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Brain signature of mild stages of cognitive and behavioral impairment in amyotrophic lateral sclerosis

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ABSTRACT

We aimed to assess the brain signature of cognitive and behavioral impairment in *C9orf72*-negative non-demented ALS patients. The study included 50 amyotrophic lateral sclerosis (ALS) patients (out of 75 initially recruited) and 38 healthy controls. High-resolution T1-weighted and spin-echo diffusion tensor images were acquired in a 3 T MRI scanner. The multi atlas-based analysis protocol and the *FreeSurfer* tool were employed for gray matter assessment, and fiber tractography for white matter evaluation. Cognitively impaired ALS patients (n = 12) had bilateral amygdalae and left thalamic volumetric reduction compared to non-impaired ALS patients. Behaviorally impaired ALS patients (n = 14) had lower fractional anisotropy (FA) at the fornix in comparison with healthy subjects. These parameters did correlate with cognitive/behavioral scores, but not with motor-functional parameters in the ALS cohort. We believe that basal ganglia and fornix damage might be related to cognitive and behavioral impairment across ALS-frontotemporal dementia continuum. Also, distinct anatomical areas seem to influence the behavioral and cognitive status of these individuals.

1. Introduction

Cognitive decline and behavioral changes are recognized features of amyotrophic lateral sclerosis (ALS), ranging from clinically recognizable dementia to mild alterations. Disorders of executive, verbal memory, social cognition and language functions, along with apathy, disinhibition and eating disorders might affect up to 50% of the individuals (Beeldman et al., 2016; Consonni et al., 2016; Woolley and Strong, 2015). These findings are in line with the proposed ALS and frontotemporal dementia (FTD) continuum (Strong et al., 2009), and the presence of cognitive decline is a poor prognostic indicator for ALS patients (Elamin et al., 2013). Fortunately, these alterations are not found in all patients. For some individuals, cognitive impairment may occur even earlier than the fulfilling of ALS diagnostic criteria, and, in contrast, some patients may have intact cognitive abilities during the entire disease course (Kasper et al., 2016). Therefore, the occurrence of these alterations may correspond to a distinct phenotype within ALS spectrum, and their anatomical correlates are not yet clarified.

Magnetic resonance imaging (MRI) based studies have yielded promising results towards the characterization of the brain correlates of cognitive decline in ALS patients. Widespread cortical thinning and white matter diffusivity alterations in demented patients have been described (Agosta et al., 2016; Ambikairajah et al., 2014; Lillo et al., 2012; Murphy et al., 2007). Frontotemporal regions are the most investigated, and have been proposed as surrogate cognitive markers (Murphy et al., 2007). The role of deep gray nuclei on the cognitive status has also been explored, and cognitively impaired patients have more intense volumetric reduction and shape alterations at these structures than cognitively intact ALS patients (Machts et al., 2015). The thalamus has also been postulated as an important discriminating structure between *C9orf72*-positive and *C9orf72*-negative ALS groups (Bede et al., 2013).

Several studies have proposed a relationship between cognitive/behavioral scores and specific brain structures in ALS cohorts. Anterior cingulate, middle and superior frontal gyrus and accumbens nucleus damage have been associated with elevated apathy scores (Machts et al., 2015; Woolley et al., 2011a; Tsujimoto et al., 2011). A recent study with a large motor neuron disease cohort has shown that some imaging parameters at frontotemporal structures may be good predictors of executive and memory deficits (Agosta et al., 2016). Also, hippocampal volume has been associated with verbal memory scores, and amygdalae diffusivity parameters with executive function (Barbagallo et al., 2014).

However, the usefulness of these findings as cognitive markers is

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still not clear. Imaging results are contradictory along different studies, and major issues are related to: cohort sizes evaluated in some studies (i.e. less than 30 patients); divergences on the criteria used for characterization of cognitive and behavioral impairment; different MRI acquisition protocols; and processing approaches employed. Also, few studies had a multimodal approach towards gray matter and white matter changes at the same cohort.

In addition, previous studies (Agosta et al., 2016; Machts et al., 2015) have bracketed behaviorally and cognitively impaired ALS patients into the same group, and the exact brain correlates of these features have not been clarified by this approach. Thus, it remains unclear which brain structures might really play a role on the cognitive and the behavioral status of these patients, and whether the MRI is able to discriminate ALS phenotypes with and without cognitive and/or behavioral changes. Therefore, we performed this MRI-based study to assess the brain signature of *C9orf72*-negative non-demented ALS patients with cognitive and/or behavioral impairment, classified according to the consensus criteria (Strong et al., 2009).

2. Methods

2.1. Subjects

Seventy-five consecutive ALS patients with diagnosis of possible, probable or definite disease according to Awaji criteria (de Carvalho et al., 2008) were initially recruited. ALS diagnosis was given by a group of neurologists. Genetic testing for hexanucleotide (GGGGCC) repeat expansion of more than 30 copies at *C9orf72* gene was performed in this cohort, and carriers were excluded. Thirty-eight healthy individuals with similar age and gender distribution were also included. Individuals under 18 years old and those who had another concomitant neurological or psychiatric disease not related to ALS were excluded, for both control and patient groups.

The Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised (ALSFRS-R) (Cedarbaum et al., 1999) was employed for disease severity evaluation. Severe dysarthria (i.e., ALSFRS-R 'Speech' score $\leq$ 1) combined with affected ability to write (i.e., ALSFRS-R 'Handwriting' score $\leq$ 2) or severe respiratory impairment (i.e., ALSFRS-R respiratory subscore $\leq$ 8) were exclusion criteria for patients, as they may compromise the cognitive assessment and/or the MRI acquisition. Suspected or confirmed FTD diagnosis on routine clinical consultation, according to core criteria (Rascovsky et al., 2011), and use of psychotropic medications were also exclusion criteria for both control and patient groups. All ALS patients were taking *Riluzole*. This study was approved by our institution Ethics Committee (Comitê de Ética em Pesquisa – Unicamp) and written informed consent was obtained from all participants.

2.2. Data acquisition

2.2.1. Clinical evaluation

We registered age, gender, familial history of ALS or FTD and educational status of all participants. Disease severity was quantified using the ALSFRS-R. Disease duration was recorded and progression rate was calculated using the following formula: (48 - ALSFRS-R score) / disease duration (months).

2.2.2. Neuropsychological assessment

2.2.2.1. Cognitive evaluation. All patients underwent clinical neurological evaluation and detailed neuropsychological assessment through a comprehensive battery which evaluated language, memory and executive functions. The neuropsychological battery included the following tests: semantic fluency test (animals), forward digit span, MoCA vigilance test, Stroop Word-Colour Interference Test, Boston Naming Test, ECAS visuospatial tests and Rey Auditory Verbal Learning Test (Abrahams et al., 2014; Kaplan et al., 1983; Lezak, 1995; Nasreddine et al., 2005). The Brazilian Portuguese version of the cognitive section of the Amyotrophic Lateral Sclerosis Cognitive Behavioral Screen (ALS-CBS-Br) was also performed. This tool is an adapted validated version of the cognitive section of the ALS-CBS, which is a cognitive screening tool developed specifically for ALS patients which includes elements of standard testing batteries focusing on executive function (Woolley et al., 2010). Normative data available for each test were used. The battery was administered either verbally or in writing to control for motor disability and adaptations were made to account for motor disability. The diagnosis of ALS with cognitive impairment was given in the presence of impaired performance on at least two distinct cognitive tests sensitive to executive functioning (Strong et al., 2009), and this group was named *ALSci*. The employed classification of cognitive impairment was similar to the previously used in another study of our group (Branco et al., 2017).

2.2.2.2. Behavioral evaluation. Patients were also evaluated regarding behavioral changes since the disease onset, through the application of the Neuropsychiatric Inventory (NPI) tool applied by our staff to the caregiver, whom were instructed that behavioral changes should not be accounted when directly caused by motor dysfunction (i.e. lack of initiative and reduced efficacy at daily activities due to motor disability). The NPI is a validated tool for screening of prevalent behavioral disorders, as following: delirium, hallucinations, agitation/aggression, depression/dysphoria, anxiety, elation/euphory, apathy, disinhibition, irritation/emotional lability, abnormal motor behavior, sleep habits, appetite/alimentary disorders. Frequency and intensity of each altered domain was recorded, and the product of them was called the domain score.

The diagnosis of ALS with behavioral impairment was made in the presence of significant alterations (i.e. score $\geq$ 3) of at least two NPI domains (Cerami et al., 2014; Strong et al., 2009; Murphy et al., 2007), necessarily including at least one altered domain suggestive of frontal dysfunction (i.e, 'Apathy/Indifference', 'Disinhibition', 'Irritability/Lability'), and this group was named *ALSbi*. 'Depression' domain was not accounted for *ALSbi* diagnosis, as it could possibly reflect normal reactional psychological status. The NPI score was obtained by summing all domain scores. ALS patients with normal cognitive and behavioral performance were classified as *ALSni*.

2.2.3. MRI

All patients performed a MRI acquisition in a 3 T Phillips ACHIEVA-Intera at the same day of the clinical and neuropsychological assessment. All healthy controls also underwent a MRI acquisition. T1- and T2-weighted images (WI) of the brain and of the cervical spinal cord were obtained to exclude unrelated conditions. We used a standard head coil of 16 channels, with the following sequences:

1. High-resolution T1-3D WI, acquired in the sagittal plane, matrix of 240*240, 180 slices, the voxel sizes were 1*1*1 mm^3, TR/TE were 7/3.2 ms and flip angle of 8°. The scan duration was 6 min.
2. Spin echo T2* diffusion tensor imaging (DTI), with voxel sizes of 2*2*2 mm^3 reconstructed with 1*1*2 mm^3, with a matrix of 256*256, 70 slices, TE/TR were 61/8500 ms, flip angle 90°, 32 gradient directions, no averages and max b-factor of 1000 s/mm^2. The total scan duration was 6 min.

2.3. Data analyses

2.3.1. MRI

Multiple MRI-based approaches were used in order to accurately assess the brain signature of cognitively and behaviorally impaired ALS patients (Fig. 1a). For gray matter evaluation, the T1 WI acquisition was used. The *FreeSurfer* software (v5.3, Linux) (surfer.nmr.mgh.harvard.edu) was employed for cortical thickness evaluation, since it is more sensitive than area and volume measurements (Hutton et al., 2009),