



Neuro-degeneration profile of Alzheimer's patients: A brain morphometry study



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ABSTRACT

Introduction: Alzheimer's disease (AD) is a primary and progressive neurodegenerative disorder, which is marked by cognitive deterioration and memory impairment. Atrophy of hippocampus and other basal brain regions is one of the most predominant structural imaging findings related to AD. Most studies have evaluated the pre-clinical and initial stages of AD through clinical trials using Magnetic Resonance Imaging. Structural biomarkers for advanced AD stages have not been evaluated yet, being considered only hypothetically.

Objective: To evaluate the brain morphometry of AD patients at all disease stages, identifying the structural neuro-degeneration profile associated with AD severity.

Material and methods: AD patients aged 60 years or over at different AD stages were recruited and grouped into three groups following the Clinical Dementia Rating (CDR) score: CDR1 (n = 16), CDR2 (n = 15), CDR3 (n = 13). Age paired healthy volunteers (n = 16) were also recruited (control group). Brain images were acquired on a 3T magnetic resonance scanner using a conventional Gradient echo 3D T1-w sequence without contrast injection. Volumetric quantitative data and cortical thickness were obtained by automatic segmentation using the Freesurfer software. Volume of each brain region was normalized by the whole brain volume in order to minimize age and body size effects. Volume and cortical thickness variations among groups were compared.

Results: Atrophy was observed in the hippocampus, amygdala, entorhinal cortex, parahippocampal region, temporal pole and temporal lobe of patients suffering from AD at any stage. Cortical thickness was reduced only in the parahippocampal gyrus at all disease stages. Volume and cortical thickness were correlated with the Mini Mental State Examination (MMSE) score in all studied regions, as well as with CDR and disease duration.

Discussion and conclusion: As previously reported, brain regions affected by AD during its initial stages, such as hippocampus, amygdala, entorhinal cortex, and parahippocampal region, were found to be altered even in individuals with severe AD. In addition, individuals, specifically, with CDR 3, have multiple regions with lower volumes than individuals with a CDR 2. These results indicate that rates of atrophy have not plateaued out at CDR 2–3, and in severe patients there are yet neuronal loss and gliosis. These findings can add important information to the more accepted model in the literature that focuses mainly on early stages. Our findings allow a better understanding on the AD pathophysiologic process and follow-up process of drug treatment even at advanced disease stages.

1. Introduction

Alzheimer's disease (AD) is a primary and progressive neurodegenerative disorder that results in cognitive impairment, memory deficits and increasing functional losses in patients (Blennow et al., 2006; Samanta et al., 2006). AD is the most common cause of dementia, accounting for 50% to 60% of cases (Blennow et al., 2006). The

worldwide prevalence of dementia due to AD is estimated to be around 1% for individuals between 60 and 64 years of age (Prince et al., 2015). In the occidental world, dementia prevalence increases exponentially with age, varying from 24 to 33% after the age of 84 years (Prince et al., 2015). The number of people aged over 60 years and suffering from dementia worldwide was estimated at 46,8 million in 2015 and this number is expected to double at every 20 years (Prince et al., 2015).

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Table 1
Demographic features of control and patient groups.

Feature/group	Controls	Patients CDR1	Patients CDR2	Patients CDR3
Sample size	16	16	15	13
Age, mean \pm std. (years)	78 \pm 4	80 \pm 4	82 \pm 4	82 \pm 5
Min. age (years)	71	71	75	75
Max. age (years)	85	87	90	89
Women (%)	63	44	67	85
MMSE score, mean \pm std. (points)	27 \pm 2	22 \pm 2	13 \pm 4	0
Disease duration, mean \pm std. (years)	0	2 \pm 1	7 \pm 3	12 \pm 1
Education, mean \pm std. (years)	7 \pm 4 ^a	6 \pm 5	5 \pm 5	3 \pm 3 ^a
Intracranial volume (l)	1.2 \pm 0.2	1.3 \pm 0.3	1.3 \pm 0.2	1.3 \pm 0.2

CDR: Clinical Dementia Rating. MMSE: Mini Mental State Exam.

^a Significantly with $p < 0.05$.

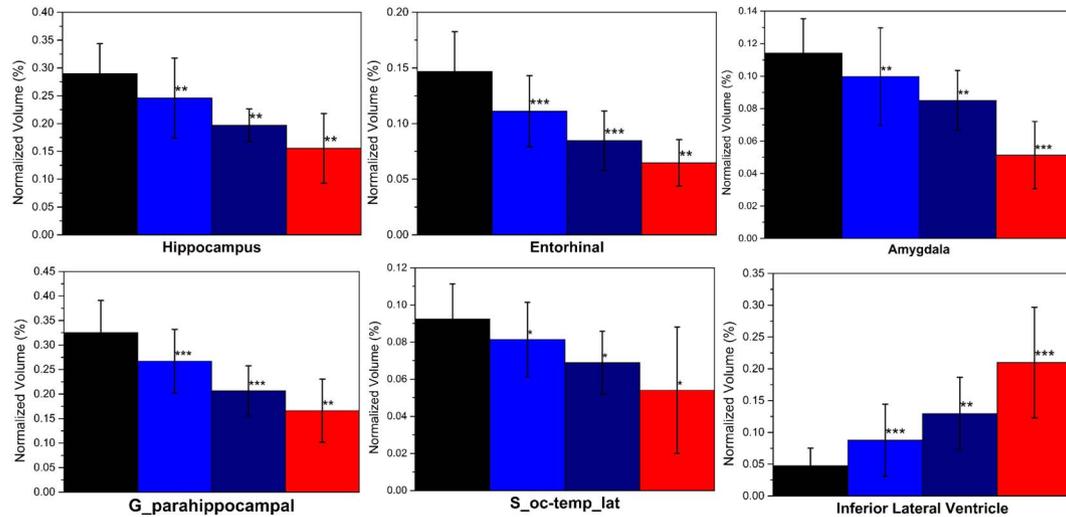


Fig. 1. Normalized brain volumes (mean \pm standard deviation) expressed as a percentage of intracranial volume for controls (black), CDR1 (light blue), CDR2 (dark blue) and CDR3 (red) patient groups, whose brain regions showed significant volume change in all AD stages (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Absolute brain region volume (mm^3) (mean \pm standard deviation) values of each group whose brain regions showed linear volume reduction towards AD progression.

Brain region	Controls	CDR1	CDR2	CDR3
Hippocampus	3480(458)	2953(556)	2473(334)	2034(605)
Entorhinal	1779(346)	1383(444)	1076(377)	867(289)
Amygdala	1376(212)	1204(260)	1069(222)	681(239)
Parahippocampal gyrus	3957(701)	3314(912)	2601(639)	2200(677)
Occipital temporal lateral	1134(265)	1003(261)	896(220)	687(310)

Although these statistics have increased the public awareness and improved the quality of health services, population aging tends to increase considerably the disease incidence. Economic and social burden turn AD into a grave public health issue, with urgent necessity for effective methods to understand its pathophysiology, thereby allowing the development of better treatments (Cummings, 2004).

The exact pathophysiology of AD is not well understood, although the amyloid cascade theory has been largely accepted. This theory proposes the precipitation of beta-amyloid proteins and formation of extracellular plaques, consequently leading to inflammatory processes and ultimately to cognitive deficits (Cummings, 2004; de Vrij et al., 2004; Morishima-Kawashima and Ihara, 2002). Other anatomo-pathological alteration of AD is the presence of neurofibrillary tangles, mainly composed of hyperphosphorylated tau protein (Blennow et al., 2006; Braak et al., 2011; Cummings, 2004). Association of these components starts a cascade of events, contributing to excitotoxicity, neuroinflammation and dysfunction of protein degradation mechan-

isms (Braak et al., 2011; Jagust et al., 2008). The order by which these events occur is yet not fully understood, and controversial evidences regarding the relationships between their causes and effects can be found in literature (Armstrong, 2013; Blennow et al., 2006). The progressive accumulation of abnormal proteins has led to the extensive investigation of imaging markers that could identify early accumulation of protein metabolites. In this context, developing biomarkers is an active research area with progress in the neuroimaging field and radiopharmaceutical analysis of β -amyloid ($A\beta$) or tau protein in cerebrospinal fluid (CSF). However, there is no current gold-standard for the clinical practice yet, and this fact highlights a lack of validation and standardization by wider studies (Ballard et al., 2011; Blennow et al., 2006). These tests would be important for detecting AD at early stages, anticipating diagnosis and treatment as well as monitoring therapeutic responses.

Some anatomical changes detected by magnetic resonance imaging (MRI) are considered fair indicators for initial changes at individual levels (Jack et al., 2013; Jagust et al., 2008). Several studies based on MRI have shown that volumetric reductions of hippocampus, entorhinal cortex, posterior cingulate gyrus, amygdala and parahippocampal gyrus are indicators of early AD (Basso et al., 2006; Bottino et al., 2002; Csernansky et al., 2005; Devanand et al., 2007; Du et al., 2001; Pennanen et al., 2004; Spulber et al., 2013; Stoub et al., 2005; Uotani et al., 2006; Wolf et al., 2004; Xu et al., 2000). The measurement of cortical thickness by MRI also may reveal reduction of these and other regions, such as medial temporal, parietal and frontal lobes (Blanc et al., 2015; Dickerson et al., 2009; Lerch, 2004; O'Brien et al., 2014). Therefore, MRI shows initial reduction at the parahippocampal gyrus,

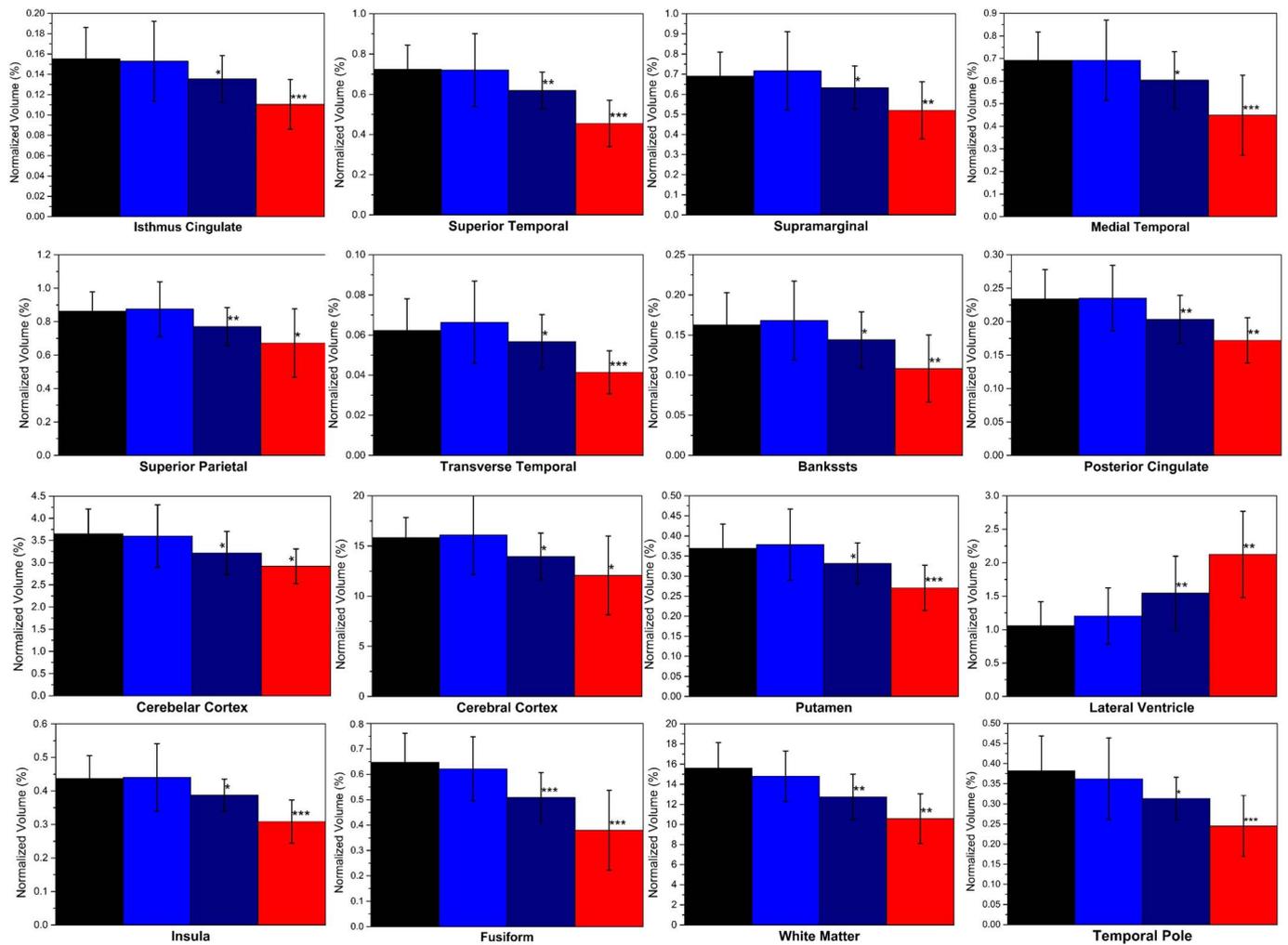


Fig. 2. Normalized brain volumes (mean ± standard deviation) expressed as a percentage of the intracranial volume for controls (black), CDR1 (light blue), CDR2 (dark blue) and CDR3 (red) patient groups, whose brain regions showed significant volume change in all AD stages, except CDR1 patients compared to the control group (*p < 0.05; **p < 0.01,***p < 0.001). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

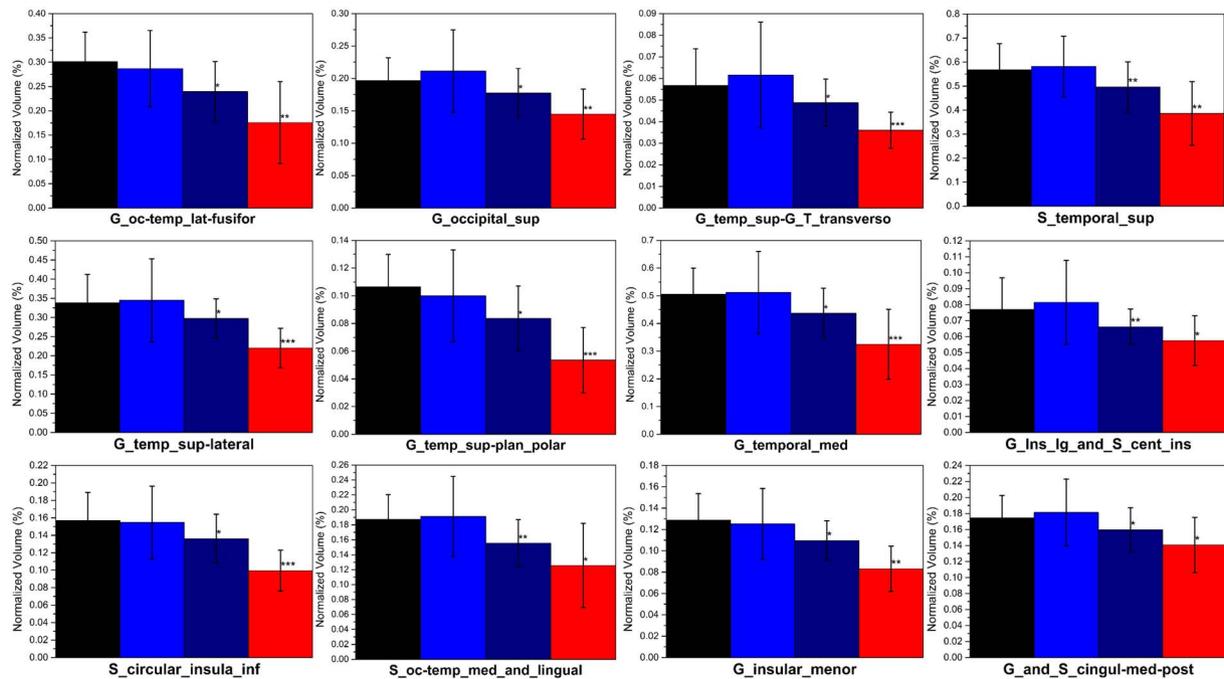


Fig. 3. Continuing Fig. 2.

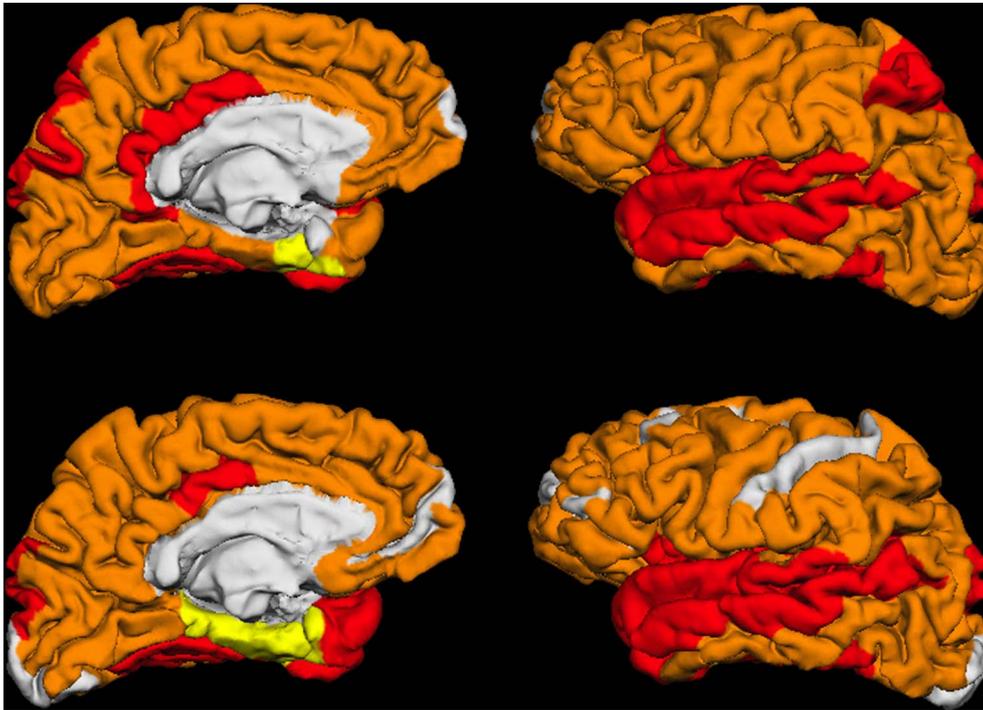


Fig. 4. Brain areas with significant atrophy over AD progression (A, B) segmentation by lobe atlas (Desikan et al., 2006) and (C, D) sulci and gyri atlas (Destrieux et al., 2010)). Colors represent regions with significant volumetric reduction ($p < 0.05$) among groups (yellow), except for control and CDR1 (red), and control, CDR1 and CDR2 (orange) [color should be used]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Multiple linear regression coefficients between brain region volumes and explanatory variables.

Regions	Intercept	MMSE slope	Age slope	Education slope	Gender slope	R ²
Hippocampus	0.4196 ± 0.1156	0.0045 ± 0.0007	−0.0030 ± 0.0014	−0.0025 ± 0.0013	−0.0355 ± 0.0139	0.35
Entorhinal	0.0926 ± 0.0558	0.0023 ± 0.0004	−0.0003 ± 0.0006	−0.0007 ± 0.0006	−0.0119 ± 0.0067	0.32
Amygdala	0.1621 ± 0.0502	0.0022 ± 0.0003	−0.0013 ± 0.0006	−0.0009 ± 0.0006	−0.0099 ± 0.0060	0.41
Inf. lat. ventricle	−0.2025 ± 0.1372	−0.0052 ± 0.0008	0.0049 ± 0.0017	−0.0001 ± 0.0002	0.0166 ± 0.0165	0.39
Parahippocampal gyrus	0.3627 ± 0.1263	0.0046 ± 0.0008	−0.0024 ± 0.0016	−0.0014 ± 0.0015	−0.0139 ± 0.0151	0.32
Occipito-temporal sulcus	0.0927 ± 0.0498	0.0015 ± 0.0003	−0.0004 ± 0.0006	−0.0007 ± 0.0005	−0.0079 ± 0.0059	0.19

followed by a widespread pattern of volume reduction involving the temporal medial lobe and posterior parietal region (Busatto et al., 2008; Jacobs et al., 2012; Karas et al., 2004; Tondelli et al., 2012). Large numbers of cross-sectional and a small number of longitudinal studies (Jack et al., 2000) have shown increased rate of atrophy (3.5% annually) of the hippocampus in patients with AD, relative to controls (1.7% annually) (Albert et al., 2004). In another study, the atrophy rate for the entorhinal cortex ($7.1 \pm 3.2\%/year$) was higher than that of the hippocampus ($5.9 \pm 2.4\%/year$) (Du et al., 2004). In later AD stages, atrophy progresses to the prefrontal cortex (Scahill et al., 2002). In general, structural MRI exams are considered biomarkers of AD progression according to the International Working Group (Dubois et al., 2014). MRI based volume measurements are correlated with Braak stage and neuronal counts (Bobinski et al., 2000; Jack et al., 2002).

Most studies on the evolution of brain atrophy in AD have focused on the earlier disease stages, i.e. the transition from health to the first phase of AD (Mueller et al., 2005). Neuroimaging studies of AD patients in later stages (CDR 3), to the best of our knowledge, have not been reported yet and could reveal details on the disease progression regarding neuronal degeneration and the follow-up of therapeutic approaches. Only theoretical models have been proposed to describe the full trajectories of different biomarkers for AD (Jack et al., 2013, 2012). This research evaluated the brain morphometry variations in AD patients from CDR 1 to CDR 3. The structural profile associated with AD

severity up to later disease stages was also identified.

2. Material and methods

2.1. Subjects

Subjects gave written informed consent, and this study was approved by the Ethics Committee of the Clinics Hospital - Ribeirão Preto Medical School (HCRP 9613-15). In case of participants with impaired autonomy and judgment due to AD, the consent form was read and signed by their carers. Controls and patients aged 60 years or older and from both genders, were included prospectively during 2015–2016 ($n = 44$). AD patients were followed by the Geriatrics Service of the Ribeirão Preto Medical School. The control group was composed of cognitively normal individuals paired by age ($n = 16$). Participants of this group were recruited from the university community, like, for instance, participants of physical activities for the third age. Inclusion criteria for the control group were having no memory complaints, no functional impairments and MMSE score above 25 for participants with at least four years of formal education and above 27 for participants with 8 years of formal education or over (Brucki et al., 2003).

The diagnosis of probable Alzheimer's disease was made using Diagnostic and Statistical Manual for Mental Disorders V. The risk of circularity was avoided considering the natural history of the patient

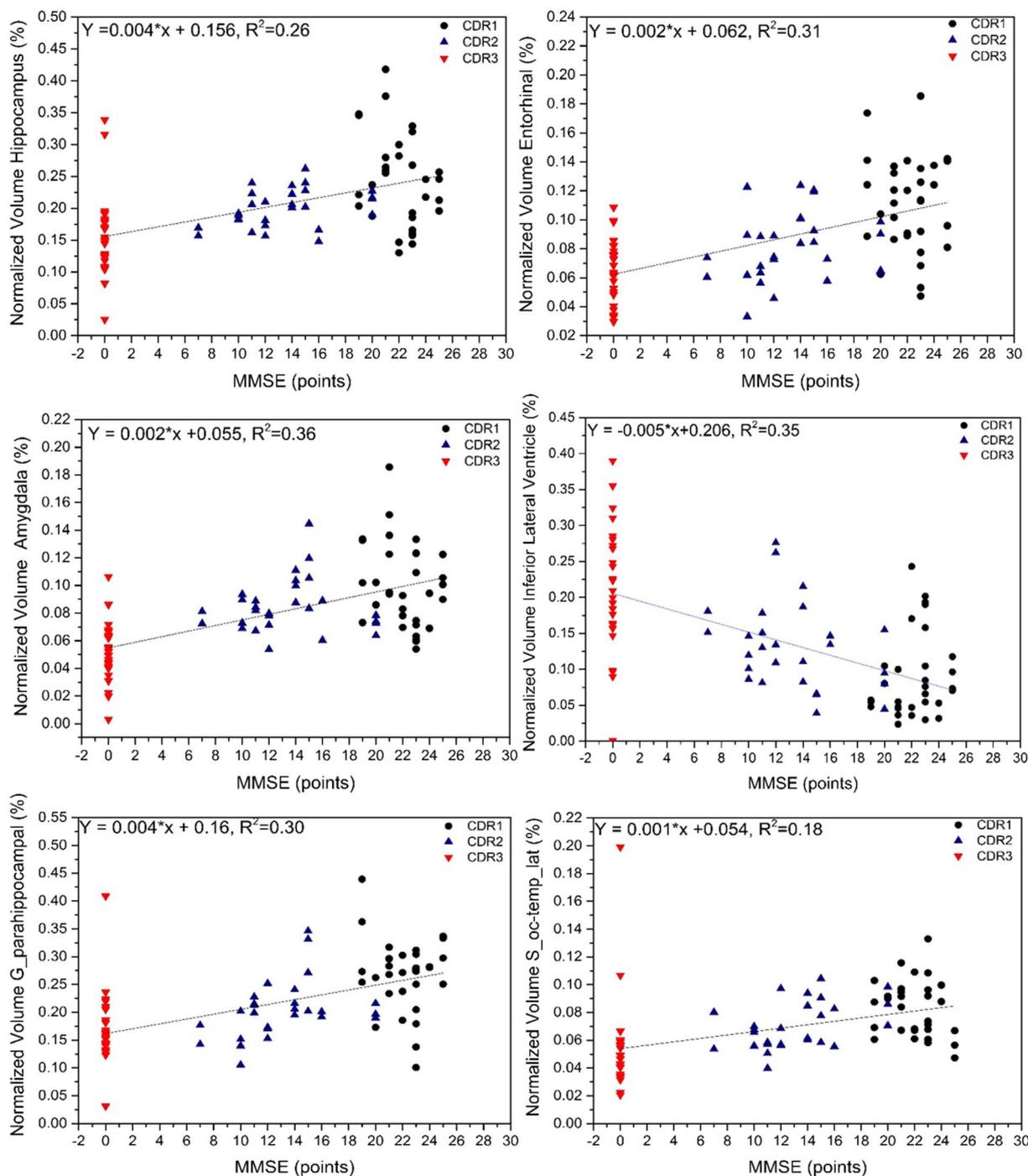


Fig. 5. Linear correlation between normalized volume and MMSE of each individual subject. Both sides included all groups indicated at the legend. Regression coefficients are inserted in the equation of each plot.

and the independent MRI exam evaluation of by two different physicians. Subjects who presented Fazekas scale (Fazekas et al., 1987) above 2 in a FLAIR MRI, even though they did not present clinical vascular dementia, were not included in the study. The following tests were applied for the exclusion of secondary causes of dementia: serology for HIV and syphilis, liver function, serum levels of Vitamin B12, Folic acid, calcium, TSH, complete blood count, renal function, electrolytes and fasting glycemia. Cognitive function was evaluated by the application of the Mini Mental State Exam (MMSE) (Folstein et al., 1975) and Clinical Dementia Rating (CDR). Using the sum of boxes of CDR test, patients were classified into low (CDR1), moderate (CDR2) and high AD severity (CDR3) (O'Bryant et al., 2008).

2.2. Image acquisition and processing

Images from all participants were acquired on a 3 T MRI (Philips Achieva) equipment. An 8-channel head coil was used to perform T1-weighted gradient echo sequences. Patients exhibiting severe AD were sedated by intravenous propofol drip infusion (4–7 mg/kg/h) for image acquisition. The following parameters were used in the acquisition of T1-weighted sequence: TR = 7 ms, TE = 3.2 ms, flip angle = 8°, FOV = 240 × 240 mm², in-plane resolution = 0.9 × 0.9 mm², slices = 170, and slice thickness = 1 mm. The time for structural image acquisition was 4 min and 27 s. No contrast agent was used in any subject.

After detailed visual inspection for the detection of motion artifacts

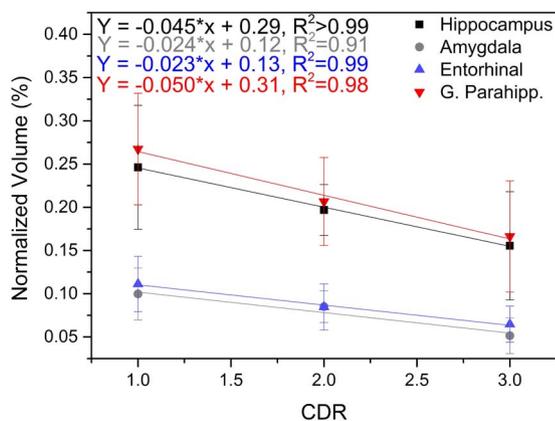


Fig. 6. Linear correlation between normalized volume and clinical dementia rate (CDR) values averaged by group. Regression coefficients are inserted in the equation of each plot.

or morphologic brain alterations that would exclude the participant from the study, volume and cortical thickness were measured automatically by the application of the Freesurfer version 5.3.0 image analysis suite, which is freely available for downloading (<http://surfer.nmr.mgh.harvard.edu/>). Images were batch processed, thus lessening repetitive work and decreasing error probability, once image processing was performed independently from the operator. Image processing included skull stripping, intensity normalization, tissue classification (parcellation), and cortical thickness and volume determinations. The intermediate step in this analysis, the automatic parcellation, relies on an intrinsic coordinate system, which stores a-priori statistics and class-conditional densities related to each location over the cortical surface. A reference set of 3D meshes, representing the manual parcellation made by specialists, allows incorporating an anatomical convention in the parcellation, especially in brain regions whose geometry is not predictive for parcel's tag. Thus, new individual brains are “warped” into the standard space and new subject's brain inherits the tags of the previously established model (Fischl et al., 2004).

Quality of segmentation and parcellation of individual brain volumes were checked independently by two specialized radiologists. No parcellation needed to be excluded or manually repaired. Quantitative volume and cortical thickness data were obtained for regions defined by two atlases comprising two brain subdivision types: the Desikan brain atlas (Desikan et al., 2006) for large areas such major brain lobes (Fig. 3) and the Destrieux brain atlas (Destrieux et al., 2010), which further divides the lobes into sulci and gyri regions (Fig. 4).

2.3. Statistical analysis

Volume of each brain region was normalized considering the total

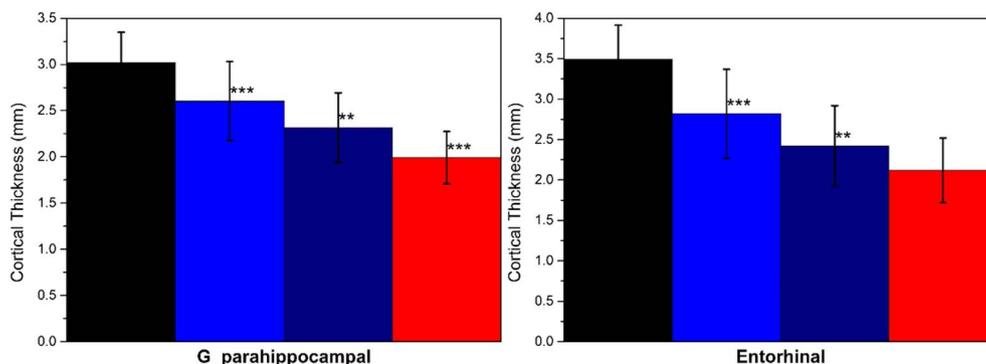


Fig. 7. Cortical thickness values (mean ± standard deviation) for bilateral regions that showed significant reduction in all AD phases (left) and the same with exception between CDR2 and CDR3 (right) (**p < 0.01; ***p < 0.001).

intra cranial volume so that inter-individual variations in head size were minimized (Whitwell et al., 2001). The estimated intra cranial volumes (eTIV) were automatically calculated by Freesurfer recon-all script, considering the volume-scaling factor derived by registration of each individual to the atlas template. Results regarding total intracranial volumes are presented in Table 1. One-way ANOVA was used to test for differences between groups with respect to age, years of education, disease duration and MMSE score (p < 0.05). Mean volume and cortical thickness with respective standard deviation were calculated for each group and brain region, and tested by one-way ANOVA (p < 0.05, uncorrected). Both simple and multiple linear regression models were used to verify the presence of correlation of independent variables like age, years of formal education, sex and MMSE with the dependent variable normalized volume or cortical thickness. Differences between groups were visualized by overlaying color scales on 3D brain mesh representations.

3. Results

Table 1 shows the demographic characteristics of the participants. Age was not significantly different between groups (p > 0.05). There were differences between groups with respect to years of education only between controls and CDR3. The intracranial volumes of each group are listed in Table 1.

Brain volume atrophy of patients suffering from AD at any stage was observed in the hippocampus, amygdala, entorhinal cortex, parahippocampal region, temporal pole and temporal lobe (Fig. 1). The absolute brain volumes for these cited regions are listed in Table 2.

Significant differences between CDR1, CDR2 and CDR3, but with no differences between the CDR1 and the control groups are showed on Figs. 2–3. The total brain volume was not significantly reduced over the AD progression, however white and gray matter volumes of CDR2 and CDR3 groups were markedly low (Fig. 2). Other brain regions presented significantly reduced volume only for patients in the CDR3 group (supplementary information - A). All brain regions showing significant differences between groups are represented in Fig. 4.

High correlations were observed between normalized volume with multiples parameters as MMSE score, age, education level and gender for hippocampus, entorhinal, amygdala, inferior lateral ventricle, parahippocampal gyrus and lateral occipito-temporal sulcus (Table 3). Age, education and gender variables showed high slope error, suggesting their fewer influence for these subjects.

Moderate linear correlation was observed between normalized volume and MMSE score for regions that decreased significantly in all stages of AD (R² < 0.36, Fig. 5). High linear correlation was observed between normalized volume and CDR values averaged by group for some regions (Fig. 6).

Figs. 7–8 show the average cortical thickness values and standard deviations of each group. Fig. 7 - left corresponds to brain regions that

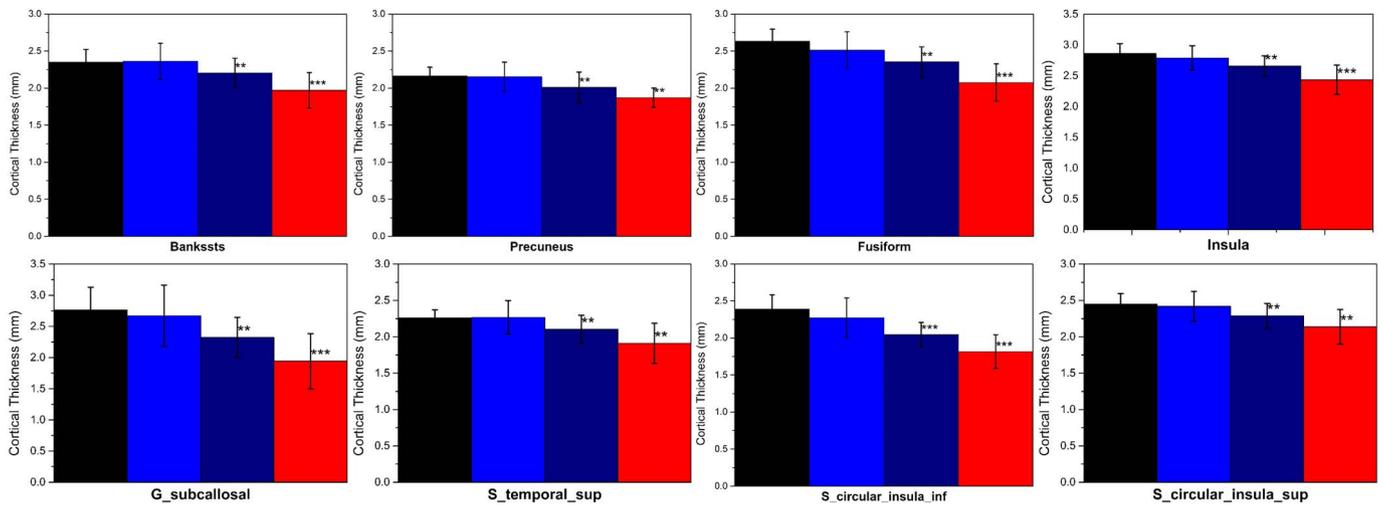


Fig. 8. Cortical thickness values (mean ± standard deviation) for bilateral regions that showed significant reduction in all AD phases, except between controls and CDR1 (*p < 0.01; ***p < 0.001).

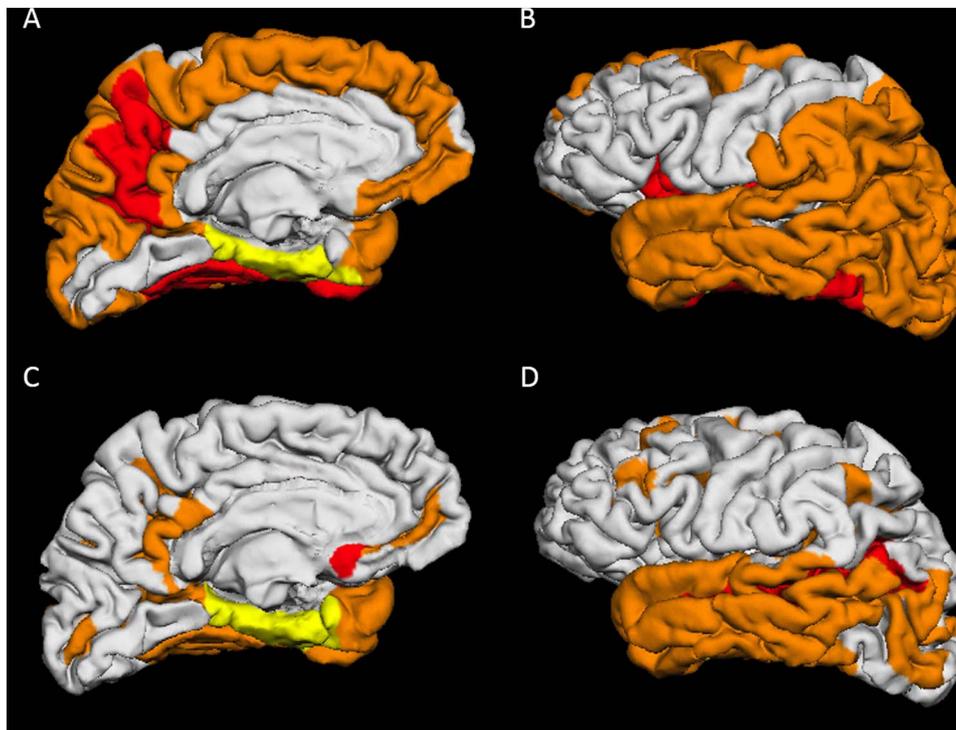


Fig. 9. Brain regions with cortical thickness reduction over the progression of AD. (A, B) Segmentation by considering Desikan template (Desikan et al., 2006) and (C, D) sulci and gyri Destrieux template (Destrieux et al., 2010). Colors represent regions with significant volumetric reduction (p < 0.01, uncorrected) among subject groups (yellow), except between control and CDR1 (red), and CDR1 and CDR2 (orange) [color should be used]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

presented significant cortical thickness reduction for all AD stages. The same trend is true for the entorhinal cortices, with exception of the difference between CDR2 and CDR3 groups (Fig. 7 - right). Fig. 8 displays brain regions with significant differences between CDR1, CDR2 and CDR3, but with no differences between CDR1 and control groups.

Other regions exhibiting significant cortical thickness reduction for specific groups have been included in supplementary information (B). Fig. 9 presents graphically the regions exhibiting significant cortical thickness reduction over AD progression.

For the parahippocampal gyrus, multiple linear regression showed only MMSE as an explanatory variable correlated with cortical thickness ($Y = 2.676 \pm 0.774 + 0.027 \pm 0.005 * (\text{MMSE}) - 0.009 \pm 0.009 * (\text{age}) - 0.001 \pm 0.009 * (\text{education}) - 0.033 \pm 0.093 * (\text{gender})$,

$R^2 = 0.30$). Moderate and high linear correlation was observed between cortical thickness of parahippocampal gyrus and MMSE ($R^2 = 0.32$) and CDR ($R^2 = 0.997$) average values by group, respectively (Fig. 10).

4. Discussion

Volumetric reduction of hippocampus, entorhinal cortex, posterior cingulate, amygdala and parahippocampal gyrus are indicators of early AD stages (Basso et al., 2006; Bottino et al., 2002; Csernansky et al., 2005; Devanand et al., 2007; Du et al., 2001; Penanen et al., 2004; Stoub et al., 2005; Uotani et al., 2006; Wolf et al., 2004; Xu et al., 2000). Studies have reported early hippocampal atrophy already in mild cognitive impairment (MCI), indicating that the atrophy rate in

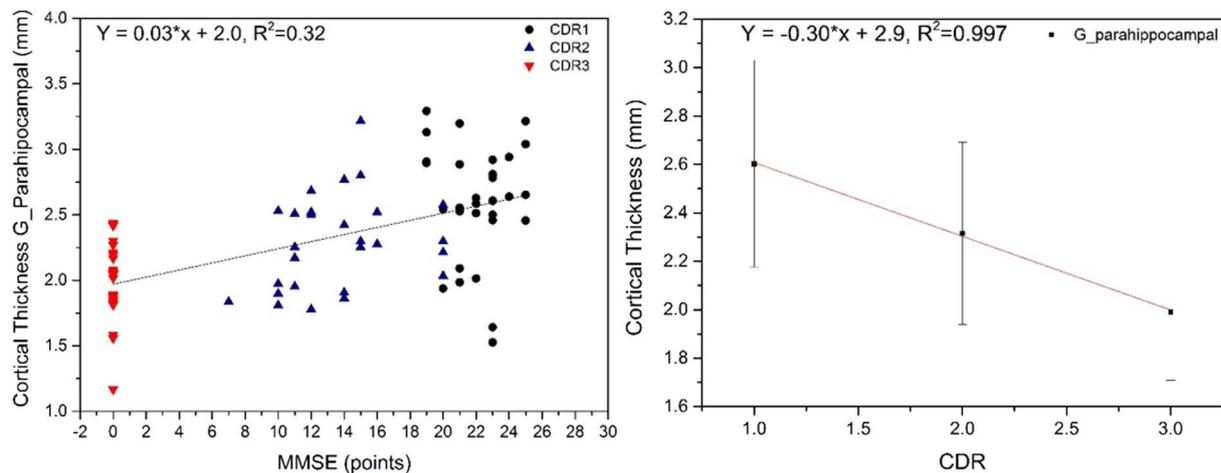


Fig. 10. Linear correlations between absolute cortical thickness and Mini Mental State Exam (MMSE) (left) and between average cortical thickness values average by group (with standard deviation) and clinical dementia rate (CDR). Regression coefficients are inserted in the equation of each plot.

this region could identify patients suffering from MCI that would be further converted to AD (Spulber et al., 2013). Other studies evaluated early and moderate AD patients and observed that the volumetric reduction is extended to frontal, parietal and temporal neocortex (Caramelli et al., 2011). These studies did not evaluate patients suffering from advanced AD stages. Such information could unveil details on the disease progression regarding neuronal degeneration and the follow-up of therapeutic approaches.

As previously reported (Basso et al., 2006; Bottino et al., 2002; Csernansky et al., 2005; Devanand et al., 2007; Du et al., 2001; Pennanen et al., 2004; Stoub et al., 2005; Uotani et al., 2006; Wolf et al., 2004; Xu et al., 2000), brain regions affected by AD during its initial stages, such as hippocampus, amygdala, entorhinal, parahippocampal regions, temporal pole and temporal lobe, were found in this work to be significantly altered, by ANOVA test, even in individuals with severe AD (CDR 3) (Figs. 1–3). These results indicate that rates of atrophy have not plateaued out at CDR 2–3, and in severe patients there is yet neuronal loss and gliosis. In addition, individuals, specifically, CDR 3, have multiple regions with lower volumes than individuals CDR 2 (Fig. 4). Moreover, normalized volume reduction was correlated mainly with AD progression (Table 3 and Figs. 5–6). Other variables like age, education and gender showed high slope error (Table 3), suggesting their lower influence for these subjects. These findings can add important information to the more accepted model in the literature that focused on early disease stages (Jack et al., 2013). The authors suggested that the atrophy quantified by MRI would be significantly larger at early AD stages and presented a plateau for severe patients. However, their patient group did not include those with extremely low MMSE values, which hindered inferences on the full AD trajectory.

Our results suggest that the volumetric atrophy (Fig. 6) begins at the parahippocampal gyrus (the entorhinal cortex), and has a widespread pattern of volume reduction involving the temporal medial lobe (hippocampus) and posterior parietal region (posterior cingulate) in agreement with literature (Busatto et al., 2008; Jacobs et al., 2012; Karas et al., 2004; Scahill et al., 2002; Tondelli et al., 2012). In the later AD stages, atrophy affects the prefrontal cortex in agreement with an experimental study with moderate AD patients (Scahill et al., 2002). The hippocampus and parahippocampal regions showed high linear correlation between average volume and cortical thickness, and CDR rates ($R^2 > 0.996$) (Figs. 6 and 10). The average volumetric reduction rate per CDR for hippocampus was 0.045%. The average thickness reduction rate for the parahippocampal gyrus was 0.30 mm per CDR. However, these results were gathered from an average of the group, and thus cannot be extrapolated for individual patients. Without a longitudinal study, it is impossible to know whether atrophy rates accelerate or decelerate in the later stages and we cannot explain why many

people with AD do not survive for 12 years.

Analysis of variance indicated that only the thickness of parahippocampal gyrus significantly decreased in all AD progression (Fig. 7) stages, in accordance with previous reports (Blanc et al., 2015; Lerch, 2004). Other regions, including the temporal, parietal and frontal lobes (Dickerson et al., 2009) showed cortical thickness reduction only at moderate and advanced stages of AD (Figs. 7–9). One fact that may explain the discrepancies between our results and those reported in literature (Blanc et al., 2015; Clerx et al., 2013; Dickerson et al., 2009; Lerch, 2004; O'Brien et al., 2014) are the number of images acquired and processing method used. Dickerson et al. (2009) acquired multiple images ($n = 4$) of the same patient to improve the contrast-to-noise ratio and the quality of segmentation (Dickerson et al., 2009). For the segmentation of a single image, Blanck and collaborators corrected manually the segmentation of 25% of the subjects (Blanc et al., 2015). The automatic segmentation is prone to error mainly at the borders of anatomical regions. Cortical thickness relies on border definition between gray and white matter and is even more dependent on the segmentation quality. In turn, segmentation quality depends on contrast-to-noise ratio, type of smoothing filter and segmentation parameters, with potential to add an error of 0.25 mm on cortical thickness values (Fischl, 2012). Lastly, acquisition of several images and manual segmentation protocols are time-consuming procedures and may lead to human errors due to fatigue.

Even though AD mainly affects individuals above 65 years, we found considerable slope error for the age variable on multiple linear regression of volumetric atrophy (Table 3). This strengthens the hypothesis that atrophy occurs by AD pathophysiological process and not only with advancing age. Also, the variable of years of education showed the same behavior (Table 3). The education time of all subjects ranged predominantly between 0 and 6 years, different from the duration of formal education of European or North American samples. The consequences of a lower educational level in this study may have implications in the “cognitive reserve” usually regarded as an important trait that enables the individual to better tolerate cognitive abnormalities in late stages of the disease. Considering that MMSE was the main variable correlated on multiple linear regression with volumetric atrophy, we suggest that duration of formal education can be considered a proxy of cognitive reserve but not exclusively so. From this point of view, an alternative for years of formal education would be literacy, which would also provide some kind of cognitive reserve (Stern, 2012).

Our results advance one step further in relation to Braak et al. (2011) results by pinpointing areas that were spared on the massive degeneration of the CDR 3 stage. Even though the CDR 3 group was not a large group ($n = 13$), we identified areas that do not change volume significantly even after a decade of disease onset. The analysis of both

Desikan and Destrieux templates allowed us to observe details about spared areas like the frontomarginal gyrus, inferior occipital gyrus, subcentral gyrus, anterior cingulate gyrus, inferior frontal orbital gyrus, inferior triangular frontal gyrus, medial frontal gyrus, lingual gyrus, orbital gyrus, superior parietal gyrus, postcentral gyrus, rectus gyrus, subcallosal gyrus (see supplemental material). The preservation of these areas may represent a background for palliative care, improving wellbeing in later stages of life.

This study has some limitations. First, the number of subjects was relatively small given the difficulty to find healthy volunteers or patients suffering from “pure” AD. Several AD patients also presented concomitant microangiopathy, which led to their exclusion. Second, the proportions of males/females between groups were unavoidably unbalanced. Previous studies indicate that women may be at higher risk of developing severe disability than men in the advanced ages due to their longer survival (von Strauss et al., 2003). Third, education level of subjects was lower than subjects evaluated by other studies (i.e. Jack et al., 2013), and this may be an explanation for the difference at neurodegeneration profile showed by Jack et al., 2013, that needs to be evaluated on future studies. Other limitation for recruiting patients, especially for the severe AD group, was the necessity of sedation during image acquisition. Ideally, the study should have a longitudinal design in order to follow the same patient over each AD phase. However, this would be impractical due to the variability in disease duration and survival among the individual subjects.

5. Conclusion

The most affected brain regions suffer atrophy without plateau phase until the later AD stages (CDR 2–3). These findings can add important information to the more accepted model in the literature that focuses mainly on early stages. Our findings permit a better understanding of the AD pathophysiology and the possible follow-up of drug effects that could modify the disease even during its late stages.

Contributions

S.R.B.S.F., J.H.O.B., C.R., A.C.S., C.E.G.S., N.K.C.L., E.F. and J.C.M. contributed to study design, supervision and interpretation of the experiments. S.R.B.S.F., A.C.S. and J.C.M. collected data. S.R.B.S.F. and J.H.O.B. analyzed the experiments. S.R.B.S.F. and J.H.O.B. and C.R. wrote the manuscript.

Conflicts of interest

The authors declare no competing financial interests. We confirm that no non-financial conflicts of interest exist for any of the authors.

Ethics approval and consent to participate

This research protocol and the statement of consent were approved by Clinics Hospital - Ribeirão Preto Medical School, in accordance with the Helsinki Declaration (Process HCRP no 9613/2015). All participants were informed about the purpose of this study. In the case of individuals with impaired autonomy and judgment due to AD, the consent form was read and signed by their carer.

Consent to publish

Written informed consent was obtained from the patient, their carers, or controls participants for anonymous publication of their individual details and accompanying images in this manuscript. The consent form is held by the authors and is available for review by the Editor-in-Chief.

Consent for publication

All authors have reviewed the contents of the manuscript being submitted, approve of its contents and validate the accuracy of the data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.nicl.2017.04.001>.

References

- Albert, M., DeCarli, C., DeKosky, S., de Leon, M., Foster, N.L., Fox, N., Frank, R., Frackowiak, R., Jack, C., Jagust, W.J., Knopman, D., Morris, J.C., 2004. No Title .
- Armstrong, R.A., 2013. What causes alzheimer's disease? *Folia Neuropathol.* 51, 169–188.
- Ballard, C., Gauthier, S., Corbett, A., Brayne, C., Aarsland, D., Jones, E., 2011. Alzheimer's disease. *Lancet* 377, 1019–1031. [http://dx.doi.org/10.1016/S0140-6736\(10\)61349-9](http://dx.doi.org/10.1016/S0140-6736(10)61349-9).
- Basso, M., Yang, J., Warren, L., MacAvoy, M.G., Varma, P., Bronen, R.A., van Dyck, C.H., 2006. Volumetry of amygdala and hippocampus and memory performance in Alzheimer's disease. *Psychiatry Res.* 146, 251–261. <http://dx.doi.org/10.1016/j.psychres.2006.01.007>.
- Blanc, F., Colloby, S.J., Philippi, N., de Pétigny, X., Jung, B., Demuyne, C., Philippis, C., Anthony, P., Thomas, A., Bing, F., Lamy, J., Martin-Hunyadi, C., O'Brien, J.T., Cretin, B., McKeith, I., Armspach, J.-P., Taylor, J.-P., 2015. Cortical thickness in dementia with Lewy bodies and Alzheimer's disease: a comparison of prodromal and dementia stages. *PLoS One* 10, e0127396. <http://dx.doi.org/10.1371/journal.pone.0127396>.
- Blennow, K., de Leon, M.J., Zetterberg, H., 2006. Alzheimer's disease. *Lancet* 368, 387–403. [http://dx.doi.org/10.1016/S0140-6736\(06\)69113-7](http://dx.doi.org/10.1016/S0140-6736(06)69113-7).
- Bobinski, M., de Leon, M.J., Wegiel, J., Desanti, S., Convit, A., Saint Louis, L.A., Rusinek, H., Wisniewski, H.M., 2000. The histological validation of post mortem magnetic resonance imaging-determined hippocampal volume in Alzheimer's disease. *Neuroscience* 95, 721–725.
- Bottino, C.M.C., Castro, C.C., Gomes, R.L.E., Buchpiguel, C.A., Marchetti, R.L., Neto, M.R.L., 2002. Volumetric MRI measurements can differentiate Alzheimer's disease, mild cognitive impairment, and normal aging. *Int. Psychogeriatr.* 14, 59–72.
- Braak, H., Thal, D.R., Ghebremedhin, E., Del Tredici, K., 2011. Stages of the pathological process in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* 70, 960–969. <http://dx.doi.org/10.1097/NEN.0b013e318232a379>.
- Brucki, S.M., Nitrini, R., Caramelli, P., Bertolucci, P.H., Ivan, H., Okamoto, I.H., 2003. Suggestions for utilization of the mini-mental state examination in Brazil. *Arq. Neuropsiquiatr.* 61, 777–781.
- Busatto, G.F., Diniz, B.S., Zanetti, M.V., 2008. Voxel-based morphometry in Alzheimer's disease. *Expert. Rev. Neurother.* 8, 1691–1702. <http://dx.doi.org/10.1586/14737175.8.11.1691>.
- Caramelli, P., Teixeira, A.L., Buchpiguel, C.A., Lee, H.W., Livramento, J.A., Fernandez, L.L., Anghinah, R., 2011. Diagnosis of Alzheimers disease in Brazil: supplementary exams. *Dement. Neuropsychol.* 167–177.
- Clerx, L., Jacobs, H.I.L., Burgmans, S., Gronenschild, E.H.B.M., Uylings, H.B.M., Echávarri, C., Visser, P.J., Verhey, F.R.J., Aalten, P., 2013. Sensitivity of different MRI-techniques to assess gray matter atrophy patterns in Alzheimer's disease is region-specific. *Curr. Alzheimer Res.* 10, 940–951.
- Csernansky, J.G., Wang, L., Swank, J., Miller, J.P., Gado, M., McKeel, D., Miller, M.L., Morris, J.C., 2005. Preclinical detection of Alzheimer's disease: hippocampal shape and volume predict dementia onset in the elderly. *NeuroImage* 25, 783–792. <http://dx.doi.org/10.1016/j.neuroimage.2004.12.036>.
- Cummings, J.L., 2004. Alzheimer's disease. *N. Engl. J. Med.* 351, 56–67. <http://dx.doi.org/10.1056/NEJMr040223>.
- Desikan, R.S., Ségonne, F., Fischl, B., Quinn, B.T., Dickerson, B.C., Blacker, D., Buckner, R.L., Dale, A.M., Maguire, R.P., Hyman, B.T., Albert, M.S., Killiany, R.J., 2006. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage* 31, 968–980. <http://dx.doi.org/10.1016/j.neuroimage.2006.01.021>.
- Destrieux, C., Fischl, B., Dale, A., Halgren, E., 2010. Automatic parcellation of human

- cortical gyri and sulci using standard anatomical nomenclature. *NeuroImage* 53, 1–15. <http://dx.doi.org/10.1016/j.neuroimage.2010.06.010>.
- Devanand, D.P., Pradhaban, G., Liu, X., Khandji, A., De Santi, S., Segal, S., Rusinek, H., Pelton, G.H., Honig, L.S., Mayeux, R., Stern, Y., Tabert, M.H., de Leon, M.J., 2007. Hippocampal and entorhinal atrophy in mild cognitive impairment: prediction of Alzheimer disease. *Neurology* 68, 828–836. <http://dx.doi.org/10.1212/01.wnl.0000256697.20968.d7>.
- Dickerson, B.C., Bakkour, A., Salat, D.H., Feczko, E., Pacheco, J., Greve, D.N., Grodstein, F., Wright, C.I., Blacker, D., Rosas, H.D., Sperling, R.A., Atri, A., Growdon, J.H., Hyman, B.T., Morris, J.C., Fischl, B., Buckner, R.L., 2009. The cortical signature of Alzheimer's disease: regionally specific cortical thinning relates to symptom severity in very mild to mild AD dementia and is detectable in asymptomatic amyloid-positive individuals. *Cereb. Cortex* 19, 497–510. <http://dx.doi.org/10.1093/cercor/bhn113>.
- Du, A.T., Schuff, N., Amend, D., Laakso, M.P., Hsu, Y.Y., Jagust, W.J., Yaffe, K., Kramer, J.H., Reed, B., Norman, D., Chui, H.C., Weiner, M.W., 2001. Magnetic resonance imaging of the entorhinal cortex and hippocampus in mild cognitive impairment and Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* 71, 441–447.
- Du, A.T., Schuff, N., Kramer, J.H., Ganzer, S., Zhu, X.P., Jagust, W.J., Miller, B.L., Reed, B.R., Mungas, D., Yaffe, K., Chui, H.C., Weiner, M.W., 2004. Higher atrophy rate of entorhinal cortex than hippocampus in AD. *Neurology* 62, 422–427.
- Dubois, B., Feldman, H.H., Jacova, C., Hampel, H., Molinuevo, J.L., Blennow, K., DeKosky, S.T., Gauthier, S., Selkoe, D., Bateman, R., Cappa, S., Crutch, S., Engelborghs, S., Frisoni, G.B., Fox, N.C., Galasko, D., Habert, M.-O., Jicha, G.A., Nordberg, A., Pasquier, F., Rabinovici, G., Robert, P., Rowe, C., Salloway, S., Sarazin, M., Epelbaum, S., de Souza, L.C., Vellas, B., Visser, P.J., Schneider, L., Stern, Y., Scheltens, P., Cummings, J.L., 2014. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. *Lancet Neurol.* 13, 614–629. [http://dx.doi.org/10.1016/S1474-4422\(14\)70090-0](http://dx.doi.org/10.1016/S1474-4422(14)70090-0).
- Fazekas, F., Chawluk, J.B., Alavi, A., Hurtig, H.I., Zimmerman, R.A., 1987. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. *AJR Am. J. Roentgenol.* 149, 351–356.
- Fischl, B., 2012. *FreeSurfer*. *NeuroImage* 62, 774–781.
- Fischl, B., van der Kouwe, A., Destrieux, C., Halgren, E., Ségonne, F., Salat, D.H., Busa, E., Seidman, L.J., Goldstein, J., Kennedy, D., Caviness, V., Makris, N., Rosen, B., Dale, A.M., 2004. Automatically parcellating the human cerebral cortex. *Cereb. Cortex* 14, 11–22.
- Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* 12, 189–198.
- Jack, C.R., Petersen, R.C., Xu, Y., O'Brien, P.C., Smith, G.E., Ivnik, R.J., Boeve, B.F., Tangalos, E.G., Kokmen, E., 2000. Rates of hippocampal atrophy correlate with change in clinical status in aging and AD. *Neurology* 55, 484–489.
- Jack, C.R., Dickson, D.W., Parisi, J.E., Xu, Y.C., Cha, R.H., O'Brien, P.C., Edland, S.D., Smith, G.E., Boeve, B.F., Tangalos, E.G., Kokmen, E., Petersen, R.C., 2002. Antemortem MRI findings correlate with hippocampal neuropathology in typical aging and dementia. *Neurology* 58, 750–757.
- Jack, C.R., Vemuri, P., Wiste, H.J., Weigand, S.D., Lesnick, T.G., Lowe, V., Kantarci, K., Bernstein, M.A., Senjem, M.L., Gunter, J.L., Boeve, B.F., Trojanowski, J.Q., Shaw, L.M., Aisen, P.S., Weiner, M.W., Petersen, R.C., Knopman, D.S., 2012. Shapes of the trajectories of 5 major biomarkers of Alzheimer disease. *Arch. Neurol.* 69, 856–867.
- Jack, C.R., Knopman, D.S., Jagust, W.J., Petersen, R.C., Weiner, M.W., Aisen, P.S., Shaw, L.M., Vemuri, P., Wiste, H.J., Weigand, S.D., Lesnick, T.G., Pankratz, V.S., Donohue, M.C., Trojanowski, J.Q., 2013. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol.* 12, 207–216.
- Jacobs, H.L.L., Van Boxtel, M.P.J., Jolles, J., Verhey, F.R.J., Uylings, H.B.M., 2012. Parietal cortex matters in Alzheimer's disease: an overview of structural, functional and metabolic findings. *Neurosci. Biobehav. Rev.* 36, 297–309.
- Jagust, W.J., Zheng, L., Harvey, D.J., Mack, W.J., Vinters, H.V., Weiner, M.W., Ellis, W.G., Zarow, C., Mungas, D., Reed, B.R., Kramer, J.H., Schuff, N., DeCarli, C., Chui, H.C., 2008. Neuropathological basis of magnetic resonance images in aging and dementia. *Ann. Neurol.* 63, 72–80.
- Karas, G.B., Scheltens, P., Rombouts, S.A.R.B., Visser, P.J., van Schijndel, R.A., Fox, N.C., Barkhof, F., 2004. Global and local gray matter loss in mild cognitive impairment and Alzheimer's disease. *NeuroImage* 23, 708–716.
- Lerch, J.P., 2004. Focal decline of cortical thickness in Alzheimer's disease identified by computational neuroanatomy. *Cereb. Cortex* 15, 995–1001. <http://dx.doi.org/10.1093/cercor/bhh200>.
- Morishima-Kawashima, M., Ihara, Y., 2002. Alzheimer's disease: beta-amyloid protein and tau. *J. Neurosci. Res.* 70, 392–401. <http://dx.doi.org/10.1002/jnr.10355>.
- Mueller, S.G., Weiner, M.W., Thal, L.J., Petersen, R.C., Jack, C., Jagust, W., Trojanowski, J.Q., Toga, A.W., Beckett, L., 2005. The Alzheimer's disease neuroimaging initiative. *Neuroimaging Clin. N. Am.* 15 (869–77), xi–xii. <http://dx.doi.org/10.1016/j.nic.2005.09.008>.
- O'Brien, J.T., Blamire, A.J., Watson, R., Colloby, S.J., 2014. Assessment of regional cortical thickness on MRI in dementia with Lewy bodies and Alzheimer's disease. *Alzheimers Dement.* 10, P242. <http://dx.doi.org/10.1016/j.jalz.2014.04.369>.
- O'Bryant, S.E., Waring, S.C., Cullum, C.M., Hall, J., Lacritz, L., Massman, P.J., Lupo, P.J., Reisch, J.S., Doody, R., 2008. Staging dementia using Clinical Dementia Rating scale sum of boxes scores: a Texas Alzheimer's research consortium study. *Arch. Neurol.* 65, 1091–1095. <http://dx.doi.org/10.1001/archneur.65.8.1091>.
- Pennanen, C., Kivipelto, M., Tuomainen, S., Hartikainen, P., Hänninen, T., Laakso, M.P., Hallikainen, M., Vanhanen, M., Nissinen, A., Helkala, E.-L., Vainio, P., Vanninen, R., Partanen, K., Soyninen, H., 2004. Hippocampus and entorhinal cortex in mild cognitive impairment and early AD. *Neurobiol. Aging* 25, 303–310. [http://dx.doi.org/10.1016/S0197-4580\(03\)00084-8](http://dx.doi.org/10.1016/S0197-4580(03)00084-8).
- Prince, M., Wimo, A., Guerchet, M., Ali, M.G.-C., Wu, Y.-T., Prina, M., 2015. *World Alzheimer report 2015. In: The Global Impact of Dementia: An Analysis of Prevalence, Incidence, Cost and Trends. Disease International (ADI), London, Alzheimer's.*
- Samanta, M.K., Wilson, B., Santhi, K., Kumar, K.P.S., Suresh, B., 2006. Alzheimer disease and its management: a review. *Am. J. Ther.* 13, 516–526. <http://dx.doi.org/10.1097/01.mjt.0000208274.80496.f1>.
- Scahill, R.I., Schott, J.M., Stevens, J.M., Rossor, M.N., Fox, N.C., 2002. Mapping the evolution of regional atrophy in Alzheimer's disease: unbiased analysis of fluid-registered serial MRI. *Proc. Natl. Acad. Sci. U. S. A.* 99, 4703–4707. <http://dx.doi.org/10.1073/pnas.052587399>.
- Spulber, G., Simmons, A., Muehlboeck, J.-S., Mecocci, P., Vellas, B., Tsolaki, M., Kloszewska, I., Soyninen, H., Spenger, C., Lovestone, S., Wahlund, L.-O., Westman, E., 2013. An MRI-based index to measure the severity of Alzheimer's disease-like structural pattern in subjects with mild cognitive impairment. *J. Intern. Med.* 273, 396–409. <http://dx.doi.org/10.1111/joim.12028>.
- Stern, Y., 2012. Cognitive reserve in ageing and Alzheimer's disease. *Lancet Neurol.* 11, 1006–1012. [http://dx.doi.org/10.1016/S1474-4422\(12\)70191-6](http://dx.doi.org/10.1016/S1474-4422(12)70191-6).
- Stoub, T.R., Bulgakova, M., Leurgans, S., Bennett, D.A., Fleischman, D., Turner, D.A., deTolledo-Morrell, L., 2005. MRI predictors of risk of incident Alzheimer disease: a longitudinal study. *Neurology* 64, 1520–1524. <http://dx.doi.org/10.1212/01.WNL.0000160089.43264.1A>.
- von Strauss, E., Agüero-Torres, H., Kåreholt, I., Winblad, B., Fratiglioni, L., 2003. Women are more disabled in basic activities of daily living than men only in very advanced ages: a study on disability, morbidity, and mortality from the Kungsholmen Project. *J. Clin. Epidemiol.* 56, 669–677.
- Tondelli, M., Wilcock, G.K., Nichelli, P., De Jager, C.A., Jenkinson, M., Zamboni, G., 2012. Structural MRI changes detectable up to ten years before clinical Alzheimer's disease. *Neurobiol. Aging* 33 (825), e25–e36. <http://dx.doi.org/10.1016/j.neurobiolaging.2011.05.018>.
- Uotani, C., Sugimori, K., Kobayashi, K., 2006. Association of minimal thickness of the medial temporal lobe with hippocampal volume, maximal and minimal hippocampal length: volumetric approach with horizontal magnetic resonance imaging scans for evaluation of a diagnostic marker for neuroimaging of Al. *Psychiatry Clin. Neurosci.* 60, 319–326. <http://dx.doi.org/10.1111/j.1440-1819.2006.01508.x>.
- de Vrij, F.M.S., Fischer, D.F., van Leeuwen, F.W., Hol, E.M., 2004. Protein quality control in Alzheimer's disease by the ubiquitin proteasome system. *Prog. Neurobiol.* 74, 249–270. <http://dx.doi.org/10.1016/j.pneurobio.2004.10.001>.
- Whitwell, J.L., Crum, W.R., Watt, H.C., Fox, N.C., 2001. Normalization of cerebral volumes by use of intracranial volume: implications for longitudinal quantitative MR imaging. *AJNR Am. J. Neuroradiol.* 22, 1483–1489.
- Wolf, H., Hensel, A., Kruggel, F., Riedel-Heller, S.G., Arendt, T., Wahlund, L.-O., Gertz, H.-J., 2004. Structural correlates of mild cognitive impairment. *Neurobiol. Aging* 25, 913–924. <http://dx.doi.org/10.1016/j.neurobiolaging.2003.08.006>.
- Xu, Y., Jack, C.R., O'Brien, P.C., Kokmen, E., Smith, G.E., Ivnik, R.J., Boeve, B.F., Tangalos, R.G., Petersen, R.C., 2000. Usefulness of MRI measures of entorhinal cortex versus hippocampus in AD. *Neurology* 54, 1760–1767.