., 2013)	7	21	3, 6	Whole brain (⇔), hippocampus (⇔)
AD	Memantine (Weiner, et al., 2011)	40[f]		12	Whole brain (⇔), ventricles (⇔), right hippocampus ⇓

⇑= treatment effect of increased atrophy (or ventricular enlargement); ⇓= treatment effect of decreased atrophy (or ventricular enlargement); ⇔=no treatment effect found. [a]Of these 231 subjects in the treatment arm, 45 were antibody responders. [b]The number of subjects enrolled in the magnetic resonance imaging substudy does not identify a division between the placebo arm and the treatment arm, thus the number in the cell indicates the total number of subjects over both arms. [c]The CAD106 study had two cohorts, where the treatment arm dosage was different (Cohort I, 50 μg; Cohort II, 150 μg). [d]One global measure of atrophy, left cerebral white matter, did show a treatment effect in Cohort I, but this did not survive correction for multiple comparisons. [e]In Cohort I, the right hippocampus showed a treatment effect and left hippocampus showed a trend towards treatment effects, but these did not survive multiple comparisons.

Diagnosis	Compound	Subjects Number		Follow-up (months)	Marker & Treatment effect
		Placebo	Treatment		
AD	Memantine (Wilkinson, et al., 2012)	144	133	12	Whole brain (⇔), hippocampus (⇔)
AD	Memantine (Schmidt, et al., 2008)	18	18	12	Whole brain (⇔), hippocampus (⇔)
AD	Rosiglitazone (Tzimopoulou, et al., 2010)	38	38	6, 12	Whole brain (⇔)
AD	Scyllo-inositol (Salloway, et al., 2011)	83	259	18	Whole brain (⇔), ventricles (⇑), hippocampi (⇔)
AD	Semagacestat (Doody, et al., 2013)	208[b]		18	Whole brain (⇔), hippocampus (⇔)
AD	Solaneuzumab (Doody, et al., 2014)	370/400	370/406	18	Whole brain (⇔), hippocampus (⇔)
AD	Donepezil (Krishnan, et al., 2003)	23	28	24	Hippocampus (⇓)
AD	Tramiprosate (Aisen, et al., 2011b,Gauthier, et al., 2009)	109	203[g]	18	Hippocampus (⇓)[h]
MCI	Donepezil and Vitamine E (Jack, et al., 2008c)	54	37 Donepezil 40 Vitamine E	36	⇔ Brain, ⇔Hippocampal, ⇔ERC rates of atrophy, ⇔ventricular dilatation rate
MCI	Donepezil (Dubois, et al., 2015)	92	82	12	⇓Whole Brain, ⇓Hippocampal rates of atrophy, ⇓ventricular dilatation rate
MCI	Donepezil (Schuff, et al., 2011)	109	125	12	⇓Whole Brain, ⇔Hippocampal rates of atrophy, ⇓ventricular dilatation rate, ⇔ERC rates of atrophy
MCI	Galantamine (Prins, et al., 2014)	188	176	24	⇓Whole Brain rate of atrophy, ⇔ Hippocampal rates of atrophy
MCI	Rivastigmine (Feldman, et al., 2007)	254	259	48	⇔ ventricular dilatation rate of change

[f]Single-group open-label study where subjects had a 24-week lead-in period, followed by 24 weeks treatment of memantine. [k]Two treatment arms: 103 subjects at 100 mg twice daily, 100 subjects at 150 mg twice daily. [h]Original model showed no treatment effects, but post-hoc analysis putting in site as a random effect and important covariates showed a treatment effect.

Three studies described stable ventricular volumes after treatment (Quinn, et al., 2010, Weiner, et al., 2011, Winblad, et al., 2012). An enlargement of brain ventricles was found in MCI patients who did not receive any treatment in clinical trials with donepezil (Dubois, et al., 2015, Schuff, et al., 2011).

Atrophy of MTL (hippocampi, entorhinal cortex) is considered one of the most specific markers in AD, and it was highly used in clinical trials on AD and MCI patients. The hippocampal atrophy was recently accepted as a marker of inclusion criteria in AD clinical trials. In the cohort of AD patients where one year of the CAD106 vaccine treatment was investigated, a hippocampal atrophy reduction was seen although this result did not survive correction for multiple comparisons (Winblad, et al., 2012). Studies investigating the effect of atorvastatin observed treatment effects in reducing hippocampal atrophy in the treated group (Feldman, et al., 2010). In the Alzheimer's Disease Cholesterol Lowering trial this finding did not reach statistical significance and the treatment effects on the hippocampus were based primarily on right hippocampal volume (Sparks, et al., 2008). Studies on memantine showed contrasting results. In a phase IV, open-label single-group study the comparison of the hippocampal rate of atrophy showed a treatment effect in right hippocampal atrophy (Weiner, et al., 2011). These results were not subsequently confirmed (Wilkinson, et al., 2012). During clinical trials with donepezil and Tramiprosate a treatment effect of these drugs was observed in the hippocampal volume of AD patients (Table 2). In MCI patients two studies investigated the effect of donepezil using neuroimaging outcomes and showed only a trend of significance of donepezil in slowing hippocampal rate of atrophy (Dubois, et al., 2015, Schuff, et al., 2011). These findings have been recently contradicted by our group investigating the donepezil effect on the annualized hippocampal rate of change

on a separate cohort of well clinically characterised MCI with the amnestic syndrome of hippocampal type (Dubois, et al., 2015).

Being the hippocampus one of the core biomarker for AD, the standardization and harmonization of this marker is essential (Frisoni, et al., 2010). Recently, a harmonized protocol for manual hippocampal segmentation was developed and validated by a collaborative project of EADC-ADNI (Frisoni, et al., 2014). This group tested the variance due to different tracers, scanners, post-processing pipeline, and manual vs. automated segmentation.

6.2 Functional Imaging Markers in Clinical Trials for AD

Longitudinal fMRI, represents a useful method for elucidating the neural changes associated with pharmacological modulation and a potential tool for monitoring intervention efficacy in clinical trials. Pharmacological effects on memory networks have been described in fMRI studies on healthy young and older subjects (Kukolja, et al., 2009, Sperling, et al., 2002, Thiel, et al., 2001). A summary of clinical trials currently published on fMRI markers applied as surrogate outcomes in AD and preventive trials are presented in Table 3. To date, only few small fMRI studies have been conducted to investigate the impact of AD drugs therapy on in vivo brain functions. To date, few fMRI studies have been conducted as typical double-blind, placebo-controlled trials. Studies investigating anticholinesterase therapy showed enhanced brain activation after acute or prolonged treatment with cholinesterase inhibitors in MCI and AD (Bokde, et al., 2009, Goekoop, et al., 2006, Saykin, et al., 2004, Shanks, et al., 2007). Others showed stabilization of medial temporal lobe regions connectivity during resting state and cognitive demand (Risacher, et al., 2013, Sole-Padulles,

et al., 2013) in AD and MCI patients. Moreover, in MCI patients an increase activation of frontal activity was found after a maximum of 24 weeks of treatment (Pa, et al., 2013, Petrella, et al., 2009, Saykin, et al., 2004). Only one study investigating 6 months of memantine treatment on the default mode network was conducted. This study showed an increased activation of the default mode network in the precuneus of treated group (Lorenzi, et al., 2011). Although few evidences are currently available, fMRI technique is now being incorporated into a small number of investigator-initiated add-on studies to ongoing Phase II and Phase III trials, which should provide some valuable information regarding the potential utility of these techniques in clinical trials. However, a series of methodological challenges are still pending for fMRI methods such as (i) the reliability and repeatability for specific task paradigms, (ii) the standardization across multiple sites, (iii) the development of validated automated quantification tools, (iv) implementation of multiple modalities (for example, merging PET and resting state-fMRI) and (v) cost optimization.

Table 3. Summary of studies using fMRI markers as imaging endpoints in Clinical Trials in mild to moderate AD and MCI patients.

Diagnosis	Compound	Subjects Number		Follow-up (months)	Marker & Treatment effect
		Placebo	Treatment		
AD	Galantamine (Shanks, et al., 2007)		9	20 weeks	Activation at fMRI in bilateral frontal regions
	Donepezil (Goveas, et al., 2011)		14	12 weeks	Stronger recovery in the network connectivity at resting state fMRI was associated with cognitive improvement
	Donepezil (Sole-Padulles, et al., 2013)	7	8	3	Stabilization of connectivity of medial temporal regions during resting state and of brain efficiency during a cognitive demand, on the whole reducing progressive dysfunctional reorganizations observed during the natural course of the disease
	Memantine (Lorenzi, et al., 2011)	9	7	6 months	Treated patients showed increased DMN activation mapping in the precuneus, while the prospective comparison in the untreated patients
	Galantamine (Goekoop, et al., 2006)		18	5 days	Acute exposure=increased activation in HP areas bilaterally; prolonged exposure= decreased activation in HP
MCI Prodromal AD	Galantamine (Goekoop, et al., 2006)		28	5 days	Acute exposure=increased activation in posterior cingulate, left inferior parietal, and anterior temporal lobe; Prolonged exposure = decreased activation in similar posterior cingulate areas, and in bilateral prefrontal areas; Effects were stronger for positive ('familiar') than for negative ('unfamiliar') decisions, indicating that the effect was specific to memory retrieval.

HP= Hippocampus DMN= Default Mode Network; MTL= medial temporal lobe

Diagnosis	Compound	Subjects Number		Follow-up (months)	Marker & Treatment effect
		Placebo	**Treatment**		
MCI Prodromal AD	Donepezil (Risacher, et al., 2013)		18	4 weeks	Normalized MTL activation and improved parietal deactivation ; increased task-related functional connectivity from the right MTL cluster seed region to a network of other sites including the basal nucleus/caudate and bilateral frontal lobes
	Donepezil (Saykin, et al., 2004)		9	5.7 ±1.7 weeks	Increased frontal activity
	Donepezil (Pa, et al., 2013)		27	3 months	Significant change associated with treatment
	Donepezil (Petrella, et al., 2009)	7	6	12 or 24 weeks	Placebo group demonstrated a decreased extent of dorsolateral prefrontal activation, whereas the donepezil group demonstrated an increased extent of activation in the ventrolateral prefrontal cortex

HP= Hippocampus DMN= Default Mode Network; MTL= medial temporal lobe

II. MAIN OBJECTIVES

In the light of literature results reported in the introductory paragraphs, several issues related to the implementation of neuroimaging markers in the context of AD trials still remain unresolved. One open issue is to understand whether imaging markers can be implemented in large cohorts in a standardized manner in order to be applied for both diagnostic purposes and AD clinical trials. Furthermore, one still open issue is to investigate the potential applications of neuroimaging techniques in AD clinical trials, particularly with respect as markers of outcomes.

These issues are addressed in the next chapters by presenting the results obtained during the studies conducted in the PhD Program. The main goals of the thesis are as follows:

(i) To test the proper implementation of Standard Operating Procedures into a multi-centre clinical environment:

Study 1: The Italian Alzheimer's Disease Neuroimaging Initiative (I-ADNI): validation of structural MR imaging

(ii) To elucidate the meaning of structural and functional neuroimaging measurements and their association with disease treatment in order to advance scientific knowledge necessary to accomplish the regulatory approval of the neuroimaging methods in clinical trials for AD:

Study 2: Donepezil decreases annual rate of hippocampal atrophy in suspected prodromal Alzheimer's disease

Study 3: Reduced regional cortical thickness rate of change in donepezil treated subjects with suspected prodromal Alzheimer's disease

Study 4: Hippocampus and basal forebrain as predictors of cognitive decline and treatment response in suspected prodromal Alzheimer's disease

Study 5: Effects of Rivastigmine on Visual Attention in Subjects with Amnestic Mild Cognitive Impairment: A Serial Functional MRI activation Pilot-Study

III. EXPERIMENTAL STUDIES

SECTION 1: Standard Operating Procedures for structural imaging markers in dementia

With the advent of neuroimaging methods – in particular the use of sMRI markers for the early diagnosis of AD – the scientific community had to deal with the improvement of methods and protocols of acquisition in order to increase the reproducibility of these markers and their application in large-scale multicentre studies. The first initiative was developed by the ADNI that implemented a common and standardised protocol of acquisition for structural MRI sequences (Jack et al., 2008). Following this first application, other multicentre studies worldwide developed and applied harmonized protocols such as the European ADNI (E-ADNI) (Frisoni et al., 2009). The following manuscript describes the application of SOPs on both the acquisition of MRI images and the use of MRI structural markers – such as the total intracranial and the hippocampus volumes – in order to assess the reliability of SOPs in reducing the variance in the quality of image acquisition and in the measurement of imaging markers.

Study 1: The Italian Alzheimer's Disease Neuroimaging Initiative (I-ADNI): validation of structural MR imaging

Cavedo E, Redolfi A, Angeloni F, Babiloni C, Lizio R, Chiapparini L, Bruzzone MG, Aquino D, Sabatini U, Alesiani M, Cherubini A, Salvatore E, Soricelli A, Vernieri F, Scrascia F, Sinforiani E, Chiarati P, Bastianello S, Montella P, Corbo D, Tedeschi G, Marino S, Baglieri A, De Salvo S, Carducci F, Quattrocchi CC, Cobelli M, Frisoni GB. "The Italian Alzheimer's Disease Neuroimaging Initiative (I-ADNI): validation of structural MR imaging".

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The Italian Alzheimer's Disease Neuroimaging Initiative (I-ADNI): Validation of Structural MR Imaging

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[1]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu/). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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Abstract.
Background: The North American Alzheimer's Disease Neuroimaging Initiative (NA-ADNI) was the first program to develop standardized procedures for Alzheimer's disease (AD) imaging biomarker collection.
Objective: We describe the validation of acquisition and processing of structural magnetic resonance imaging (MRI) in different Italian academic AD clinics following NA-ADNI procedures.
Methods: 373 patients with subjective memory impairment ($n = 12$), mild cognitive impairment ($n = 92$), Alzheimer's dementia ($n = 253$), and frontotemporal dementia ($n = 16$) were enrolled in 9 Italian centers. 22 cognitively healthy elderly controls were also included. MRI site qualification and MP-RAGE quality assessment was applied following the NA-ADNI procedures. Indices of validity were: (i) NA-ADNI phantom's signal-to-noise and contrast-to-noise ratio, (ii) proportion of images passing quality control, (iii) comparability of automated intracranial volume (ICV) estimates across scanners, and (iv) known-group validity of manual hippocampal volumetry.
Results: Results on Phantom and Volunteers scans showed that I-ADNI acquisition parameters were comparable with those one of the ranked-A ADNI scans. Eighty-seven percent of I-ADNI MPRAGE images were ranked of high quality in comparison of 69% of NA-ADNI. ICV showed homogeneous variances across scanners except for Siemens scanners at 3.0 Tesla ($p = 0.039$). A significant difference in hippocampal volume was found between AD and controls on 1.5 Tesla scans ($p < 0.001$), confirming known group validity test.
Conclusion: This study has provided standardization of MRI acquisition and imaging marker collection across different Italian clinical units and equipment. This is a mandatory step to the implementation of imaging biomarkers in clinical routine for early and differential diagnosis.

Keywords: Alzheimer's disease, hippocampus, intracranial volume, magnetic resonance imaging, mild cognitive impairment, standardized operating procedures

INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disorder. Over the last few years, there has been strong interest in identifying individuals at earlier stages of AD, before AD dementia criteria are met [1]. Several biomarkers, both biological and imaging, have been introduced in the new diagnostic NIA-AA criteria for AD [2, 3]. These are indicators of specific changes characterizing the in vivo neuropathological cascade that occurs during different clinical stages of AD [4, 5].

Although sophisticated quantitative methods to analyze neuroimaging markers do exist, it should be underlined that standardization of these imaging markers is currently limited, and results often vary from laboratory to laboratory [2]. Heterogeneity of biomarker collection and measurement is a barrier to their translation into daily clinical practice. For these reasons, several worldwide initiatives are developing standard operating procedures to minimize the variability of biomarkers collection. The North American Alzheimer's Disease Neuroimaging Initiative (NA-ADNI) [6, 7] represents the flagship program that established a platform for biomarker collection and measurement with standardized procedures. The major goals of the NA-ADNI are: (i) to develop improved methods that will lead to uniform standards for acquiring longitudinal multisite magnetic resonance imaging (MRI) and positron emission tomography (PET) data on patients with AD, patients with mild cognitive impairment (MCI), and elderly controls, and (ii) to build an accessible data repository that describes the biomarkers longitudinal changes [8]. After the development of the NA-ADNI, other ADNI initiatives were established. In Europe, the pilot European-ADNI (E-ADNI) and AddNeuroMed were the first initiatives to implement the MRI protocol of acquisition compatible to the NA-ADNI [9–11]. ADNI initiative have also been launched in South America (Argentina-ADNI) and Asia (Japan, Korea, Taiwan, and China) [12, 13]. The Australian ADNI, also known as the Australian Imaging Biomarkers and Lifestyle Flagship Study of Aging (AIBL), was launched in 2006 with the intention of recruiting 1,000 individuals (over the aged 60) who underwent neuroimaging biomarkers in order to assess their utility as AD indicators [14]. All of these worldwide initiatives have been set up to develop AD biomarkers using the same ADNI standardized MRI and clinical protocols.

This paper illustrates the design and early results of Italian ADNI (I-ADNI). The NA-ADNI platform for structural MRI was implemented in nine Italian academic outpatient memory clinics covering the national territory with the main aim to promote the use of structural MRI ADNI sequences. I-ADNI applied the NA-ADNI imaging protocol on a naturalistic series of patients with cognitive disorders. The validity

of acquisition and processing was estimated detecting: signal-to-noise ratio and contrast-to-noise ratios; quality control pass; comparability of craniometric features (intracranial volume) across scanners; and known-group validity of brain structural features (hippocampal volume).

MATERIALS AND METHODS

Nine Italian academic Memory Clinics have been involved in the study. The Coordinating Center was IRCCS Centro San Giovanni di Dio, Brescia (OU1, PI: G.B. Frisoni). The academic Memory clinics involved in the project were: IRCCS Santa Lucia Foundation, Rome (OU2, PI: U. Sabatini); SDN Foundation Naples, Naples (OU3, PI: A. Soricelli); Campus Bio_Medico University, Rome (OU4, PI: F. Vernieri); University of Foggia (OU5, PI: C. Babiloni); Fond. IRCCS Istituto Neurologico Besta, Milan (OU6, PI: M.G: Bruzzone); IRCCS Mondino National Institute of Neurology Foundation, Pavia (OU7, PI: E Sinforiani); Second University of Naples, Naples (OU8, PI: G. Tedeschi); and Centro Neurolesi "Bonino-Pulejo ", Messina (OU9, PI: P. Bramanti).

The Coordinating Center was responsible for clinical and MRI data including: case report form development, implementation of clinical and neuropsychological database, implementation of ADNI platform for structural MRI, MR sequences storage, MRI quality control. In addition, the overall data analysis was carried out by IRCCS Centro San Giovanni di Dio Fatebenefratelli. The project management was taken by Giovanni B. Frisoni. The study protocol was approved by the local ethics committee and all participants signed informed participation consents.

Patients

The nine Italian academic Memory Clinics enrolled 395 outpatients between 1 January 2009 and 31 October 2011. Twelve of them were subjective memory impairment (SMI) individuals, 92 MCI patients, 253 AD patients, and 16 frontotemporal dementia (FTD) patients. In addition, a group of 22 cognitive intact persons (CTRL) participated voluntarily in the study. The exclusion criteria were: stroke, psychiatric diseases, neurological diseases other than cognitive impairment. Clinical criteria for each diagnosis were: NINCDS-ADRDA criteria for probable AD [15] and Neary Criteria for FTD [16]; MCI was defined as the presence of objective impairment in memory or other cognitive domains (performance lower than the fifth percentile on neuropsychological tests as detailed below) in the absence of functional impairment. SMI individuals were persons worried about their memory performances without any objective cognitive deficit. All participants underwent a clinical and neuropsychological assessment.

Clinical and neuropsychological assessment

We assessed global cognition with the Mini-Mental State Examination (MMSE) [17] and depressive symptoms using the pertinent subscales of the Brief Symptom Inventory (BSI) [18]. BSI subscores range from 0 to 4, higher scores indicating more severe symptoms. The Clinical Dementia Rating scale was used to quantify the severity of symptoms of dementia. We administered a set of neuropsychological tests to assess long term memory (Story Recall Test, Rey–Osterrieth complex figure, recall), attention and executive functions (Trail Making Test A and B), language abilities (Letter and Category Fluency Test), and visuo-spatial skills (Rey–Osterrieth complex figure, Copy). We corrected the results for age and education, according to the Italian normative populations. Moreover, hypertension, heart disease, diabetes mellitus, and hypercholesterolemia were investigated based on clinical history. Finally, the Instrumental Activities of Daily Living were collect to assess the functional status of subjects [19].

Table 1
Demographic features of the Italian ADNI sample

Diagnosis		Controls	p	SMI	p	MCI	p	AD	p	FTD
	n 1.5 T/3 T	18/4		6/6		69/23		152/101		13/3
	n total	22		12		92		253		16
General features	Age, y	70 ± 7	–	68 ± 12	–	70 ± 7	–	71 ± 27	–	69 ± 9
	Education, y	9 ± 5	–	10 ± 5	–	8 ± 4	–	8 ± 5	*	12 ± 6
	Women	16 (73%)	–	8 (66%)	–	50 (54%)	–	160 (63%)	*	4 (25%)
Clinical features	MMSE	29 ± 1	–	29 ± 1	–	27 ± 2	*	20 ± 5	–	20 ± 6

*p values <0.05 between adjacent groups on ANOVA, Chi-square, or Fisher tests. n 1.5 T/3 T, number of subjects scanned at 1.5 or 3.0 Tesla; SMI, subjective memory impairment; MCI, mild cognitive impairment; AD, Alzheimer's disease; FTD, frontotemporal dementia; MMSE, Mini-Mental State Examination. Values denote mean ± standard deviation or number (%).

Table 2
Technical features of ADNI-qualified scanners and number of MPRAGE scans acquired during the study

Center (OU)/Scanner location	Manufacturer/model	Field strength	Coil	MP-RAGE (n)
IRCCS Centro S. Giovanni di Dio (OU1), Brescia	GE Signa Excite	1.5 T	8 channels head	23
IRCCS Fondazione S. Lucia (OU2), Rome	Siemens Allegra	3 T	8 channels head	27
Fond. SDN Naples (OU3), Naples	Philips Achieva	3 T	8 channels head	63
University Campus Bio_Medico (OU4), Rome	Siemens, Avanto	1.5 T	8 channel head	63
University of Foggia/"La Sapienza" University (OU5), Rome	Siemens Sonata	1.5 T	Body	27
Fond. IRCCS Istituto Neurologico Besta (OU6), Milan	Siemens Avanto	1.5 T	12 channels head	25
Fond. IRCCS Mondino (OU7), Pavia	Philips Intera	1.5 T	8 channel head	48
University of Naples (OU8), Naples	GE Signa HDx 14.0_M5A	3 T	8 channel head	43
Centro Neurolesi "Bonino-Pulejo" (OU9), Messina	Siemens Sonata	1.5 T	8 channel head	24

MR imaging

Data acquisition

Three hundred and forty-three MR scans from routine patients were acquired, of which 210 at 1.5 and 133 at 3.0 Tesla respectively. MRI acquisition activities were divided into: i) a preparatory phase, ii) site qualification, iii) experimental subjects scanning, and iv) MP-RAGE quality ranking of overall images acquired.

The preparatory phase involved the collection of the I-ADNI MRI scanner features in terms of manufacturer, coils adopted, and magnetic strength (see Table 2). The description of the practical procedures concerning the image transmission of the data collected in this study on a centralized repository at the FBF has been detailed to every partner of the I-ADNI consortium.

The site qualification phase entailed the successful installation of the ADNI sequences. This step consisted in setting up and checking the correct configuration of the official ADNI1 protocol parameters (http://adni.loni.usc.edu/methods/documents/mri-protocols/) on every scanner of the project according to the features collected. The scan protocol included: 1) Localizer Scan (20 s); 2) Straight Sagittal 3D Magnetization-Prepared Rapid Acquisition Gradient-echo (MPRAGE) (8–10 min); 3) Straight Sagittal 3D MPRAGE – REPEAT – (8–10 min); 4) B1 Calibration Scan Phase Array Coil (if applicable) (30 s); 5) B1 Calibration Scan Body Coil (if applicable) (30 s); and 6) Axial Dual Echo T2 Fast Spin Echo (FSE) (5 min). Furthermore, OU2-OU3 and OU8 acquired diffusion tensor imaging and resting state functional MRI sequences (not reported here).

To test if MRI passed the qualification phase, each I-ADNI center acquired the whole scan protocol on a local volunteer subject to verify the adherence to the official ADNI protocol and the absence of artifacts (e.g., movement, ringing, wrap around, metal artifacts). The MPRAGE images were corrected following the image correction steps (i.e., N3, B1, and Grad-Warp) provided by ADNI (http://adni.loni.usc.edu/methods/mri-analysis/mri-pre-processing/) and specific indexes of MRI signal quality (i.e., signal to noise ratio (SNR) and peak signal to noise ratio (PSNR)) were derived considering the whole brain slices of the 3D stack. Then, the above parameters have been compared with ranked-A ADNI reference scans in order to define the goodness of the I-ADNI site acquisition. The main software adopted during the site qualification phase were ImageJ, MIPAV, MRIcro, MNI tools, and the Gradient Non-linearity Unwarping Tool.

Starting from year 2 onward, the NA-ADNI MagPhan® phantom was circulated among all I-ADNI sites to measure *post-hoc* inter-scanner signal repeatability. The ADNI phantom consists of spherical inclusions inside a 20 cm diameter water-filled clear urethane shell. Inclusions are copper sulfate filled polycarbonate spherical shells: four 3.0 cm spheres with copper sulfate concentrations of 0.9, 1.2, 1.7, and 2.4 mM are used for SNR and contrast noise ratio (CNR) information measurements.

During the whole lifetime of the project, each MPRAGE sequence was graded for artifacts in a qualitative manner by an experienced individual at the Coordinating Centre. In line with NA-ADNI procedures (http://www.adni-info.org/Scientists/Pdfs/adniproceduresmanual12.pdf), a scan with a 1 ranking was considered a high quality scan, a 2 ranking was considered a medium quality scan, and a 3 ranking was considered a low quality scan. From the NA-ADNI database, we extracted MPRAGE ranking of 791 scans acquired by ADNI1 protocol and compared the proportion of MPRAGE assessed of high quality between the I-ADNI and the NA-ADNI. All the I-ADNI MPRAGE images used in the present article are those with a high-quality evaluation.

Automated intracranial volume

The intracranial volumes (ICV) of the I-ADNI cohort were segmented with the Freesurfer (5.1 version) image analysis package (http://surfer.nmr.mgh.harvard.edu/). The automated procedure for image segmentation and volume measurement includes the following steps [20]: intensity normalization [21], removal of non-brain tissue [22], and transformation to Talairach space. This technique has been widely validated against manual tracings in healthy individuals and patients with neurologic diseases [20, 23]. Moreover, from the NA-ADNI database (https://ida.loni.usc.edu/login.jsp), we selected the ICVs measured by Freesurfer of 745 subjects matched by age, gender, strength field, protocol of MPRAGE acquisition (ADNI1), and scanner models to our I-ADNI subjects. Subsequently, we compared the homogeneity variances of ICV among scanners of the two different cohorts stratified by strength field.

Information on the ADNI

Data used in the preparation of this article were obtained from the ADNI database (http://adni.loni.usc.edu/). The NA-ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies, and nonprofit organizations, as a $60 million, 5-year public private partnership. The primary goal of ADNI has been to test whether serial MRI, PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials.

The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California-San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 subjects but ADNI has been followed by ADNI-GO and ADNI-2. To date, these three protocols have recruited over 1,500 adults, ages 55 to 90, to participate in the research, consisting of cognitively normal older individuals, people with early or late MCI, and people with early AD. The follow up duration of each group is specified in the protocols for ADNI-1, ADNI-2, and ADNI-GO. Subjects originally recruited for ADNI-1 and ADNI-GO had the option to be followed in ADNI-2. For up-to-date information, see http://www.adni-info.org/.

Manual hippocampal segmentation

Hippocampal boundary

All the hippocampi were manually segmented according to a prototype of the Harmonized Protocol for the Hippocampal Volumetry [24]. The most rostral slice was considered where the hippocampus was separated from the amygdala by the alveus. The boundaries of the hippocampal head were considered the most medial gray matter (GM), i.e., the visible morphological boundary of the structure, adjacent to liquor of the ambient cistern. The ventral boundary of the hippocampus was defined by the white matter (WM) of the parahippocampal gyrus. The medial boundary was the boundary with the WM of the parahippocampal gyrus and/or the liquor of the ambient and the perimesencephalic cistern. In the most caudal slices, the medial boundaries consisted of the GM belonging to the isthmus, or to the vestigial hippocampal tissue that has been excluded from the tracing. The software used for the manual segmentation was the Multitracer V1.0 software.

Learning phase

Ten NA-ADNI MPRAGE images with different medial temporal lobe atrophy at Medial Temporal Atrophy Scale [25] acquired twice (both 1.5 and 3 Tesla) on the same subjects were selected. An expert tracer from the coordinating center segmented the hippocampi of these images according to the protocol described above. These segmentations were considered the reference standard. A tracer, from each operative unit, segmented the 10 NA-ADNI MPRAGE images twice according to the strength field of each MRI scan. The learning phase was considered concluded once each tracer achieved an intra-class coefficient of correlation (ICC) greater than 0.80. Reliability results on manual hippocampal segmentation showed an ICC between 0.85 and 0.99 (Supplementary Table 1, test-retest reliability). The inter-rater reliability versus the reference standard tracing ranged from 0.86 to 0.99 at 3T, and from 0.82 to 0.97 at 1.5T scanners (Supplementary Table 1, inter-rater reliability versus reference). Considering only OUs at 3T, we found an ICC of 0.93 (CI 95% 0.81 ± 0.95) for the right hippocampus, and of 0.92 (CI 95% 0.78 ± 0.98) for the left one, while 1.5 Tesla scanners showed an ICC of

0.87 (CI 95% 0.66 ± 0.98) for the right and 0.93 (CI 95% 0.76 ± 0.99) for the left hippocampus.

After the learning phase, all the MPRAGE images were manually oriented along the anterior-posterior commissural line by the MRIcro software and the hippocampi of the entire sample were manually traced from each OU.

Statistical analysis

After the assessment of the homoscedasticity with the Bartlett test, MPRAGE phantom acquisition scans were compared among different I-ADNI sites using the analysis of the variance (ANOVA).

Sociodemographic and clinical features, neuropsychological performances, and ICVs were compared among clinical groups with the ANOVA for continuous variables (*post-hoc* analysis were done using Bonferroni correction), and with the χ^2 test for dichotomous variables. The Levene's test was applied to verify the homogeneity of variances of ICVs acquired using the same scanner model between I-ADNI and NA-ADNI cohort. For each center, the intra-class correlation coefficient (absolute model) of hippocampal tracings was computed to assess the intra- and inter-reliability. A test of linear trend was executed to test whether the right/left hippocampal volumes, manually traced, were linearly related to the disease progression (from CTRL to AD), across different field strengths. Moreover, known group validity by the Mann-Whitney U-test was executed comparing the hippocampal volume of controls and AD patients. All statistical analyses were performed using SPSS software version 12.0.

RESULTS

Sociodemographic features revealed no significant difference among groups except for the educational level, where MCI and AD were less educated than FTD patients ($p = 0.028$ and $p = 0.007$, respectively). Moreover, FTD were mainly men than AD patients ($p = 0.003$, Table 1). The activities of daily living were more compromised in AD than MCI patients ($p < 0.001$, Supplementary Table 2). Neuropsychological performances showed a significant global cognitive deterioration in AD than MCI ($p < 0.001$). Moreover, MCI performed worse than SMI ($p < 0.001$) and, as expected, AD patients were more impaired than MCI ($p < 0.001$) in long term memory performances. Furthermore, AD patients showed significantly lower language and psychomotor speed abilities than MCI patients ($p < 0.001$ and $p = 0.019$ respectively, Supplementary Table 2). As expected, the main clinical features of SMI were memory complaints in absence of objective cognitive deficits and functional impairment. Instead, MCI patients were characterized by the presence of cognitive decline in one or more domain without functional impairment. AD patients showed a severe cognitive impairment with a greater functional deterioration (Supplementary Table 3).

ADNI phantom's SNR and CNR results

Table 2 reports the technical features of each scanner and the number of MPRAGE sequences acquired in each OU. Details of the protocols adopted by the different I-ADNI sites are represented in Supplementary Table 4a. Test-retest scanning sessions were acquired from a group of 9 volunteers (56% male and 44% female, mean age 42 ± 18 years). From each volunteer, two T13D (scan-rescan) and one axial-PD/T2-FSE volumes (8 sites, 1 subject per site, 16 T13D acquisitions, 8 Dual Echo FSE acquisitions) were obtained. The nine scanners showed good adherence to the original NA-ADNI protocol with negligible changes in terms of both spatial and temporal resolution. During the site qualification phase, every I-ADNI MPRAGE scan was rated by SNR and PSNR. These metrics were equal and even better than those of the ranked-A scans acquired in the original NA-ADNI study, indicating good acquisition quality. All the I-ADNI sites successfully performed the site qualification phase (Supplementary Table 4b). The inter scanner signal repeatability results from post-hoc analysis on the MagPhan® phantom are summarized in Fig. 1. The SNR analyses consist of extracting from the image data the spherical regions of the four copper sulfate shells. SNR intensity values were taken as the mean intensity of the voxels in that area. The mean intensity of a subsection of 35×35 voxels band immediately outside the phantom shell was taken as estimation of the background signal. CNR intensity values were then computed as signal differences among all pairs of spherical shells. Seven out of nine I-ADNI sites exhibited similar SNR and CNR patterns to each other ($p > 0.05$). Only one center (OU8) displayed slight warping effect distortion. The remaining two I-ADNI centers (OU2 and OU4) acquired the phantom but the data were not analyzed due to technical issues (i.e., phantom rotated; not fitted into the coil; not positioned properly; not identical location with respect to the isocenter).

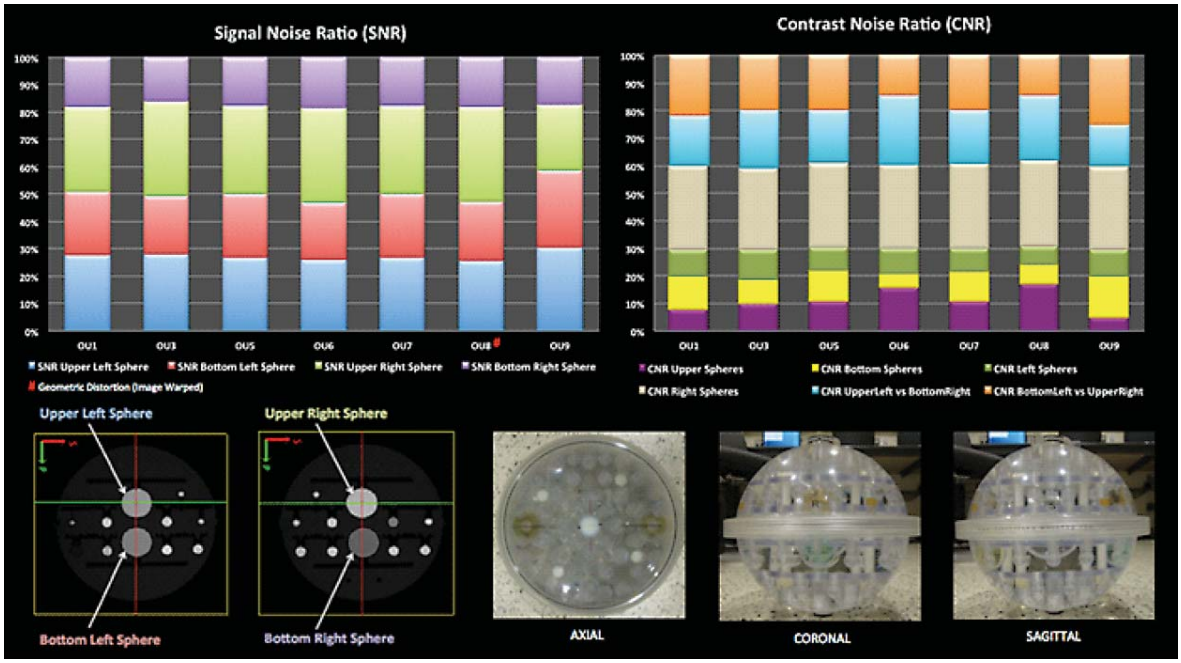

Fig. 1. Post-hoc inter-scanner signal repeatability. Relative stacked column graphs show comparable SNR and CNR values in I-ADNI qualified centers. OU8 showed geometric distortion. OU2 and OU4 phantom data are not available. Photographs of the MagPhan® phantom and typical acquisition slices are also shown in the bottom. Each phantom's sphere is filled with a copper sulfate solution that generates different levels of signal intensity. OU1, IRCCS Centro S. Giovanni di Dio, Brescia; OU2, IRCCS Fondazione S. Lucia, Rome; OU3, Fond. SDN Naples, Naples; OU4, University Campus Bio_Medico, Rome; OU5, University of Foggia/"La Sapienza" University, Rome; OU6, Fond. IRCCS Istituto Neurologico Besta, Milan; OU7, Fond. IRCCS Mondino, Pavia; OU8, University of Naples, Naples; OU9, Centro Neurolesi "Bonino-Pulejo", Messina.

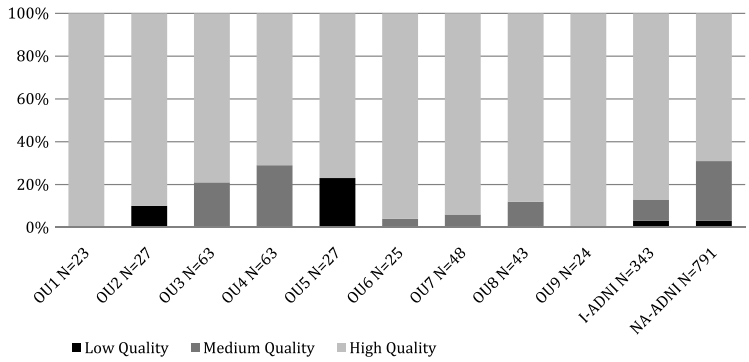

Fig. 2. Proportion of MP-RAGE scans assessed with low, medium, and high quality across I-ADNI operative units (OU) and in the I-ADNI and NA-ADNI total sample.

MPRAGE quality controls results

Considering each I-ADNI OU, 70% or higher of MPRAGE acquired were assessed of high quality. This percentage reached 87% considering the total sample of MPRAGE acquired during the study. This proportion is higher than NA-ADNI MPRAGE images that showed high quality scans of 69%. Moreover, I-ADNI cohort showed a lower proportion of medium quality MPRAGE scans in comparison to NA-ADNI (10% and 28%, respectively), while the percentage of low quality MPRAGE scans were the same between cohorts (Fig. 2).

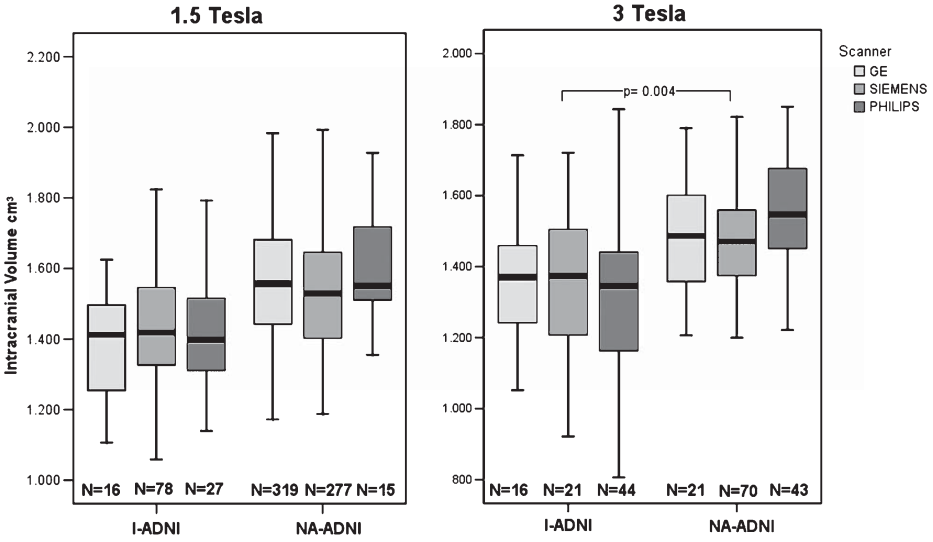

Fig. 3. Stability of automated ICV measurements across different scanners. *p* denotes significant value at Levene's test. The only statistical difference is between Siemens scanner at 3T.

Automated intracranial volume estimates across scanners

We found homogeneous variances in the automated ICV between NA-ADNI and I-ADNI scanners ($p > 0.05$). When we compared the ICV between scanner models we found homogeneous variances in the ICV of GE and Philips scanners ($p > 0.05$). Siemens scanners showed homogeneous variances in ICV at 1.5T but not at 3T ($p > 0.05$ and $p = 0.039$ respectively, Fig. 3). Furthermore, considering exclusively I-ADNI scanners, *post-hoc* comparisons revealed a significant higher ICV for the UO6 ($p < 0.05$ with Bonferroni correction) compared to that obtained from all other I-ADNI units, with the 1.5T. No ICV differences were found among 3T scanners (Supplementary Figure 1).

Known group validity of manual hippocampal volumetry

Box plots in Fig. 4 show the hippocampal volume and the interquartile variability according to diagnosis and field strength. There is a proportional increase of hippocampal atrophy according to the disease progression confirmed by the linear trend analysis at 1.5T ($p < 0.001$). Hippocampal volumes resulted significantly different between controls and AD patients ($p < 0.001$); the former showed an hippocampal atrophy of 26% at 1.5 Tesla. Known group validity was not performed in the 3.0 Tesla groups due to the lack of enough control subjects.

DISCUSSION

In this manuscript, we described the validation of procedures of acquisition and processing of structural MRI in different Italian academic AD clinics following NA-ADNI procedures. This is the first Italian study applying standardized procedures for the collection and analysis of MR imaging for AD. I-ADNI has built a platform, capitalizing on academic Italian National Health Service Centers, for the use of harmonized parameters for structural MRI acquisition and processing. The sample of patients enrolled in the I-ADNI can be considered representative of those attending memory clinics [26, 27].

The analysis of volunteer scans showed no quality difference among sites. Indeed, the mean SNR found in each site was higher than the mean SNR obtained from NA-ADNI MPRAGE images at 1.5 and 3 Tesla. The phantom analysis provided precise estimates of intensity and linear geometrical scale factors distortion. Based on field experience to date, the greatest practical value of incorporating the MagPhan phantom measurements was identify scanner errors through central monitoring and harmonize scanner acquisition differences. Results obtained from MRI phantom, circulated among I-ADNI centers, showed that the overall SNR and CNR metrics were equal among centers ensuring high reliability. Finally, it is reassuring to note that 86% of I-ADNI MPRAGE images were assessed of high quality; this percentage was higher of those obtained from NA-ADNI ranking.

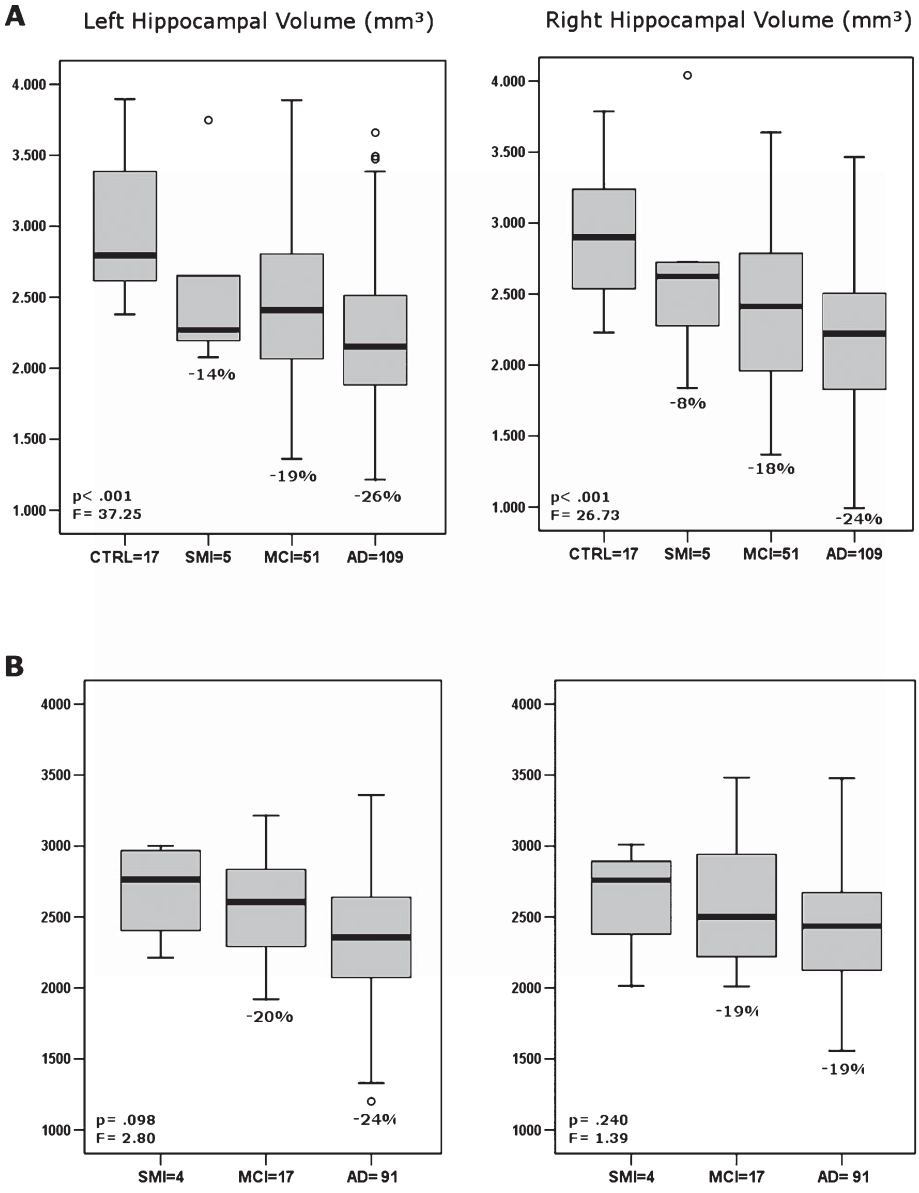

Fig. 4. Known-group validity of manual hippocampal segmentation on 182 magnetic resonance images at 1.5T (A) and on 112 at 3T (B). Volumes are normalized to total intracranial volume. Percentages indicate the proportion of hippocampal atrophy in each group versus controls or SMI subjects. *p* and F values denote the significance of the linear test for trend. AD, Alzheimer's disease; CTRL, controls; MCI, mild cognitive impairment; SMI, subjective memory impairment.

Findings on ICV confirmed the stability of MRI quantification related to the implementation of the same MRI acquisition protocol. Indeed, although there was an intrinsic variance due to different population characteristics, when we compared ICVs computed from scans acquired with the same scanner model from different subjects we found homogeneous variances between the two cohorts (I-ADNI and NA-ADNI). Moreover, exclusively considering the I-ADNI sample, *post-hoc* analysis of variance revealed no differences between memory clinics except for one operative unit. These results indicated a reduced ICVs variability between centers and the possibility to compare the MRI data between different Italian academic clinical centers.

The goodness of our hippocampal results have been also highlighted by the known group validity method that showed significant hippocampal atrophy in AD

patients compared to their controls. Moreover, MCI hippocampal volume resulted between the volume of individuals with subjective memory impairment and that of the patients with AD. AD patients showed the greatest level of atrophy when compared to the other groups of subjects enrolled in the study.

Limitations

Ideally, in order to minimize error variance, an ADNI like multi-site reproducibility using a large sample of volunteers scanned repeatedly at all sites and within a short period of time, should be used. Such a study is extremely challenging for the associated huge cost and need of great coordination. On this prospective, our study has some limitations relative to this ideal scenario: a) in the site qualification phase, useful to fine tuning all the acquisition parameters according to the NA-ADNI standard, each MRI site scanned a different set of subjects preventing direct comparison of the scanners; and b) only one phantom acquisition has been completed in all the I-ADNI centers, mainly due to the lack of time, preventing the acquisition repeatability and stability of the scanners in the longitudinal period.

In addition, more efforts should be done in the future to collect standardized biological AD markers (amyloid-β and tau levels in cerebrospinal fluid, measure of glucose hypometabolism, and *in vivo* brain amyloid burden) as done from other ADNI initiatives.

One more limitation was the harmonization of MPRAGE images only. Other studies such as Pharmacog (http://www.alzheimer-europe.org/Research/PharmaCog) included harmonization of diffusion tensor imaging and resting state functional MRI sequences [28].

CONCLUSIONS

This study reports the harmonization of the ADNI sequences of structural MR in nine academic Memory Clinics in Italy. The aim of the I-ADNI was to demonstrate the feasibility of implementing the NA-ADNI methods enrolling and assessing a naturalistic population. It will now be possible to compare the brain structural features of patients studied in these Memory Clinics with those of patients studied in other worldwide ADNI initiatives.

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Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=2086).

SUPPLEMENTARY MATERIAL

Supplementary tables and figure are available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-132666.

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SECTION 2: Structural and functional imaging correlates of cholinesterase inhibitors use

The qualification and validation of markers of AD are assumed to lead to important results in the future development of novel drugs for AD. Regulatory guidelines have already included some biomarkers in the process of implementation as outcome variables. For instance, regarding phase II, some biomarkers are integrated in drug development programs as outcome measures in proof-of-concept or in dose-finding studies (Broich, 2012). Large-scale international controlled multicentre studies are needed in order to validate specific biomarkers before they can be accepted as outcome measures in pivotal phase III clinical trials. To date, no biomarker is sufficiently validated to be acceptable as a surrogate endpoint. Since traditional clinical outcome measures might be too subtle to be sensitive to change or need impractical treatment durations for clinical trial conditions, one of the most important aims in the early AD research is to establish reliable surrogate endpoints.

In this section, first of all, it was examined the effect of one-year donepezil treatment on surrogate outcome measures of AD, including the hippocampus and the cortical thickness, in a population of suspected prodromal AD patients enrolled in the context of the Hippocampus Study Clinical Trials. Moreover, on the same population, it was assessed the value of the hippocampus and the cholinergic basal forebrain volumes to predict the rate of cognitive decline and the response to cholinergic treatment. Finally, the results conducted on a pilot study aimed to explore the effect of anticholinesterase treatment on brain activation changes during a visual perception functional MRI task in patients with MCI were described.

Study 2: Donepezil decreases annual rate of hippocampal atrophy in suspected prodromal Alzheimer's disease

Dubois B, Chupin M, Hampel H, Lista S, **Cavedo E**, Croisile B, Tisserand GL, Touchon J, Bonafe A, Ousset PJ, Ait Ameur A, Rouaud O, Ricolfi F, Vighetto A, Pasquier F, Delmaire C, Ceccaldi M, Girard N, Dufouil C, Lehericy S, Tonelli I, Duveau F, Colliot O, Garnero L, Sarazin M, Dormont D; "Hippocampus Study Group"

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Research Article

Donepezil decreases annual rate of hippocampal atrophy in suspected prodromal Alzheimer's disease

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Abstract

Background: To study the effect of donepezil on the rate of hippocampal atrophy in prodromal Alzheimer's disease (AD).

Methods: A double-blind, randomized, placebo-controlled parallel group design using donepezil (10 mg/day) in subjects with suspected prodromal AD. Subjects underwent two brain magnetic resonance imaging scans (baseline and final visit). The primary efficacy outcome was the annualized percentage change (APC) of total hippocampal volume (left + right) measured by an automated segmentation method.

Results: Two-hundred and sixteen only subjects were randomized across 28 French expert clinical sites. In the per protocol population (placebo = 92 and donepezil = 82), the donepezil group exhibited a significant reduced rate of hippocampal atrophy (APC = −1.89%) compared with the placebo group (APC = −3.47%), $P < .001$. There was no significant difference in neuropsychological performance between treatment groups.

Conclusions: A 45% reduction of rate of hippocampal atrophy was observed in prodromal AD following 1 year of treatment with donepezil compared with placebo.

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1552-5260/

Keywords: Randomized controlled trial; Amnestic MCI; MRI; Volumetric imaging; Donepezil; Rate of atrophy; Hippocampus; Whole brain analysis; Alzheimer's disease; Prodromal Alzheimer's disease; Mild cognitive impairment; Biomarker; Therapy

1. Background

The concept of prodromal Alzheimer's disease (AD) was recently introduced by the International Working Group on the New Criteria for the diagnosis of AD [1] to describe the stage of AD where clinical symptoms, including episodic memory disorders of the hippocampal type, are present but not sufficiently severe to impact significantly on activities of daily living and where biomarker evidence is supportive of the presence of Alzheimer pathology. Detection and identification of AD at the prodromal stage may allow delaying disease progression through appropriate treatment intervention [2]. AD phenotypical prodromes fall within the set of symptoms associated with mild cognitive impairment (MCI), a heterogeneous clinical condition that may be caused by different disorders. The use of specific memory tests, such as the Free and Cued Selective Reminding Test (FCSRT), significantly increases the capability to identify prodromal AD within the group of MCI subjects [3]. Moreover, the recall performance of the FCSRT has been significantly correlated with hippocampal volume and with cerebrospinal fluid (CSF) biomarker changes of the Alzheimer type [4,5].

In patients diagnosed with AD, either at prodromal or at dementia stages, the association between rates of brain atrophy and cognitive decline has been explored [6–8]. MCI subjects who progress to AD dementia frequently demonstrate a faster rate of hippocampal atrophy and increased ventricular expansion relative to healthy controls and subjects with stable nonprogressive MCI. Greater hippocampal atrophy has also been observed in patients with rapidly progressing AD relative to those exhibiting slower progression [6]. These results indicate a continuum of increased hippocampal atrophy as patients evolve from prodromal to mild, moderate, and severe AD dementia. Therefore, it is important to determine if subjects with amnestic MCI may experience clinical benefits, such as delayed emergence of dementia or preservation of functional activities, through treatment with interventions that have a disease modifying effect on established core biomarkers brain structure and morphology, such as hippocampal and whole brain atrophy. To answer this question, the first step is to investigate and potentially identify interventions that significantly reduce hippocampal and whole brain atrophy in a carefully characterized and selected prodromal AD target population. After the identification of such candidate treatments, it can be determined if effective early modification and prevention of atrophy may then modify the progression of clinical and functional disease related symptoms. An interrelation of neuroanatomical brain changes with the clinical phenotype of AD may well be nonlinear and is not yet fully understood and needs to be elucidated.

Donepezil hydrochloride (HCl) is a chemically unique, piperidine-based acetylcholinesterase inhibitor that has shown cognitive and functional benefit in the treatment of mild, moderate, and severe AD dementia in multiple randomized controlled trials [9]. In addition to its symptomatic effects on memory and cognition, donepezil has demonstrated some effects on the cellular and molecular system level associated with AD in nonclinical studies that may contribute to the significant changes observed on hippocampus in patients with mild moderate AD dementia treated with donepezil [10,11]. In subjects with MCI, the effect of donepezil is less clearly understood. Evidence from several large-scale clinical trials failed to demonstrate a statistically significant benefit on symptomatic outcome in this heterogeneous population [12,13]. A possible effect on brain structures in subjects with MCI is controversial with one study showing no effect on hippocampal, entorhinal cortex, whole brain, or ventricular volume [14] and another study showing a reduction in ventricular, cortical, and whole brain atrophy relative to placebo. [15]

To examine the hypothesis of a disease modifying effect of donepezil on AD-related brain structural alterations derived from the pilot trial we constructed a large-scale multicenter study. In this double-blind, randomized, placebo-controlled study in subjects with amnestic MCI, hippocampal volume was used as the primary outcome criterion to determine whether donepezil slows the progression of atrophy. Subjects with prodromal AD were isolated from the broader group of MCI subjects based on identification of an amnestic syndrome of the hippocampal type characterized by a significant impairment of memory recall that does not benefit from cueing [4,16]. In this well targeted and specific subset of MCI subjects, it was hypothesized that donepezil would decrease the rate of hippocampal atrophy relative to placebo and that this decrease would be associated with reduced decline on neuropsychological assessments.

2. Methods

2.1. Study population

The protocol of the 'Hippocampus study' and informed consent forms were approved by the Ethics Committee of Salpêtrière Hospital. A total of 332 patients were screened within the national network of Memory Resources and Research Centers (CMRR) consisting of 28 regional university expert centers with neurologists, geriatricians, neuropsychologists, biological, and neuroimaging resources in each center.

During visit 0, the clinical diagnosis of MCI was evaluated through clinical, neurological, and neuropsychological evaluation including the FCSRT, Hamilton Depression Scale, clinical dementia rating (CDR), Mini-Mental State Examination (MMSE), and Instrumental Activities of Daily Living. To be eligible for enrollment, patients should have met the following criteria: (1) more than 50 years of age; (2) a progressive hippocampal amnestic syndrome defined by free recall $\leq$17 or total recall <40 on the FCSRT; and (3) no dementia with a CDR stage of 0.5 and preserved cognition and functional performance. Subjects who met the eligibility criteria were enrolled in the randomization phase beginning with visit 1.

2.2. Study design and randomization

This was a multicenter double-blind, randomized, placebo-controlled, parallel group study consisting of a 4-week selection period (visit 0) and a 12-month randomized double-blind treatment period (visit 1 to visit 4) followed by an open label extension period (visit 4 to visit 5) for a total study duration of up to 18 months (Figure 1). At visit 1, patients underwent baseline magnetic resonance imaging (MRI) evaluation and a battery of secondary efficacy measure neuropsychological and cognitive evaluations including the Alzheimer's Disease Assessment Scale-cognitive subscale, MCI version (ADAS-COG-MCI), MMSE, Modified Isaacs test score, California Verbal Learning Test (CVLT), Trial Making Tests (TMT) A and B, and the Benton Test. After baseline evaluation, patients were randomly assigned to one group out of two, corresponding to either active treatment or placebo [one capsule of 5 mg donepezil daily for weeks 0 to 6, then two capsules of 5 mg donepezil (i.e., 10 mg) daily from week 6 to month 12 for double-blind treatment; or 1 placebo capsule daily for weeks 0 to 6, then two capsules daily from week 6 to month 12 for double-blind treatment, respectively].

Adverse events and vital signs were evaluated 6 weeks after baseline evaluations (visit 2) and at month 6 (visit 3). At month 12 (visit 4), patients underwent their second MRI evaluation along with the battery of secondary efficacy measure neuropsychological and cognitive evaluations as conducted at visit 1. Patients who withdrew after the end of month 3, but before visit 3 did not undergo an MRI but underwent all secondary efficacy measure, neuropsychological and cognitive evaluations. Patients who withdrew at or after month 6 received an MRI and underwent all secondary efficacy measure, neuropsychological, and cognitive evaluations.

2.3. Acquisition of MRI data

MRI was performed in each center with the same acquisition procedure. Patients underwent their first brain MRI scan before the baseline visit (visit 1). This scan was validated by a central reading structure. Patients underwent a second MRI scan at the final visit (defined as visit 4 at month 12 of double-blind treatment or at a time point after month 6 in case of early withdrawal).

Brain MRI scans were performed using 1.5 Tesla or 3 Tesla MRI scanners qualified by the central MRI analysis core at the Cogimage team, **Institut du Cerveau et de la Moelle épinière**, to confirm compatibility with the segmentation software to be used in the study. Equipment-related variability in MRI measurements was reduced by evaluating all patients enrolled with the same scanner at both measurements. Sequences used included 3D T1-weighted, 2D fluid attenuated inversion recovery, and 2D T2-weighted volumes of the entire brain, and a diffusion-weighted sequence.

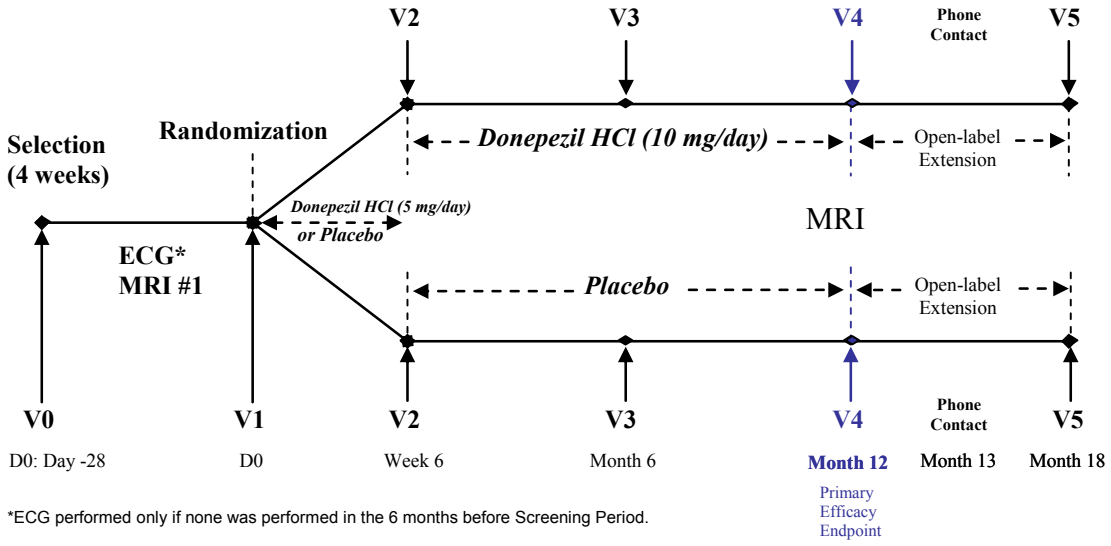

Fig. 1. Study diagram. Magnetic resonance imaging (MRI) scans were performed and validated by the central MRI analysis core before baseline and at final visit. Cognitive and neuropsychological assessments were performed at screening, randomization visit (visit 1) and month 12 (visit 4), and at premature discontinuation between months 3 and 12. At month 6 (visit 3), the Mini-Mental Status Examination (MMSE) and Alzheimer's Disease Assessment Scale–Cognitive Subscale–Mild Cognitive Impairment (ADAS-COG-MCI) tests were also performed. Safety was evaluated through patient interviews and adverse events.

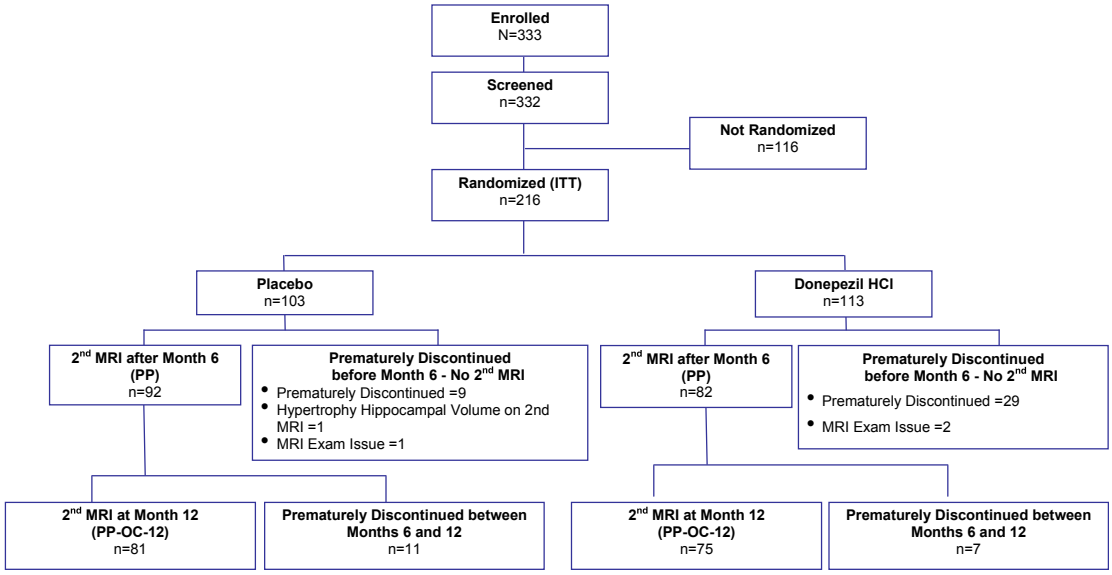

Fig. 2. Patient disposition flow chart showing the total number of patients randomized to each treatment group (ITT population); number of patients who prematurely discontinued before month 6; number of patients with a second magnetic resonance imaging (MRI) after month 6 (PP population); number of patients who prematurely discontinued between months 6 and 12, and the number of patients with a second MRI at month 12 (PP-OC-12 population). Similar proportions of patients in the donepezil and placebo groups completed the study. ITT: intent-to-treat population; PP: per protocol population; PP-OC-12: per protocol-observed case at month 12 population.

2.4. Evaluation of MRI data

To further increase sensitivity to actual change between the two time points, hippocampal volumes were computed with a longitudinal extension of the automatic version of **Segmentation Automatique Compétitive de l'Hippocampe et de l'Amygdale** [17] software which uses information from both time points at the same time. The extension relied first on a preliminary registration of the baseline and follow-up MRI scans in a common space. Intensities of both scans were then normalized. These two preprocessing steps allowed a direct comparison of both acquisitions; the same kind of preprocessing steps have already been used for longitudinal analyses (Figure 3) [18,19]. The baseline and final visit MRI scans were first segmented jointly (i.e., considered as identical and leading to a single segmentation). The resulting segmentation was then used as an initialization of separate segmentations while keeping the two segmentations consistent between the two time-points. Taking into account both acquisitions at the same time in longitudinal analyses allows obtaining results that are more sensitive to actual change [20].

2.5. Efficacy measures

The primary outcome was the APC of total hippocampal volume (THV) from baseline to final visit. Secondary MRI outcomes included left and right hippocampal volume, global cerebral volume, and ventricular volume APCs from baseline to final visit. Additional secondary outcomes included the ADAS-COG-MCI, MMSE, Isaacs verbal fluency and lexical fluency tests, CVLT, TMT-Part A, and TMT-Part B and Benton test. Neuropsychological assessments were conducted in the same order and by the same evaluator at each visit to minimize variability in patient and caregiver responses. In this report, all primary and secondary outcomes were evaluated for the per protocol population, which consisted of all randomized patients who took at least one dose of study drug, had a second MRI, and did not have any major protocol deviations.

2.6. Statistical analysis

2.6.1. Power analysis

The required sample size of 100 patients per group was calculated based on primary efficacy criterion of APC in THV from baseline to final visit, with a bilateral test performed with $\alpha = 0.05$ and $\beta = 0.20$ (80% power) based on the following assumptions from data reported in Jack et al. (2004) [7]:

- An estimated standard deviation of the percentage of variation of the hippocampal formation between baseline and the last value of the patient estimated of 2.5%.
- An observed decrease of 3.3% of the hippocampal volume in MCI subjects treated with placebo (Jack et al. [2004]) [7].
- An expected decrease of 2.3% of the hippocampal volume in subjects treated with donepezil. A total of 240 patients were planned to be randomized based on an expected withdrawal rate of 20% to achieve the required 100 assessable patients per treatment group.

2.6.2. Demographic, clinical and MRI volumetric variables

Baseline demographic, clinical, neuropsychological, and MRI volumetric variables were compared between patient

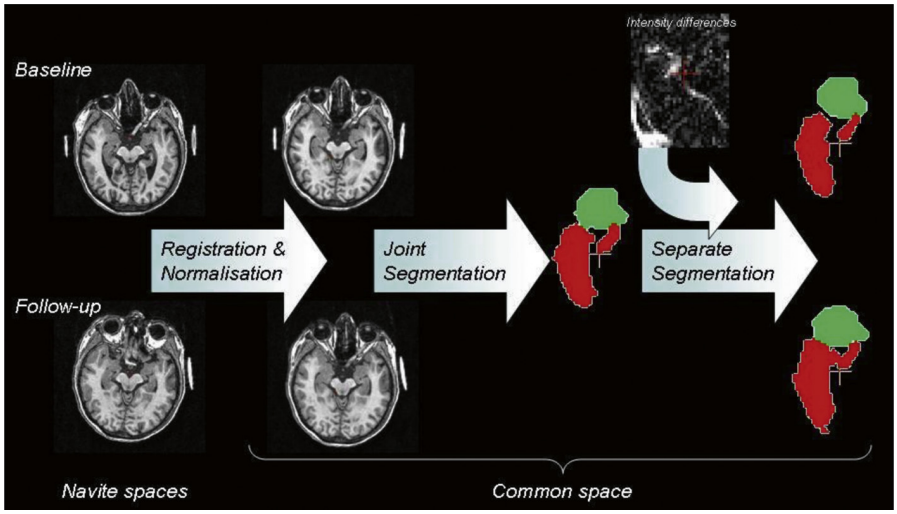

Fig. 3. Hippocampus longitudinal segmentation method illustrating preliminary registration of the baseline and final visit magnetic resonance imaging (MRI) scans in a common space followed by normalization of the intensities of both scans. The baseline and final visit MRI scans were then segmented jointly. The resulting segmentation was then used as an initialization of separate segmentations while keeping the two segmentations consistent between the two time-points.

groups (placebo vs. donepezil). Fisher Exact Test was performed on categorical variables, whereas the analysis of variance (ANOVA) was used for continuous variables.

2.6.3. Efficacy

In this report, all efficacy criteria were evaluated using the per protocol population, defined as all randomized patients who took at least one dose of study medication, had a baseline and final visit MRI, and did not have any major protocol deviations. The primary efficacy outcome was the APC of THV from baseline to final visit. APC for primary and secondary MRI outcomes were analyzed using ANOVA. APC was computed as follows:

$$APC = \frac{change\ from\ baseline}{value\ at\ baseline} \times \frac{365}{MRI\ delay} \times 100$$

Clinical and neuropsychological assessments were analyzed for the per protocol population using a change from baseline model and descriptive statistics.

2.6.4. Safety

All safety analyses were performed using the safety population comprising all randomized subjects who took at least one dose of study medication and had at least one postbaseline safety assessment.

3. Results

3.1. Study population

Of the 332 patients screened in 28 French CMRR, 216 were randomized to placebo (n = 103), or donepezil (n = 113), forming the intent-to-treat (ITT) population (Figure 2). In the placebo ITT group, a total of 11 patients discontinued before month 6 or had MRI exclusion criteria that led to their exclusion from the per protocol population (n = 92). In the donepezil ITT group, 31 patients were excluded from the per protocol population (n = 82). A total of 11 patients in the placebo group and seven in the donepezil group discontinued between month 6 and month 12.

At the start of the double-blind treatment period (visit 1), age, sex, level of education, well-being, and cognitive ability of patients did not differ significantly between the placebo and donepezil groups (Table 1). Apolipoprotein E (*APOE*) data were available for 43 patients in the placebo group (41.7%) and 39 patients in the donepezil group (34.5%). Within these subsets, 20 (46.5%) patients in the placebo group and 23 (58.9%) in the donepezil group were *APOE ε4* positive. No substantial differences were found in terms of *APOE ε4* profile ($P = .279$) between the two groups of patients. There were no significant differences between the placebo and treatment groups at baseline for the hippocampal volume (total, left, or right), global cerebral volume, or ventricular volume (Table 2).

3.2. Primary outcome measures

In the per protocol population, a significant difference was observed between the treatment groups for the primary endpoint of APC in THV (Table 3) with reduced rate of atrophy observed in the donepezil group ($P < .001$). The donepezil group exhibited a slower rate of hippocampal atrophy versus placebo over a 1-year period for the per protocol population (APC = -1.89% [SE = 0.34] vs −3.47% [SE = 0.32], respectively, n = 174, $P < .001$). The results showed a difference of 1.58 percentage points (size effect) of hippocampal volume APC (N = 92 and 82 for the placebo group and donepezil group, respectively). Considering only the sample of patients who performed MRI at 12 months, the donepezil group showed again a significant reduced rate of hippocampal atrophy (APC = −1.78%) compared with

Table 1
Screening and baseline demographic and patient characteristics: ITT population

	Placebo (n = 103)	Donepezil (n = 113)	*P*-value
Screening characteristics			
Duration of memory disorders (months)			.175
N	103	113	
Mean (SD)	33.02 (25.30)	37.98 (28.09)	
Free recall			.281
N	103	113	
Mean (SD)	11.34 (5.55)	12.14 (5.34)	
Total recall			.359
N	103	113	
Mean (SD)	29.65 (9.78)	30.82 (8.96)	
Baseline characteristics			
Age, years, mean ± SD	73.67 ± 6.61	74.13 ± 6.40	.607
Sex			1.000
Male (%)	49 (47.6)	54 (47.8)	
Female (%)	54 (52.4)	59 (52.2)	
APOE genotype, positive for *APOE ε4*			.279
n (%)	20 (46.5)	23 (58.9)	
Missing	60	74	
Education, n (%)			.096
No schooling	1 (1.0)	0 (0.0)	
Primary	7 (6.8)	9 (8.0)	
Certificate of primary education	47 (45.6)	44 (39.3)	
Secondary (baccalaureate)	17 (16.5)	34 (30.4)	
Higher education	31 (30.1)	25 (22.3)	
Missing	0	1	
Hamilton Rating Scale for Depression			.488
n/N	58/103	72/113	
Mean (SD)	3.05 (2.82)	2.72 (2.57)	
ADAS-COG-MCI			.563
n/N	103/103	113/113	
Mean (SD)	12.20 (4.22)	11.87 (4.18)	
MMSE			.420
n/N	103/103	113/113	
Mean (SD)	25.83 (2.57)	26.09 (2.21)	

Abbreviations: SD, standard deviation; *APOE*, apolipoprotein E; ITT, intent-to-treat; ADAS-COG-MCI, Alzheimer's Disease Assessment Scale-cognitive subscale, MCI version; MMSE, Mini-Mental State Examination.
NOTE. *P*-value denotes a significant difference at the Fisher Exact Test and the analysis of variance for categorical and continuous variables, respectively.

the placebo group (APC = −3.08%, $P = .02$) of the same amplitude with a size effect of 1.30 percentage points.

No significant difference was found comparing APC in THV between placebo and donepezil *APOE ε4* carriers ($P = .424$.)

3.3. Secondary outcome measures

Significant differences were observed between placebo and donepezil groups for all secondary MRI outcome measures in the per protocol population (Table 3). APC of both left and right hippocampal volumes demonstrated significantly reduced atrophy in the donepezil treatment group relative to the placebo group (−1.81% vs −3.64%, $P = .001$ and −2.02% vs 3.45%, $P = .008$, respectively). In addition, both APC of global cerebral volume and APC of ventricular volume differed significantly between placebo and donepezil groups (−0.71% vs −0.41% $P = .005$ and 4.87% vs 3.16%, $P < .001$, respectively). *APOE ε4* carriers of both groups showed the same APC in the right, left hippocampus ($P = .743$ and $P = .339$, respectively) and in the global cerebral and ventricular volumes ($P = .181$ and $P = .239$, respectively).

Table 2
Baseline volumetric measures (cubic centimeters) per protocol population

	Placebo (n = 92)	Donepezil (n = 82)	*P*-value
Total hippocampal volume			.743
n/N	92/92	82/82	
Mean (SD)	4.84 (0.88)	4.88 (0.84)	
Left hippocampal volume			.753
n/N	92/92	82/82	
Mean (SD)	2.37 (0.47)	2.35 (0.47)	
Right hippocampal volume			.350
n/N	92/92	82/82	
Mean (SD)	2.47 (0.48)	2.53 (0.44)	
Global cerebral volume			.862
n/N	92/92	80/82	
Mean (SD)	980.33 (108.64)	983.21 (107.94)	
Ventricular volume			.916
n/N	92/92	80/82	
Mean (SD)	52.10 (18.74)	51.79 (20.25)	

NOTE. *P*-value denotes a significance difference at analysis of variance.

Finally, the neuropsychological scores at baseline did not reveal any significant difference between the placebo group and the donepezil treatment group for each neuropsychological test (ADAS-COG-MCI, MMSE, Isaacs verbal and lexical fluency tests, CVLT total score, TMT A and B, and the Benton Test) in the per protocol population.

3.4. Adverse events

Overall, the number of patients experiencing treatment emergent adverse events (TEAEs) was higher in the donepezil treatment group (88 patients; 77.9%) relative to the placebo group (67 patients; 65.0%). A total of 32 patients in the donepezil group (28.4%) experienced TEAEs considered serious or severe, compared with 18 (17.5%) in the placebo group. Discontinuations due to TEAEs also occurred at higher frequency in the donepezil treatment group (20 patients; 17.7%) compared with the placebo group (seven patients; 6.8%). The most common TEAEs (>5%) that occurred with greater frequency in the donepezil group included muscle spasms, nightmares, diarrhea, headache, nausea, sleep disorder, abdominal pain, and vertigo. Adverse events reported in the study are in relation with the cholinergic properties of the drug and were expected at the notable exception of the pyrexia. They were observed at the same rate and in the same proportion than in the existing literature except for muscle spasms that were more frequent. No death has been recorded during the overall length of the study period.

Table 3
APC in volumetric measures (%) in per protocol population

	Placebo (n = 92)	Donepezil (n = 82)	Treatment difference (95% CI)	*P*-value
APC of total hippocampal volume			−1.58 (−2.51, −0.65)	$P < .001$
n/N	92/92	82/82		
Mean (SE)	−3.47 (0.32)	−1.89 (0.34)		
APC of left hippocampal volume			−1.83 (−2.94, −0.71)	$P = .001$
n/N	92/92	82/82		
Mean (SE)	−3.64 (0.39)	−1.81 (0.41)		
APC of right hippocampal volume			−1.43 (−2.47, −0.38)	$P = .008$
n/N	92/92	82/82		
Mean (SE)	−3.45 (0.36)	−2.02 (0.39)		
APC of global cerebral volume			−0.30 (−0.51, −0.09)	$P = .005$
n/N	92/92	80/82		
Mean (SE)	−0.71 (0.07)	−0.41 (0.08)		
APC of ventricular volume			1.71 (0.75, 2.67)	$P < .001$
n/N	92/92	80/82		
Mean (SE)	4.87 (0.33)	3.16 (0.35)		

Abbreviations: APC, annualized percentage change; SE, standard error.
NOTE. *P*-value denotes a significant difference at analysis of variance.

4. Discussion

This large-scale, randomized, double-blind, multicenter study demonstrates a statistically significant reduction of 45% in the APC of THV in a selected subgroup of MCI subjects on 10 mg/day of donepezil. The donepezil HCl group exhibited a slower rate of hippocampal atrophy versus placebo over a 1-year period for the per protocol population (APC = −1.89% [SE = 0.34] vs −3.47% [SE = 0.32], respectively, n = 174, $P < .001$). These findings were maintained also in the subsample of patients who had their second MRI at 12 months. Secondary neuroimaging efficacy parameters also showed significant differences between treatment groups in favor of the donepezil group for the left hippocampal volume APC ($P = .001$), the right hippocampal volume APC ($P = .008$), the global cerebral volume APC ($P = .005$), and the ventricular volume APC ($P < .001$). Moreover, there was no effect of *APOE ε4* status on any these MRI measures. No significant difference between treatment groups was observed in any of the neuropsychological tests. Adverse events in the donepezil 10 mg/day group consisted mainly of expected acetylcholinesterase inhibitor effects including abdominal pain, sleep disorders, nausea, and diarrhea.

Previous studies have shown some evidence of structural changes in the brain of AD patients under donepezil. A small decrease in left hippocampal volume was reported after 24-week of donepezil compared with the placebo-treated subjects in a randomized double-blind, placebo-controlled monocenter study [10]. More recently, a randomized, double-blind placebo-controlled monocenter study reported significant differences favoring the donepezil group for cortical region and whole brain volumes although the primary MRI outcome measure HC volumes was statistically nonsignificant [15]. In contrast to the current study, the former two pilot studies none of these studies was performed in a large-scale community-based multicenter cohort and subjects were not included on the basis of the FCSRT, a memory test that was reported to be correlated with hippocampal volume and CSF changes of the Alzheimer type [4,5].

Our results obtained on the primary structural outcome despite enrollment of less than 120 patients/treatment arm may be explained by selection of a specific study population and sensitive and highly controlled measurement of hippocampal volume. The selection of MCI subjects with an amnestic syndrome of the hippocampal type (defined by a low free score not normalized with cueing at the FCSRT) identifies the right target population (i.e., prodromal AD patients) with a high specificity because it assesses verbal episodic memory with semantic cueing that allows one to control for encoding and to facilitate retrieval to isolate the storage capacities of the patients. In addition, the use of stringent cutoff scores (free recall below ≤17 or a total recall score below <40) permitted specific selection of MCI progressors who may convert to dementia in a short period of time.

For the first time to our knowledge, the rate of hippocampal atrophy was the primary outcome of a large-scale community based multicenter clinical trial in prodromal AD. Hippocampal volume was chosen for several reasons: (1) it is central to the pathophysiology of AD as it is one of the earlier and more severely affected regions in AD; (2) it is well delimited with rather well-defined boundaries, validated, localized, and central to the neurodegenerative pathophysiology. This region can be analyzed with 3D-MRI.

Another strength of the study is that it was performed in a single country. This significantly reduced the variability of the data and also facilitated a centralized neuroimaging network with a centralized reading for MRI quality and analysis of images supervised by a single investigator. The choice of an automated segmentation method for assessing hippocampal volume also decreased human intervention compared with manual segmentation. This allows more sensitive volume measures to be obtained because it reduces variability caused by noise and position differences. This procedure provides a high reproducibility and it is specifically adapted for

longitudinal studies with for repeated investigations. All these controls may have contributed the strong statistical effect of donepezil on hippocampal rate of atrophy.

Despite the highly significant effect on hippocampal atrophy, no significant difference between treatment groups was observed in the cognitive evaluations for the Per Protocol and ITT populations. It should be noted, however, that the patients were at very mild stage of the disease as expected based on the inclusion criteria (with a mean score of 11.87 at the ADAS-COG at baseline in the treated group, see Table 1) indicating that their cognitive problems were mostly restricted to memory disorders. Furthermore, it has been demonstrated that the number of patients per arm necessary to detect a given effect based on hippocampal atrophy rate is much smaller than that needed to detect the same effect based on cognitive assessment variations [21]. The absence of clinical relevance and of significant changes on the neuropsychological performance of the structural effect prevents us to conclude to any disease modifying effects of the donepezil in prodromal AD.

Some limitations of the present study should also be considered. The protocol of the study did not include information on the settings of the subjects of the population or on race and ethnicity characteristics or data in terms of lifestyle. The latter, in particular, with its practical aspects—nutrition, hydration, alcohol consumption, smoking, and physical activity—has become significant in terms of AD prevention [22]. Although it is most likely that these factors were matched between the two groups of patients, further studies are needed to elucidate the impact of these factors in the prodromal or even in the asymptomatic stages of AD. We should also mention the number of drop-outs and the lack of *APOE* data on the entire group as potential limitations of the study.

In summary, our study showed a 45% reduction of the rate of hippocampal atrophy after one of treatment with donepezil in patients suspected to have prodromal AD. The result was obtained in a relatively small number of patients, underlying the interest of a well-selected population and centralized procedures. This is the first large-scale multicenter study of a treatment in subjects with MCI to show a positive result for a biological (morphological) primary efficacy variable. This is also the first time that a statistically significant effect of a drug is reported on rate of hippocampal atrophy in subjects with MCI. The clinical significance of this result is unclear and additional research will be needed to determine if the specific subset of MCI subjects with prodromal AD will benefit from donepezil treatment as a preventive measure to maintain memory and autonomy. Longer observation periods and longitudinal studies are warranted to evaluate the association between reduced rate of hippocampal atrophy and protective effects on cognition, such as memory and other clinically relevant domains.

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RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the literature using traditional (e.g., PubMed) sources. A stabilization of cognitive changes was reported in a few studies in patients with mild cognitive impairment (MCI) treated with donepezil. Only two studies have challenged a potential disease-modifying effect of donepezil in subjects with MCI, none of these showing a significant effect on specific brain structures.
2. Interpretation: Several features may account for the reduction of 45% in the rate of hippocampal atrophy reported here: the population chosen (amnestic MCI), the structure chosen (hippocampus), the method chosen (automated segmentation), and the procedures chosen that decreased variability (one country, a centralized neuroimaging network …). Besides these elements, the question of a specific effect of donepezil on AD brain lesions is raised.
3. Future direction: There is a need to replicate the results in prodromal AD to understand the basic mechanism through which donepezil impact morphology and/or structure of brain regions affected by AD.

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Study 3: Reduced regional cortical thickness rate of change in donepezil treated subjects with suspected prodromal Alzheimer's disease - A longitudinal multi-centric double-blind, randomized, placebo-controlled trial

Cavedo E, Dubois B, Colliot O, Lista S, Croisile B, Tisserand GL, Touchon J, Bonafe A , Ousset PJ, Ameur AA, Rouaud O, Ricolfi F, Vighetto A, Pasquier F, Delmaire C, Ceccaldi M, Girard N, Lehericy S, Tonelli I, Duveau F, Chupin M, Garnero L, Sarazin M, Dormont D, and Hampel H for The "Hippocampus Study Group"

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Reduced regional cortical thickness rate of change in donepezil treated subjects with suspected prodromal Alzheimer's disease

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Abstract

OBJECTIVES: Cortical thinning, previously identified during prodromal stages of AD, is a "candidate" biomarker implemented in AD clinical therapy trials. We have investigated the effect of donepezil treatment on cortical thickness in mild cognitively impaired subjects with the amnestic syndrome of the hippocampal type, a prodromal at risk group for progression to AD dementia.

METHODS: Longitudinal analysis of a community-based multi-centric suspected prodromal AD cohort (92 placebo versus 81 donepezil) enrolled in a double-blind, randomized, placebo-controlled parallel group design using donepezil (10mg/day) has been conducted. All subjects underwent two brain structural Magnetic Resonance Imaging (MRI) scans, at baseline and at the end of the trial. Structural MRI images have been processed using the automated pipeline for longitudinal segmentation and surface reconstruction implemented in Free Surfer. The primary outcome measure of this post-hoc study was the cortical thickness annualized percentage change (APC).

RESULTS: Donepezil group exhibited reduced APC cortical thinning compared to placebo in the Rostral Anterior Cingulate, the Orbitofrontal, and the right Inferior Frontal Cortices and in the right Insula.

CONCLUSIONS: Our findings support the hypothesis that donepezil might be a candidate surrogate outcome in pre-dementia AD trials. In addition, donepezil may have an impact on cortical morphology of areas innervated by the medial and lateral cholinergic pathways.

Introduction

The current approved treatments with cholinesterase inhibitors are generally considered as moderately and only symptomatically beneficial for late-stage AD dementia patients (Johannsen, et al., 2006). Evidence of potential biological and disease-modifying properties of cholinesterase inhibitors is controversially and critically discussed. No agent tested in randomized clinical trials in Mild Cognitive Impairment (MCI) has met its primary efficacy objectives, measured by clinical rating instruments (Crane and Doody, 2009,Doody, et al., 2010,Doody, et al., 2009,Feldman, et al., 2003,Feldman, et al., 2001,Feldman, et al., 2005,Gauthier, et al., 2002,Petersen, et al., 2005,Salloway, et al., 2004).

To date, only one study investigated the use of in vivo biomarkers of AD in donepezil clinical trial has been conducted (Teipel, et al., 2006). On the other hand, further clinical studies on structural imaging markers in MCI subjects have reported conflicting results (Dubois, et al., 2015b, Jack, et al., 2008, Schuff, et al., 2011). Although cortical-thickness represents a promising structural imaging end point in clinical trials, as it offers a direct assessment of effect sizes expressed in a meaningful metric (Hampel, et al., 2008,Teipel, et al., 2013,Teipel, et al., 2008) no study has investigated the donepezil effect on the cortical mantle yet.

This is a post hoc study based on the data collected in the context of the Hippocampus Study Clinical Trial (NCT00403520). The first aim of the trial was to investigate the effect of donepezil treatment using the hippocampal rate of changes as primary outcome (Dubois, et al., 2015b). Here, we are going to consider the impact of donepezil treatment on the thickness rate of change in suspected prodromal AD patients diagnosed using the Free and

Cued Selective Reminding Test (FCSRT), a test allowing prognostication of prodromal AD within the group of MCI individuals (Sarazin, et al., 2010, Wagner, et al., 2012).

Methods

Study Population

Participants included in the present study were all the patients who performed a baseline and a follow-up MRI scan during the Hippocampus Study Clinical Trial (NCT00403520)(Dubois, et al., 2015a). The primary efficacy outcome of the Hippocampus Study was the annualized percentage change (APC) of total hippocampal volume measured by an automated segmentation method. In this post hoc analysis we explored the effect of donepezil on the cortical thickness. Each patients received at least 1 dose of double-blind study medication, and had baseline and follow-up clinical and Magnetic Resonance Imaging (MRI) assessment.

The details of the patient characteristics have been previously described (Dubois, et al., 2015b). Briefly, a total of 332 patients were screened within the national network of Memory Resources and Research Centres (MRRC) consisting of 28 regional university expert centres with neurologists, geriatricians, neuropsychologists, biological and neuroimaging resources in each centre.

Inclusion criteria were: 1) more than 50 years of age; 2) a progressive hippocampal amnestic syndrome defined by Free Recall ≤ 17 or Total Recall < 40 on the FCSRT; and 3) no dementia with a CDR stage of 0.5 and preserved cognition and functional performance. Subjects who met the eligibility criteria were enrolled in the randomization phase beginning with Visit 1. From the total population of individuals randomized in the clinical trial (103 Placebo and 113 Donepezil), for the present study we considered exclusively patients who performed MRI at baseline and at the end of the treatment (Placebo=92 and Donepezil= 82).

Study Design

This was a multi-centre double-blind, randomized, placebo-controlled trial with a treatment period of 12 months. The sample power of the study was calculated according to the primary aim of the Hippocampus Study Clinical Trial, and not for the aim of the present study. At baseline visit, patients underwent a baseline MRI scan and a cognitive evaluation including: the Alzheimer's Disease Assessment Scale-cognitive subscale, MCI version (ADAS-COG-MCI), Mini-Mental State Examination (MMSE), Modified Isaacs test score, California Verbal Learning Test (CVLT), Trial Making Tests (TMT) A and B, and the Benton Test. Following baseline evaluation, patients were randomly assigned to one group out of two, corresponding to either active treatment or placebo (1 capsule of 5-mg donepezil daily for Weeks 0 to 6, then 2 capsules of 5‑mg donepezil [ie, 10 mg] daily from Week 6 to Month 12 for double-blind treatment; or 1 placebo capsule daily for Weeks 0 to 6, then 2 capsules daily from Week 6 to Month 12 for double-blind treatment, respectively). The study protocol was approved by the institutional review board of each site, and informed consent was obtained from all subjects.

Acquisition of MR images

Brain MRI scans were acquired in each centre at baseline and after at the end of treatment period. All the MRI were performed using 1.5 Tesla or 3 Tesla MRI scanners qualified by the central MRI analysis core at the Cogimage team, Centre de Recherche de l'institut d Cerveau et de la Moelle Épinière (CRICM). Sequences used included 3D T1-weighted, 2D fluid attenuated inversion recovery (FLAIR), and 2D T2‑weighted volumes of

the entire brain, and a diffusion-weighted sequence. After quality control of images, one 3D T1-weighted scan was identified as corrupted and it was excluded from the all statistical and the neuroimaging analysis.

Cortical Thickness Reconstruction and Surface Analysis

To extract reliable thickness estimate, images were automatically processed with the longitudinal stream (Bernal-Rusiel, et al., 2013) in FreeSurfer (http://surfer.nmr.mgh.harvard.edu/). Specifically an unbiased within-subject template space and image is created using robust, inverse consistent registration (Reuter, et al., 2010). Several processing steps, such as skull stripping, Talairach transforms, atlas registration as well as spherical surface maps and parcellations are then initialized with common information from the within-subject template, significantly increasing reliability and statistical power (Reuter, et al., 2012). Based on gyral and sulcal anatomy, the cortex was segmented using the Desikan–Killiany Atlas (Desikan, et al., 2006). For each of cortical regions, mean cortical thickness was calculated as the distance (in mm) between the pial and gray/white matter surfaces.

Statistical Analysis

Demographic, clinical and neuropsychological features were compared between subject groups (Placebo vs Donepezil). Chi-square test was performed on categorical variables, while the one-way analysis of variance (ANOVA) was utilized for continuous variables. Since the assumption of normality was violated, baseline cortical thicknesses measures were

transformed according to the method of Templeton, G.F. 2011 (G.F.Templeton, 2011) and compared between groups using the ANOVA .

Annualized Percent Change (APC) of cortical thickness, in all the regions extracted from FreeSurfer pipeline, were computed as follows:

$$APC = \frac{change\ from\ baseline}{value\ at\ baseline} \times \frac{365}{MRI\ delay} \times 100$$

Then, the cortical thickness APC were compared between placebo and donepezil using the Wilcoxon-Mann-Whitney test.

The cortical thickness APC comparisons were supplemented post hoc by the linear mixed effects regression models with random intercepts to compare the difference in the cortical thickness change between placebo and donepezil patients in relation to treatment. MRI scans was included as covariate. Bonferroni correction for multiple comparisons was applied to the cortical thickness APC comparisons and to the mixed effect regression model results. The statistical analyses were performed using SPSS v.22.00.

Surface analyses were performed using MATLAB (http://fr.mathworks.com) and the QDEC toolbox of FreeSurfer (www.surfer.nmr.mgh.harvard.edu). One-year changes in bilateral cortical structures were investigated using the Linear Mixed Effects Models. The results were projected onto the template. The surface analyses were re-thresholded using a two-stage false discovery rate of 0.05 (FDR2 < 0.05).

Results

From the total sample of 316 individuals (103 Placebo and 113 Donepzil) randomized in the trial, 174 individuals (92 Placebo and 82 Donepezil) underwent both baseline and follow-up MRI scans. One subject of them was excluded from analysis due to a corrupted scan.

No significant differences were found at baseline sociodemographic as well as in cognitive features between groups (Table 1). The Apolipoprotein E (*APOE*) genotype was present in 18 (47.3%) patients in the placebo group and 18 (60%) in the donepezil showing no substantial differences (p = 0.215) between the two groups.

The baseline cortical thickness, for all brain regions considered, did not show any significant statistical differences between the two groups (Table 2).

As detailed in the Figure 1, a significant difference in the regional cortical thickness APC was observed between groups. In particular, the Placebo group compared with the donepezil showed a higher APC in the right and left Rostral Anterior Cingulate Cortex -1.14% *vs* -0.38% (p = 0.048) and -1.28% *vs* 0.07% (p = 0.032) respectively, in the left Caudal Anterior Cingulate Cortex -1.07% *vs* 0.16% (p= 0.033), in the right and left Orbitofrontal Cortex -1.04% *vs* -0.001% (p = 0.012) and -0.54% *vs* 0.43% (p < 0.048) respectively, in the right Inferior Frontal Cortex -0.94% *vs* 0.23% (p = 0.022), and in the right Insula -1.06% *vs* 0.013% (p = 0.010).

Post-hoc analysis by the mixed effects model (Figure 2) revealed that, during the 12 months of treatment period, cortical thickness was significantly decreased in the Placebo group compared with the donepezil in the right Lateral Orbitofrontal cortex (difference in slope 0.0023; p = 0.026), in the right Middle temporal Cortex (difference in slope 0.0027; p =

0.027) and in the right Insula (difference in slope 0.0027; p = 0.015). Both the cortical thickness annualized percent change comparisons and the results on the mixed effect model did not survived after Bonferroni correction.

Surface differences were described in Figure 3 revealing a cortical thinning of Placebo group compared to the Donepezil in the Left Superior Temporal, Left Orbitofrontal, Right Supramarginal and Right Insula cortices. This result did not survive after False Discovery Rate (FDR) correction.

Discussion

To our knowledge, this is the first study investigating the effect of donepezil treatment on regional brain cortical thickness in suspected prodromal AD patients with amnestic syndrome of hippocampal type. Our results highlight how the cortical thickness APC remains stable in the Anterior Cingulate Cortex, in the left Orbitofrontal Cortex, in the Inferior Frontal Cortex, and in the right Insula in suspected prodromal AD patients treated with donepezil. These findings have been strengthened by data obtained from the Longitudinal Mixed Model. In addition our findings on cortical surface support the results found from the comparison of cortical thickness APC between groups. Indeed, we have found a trend of reduced cortical surface in the placebo group compared with the donepezil after one year of donepezil treatment in the following regions: Orbitofrontal, Superior, and Caudal Middle Frontal Cortices, Superior Temporal, Supra Marginal, Precuneus, Inferior Parietal, and Insula. Some of these areas have been recognised to be of key importance since they are supposed to represent the cortical signature of Prodromal AD patients (Bakkour, et al., 2009,Dickerson, et al., 2012). Although our results did not survive after multiple comparison, they still remain promising since they have showed a statistical trend toward a significant impact of donepezil treatment in preserving cortical thickness in suspected prodromal AD patients. One possible reason why our results did not survive to multiple comparison corrections might due to the fact that the sample power of the current study was not calculated for the present purpose but for the original aim of the Hippocampus Study Trial.

Notably, in line with a previous study revealing an increased resting state metabolism in the left prefrontal cortex (and in the right hippocampus) of AD patients treated with

donepezil (Teipel, et al., 2006), we also found a significant reduced cortical thinning in left Orbitofrontal Cortex and Anterior Cingulate Cortex in this group.

Previous studies investigating the donepezil effect on different structural imaging markers, such as the hippocampus, the brain lateral ventricles, and the whole brain volumes, showed contrasting results. A randomized double-blind, placebo-controlled mono-center study on 67 patients with mild-to-moderate AD found a small decrease in left hippocampal volume after 24-week of donepezil compared with the placebo-treated subjects (Krishnan, et al., 2003). Results from the Alzheimer's Disease Cooperative Study (ADCS) MCI Donepezil/Vitamin E study conducted on 131 MCI patients showed a statistical trend towards a possible slowing of the hippocampal atrophy rate by donepezil (Jack, et al., 2008). A more recent study conducted by Schuff and colleagues (Schuff, et al., 2009) on aMCI subjects reported significant differences in favour of the donepezil group for the total ventricular region and the cortical region (whole brain volumes) but not for the hippocampal volume. Recently, our group confirmed the data by Schuff and colleagues (Schuff, et al., 2009) for ventricular and whole brain volumes. In addition, we observed a significant reduction in the annual rate of hippocampal atrophy in suspected prodromal AD patients treated with donepezil (Dubois, et al., 2015a).

Regional cortical thickness reduction is used as a predictive indicator of AD-related neurodegeneration in MCI subjects (Dickerson, et al., 2012, Lerch, et al., 2008). Our results revealed unilateral treatment differences in several brain regions that might be due to normal variation and specialization of function and structure. Brain asymmetry is believed to be evolutionally adaptive, reducing possible interference between hemispheres (Toga and Thompson, 2003).

Based on the hypothesis that donepezil may attenuate amyloid-induced neuronal toxicity (Svensson and Nordberg, 1998, Wolf, et al., 1995), it is conceivable that 12 months of donepezil treatment might have a neuroprotective effect on the cortex. In vitro studies demonstrated that lesions of the cholinergic nucleus basalis of Meynert promote the ex vivo synthesis of Aβ precursor protein (AβPP) in the cerebral cortex (Wallace, et al., 1993). Recently, an in vivo neuroimaging study described a correlation between basal forebrain atrophy and elevated cortical amyloid load in preclinical and pre-dementia stages of AD (Grothe, et al., 2014), thus reflecting the association found in several human autopsy studies between amyloid pathology and cholinergic atrophy in AD (Beach and McGeer, 1992,Perry, et al., 1978), (Arendt, et al., 1985). Cortical amyloid accumulation might induce cholinergic cell death involving alterations in the levels of intracellular calcium and/or production of toxic and inflammatory mediators such as nitric oxide, cytokines, and reactive oxygen intermediates (Kar, et al., 2004). On the other hand, a cholinergic stimulation alters positively the mechanisms of amyloid processing, thus protecting neurons from neurodegeneration induced by Aβ (Svensson and Nordberg, 1998).

In this study, we found a trend toward a statistical significance for cortical thinning reduction in the cortical areas innervated by the medial and lateral cholinergic pathways (Selden, et al., 1998) in suspected prodromal AD patients receiving one year of donepezil treatment.

One of the main strengths of the present study is the refined target population deriving from a large-scale community-based multi-centre cohort of subjects included on the basis of the FCSRT, a memory test reported to be highly correlated with hippocampal volume and CSF levels changes of the Alzheimer type (Sarazin, et al., 2010,Wagner, et al., 2012).

The study presents potential limitations. First of all, the data used in the present research were not specifically powered for the aims of the present study, thus reducing the significance of the results. The protocol of the study did not include information on the settings of the subjects of the population or on race and ethnicity characteristics or data in terms of lifestyle. The latter, in particular, given its practical aspects – nutrition, hydration, and physical activity – has become significant in terms of AD prevention. We did not collect sufficient data on APOE ε4 genotype to exclude the hypothesis that our results were not significantly impacted by the presence of APOE ε4 genotype. Finally, the subjects have not been followed for a long enough period of time in order to determine the incidence of incipient AD in each group.

Overall, our findings support the hypothesis that cortical thickness might be used as surrogate outcome in pre-dementia AD clinical trials. Moreover, our findings suggest that donepezil may have an impact on cortical morphology in prodromal AD patients. A potentially disease-modifying effect of approved cholinesterase inhibitors represents a result of pivotal clinical interest. Further studies are needed to confirmed this first investigation. Moreover, established measurement of the basal forebrain cholinergic nuclei volume, such as the nucleus basalis of Meynert and its subnuclei, as well as their in vivo white matter connections to subcortical and cortical areas, are of interest to detect the specific donepezil effect on the brain's cholinergic system.

Table 1: Baseline Demographic and Clinical Characteristics of patients performing baseline and follow-up MRI.

	Placebo (n = 92)	**Donepezil (n = 81)**	**p-value**
Age, years	73.17 (6.63)	73.24 (6.67)	0.966
Sex, n (%)			
Male	44 (47.8)	39 (48.1)	0.544
Female	48 (52.1)	42 (51.8)	
***APOE ε4 carriers, n* (%)**	18 (47.3)	18 (60)	0.215
Missing	54	43	
Education, n (%)			
No education	1 (0.01)	0 (0.0)	0.330
Primary	7 (7.6)	8 (9.8)	
Certificate of Primary Education	43 (46.7)	30 (37)	
Secondary	14 (15.2)	21 (25.9)	
Higher education	27 (29.3)	22 (27.1)	
Follow-up MRI (months)	9.65 (4.78)	10.07 (4.43)	0.537
FCSRT (Free recall)	11.32 (5.61)	12.21 (5.40)	0.289
FCSRT (Total recall)	29.55 (9.93)	31.37 (8.91)	0.210
Hamilton Rating Scale for Depression	3.22 (2.94)	2.83 (2.49)	0.352
ADAS-COG-MCI	12.32 (4.40)	12.32 (4.61)	0.997
MMSE	25.86 (2.79)	25.94 (2.26)	0.838
3 Tesla MRI (%)	22 (23%)	23 (28%)	0.534

Means and standard deviations are reported for continuous variables, numbers and percentages for the dichotomous ones. P-values denote significant differences at ANOVA and Chi-square tests. Abbreviations: FCSRT= Free and Cued Selective Reminding Test, APOE= Apolipoprotein E, MRI= Magnetic Resonance Imaging.

Table 2. Baseline Right and Left Cortical thickness (mm) of 173 patients.

	Left Hemisphere			Right Hemisphere		
	Placebo (n = 92)	**Donepezil (n = 81)**	**p-value**	**Placebo (n = 92)**	**Donepezil (n = 81)**	**p-value**
Cingulate Cortex						
Rostral Anterior	2.74±0.02	2.80±0.02	0.113	2.72±0.02	2.75±0.03	0.51
Caudal Anterior	2.57±0.03	2.60±0.04	0.820	2.57±0.03	2.68±0.03	0.05
Posterior	2.31±0.01	2.34±0.02	0.452	2.31±0.02	2.34±0.02	0.35
Isthimus	2.15±0.02	2.17±0.01	0.337	2.12±0.01	2.14±0.02	0.59
Frontal Lobe						
Pre-central	2.32±0.01	2.35±0.01	0.355	2.34±0.01	2.35±0.01	0.80
Superior Frontal	2.49±0.01	2.50±0.01	0.996	2.51±0.01	2.51±0.01	0.73
Middle Frontal Caudal	2.33±0.01	2.35±0.01	0.350	2.37±0.01	2.38±0.01	0.95
Middle Frontal Rostral	2.20±0.01	2.22±0.01	0.472	2.21±0.01	2.23±0.01	0.77
Inferior Frontal Pars Orbitalis	2.51±0.02	2.54±0.02	0.255	2.49±0.02	2.45±0.02	0.20
Inferior Frontal Pars Triangularis	2.27±0.01	2.27±0.01	0.941	2.26±0.01	2.24±0.01	0.25
Inferior Frontal Pars Opercularis	2.38±0.01	2.39±0.01	0.557	2.40±0.01	2.40±0.01	0.87
Frontal Pole	2.61±0.03	2.65±0.03	0.192	2.67±0.03	2.66±0.03	0.70
Orbitofrontal Lateral	2.45±0.01	2.44±0.01	0.892	2.44±0.01	2.44±0.01	0.80
Orbitofrontal Medial	2.36±0.02	2.36±0.01	0.308	2.34±0.01	2.36±0.01	0.19
Parietal						
Post-Central	1.95±0.01	1.99±0.02	0.460	1.98±0.01	2.00±0.01	0.41
Supra Marginal	2.31±0.01	2.32±0.01	0.885	2.33±0.01	2.34±0.01	0.62
Superior Parietal	2.01±0.01	2.04±0.01	0.182	2.05±0.01	2.06±0.01	0.75
Inferior Parietal	2.22±0.01	2.26±0.01	0.166	2.22±0.01	2.25±0.01	0.11
Precuneus	2.12±0.01	2.14±0.01	0.541	2.12±0.01	2.13±0.01	0.76
Temporal						
Inferior Temporal	2.56±0.01	2.58±0.02	0.447	2.57±0.01	2.57±0.01	0.78
Superior Temporal	2.5±0.1	2.5±0.1	0.980	2.5±0.1	2.5±0.1	0.99

Transverse Temporal	2.25±0.02	2.22±0.02	0.770	2.26±0.02	2.27±0.02	0.99
Middle Temporal	2.59±0.01	2.60±0.01	0.778	2.56±0.01	2.55±0.01	0.89
Temporal Pole	3.3±0.3	3.2±0.3	0.376	3.32±0.04	3.31±0.03	0.88
Entorhinal	2.94±0.04	2.88±0.04	0.554	2.84±0.05	2.87±0.04	0.75
Para-hippocampal	2.38±0.03	2.41±0.03	0.936	2.34±0.02	2.28±0.04	0.42
Fusiform	2.43±0.01	2.45±0.01	0.322	2.41±0.01	2.43±0.01	0.33
Occipital						
Lateral Occipital	2.03±0.01	2.05±0.01	0.713	2.02±0.01	2.03±0.01	0.75
Cuneus	1.75±0.01	1.78±0.01	0.250	1.74±0.01	1.75±0.01	0.61
Peri-Calcarine	1.56±0.01	1.56±0.01	0.940	1.51±0.01	1.51±0.01	0.61
Lingual	1.85±0.01	1.86±0.01	0.718	1.83±0.01	1.82±0.01	0.99
Insula	2.81±0.01	2.80±0.01	0.748	2.86±0.01	2.82±0.02	0.18

Means and standard deviations are reported, P Values denote significant differences at ANOVA.

Figure 1. Significant differences in the Left and Right Brain Cortical Thickness Annualized Percent Changes. Patients treated with Donepezil showed an increase or stable change of the frontal and insula cortical thicknesses compared to the Placebo group revealing a cortical thinning in all the cortical regions. Numbers indicate means (errors standard) of Annual Percent Change; p values denote the significance at Wilcoxon-Mann-Whitney test.

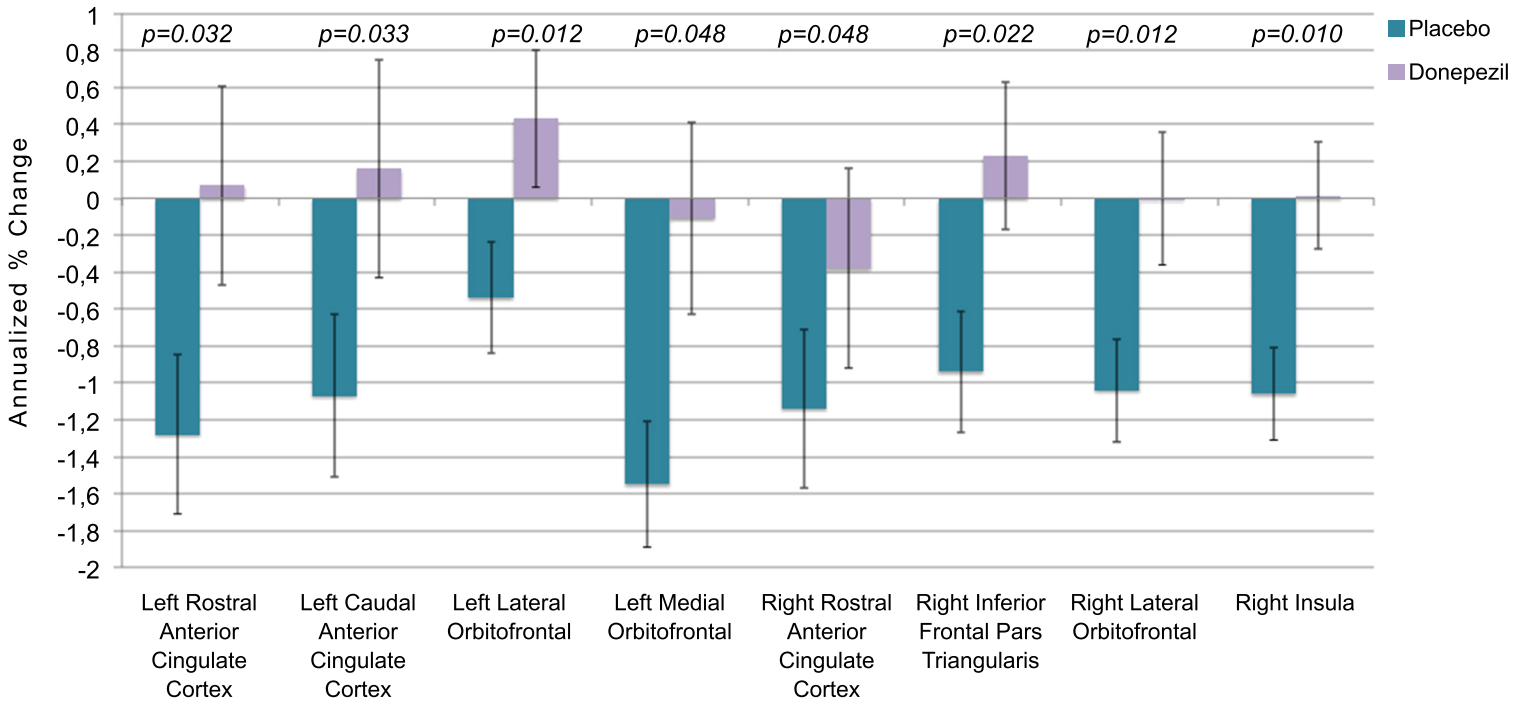


Figure 2. Individual trajectories of Cortical Thickness change. Graphs display significant changes over time resulted from the Linear Mixed Effects regression Models with random intercepts in the donepezil and placebo groups based on cortical thickness measurements from baseline (Month 0) to follow-up scan.

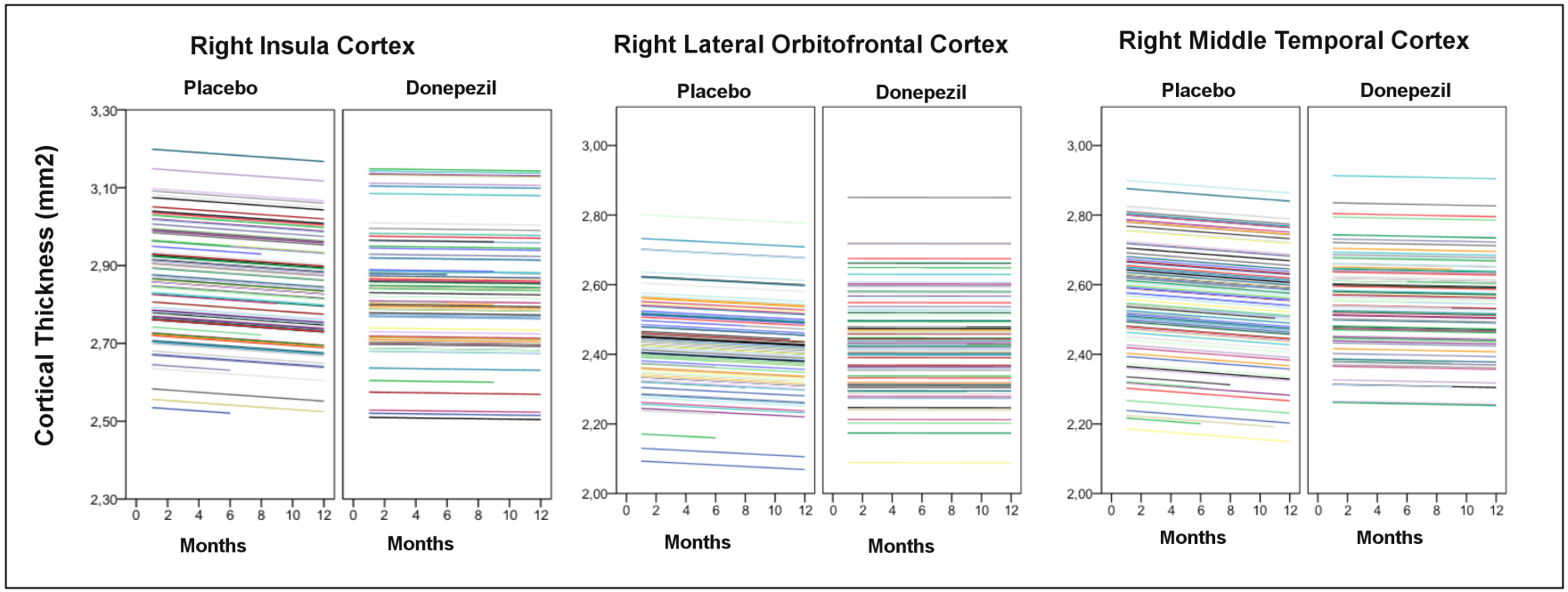
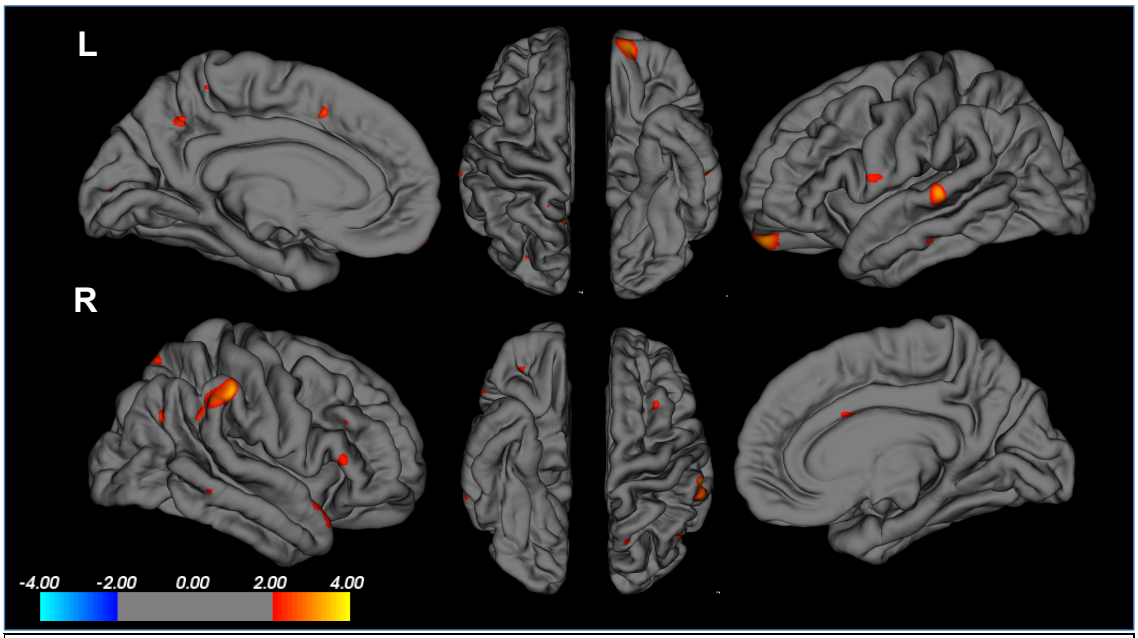


Figure 3. Surface differences between placebo and donepezil groups detected by the Linear Mixed Effects Model, results are FDR uncorrected.



Cortical Surface	Clusters Size (mm2)	Coordinates			p-value
		X	Y	Z	
Left Superior Temporal	70.02	-64.6	-27.4	4.2	< 0.001
Left Orbitofrontal	113.19	-11.4	51.7	-17.9	< 0.001
Left Superior Frontal	41.01	-10.5	14.0	41.2	0.002
Left Precuneus	61.98	-8.9	-52.0	40.3	0.003
Right Supramarginal	279.94	58.7	-27.8	38.8	< 0.001
Right Insula	43.70	30.1	20.9	7.5	< 0.001
Right Inferiorparietal	61.87	46.1	-61.0	29.3	0.002
Right Caudal Middle Frontal	44.26	33.0	18.8	43.5	0.003

FDR=False Discovery Rate

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Study 4: Hippocampus and basal forebrain as predictors of cognitive decline and treatment response in suspected prodromal Alzheimer's disease

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Neuropharmacology, *Under Review*

Hippocampus and basal forebrain as predictors of cognitive decline and treatment response in suspected prodromal Alzheimer's disease

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Abstract

Importance: Prediction of the individual course of cognitive decline and response to anti-dementing treatment is critical for adequate resource allocation, patient care, and avoidance of side effects in case of ineffective therapy.

Objective: The primary objective was to determine the value of hippocampus (Hp) and basal forebrain (BF) volumes in predicting cognitive decline in suspected prodromal Alzheimer's disease (AD) patients. The secondary objective was to determine the value of both Hp and BF in predicting the response to cholinergic treatment in suspected prodromal AD patients.

Design: A double-blind, randomized, placebo-controlled, parallel group phase 4 study in patients with suspected prodromal AD (amnestic mild cognitive impairment [MCI]) over 12 months and 6 months open label follow-up, conducted between 2007 and 2013, the 'Hippocampus Study'.

Setting: Multicenter trial across 28 university expert centers.

Participants: From a consecutively screened sample of 332 patients, 216 cases were enrolled based on a clinical diagnosis of amnestic mild cognitive impairment (MCI), and 174 participants completed the trial per protocol. The ITT sample analyzed comprised 215 cases, as one baseline MRI scan did not meet quality criteria.

Intervention: Donepezil 10 mg daily or placebo over 12 months and 6 months open label follow-up with Donepezil 10 mg daily.

Main outcome measure: Rates of global and domain specific cognitive decline as non-primary efficacy endpoint. The research question was formulated after completion of data collection.

Results: Baseline Hp volume was a significant predictor of rates of change in global cognitive function as well as cued delayed recall in the prodromal AD cohort after controlling for age,

sex, and total intracranial volume in linear mixed effects models. This effect was independent of treatment. Baseline performance in global cognition and memory was significantly associated with Hp and BF volumes. In addition, BF volume was associated with baseline performance in executive function.

Conclusions: Only Hp, but not BF volume was a useful predictor of cognitive decline in suspected prodromal AD patients. Both Hp and BF volumes were poor predictors of treatment response, questioning previous approaches on predicting treatment response without placebo control.

Trial registration: clinicalTrials.gov Identifier NCT00403520.

Introduction

Treatment with cholinesterase inhibitors has shown modest, but clinically relevant effects on rates of global cognitive decline in mild and moderate stages of Alzheimer's disease (AD) dementia (Tan et al, 2014). Accurate prediction of the individual course of disease and response to anti-dementing cholinergic treatment is critical for adequate resource allocation, patient care, and avoidance of side effects in case of ineffective therapy.

The hippocampus (Hp) represents a key structure for the consolidation of long-term declarative memory (Carr et al, 2010; Deweer et al, 2001). It is recognized as one of the core in vivo neuroimaging biomarkers of AD and undergoes neurodegeneration and atrophy already during pre-dementia stages of AD (Kaye et al, 1997; Price and Morris, 1999). Hp volume represents the first clinical imaging biomarker qualified by regulators for implementation in clinical trials as marker of selection (Hill et al, 2014; Merlo Pich et al, 2014). It can be measured in vivo using established volumetric protocols (Frisoni et al, 2015).

In addition to Hp, the basal forebrain (BF) has evolved as another relevant brain region, being substantially affected by neurodegeneration and atrophy at early AD stages (Schliebs and Arendt, 2011). The cholinergic BF is the main source of neocortical acetylcholine (Mesulam et al, 1983), and subserves mnemonic and attentional processes, involving immediate recall and executive function (Bracco et al, 2014). Loss and shrinkage of cholinergic BF neurons have been repeatedly documented in early clinical stages of AD based on autopsy data (Cullen et al, 1997; Mufson et al, 2003). In recent years, MRI-based protocols for an automated measurement of cholinergic BF volumes have been established that make use of the combined information from post-mortem MRI and histology (Kilimann et al, 2014; Teipel et al, 2005; Zaborszky et al, 2008).

MRI volumetric studies have shown a consistent pattern of atrophy of Hp and cholinergic BF

in AD dementia (Grothe et al, 2012; Teipel et al, 2005) and patients with amnestic mild cognitive impairment (MCI) (Grothe et al, 2010; Muth et al, 2010) or amyloid positive MCI (Teipel et al, 2014). Based on these findings, both Hp and cholinergic BF volumes have been studied as biomarkers for the prediction of rates of cognitive decline and response to cholinergic treatment in AD dementia and prodromal AD. In a previous study on 82 AD dementia patients, a smaller thickness of the substantia innominata, as a proxy for cholinergic BF integrity (Hanyu et al, 2002), was associated with slower trajectories of global cognitive decline during treatment with donepezil over nine months (Tanaka et al, 2003). Hp volume has shown a moderately predictive value for subsequent decline in MCI (Apostolova et al, 2006; Erten-Lyons et al, 2006; Ewers et al, 2012; Jack et al, 1999; Macdonald et al, 2013). In addition, in a study conducted on 37 patients with mild AD dementia, smaller Hp volume predicted cognitive decline during donepezil therapy as measured by the Alzheimer's disease assessment scale total score (ADAScog) (Csernansky et al, 2005). Notably, the predictive effect of Hp and BF volumes for cognitive decline during cholinergic treatment compared with placebo has not yet been investigated.

The aim of the present study was to assess the value of Hp and cholinergic BF volume measurements to predict (i) the rates of cognitive decline and (ii) the response to cholinergic treatment in suspected prodromal AD patients. For this purpose, we used the intent-to-treat (ITT) population of the prospective 'Hippocampus study'(Dubois et al, 2015), a two-arm randomized, double-blinded clinical trial on 216 well phenotyped amnestic MCI participants who were treated with donepezil or placebo over 12 months, followed by a 6 months open label extension phase (Dubois et al, 2015).

Methods

Study population

We used the baseline MRI data and the baseline and follow-up neuropsychological data of the participants of the 'Hippocampus study'. The design of the study has been previously described in detail (Dubois et al, 2015). Briefly, a total of 332 individuals with a progressive hippocampal amnestic syndrome defined by Free Recall ≤ 17 or Total Recall < 40 on the Free and Cued Selective Reminding Test (FCSRT) were selected (Sarazin et al, 2010; Wagner et al, 2012). All individuals enrolled were screened within the national network of Memory Resources and Research Centers (Centres Mémoire de Ressources et de Recherche, CMRR) consisting of 28 university expert centers in France. The protocol of the 'Hippocampus study' and informed consent forms were approved by the Ethics Committee of Salpêtrière Hospital. The study was registered at www.clinicalTrial.gov (identifier: NCT00403520).

We used the ITT population; therefore, all included cases had at least a baseline measure of MRI and neuropsychology. Of the original population of 216 participants, one case was excluded based on the covariance check of the segmentation output (see MRI preprocessing below). Participants were treated in a double blind fashion over 12 months with 10 mg donepezil or placebo, followed by a six months open-label extension study.

Neuropsychological tests

We considered neuropsychological tests used as secondary outcome variables of the study, i.e. ADAScog-MCI, California Verbal Learning Test (CVLT) immediate recall, CVLT delayed free and cued recall, Trail Making Test (TMT)-Part A, and TMT-Part B.

These tests are indices of overall cognitive function (ADAScog-MCI), episodic long term memory (CVLT delayed free and cued recall) believed to be mainly sustained by Hp

function,(Carr et al, 2010; Deweer et al, 2001) short term memory (CVLT immediate recall), and executive function (TMT-A, TMT-B, as well as TMT-B – TMT-A [δBA], and TMT-B/TMT-A [B/A]), believed to be mainly sustained by cholinergic system integrity (Bracco et al, 2014). Neuropsychological assessments were conducted in the same order and in each center by the same evaluator at each visit to minimize variability in patient responses.

MRI acquisition

MRI was performed in each center with the same acquisition procedure as previously described (Dubois et al, 2015). The MRI scans were validated by a central reading structure. Brain MRI scans were performed using 1.5 Tesla or 3 Tesla MRI scanners qualified by the central MRI analysis core at the Cogimage Team, Institut du Cerveau et de la Moelle Épinière. Sequences acquired during the project included: 3D T1-weighted, 2D fluid attenuated inversion recovery, and 2D T2-weighted volumes of the entire brain, and a diffusion-weighted sequence.

MRI data processing

The processing of structural MRI scans was implemented through statistical parametric mapping, SPM8 (Wellcome Dept. of Imaging Neuroscience, London) and the VBM8-toolbox (available at http://dbm.neuro.uni-jena.de/vbm/) implemented in MATLAB 7.1 (Mathworks, Natick). First, images were segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) partitions using the tissue prior free segmentation routine of the VBM8-toolbox. The GM and WM partitions of each subject were then high-dimensionally registered to a crisp template of average anatomy in MNI space (IXI-template) using the Diffeomorphic Anatomical Registration using Exponentiated Lie algebra (DARTEL) algorithm

(Ashburner, 2007). The IXI-template is part of the VBM8-toolbox and was derived by DARTEL inter-subject alignment of 550 healthy control subjects of the publicly available IXI-Database (http://www.brain-development.org).

Flow-fields resulting from the DARTEL registration to the IXI-template were used to warp the GM segments, and voxel-values were modulated for non-linear and linear effects of the high-dimensional normalization. This preserves the total amount of GM in native space. Modulated warped GM segments were resliced to an isotropic voxel-size of 1.5 mm3.

GM volumes of the Hp and BF cholinergic nuclei were automatically extracted by summing up the modulated GM voxel values within the respective regions of interest (below). The total intracranial volume (TIV) was calculated as the sum of the total segmented GM, WM, and CSF.

The mask for the Hp was obtained by manual delineation of the Hp in the IXI standard space template used for high-dimensional image normalization in the VBM8 toolbox. Tracing of the Hp outlines followed recently developed international consensus criteria for manual Hp segmentation on MRI (Boccardi et al, 2014) and was performed by a certified tracer (MJG) using MultiTracer 1.0 software (available at http://www.loni.usc.edu/Software/MultiTracer).

The delineation and localization of the cholinergic BF nuclei according to Mesulam's nomenclature was based on the histological serial coronal sections and post-mortem MRI scan of a brain from a 56-year-old man, as previously described (Kilimann et al, 2014). In short, subregions of the cholinergic nuclei were identified from digitalized stained sections of the BF and manually transferred into the corresponding slices of the post-mortem MRI of the dehydrated brain in alcohol space. Employing SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK; available at www.fil.ion.ucl.ac.uk/spm) the MRI brain volume in alcohol space was first transferred into the post-mortem in cranio MRI space applying a 12-

parameter affine transformation followed by high dimensional normalization (Ashburner et al, 1999). In a further step, the post-mortem in cranio MRI was transferred into IXI template standard space using the DARTEL registration method (Ashburner, 2007). The linear and non-linear transformations from alcohol through in cranio to MNI space were combined to spatially transform the BF mask into the IXI template standard space. Hp and BF volumes were transformed to z-standardized values for subsequent statistical analysis.

Statistical analysis

Differences in participants' characteristics between treatment groups were determined using Student's t-test for age, Mann-Whitney U test for Mini-Mental State Examination (MMSE) scores and education, and Chi-square statistics for sex distribution.

We determined the main effects and the two- and three-way interactions of baseline volumes by time by treatment on neuropsychological performance as dependent variable, using linear mixed effects models with subject-related random effects for intercept and time, controlling for age, sex, and TIV. The model fit was compared between nested models (random intercept only vs. uncorrelated random intercept and slope vs. correlated random intercept and slope) using Akaike's information criterion (AIC) (Sakamoto et al, 1986). Significance of parameters was determined using t-statistics with degrees of freedom determined according to the Satterthwaite approximation.

Analyses were performed with RStudio, version 0.98.1102, a user interface of R Project for Statistical Computing including the libraries "lme4" and "lmerTest", available at http://cran.r-project.org/web/packages.

Results

The participant's baseline characteristics reported in Table 1 showed no differences in age, sex, MMSE score, or education between treatment groups.

Table 2 provides a detailed summary of the main and interaction effects of Hp and BF volumes.

For ADAScog-MCI, the best fit was achieved with a model allowing for a random intercept and slope; a model including a random effects term for the correlation between intercept and slope, however, was not superior (AIC = 3818.6) to the simpler model without correlation between intercept and slope (AIC = 3818.8). In a mixed effect model with uncorrelated random intercept and slope, controlling for age, sex, and TIV, higher left and right Hp volumes predicted a significantly slower increase of ADAScog-MCI over time (Table 2, Figure 1). Effects amounted to 0.80 (0.72) points less increase in ADAScog-MCI per year of follow-up when left (right) Hp volume was one standard deviation higher. There was no main or interaction effect with treatment. BF volume had no effects on rates of change of ADAScog-MCI. Significance of effects was unchanged when the models only included observations during the first 12 months of the trial (the blinded phase).

For TMT-B, δ(BA), and B/A, there was no significant change over time, no effect of treatment and no main or interaction effects of Hp and BF volumes.

For TMT-A, the best fit was achieved with a model allowing for a correlated random intercept and slope (AIC = 4565) compared to the simpler model without correlation between intercept and slope (AIC = 4571) and a model with only a random intercept (AIC = 4570). TMT-A showed no significant change over time (t= 1.6, 256 df, p = 0.12), and no effect of treatment. BF volume showed a trend of association with baseline TMT-A performance (p = 0.074) with higher volume associated with shorter time for TMT-A performance (Table 2),

but neither BF nor Hp volume were significant predictors of rates of change in TMT-A performance.

All models with CVLT endpoints were best fitted with a model with only a random effect for intercept, but not for slope.

CVLT immediate recall showed a strong decline with time (t= -4.1, 178 df, $p < 0.001$), with no effect of treatment. Left and right Hp and BF volumes were significantly associated with baseline CVLT immediate recall performance, indicating higher recall with higher Hp and BF volumes, but not with longitudinal rates of change (Table 2).

CVLT delayed free recall showed no significant decline over time (t= -1.3, 185 df, $p = 0.19$), but a significant treatment effect, with higher baseline performance (t= 2.4, 281 df, $p = 0.017$) and faster rate of decline (t= -1.9, 190 df, $p = 0.057$) in the verum treated group. Left and right Hp and BF volumes were significantly associated with baseline performance, indicating higher recall with higher volumes, but not with longitudinal rates of change (Table 2).

CVLT delayed cued recall showed a significant decline over time (t= -2.5, 187 df, $p = 0.013$), with no effect of treatment. Baseline volumes of left and right Hp were significantly associated with baseline performance in CVLT delayed cued recall, indicating higher recall with higher volumes (Table 2). In addition, there was a tendency for a smaller rate of decline of recall over time with higher left and right Hp volume ($p = 0.01$; Table 2). Effects amounted to 0.41 (0.42) points more recall per year of follow-up when left (right) Hp volume was one standard deviation higher (Figure 2). BF volume was significantly associated with baseline performance, indicating higher recall with higher volume, but there was no effect of BF volume on longitudinal rates of change (Table 2).

Discussion

We found a significant decline of global cognitive function as well as of memory and executive functions in suspected prodromal AD cases during follow-up. Higher left and right Hp volumes predicted reduced worsening of global cognitive function and delayed recall performance independently of treatment. We did not find an effect of treatment on rates of global or domain specific cognitive changes with exception of delayed free recall rates that declined significantly faster in the donepezil group. We used a mixed effects model to take inhomogeneous number and spacing of observations into account.

Decline of global cognitive function as assessed by ADAScog-MCI in our sample of suspected prodromal AD patients is consistent with previous studies on the course of cognitive decline in prodromal stages of AD (Raghavan et al, 2013). In addition, our findings on 113 suspected prodromal AD cases treated with donepezil and 102 placebo treated cases agree with previous studies showing no significant effect of cholinesterase inhibitor treatment on rates of global cognitive decline in this population (Birks and Flicker, 2006; Salloway et al, 2008). However, the 'Hippocampus study' by design was not powered to detect treatment effects on neuropsychological endpoints. We found a significantly faster decline in delayed recall in the donepezil compared with the placebo arm. One reason for this observation may be that the donepezil arm started from a significantly higher baseline, leaving more room for subsequent decline.

Previous studies have shown that TMT-B was a strong predictor of conversion from MCI to AD dementia (Ewers et al, 2012). However, Cohen's d effect size of rates of change in TMT-B was only 0.15 even after 2 years of follow-up in a MCI sample from the ADNI cohort (Gomar et al, 2011) so that our sample was not powered to detect decline in TMT-B performance over 18 months.

In our suspected prodromal AD patients, left and right Hp volumes were significant predictors of rates of global cognitive decline. MCI cases with an Hp volume one standard deviation higher had on average 0.8 less worsening in ADAScog-MCI score per year. This effect is modest, but supports previous studies describing the Hp as an imaging marker of cerebral reserve capacity in the prodromal stage of AD. Our findings agree with previous evidence that Hp volume can predict future conversion into dementia in MCI (Apostolova et al, 2006; Erten-Lyons et al, 2006; Ewers et al, 2012; Jack et al, 1999; Macdonald et al, 2013). In a previous study conducted in a small sample of 37 very mild AD patients, resembling rather the MCI stage than the dementia stage of AD, the authors found results in line with our findings on a larger cohort, namely a higher Hp volume associated with less worsening of ADAScog score over 0.5 to 2 years of follow-up (Csernansky et al, 2005). In this previous study, all patients had received donepezil during follow-up. Our findings now suggest that the Hp volume is a predictor of cognitive decline irrespective of cholinergic treatment in MCI. In addition to global cognitive decline, higher baseline volumes of left and right Hp predicted lower decline in cued delayed recall in the MCI cases. Again, the predictive effect was independent of treatment, and would agree with a role of the Hp as a surrogate measure of cerebral reserve capacity in suspected prodromal AD.

In the cross-sectional analysis, baseline volumes of left and right Hp were associated with global cognitive function as well as memory performance, as assessed by delayed cued and free recall. These findings are consistent with previous evidence for an association of Hp volume with episodic memory performance and global cognitive function in AD. In addition, Hp volume was associated with immediate recall in the CVLT. BF volume was associated with measures of episodic memory as well, but also with measures of executive function as assessed by the TMT-A, and with immediate recall in the CVLT as a measure of short-term

memory. This agrees with the important role of cholinergic function for executive function and attention as documented in trials on anticholinergic treatment effects (Dumas and Newhouse, 2011; Pomara et al, 1995; Snyder et al, 2014). A recent study showed slowing of cognitive decline primarily in attention and executive function with cholinergic treatment in AD dementia (Bracco et al, 2014). Together, these differential associations of Hp and BF volume with memory and executive function deficits also agree with findings from our recent study on MCI cases from the ADNI cohort, where BF volume was found to correlate with both memory and executive function deficits, whereas Hp volume showed a selective association with memory deficits (Grothe et al, 2015).

As a limitation of our study, MRI data were derived from different scanners. Previous multicenter studies, however, suggest stable estimates of Hp and BF volumes from multicenter MRI data as long as minimum requirements of data acquisition are met (Ewers et al, 2006; Kilimann et al, 2014). In addition, follow-up times in the ITT population were significantly different between placebo and verum treated groups. This agrees with the higher number of drop outs during the study in the verum vs. the placebo group: premature discontinuations not due to MRI related reasons occurred in the verum group in 36 cases, and in the placebo group in 20 cases, as previously described (Dubois et al, 2015).

In conclusion, Hp volume, but not BF volume, was a predictor of global cognitive decline and memory decline in our suspected prodromal AD cohort. Our data from a controlled treatment trial indicate that Hp and BF are not useful as biomarkers for predicting response to cholinergic treatment in prodromal AD, at least within the studied timeframe. The use of Hp and BF volumes to predict response to cholinergic therapy in AD dementia still needs to be studied in dementia cohorts with controlled treatment.

Table 1: Baseline characteristics of the ITT population of the Hippocampus Cohort.

	Placebo	Verum
N (women)[1]	102 (54)	113 (59)
Age, mean (SD) in years[2]	73.67 (6.64)	74.13 (6.40)
MMSE, mean (SD)[3]	25.82 (2.58)	26.09 (2.21)
Education, mean (SD)[4]	4.68 (1.02)	4.63 (0.97)
Follow-up (SD) in months[5]	14.8 (4.2)	12.5 (7.0)

[1]not significantly different between treatment groups, $Chi^2 = 0$, 1 df, $p = 1$

[2]not significantly different between treatment groups, Student's $t = -0.52$, 209 df, $p = 0.60$

[3]not significantly different between treatment groups, Mann Whitney U test, $p = 0.62$

[4]not significantly different between treatment groups, Mann Whitney U test, $p = 0.94$

[5]significantly different between treatment groups, Student's $t = 2.8$, 213 df, $p = 0.005$

Education was scored from 1=No education, 2= primary school, 3=secondary school, education, 4= high school, 5= university.

Table 2: Summary of predictor effects

		left Hp t; df; p	right Hp t; df; p	BF t; df; p
ADAScog-MCI	Volume	-	-2.15; 255; 0.033	-
	Time*Volume	-2.48; 188; < 0.015	-2.19; 193; < 0.03	-
	Treatment*Time*Volume	-	-	-
TMT-A (Time)	Volume	-	-	-1.80; 216; 0.074
	Time*Volume			
	Treatment*Time*Volume	-	-	-
TMT-B (Time)	Volume	-	-	-
	Time*Volume			
	Treatment*Time*Volume	-	-	-
CVLT Immediate Recall	Volume	2.75; 213; 0.007	4.16; 213; <0.001	2.79; 212; 0.006
	Time*Volume			
	Treatment*Time*Volume	-	-	-
CVLT Delayed Free Recall	Volume	4.91; 268; <0.001	3.87; 265; <0.001*	2.54; 266; 0.012
	Time*Volume			
	Treatment*Time*Volume	-	-	-
CVLT Delayed Cued Recall	Volume	3.67; 276; < 0.001	4.78; 281; <0.001	3.26;280;< 0.0013
	Time*Volume			
	Treatment*Time*Volume	1.66; 221; 0.099	1.65; 223; 0.10	-

Reported are the main effect of volume and the interaction effects of volume by time and volume by time by treatment.
Data are given as: t-values (t); number of degrees of freedom (df); p-value (p).
Please note: the sign of the t-value gives the direction of the effect, i.e. a positive t-value indicates that test scores were higher with higher volume and vice versa. Hp – hippocampus; BF – basal forebrain

Figure 1: Hippocampus volume and rates of change in ADAScog-MCI in the Hippocampus cohort

Plot of z-standardized left hippocampus (upper row) and right hippocampus (lower row) volume on mixed effects linear model estimates of rates of change in ADAScog-MCI score, controlling for age and sex, with linear regression lines for placebo (blue) and verum (pink) treatment.

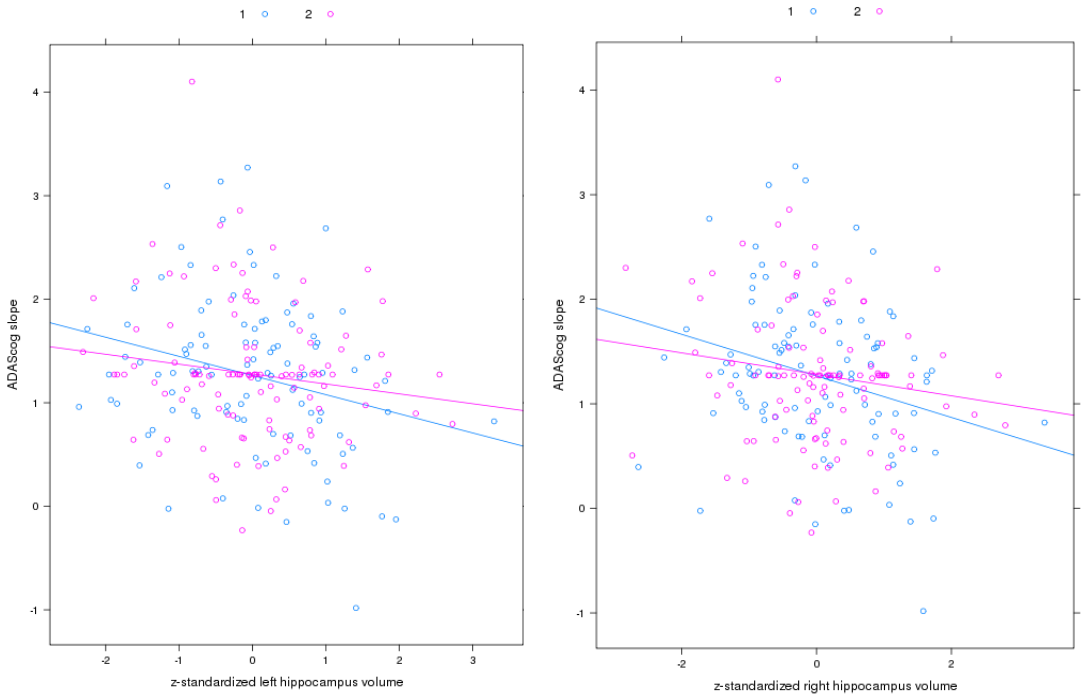
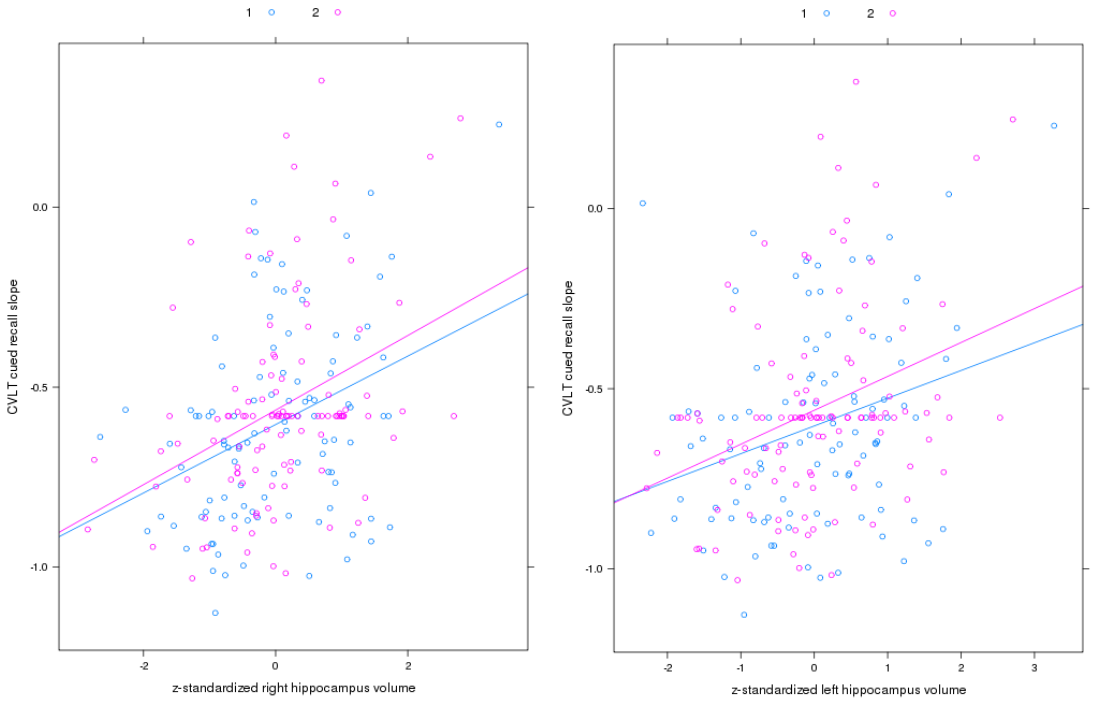


Figure 2: Hippocampus volume and rates of change in delayed cued CVLT recall in the Hippocampus cohort

Plot of z-standardized left hippocampus (upper row) and right hippocampus (lower row) volume on mixed effects linear model estimates of rates of change in delayed cued CVLT recall, controlling for age and sex, with linear regression lines for placebo (blue) and verum (pink) treatment.



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Study 5: Effects of Rivastigmine on Visual Attention in Subjects with Amnestic Mild Cognitive Impairment: A Serial Functional MRI activation Pilot-Study

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***Psychiatric Neuroimaging**, 1st Revision*

Effects of Rivastigmine on Visual Attention in Subjects with Amnestic Mild Cognitive Impairment: A Serial Functional MRI activation Pilot-Study

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Abstract

A pilot study to investigate the effects of rivastigmine on the brain activation pattern due to visual attention tasks in a group of amnestic Mild Cognitive Impaired patients (aMCI). The design was an initial three-month double blind period with a rivastigmine and placebo arms, followed by a nine-month open-label period. All patients underwent serial functional magnetic resonance imaging (fMRI) at baseline, and after three and six months of follow-up. Primary endpoint was the effect of rivastigmine on functional brain changes during visual attention (face and location matching) tasks. There were 13 aMCI enrolled in study, seven aMCI completed the study – five in the rivastigmine arm and two in the placebo arm.

Changes in the activation pattern after treatment were different for both tasks. The face matching task showed higher activation of visual areas after three months of treatment but no differences compared to baseline at six months. The location matching task showed a higher activation along the dorsal visual pathway at both three and six months follow ups. Due to its small size, the placebo arm was not analysed.

Treatment with rivastigmine demonstrates a significant effect on brain activation of the dorsal visual pathway during a location matching task in patients with amnestic MCI. Our data support the potential use of task fMRI to map specific treatment effects of cholinergic drugs during prodromal stages of AD.

Keywords: visual processing, mild cognitive impairment, Alzheimer's disease, cholinergic system, pharmaceutical therapy, Rivastigmine, Cholinesterase-inhibitor, fMR

1 Introduction

Cholinesterase-inhibitor therapy is currently approved as a treatment for the mild to moderate stages of Alzheimer's disease (AD) dementia. The potential beneficial use in patients with mild cognitive impairment (MCI) or prodromal AD, however, is still an unresolved issue and awaits evidence-based confirmation using improved clinical trial designs. Over the last few years, the identification of potentially efficacious compounds for treatment during the earlier stages of AD and cognitive decline has been advanced. In this context, a general scientific consensus has been reached on suitable functions of magnetic resonance imaging (MRI) biomarkers for improving novel drug development and discovery (Merlo Pich, et al., 2014). Several neuroimaging studies report evidence of statistically significant effects of cholinesterase inhibitors on in vivo neuroimaging markers in memory impaired individuals (Apostolova, et al., 2013,Dubois, et al., 2015,Goekoop, et al., 2004,Goekoop, et al., 2006,Jack, et al., 2008,Miettinen, et al., 2011,Petrella, et al., 2009,Risacher, et al., 2013,Saykin, et al., 2004,Schuff, et al., 2011).

In addition to the memory impairments that are characteristic of MCI and AD patients, early work in AD also found impairments in attention (Baddeley, et al., 1986,Greenwood, et al., 1997,Parasuraman, et al., 2000,Parasuraman, et al., 1992) and recent work has extended the findings to MCI (Alegret, et al., 2009,Bublak, et al., 2011,Okonkwo, et al., 2008,Saunders and Summers, 2011). The characteristics of the impairment in MCI are in deficits in complex visual-perceptual perception (Alegret, et al., 2009), in pre-attentive visual processing in MCI followed by attentive processing in mild AD patients (Bublak, et al., 2011). Further support for attentional deficits in MCI was found by Okonkwo, et al., 2008 where they tested MCI for impairments in simple, divided and selective attention and found that greatest number of

MCI subjects were impaired in divided attention while impairments in simple attention were present in the fewest MCI subjects. Another study that examined amnestic MCI and non-amnestic MCI (naMCI) subjects found that over a 10 month period there was a significant decline in divided attention in the aMCI group whereas there was decline in sustained attention in both naMCI and aMCI groups (Saunders and Summers, 2011). The effects in the attentional domain have been found not only in MCI but also in healthy non-demented middle and older individuals that carry the APOE e4 allele, where e4 carriers had decreased performance in redirecting visuospatial attention, retention of memory for location, and attentional modulation of memory of target location (Greenwood, et al., 2005, Greenwood, et al., 2000). At the same time that different attentional impairments domains are being detailed in various risk groups, the neurochemical innervation are also being investigated (Davidson and Marrocco, 2000, Witte, et al., 1997). Lesions of the cholinergic system in monkeys led to impairments of attention but not memory and learning (Voytko, et al., 1994). Various studies have examined the effect of acute cholinergic enhancement using physostigmine in AD patients. They have found selective increases in activation in the perceptual areas during the encoding phase in working memory (Petrella, et al., 2009); moreover, activation in the visual cortex was modulated across a range of attentional and memory tasks (Bentley, et al., 2008,Bentley, et al., 2004,Furey, et al., 2000c,Furey, et al., 1997,Furey, et al., 2008). During periods of high attentional demand, acetylcholine is diffusely released throughout the neocortex to modulate processing in the visual cortices and in parietal and frontal lobe (Sarter and Bruno, 1997). Cholinergic input to visual cortex has been shown to sharpen stimulus representations through a combination of signal amplification and noise suppression (Sato, et al., 1987). The objective of this study was to perform a pilot study to investigate the effects of rivastigmine on the brain activation

pattern due to visual attention tasks in a group of amnestic Mild Cognitive Impaired patients. The design was an initial three-month double blind period with rivastigmine and placebo arms, followed by a nine-month open-label period. All patients underwent serial functional magnetic resonance imaging (fMRI) at baseline, and after three and six months of follow-up. Primary endpoint was the effect of rivastigmine on functional brain changes during visual attention (face and location matching) tasks. Of interest also was the feasibility of performing such a study over a 1 year period that included multiple neuroimaging scans. To our knowledge, this is the first open-label study investigating the effect of cholinergic treatment with rivastigmine on brain activation changes during a visual perception functional MRI (fMRI) task in MCI patients.

2 Methods

2.1 Patients

A total of 12 aMCI patients diagnosed according to Petersen criteria (Petersen, et al., 2001) were enrolled in study at an academic expert memory clinic and were randomly assigned to either verum or placebo arm. Five patients in the verum arm and two patients in the placebo arm completed the study. Of the 3 patients in the verum arm that did not complete study one discontinued during double blind phase and 2 discontinued during open label phase. There were four patients assigned into placebo arm, one discontinued during the placebo phase, the second one discontinued during the open label phase. The results from the placebo arm will not be presented due to small numbers. Any comorbidity, such as cerebrovascular disease, was excluded by medical history, EEG, ECG, psychiatric evaluation, laboratory tests, and MRI examination. The study was approved by the Ethics Review Board of the Faculty of Medicine of the Ludwig-Maximilian-University, Munich, Germany. All patients signed a consent form after the study was explained to them. The study was performed from August 2002 to June 2006. The subjects included in this study overlap with those of previous publications and the overlap is only with the baseline measurements (Bokde, et al., 2008, Bokde, et al., 2006).

2.2 Design

The design of the study was one year in length with an initial three months double-blind phase where the participants received either rivastigmine or placebo. After the initial phase,

all patients received rivastigmine (open label) for nine months. The titration for the Rivastigmine was 3 mg/day in the first month, 6 mg/day in the second month, and 9 mg/day in the third month, in two daily doses. All patients remained at 9 mg/day until end of the study. In the open label phase, all patients underwent the above reported titration process.

The primary outcome variables were represented by the fMRI measured brain activation changes; the secondary outcome variables were represented by the neuropsychological measures. Notably, the practicality of performing a drug-fMRI study over one-year period was also investigated.

At entry into the study, all patients underwent the fMRI tasks and psychometric assessment. The CERAD (Consortium to Establish a Registry for Alzheimer's Disease) test battery was carried out to detect the neuropsychological performance. The fMRI cognitive tasks were performed at entry, three months (end of the double blind phase), and six months after the inclusion in the study. The CERAD battery was performed on the entire sample on the same day of fMRI scans (baseline, 3 months, 6 months) and 1 year follow-up from baseline.

2.3 Stimuli and tasks

The two tasks were a face and location matching task with a common control task (Bokde, et al., 2008). The face matching task consisted of two faces presented simultaneously and participants were asked to decide on each trial if a pair of faces was identical or not. If they were, the subject had to respond by pressing a button in the right hand. No response was required if the faces were dissimilar. The faces were grey scale stimuli where only the face was visible. Each trial was 2.8 sec long with an interval between pairs of faces of 0.318 sec. There were 8 trials per block and there were 3 blocks of the task in each run (each

block was 24.944 sec long). The number of matched pair of faces was 80%. The faces are from the Max Planck Institute for Biological Cybernetics database (Blanz and Vetter, 1999). All the faces were seen only once by the study subject except in trials were both faces matched.

The location matching task consisted of two abstract images located within a smaller square. The smaller square was located within a large square. The subject had to decide if the relative location of the small square relative to the larger one was the same. The subject would press a button if the relative locations were identical.

In the control task, the subject had to press the button every time a pair of abstract images appeared. There were 4 blocks of the control task and the parameters for the presentation of the images were identical to the face and location matching task. There was a single run of the face and location matching tasks per subject. The order of the face and location matching tasks were randomized across subjects in each group. At the beginning of each block there was a 7.2 sec task instruction. Performance was monitored and the percentage correct and reaction times measured.

2.4 Scanning

The imaging sequence was an interleaved T2* weighted echoplanar (EPI) sequence with 28 axial slices and 69 volumes acquired per run (each volume was measured in 2.8 sec with 0.8 sec gap between volumes) on a 1.5 Tesla Siemens Magnetom Vision scanner (Erlangen, Germany). For anatomical reference in each subject, a T-1 weighted sequence with 28 slices was acquired in the same orientation as the EPI sequence and a high resolution T1-weighted 3D Magnetization Prepared Rapid Gradient Echo (MPRAGE) structural image was acquired. Further details are given in (Bokde, et al., 2008).

2.5 Data Analysis

A whole brain analysis was performed using AFNI (available at http://afni.nimh.nih.gov/afni/) and FSL (available at http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) (Bokde, et al., 2008). The first 4 volumes of each scan were deleted to remove the initial T1 magnetic transients, the remaining time series was corrected for timing differences between each slice using Fourier interpolation and then corrected for motion effects (6-parameter rigid body).

Each run for each subject was analyzed using a fixed effects general linear model using FSL. Each model was composed of the regressor modeling the task of interest, the instructions, the time derivatives of the two previous regressors, and regressors for motion during the run. The task and instruction models were square wave-forms (on-off). The regressors for the task of interest and instructions were convolved with a standard double gamma hemodynamic response function. The data were smoothed (Gaussian filter at full width at half maximum = 8 x 8 x 8 mm) and high pass filtered with a cutoff at (1/100) Hz. The statistical results were normalized to the Montreal Neurological Institute/International Consortium for Brain Mapping 152 standard (MNI/ICBM). The location of the activation in the brain was done with reference to the Talairach and Tournoux template (Talaraich and Tournoux, 1988). To convert the MNI/ICBM coordinates to the Talairach and Tournoux coordinates, we utilized a non-linear transformation developed by M. Brett for transforming coordinate location between both stereotactic spaces (see online at http://www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html).

The group statistical analyses were based on a mixed effects model with a voxel wise threshold of $Z = 2.33$ ($p < 0.01$ uncorrected) and was corrected for multiple comparisons at

the $p < 0.05$ level using random field theory (cluster size correction) (Friston, et al., 1994). The subject-level activation maps were from fixed effects model, whereas at the group level t-tests are used (random effects model). The contrast between time points was a paired t-test. The t-tests performed were to test for statistically significant differences between 3 month vs baseline and 6 month vs baseline.

The structural images were first edited of the non-brain tissue using BET (Smith, 2002). The EPI images were co-registered to the 28-slice T1 weighted image (7-parameter rigid body), the 28-slice T1 weighted image was registered to the MPRAGE image, and the MPRAGE image was registered to the MNI/ICBM template (12 parameter). The statistical results from each subject were transformed into the MNI/ICBM space for group analysis. The Statistical Parametric Mapping (SPM8, http://www.fil.ion.ucl.ac.uk/spm) anatomy toolbox (V2.0) was used to localize activation clusters (Eickhoff, et al., 2005).

Statistical analysis of the neuropsychological data was performed using IBM SPSS Statistics v. 22 (Armonk, NY, USA) and paired t-tests were performed comparing performance at the follow-up time point compared to baseline. Statistical significance (two tailed) was set at $p < 0.0167$ ($p<0.05$ corrected for 3 multiple tests).

3 Results

3.1 Neuropsychological and Behavioral Performance

The demographics of the study subjects were in the verum arm average age of 68.7 years (standard deviation of 8.5 years) with sex distribution being 3M/2F. The placebo arm had 2 subjects of average age 50.7 years (2.6 years) with males only in group. Given the age difference between the two arms, and small size of the placebo arm, the analysis proceeded only with the verum arm. In the neuropsychological tests (sub-scores in CERAD) there were statistically significant differences only in verbal fluency (baseline vs 3 month follow-up) (Table 1). Task performance did not change (no statistically significant difference detected) across the length of the study (Table 2). The statistical analyses were comparisons between the follow-up time point to the baseline measurements.

The task performance was high – for the face matching task it meant that most participants had about 2 incorrect trials in baseline and first follow-up whereas for the 6 month follow-up the performance would have, on average, about 1 incorrect trial for the task. In the case of the location matching task, the performance throughout the trails would indicate that on average task performance led to about 3 incorrect trials. The performance level was high for both tasks, with reaching ceiling level for the face matching task at 6 month follow-up.

3.2 Differences in Activation After Rivastigmine Treatment

The statistical analyses between time points were paired t-tests testing for significant differences in activation (2-sided). After three months of rivastigmine treatment, statistically significant increases during the face matching task at 3 months follow-up compared to baseline were found bilaterally in lingual and fusiform gyri, left angular gyrus and cerebellum ($p < 0.05$ corrected for multiple comparisons, Figure 1A and Table 3). During the location matching task, at 3 months follow-up compared to baseline, there was statistically significant increase of activation was observed in early visual areas, left inferior temporal gyrus, right precuneus and angular gyri, and right inferior frontal gyrus ($p < 0.05$ corrected for multiple comparisons, Figure 1B and Table 4). We did not detect any statistically significant decrease in activation at the 3 month period compared to baseline measurements.

After six months of treatment, in the face matching task there were no statistically differences in activation detected between 6 month follow-up andcompared baseline. In the location matching task, there were statistically significant increases in activation at 6 month follow-up compared to baseline in right inferior parietal lobule and supramarginal gyrus, left precuneus and paracentral lobule, and left medial frontal gyrus ($p < 0.05$ corrected for multiple comparisons, Figure 1C and Table 5). We did not detect any statistically significant decrease in activation at the 6 month period compared to baseline measurements.

4 Discussion

In terms of the primary outcome variable, i.e. brain activation changes pre-and post-treatment, we found that continuous rivastigmine treatment of amnestic MCI patients led to statistically significant changes compared to baseline in brain activation in the visual cortex during the face and location matching tasks. The statistically significant increased activation compared to baseline in the temporal and parietal lobes remained after six months of treatment during the location matching task. There were no statistically significant differences in task performance over the course of the study, thus performance differences cannot account for the changes in activation in either task.

The increased activation observed in our study may be due to more efficient processing of the visual stimulus along the visual pathways. For instance, studies assessing the cholinergic modulation during a selective attention task, found that early visual cortex is modulated and correlated with task performance (Furey, et al., 2000b,Furey, et al., 2000c,Furey, et al., 2008). In our study, the face matching task revealed an increase of activation after three months of treatment compared to baseline in visual area - the fusiform and lingual gyrii. Previous studies investigating the effects of acute cholinergic enhancement on working memory with face stimuli in healthy younger subjects found that activation in the early visual cortex was modulated and correlated with task performance (Furey, et al., 2000a). Given that perceptual deficits can lead to further deterioration of higher cognitive function (Mielke, et al., 1995), cholinergic enhancement could lead to improved cognition by increasing the selectivity in the visual cortex. A study of Bentley and colleagues (Bentley, et al., 2008) demonstrated that one mechanism of action of cholinesterase inhibitors is represented by the modulation of visual cortex during encoding of stimuli in healthy

cognitive individuals, but not in AD patients.

Previous studies investigating the activation pattern for the face- and location-matching tasks on healthy controls showed that they differentially activate the ventral and dorsal pathways of the visual system, respectively (Bokde, et al., 2010, Bokde, et al., 2008). Areas that respond more during the presentation of faces were the middle lateral fusiform gyrus (Kanwisher, et al., 1997) and the inferior occipital gyrus (Halgren, et al., 1999) showing a right hemispheric predominance. In contrast, brain areas related to object visual processing correspond to the activation of lateral superior parietal cortex (Haxby, et al., 1991) and the left occipito-temporal cortex and did not involve the right hemisphere regions specifically activated during the face-identity task (Sergent, et al., 1992). Episodic encoding and delayed-response visual memory for novel faces in MCI patients, disclosed an increased activation in medial temporal lobe regions (Goekoop, et al., 2004,Pa, et al., 2013,Risacher, et al., 2013) and in the left inferior frontal gyrus during memory processing (Petrella, et al., 2009).

In current study we showed a statistically significant increase in activation compared to baseline during the location matching task, with the differences mainly located in the temporal and parietal lobes sample after six months of treatment. These results are partially in line with a previous study evaluating the effect of cholinesterase inhibitors on visuospatial perception tasks in AD patients. Indeed, findings revealed brain activation in the precuneus, left cuneus, left supramarginal gyrus, right parieto-temporal cortex, and right inferior parietal lobule (Thiyagesh, et al., 2010). In contrast, in a previous study with AD patients, decreased activation changes in AD patients after a three-month treatment with galantamine were reported (Bokde, et al., 2009). The differing response to cholinesterase inhibitors in the two groups may reflect the level of pathology in the brain, assuming that the putative neuropathology underlying the aMCI is AD related. We know that aMCI is a

heterogeneous group and the differences may reflect those found between groups.

For the secondary outcome variables, i.e. the scores from the CERAD, there were no significant changes in cognitive performance. Given the small size of the group, it is underpowered to detect statistically significant differences.

One finding of this pilot study was related to the feasibility of conducting a one-year trial with multiple scanning sessions with a double-blind stage followed by an open label treatment stage. This trial design is challenging given the 3 MRI sessions, which even though were only 30 minutes in length, were demanding of the participants. One potential simplification would be to remove the 6 month follow-up scanning session and keep only the 3 month follow-up scanning session to measure the effects over the double-blind period. Notably, one issue of interest before starting the study was whether our population would have participated in all imaging sessions or simply discontinued the study given the frequency of scanning sessions. None of the patients reported scanning sessions as a significant issue, in particular, none of the patients that discontinued the study reported that scanning was an important factor in their decision. The performance of the fMRI tasks were very high thus the tasks that were clearly doable by the study patients. It is an important experimental issue as a too-difficult task may make the study participants unwilling to participate in the follow-up scanning sessions, but also low performance may decrease the value of the scanning data. These challenges exist not only in studies with cognitively impaired patients but also relevant in studies of normal cognition and aging. An important feasibility issue with fMRI are the artifacts that may arise in the fMRI data from excessive motion during the scan – motion artifacts cannot be removed completely and if it is excessive (usually defined by more than 1 mm in any direction) may render the data unusable. In the present study none of the data had to be removed because of excessive

motion during the scan. Thus we believe that these types of studies would be feasible with larger numbers, with perhaps the simplification of 2 scanning sessions as opposed to 3 as performed in the present study.

Some limitations of the present study should be underlined. Firstly, although the present results are very promising, they need to be replicated in a larger cohort of patients. Secondly, the very underpowered placebo arm has reduced our ability to assess if the change may occur in the absence of treatment.

5 Conclusions

Despite the limitations inherent to this pilot study, our results identify for the first time a statistically significant increase of brain activation in visual regions, parietal cortices and frontal lobes as a possible neural response to rivastigmine therapy. A follow up study testing in a larger group would provide stronger information about the potential of using the tasks used in this study to measure the effects of cholinesterase treatment. Longitudinal fMRI represents a useful method for elucidating the neural changes associated with pharmacological modulation and a potential tool for monitoring intervention efficacy in clinical trials.

Table 1. Neuropsychological performances of 5 MCI patients who underwent Rivastigmine treatment.

	Entry	3 months	6 months	12 months
MMSE [30]	25.2 (3.6)	26.2 (2.8)	26.8 (1.3)	26.7 (1.5)
Learning 1-3 [30]	16.3 (3.9)	15.4 (6.1)	15.3 (7.6)	17.0 (2.9)
Word Recall [10]	3.8 (2.4)	3.0 (1.9)	4.0 (2.9)	2.7 (2.3)
Word Recognition [10]	10.8 (3.8)	8.0 (2.9)	7.0 (3.8)	6.8 (3.0)
Word Fluency	20.0 (6.6)	16.4* (5.4)	17.0 (2.8)	15.6 (4.2)
Word Naming [15]	13.4 (2.1)	13.4 (1.1)	14.2 (1.9)	13.0 (2.5)
Drawing [11]	9.6 (1.3)	10.0 (1.4)	10.5 (1.0)	10.6 (0.6)

Values in brackets [ ] indicate maximum possible score for each specified test except Verbal Fluency for which a maximum score does not exist.
Values are mean (standard deviation)

Table 2. Behavioral performances of the face and location matching tasks of 5 MCI patients who underwent Rivastigmine treatment.

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	Entry	3 Months	6 Months
Face Matching			
Correct rate	92.7 (8.6)	90.0 (6.3)	94.8 (2.3)
Response time	1.59 (0.3)	1.43 (0.2)	1.59 (0.2)
Location Matching			
Correct rate	87.5 (12.1)	86.7 (1.5)	87.5 (12.5)
Response time	1.54 (0.4)	1.53 (0.2)	1.54 (0.4)

Values are mean (standard deviation)
Correct rate values are in percent correct
Response time unit is second

Table 3. Statistically significant differences in the face matching task between the baseline and 3 month follow up. The positive t-values indicate greater activation for the 3 month follow up.

Region	Coordinates (x/y/z)	t-value
R Lingual Gyrus	20 -72 -12	4.00
L Lingual Gyrus	-18 -84 -8	3.68
R Fusiform Gyrus	32 -72 -10	3.18
L Fusiform Gyrus	-28 -66 -12	2.72
L Angular Gyrus	-38 -64 26	2.72
L Cerebellum (Crus 1)	-10 -84 -20	2.87

Single cluster (size of 1775 voxels). Coordinates in MNI space.

Table 4. Statistically significant differences in the location matching task at 3 months follow-up compared to baseline. The positive t-values indicate greater activation for the 3 month follow up.

Region	Coordinates (x/y/z)	t-value
Cluster 1 (3269 voxels)		
R Lingual Gyrus	22 -82 -2	4.18
R Fusiform Gyrus	30 -62 -4	3.91
R Cerebellum (Crus 1)	10 -84 -26	3.34
L Cerebellum (VIIIa)	-4 -62 -36	3.21
Cluster 2 (2804 voxels)		
L Inferior Occipital Gyr	-30 -76 -10	3.73
L Lingual Gyrus	-32 -84 -16	3.27
L Inferior Temporal Gyr	-42 -40 -16	3.07
Cluster 3 (2443 voxels)		
R Precuneus	18 -60 32	3.40
L Posterior Cingulate Gyr	-4 -32 32	3.32
R Angular Gyr	44 -74 34	3.06
Cluster 4 (1799 voxels)		
R Inferior Frontal Gyr	52 26 10	3.99
	42 14 26	2.92
R Anterior Cingulate Gyr	4 44 25	3.15
Cluster 5 (525 voxels)		
L Middle Occipital Gyr	-32 -84 42	3.77
L Superior Occipital Gyr	-16 -92 38	3.68
Cluster 6 (273 voxels)		
L Inferior parietal Lobule	-50 -38 50	2.72

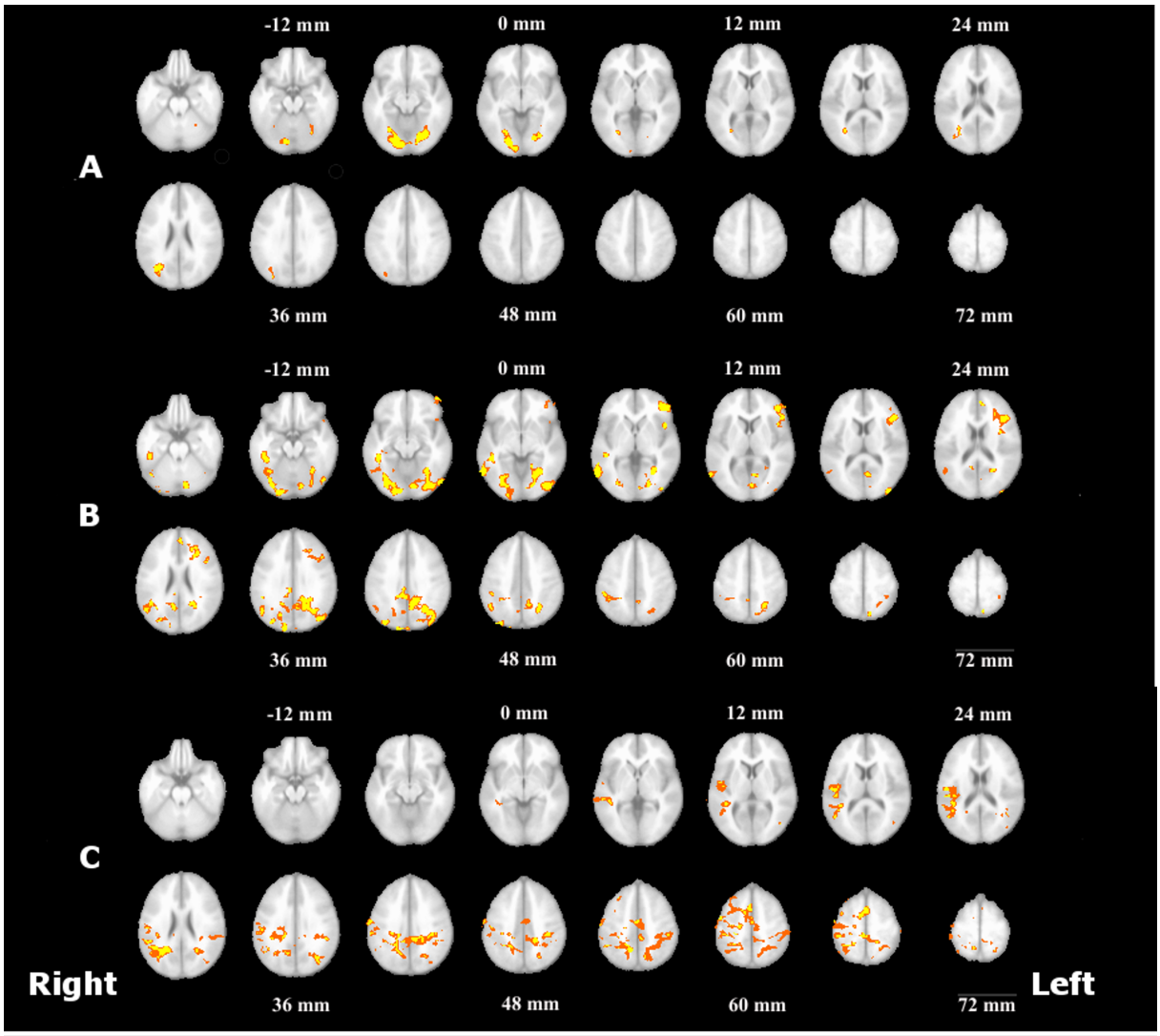
Coordinates in MNI space.

Table 5. Statistically significant differences in the location matching task at 6 months compared to the baseline. The positive t-values indicate greater activation for the 6 month follow up.

Region	Coordinates (x/y/z)	t-value
R Inferior Parietal Lobule	44 -42 38	4.98
L Paracentral Lobule	-4 -34 60	4.11
R Supramarginal Gyrus	48 -36 28	4.00
L Precuneus	-10 -56 48	3.98
L Medial Frontal Gyrus	-2 6 57	3.90

Single cluster of 9099 voxels. Coordinates in MNI space.

Figure 1. Increase in activation after Rivastigmine treatment **(A)** after 3 months in the face matching task (contrast [face (3 months) – face (baseline)]); **(B)** after 3 months in the location matching task (contrast [location (3 months) – location (baseline)]), **(C)** after 6 months in the location matching task (contrast [face (6 months) – face (baseline)]).



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IV. GENERAL DISCUSSION

The studies representing the body of this PhD thesis are intended to address the issues on the application of SOPs of imaging markers and to expand the knowledge on the implementation of structural and functional imaging markers in AD clinical treatment trials.

Implementation of Standard Operating Procedures in a multicentre setting

In the first section of the thesis, it was described the process of implementation of harmonized procedures for the acquisition of structural magnetic resonance images and their related markers for AD in ten Italian Outpatients Memory Clinics. This project represents the first Italian Initiative applying ADNI procedures for the implementation of standard MRI protocols. The main aim was to establish, at national level, globally recognized standards for the acquisition of MRI protocols. Thank to this initiative, sMRI sequences acquired in different Italian centres are now comparable to sMRI sequences acquired in other European and/or North America countries involved in the same initiative.

According to the results presented in the Study 1, a good adherence was found between the Italian ADNI MPRAGE image protocol and the original North American ADNI protocol. Indeed, insignificant changes were detected in terms of spatial and temporal resolution. Moreover, we found a higher mean SNR in the MPRAGE images acquired in each Italian site (both 1.5T and 3T scanners) compared to the mean SNR obtained from the same scanner at North American ADNI. This result describes the good quality of MRI acquisition obtained from the 9 scanners where the ADNI protocol was implemented. In addition, the results on

the structural imaging markers, such as the total intracranial volume and the hippocampal volume, confirmed the stability of MRI quantification.

As for other ADNI initiatives, the Italian ADNI project was not a treatment trial; actually, this was a naturalistic multisite observational study designed to mimic or stimulate treatment trials. Since the data collected during the Italian ADNI and the other worldwide initiatives are available in public databases, as a result, pharmaceutical companies, government funded investigators, or other research entities might employ these data to design improved treatment trials (Hendrix, et al., 2015).

Implementation of neuroimaging markers in pre-dementia prevention trials for Alzheimer's disease

In the second part of the thesis, evidences on the role of structural and functional imaging markers in anticholinesterase therapies were elucidated. First of all, Studies 2 and 3 employed the most common structural imaging markers for AD – such as the whole brain atrophy, the hippocampal atrophy, the ventricle expansion, and the cortical thinning –as surrogate outcomes in the Hippocampus Study Clinical Trial. This trail was a large-scale, randomized, double-blind, multicentre study conducted in a population of mild cognitive impairment individuals presenting the amnestic syndrome of hippocampal type detected by the FCSRT test, a memory test highly correlated with hippocampal atrophy and CSF levels changes (Sarazin, et al., 2010, Wagner, et al., 2012). Originally, the trial was designed to test as primary outcome of efficacy: the annualized percent change (APC) of total hippocampal volume. The hippocampal volume was selected as primary outcome since it represents one of the key brain regions affected by the pathophysiology of Alzheimer's disease and its boundaries are well delimited in order to allow a reliable segmentation. Results from Study 2 highlighted a slower rate of hippocampal atrophy in the group of patients receiving 1-year donepezil treatment compared to the placebo group (APC = -1.89% [SE= 0.34] vs -3.47% [SE= 0.32] respectively, $p < 0.001$). In addition, it was also found a reduced whole brain volume ($p= 0.005$) and ventricular annualized percent changes ($p< 0.001$) in favour of the donepezil treated group. Such results are partially in line with previous evidences of structural alterations in the brain of AD patients under donepezil treatment (Krishnan, et al., 2003, Schuff, et al., 2011).

Notably, even though the design of the Hippocampus Study Clinical Trial was not powered to test other neuroimaging markers for AD, the Study 3 was performed to explore the impact of donepezil treatment on the cortical thickness APC, in the same population of suspected prodromal AD patients. Cortical thickness represents a candidate surrogate outcome in AD trials since it is able to detect a biological process that is dynamic (Thompson, et al., 2003). Indeed, it is sensitive to alterations to short-term follow-ups and it correlates with clinical changes (Thompson and Apostolova, 2007). Interestingly, a study using the Freesurfer pipeline demonstrated the features of reliability and repeatability of this software in segmenting the cortical surface in multicentre studies (Han, et al., 2006). In this post-hoc study (i.e., the Study 3), it was observed how suspected prodromal AD individuals receiving the placebo compared to the patients receiving the donepezil showed a trend of significant annual thickness reduction in the Anterior Cingulate Cortex bilaterally, in the left Orbitofrontal Cortex, in the Inferior Frontal Cortex, and in the Right Insula. These promising results support the hypothesis that cortical thickness might represent a candidate surrogate marker in pre-dementia trials for AD. Despite the findings obtained on the structural imaging markers investigated in the Studies 2 and 3, no difference between treatment groups was observed in the cognitive evaluations. It should be noted, however, that the patients were at very mild stage of the disease, as expected, based on the inclusion criteria. Indeed, the significance of the imaging results obtained in the Studies 2 and 3 are related to the strength of the clinical cohort recruited in the Hippocampus Study Clinical Trial. In comparison to the cohorts investigating the effect of donepezil treatment in MCI individuals, the data collected using the Hippocampus Study Clinical Trial were gathered in a single country reducing the variability of data and facilitating a centralized neuroimaging network for the quality control of MRI images and their analysis.

The results found in the Studies 2 and 3 support the hypothesis that donepezil treatment might significantly influence the brain morphology. However, the absence of substantial clinical changes between the two groups prevents from developing any assumption on the disease-modifying effect of the donepezil in the pre-dementia stages of AD. Further studies investigating more candidate surrogate outcome measures related to the cholinergic circuit, such as the basal forebrain volume and the structural network connectivity of cholinergic pathways are needed. These studies will increase our knowledge on the effects of acetylcholinesterase inhibitors on brain morphology and networks related to the treatment and its clinical effects.

The prediction of the individual cognitive decline and the response to anti-dementing treatment using imaging markers of AD represent an essential goal to allocate clinical resources and to reduce side effects of ineffective therapies. For these reasons, in the Study 4, it was investigated the value of the hippocampus and the cholinergic basal forebrain volumes in predicting the cognitive deterioration and the response to the cholinergic treatment in the population of MCI with the amnestic syndrome of hippocampal type enrolled in the Hippocampus Study Clinical Trial. Results revealed that the hippocampal volume, but not the cholinergic basal forebrain volume, was a predictor of global cognitive and memory decline in suspected prodromal AD patients. The predictor effect found on global cognitive and memory performance was independently related to the cholinergic treatment received during the clinical trial. These results suggested that both the hippocampus and the cholinergic basal forebrain volumes were not useful biomarkers for predicting response to cholinergic treatment in suspected prodromal AD patients within the timeframe considered for the study (18 months maximum). Further analyses are needed to

better examine the role of these imaging markers as predictors of cognitive decline and treatment response in AD pre-dementia controlled treatment trials.

Finally, in the Study 5 was investigated the effect of cholinesterase inhibitors in the pattern of activation for the face-and location matching tasks. The ventral and dorsal spatial vision pathways project to different regions in the parietal, temporal and prefrontal cortices. These circuits were shown to play a critical role in high-order cognitive function, namely the working memory (Fuster, 1990, Goldman-Rakic, 1990). A reduced activation of these pathways was previously found in AD patients (Bokde, et al., 2010). Moreover, functional imaging task related markers investigating the dysfunction of the dorsal and ventral spatial vision pathways might represent a candidate surrogate outcome to elucidate the neural changes associated with pharmacological modulation. Results from study 5 revealed a significant activation of the visual cortex during the face and location tasks after 3 months of rivastigmine treatment in comparison to the baseline performance. In addition, an activation of the parietal lobe was found limited to the location matching task. After 6 months of rivastigmine treatment, increase activation in the temporal and parietal lobes was found exclusively during the location-matching task. Notably, these results suggest that rivastigmine treatment might increase the efficiency of the visual stimuli processing along the visual pathways in MCI individuals. Cholinergic enhancement could lead to enhanced cognition by increasing selectivity in the visual cortex. Further investigations using larger sample size of prodromal AD patients and randomized control-based design studies are needed to support the encouraging findings detected in this pilot study.

Conclusions and Future Perspectives

The discovery and validation of a broad spectrum of interventions for AD, including pharmacological, behavioural and life-style treatments, remains a crucial global public policy objective for the AD research.

The use of SOPs for the acquisition of MRI images and for detecting structural and functional imaging markers, at multiple centric levels, will be one of the main challenges in the field of AD research in the next years. The implementation of SOPs will increase the reliability and the repeatability of imaging methods. Moreover, it will lead to an increased comparability of the results obtained across multiple sites and to develop validated quantification tools for efficiently discovering structural and functional imaging markers. This step will be of key importance for better applying imaging surrogate outcomes in prevention trials.

Since the cellular and molecular alterations responsible for the neurodegeneration process in AD are assumed to start at least 10-20 years before the onset of clinical symptoms, reliable surrogate markers of outcome reflecting the *in vivo* neuropathological processes of AD are eagerly required. The studies presented along this thesis have highlighted that therapies using acetylcholinesterase inhibitor drugs may have an effect in reducing brain atrophy and increase the efficiency of functional networks in pre-dementia stage of AD. These results are a small piece of evidence of a bigger scenario that require understanding why similar evidences are not detected at clinical level. Secondly, the candidate surrogate markers described in the present manuscript should be investigated also at earliest stage of AD such as the asymptomatic phase. In this context, several recent pharmacological

prevention trials have been started on different populations: asymptomatic individuals with biomarker evidence of AD pathology (i.e., late onset) and presymptomatic individuals with dominant mutations or other genetic risk factors for AD (i.e., early onset) (Carrillo, et al., 2013). Among the most significant trials, it is worthwhile to mention: (i) the Anti-Amyloid Treatment for Asymptomatic AD Trial consisting of a secondary prevention trial in clinically normal, Abeta-positive individuals who receive an anti-amyloid drug for a 3-year period (Sperling, et al., 2012, Sperling, et al., 2014); (ii) the Alzheimer's Prevention Initiative, aiming at evaluating the anti-amyloid treatment on asymptomatic individuals carrying a rare autosomal-dominant mutation in the *PSEN1* gene, which confers certainty of developing AD symptoms by approximately age 45 (Reiman, et al., 2011, Reiman, et al., 2010); (iii) the Dominantly Inherited Alzheimer's Network (DIAN), an international research partnership aiming at studying the adult children of parents with forms of dominantly inherited AD (Morris, et al., 2012). The primary outcome of the initial phase of the DIAN will be represented by the rate of change for brain amyloid deposition and other biomarkers as secondary outcomes. Strictly linked to the DIAN study, is the DIAN Trial Unit launched in the same population to assess the safety, tolerability, and biomarker efficacy of two monoclonal antibodies, gantenerumab and solanezumab. An additional currently ongoing initiative is a Phase III primary prevention study in cognitively normal elderly subjects. Individual are stratified by a genetic algorithm based on the presence of the *TOMM40* genotype, the *APOE* $\varepsilon 4$ allele, and age of the individuals at entry. In this trial, high-risk subjects will be randomized to receive placebo or pioglitazone, an anti-inflammatory drug associated with a lower dementia incidence (Heneka, et al., 2015), within the next 5 to 7 years (Roses, et al., 2010).

Moreover, the European Dementia Prevention Initiative, focused on multiple domain interventions including the reduction of lifestyle risk factors for AD, was recently launched in Europe. The study of lifestyle risk factors for AD is becoming crucial since it was demonstrated that some individuals, showing brain plaques and neurofibrillary tangles comparable to the ones of AD patients (Snowdon, et al., 1997), are able to "mask" the cognitive loss due to the ability of the brain to cope with neurodegeneration (Stern, 2012). Notably, life experiences such as education, occupation, social interactions, leisure activities, physical exercise and a healthy balanced diet appear to increase the cognitive reserve, in such a way that an individual is better able to tolerate brain damages or pathologies. Vascular risk factors monitoring and management is another important aspect to consider for preventing dementia. A systematic review of the literature found that dementia risk was decreased by 46% in persons with high cognitive reserve, where complex mental activities have showed the greatest effect (Valenzuela and Sachdev, 2006).

The shift to presymptomatic trials and prevention therapies investigating the lifestyle risk factors for AD, increases the need of employing imaging modalities and biomarkers as markers of efficacy because conventional clinical outcomes have limited ability to detect treatment affects in these early stages. In addition, several evidences are demonstrating how sMRI markers, already validated at the multicentre study phase, can become part of clinical trials assessing downstream effect of neurodegeneration such as brain atrophy (Teipel, et al., 2015). Whereas, functional MRI and DTI markers are still in the single centre study phase, representing an initial proxy for an assessment of their potential clinical usefulness as downstream markers.

Imaging measures, which provide an *in vivo* information on the underlying disease process, could be of benefit in providing evidence of a specific treatment. In line with this perspective will be based the implementation of imaging endpoints in the non-pharmacological trial that I was recently granted by the Italian Ministry of Health. The study aimed at investigating the effect of a multiple domain non-pharmacological intervention on structural and functional imaging markers in elderly individuals with subjective memory complaints, a category at risk for objective and progressive cognitive impairment (Lista S., 2015). In this study, base (sMRI) and advanced (fMRI and DTI) imaging markers will be used as surrogate markers of efficacy after one year of multi domain non-pharmacological treatment.

Although, a series of clinical trials for treating AD dementia have failed during the last two decades, these impediments have not discouraged the confidence of investigators in pursuing the strategic goal of developing disease-modifying treatments, which would inhibit and potentially halt the progression of neurodegeneration in AD patients with the aim of preventing the onset of symptoms (Cavedo, et al., 2014). The optimism of the scientific community, regarding the technical feasibility of discovering strategies to slow or halt neurodegenerative process is conditional, predicated by the availability of adequate resources and our capabilities to surmount the major barriers that are hindering progress of research on prevention. In such scenario the role of neuroimaging and biological markers for AD will be crucial.

Ultimately, most of the challenges described along the thesis can be exclusively accomplished in the context of large consortia involving collaborations between academia

and industries as well as research organizations and technology-oriented academic centres of excellence.

Annex 1: Supplementary Material Study 1

Supplementary Table 1. Stability of manual hippocampal segmentation among different human tracers. Hippocampal segmentation of Tracer1 has been considered the reference standard. Numbers denote intra-class correlation coefficient (95% confidence interval).

Tracer	Field strength	Test-Retest Reliability		Inter-rater Reliability versus Reference	
		Right	Left	Right	Left
2		0.96(0.87-0.99)	0.97(0.89-0.99)	0.88(0.61-0.97)	0.86(0.58-0.96)
3	**3T**	0.99(0.97-0.99)	0.98(0.95-0.99)	0.99(0.97-0.99)	0.99(0.99-1.00)
8		0.88(0.56-0-97)	0.91(0.70-0.97)	0.90(0.0-0.98)	0.94(0.79-0.98)
1		0.99 (0.98–0.99)	0.99 (0.98–0.99)		
4		0.85 (0.53-0.96)	0.95(0.82-0.99)	0.89(0.13-0.97)	0.85(0.21-0.96)
5	**1.5T**	0.99(0.96-0.99)	0.98(0.94-0.99)	0.95(0.81-0.98)	0.97(0.86-0.99)
6		0.98(0.81-0.99)	0.94(0.59-0.99)	0.82(0.18-0.98)	0.84(-0.59-98)
7		0.94(0.80-0.98)	0.95(0.85-0.98)	0.88(0.59-0.97)	0.91(0.70-0.97)
9		0.96(0.87-0.99)	0.95(0.84-0.98)	0.88(0.18-0.97)	0.94(0.73-0.98)

Supplementary Table 2. Demographic, clinical, and neuropsychological features of the I-ADNI sample.

Diagnosis		Controls	SMI	MCI	AD	FTD
	n 1.5T/3T	18/4	6/6	69/23	152/101	13/3
	n total	22	12	92	253	16
General Features	Age, y	70±7	68±12	70±7	71±27	69±9
	Education, y	9±5	10±5	8±4	8±5	12±6
	Women	16 (73%)	8 (66%)	50 (54%)	160 (63%)	4 (25%)
	Family history of dementia	7/20 (35%)	4/10 (40%)	26/77 (34%)	74/214 (35%)	4/16 (25%)
Clinical Features	MMSE	29±1	29±1	27±2	20±5	20±6
	Depressive ss	17/22 (77%)	2/5(40%)	22/66 (33%)	92/193 (48%)	5/16 (31%)
	Risk factors	11/20 (55%)	6/10 (60%)	43/76 (56%)	148/214 (69%)	13/16 (81%)
	IADL lost	0	0	1/77 (1%)	193/218 (88%)	12 (75%)
Memory	Babcock test	11±3	12±4	6±4	3±3	5±5
	Rey fig recall	16±4	18±6	11±7	7±8	9±6
Verbal fluency	Letter	30±6	30±9	28±9	21±10	14±12
	Category	25±11	38±13	33±11	24±10	19±14
Psychomotor speed	Trail-making A	43±16	36±15	76±108	144±211	86±14
Visuo-spatial function	Rey figure	33±2	35±2	28±9	21±65	24±13

Values denote means ± standard deviation or number (%).*p values <0.05 between adjacent groups on ANOVA, Chi-square, or Fisher tests. n 1.5T/3T, number of subjects scanned at 1.5 or 3.0 Tesla; SMI, subjective memory impairment; MCI, mild cognitive impairment; AD, Alzheimer's disease; FTD, frontotemporal dementia; MMSE, Mini-Mental State Examination, Depressive ss, depressive symptoms rated with pertinent subscale of the Brief Symptoms Inventory; Risk factors, hypertension, diabetes, or heart disease; IADL, 1 or more functions lost on the Instrumental Activity of Daily Living questionnaire.

Supplementary Table 3. Clinical core features of the major groups.

		SMI (n=12)	MCI (n=92)	AD (n=253)
Subjective memory complaints		88%	71%	67%
Cognitive deficits (memory, language, executive functions)	0	100%	8%	0%
	1	0	44%	17%
	2 or more	0	58%	83%
IADL lost ≤ 1		100%	98%	23%
MMSE ≥ 25		100%	86%	7%

IADL, 1 or more functions lost at Instrumental Activity of Daily Living questionnaire; MMSE, Mini-Mental State Examination; SMI, subjective memory complaints; MCI, mild cognitive impairment; AD, Alzheimer's disease.

Supplementary Table 4. I-ADNI MPRAGE parameters and comparison with NA-ADNI protocols (A); SNR and PSNR comparison in I-ADNI and NA-ADNI MPRAGE scans (B).

(A)

	Number of volumes	Field Strength (T)	Voxel size (mm)	Slice thickness (mm)	TE (ms)	TI (ms)	TR (ms)
OU1	2	1.5	0.94 x 0.94	1.2	3.96	1000	9.12
GE Excite 1.5T ADNI1 Protocol	2	1.5	1.30 x 1.30	1.2	3.54	1000	Shortest
OU2	2	3	1 x 1	1.2	3.28	900	2300
SIEMENS Allegra 3.0T ADNI1 Protocol	2	3	1 x 1	1.2	2.91	900	2300
OU3	2	3	1 x 1	1.2	3.13	0	6.76
Philips Achieva 3.0T ADNI1 Protocol	2	3	1 x 1	1.2	Shortest	0	Shortest
OU4	2	1.5	1.45 x 1.45	1.2	3.44	1000	2400
SIEMENS Avanto 1.5T ADNI1 Protocol	2	1.5	1.30 x 1.30	1.2	3.5	1000	2400
OU5	2	1.5	1.25 x 1.25	1.2	4.38	1100	3000
SIEMENS Sonata 1.5T ADNI1 Protocol	2	1.5	1.30 x 1.30	1.2	3.54	1000	3000
OU6	2	1.5	1.25 x 1.25	1.2	3.61	1000	2400
SIEMENS Avanto 1.5T ADNI1 Protocol	2	1.5	1.30 x 1.30	1.2	3.5	1000	2400
OU7	2	1.5	0.94 x 0.94	1.2	3.99	0	8.53
Philips Intera 1.5T ADNI1 Protocol	2	1.5	1 x 1	1.2	4	0	Shortest
OU8	2	3	1.02 x 1.02	1.2	2.848	650	6.988
GE Signa 3.0T ADNI1 Protocol	2	3	1 x 1	1.2	Shortest	650	Shortest
OU9	2	1.5	1.25 X 1.25	1.2	3.54	1000	2400
SIEMENS Sonata 1.5T ADNI1 Protocol	2	1.5	1.30 x 1.30	1.2	3.5	1000	2400

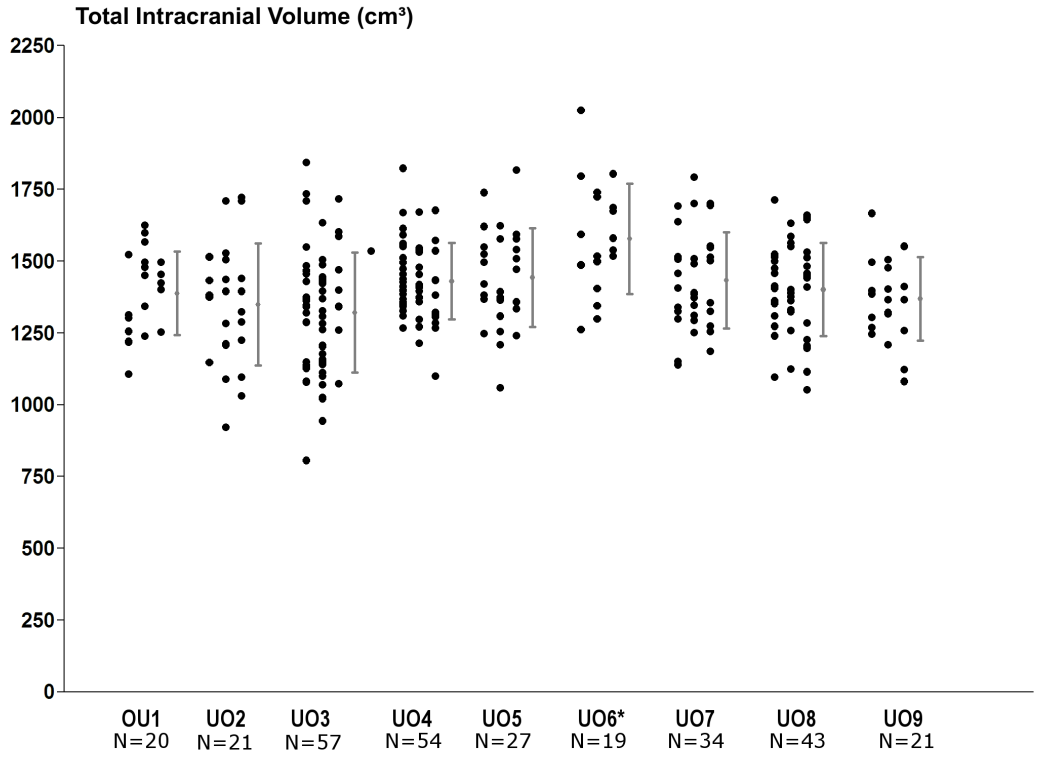
(B)

	T13d MPRAGE	
	SNR average	PSNR average
OU1	17.11	10.95
OU2	15.31	30.76
OU3	17.87	30.89
OU4	16.02	28.84
OU5	21.49	33.83
OU6	19.03	31.69
OU7	21.49	31.82
OU8	16.36	28.45
OU9	18.08	30.05
1.5T ADNI1	8.26	21.43
3.0T ADNI1	9.24	22.37

TE, Echo Time; TI, Inversion Time; TR, Repetition Time; SNR, signal to noise ratio; PSNR, peak signal to noise ratio and image noise; OU1, IRCCS Centro S. Giovanni di Dio, Brescia; OU2, IRCCS Fondazione S. Lucia, Rome; OU3, Fond. SDN Naples, Naples; OU4, University Campus Bio_Medico, Rome; OU5, University of Foggia/"La Sapienza" University, Rome; OU6, Fond. IRCCS Istituto Neurologico Besta, Milan; OU7, Fond. IRCCS Mondino, Pavia; OU8, University of Naples, Naples; OU9, Centro Neurolesi "Bonino-Pulejo", Messina.

Supplementary Figure 1. Mean and Standard Deviation (red line) of total intracranial volume of each Operative Units (OU). The sample of each OU is composed of different cases among CTRL, SMI, MCI, and AD patients.).*Means the significance at Bonferroni correction.

OU1, IRCCS Centro S. Giovanni di Dio, Brescia; OU2, IRCCS Fondazione S. Lucia, Rome; OU3, Fond. SDN Naples, Naples; OU4, University Campus Bio_Medico, Rome; OU5, University of Foggia/"La Sapienza" University, Rome; OU6, Fond. IRCCS Istituto Neurologico Besta, Milan; OU7, Fond. IRCCS Mondino, Pavia; OU8, University of Naples, Naples; OU9, Centro Neurolesi "Bonino-Pulejo", Messina



Annex 2: Supplementary Material Study 5

The following tables contain the data from the CERAD in the subjects in the verum arm. All subjects were native German speakers except for subject 2; for this person the verbal learning, recall and recognition were not performed. The data from subject 4, at 6 month time period, is missing

Subj	MMSE	Baseline	3 month	6 month	12 month
1		26	28	25	26
2		19	22	27	27
3		26	26	27	28
4		30	28		27
5		25	23	28	24

Subj	Word Learning	Baseline	3 month	6 month	12 month
1		14	18	20	14
2					
3		20	21	22	17
4		19	17		21
5		12	16	14	16

Subj	Word Recall	Baseline	3 month	6 month	12 month
1		2	2	5	2
2					
3		7	5	7	4
4		4	5		5
5		2	2	4	0

Subj	Word Recognition	Baseline	3 month	6 month	12 month
1		16	10	6	10
2					
3		10	10	10	2
4		10	9		8
5		7	8	2	8

Subj	Verbal Fluency	Baseline	3 month	6 month	12 month
1		17	15	19	17
2		11	8	13	11
3		22	20	19	11
4		27	22		21
5		23	17	17	15

Subj	Naming	Baseline	3 month	6 month	12 month
1		15	15	15	15
2		13	13	14	9
3		10	12	11	11
4		15	13		14
5		14	14	15	14

Subj	Drawing	Baseline	3 month	6 month	12 month
1		11	11	11	11
2		8	11	11	11
3		9	8	9	11
4		11	11		10
5		9	9	11	10

List of Publications Related to the Thesis

Original Articles:

1. **The Italian Alzheimer's Disease Neuroimaging Initiative (I-ADNI): validation of structural MR imaging.**

   Cavedo E, Redolfi A, Angeloni F, Babiloni C, Lizio R, Chiapparini L, Bruzzone MG, Aquino D, Sabatini U, Alesiani M, Cherubini A, Salvatore E, Soricelli A, Vernieri F, Scrascia F, Sinforiani E, Chiarati P, Bastianello S, Montella P, Corbo D, Tedeschi G, Marino S, Baglieri A, De Salvo S, Carducci F, Quattrocchi CC, Cobelli M, Frisoni GB. **J Alzheimers Dis**. 2014;40(4):941-52.

2. **Donepezil decreases annual rate of hippocampal atrophy in suspected prodromal Alzheimer's disease.**

   Dubois B, Chupin M, Hampel H, Lista S, **Cavedo E**, Croisile B, Tisserand GL, Touchon J, Bonafe A, Ousset PJ, Ait Ameur A, Rouaud O, Ricolfi F, Vighetto A, Pasquier F, Delmaire C, Ceccaldi M, Girard N, Dufouil C, Lehericy S, Tonelli I, Duveau F, Colliot O, Garnero L, Sarazin M, Dormont D; "Hippocampus Study Group". ***Alzheimers Dement. 2015*** Jan 14.

3. **Reduced regional cortical thickness rate of change in donepezil treated subjects with suspected prodromal Alzheimer's disease - A longitudinal multi-centric double-blind, randomized, placebo-controlled trial.**

   Enrica Cavedo; Bruno Dubois; Olivier Colliot; Simone Lista; Bernard Croisile ; Guy Louis Tisserand ; Jacques Touchon ; Alain Bonafe ; Pierre Jean Ousset ; Amir Ait Ameur; Olivier Rouaud; Fréderic Ricolfi; Alain Vighetto; Florence Pasquier; Christine Delmaire; Mathieu Ceccaldi; Nadine Girard; Carole Dufouil; Stéphane Lehericy; Isabelle Tonelli; Françoise Duveau; Marie Chupin; Line Garnero; Marie SARAZIN; Didier Dormont; Harald Hampel "Hippocampus Study Group". ***The Journal of Clinical Psychiatry*** *Under review*

4. **Hippocampus and basal forebrain as predictors of cognitive decline and treatment response in suspected prodromal Alzheimer's disease.**

   Teipel SJ, **Cavedo E**, Grothe MJ, Lista S, Galluzzi S, Colliot O, Chupin M, Bakardjian H, Dormont D, Dubois B, and Hampel H for the Hippocampus Study Group. ***Neuropharmacology,*** *Under Review*

5. **Effects of Rivastigmine on Visual Attention in Subjects with Amnestic Mild Cognitive Impairment: A Serial Functional MRI activation Pilot-Study.**

   Bokde ALW, **Cavedo E**, Lopez-Bayo P, Lista S, Meindl T, Born C, Galluzzi S, Faltraco F, Dubois B, Teipel SJ, Reiser M, Möller HJ, and Hampel. ***Psychiatry Research: Neuroimaging,*** *1st Revision*

Reviews:

6. **The Road Ahead To Cure Alzheimer'S Disease: Development Of Biological Markers And Neuroimaging Methods For Prevention Trials Across All Stages And Target Populations.**

   Cavedo E, Lista S, Khachaturian Z, Aisen P, Amouyel P, Herholz K, Jack Jr CR, Sperling R, Cummings J, Blennow K, O'Bryant S, Frisoni GB, Khachaturian A, Kivipelto M, Klunk W, Broich K, Andrieu S, Thiebaut de Schotten M, Mangin JF, Lammertsma AA, Johnson K, Teipel S, Drzezga A, Bokde A, Colliot O, Bakardjian H, Zetterberg H, Dubois B, Vellas B, Schneider LS, Hampel H. ***J Prev Alz Dis*** **2014**;1(3):181-202

7. **Multimodal imaging in Alzheimer's disease: validity and utility for early detection.**

   Teipel S, Drzezga A, Grothe MJ, Barthel H, Chetelat G, Schuff N, Skudlarski P, **Cavedo E**, Frisoni GB, Hoffmann W, Thyrian JR, Fox C, Minoshima S, Sabri O, Fellgiebel A. ***Lancet Neurology*****.** 2015 Aug 26 Lancet Neurol. 2015 Aug 26. pii: S1474-4422(15)00093-9. doi: 10.1016/S1474-4422(15)00093-9

8. **Evolving Evidence for the Value of Neuroimaging Methods and Biological Markers in Subjects Categorized with Subjective Cognitive Decline.**

   Lista S, Molinuevo JL, **Cavedo E**, Rami L, Amouyel P, Teipel SJ, Garaci F, Toschi N, Habert MO, Blennow K,Zetterberg H, O'Bryant SE, Johnson L, Galluzzi S, Bokde ALW, Broich K, Herholz K, Bakardjian H, Dubois B, Jessen F, Carrillo MC, Aisen PS, Hampel H. ***J Alzheimers Dis 2015****. In press doi: 10.3233/JAD-150202*

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