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A diffusion tensor MRI study of patients with MCI and AD with a two year clinical follow up

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Abstract

Objective. We assessed whether brain changes detected by diffusion tensor (DT) MRI can improve the understanding of structural damage in Alzheimer's disease (AD), and are associated with different risks of conversion to AD in amnesic mild cognitive impairment (aMCI).

Methods. Twenty-one aMCI patients, 21 AD patients, and 20 healthy subjects underwent conventional and DT MRI at baseline. All subjects were clinically followed up over two years; at the end of follow up, aMCI were grouped into converters to AD (aMCI-C) and nonconverters (aMCI-NC). Mean diffusivity (MD) and fractional anisotropy (FA) were obtained from total grey (GM) and white matter (WM), and from several GM and WM regions of interest (ROIs). **On T1-weighted images, normalised volumes of the whole brain (NBV), GM (NGMV) and WM were measured.**

Results. A significant "trend" of worsening with a trajectory "normal/aMCI/AD" was found **for NBV and NGMV**, total GM and WM MD, total WM FA, as well as for diffusivity abnormalities in several GM and WM ROIs, mainly located in posterior brain regions. aMCI-C had GM and WM changes similar to those seen in AD, whereas aMCI-NC showed a DT MRI pattern similar to that of healthy subjects. DT MRI metrics that better distinguished aMCI-C from aMCI-NC were MD of total GM and WM, hippocampi, anterior insulae, frontal and parietal WM, and occipital GM and WM, and FA of temporal WM. **Volumetric variables were not able to distinguish the two aMCI subgroups (aMCI-C and aMCI-NC).**

Conclusions. Subtle brain diffusivity changes occur from the prodromal stages of AD, mainly in posterior brain regions, and spread over the course of the disease to involve the frontal lobe. In aMCI, the severity of **microstructural** damage within and beyond the medial temporal lobe is associated with an increased short-term risk to develop AD.

INTRODUCTION

Mild cognitive impairment (MCI) is considered to be a transitional stage between normal aging and dementia. Amnesic MCI (aMCI) subjects convert to Alzheimer's disease (AD) at a rate three-six times higher than normal elderly population. However, not all patients with aMCI go on to develop AD. In some patients, the cognitive deficits may stabilise; in others, they may improve or progress to other phenotypes of dementia.[1] Compared to nonconverters, aMCI who evolve to AD typically show a more severe atrophy of the medial temporal lobe (MTL), the inferior and middle temporal gyrus, the posterior cingulate and the precuneus.[2]

Diffusion tensor (DT) magnetic resonance imaging (MRI) has made possible to detect microstructural brain changes in patients with AD, which go undetected when using conventional MRI.[3-5] Increased diffusivity has been demonstrated in the grey matter (GM) of patients with AD compared to healthy elderly subjects mainly in the hippocampi and in the temporal and parietal cortices.[4, 5] Most likely, increased GM diffusivity reflects loss of neurons and dendrites, which causes an enlargement of the extracellular spaces and, hence, reduces the "barriers" limiting water molecular motion.[5] DT MRI studies also showed AD-related subtle changes in the white matter (WM) of the temporal, parietal and frontal lobes, the corpus callosum, and the cingulum.[3, 5-7] These findings agree with neuropathological data indicating a loss of myelin sheaths, axons and oligodendrocytes in AD brains.[8]

DT MRI studies have also attempted to elucidate the earliest point at which such changes can be detected by focusing on patients with aMCI.[5, 9, 10] aMCI patients were found to harbour diffusivity alterations in brain regions that are typically involved in early AD, especially in the hippocampi.[5] Previous DT MRI studies, which assessed the predictive value of structural brain changes for conversion to AD, limited their analysis to the MTL. Hippocampal diffusivity measurements have been found to be more sensitive than hippocampal atrophy in predicting conversion to AD in these patients.[11, 12] To date, no prospective studies have considered the prognostic value of brain DT MRI measures beyond the MTL.

The aim of this longitudinal study was to assess whether GM and WM microstructural changes detected by DT MRI can improve the understanding of structural damage in clinically probable AD and have the ability to predict the conversion to AD in aMCI patients. Patients with aMCI were scanned at baseline, then entered a longitudinal clinical follow up of two year duration, which allowed them to be classified as aMCI converters (aMCI-C) and nonconverters (aMCI-NC). First, we tested whether DT MRI variables from total GM and WM as well as from several specific regions-of-interest (ROIs) in both GM and WM were characterised by a significant worsening over the trajectory “normal/aMCI/AD”. Then, we evaluated the ability of DT MRI metrics, **volumetric MRI and neuropsychological tests** at baseline in distinguishing aMCI-C from aMCI-NC.

MATERIALS AND METHODS

Patients. **Patients were enrolled consecutively from the population attending the Outpatient Dementia Clinic of our Institution.** Clinical diagnosis was based on a multi-disciplinary evaluation including neurological examination and a neuropsychological test battery. Inclusion criteria for all patients were: a) no evidence of depression or dysthymia; b) normal laboratory tests, including vitamin B12, folate and thyroid hormone levels; c) no metabolic diseases or substance abuse that could interfere with cognitive functioning; d) no other major systemic, psychiatric or neurological illnesses. **History of type 2 diabetes, hypertension and heart disease was explored. Heart disease included a history of atrial fibrillation, other arrhythmias, congestive heart failure, myocardial infarction, and angina pectoris.** In addition, as suggested in the past [3] and in order to minimise the inclusion of subjects with significant cerebrovascular disorder, individuals with evidence of concomitant cerebral vascular disease on conventional MRI scans (i.e., one hyperintense area with a diameter equal or greater than 5 mm, or more than four hyperintensities smaller than 5 mm in diameter on T2 weighted scans) were excluded.

Twenty-one patients with aMCI, 21 patients with clinically probable AD, and 20 healthy subjects were recruited (Table 1). Patients with aMCI reported a subjective memory complaint,

showed abnormal memory performance for their age, and did not display functional impairment in activities of daily living according to caregiver report.[1] Patients with probable AD met the criteria established by the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRA), and were in the mild range of the disease based on Clinical Dementia Rating (CDR) scale scores,[13] which ranged from 1 to 2 (i.e., with mild or moderate cognitive deficits). Healthy controls had no memory complaints and no evidence of cognitive deficits on formal neuropsychological testing, no history of systemic, neurological, and psychiatric disorders, and a MRI which was normal or where evidence of cerebrovascular disease met the exclusion criteria described above. **Two subjects (one aMCI and one AD) had a history of type 2 diabetes, 17 subjects (four controls, seven aMCI and six AD) suffered from hypertension, and three aMCI patients had a history of atrial fibrillation.**

Table 1 near here

At baseline, within 48 hours from acquisition of the MR images, an extensive neuropsychological battery was administered by a trained neuropsychologist. General intellectual functioning was assessed using the Mini Mental State Examination (MMSE),[14] and the total functional assessment was measured using the CDR.[13] Memory assessment included an episodic long-term (Delayed Recall Test) [15] and two short-term (Forward Digit Span Test and Corsi Test) [16, 17] memory tests. The Raven's Progressive Matrices [18] were used to evaluate problem solving and reasoning, and the Attention Matrices [19] to assess attention. Visuospatial abilities were investigated using the Copying of Geometrics Figures.[20] The Token Test [19] was used to assess language comprehension, while semantic verbal fluency was assessed using the Semantic Verbal Test.[15] The Activities of Daily Living [21] and the Instrumental Activities of Daily Living [22] scales were also administered. All neuropsychological testing scores were corrected for age and level of education.

aMCI patients were re-evaluated every six months and the assessment of whether subjects had progressed to clinically probable AD was made through a consensus committee meeting, as previously described.[23] The diagnosis of probable AD was made according to the NINCDS-ADRDA criteria.[24] The study was approved by the Local Ethics Committee. All subjects provided written informed consent before entering the study.

MRI data acquisition. Brain MR scanning was performed on a magnet operating at 1.5 T (Vision, Siemens, Erlangen, Germany). On a single occasion, the following pulse sequences were obtained from all subjects without moving them from the scanner: 1) dual echo (DE) turbo spin echo (SE) (repetition time [TR]=3300 ms; echo time [TE]=16/98 ms; echo train length=5; 24 contiguous axial slices, 5 mm slice thickness, no gap; 256x256 matrix size; 250x250 mm² field of view [FOV]); 2) T1-weighted conventional SE (TR=768 ms; TE=14 ms; 24 contiguous axial slices, 5 mm slice thickness, no gap; 256x256 matrix size; 250x250 mm² FOV); and 3) pulsed gradient spin echo (PGSE) echo planar pulse (interecho spacing=0.8 ms; TE=123 ms; 18 contiguous axial slices, 6 mm slice thickness, no gap, covering the entire brain; 128x128 matrix size; 250x250 mm² FOV), with diffusion gradients applied in eight non-collinear directions, chosen to cover three dimensional space uniformly.[25] The duration and maximum amplitude of the diffusion gradients were respectively 25 ms and 21 mTm⁻¹, giving a maximum b factor in each direction of 1044 s mm². Fat saturation was performed using a four radio-frequency binomial pulse train to avoid chemical shift artifacts. For the DE and T1-weighted images, the slices were positioned to run parallel to a line that joins the most infero-anterior and infero-posterior parts of the corpus callosum. The PGSE images were acquired with the same orientation as the DE scans, and with the second-last caudal slice positioned to match exactly the central slices of the DE set.

MRI analysis and postprocessing. MR images were transferred to a workstation (Sun Sparcstation; Sun Microsystem, Mountain View, CA, USA) for analysis, which was performed by consensus by two experienced observers unaware of subjects' identity. An experienced radiologist reviewed the severity of WM hyperintensities according to the Wahlund rating scale.[26] On T1-

weighted images, normalised volumes of the whole brain (NBV), GM (NGMV) and WM (NWMV) were measured using the cross-sectional version of the Structural Image Evaluation of Normalised Atrophy (SIENAx) software.[27]

The computation of the DT was performed using an in-house developed software. First, the distortion in the diffusion-weighted (DW) images caused by eddy currents was corrected.[28] Then, the DT was estimated by linear regression,[29] and MD and FA maps calculated.[30] The b=0 images of the PGSE scans (T2, but non-DW-weighted) were then coregistered with the corresponding T2-weighted images,[28] and the same transformation parameters applied to the MD and FA maps. GM and WM were segmented as previously described.[31] This generated mutually exclusive masks for each tissue, which were superimposed onto the MD and FA maps. MD histograms of the total GM and WM, and FA histograms of the WM were produced.[32] FA histograms were derived only for the WM, since no preferential water molecular motion is expected to occur in the GM.

DT MRI metrics were also derived from regions of interest (ROIs) within GM and WM. First, rectangular ROIs of variable size (range=8.6-15.3 mm²), depending on the anatomical region studied, were carefully placed on the T2-weighted images to avoid partial volume averaging from the cerebrospinal fluid (CSF). ROIs were drawn bilaterally in the following regions: amygdala, hippocampus, head of the caudate nucleus, thalamus, posterior limb of the internal capsule, genu and splenium of the corpus callosum, anterior and posterior cingulate gyri, and anterior insular cortex (Figure 1). ROIs were also placed in the GM and WM of the four cerebral lobes (Figure 1). Amygdala and hippocampus ROIs were positioned on the slice including the mesencephalus where these two structures are easily identifiable. Thalamus was sampled in its medial portion, where fibers associated to temporal lobes are preferentially located. Anterior and posterior cingulate ROIs were positioned on the slice showing the genu and the splenium of the corpus callosum, anteriorly and posteriorly to these structures, respectively. Anterior insular cortex ROIs were placed on the slice including the trigone of the lateral ventricle, the basal ganglia, and the Sylvian fissure. In the

frontal lobe, GM ROIs were drawn on the cortex of the middle frontal gyrus, while the frontal WM ROIs were placed on the boundary between the superior and the middle frontal gyri. Parietal GM and WM ROIs were positioned, respectively, on the postcentral gyrus, and posterior to the central sulcus in the centrum semiovale above the ventricles. Temporal GM ROIs were placed on the middle temporal gyrus, while WM ROIs were positioned anterior-laterally to the temporal horn of the lateral ventricle. Finally, occipital GM ROIs were placed on the calcarine cortex medially to the occipital horn of the lateral ventricle, and occipital WM ROIs laterally to the optic radiations. The ROIs were then superimposed onto the corresponding coregistered MD (Figure 1) and FA maps of each subject. Average MD was calculated for GM and WM ROIs; for the reason given above average FA was measured for WM ROIs, only.

Statistical analysis. To assess intrarater reproducibility of ROI analysis, DT MRI measurements were obtained twice in a blinded fashion in nine randomly selected subjects (three from each of the three groups). For each pair of measurements (separated by a period of one week), the intra-class correlation coefficients were calculated. Left-right differences of DT-MRI values derived from ROI analysis were assessed using a t-test for paired data; since no left-right difference was found (data not shown), the mean of the values obtained from the two hemispheres entered the statistical analysis. An analysis of variance (ANOVA) model with each of the neuropsychological, volumetric and DT MRI metrics as the dependent variable and a three-level variable (“trend-variable”; i.e., healthy controls, aMCI patients, and AD patients) as the independent covariate (“test for trend”) was used to investigate the trajectory “normal/aMCI/AD”. Those variables showing a trend to worsen along such a trajectory were then tested at a second-level analysis. For each neuropsychological and MRI metric selected in the first step, a multivariate model with a stepwise selection procedure was performed including two different variables: 1) the three-level variable used for the trend analysis (“trend-variable”), and 2) a two-level stratification, where aMCI-NC were grouped with healthy controls and aMCI-C with AD patients (“split-group variable”). The stepwise selection procedure investigated whether aMCI could be considered, on the basis of the

neuropsychological and MRI variables, as an intermediate condition between normality and AD (including in the final model the “trend-variable”, only), or as consisting of two different subgroups, being one similar to controls and the other to AD patients (including in the final model the “split-group variable”, only). Statistical analyses were repeated by entering subject’s age, and brain volumes in case of DT MRI measures, as covariates.

RESULTS

Clinical and neuropsychological data. No healthy control converted to aMCI or AD during the follow up period. Two aMCI patients refused to come back to the follow up visits, and were excluded from further analyses. Of the remaining 19 aMCI patients, 11 (58%) progressed to AD over two years (aMCI-C; 6 women and 5 men, mean age 71 years [range=57-79 years], mean disease duration 20 months [range=6-36]), while eight patients did not (aMCI-NC; 2 women and 6 men, mean age 69 [range=52-79], mean disease duration 26 months [range=12-56]). No difference in demographic and clinical findings were found between aMCI-NC and aMCI-C patients. In Table 2, neuropsychological tests scores for each group are reported. A significant trend over the trajectory “normal/aMCI/AD” was found for all the neuropsychological tests, except the Forward Digit Span Test score (Table 2). The multivariate model with a stepwise selection procedure showed that aMCI group better fitted a condition where it is considered to be made up by two different subgroups (see Statistical analysis) in terms of Delay Recall Test and the Raven’s Progressive Matrices scores (Table 2).

Table 2 near here.

Neuroimaging results. Wahlund rating scale score was 0 in all subjects. Tables 3 shows volumetric measurements of total brain, GM and WM and DT MRI-derived metrics of total GM and WM from the subject groups.

Table 3 near here.

Mean intra-class correlation coefficient for DT MRI-based ROI analysis was 0.87 (SD=0.04). Table 4 shows the DT MRI-derived metrics of selected ROIs from the subject groups.

Table 4 near here.

A significant “trend” over the trajectory “normal/aMCI/AD” was found for NBV and NGMV, as well as for total GM and WM MD (Figure 2), and total WM FA (Table 3). Moreover, a significant “trend” was shown for MD and FA values of several ROIs. In particular, a significant “trend” toward increased MD values was found in the hippocampus, thalamus, posterior cingulate gyrus, anterior insula, genu of the corpus callosum, frontal WM, and parietal and occipital GM and WM (Table 4). A “trend” toward decreased FA values was found in the frontal, temporal and occipital WM (Table 4). After adjustment for subject’s age and brain volumes, a significant “trend” was found for total WM MD, MD of the parietal GM, MD of the frontal and occipital WM, and FA values of the temporal WM.

The multivariate model with a stepwise selection procedure showed that volumetric variables were not able to distinguish the two aMCI subgroups (aMCI-C and aMCI-NC). On the contrary, DT MRI variables that better distinguish the two aMCI subgroups included MD of total GM and WM, hippocampus, anterior insula, frontal and parietal WM, and occipital GM and WM, and FA values of temporal WM (Tables 3 and 4, Figure 2).

DISCUSSION

The main aims of this two-year prospective study were to improve the understanding of structural damage in clinically probable AD, and to investigate whether brain microstructural

changes detected by DT MRI could offer insight into the development of AD in patients with aMCI. First, we found a trend toward increased MD and decreased FA values along the trajectory “normal/aMCI/AD”. Second, in aMCI patients, the severity of brain diffusivity and anisotropy changes within and beyond the MTL were associated with an increased short-term risk of conversion to AD. **Conversely, volumetric measurements were not able to distinguish between aMCI-C and aMCI-NC patients.** This suggests that DT MRI has the potential to provide useful markers to predict subsequent progression to AD in patients with aMCI.

The pattern of GM damage along the trajectory “normal/aMCI/AD” is typical of that observed in previous pathological and neuroimaging studies.[2, 5, 33] **As expected [2-5, 33], a significant “trend” of worsening was found not only for total GM atrophy but also for global GM DT MRI metrics. Regional DT MRI analysis also showed that,** when subjects have a clinical diagnosis of AD, the pattern of **GM** damage is diffuse, since it does not involve only the MTL, but also the parieto-occipital association cortices, as well as the frontal lobes. Increased GM MD in aMCI and AD patients may result from loss of neuron cell bodies, synapses and dendrites, which in turn may cause an increase of extracellular spaces,[5] and hence abnormal diffusivity values. The MTL is known to be one of the first structures to be involved by AD pathology.[34] Postmortem studies showed that most patients with MCI already have the pathological hallmarks of AD – neocortical senile plaques, neurofibrillary tangles, atrophy, and neuronal loss – in the hippocampus [35] and entorhinal cortex.[36] Our finding of an increased GM damage beyond the MTL also agrees with the previous pathological and imaging literature.[5, 37, 38] Indeed, this fits well with the Braak and Braak neurofibrillary pathological staging scheme in AD, which indicates that the disease starts mainly in the hippocampus and entorhinal cortex, and subsequently spreads throughout temporal, posterior cingulate, and frontal cortices.[34]

Another intriguing finding of our study is that WM changes, which occur before the onset of clinically overt dementia, also progress along the trajectory “normal/aMCI/AD”, and that they may play a role in the cognitive decline leading to AD. There are several possible explanations for

changes of WM integrity in aMCI and AD. First, the early deposition of amyloid around neuronal cell bodies and accumulation of tau in neuronal cells in GM may lead to anterograde Wallerian degeneration, especially in regions close to cortical areas with the greatest pathological burden. Second, the occurrence of vascular disease with AD, either due to amyloid angiopathy and/or arteriosclerosis, can cause axonal damage and gliosis. Third, as it has recently been suggested,[39] myelin breakdown is likely to be an important component of the disease process in AD, which may also constitute a critical initiating step. Our finding of a posterior WM involvement agrees with many previous DT MRI studies in AD patients.[9, 10] Regions located in the posterior WM, such as the parietal and temporal lobes, have being consistently found affected also in patients with MCI.[7] In agreement with the notion that the disease progresses to a multidomain clinical and cognitive syndrome from aMCI to AD, we found that WM damage increased also in the anterior brain regions, such as the genu of the corpus callosum and the frontal lobe. More controversial is the observation of diffusivity abnormalities in the occipital lobe. Previous DT MRI studies in AD and MCI patients have resulted in mixed observations.[3, 9, 40-42] However, recent postmortem studies demonstrated occipital pathological changes early in the course of the disease,[43] and PET studies of AD patients showed amyloid deposition and reduced cerebral glucose metabolism in posterior associative regions, including the occipital cortex.[37] The discrepancy of findings across different DT MRI studies may be due to methodological and subject selection differences.

The major result of this study was the one which showed that DT MRI findings are useful in predicting short-term conversion to AD in patients with aMCI **while volumetric measurements did not**. More than half (58%) of our aMCI patients deteriorated during the follow up and fulfilled the criteria for diagnosing clinically probable AD. This is in agreement with previous studies on the conversion rate of aMCI.[1] When compared to aMCI-NC, aMCI-C showed greater structural abnormalities in both GM and WM. Moreover, the stepwise selection procedure showed that aMCI-C had a pattern of **DT MRI** abnormalities similar to that seen in patients with AD, whereas aMCI-NC showed DT MRI values similar to those of healthy subjects. Our finding of increased diffusivity

changes in aMCI patients who progressed to AD strengthen and expand the results from previous neuroimaging studies.[12, 44] Indeed, most of the previous MRI studies limited their analysis to the assessment of pathology within the MTL, whereas our study showed that the presence and severity of tissue damage beyond the MTL is also associated with a short-term risk of developing clinically probable AD in aMCI patients.

Several studies demonstrated that measures of hippocampal volume are associated with time to progression from MCI to AD (see [45] for review). In a recent MRI study of 190 aMCI subjects, the rate of progression of MTL atrophy was associated with a significantly increased risk of developing dementia within three years.[46] In addition, previous DT MRI studies showed that aMCI patients have increased MD values in brain regions that are typically involved in early AD, especially in the hippocampi,[5] and two other studies found that increased hippocampal diffusivity predicts progression to AD in aMCI equally, if not better, than hippocampal atrophy,[11, 12] thus indicating that DT MRI is sensitive to hippocampal microscopic alterations, which may be apparent earlier than the atrophy changes measured with volumetric approaches.

In the present study, we showed, using regional between-group analyses, that MD values are diffusely altered, while the anatomical pattern of WM anisotropy changes is greater in the posterior than in the anterior regions of the brain. More interestingly, a more severe damage in the insular and occipital cortices and the lobar WM was found in aMCI-C when compared to aMCI-NC. As a consequence, one may speculate that an higher risk for conversion to AD in aMCI patients is associated with both a greater damage in disease-specific regions and to an early involvement of regions usually damaged later in the course of AD.

This study is not without limitations. The main of which is the relatively small sample size, which was nevertheless comparable to those of previous reports.[5, 9, 11, 12] **Due to the exploratory nature of our study, we did not correct for multiple comparisons, and therefore, some of the significances might have been overestimated. However, our findings offer *a priori* hypotheses to be tested in future larger studies. The presence of brain atrophy is likely to have increased the**

impact of partial volume effect from the CSF on DT MRI findings, especially because of the use of 6 mm thick slices. This choice was dictated by an attempt to reduce the length of the acquisition time – which was deemed necessary in not-fully compliant patients, such as those with moderate AD- while still achieving a good signal-to-noise ratio and covering the entire brain. However, in our analysis, particular attention was paid to minimise this issue. ROIs were carefully placed on the T2-weighted images to keep away from the CSF and then moved to the coregistered DT MRI maps. The good quality of ROI placement and registration can be appreciated in Figure 1. When we ran the analysis of DT MRI changes with correction for GM and WM volumes, the data demonstrated that GM and WM atrophy did account for differences in some areas. However, significant MD increase and FA decrease remain across several regions including the parietal cortex and temporal lobe which are known to be among the structures to be mostly involved by AD pathology. Since we did not acquire high-resolution T1-weighted images and, as a consequence, we did not investigate the topographical distribution of GM and WM tissue loss, we can not completely rule out that a systematic increase of MD and a systematic decrease of FA might have occurred in small structures. Future studies using high-resolution DT MRI and T1-weighted MRI to measure volumes of specific brain structures in larger patient sample are necessary to validate our findings.

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Table 1. Main demographic and clinical characteristics from patients and healthy controls at baseline.

	Controls n = 20	aMCI n = 19	AD n = 21
Sex, F/M	13/7	8/11	11/10
Mean age (range), years	65.2 (56-75)	68.2 (52-79)	69.9 (56-79)
Median disease duration (range), months	NA	24 (6-56)	29 (12-96)
Median CDR (range)	0.0 (0.0-0.0)	0.5 (0.5-0.5)	1.0 (1.0-2.0)
Median ADL (range)	NA	6 (6-6)	5 (4-6)
Median IADL (range)	NA	7 (4-8)	5 (3-7)

Abbreviations: AD= Alzheimer's disease; aMCI= amnesic mild cognitive impairment; NA= not applicable; CDR=Clinical Dementia Rating scale score; ADL= Activities of Daily Living; IADL= Instrumental Activities of Daily Living.

Table 2. Baseline neuropsychological data from patients with Alzheimer’s disease (AD), patients with amnesic mild cognitive impairment (aMCI), and healthy controls. Within aMCI group, values from patients who progressed to AD during follow up (aMCI-C) and those who did not (aMCI-NC) are also shown.

	Controls n = 20	aMCI n = 19		AD n = 21	p [†]
		aMCI-NC n = 8	aMCI-C n = 11		
Mini Mental State Examination score	27.4 (1.3)	25.5 (1.7)		20.4 (6.3)	<0.001
		25.5 (1.8)	25.9 (1.8)		
Memory					
Delayed Recall Test score	14.7 (3.6)	8.1 (4.3)		7.0 (3.7)	<0.001*
		10.7 (4.7)	6.9 (2.4)		
Forward Digit Span Test score	5.5 (0.8)	5.4 (1.0)		5.2 (0.9)	0.36
		4.9 (0.6)	5.7 (1.0)		
Corsi Test score	5.1 (1.0)	4.3 (0.8)		3.7 (0.7)	0.001
		4.6(1.4)	4.2 (0.6)		
Problem solving and reasoning					
Raven’s Progressive Matrices score	28.4 (5.0)	26.9 (4.0)		23.5 (5.7)	0.004*
		28.8 (2.9)	25.6 (4.2)		
Attention					
Attention Matrices score	49.1 (5.7)	42.5 (6.8)		34.0 (10.5)	<0.001
		42.4 (9.5)	42.6 (4.9)		
Visuospatial abilities					
Copying of Geometrics Figures score	11.8 (1.8)	10.7 (1.4)		9.5 (2.9)	0.03
		10.3 (1.5)	11.6 (1.3)		
Language					
Token Test score	32.2 (1.6)	31.0 (1.9)		26.5 (4.6)	<0.001
		30.4 (2.4)	31.4 (1.6)		
Semantic Verbal Test score	39.7 (5.0)	32.1 (6.8)		20.7 (7.1)	<0.001
		34.0 (5.8)	31.0 (7.4)		

Results are means, with standard deviation in parenthesis. †p values refer to “trend-analysis” (see text for further details). *Variables that better distinguish the two aMCI subgroups (aMCI-C and aMCI-NC) (multivariate model with a stepwise selection procedure) (see text for further details).

Abbreviations: AD=Alzheimer’s disease, aMCI= amnesic mild cognitive impairment,

FA=fractional anisotropy, aMCI-C= aMCI converters, aMCI-NC= aMCI nonconverters, MD= mean diffusivity, GM=grey matter, WM=white matter.

Table 3. Baseline **volumetric** and diffusion tensor MRI-derived metrics of total grey matter and white matter from patients with Alzheimer’s disease (AD), patients with amnesic mild cognitive impairment (aMCI), and healthy controls. Within aMCI group, values from patients who progressed to AD during follow up (aMCI-converters) and those who did not (aMCI-nonconverters) are also shown.

	Controls n = 20	aMCI n = 19		AD n = 21	p [†]
		aMCI-NC n = 8	aMCI-C n = 11		
NBV	1564 (108)	1526 (144)		1487 (48)	0.03
		1526 (174)	1526 (119)		
NGMV	773 (47)	738 (71)		713 (36)	0.002
		731 (85)	745 (58)		
NWMV	791 (64)	788 (76)		774 (26)	0.32
		794 (91)	781 (64)		
GM average MD	1.17 (0.08)	1.25 (0.11)		1.29 (0.07)	<0.001*
		1.21 (0.146)	1.27 (0.08)		
WM average MD	0.94 (0.05)	0.99 (0.09)		1.02 (0.06)	<0.001*
		0.94 (0.07)	1.02 (0.09)		
WM average FA	0.29 (0.02)	0.28 (0.02)		0.27 (0.03)	0.003
		0.28 (0.02)	0.28 (0.02)		

Results are means, with standard deviation in parenthesis. **Normalised volumes are expressed in ml.** MD is expressed in units of $\text{mm}^2 \times \text{s}^{-1} \times 10^3$. †p values refer to “trend-analysis”. *Variables that better distinguish the two aMCI subgroups (aMCI-C and aMCI-NC) (multivariate model with a stepwise selection procedure) (see text for further details). Abbreviations: AD=Alzheimer’s disease, aMCI= amnesic mild cognitive impairment, aMCI-C= aMCI converters, aMCI-NC= aMCI nonconverters, FA=fractional anisotropy, GM=grey matter, NBV=**normalised brain volume**, NGMV= **normalised grey matter volume**, NWMV= **normalised white matter volume**, MD= mean diffusivity, WM=white matter.

Table 4. Baseline diffusion tensor MRI-derived metrics of selected regions of interest (ROIs) within grey matter and white matter from patients with Alzheimer's disease, patients with amnesic mild cognitive impairment (aMCI), and healthy controls. Within aMCI group, values from patients who progressed to AD during follow up (aMCI-converters) and those who did not (aMCI-nonconverters) are also shown.

ROI anatomical location	Controls n = 20	aMCI n = 19		AD n = 21	p†
		aMCI-NC n = 8	aMCI-C n = 11		
MD					
Amygdala	1.10 (0.21)	1.22 (0.22)		1.19 (0.17)	0.15
		1.20 (0.29)	1.24 (0.16)		
Hippocampus	1.15 (0.10)	1.29 (0.18)		1.31 (0.22)	0.005*
		1.23 (0.22)	1.34 (0.15)		
Head of caudate nucleus	1.13 (0.22)	1.27 (0.29)		1.22 (0.19)	0.14
		1.24 (0.35)	1.29 (0.25)		
Thalamus	1.05 (0.18)	1.08 (0.13)		1.16 (0.17)	0.01
		1.06 (0.16)	1.09 (0.11)		
Posterior limb of internal capsule	0.86 (0.07)	0.84 (0.04)		0.85 (0.04)	0.58
		0.82 (0.03)	0.85 (0.05)		
ACC	1.21 (0.19)	1.29 (0.22)		1.29 (0.27)	0.47
		1.30 (0.19)	1.27 (0.25)		
PCC	1.08 (0.18)	1.13 (0.16)		1.17 (0.21)	0.04
		1.13 (0.15)	1.13 (0.16)		
CC genu	1.17 (0.21)	1.33 (0.32)		1.38 (0.27)	0.01
		1.33 (0.40)	1.32 (0.27)		
CC splenium	0.93 (0.12)	0.99 (0.28)		0.99 (0.15)	0.09
		0.90 (0.14)	1.06 (0.35)		
Anterior insula	1.16 (0.12)	1.23 (0.15)		1.31 (0.19)	0.006*
		1.18 (0.13)	1.27 (0.16)		
Frontal GM	1.33 (0.26)	1.28 (0.28)		1.36 (0.21)	0.53
		1.14 (0.28)	1.38 (0.25)		
Frontal WM	0.85 (0.06)	0.89 (0.11)		0.93 (0.09)	0.001*
		0.85 (0.06)	0.92 (0.13)		
Parietal GM	1.12 (0.15)	1.25 (0.22)		1.34 (0.21)	0.001
		1.22 (0.18)	1.28 (0.25)		

Parietal WM	0.85 (0.06)	0.87 (0.09)		0.90 (0.05)	0.002*
		0.84 (0.03)	0.90 (0.11)		
Temporal GM	1.12 (0.14)	1.14 (0.19)		1.15 (0.18)	0.54
		1.10 (0.24)	1.17 (0.16)		
Temporal WM	0.97 (0.08)	1.02 (0.10)		1.02 (0.11)	0.17
		1.01 (0.13)	1.02 (0.07)		
Occipital GM	1.08 (0.18)	1.23 (0.20)		1.23 (0.17)	0.01*
		1.19 (0.15)	1.27 (0.21)		
Occipital WM	0.79 (0.07)	0.88 (0.17)		0.91 (0.13)	<0.001*
		0.82 (0.06)	0.92 (0.21)		
FA					
Posterior limb of internal capsule	0.41 (0.08)	0.43 (0.08)		0.42 (0.07)	0.69
		0.42 (0.10)	0.43 (0.06)		
CC genu	0.47 (0.15)	0.44 (0.14)		0.42 (0.14)	0.13
		0.43 (0.17)	0.44 (0.13)		
CC splenium	0.70 (0.07)	0.69 (0.13)		0.66 (0.09)	0.27
		0.73 (0.11)	0.66 (0.14)		
Frontal WM	0.28 (0.08)	0.25 (0.07)		0.23 (0.07)	0.01
		0.24 (0.07)	0.25 (0.07)		
Parietal WM	0.32 (0.06)	0.33 (0.05)		0.33 (0.09)	0.37
		0.32 (0.05)	0.33 (0.04)		
Temporal WM	0.30 (0.05)	0.27 (0.05)		0.24 (0.05)	0.001*
		0.29 (0.06)	0.26 (0.04)		
Occipital WM	0.34 (0.06)	0.31 (0.04)		0.31 (0.05)	0.03
		0.32 (0.03)	0.31 (0.05)		

Results are means, with standard deviations in parenthesis. MD is expressed in units of $\text{mm}^2 \times \text{s}^{-1} \times 10^3$. †p values refer to “trend-analysis”. *Variables that better distinguish the two aMCI subgroups (aMCI-C and aMCI-NC) (multivariate model with a stepwise selection procedure) (see text for further details). Abbreviations: AD=Alzheimer’s disease, aMCI= amnesic mild cognitive impairment, aMCI-C= aMCI converters, aMCI-NC= aMCI nonconverters, MD= mean diffusivity, GM=grey matter, WM=white matter CC= corpus callosum; ACC= anterior cingulate cortex; PCC= posterior cingulate cortex, FA=fractional anisotropy.

Figure legends

Figure 1. This figure shows the anatomical locations of the regions of interest (ROIs) on T2-weighted images (top row) and coregistered mean diffusivity maps (bottom row) from a healthy control: A) ROI 1 = temporal grey matter (GM), ROI 2 = temporal white matter (WM); B) ROI 3 = amygdala, ROI 4= hippocampus; C) ROI 5 = occipital WM, ROI 6 = occipital GM, ROI 7 = head of the caudate nucleus, ROI 8 = posterior limb of the internal capsule, ROI 9 = anterior insula, ROI 10 = frontal GM, ROI 11 = frontal WM; D) ROI 12 = thalamus, ROI 13 = genu of the corpus callosum, ROI 14 = splenium of the corpus callosum, ROI 15= anterior cingulate gyrus, ROI 16 = posterior cingulate gyrus; E) ROI 17 = parietal GM, ROI 18 = parietal WM.

Figure 2. Plots describing mean diffusivity (MD) of total grey matter and white matter of Alzheimer's disease (AD) patients, amnesic mild cognitive impairment (aMCI) patients who progressed to AD (aMCI-converters = aMCI-C), aMCI patients who did not progress (aMCI nonconverters=aMCI-NC) and healthy controls. MD is expressed in units of $\text{mm}^2 \times \text{s}^{-1} \times 10^3$. Each box represents the interquartile range, which contains 50% of individual subjects' values. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. A line across the box indicates the median value.

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