Progressive alterations in brain connectivity patterns revealed by diffusion-tensor brain networks across severity stages in Alzheimer's disease

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Abstract. Alzheimer's disease (AD) is a chronically progressive neurodegenerative disease highly correlated to aging. Whether AD originates by targeting a localized brain area and propagates to the rest of the brain across disease-severity progression is a question with an unknown answer. Here, this question is addressed at the group-level by looking to differences in diffusion-tensor brain networks. In particular, making use of data from Alzheimer's Disease Neuroimaging Initiative (ADNI), 4 different groups were defined (all of them matched by age, sex and education level): $\boldsymbol{G_1}(\boldsymbol{N_1 = 36}$, healthy control participants, HC), $\boldsymbol{G_2}$ ($\boldsymbol{N_2 = 36}$, early mild cognitive impairment, EMCI), $\boldsymbol{G_3}$ ($\boldsymbol{N_3 = 36}$, late mild cognitive impairment, LMCI) and $\boldsymbol{G_4}(\boldsymbol{N_4 = 36}$, AD). We built diffusion-tensor brain networks and performed group comparison across 3 disease stages: stage I (HC vs EMCI), stage II (HC vs LMCI) and stage III (HC vs AD). The group comparison was performed using the multivariate distance matrix regression analysis, a technique that was born in genomics and was recently proposed to handle functional network data, but here was applied to diffusion-tensor data. The results were three-fold: First, no significant differences were found in stage I. Second, in stage II, statistically significant differences were found in the connectivity pattern of a subnetwork strongly associated to memory function (including part of the hippocampus, amygdala, entorhinal cortex, fusiform gyrus, inferior and middle temporal gyrus, parahippocampal gyrus and temporal pole). Third, a widespread disconnection across the entire AD brain was found in stage III, affecting stronger to the same memory subnetwork appearing in stage II plus to other subnetworks, including the default mode network, the medial visual network, frontoparietal regions, and subnetworks encompassing mainly subcortical structures (including part of the hippocampus, amygdala and putamen). The novelty of the approach lies in the fact that group differences were approached across severity progression. A better possibility would have been to analyze well time-resolved longitudinal data, building diffusion-tensor networks belonging to the same patient across all disease stages (from control to AD), but such data (to the best of our knowledge) are not available yet.

Introduction

Alzheimer's disease (AD), the most common form of dementia, is a chronically progressive neurodegenerative disease highly correlated to aging; indeed, although the prevalence of clinically manifested AD is about 2% at the age of 65 years, it increases to about 30% at the age of 85 years (Wimo et al. 1997).

AD is characterized by an accumulation of beta-amyloid plaques and neurofibrillary tangles composed of tau amyloid fibrils (Hardy 2006) associated with synapse loss and neurodegeneration leading to long-term memory impairment and other cognitive problems. There is currently no known treatment that slows down the progression of this disorder.

The initial AD pathology develops many years before the cognitive and functional impairments are evident. Different terms have been used to describe this disease-starting condition, including pre-dementia and prodromal AD or MCI (mild cognitive impairment). The concept of MCI, a disorder situated in the spectrum between normal age-related cognitive decline and dementia, has varied over the past 2 decades. Indeed, MCI has been classified into different broad categories depending on memory performance and the number of impaired cognitive functions (Mueller *et al.* 2005).

An accurate prediction of conversion from MCI to AD can help to clinicians to evaluate AD risk pre-symptomatically, initiate treatments at early stage, and monitor their effectiveness (Cheng *et al.* 2015, Li *et al.* 2014). However, the group of MCI is highly heterogeneous, and not all MCI patients convert to AD (Ritter *et al.* 2015). Indeed, the annual rate in which MCI progresses to dementia varies between 8% and 15% per year (Mitchell and Shiri-Feshki 2009). The amnestic subtype of MCI is more prevalent than non-amnestic one (Petersen *et al.* 2010), and it has a significantly higher annual conversion rate to AD, between 30% (Schmidtke and Hermeneit 2008, Rozzini *et al.* 2007) to 40% (Geslani *et al.* 2005).

This study aims to search for neuroimaging biomarkers that can account for differences with respect to a healthy control (HC) population from the early to the final stages of AD. Multitude of different neuroimaging studies has addressed the conversion from MCI to AD, see (Zhang *et al.* 2014) and references therein. In relation to structural magnetic resonance imaging (MRI), it was shown that the hippocampus volume and the volume from other subcortical structures at MCI were well correlated to a worse progression to AD, with accuracy of about 65% in the prediction from MCI to AD (Teipel *et al.* 2015).

Rather than assuming that specific brain regions are going to be affected by AD, other authors achieved a better accuracy in the prediction from MCI to AD (achieving values of about 80% accuracy) by performing a blind approach including multiple regions of interest (Westman *et al.* 2011, Eskildsen *et al.* 2013, Liu *et al.* 2013). The use of Tensor Diffusion MRI in combination with structural MRI has provided better results as compared to solely structural MRI, finding that white-matter integrity of the fornix, cingulum, and parahippocampal gyrus provided accuracy varying from 80% to even 95% (Wee *et al.* 2013, Mielke *et al.* 2012, Douaud *et al.* 2013).

Initiatives like the Alzheimer's Disease Neuroimaging Initiative (ADNI) provide open access to the research community to important material and resources (demographic data, imaging datasets, cognitive tests, etc.) to study AD, pushing forward studies correlating different imaging modalities to the neuropsychological disease's status. Interestingly, ADNI also allows the possibility of studying variations in the images across disease's progression, as brain images are categorized in different groups ranging from HC to AD, with two intermediate stages, early and late mild cognitive impairment, EMCI and LMCI, respectively. Albeit EMCI and LMCI patients have some memory impairment beyond the standard dysfunction associated exclusively to aging or education level (Medina *et al.* 2006), the conversion rate to AD is only of 5-10% per year (Mitchell and Shiri-Feshki

2009). Thus, this group of individuals become of special importance in the searching for new imaging markers that can correlate with disease progression.

Despite extensive research shedding light into this problem, the precise mechanisms and clinical variables responsible for the progression from MCI to AD have not been fully characterized, mainly due to the lack of time-resolved longitudinal studies in large populations. Here, motivated by previous work (Khedher *et al.* 2015, Douaud *et al.* 2011, Bosch *et al.* 2012, Liu *et al.* 2013, Acosta-Cabronero *et al.* 2012, Preti *et al.* 2012), the study focuses on the variations of brain networks across the AD progression, and it is hypothesized that, if in the transition from HC to MCI the connectivity pattern of some subnetworks is altered, it will propagate to the rest of the AD brain.

Material and Methods

2.1 Alzheimer's Disease Neuroimaging Initiative (ADNI)

The DTI images used in this paper were obtained from ADNI database http://adni.loni.usc.edu. ADNI was launched in 2003 by the Nat. Inst. on Aging (NIA), the Nat. Inst. Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations, as a $60 million, 5-year public-private partnership. ADNI's main goal has been to test whether serial MRI, positron emission tomography (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 to lessen the time and cost of clinical trials. The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and Univ. California – San Francisco.

ADNI subjects have been recruited from over 50 sites across the U.S. and Canada. Currently, around 1500 adults were recruited in the different ADNI initiatives, ages 55 to 90, consisting of cognitively normal older (NC), early/late MCI (EMCI/LMCI), significant memory concern (SMC) and early AD (AD) individuals. The follow up duration of each group is specified in the protocols for ADNI-1, ADNI-2 and ADNI-GO, see further information in www.adni-info.org .

2.2 Demographic Data

A total number of N=144 subjects were used in this study (Table I). This number was chosen in order to get the biggest 4 groups as possible (HC, EMCI, LMCI and AD), balanced by size, age and sex. DTI images were selected and downloaded from ADNI database, belonging to 4 different groups: HC (N_1=36), EMCI (N_2=36), LMCI (N_3=36) and AD (N_4=36). Age and sex were balanced across groups (Table II), respectively, using a t-test and chi-squared test. In addition, it is important to remark that the "years of education" variable was already controlled by the ADNI group classification, for details see Inclusion criteria in page 31 of https://adni.loni.usc.edu/wp-content/uploads/2008/07/**adni2-procedures-manual**.pdf

2.3 ADNI group classification

The group labels HC, EMCI, LMCI and AD are based on several test scores, such as the Logical Memory II subscale (LMIIS) from the Wechsler Memory Scale, the Mini-Mental State Examination (MMSE) and the Clinical Dementia Rating (CDR), as well as National Institute of Neurological and Communicative Disorders and Stroke and the AD and Related Disorders Association

(NINCDS/ADRDA) criteria in AD cases. In the procedures manual each of the criteria are cited (http://adni.loni.usc.edu/wp-content/uploads/2008/07/adni2-procedures-manual.pdf).

HC subjects are free of memory complaints (beyond what expected due to age), verified by a study partner. EMCI, LMCI and AD must have a subjective memory concern as reported by subject, study partner, or clinician. Details of specific groups are given in Table III.

2.4 Group-level stages for AD progression

AD progression was defined by three different stages: stage I (control vs EMCI), stage II (control vs LMCI) and stage III (control vs AD). More details in Figure 1.

2.5 DTI acquisitions

All subjects in this study had the same ADNI imaging protocol, explained in http://adni.loni.usc.edu/methods/documents/mri-protocols/ and consisting in whole-brain MRI 3T scanners and Diffusion Weighted Images (DWI) images of the axial DTI series. The DTI images were acquired using spin echo pulse sequence echo-planar-imaging (SE-EPI) with the following parameters: TR = 9050.0 ms; TE set to minimum (values ranging from 60 ms till 69 ms); 59 slices with thickness of 2.7 mm with no gap among slices; 128x128 matrix with a FOV of 35.0 cm; with matrix pixels 256x256x2714 and voxel size 1.36x1.36x2.7 mm^3, flip angle = 90^o. A diffusion gradient was applied along 41 non-collinear directions with a b value of 1000 s/mm2. Additionally, one set of images was acquired with no diffusion weighting (b= 0 s/mm2).

152 **2.6 Diffusion tensor brain networks**

153 To build diffusion tensor brain networks a similar methodology as in previous work was followed
154 (Diez *et al.* 2015, Alonso-Montes *et al.* 2015, Amor *et al.* 2015) using FSL (FMRIB Software
155 Library v5.0) and the Diffusion Toolkit. First, all the selected images were downloaded in DICOM
156 and transformed to Nifti format for further analysis. Next, an eddy current correction was applied to
157 overcome the artifacts produced by variation in the gradient field directions, together with the
158 artifacts produced by head movements. Next, using the corrected data, a local fitting of the diffusion
159 tensor was applied to compute the diffusion tensor model for each voxel. Next, a Fiber Assignment
160 by Continuous Tracking (FACT) algorithm was applied (Mori *et al.* 1999). Then a transformation
161 from the Montreal Neurological Institute (MNI) space to the individual-subject diffusion space was
162 applied and projected the brain hierarchical atlas (available to download at
163 http://www.nitrc.org/projects/biocr_hcatlas/). In particular, M=20 modules were considered that was
164 shown in (Diez *et al.* 2015) to best-match functional connectivity modules with structural ones. This
165 allowed building 20 x 20 structural connectivity (SC) matrices, each per subject, by counting the
166 number of white matter streamlines connecting all module pairs. Thus, the element matrix *(i,j)* of SC
167 is given by the streamlines number between modules *i* and *j*. As a result, SC is a symmetric matrix,
168 where connectivity from *i* to *j* is equal to that from *j* to *i*.

169

170 **2.6 Cross-group analysis: Multivariate Distance Matrix Regression**

171 The cross-group analysis has been performed using the Multivariate Distance Matrix Regression
172 (MDMR) approach proposed in (Shehzad *et al.* 2014). Based on the hypothesis that at the early
173 stages of the AD progression, if some subnetworks disconnect will propagate at further stages to the
174 rest of the brain, a multivariate distance regression was chosen to apply, which allows testing the

variation of distance in connectivity patterns between groups as a response of the Alzheimer's progression as compared to the HC state. For a fixed brain module *i*, the distance between connectivity patterns of module *i* to the rest of the brain was calculated per pair of subjects (u,v) --by calculating Pearson correlation between connectivity vectors of subject pairs--, thus leading to a distance matrix in the subject space for each module *i* investigated. In particular, the following formula was calculated

$$d_{uv}^{i} = \sqrt{2 \cdot (1 - r_{uv})} \qquad \text{(Eq. 1)}$$

where r_{uv} is the Pearson correlation between connectivity patterns of *i* for subjects *u* and *v*. After repeating the same procedure for all subjects, as many distance matrices as partition modules $(i = 1, \ldots, 20)$ were obtained. Next, MDMR was applied to perform cross-group analysis as implemented in R (McArtor 2016).

It is important to emphasize that NDMR does not look to how individual modules are locally organized or connected, but to the integration connectivity pattern between those segregated modules to the rest of the brain. Therefore, when NDMR finds group differences in a given module, this means that the connectivity alterations from that module are being propagated to the rest of the brain.

MDMR yielded a pseudo-F estimator (analogous to that F-estimator in standard ANOVA analysis), which addresses significance of disease strength due to between-group variation as compared to within-group variations (McArdle and Anderson 2001). To compare between groups when the regressor variable is categorical (*i.e.* the group label), given a distance matrix, one can calculate the total sum of squares as

$$SS_T = \frac{1}{N} \sum_{u=1} \sum_{v=1+1} d_{uv}^2, \qquad \text{(Eq. 2)}$$

with N being the total number of subjects. Notice that, from here on, we will consider $d_{uv} \equiv d_{uv}^{i}$. Thus, we got a different SS_T for each module *i*. Similarly, the within-group sum of squares can be written as

$$SS_W = \sum \frac{1}{n_g} \sum_{u=1} \sum_{v=u+1} d_{uv}^2 \, \varepsilon_{uv}^{g}, \qquad \text{(Eq. 3)}$$

where n_g is the number of subjects per group and ε_{uv}^{g} a variable equal to 1 if subjects *u* and *v* belong to group g and 0 otherwise. The between-group variation is simply $SS_A = SS_T - SS_W$, which leads to a pseudo-F statistic that reads

$$F = \frac{SS_A/(m-1)}{SS_W/(N-m)} \qquad \text{(Eq. 4)}$$

where *m* is the number of groups. As it was acknowledged in (Zapala and Schork 2006), the pseudo-F statistic is not distributed like the usual Fisher's F-distribution under the null hypothesis. Accordingly, we randomly shuffled the subject indices and computed the pseudo-F statistic for each time. A p-value is computed by counting those pseudo F-statistic values from permuted data greater than that from the original data respect to the total number of performed permutations.

Finally, we controlled for type I errors due to the 20 independent statistical performed tests by false discovery rate corrections (Benjamini and Hochberg 1995). Corrected whole-brain connectivity patterns of modules are the ones related to AD progression at the different stages. An schematic overview of the method can be found in Figure 2.

3. Results

Results are summarized in Table IV and modules involved in the disease progression are shown in Figure 3. See also Table V for some examples of the different terms participating in the statistical test.

3.1 Stage I: HC vs EMCI

A total number of 36 images per each group were selected to perform group comparison. No significant differences were found in terms of module connectivity patterns to the whole brain.

3.2 Stage II: HC vs LMCI

A total number of 36 images per each group were selected to perform group comparison. Significant differences were found for the connectivity between the module 18 and the rest of the brain (p=0.007). As detailed in (27), the module 18 of the brain hierarchical atlas consisted in part of the hippocampus, amygdala, entorhinal cortex, fusiform gyrus, inferior temporal gyrus, middle temporal gyrus, parahippocampal gyrus and temporal pole.

3.3 Stage III: HC vs AD

A total number of 36 images per each group were selected to perform group comparison. For this situation, the number of significantly different connectivity patterns was found in multiple modules from the brain hierarchical atlas:

Module 1 (p=0.023); including part of the posterior cingulate.

Module 2 (p=0.049); including part of the putamen, anterior cingulate, rostral pars of the middle frontal gyrus, superior parietal gyrus, supramarginal gyrus, insula, inferior parietal gyrus, precentral gyrus and superior frontal gyrus.

Module 3 (p=0.049); part of the paracentral lobe, precentral gyrus, postcentral gyrus, precuneus, superior frontal gyrus, superior parietal gyrus, superior temporal gyrus, supramarginal gyrus and insula.

Module 4 (p=0.031); part of the cuneus, lateral occipital sulcus, lingual gyrus, pericalcarine cortex and precuneus.

Module 8 (p=0.031); part of the caudate nucleus and putamen.

Module 12 (p=0.031); part of the inferior parietal gyrus, inferior temporal gyrus, lateral frontal orbital gyrus, pars orbitalis, pars triangularis, rostral pars of the middle frontal gyrus, superior frontal gyrus, caudate nucleus and anterior cingulate.

Module 14 (p=0.006); part of the thalamus, hippocampus, amygdala, putamen, ventral diencephalon, banks of the superior temporal sulcus, parahippocampal gyrus, superior temporal gyrus, insula, middle temporal gyrus and temporal pole.

Module 15 (p=0.031); part of the thalamus, putamen, pallidum, brainstem, hippocampus, amygdala, accumbens nucleus, ventral diencephalon, orbital gyrus and insula.

Module 16 (p=0.031); part of the cerebellum, banks of the superior temporal sulcus, inferior parietal gyrus, cingulate isthmus, middle temporal gyrus, precuneus and superior temporal gyrus.

Module 18 (p=0.002); see previous 3.2 section for the anatomical description, but notice a reduction in p value from 0.007 (HC vs LMCI) to 0.002 (HC vs AD).

3.4 Common affected modules between stages

Connectivity pattern of module 18 to the rest of the brain was found at stage II (p=0.007) and at stage III (p=0.002), indicating that the further the disease progresses, the greater the connectivity of module 18 is altered to the rest of the brain.

4. Discussion

The aim of the study was to identify differences in brain connectivity patterns between three groups with different disease severity and a control group. For this purpose, diffusion tensor brain networks were built allowing determining connectivity differences at three consecutive disease stages: stage I (HC vs EMCI), stage II (HC vs LMCI) and stage III (HC vs AD).

The results showed an absence of significant changes in connectivity patterns in stage I, that is, between patients with early mild cognitive impairment and healthy individuals. The approach we have applied, the Multivariate Distance Matrix Regression (MDMR) analysis, finds group differences in the connectivity patterns from different modules to the rest of the brain. Therefore, at very early mild cognitive impairment, although possible structural damages might occur locally, they are not capable to produce global inter-module network reorganization/redistribution that can be measured by the NDMR analysis.

However, significant differences were found by the MDMR method in stage II to a network involved with memory (module 18), which includes the hippocampus, amygdala, entorhinal cortex, fusiform gyrus, inferior temporal gyrus, mean temporal gyrus, parahippocampal gyrus and the temporal pole, among patients with late mild cognitive impairment and healthy individuals. This change in

connectivity in module 18 becomes more evident in patients with AD. Finally, a multitude of different modules are connected differently in AD as compared to healthy subjects (modules 1-4, 8, 12, 14-16 and 18), including the default mode network, the sensory-motor network, the medial visual network, frontoparietal regions and networks that mainly encompass subcortical structures (including part of the hippocampus, amygdala and putamen).

The brain connectivity changes found in this study in stage II might be related to the appearance of several cognitive manifestations, which are typical of AD. For example, many studies have determined the main cognitive impairment in the preclinical phase of AD is episodic memory (Almkvist, 1996, Arnaiz et al., 2003; Albert et al., 2001; Bäckman et al., 2004, 2005; Grober et al., 2008), in which hippocampal formation, entorhinal cortex, amygdala and hypothalamus are involved; that is, regions of the temporal-medial lobe which are regions affected before AD can be clinically diagnosed (Almkvist, 1996; Bäckman et al., 2004, 2005; Small et al., 1999; Estévez-González et al., 2003; Small et al., 2003). In addition, it is known that the initial neuronal lesions in AD also begin in the entorhinal region (included in module 18, therefore, in agreement with our results) with the accumulation of neurofibrillary tangles and neuritic plaques (Gómez-Isla et al., 1996).

Although alterations of the episodic memory are considered the most critical ones at the preclinical phase (Small, et al., 2003; Storandt, 2008) and tasks that measure episodic memory have been shown to be particularly effective at identifying people at risk for developing AD (Elias et al., 2000; Tierney et al., 1996), studies have shown that people with mild cognitive impairment who have altered episodic memory and other cognitive areas such as verbal ability (Apostolova et al., 2008; Arnaiz et al et al., 2003; Bäckman et al., 2004, 2005; Joubert et al., 2010), executive functions (Albert et al., 2001; Bäckman et al., 2004, 2005; Dickerson et al., 2007; Grober et al., 2008; Storandt, 2008; Blacker et al., 2007; Rapp et al., 2005), perceptual speed (Bäckman et al., 2005), Visuo-spatial / visuoperceptive skills (Almkvist, 1996, Arnaiz et al., 2003; Bäckman et al., 2004, 2005; Alegret et

al., 2009), attention (Bäckman et al., 2005; Rapp et al., 2005), etc. are more likely to convert to AD than those with only memory impairment (Bozoki et al., 2001). As indicated by Bäckman et al. (2004, 2005), some studies suggest that other areas in addition to the ones in temporal-medial lobe are altered prior to the diagnosis of AD, such as volume reduction of anterior cingulate and temporal sulcus, posterior cingulate, temporoparietal regions, frontal regions; decreased blood flow in the posterior cingulate and precuneus, reduced glucose in temporoparietal regions and deposition of amyloid plaques in the temporal and frontal cortex. This may explain why studies attempting to find cognitive markers of the AD preclinical stage find alterations in other cognitive functions apart from episodic memory.

As the disease progresses, not only the disconnection pattern of module 18 becomes more evident (increasing the distance between AD and controls, Table IV), but such significant changes extend to other brain regions. For example, areas of the hippocampus affected by module 14 are well known to suffer a very severe cognitive degeneration, a fact also confirmed by functional connectivity studies (Zhou *et al.* 2008). The results also indicate a significant connectivity change with temporal medial areas, as revealed by module 16, as shown in Tract Based Spatial Statistics at (Stricker *et al.* 2009, Acosta-Cabronero *et al.* 2010, Salat *et al.* 2010). The authors of (He *et al.* 2007) demonstrated, through a combined structural and functional analysis, changes in connectivity between the lingual and cuneus, confirming the results of this study obtained exclusively from structural connectivity data. At the same time, the results indicate a significant change in the connectivity of the entire brain to the areas provided by module 4, mainly affected by visual functions. For example, a decrease in virtual capacity due to AD is well known, especially in those areas involving movement blindness, depth perception, color perception and contrast sensitivity (Whittaker *et al.* 2002). Again, this damage expansion to other brain regions also agrees with the extent and worsening of cognitive aspects (e.g., memory, attention, language; Weintraub *et al.* 2012) and neurobehavioral problems

(e.g. personality changes, anxiety, depression, agitation, hallucinations; Chung & Cummings, 2000, Bassiony et al., 2000, Senanarong et al., 2003) of patients with AD.

Previous studies have analyzed the connectivity differences from tensor diffusion networks in AD and have found significant alterations in the inferior longitudinal fasciculus for patients at risk of AD (Smith *et al.* 2010), which could correspond to LMCI. Similarly, a voxel-based analysis in (Honea *et al.* 2009) showed a significant decrease in FA for fibers connecting the parahippocampal gyrus. In addition, patients diagnosed in the early stages of AD (corresponding to early or late mild cognitive impairment in this study) had a significant reduction in white matter in the upper longitudinal fasciculus, which also connects part of module 18 in the brain hierarchical atlas with the frontal lobe (Rose *et al.* 2000). The authors (Hanyu *et al.* 1998) found significant changes in apparent diffusion coefficients and diffusion anisotropy in patients with recent progressive cognitive impairment, suggesting an early decrease in temporal fiber density, a region included in the module 18, therefore in concordance to our results.

Implications

In recent years a great deal of emphasis has been placed on the early AD detection (Albert et al., 2001). The results of this study have great clinical implications since the analysis of cerebral connectivity could help in the early diagnosis of the disease. This early detection would help to look for pharmacological or non-pharmacological treatment methods to help delay the age of disease onset or to slow down the clinical disease progression.

On the other hand, identifying the brain connection patterns in those patients who have not yet developed AD, will allow us to know the changes that occur in these connectivity patterns over time.

In addition, it will be possible to associate those connectivity patterns with clinical patient's changes present at each disease stage. This might help to better understand the relationship between deterioration in brain functioning and the clinical patient's characteristics.

Limitations

The results of the present study should be interpreted in light of the following limitations. First, it is a cross-sectional study with different groups of people in each experimental group and with a small sample size, so future studies should try to extend to bigger cohorts and follow the same group of people over time as the disease progresses. Second, the patients included in the study have a probable AD, which means that the definitive diagnosis of AD can only be performed post-mortem (Fearing et al., 2007). The use of patients with familial AD could help to know in depth the evolution of the disease and the changes in cerebral connectivity from many years back to its onset. Third, there are a number of risk factors associated with the decline of mild cognitive impairment which can affect brain connectivity such as advanced diabetes, symptomatology depressive disorder, hypertension, hypotension, obesity, history of traumatic brain injury and APOE genotype, that have not been taken into account in this study. Future studies should take into account the possible influence of these variables on the processes of cerebral connectivity.

In conclusion, the deterioration of cerebral connectivity is evident and is in line with the evolution of AD from both the neuropathological and neuropsychological point of view. That is, the first cerebral connectivity changes occur in the regions of the middle temporal lobe (hippocampus and entorhinal), which coincides with the first symptoms of altered episodic memory in the preclinical stage and in mild cognitive impairment. As the disease progresses, the brain damage and its disconnection of these regions become more evident and expands to other areas, which coincides with the expansion

370 and/or worsening of other cognitive functions and neurobehavioral aspects seen in the individuals
371 with AD.

Author Contributions

JR, CAM and ID had equal first-author contribution; JR, CAM and ID analyzed the data and made the figures; LOL and JCAL connected results to cognitive deficits in AD; LR, IE, BM, PB, MF, JCAL, SS and JMC designed the research; all the authors wrote the manuscript and agreed in its submission; SS and JMC had equal last author contributions

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References

Acosta-Cabronero, J., Alley, S., Williams, G. B., Pengas, G. & Nestor, P. J. Diffusion Tensor Metrics as Biomarkers in Alzheimer's Disease. PLOS ONE 7, e49072 (2012).

Acosta-Cabronero, J., Williams, G. B., Pengas, G. & Nestor, P. J. Absolute diffusivities define the landscape of white matter degeneration in Alzheimer's disease. Brain 133, 529–539 (2010).

Albert, M. S., Moss, M. B., Tanzi, R., & Jones, K. (2001). Preclinical prediction of AD using neuropsychological tests. Journal of the International Neuropsychological Society, 7(05), 631-639.

Alegret, M., Boada-Rovira, M., Vinyes-Junqué, G., Valero, S., Espinosa, A., Hernández, I., ... & Tárraga, L. (2009). Detection of visuoperceptual deficits in preclinical and mild Alzheimer's disease. Journal of clinical and experimental neuropsychology, 31(7), 860-867.

Almkvist, O. (1996). Neuropsychological features of early Alzheimer's disease: preclinical and clinical stages. Acta Neurologica Scandinavica, 94(S165), 63-71.

Alonso-Montes, C. et al. Lagged and instantaneous dynamical influences related to brain structural connectivity. Front. Psychol. 6, (2015).

Amor, T. A. et al. Extreme brain events: Higher-order statistics of brain resting activity and its relation with structural connectivity. EPL Europhys. Lett. 111, 68007 (2015).

Apostolova, L. G., Lu, P., Rogers, S., Dutton, R. A., Hayashi, K. M., Toga, A. W., ... & Thompson, P. M. (2008). 3D mapping of language networks in clinical and pre-clinical Alzheimer's disease. Brain and language, 104(1), 33-41.

Arnáiz, E., & Almkvist, O. (2003). Neuropsychological features of mild cognitive impairment and preclinical Alzheimer's disease. Acta Neurologica Scandinavica, 107(s179), 34-41.

409 Bassiony, M. M., Steinberg, M. S., Warren, A., Rosenblatt, A., Baker, A. S., & Lyketsos, C. G.
410 (2000). Delusions and hallucinations in Alzheimer's disease: prevalence and clinical correlates.
411 International journal of geriatric psychiatry, 15(2), 99-107.

412 Benjamini, Y. & Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful
413 Approach to Multiple Testing. J. R. Stat. Soc. Ser. B Methodol. 57, 289–300 (1995).

414 Blacker, D., Lee, H., Muzikansky, A., Martin, E. C., Tanzi, R., McArdle, J. J., ... & Albert, M.
415 (2007). Neuropsychological measures in normal individuals that predict subsequent cognitive
416 decline. Archives of neurology, 64(6), 862-871.

417 Bosch, B. et al. Multiple DTI index analysis in normal aging, amnestic MCI and AD. Relationship
418 with neuropsychological performance. Neurobiol. Aging 33, 61–74 (2012).

419 Bozoki, A., Giordani, B., Heidebrink, J. L., Berent, S., & Foster, N. L. (2001). Mild cognitive
420 impairments predict dementia in nondemented elderly patients with memory loss. Archives of
421 Neurology, 58, 411–416.

422 Bäckman, L., Jones, S., Berger, A. K., Laukka, E. J., & Small, B. (2004). Multiple cognitive deficits
423 during the transition to Alzheimer's disease. Journal of internal medicine, 256(3), 195-204.

424 Bäckman, L., Jones, S., Berger, A. K., Laukka, E. J., & Small, B. J. (2005). Cognitive impairment in
425 preclinical Alzheimer's disease: a meta-analysis. Neuropsychology, 19(4), 520.

426 Cerhan, J. H., Ivnik, R. J., Smith, G. E., Machulda, M. M., Boeve, B. F., Knopman, D. S., ... &
427 Tangalos, E. G. (2007). Alzheimer's disease patients' cognitive status and course years prior to
428 symptom recognition. Aging, Neuropsychology, and Cognition, 14(3), 227-235.

429 Cheng, B. et al. Multimodal manifold-regularized transfer learning for MCI conversion prediction.

430 Brain Imaging Behav. 9, 913–926 (2015).

431 Chung, J. A., & Cummings, J. L. (2000). Neurobehavioral and neuropsychiatric symptoms in
432 Alzheimer's disease. Neurologic clinics, 18(4), 829-846.

433 Daniel B. McArtor, D. B. M. MDMR: Multivariate Distance Matrix Regression. (2016).

434 Dickerson, B. C., Sperling, R. A., Hyman, B. T., Albert, M. S., & Blacker, D. (2007). Clinical
435 prediction of Alzheimer disease dementia across the spectrum of mild cognitive impairment.
436 Archives of General Psychiatry, 64(12), 1443-1450.

437 Diez, I. et al. A novel brain partition highlights the modular skeleton shared by structure and
438 function. Sci. Rep. 5, 10532 (2015).

439 Douaud, G. et al. Brain Microstructure Reveals Early Abnormalities more than Two Years prior to
440 Clinical Progression from Mild Cognitive Impairment to Alzheimer's Disease. J. Neurosci. 33, 2147–
441 2155 (2013).

442 Douaud, G. et al. DTI measures in crossing-fibre areas: increased diffusion anisotropy reveals early
443 white matter alteration in MCI and mild Alzheimer's disease. NeuroImage 55, 880–890 (2011).

444 Elias, M. F., Beiser, A., Wolf, P. A., Au, R., White, R. F., & D'Agostino, R. B. (2000). The
445 preclinical phase of Alzheimer's disease: A 22-year prospective study of the Framingham cohort.
446 Archives of Neurology, 57, 808–813.

447 Eskildsen, S. F. et al. Prediction of Alzheimer's disease in subjects with mild cognitive impairment
448 from the ADNI cohort using patterns of cortical thinning. NeuroImage 65, 511–521 (2013).

449 Estevez Gónzalez, A., Kulisevsky, J., Boltes, A., Otermin, P., García-Sánchez, C. Rey. Verbal
450 Learning test is a useful tool for differential diagnosis in the preclinical phase of Alzheimer's disease:

451 comparison with mild cognitive impairment and normal aging. Int J Geriatr Psychiatry 2003; 18 (11):
452 1021-8.

453 Fearing, M.A., Bigler, E.D., Norton, M., Tschanz, J.A., Hulette, C., Leslie, C., Welsh-Bohmer, K., &
454 Cache County Investigators (2007). Autopsy-confirmed Alzheimer's disease versus clinically
455 diagnosed Alzheimer's disease in the Cache County Study on Memory and Aging: a comparison of
456 quantitative MRI and neuropsychological findings. Journal of Clinical and Experimental
457 Neuropsychology, 29(5): 553-560

458 Geslani, D. M., Tierney, M. C., Herrmann, N. & Szalai, J. P. Mild cognitive impairment: an
459 operational definition and its conversion rate to Alzheimer’s disease. Dement. Geriatr. Cogn. Disord.
460 19, 383–389 (2005).

461 Gomez-Isla, T., Price, J., McKeel, D., Morris, J., Growdon, J., & Hyman, B. (1996). Profound loss of
462 layer II entorhinal cortex neurons occurs in very mild Alzheimer’s disease. Journal of Neuroscience,
463 16, 4491– 4500.

464 Grober, E., Hall, C. B., Lipton, R. B., Zonderman, A. B., Resnick, S. M., & Kawas, C. (2008).
465 Memory impairment, executive dysfunction, and intellectual decline in preclinical Alzheimer's
466 disease. Journal of the International Neuropsychological Society, 14(02), 266-278.

467 Hanyu, H. et al. Diffusion-weighted MR imaging of the hippocampus and temporal white matter in
468 Alzheimer’s disease. J. Neurol. Sci. 156, 195–200 (1998).

469 Hardy, J. Alzheimer’s disease: the amyloid cascade hypothesis: an update and reappraisal. J.
470 Alzheimers Dis. JAD 9, 151–153 (2006).

471 Honea, R. A., Vidoni, E., Harsha, A. & Burns, J. M. Impact of APOE on the Healthy Aging Brain: A
472 Voxel-Based MRI and DTI Study. J. Alzheimers Dis. JAD 18, 553–564 (2009).

473 Joubert, S., Brambati, S. M., Ansado, J., Barbeau, E. J., Felician, O., Didic, M., ... & Kergoat, M. J.
474 (2010). The cognitive and neural expression of semantic memory impairment in mild cognitive
475 impairment and early Alzheimer's disease. Neuropsychologia, 48(4), 978-988.

476 Khedher, L., Ramírez, J., Górriz, J. M., Brahim, A. & Segovia, F. Early diagnosis of Alzheimer′s
477 disease based on partial least squares, principal component analysis and support vector machine
478 using segmented MRI images. Neurocomputing 151, Part 1, 139–150 (2015).

479 Li, H. et al. Hierarchical Interactions Model for Predicting Mild Cognitive Impairment (MCI) to
480 Alzheimer's Disease (AD) Conversion. PLOS ONE 9, e82450 (2014).

481 Liu, J. et al. White Matter Changes in Patients with Amnestic Mild Cognitive Impairment Detected
482 by Diffusion Tensor Imaging. PLOS ONE 8, e59440 (2013).

483 Liu, Y. et al. Predicting AD Conversion: Comparison between Prodromal AD Guidelines and
484 Computer Assisted PredictAD Tool. PLOS ONE 8, e55246 (2013).

485 McArdle, B. H. & Anderson, M. J. Fitting Multivariate Models to Community Data: A Comment on
486 Distance-Based Redundancy Analysis. Ecology 82, 290–297 (2001).

487 Medina, D. et al. White matter changes in mild cognitive impairment and AD: A diffusion tensor
488 imaging study. Neurobiol. Aging 27, 663–672 (2006).

489 Mielke, M. M. et al. Fornix integrity and hippocampal volume predict memory decline and
490 progression to Alzheimer's disease. Alzheimers Dement. J. Alzheimers Assoc. 8, 105–113 (2012).

491 Mitchell, A. J. & Shiri-Feshki, M. Rate of progression of mild cognitive impairment to dementia –
492 meta-analysis of 41 robust inception cohort studies. Acta Psychiatr. Scand. 119, 252–265 (2009).

493 Mori, S., Crain, B. J., Chacko, V. P. & van Zijl, P. C. Three-dimensional tracking of axonal

494 projections in the brain by magnetic resonance imaging. Ann. Neurol. 45, 265–269 (1999).

495 Mueller, S. G. et al. The Alzheimer's disease neuroimaging initiative. Neuroimaging Clin. N. Am.
496 15, 869–877, xi–xii (2005).

497 Petersen, R. C. et al. Prevalence of mild cognitive impairment is higher in men The Mayo Clinic
498 Study of Aging. Neurology 75, 889–897 (2010).

499 Preti, M. G. et al. Assessing Corpus Callosum Changes in Alzheimer's Disease: Comparison between
500 Tract-Based Spatial Statistics and Atlas-Based Tractography. PLOS ONE 7, e35856 (2012).

501 Rapp, M. A., & Reischies, F. M. (2005). Attention and executive control predict Alzheimer disease
502 in late life: results from the Berlin Aging Study (BASE). The American Journal of Geriatric
503 Psychiatry, 13(2), 134-141.

504 Ritter, K. et al. Multimodal prediction of conversion to Alzheimer's disease based on incomplete
505 biomarkers∗. Alzheimers Dement. Diagn. Assess. Dis. Monit. 1, 206–215 (2015).

506 Rose, S. et al. Loss of connectivity in Alzheimer's disease: an evaluation of white matter tract
507 integrity with colour coded MR diffusion tensor imaging. J. Neurol. Neurosurg. Psychiatry 69, 528–
508 530 (2000).

509 Rozzini, L. et al. Conversion of amnestic Mild Cognitive Impairment to dementia of Alzheimer type
510 is independent to memory deterioration. Int. J. Geriatr. Psychiatry 22, 1217–1222 (2007).

511 Salat, D. H. et al. White matter pathology isolates the hippocampal formation in Alzheimer's disease.
512 Neurobiol. Aging 31, 244–256 (2010).

513 Schmidtke, K. & Hermeneit, S. High rate of conversion to Alzheimer's disease in a cohort of
514 amnestic MCI patients. Int. Psychogeriatr. 20, 96–108 (2008).

Senanarong, V., Cummings, J. L., Fairbanks, L., Mega, M., Masterman, D. M., O'connor, S. M., & Strickland, T. L. (2003). Agitation in Alzheimer's disease is a manifestation of frontal lobe dysfunction. Dementia and geriatric cognitive disorders, 17(1-2), 14-20.

Shehzad, Z. et al. A multivariate distance-based analytic framework for connectome-wide association studies. NeuroImage 93, Part 1, 74–94 (2014).

Small, B. J., Mobly, J. L., Laukka, E. J., Jones, S., & Bäckman, L. (2003). Cognitive deficits in preclinical Alzheimer's disease. Acta Neurologica Scandinavica, 107(s179), 29-33.

Small, S. A., Perara, G., DeLaPaz, R., Mayeux, R., & Stern, Y. (1999). Differential regional dysfunction of the hippocampal formation among elderly with memory decline and Alzheimer's disease. Annals of Neurology, 45, 466–472.

Smith, C. D. et al. White matter diffusion alterations in normal women at risk of Alzheimer's disease. Neurobiol. Aging 31, 1122–1131 (2010).

Storandt, M. (2008). Cognitive deficits in the early stages of Alzheimer's disease. Current Directions in Psychological Science, 17(3), 198-202.

Stricker, N. H. et al. Decreased white matter integrity in late-myelinating fiber pathways in Alzheimer's disease supports retrogenesis. NeuroImage 45, 10–16 (2009).

Teipel, S. et al. Multimodal imaging in Alzheimer's disease: validity and usefulness for early detection. Lancet Neurol. 14, 1037–1053 (2015).

Tierney, M. C., Szalai, J. P., Snow, W. G., & Fisher, R. H. (1996). The prediction of Alzheimer disease: The role of patient and informant perceptions of cognitive deficits. Archives of Neurology, 53, 423–427

536 Wee, C.-Y., Yap, P.-T., Shen, D. & Alzheimer's Disease Neuroimaging Initiative. Prediction of
537 Alzheimer's disease and mild cognitive impairment using cortical morphological patterns. Hum.
538 Brain Mapp. 34, 3411–3425 (2013).

539 Westman, E. et al. Sensitivity and Specificity of Medial Temporal Lobe Visual Ratings and
540 Multivariate Regional MRI Classification in Alzheimer's Disease. PLOS ONE 6, e22506 (2011).

541 Whittaker, K. W., Burdon, M. A. & Shah, P. Visual field loss and Alzheimer's disease. Eye 16, 206–
542 208 (2002).

543 Wimo, A., Ljunggren, G. & Winblad, B. Costs of dementia and dementia care: a review. Int. J.
544 Geriatr. Psychiatry 12, 841–856 (1997).

545 Y. et al. Regional coherence changes in the early stages of Alzheimer's disease: A combined
546 structural and resting-state functional MRI study. NeuroImage 35, 488–500 (2007).

547 Zapala, M. A. & Schork, N. J. Multivariate regression analysis of distance matrices for testing
548 associations between gene expression patterns and related variables. Proc. Natl. Acad. Sci. U. S. A.
549 103, 19430–19435 (2006).

550 Zhang, S. et al. in Cochrane Database of Systematic Reviews (John Wiley & Sons, Ltd, 2014).

551 Zhou, Y. et al. Abnormal connectivity in the posterior cingulate and hippocampus in early
552 Alzheimer's disease and mild cognitive impairment. Alzheimers Dement. 4, 265–270 (2008).

553 **Table I: ADNI subjects within each group**

554 HC=Healthy controls, EMCI: Early mild cognitive impairment; LMCI=Late mild cognitive
555 impairment; AD= Alzheimer disease; M=Male, F=Female.

556

HC			**EMCI**			**LMCI**			**AD**		
SubjectId	*Sex*	*Age*	*SubjectId*	*Sex*	*Age*	*SubjectId*	*Sex*	*Age*	*SubjectId*	*Sex*	*Age*
003_S_4119	M	79	003_S_2374	F	81	003_S_0908	F	70	003_S_4373	F	71
003_S_4839	M	66	007_S_2394	M	69	003_S_4354	M	76	003_S_5165	M	79
007_S_4488	M	73	016_S_4575	F	62	016_S_4584	F	78	003_S_5187	F	62
007_S_4516	M	72	021_S_2077	M	81	016_S_4646	F	61	005_S_4707	M	68
007_S_4620	M	77	021_S_2100	F	88	016_S_4902	F	75	005_S_4910	F	82
016_S_4121	M	89	021_S_2125	F	78	021_S_4402	F	73	005_S_5038	M	82
021_S_4558	F	71	021_S_2142	F	83	021_S_4633	F	73	005_S_5119	F	77
029_S_4279	M	84	021_S_4419	F	65	021_S_4857	M	68	007_S_4568	F	71

029_S_4290	M	74	021_S_4659	M	86	027_S_4729	F	78	007_S_4911	M	75
029_S_4384	M	62	021_S_4744	F	73	027_S_4757	F	63	007_S_5196	F	73
029_S_4385	F	68	029_S_2370	F	64	027_S_4804	M	80	016_S_4591	F	66
029_S_4585	M	66	029_S_2395	M	73	027_S_4869	M	77	016_S_4887	M	75
029_S_4652	M	79	029_S_4327	M	83	027_S_4873	M	83	016_S_4963	F	72
057_S_0934	F	77	029_S_5135	M	77	027_S_4936	M	78	016_S_5057	M	75
094_S_4234	M	70	094_S_2201	F	64	027_S_4943	M	76	016_S_5251	F	66
094_S_4459	F	68	094_S_2216	M	69	027_S_4955	M	72	021_S_4718	M	79
094_S_4460	F	67	094_S_2238	M	69	052_S_4626	M	69	021_S_4924	M	77
094_S_4503	F	72	094_S_2367	M	75	052_S_4807	F	72	027_S_4801	M	78
094_S_4649	M	66	094_S_4434	M	68	052_S_4945	M	57	027_S_4802	M	83
098_S_4002	F	74	098_S_2052	M	74	057_S_4888	M	75	027_S_4938	M	71
098_S_4003	F	72	098_S_2071	M	85	057_S_4909	F	78	027_S_4962	F	80

098_S_4018	M	76	099_S_4205	F	84	094_S_4162	F	71	027_S_4964	M	81
098_S_4050	M	77	099_S_4498	F	80	094_S_4295	F	70	052_S_5062	F	71
098_S_4275	M	73	109_S_2110	F	68	094_S_4630	F	66	094_S_4089	M	74
098_S_4506	M	72	109_S_2111	M	72.	109_S_4471	M	73	094_S_4737	F	74
099_S_4076	F	75	109_S_2200	F	76	109_S_4531	M	74	098_S_4201	F	64
127_S_4148	M	73	109_S_4380	M	72	126_S_4458	F	76	098_S_4215	M	82
127_S_4198	F	78	109_S_4455	M	64	126_S_4507	M	78	109_S_4378	M	80
127_S_4604	M	65	109_S_4594	M	62	126_S_4675	M	80	126_S_4494	M	71
127_S_4645	F	76	126_S_2360	M	64	126_S_4712	M	74	127_S_4749	F	78
127_S_4843	F	73	126_S_4891	M	60	126_S_4743	M	70	127_S_4992	F	64
129_S_0778	M	80	127_S_4301	M	75	126_S_4896	M	68	127_S_5028	M	62
129_S_4369	M	70	127_S_4624	F	78	127_S_4197	M	79	127_S_5056	M	85
129_S_4371	M	70	127_S_4765	M	76	127_S_4210	M	64	127_S_5058	M	62

129_S_4396	F	81	129_S_2347	M	73	127_S_4240	M	71	127_S_5067	M	81
131_S_0123	M	81	129_S_4220	F	73	129_S_4287	F	73	127_S_5095	M	66

557

558

559 **Table II: t-test and Chi^2 test across groups**

560 HC=Healthy controls, EMCI: Early mild cognitive impairment; LMCI=Late mild cognitive
561 impairment; AD= Alzheimer disease.

562

	HC VS EMCI		**HC VS LMCI**		**HC VS AD**	
	test value	*p-value*	*test value*	*p-value*	*test value*	*p-value*
Age (t-test)	0.0349	0.9722	0.5539	0.5814	0.2071	0.8365
Sex (Chi^2 test)	0.2338	0.6287	0.2338	0.6287	0.2338	0.6287

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567 **Table III: Further information about ADNI group classification.**

568 HC=Healthy controls, EMCI: Early mild cognitive impairment; LMCI=Late mild cognitive
569 impairment; AD= Alzheimer disease; LMIIS=Logical Memory II subscale; MMSE= Mini Mental
570 State Examination; CDR= Clinical Dementia Rating.

	HC	**EMCI**	**LMCI**	**AD**
LMIIS (maximum of 25 points)				
Education ≥16 years	≥ 9	[9-11]	≤ 8	≤ 8
Education [8-15] years	≥ 5	[5-9]	≤ 4	≤ 4
Education [0-7] years	≥ 3	[3-6]	≤ 2	≤ 2
MMSE (Maximum of 30 points)	[24-30]	[24-30]	[24-30]	[20-26]
CDR	0	0.5	0.5	0.5 or 1
Memory Box Score (subpart of CDR)	0	at least 0.5	at least 0.5	NA

571

572

573 **Table IV: p-values associated to each module from the brain hierarchical atlas.**

574 HC=Healthy controls, EMCI: Early mild cognitive impairment; LMCI=Late mild cognitive
575 impairment; AD= Alzheimer disease; * $0.01<p<0.05$; ** $0.005 < p < 0.01$: *** $p<0.005$.

576

Module	HC vs EMCI	HC vs LMCI	HC vs AD
1	0.956	0.753	0.023*
2	0.956	0.466	0.049*
3	0.956	0.441	0.049*
4	0.880	0.532	0.031*
5	0.859	0.689	0.973
6	0.859	0.438	0.546
7	0.956	0.900	0.503
8	0.859	0.449	0.031*
9	0.859	0.600	0.591

10	0.956	0.900	0.627
11	0.956	0.438	0.759
12	0.956	0.466	0.031*
13	0.859	0.600	0.531
14	0.956	0.438	0.006**
15	0.956	0.753	0.031*
16	0.956	0.986	0.031*
17	0.890	0.898	0.546
18	0.399	0.007**	0.002***
19	0.956	0.438	0.109
20	0.956	0.986	0.972

577

578

579 **Table V: Examples of pseudo F-statistics, between-group and within-group sum of squares.**
580 Three different situations: Node 10, that does not provide any significant change in pattern
581 connectivity; Node 16, significantly different in stage III; and Node 18, with pattern connectivity
582 significantly different in stages II and III.

583 HC=Healthy controls, EMCI: Early mild cognitive impairment; LMCI=Late mild cognitive
584 impairment; AD= Alzheimer disease

Module	**HC vs EMCI**			**HC vs LMCI**			**HC vs AD**		
	F	*SSA*	*SSW*	*F*	*SSA*	*SSW*	*F*	*SSA*	*SSW*
10	0.527	0.008	1.028	0.630	0.008	0.940	0.894	0.012	0.941
16	0.380	0.019	3.457	0.285	0.014	3.404	3.410	0.173	3.550
18	3.057	0.024	0.558	5.854	0.049	0.595	6.018	0.051	0.588

585

586

Figure 1: Details of group size and population demographic data. Alzheimer's disease progression is addressed across three stages. 4 groups of 36 subjects each show different stages of AD (Healthy, Early and Late MCI, Alzheimer) under ADNI criterion. All groups have been balanced with regard to age, sex and years of education. Brain connectivity patterns and its relation with disease progression is accomplished by comparing the healthy control group with the rest of groups, i.e. NC vs EMCI (bottom-left, stage I), NC vs LMCI (bottom-center, stage II) and NC vs AD (bottom-right, stage III).

595 **Figure 2: Schematic description of the method used to find brain modules connectivity patterns**
596 **that are statistically different between groups.** In a first step (top-red), brain images are prepared
597 for preprocessing by using standard techniques (Eddy current and correction for head motion) and
598 fibers connectivity matrices are computed by Continuous Tracking (FACT) algorithm. In the next
599 step (Distance matrix calculation in green), the connectivity patterns of subjects for a given module
600 are used to construct the distance matrix by means of Pearson correlation coefficients. Once the
601 distance matrix for a given module is calculated, we test (bottom-blue) whether the variability in
602 distance among different groups is statistically related with disease, for which we compare the
603 observed results with a simulated distribution given by N_perm permutations of the labels. We repeat
604 this operation for every module. We finally apply a multiple comparison correction to control the rate
605 of false discoveries (bottom-black)

606

Figure 3: Brain mapping of p-values corresponding to modules that become statistically different with regard to connectivity profile as disease progresses. Brain disconnection as disease progresses is quantitatively addressed by looking at the p-values of the modules. At first stages (HC vs EMC, top), fibers deterioration is not sufficient to yield significant changes in modules connectivity patterns. In a later stage (Control vs LMCI - middle), the connectivity pattern of module 18, which involves parts of the hippocampus, entorhinal cortex, amygdala and other memory-related areas, disconnects statistically with respect to healthy subjects (p-val = 0.007). Such connectivity differences are after spread to the rest of the brain at final stages (Control vs AD, bottom), showing a clear deterioration given by a stronger decrease in p-values.

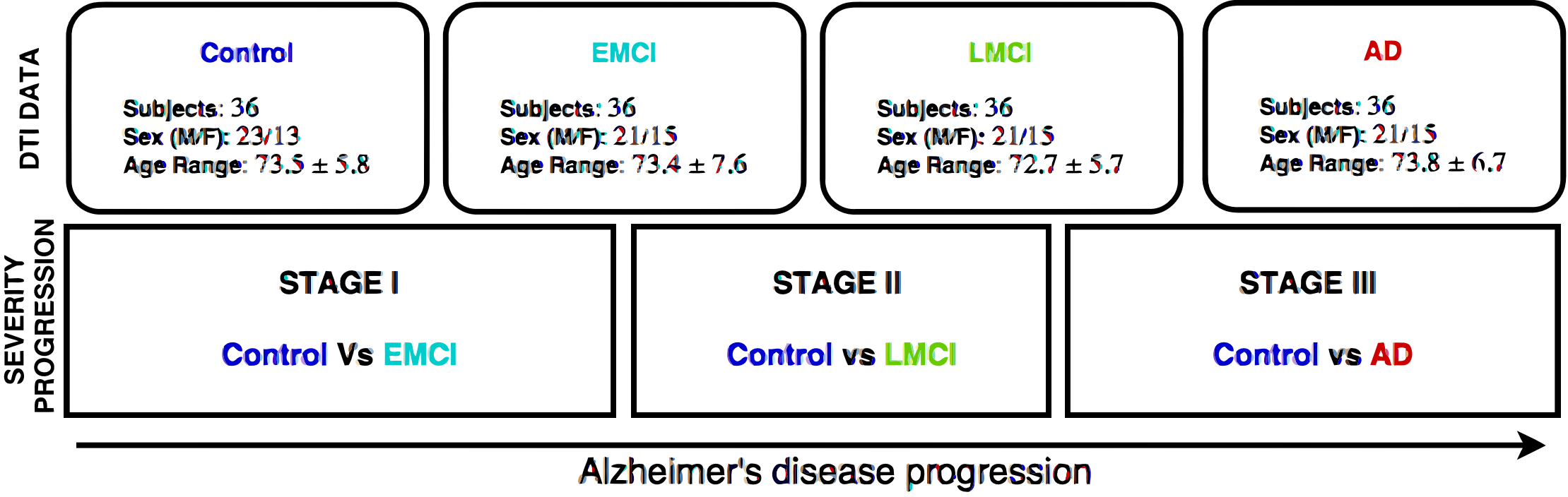
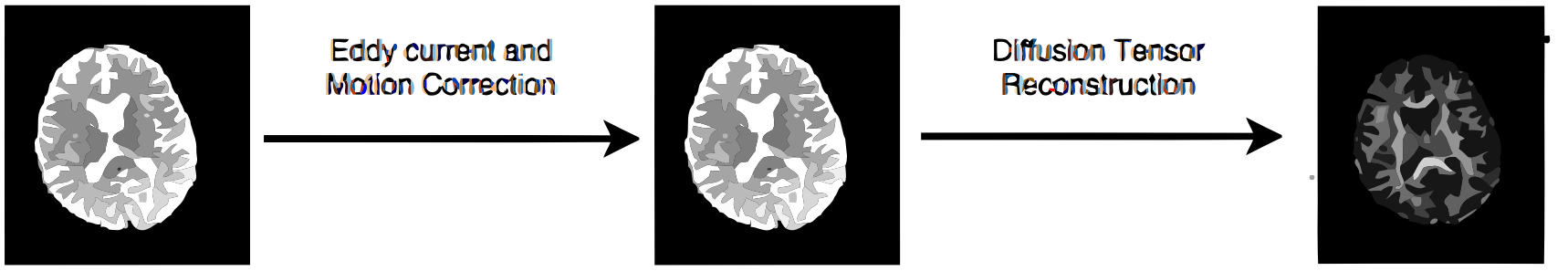
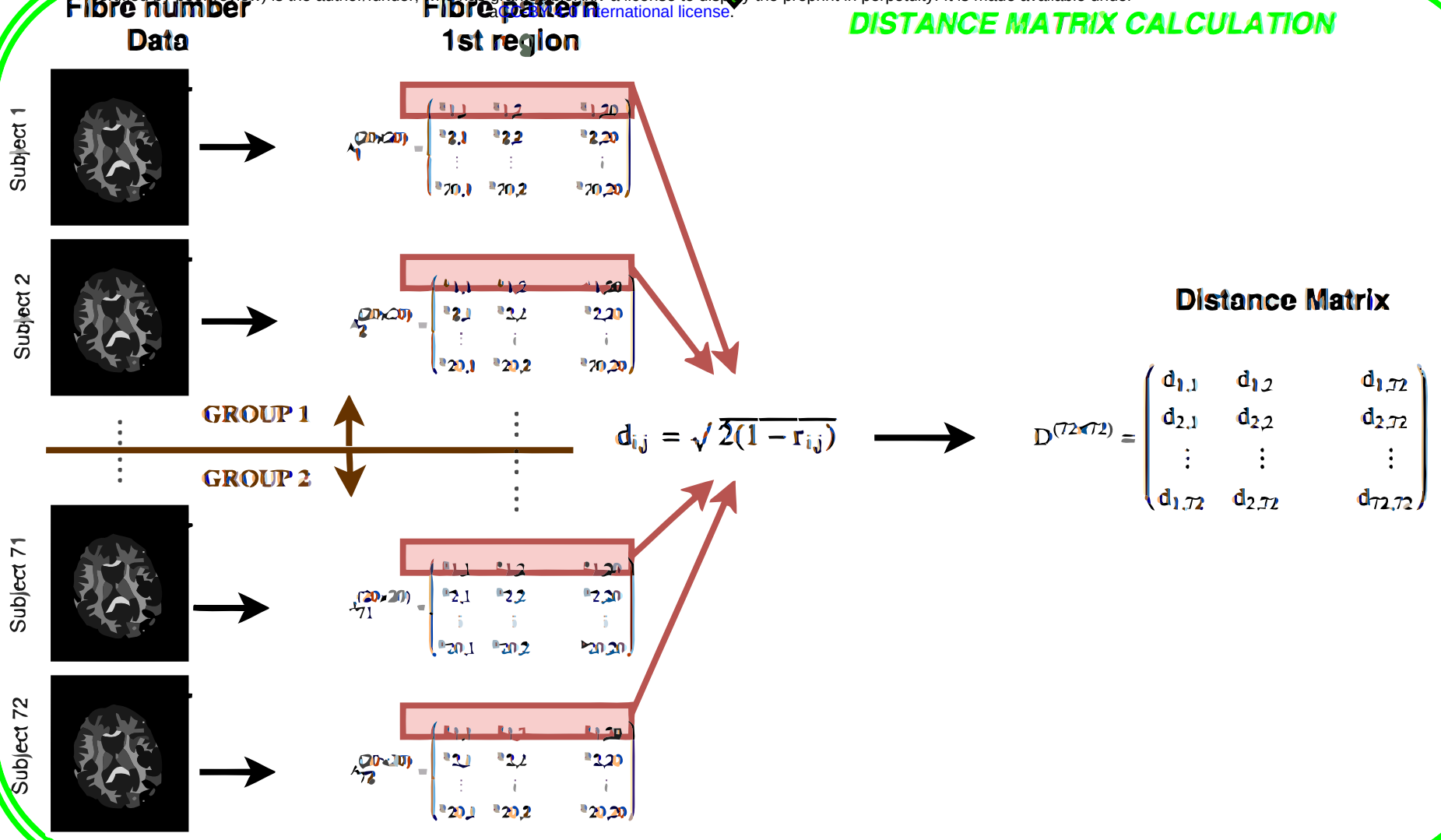

DTI DATA
Control
Subjects: 36
Sex (M/F): 23/13
Age Range: 73.5 ± 5.8
EMCI
Subjects: 36
Sex (M/F): 21/15
Age Range: 73.4 ± 7.6
LMCI
Subjects: 36
Sex (M/F): 21/15
Age Range: 72.7 ± 5.7
AD
Subjects: 36
Sex (M/F): 21/15
Age Range: 73.8 ± 6.7
SEVERITY PROGRESSION
STAGE I
Control Vs EMCI
STAGE II
Control vs LMCI
STAGE III
Control vs AD
Alzheimer's disease progression

IMAGE PREPROCESSING



DISTANCE MATRIX CALCULATION



REPEAT WITH ALL REGIONS

MULTIVARIATE REGRESSION

Pseudo F-stat measured:

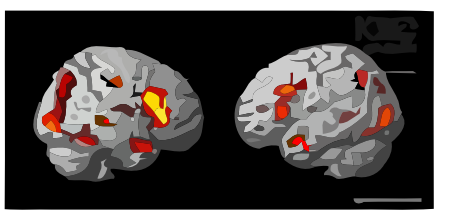
$$F \sim \frac{\sum_{i=1}^{N-1} \sum_{j=i+1}^{N} d_{ij}^2}{\sum_{i=1}^{N-1} \sum_{j=i+1}^{N} d_{ij}^2 \epsilon_{ij}} - 1$$

Recalculate F from permuted data

$$p_{val} = \frac{\#F_{permuted} > F}{N_{permutations}}$$

FALSE DISCOVERY RATE CORRECTIONS

$$FDR = < \frac{V}{V+S} > = < \frac{V}{R} >$$



Control Vs EMCI

NO significant modules

Control Vs LMCI

Module 18

P-vals (FDR corrected)

0.002
0.006
0.007
0.023
0.031
0.049

Control Vs AD

Module 18

Module 1

Module 2

Module 12

Module 3

Module 14

Module 4

Module 15

Module 8

Module 16