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DOCTORAL THESIS

"Progression over time of cognitive, behavioral and social deficit in a population- based series of ALS patients"

Thesis' Author: Barbara Iazzolino , PsyD.

Supervisor: Adriano Chiò, Prof., M.D., FAAN

PhD Program Co-ordinator: Sassoè-Pognetto Marco, Prof., PhD

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Chapter I: *Amyotrophic Lateral Sclerosis*

9. Introduction

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder characterized by the progressive impairment of motor neurons at cerebral cortex level, brain stem and anterior horns of the spinal cord, which causes the gradual loss of function of voluntary muscles.

The first clinical description of the disease can be attributed to François-Amilcar Aran, who in 1848 reported the description of a patient with progressive motor impaired upper limbs associated with atrophy. He called it "Progressive Muscular Atrophy".

The term "Amyotrophic Lateral Sclerosis" (ALS) was used for the first time in 1874 by Jean Marie Charcot, a neurologist who distinguished it from the heterogeneous group of spinal muscular atrophy (Aran, 1850), specifying their clinical characteristics and anatomopathological features, With his new definition, Charcot reported the clinical characteristics of ALS: the expression "Lateral Sclerosis" refers to sclerosis (a gliotic reaction secondary to degeneration) of the lateral portion of the spinal cord, where the nerve fibers of the upper motor neuron are located. The term "amyotrophic" refers to muscle atrophy, caused by denervation, one of the typical clinical signs of the disease[5,7].

Only in 1934 the definition "Motor Neuron Disease (MND)" was used to highlight the existence of other neurodegenerative diseases affecting motor neurons, characterized by the presence of motor hyperactivity (spasticity, cramps) or functional deficiency (asthenia, muscular atrophy). ALS is clinically characterized by the combination of signs of the upper and/or the lower motor neuron, and represents the most frequent MND.

2. Epidemiology

The incidence of ALS in the European population is 2.16/100,000 per year (Chiò et al., 2009)[13]. The incidence is higher among men (3.0/100,000 individuals per year, CIs 95% 2.8-3.3) compared to women (2.4/100,000 individuals per year, CI 95% 2.2-2.6) with a men to women ratio ranging from 1.04 (in 1997) to 1.71 (in 2000) (Chiò et al., 2009)[12]. The incidence of ALS increases with age, with a peak at between 65 and 74 years (McGuire et al., 1996)[20,21]. Despite these premises, it has been

recently hypothesized that the worldwide incidence of the disease will increase by 32% in 2040, with an increase of 40% in the female population (Arthur et al, 2016)[2].

3. Etiopathogenesis and risk factors

The causes of ALS are not yet fully understood; ALS is considered a complex disease, characterized by the interaction between genetic, personal and environmental factors (Simpson & Al-Chalabi, 2006)[31].

Recently, multiple endogenous and exogenous factors have been considered in as possible risk factors; nevertheless, for most of these factors, there is no clear and certain evidence of a positive association with the incidence of the disease (Angelini et al., 1983; Chiò et al., 1991)[1,9]. Although research is increasingly focusing on genetic aspects, as for today, the only definitive risk factors remain the age, sex, and heredity of the pathology.

About 5-10% of ALS cases are familial mostly with an autosomal-dominant transmission. The main and most frequently mutated genes involved in the onset of familial ALS are: SOD1, TARDBP, FUS, C9ORF72. It should be emphasized, however, that the identification of an alteration of these genes, although evident in a small but significant proportion of patients with sporadic ALS, is not an essential element for the diagnosis of fALS (Turner et al., 2017)[36]. Indeed, the definition of familial ALS (fALS) is based on the presence of the disease also in other family members.

Research on environmental risk factors has shown no significant results. Nevertheless, physical trauma, exposure to electromagnetic fields, participation in intense physical activities, prolonged working contact with metals and pesticides, smoking and some dietary factors should be included.

4. Familial forms of ALS

As in other neurodegenerative conditions, about 10% of ALS cases are classified as familial, while the remaining 90% are considered 'apparently' sporadic.

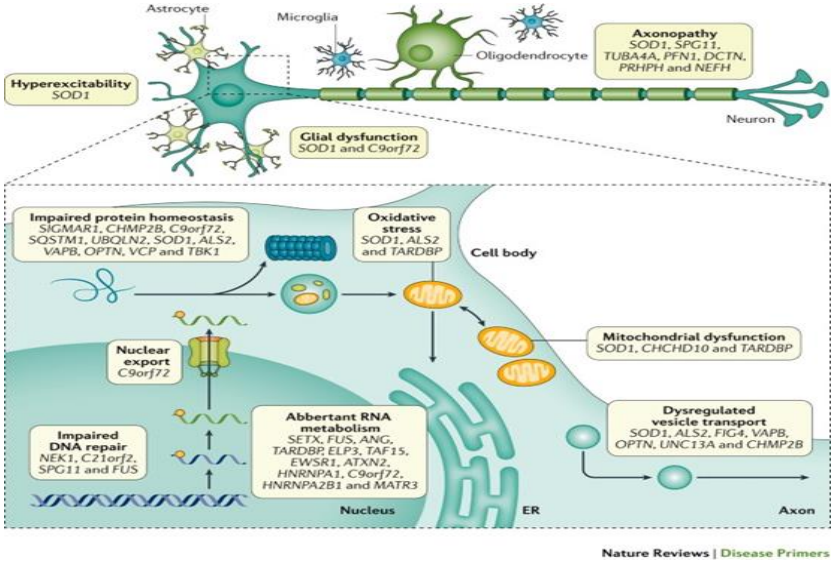
The most frequent genes associated with familial ALS forms are as follows:

1. ***C9orf72*** is the gene most commonly associated with familial ALS, causing about 40% of cases.

It also causes about 25% of family cases of Frontotemporal Dementia. This mutation represents the most important link between these two conditions. The pathogenic mutation is an hexanucleotide expansion in the first intron of the gene.

2. ***SOD1***, a gene that encodes superoxide dismutase 1. It is the second most common gene accounting for about 12% of familial cases and about 2% of sporadic cases.
3. ***TARDBP***, which encodes the TAR DNA-binding protein (TDP43), accounts for 1-5% of cases of familial ALS and less than 1% of sporadic forms of ALS.
4. ***FUS***, codes for the protein "Fused in sarcoma". It accounts for 1-5% of familail ALS and less than 1% of sporadic forms. FUS is an RNA-binding protein with a function similar to that of TDP-43.

The most recognized pathogenic hypotheses are the following:



- Anomalies in processing of RNA
- Protein misfolding
- Alterations in response Inflammatory
- Ca-dependent eccitotoxicity
- Citscheletric alterations
- Mitochondrial dysfunction
- Changes in growth factors

5. Pathological aspects

The pathological alterations described in ALS patients present multiple interpretative problems. Since analyses are carried out when the disease is now in its advanced stages, their findings could be the effect of reactive processes rather than of the pathology itself.

Despite these methodological limitations, the most relevant anatomopathological findings can be visualized at cerebral cortex level, brainstem and spinal cord.

The cerebral cortex of ALS patients shows no obvious signs at the macroscopic level; however, an atrophy of the frontal cortex can be observed in some patients who clinically present a prevalent upper motor neuron diseases .At the microscopic level, however, a loss or reduction in the size of giant Betz cells, (pyramidal neurons of the primary cerebral cortex) is visible.

The brainstem, the pons and the bulb, usually show a loss of volume; some specific nuclei appear more affected, such as the hypoglossus, trigeminal and facial nuclei, which can be atrophic as a result of the loss of neurons and reactive gliosis (Lawyer and Netsky, 1953; Bonduelle, 1975; Rossi, 1994)[4,19,29,].

The pallor found in the lateral cords testifies the demyelination of the corticospinal fibers, which leads to the degeneration of the axles of cortical motor neurons.

Microscopically, there is a marked reduction in the coloration of myelin and the number of axions. In the gray substance of the front horns the loss of peripheral motor neurons is visible, although the extent of such loss is variable.

Finally, a modest degeneration of substantia nigra, which presents itself as an atypical degeneration of dopaminergic neurons, has been observed in some cases of ALS.

6. Clinical Description

ALS is a syndrome characterized by the impairment of central and/or peripheral motor neurons. Oculomotion and sphincter functions are usually spared.

The interest impairment of central motor neurons causes:

- *loss of muscle strength*:(mostly evident in the extensor muscles of the upper limbs and flexors of the lower limbs);
- *spasticity*:, i.e.increased muscle tone (muscle flexors upper limbs and lower limb extenders);
- *hyperreflexion, i.e.*exaggerated proprioceptive receptivity resulting from the reduction of the inhibitory polysynoptic impulse;
- *pathological reflexes* (Babinski reflex, Oppenheim sign, Chaddock sign, Hoffmann sign);
- *pseudobulbar signs, including dysphagia, dysarthria and* emotional lability ("laughs and spastic crying").

The impairment of the lower motor neuron causes:

- *atrophy*:, i.e. a volumetric reduction of the affected muscle mass;
- *hypotonia or flaccidity*;

- *fasciculaitons* i.e. muscle flicks that occur irregularly in time and localization, caused by the short contraction of a group of muscle fibers (frequent in the early stages of the disease, usually decrease in the more advanced stages);
- *cramps*.

In the case of bulbar onset, in addition to tongue atrophy, they appear as symptoms:
- *disarthria*: progressive difficulty in the articulation of the word,
- *disphagia*: difficulty in chewing and swallowing,
- *siaallorhea*: the inability to effectively swallow the saliva produced.

Respiratory symptoms such as dyspnea normally appear in advanced stages of the pathology and can progress to respiratory failure and the need of mechanical support for ventilation, such as NIV (non-invasive ventilation) or tracheostomy. Although rarely, there are cases of respiratory onset with respiratory symptomatology (Scelsa et al., 2002)[30].

Despite these supportive interventions, respiratory failure is the most common cause of death in ALS patients.

It is now widely accepted that the disease begins years before the clinical onset. As the loss of the motor neurons begins, a process of reinnervation compensates for the progressive initial denervation, until the loss of motor neurons reaches 50% .To date, there is still little knowledge of the preclinical phase of the disease; however, studies show that the ability to reinnervation tends to decrease as the disease progresses (Swash and Scwartz, 1982)[34]. Median survival is 30.5 months from onset, and 19.3 months from diagnosis (Chiò et al., 2002)[11].

To date, he only disease-modifying drug is riluzole, an benzothiazolic derivative that counteracts the neurotoxic effect of excess glutamate, reducing the extracellular accumulation of the neurotransmitter through the inhibition of presynaptic release. The assumption of riluzole at a dose of 50 mg twice a day increases overall survival by about 3 months (Bensimon et al., 1994; Chiò et al., 2002; Traynor et al., 2003; Mitchell et al., 2006)[3,11,35,22].

Multiple studies have also shown that the presence of anxious and depressive symptomatology is closely related to a faster progression of the pathology and, therefore, to a decreased survival.

In addition, patients with ALS-FTD show a worse prognosis compared to cognitively healthy patients. Studies confirm that patients with cognitive disorders demonstrate less compliance and greater difficulty in adapting to both NIV and endoscopic percutaneous gastrostomy (PEG), thus leading these patients to a more inauspicious prognosis.

7. Diagnosis

The absence of diagnostic markers and clinical variability often make the diagnosis of ALS quite difficult. At present there are no specific tests to identify the disease. In order to diagnose ALS, it is necessary to find a combination of signs of motor neuron I and II and their spread within one or more regions of the body.(bulbar,, cervical, thoracic and lumbosacral).

In 1989 the World Federation of Neurology established the El Escorial criteria, useful for placing the diagnosis of ALS in clinical research. Subsequently, these criteria were revised and updated (Brooks et al., 2000)[6]. With this revision, the category of "Probable ALS with Laboratory Support" was also added in order to allow for a faster diagnosis (Ross et al., 1998)[28]. With this change it is in fact possible to put the diagnosis of ALS more quickly: currently it is attested that the average time is 9.7 months from the onset of symptoms, in contrast to the 12 months of diagnostic delay demonstrated by the previous literature.

- *Defined or certain ALS*: signs of the upper and lower motor neurons in the bulbar region and in at least two other spinal regions or in three spinal regions
- *Probable ALS*: signs of the upper and lower motor neuron in at least two regions (the signs of the upper motor neuron must be at a more rostral level than the signs of the lower motor neuron)
- *Probable ALS with laboratory support*: clinical signs of the upper and lower motor neurons in a region, or signs of the upper motor neuron present in a single region and signs of the lower motor neuron present in at least two limbs
- *Possible ALS*: signs of the upper or lower motor neuron simultaneously present in a single region, or signs of the upper motor neuron in two or more regions

In order to improve diagnostic times, in 2015, a study was carried out on ALS patients and healthy controls in which 18F-2-fluoro-2-deoxy-D-glucose (18F-FDG) PET was used as a biomarker to distinguish patients (5 months after diagnosis) from controls; subgroups of spinal and bulbar ALS (Cistaro et al., 2012)[14] were also examined.

This research, in which 25 bilateral cortical and subcortical volumes of interest and cerebellum were taken into account, revealed, in ALS patients, a hypometabolism in the frontal, motor and occipital cortex and a hypermetabolism in the midbrain, temporal pole and hippocampus. A similar metabolic model has also been found in the two subgroups (spinal and bulbar).

8. Therapy

In the current state of knowledge about ALS there is still no etiological therapy; therefore, the purpose of treatment is to improve the patient's quality of life by intervening on the most disabling symptoms. To carry out this support treatment, it is necessary to involve a multidisciplinary team that takes care of the clinical, psychological, and social aspects of the patient through specific professional skills (Chiò et al, 2001)[10].

As far as drug therapy is concerned, as mentioned above, the only neuroprotective therapy used today is Riluzole. It is also pharmacologically useful to intervene to reduce the most disabling symptoms such as sialorrhea, emotional lability, depression.

In addition to pharmacological therapies, the therapeutic management of the patient suffering from ALS includes multidisciplinary support interventions, with the involvement of numerous health professionals:

- *Physiatrist and Physiotherapist*: indication of the path of taking charge of physiotherapy for the purpose of limit the damage caused by the loss of motility and training, including for caregivers, to allow safe travel; prescription of aids.
- *Pulmonologist*: indication for respiratory physiotherapy and / or prescription of devices dedicated to management of respiratory disorders, in particular: cough assistance device and non-invasive ventilation instruments or invasive ventilation via tracheostomy.
- *Nutritionist*: weight control and adaptation of the diet to swallowing problems.
- *Speech therapist and speech therapist*: for the management of swallowing and communication problems. Indication of the positioning of PEG.

Considering the unfortunate evolution of the disease, the ultimate goal is therefore to improve the quality of life of both the patient and the caregiver.

Chapter II: FTD

1. Description of the Disease Profile of FTD

The predominant symptoms of FTD is a worsening impairment of cognition, social behaviour and language. The term Frontototemporal Dementia is a consequence of the different cerebral areas which undergo a neurodegenerative process: the frontal and temporal lobes. In the 1998 Consensus Conference (Neary et al, 1998)[23] at least two variants were identified. The most common manifestation is an alteration of social conduct and personality characterized either by inertia, loss of insight and attenuation of emotions, or social disinhibition, distractibility with fairly preserved mnestic functions which cause behavioral problems and personality disorders (behavioural-FTD- bvFTD). The other two prototype variants are semantic variants: primary progressive aphasia characterized by severe deficit in word understanding and in naming objects, in the context of a fluent, grammatically correct eloquence with loss of word meaning (svPPA) and nonfluent agrammatic primary progressive aphasia characterized by difficult production of language, phonological and grammatical errors, difficulty in recalling words and non-fluent agrammatic eloquence (naPPA) .

Diagnostic criteria include executive deficits, personality change, and apathy for bvFTD (Rascovsky et al. 2011)[27]and a discrete impairment in word comprehension and agrammatism for svPPA and naPPA, respectively (Gorno-Tempini et al. 2011)[17]. The bvFTD syndrome is roughly four times more prevalent than naPPA or svPPA (Hogan et al. 2016)[18]. Age of onset, in most cases ranges between 40-64 years with an incidence of 2.7-4.1/100,000 (Onyike and Diehl-Schmid 2013)[24]. FTD is thus the 2nd most common form of dementia among individuals less than age 65.

Prevalence estimates of the variant of behavior and semantic language are higher among males, while those of the non-fluent language variant are higher among women; however, the FTD affects men and women indiscriminately. The disease is gradually progressive, with an average survival of 6-11 years after the onset of symptoms and 3-4 years after diagnosis.

2. Etiopathogenesis and risk factors

Although the causes of FTD are still unknown, about 40% of patients have a family history of early onset cognitive disorder, and about 10% reveal a dominant autosomal inheritance.

To date some genetic mutations have been identified, the mutation in the *C9orf72* genebeing one of the more frequent. This mutation was also found in 2011 in patients with ALS. Hexanucleotidic expansion of the *C9orf72* gene is found in 80% of patients with ALS and FTD.

bvFTD (Pan et al. 2012)[27] is characterized by atrophy in the orbital and dorsolateral prefrontal cortex, anterior cingulate cortex, and insula; an atrophy in the left anterior inferior temporal lobe are observed in svPPA, and in the left inferior frontal cortex and insula in naPPA (Gorno-Tempini et al. 2011)[17]. All three syndromes include the progressive atrophy of subcortical structures as the thalamus, basal ganglia and amygdala increasingly involved in bvFTD (Devenney et al. 2015)[15] and an increasing left and right hemispheric atrophy in svPPA and naPPA (Placek et al 2017)[26].

3. Symptoms and therapy

The symptomatology of FTD changes in the variants. In the bvFTD variant the main clinical characteristics are personality changes that result in impaired interpersonal social conduct, emotional flattening, lack of insight, absence of marked anterograde amnesia.

In the svPPA variant there is a significant loss of vocabulary, an increase in anomalies affecting eloquence, lack of understanding of word meaning in the context of fluent production and absence of marked anterograde amnesia. In the naPPA variant there is a marked difficulty in the production of eloquence, characterized by poor fluency, hesitation, difficulty in finding the appropriate vocabulary, difficult eloquence, articulatory apraxia, phonic paraphrase and agrammatism along with absence of a marked anterograde amnesia.

Actually there is no specific therapy for FTD; but current treatments include antidepressant and antipsychotic medications only for symptoms management.

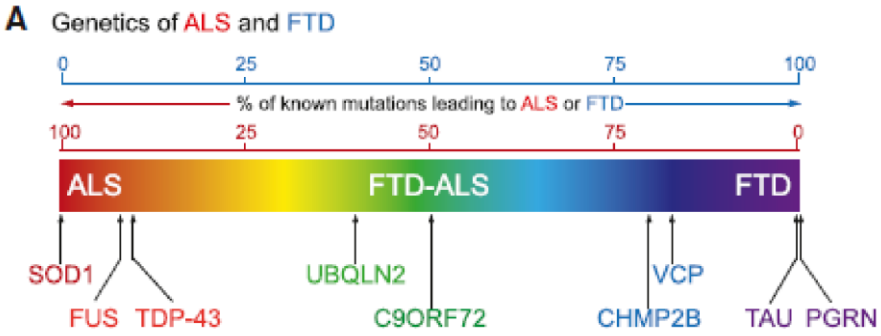
Chapter III: *ALS-FTD*

1. Introduction

ALS can be phenotypically described by separating its phenotypic aspects on two axes:

- The *first axis* depends on the greater or lesser involvement of the 1st or 2nd motor neuron: according to this level of description, ALS constitutes an entity located at the center of a spectrum of clinical phenotypes in which at one extreme there is Progressive Muscular Atrophy (PMA- characterized by prevalent involvement of the second motor neuron) and at another Primary Lateral Sclerosis (PLS- characterized by an exclusive involvement of the first motor neuron).

- The *second level* of description instead foresees, at one extreme, cognitively intact ALS patients and at the opposite extreme there are ALS patients suffering from fronto-temporal dementia.



FTD and ALS have a considerable phenotypic overlap on a continuous disease spectrum. Although the phenotypic overlap between ALS and FTD is detectable in the scientific and medical literature from the 80s and 90s (Neary et al. 1990)[25], it is possible to find some cases of this phenotypic overlap already described in the 1920s. The first description is about a French male patient with ALS diagnosis who developed impaired cognition late in disease course; another case is about a 25 year-old Brazilian female patient with apathy and executive dysfunction that died after severe muscular atrophy.

The recent studies indicate 10-15% of patients with ALS meet criteria for a diagnosis of FTD and that nearly half manifest executive or language or deficit in social cognition or/and behavior impairment coherent with extra-motor frontal and temporal lobe neurodegeneration. Behavioral impairment occurs in ~40% of patients, and 20-30% of patients show executive, verbal fluency,

and language deficits; at the same time, alteration of memory and visuospatial function affect only 9-12% of ALS patients (Crockford et al. 2018)[15].

2. Strong's criteria (2009)

In 2009 Strong et al. proposed a classification of the cognitive and behavioral status of ALS patients: these criteria were used for the diagnosis of frontotemporal cognitive and behavioral syndromes in ALS (Strong et al. 2009)[32]. Categories were defined as follows:

- ***ALS Pure MND***: ALS with only the involvement of motor areas
- ***ALSci*** (ALS cognitive impairment): SLA with executive deficits;
- ***ALSbi*** (ALS behavioral impairment): SLA with behavioral impairment;
- ***ALS-FTD***: ALS with cognitive-behavioral disorder;
- ***FTD-MND-like****:* frontotemporal lobar degeneration in which there is neuropathological evidence of motor neuron degeneration
- ***ALS-dementia***: ALS with non-FTD dementia (concomitant AD or vascular dementia).

The uninhibited subtype of bvFTD was included in the ALS-bi category, that is restless, hyperactive, distracted, with a profound alteration of social conduct, a lack of concern for one's disability, social disinhibition; but also the subtype behavioral alterations such as flattening of emotions, apathy, gluttony, stereotypies (Gibbons et al., 2008)[16]. At the same time, those who show alterations in executive and attentional functions were included in the ALS-ci category (Strong et al., 2009)[32]. Furthermore, those who, in addition to meeting the El Escorial diagnostic criteria for ALS, also met Neary criteria for FTD, were included in the SLA - FTD category (Wooley et al, 2015)[37].

3. Revised Strong's criteria for ALS-FTD (2017)

In 2017 Strong et al.(Strong et al, 2017) [33] published a new classification with a revised criteria for ALS-FTD (Tab. 1).

The first innovation of this new classification was the introduction of a new category:

-***ALS-cbi***: ALS meeting both cognitive and behavioral impairment criteria.

Furthermore, the new classification underlines the importance of impairment of language (not only as a deficit in verbal fluency) and impairment in social cognition (overlapping with executive function). For a diagnosis of ALS –FTD he introduced the presence of 2 or 3 cognitive /behavioral symptoms of Raskowsky criteria highlighting the importance of psychiatric symptoms. Moreover, for ALS bi classification apathy has become a fundamental, if not exclusive, criterion.

Another function that has gained greater importance in this new classification is Social Cognition; that is, the set of cognitive processes that make it possible to recognize and / or infer the emotional states and mental contents of others. On DSM 5, Social Cognition has been defined, and has acquired the same importance as the other better known cognitive domains (Language, Memory, Attention, Executive Functions, Visuospatial Skills). For a long time, social cognition was thought to be an aspect of executive functions. Currently it is considered a further domain certainly very correlated to the other domains. Some executive functions seem to support some aspects of social cognition such as the ability to modulate one's mental state in relation to the mental state of others rather than in relation to the social context or the burst that you want to achieve. Social cognition correlates with: language allowing us to make explicit the mental and emotional representation of ourselves and others; working memory allowing us to represent at the mental level of the contents; visual spatial functions allowing us a graphic representation of certain contents.

Tab. 1

	CRITERIA 2009	**CRITERIA 2017**
ALS bi	2 non-overlapping diagnostic features from: ● **Neary criteria (1998):** decline in personal hygiene and grooming, mental rigidity and inflexibility, distractibility and impersistence, hyperorality and dietary changes, perseverative and stereotyped behaviour, utilization behaviour ● **Hodge's criteria (1999)**: loss of insight, disinhibition, restlessness, distractibility, reduced empathy or unconcern for others, lack of foresight or planning, impulsiveness, social withdrawal, apathy or loss of spontaneity, reduced verbal output, verbal stereotypes or echolalia, verbal or motor perseveration, poor self care, gluttony, sexual hyperactivity The presence of at least 2 abnormalities should be supported by at least 2 sources among: ● Patient interview/observation ● Caregiver report ● Caregiver structured interview/questionnaire	● Apathy with or without other behavioural change OR ● 2 non-overlapping supportive diagnostic features from **Raskovsky criteria (2011)**: disinhibition, loss of sympathy and empathy, perseverative, stereotyped or compulsive behaviour, hyperorality/dietary change, loss of insight, psychotic symptoms (hallucinations, irrational believes, somatic delusions)*
ALS ci	At least 2 distinct cognitive tests of executive functions below the 5th percentile. ● Assess domains other than executive functioning (memory/learning, attention, language, visuospatial and premorbid IQ) ● Assess for other medical condition that could affect cognition (pseudobulbar affect, respiratory dysfunction, disrupted sleep, delirium, fatigue, pain, psychotropic medication)	Executive impairment AND/OR language impairment. Executive impairment: ● Impaired verbal fluency (letter) OR ● Impairment on 2 non-overlapping measures of executive function (which may include social cognition) Language impairment: ● Impairment in 2 non overlapping tests, in which language impairment is not solely explained by verbal fluency deficits
ALS cbi		Patients who meet the criteria for both ALSci and ALSbi
ALS - FTD **ALS - bvFTD**	Behavioural symptoms: Behavioural symptoms: ● Disinhibition (impulsivity, distractability, impaired social interaction) ● Apathy ● Irritability ● Selfishness/disinterest in others ● Rigid/inflexible thinking ● Hyperorality/food stuffing ● Stereotyped behaviour ● Frontal release signs Functioning level affected in: ● Decline in social interpersonal conduct ● Impairment in regulation of personal	Diagnosis of FTD requires: ● Evidence of progressive deterioration of behaviour and/or cognition by observation or history AND ● Presence of at least 3 of behavioural/cognitive symptoms of Raskovsky (2011): disinhibition, apathy or inertia, loss of sympathy and empathy, perseverative, stereotyped or compulsive behaviour, hyperorality/ dietary change, executive deficit with sparing of episodic memory and visuospatial skills* OR

ALS - PNFA (progressive non-fluent aphasia) **ALS - SD**	conduct ● Loss of insight (marked changes from premorbid levels) ● Supportive features; impaired executive functioning (without posterior visuospatial dysfunction or amnestic disorder) May be present speech and language impairment (but not as a dominant feature, in that case SD or PPA). May be present speech and language impairment (but not as a dominant feature, in that case SD or PPA).	● Presence of at least 2 of behavioural/cognitive symptoms of Raskovsky (2011) together with loss of insight and/or psychotic symptoms OR ● Presence of language impairment meeting criteria for semantic dementia or non-fluent variant PPA. this may co-exist with behavioural/cognitive symptoms.

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Validation of the revised classification of cognitive and behavioural impairment in ALS

Iazzolino B, Pain D, Laura L, Calvo A, Moglia C, Canosa A, Manera U, Ilardi A, Bombaci A, Zucchetti JP, Mora G, Chio A.

Abstract

Objective In 2017, the diagnostic criteria for cognitive and behavioural impairment in amyotrophic lateral sclerosis (ALS) with frontotemporal dementia (ALSFTD-1) have been modified (ALSFTD-2) with the inclusion of a novel category (ALS with combined cognitive and behavioural impairment, ALScbi) and with changes of operational criteria of the other categories (ALS with cognitive impairment (ALSci), ALS with behavioural impairment (ALSbi) and ALS with frontotemporal dementia (ALS-FTD)). We compared the two sets of criteria to assess the effect of the revised criteria on the cognitive classification of patients with ALS.

Methods Two cohorts of patients with ALS were included in this study: a population-based cohort including patients identified through the Piemonte/ Valle d'Aosta register for ALS in the 2014–2017 period (n=321), and a referral cohort recruited at the Turin ALS centre and at the ALS centre of the Maugeri Institute in Milan in the same period (n=205). Cognitive function was classified in blind by two neuropsychologists expert in ALS.

Results ALSFTD-2 criteria determined a shift of about 15% of patients from their original category to a new one. In both cohorts, about 9% of patients were reclassified to the novel category ALScbi. Among patients previously classified as cognitively normal, 14 (4.3%, population-based cohort) and 19 (9.3%, referral cohort) were reclassified as ALSbi or ALSci. The median survival of the different categories was significantly different with both with sets of criteria.

Conclusions The new ALSFTD-2 criteria, compared with the old ones, have positive effects on the clinical practice being more sensitive to the early cognitive impairment and having a better prognostic yield.

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, characterised by loss of motor neurons at cortical, bulbar and spinal levels causing a progressive paresis of voluntary muscles, being fatal within 2–5 years from onset usually due to respiratory failure. The motor symptomatology of ALS is associated in about 50% of cases to a cognitive impairment ranging from isolate executive or behavioural deficits to frontotemporal dementia (FTD), a comorbidity which has profound effects on ALS prognosis.[1 2] Until recently, the diagnosis of cognitive impairment in ALS has been based on the consensus criteria proposed in 2009 (ALSFTD consensus criteria, ALSFTD-1) which classified patients in ALS with comorbid FTD (ALS-FTD), ALS with behavioural impairment (ALSbi), ALS with cognitive impairment (ALSci) and ALS with normal cognition (ALS-CN).[3] In 2017, the diagnostic criteria have been partially modified (ALSFTD-2) with the inclusion of a novel category (ALS with combined cognitive and behavioural impairment, ALScbi) and with changes of the operational criteria of the other categories.[4] The frequency and characteristics of

the new cognitive subgroups in comparison to those based on the original criteria remain to be described. The aim of this paper is to compare the ALSFTD-1 and ALSFTD-2 criteria in a population-based cohort of ALS patients and in two clinical series enrolled in referral (tertiary) ALS centres in Italy in order to assess [1] the effect of the revised criteria on the cognitive classification of patients with ALS and [2] their prognostic value.

Methods

Patients

Two cohorts of patients were included in this study: 1. a population-based cohort including patients identified through the Piemonte and Valle d'Aosta register for ALS (PARALS) in the 2014–2017 period. The PARALS is a prospective epidemiological register established in 1995, whose characteristics have been already published.[5] All patients of this cohort were evaluated at the Turin ALS centre. 2. Two referral cohorts, one enrolled at the ALS centre at the Maugeri Institute in Milan between 2014 and 2017 and the second including the patients not resident in Piemonte evaluated at the ALS centre in Turin in the same period. For the purpose of this study, the two referral cohorts have been combined.

Neuropsychological battery

In both centres, patients with ALS underwent a battery of neuropsychological tests encompassing executive function, memory, visuospatial function, social cognition and language, selected according to the Diagnostic Criteria for the Behavioural variant of Frontotemporal Dementia6 and ALS-FTD Consensus Criteria.[3 4] All patients underwent the following neuropsychological battery (online supplementary E-Table 1): Mini Mental State Examination (MMSE); Edinburgh Cognitive and Behavioural ALS Screen (ECAS);7 Wisconsin Card Sorting Test (WCST); Trail Making Test A and B (TMT A-B); Digit Span Forward and Backward; Letter and Category fluency test; Boston Naming Test (BNT); Rey Auditory Verbal Learning Test (RAVLT); Babcock Story Recall Test (BSRT); Rey-Osterrieth Complex Figure Test (ROCF); Raven's Colored Progressive Matrices (CPM47); Frontal Assessment Battery (FAB). Neurobehavioral dysfunction was determined both with the direct observation by the neuropsychologist and patient's history,[6 8] with the behavioural screening section of the ECAS and with the Frontal Systems Behaviour Scale (FrSBe),[9] using the Family-form evaluated by a close relative/caregiver (scores: normal ≤59, borderline 60–64; pathological ≥65). If a subject had scores reflecting a frontal systems abnormality both in the premorbid and in the

post-illness forms, he/she was considered pathological only if there was an increase of ≥10 points at the T-score between the two forms.[2] Anxiety and depression were assessed with the Hospital Anxiety and Depression Scale; the item 'I feel slowed down' was discussed with patients in order to have him/her not to refer to physical disability.[10] The battery was administered following the same sequence in order to avoid the possible differential interference of the answers of one test over the others. The administration of the battery required a median of 105 min (IQR 84–140) and was generally performed in the morning. If the subject felt too tired, a further session was scheduled to complete the battery, within 2 weeks after the first one. Patients' O2 saturation at the time of the neuropsychological testing was measured with a pulse oximeter; none of the patients had evidence of hypoxemia (oxygen saturation)

E-Table 1. Tests included in the battery and corresponding cognitive domains

Cognitive domain	**Test**
Multidomain tests	ECAS
	MMSE
Psychomotor speed	TMT part A
Language	Boston Naming Test
Fluency	Letter fluency
	Category fluency
Social cognition	ECAS – Social Cognition Part A and B
Executive functions	CPM47
	FAB
	TMT part B
	TMT B-A
	WCST
Attention	Span Forward and Backward
Immediate and delayed verbal memory	BSRT
	RAVLT
Visual memory and visual-perceptive functions	ROCF
Neurobehavioral function	FrSBe

Cognitive classification

The original criteria (ALSFTD-1)[3] classified the patients in three main categories, besides those with normal cognition: (1) patients with ALS with a FTD syndrome (ALS-FTD), who met either the Neary criteria or the Hodges criteria for FTD;[8 11] (2) patients who showed some degree of cognitive impairment, but did not meet the criteria for FTD were classified either as ALSbi meeting at least

two non-overlapping supportive diagnostic features from either the Neary criteria or Hodges criteria for FTD or as ALSci, with evidence of cognitive impairment at or below the 5th percentile on at the least two distinct tests of cognition that are sensitive for executive functioning. The 2017 revised criteria (ALSFTD-2)[4] made several modifications of the classification: first, it has established the novel category of ALScbi, which includes patients who fulfils criteria for both ALSci and ALSbi; second, it has to some extent modified the criteria for the other three original cognitive categories. A comparison of the two sets of criteria is reported in table 1.

Table 1. Comparison of the two sets of criteria for the diagnosis of cognitive and behavioral impairment in ALS

ALSFTD-1[3]	**ALSFTD-2[4]**
ALSbi A diagnosis of ALSbi requires meeting at least two non-overlapping supportive diagnostic features from either the Neary criteria or Hodges criteria for FTD	**ALSbi** A diagnosis of ALSbi requires: 1. The identification of apathy with or without other behavior change OR 2. meeting at least two non-overlapping supportive diagnostic features from the Rascovsky criteria
ALSci A diagnosis of ALSci depends on evidence of cognitive impairment at or below the 5th percentile on at the least two distinct tests of cognition that are sensitive executive functioning	**ALSci** A diagnosis of ALSci depends on evidence of either executive dysfunction (including social cognition) or language dysfunction or a combination of the two. Executive impairment is defined as: 1. Impaired verbal fluency (letter). OR 2. Impairment on two other non-overlapping measures of executive functions (which may include social cognition) Language impairment is defined as:

	1. Impairment on two non-overlapping tests and in which language impairment is not solely explained by verbal fluency deficits.
	ALScbi Patients who meet the criteria for both ALSci and ALSbi
ALS-FTD ALS-bvFTD ALS-dementia (ALS-D)*, FTD-MND All patient meeting either the Neary criteria or Hodges criteria for FTD	**ALS-FTD, ALS-dementia (ALS-D)*, FTD-MND** A diagnosis of ALS-FTD requires: 1. Evidence of progressive deterioration of behavior and/or cognition by observation or history AND 2. The presence of at least 3 of the behavioral/cognitive symptoms outlined by Rascovsky et al 2011 OR 3. The presence of at least 2 of those behavioral/cognitive symptoms, together with loss of insight and/or psychotic symptoms OR 4. The presence of language impairment meeting criteria for semantic dementia/ semantic variant PPA or non-fluent variant PPA. This may co-exist with behavioral/ cognitive symptoms as outlined above.

All patients were classified in blind by two neuropsychologists expert in ALS. When there was disagreement, the case was discussed until a final diagnosis was agreed. Statistical methods Comparisons between means were made with Student's t-test or analysis of variance; comparisons between categorical variables were made with $\chi 2$ test. All tests were two-tailed. Rater agreement was calculated via the k statistic, which is the rate of observed agreement between all possible pairs of ratings adjusted for the proportion of agreement expected to occur

by chance.[12] Survival was calculated from onset to death/tracheostomy or censoring date (31 December 2017) using the Kaplan Meier method and compared with the log-rank test. No patients were lost to follow-up. Multivariable analysis for survival was performed with the Cox proportional hazards model (stepwise backward) with a retention criterion of p In the clinical-based cohort, according to the ALSFTD-1 criteria, 8 patients (3.9%) were classified as ALS-FTD, 37 (18.0%) as ALSci, 12 (5.9%) as ALSbi and 148 (72.2%) as cognitively normal. According to the ALSFTD-2 criteria, 17 (8.3%) patients were included in the novel category ALScbi: of these 14 had been previously classified as ALSci and 3 as ALSbi. Moreover, among patients who were previously classified as cognitively normal, 19 (9.3%) were reclassified as ALSci and 1 as ALSbi. No patients were reclassified as ALS-FTD. Overall, 37 patients (18.0%) had their cognitive category changed. The change of category was due to the same reasons reported for the population-based cohort. The shift of some patients from the previous categories to the novel ones has had some implications on the clinical characteristics of the cognitive categories (table 3).

E-Table 2. Interrater agreement between the two blinded raters. Population- based cohort, *k* value 0.91 (95% c.i. 0.87-0.95); referral cohort, *k* value 0.87 (95% c.i. 0.81-0.94)

Population-based cohort		**Rater 1**				
		CN	**ALSbi**	**ALSci**	**ALScbi**	**FTD**
Rater 2	**CN**	164	2	2	0	0
	ALSbi	4	17	0	3	0
	ALSci	2	0	47	2	0
	ALScbi	0	1	3	27	0
	FTD	0	0	0	0	47

Referral cohort		**Rater 1**				
		CN	**ALSbi**	**ALSci**	**ALScbi**	**ALS-FTD**
Rater 2	**CN**	125	1	2	0	0
	ALSbi	1	9	0	9	0
	ALSci	4	0	35	3	0
	ALScbi	0	1	2	13	0
	ALS-FTD	0	0	0	0	8

E-Table 3. *k* values (95% c.i.) for each cognitive diagnosis in the two cohorts

	Population-based cohort	**Referral cohort**
Cognitively normal	0.94 (0.90 to 0.98)	0.92 (0.87 to 0.98)
ALSbi	0.76 (0.61 to 0.90)	0.85 (0.682 to 1.000)
ALSci	0.90 (0.829 to 0.963)	0.83 (0.734 to 0.927)
ALScbi	0.84 (0.740 to 0.943)	0.80 (0.639 to 0.955)
ALS-FTD	1.0	1.0

Table 2. Characteristics of the population-based and the referral cohorts.

	Population-based cohort (n=321)	**Referral cohort (n=205)**	**p**
Male (%)	181 (55.0%)	120 (58.3%)	0.65
Bulbar onset (%)	98 (29.8%)	45 (22.0%)	0.03
Mean age at onset (years, SD)	66.4 (10.1)	59.9 (11.7)	<0.0001
Median survival (years, IQR)	3.15 (1.95-7.10)	5.13 (3.12-8.21)	<0.0001

Table 3. Clinical characteristics of patients according to the two cognitive classifications in the population-based cohort.

	ALSFTD-1					**ALSFTD-2**					
	ALS-CN (n=182)	**ALSbi (n=19)**	**ALSci (n=75)**	**ALS-FTD (n=45)**	**p**	**ALS-CN (n=168)**	**ALSbi (n=24)**	**ALSci (n=51)**	**ALScbi (n=31)**	**ALS-FTD (n=47)**	**p**
Mean age at onset (years, SD)	64.3 (10.4)	67.1 (8.2)	69.7 (8.2)	69.7 (8.5)	0.001	64.7 (10.1)	64.8 (12.7)	66.8 (10.2)	72.1 (6.7)	69.9 (8.4)	0.001
Gender (female, %)	75 (41.2%)	7 (36.8%)	32 (42.7%)	30 (66.7%)	0.016	71 (42.3%)	8 (33.3%)	24 (47.1%)	11 (35.5%)	30 (63.8%)	0.04
Onset (bulbar, %)	37 (20.3%)	10 (52.6%)	21 (28.0%)	25 (55.6%)	0.0001	33 (19.6%)	10 (41.7%)	16 (31.4%)	8 (25.8%)	26 (55.3%)	0.0001

Mean education (years, SD)	10.1 (4.0)	9.6 (4.4)	7.5 (3.4)	7.8 (3.4)	0.0001	10.0 (4.1)	10.5 (4.3)	7.2 (3.2)	8.7 (3.5)	7.8 (4.8)	0.0001
Median survival (years, IQR) *	4.0 (3.1-4.8)	3.4 (2.1-4.7)	2.9 (2.3-3.4)	2.0 (1.5-2.4)	0.0001	4.0 (3.2-4.8)	5.4 (1.4-9.4)	3.1 (2.3-3.8)	2.7 (2.1-3.3)	2.1 (1.7-2.4)	0.0001

* p<0.0001 (ALSFTD-1); p<0.0001 (ALSFTD-2)

In particular, the new ALScbi category is characterised by a higher age at onset (72.1 years, SD 6.6) than all other cognitive categories and has a median survival (2.6 years, 95% CI 2.1 to 3.3), which is intermediate between that of ALS-FTD (2.1 years, CI 1.7 to 2.4) and ALSci (3.1, CI 2.3 to 3.8). Overall, the median survival of the different categories remains significantly different both with the ALSFTD-1 and ALSFTD-2 criteria (figure 2). The other characteristics of cognitive subgroups did not modify significantly. P values of posthoc paired comparisons are reported in online supplementary E-Table 4.

Figure 1. Category change between the ALSFTD-1 and ALSFTD-2 criteria in the population-based cohort.

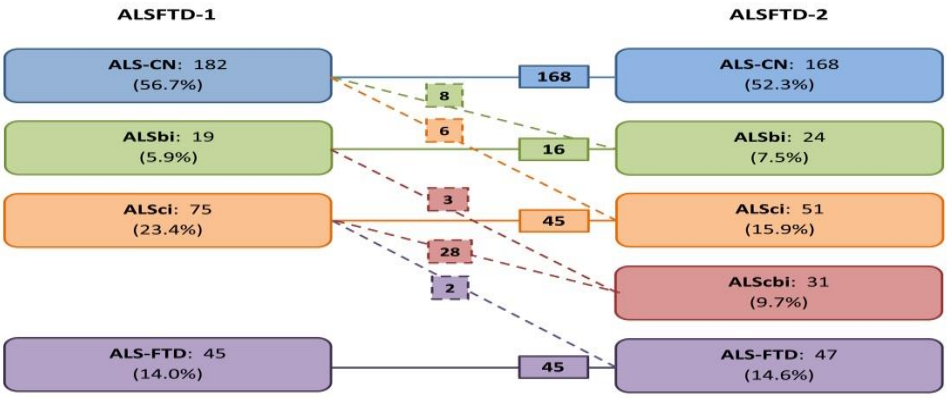
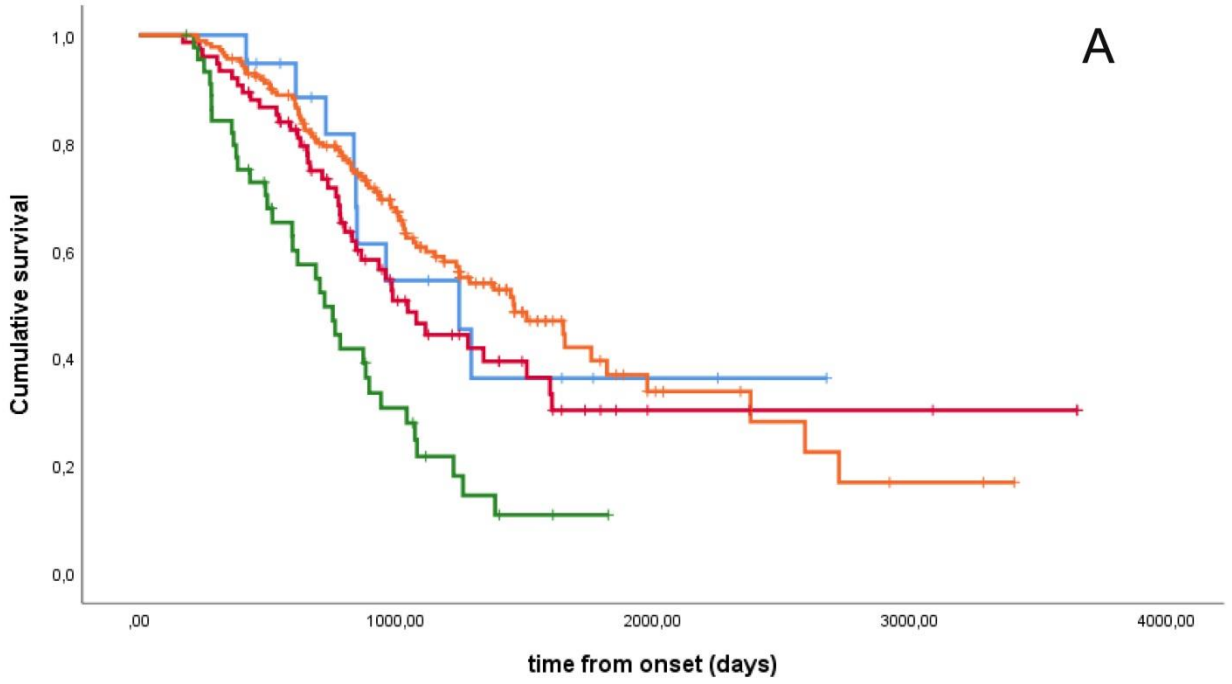
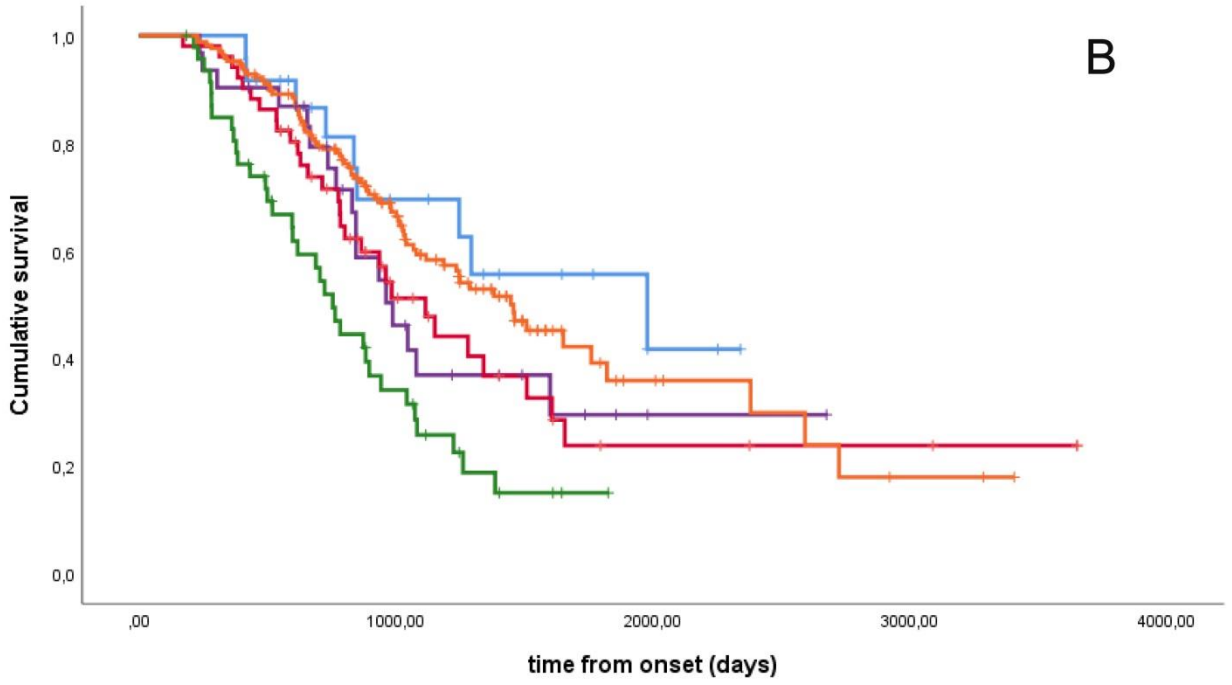


Figure 2. Survival of population-based cohort according to cognitive classification. **A.** ALSFTD-1 criteria (p<0.0001). **B.** ALSFTD-2 criteria (p<0.0001). Orange, cognitively normal; blue, ALSbi; red, ALSci; green, ALS-FTD; violet, ALScbi.





E-Table 4. P values of comparisons between demographic and clinical characteristics of patients according to their cognitive classification (Table 3). Right, ALSFTD-1; left, ALSFTD-2. Not corrected p values are reported. Bonferroni correction for ALSFTD-1 is p=0.002, for ALSFTD-2 is 0.001.

Age at onset	ALS-CN		
ALSbi	n.s.	ALSbi	
ALSci	0.0001	n.s.	ALSci
ALS-FTD	0.002	n.s.	n.s

Age at onset	AL-CN			
ALSbi	n.s.	ALSbi		
ALSci	n.s.	n.s.	ALSci	
ALScbi	0.0001	n.s.	0.01	ALScbi
ALS-FTD	0.001	0.001	n.s.	n.s.

Gender	ALS-CN		
ALSbi	n.s.	ALSbi	
ALSci	n.s	n.s.	ALSci
ALS-FTD	0.0002	0.03	0.0009

Gender	AL-CN			
ALSbi	n.s.	ALSbi		
ALSci	n.s.	n.s.	ALSci	
ALScbi	n.s.	n.s.	n.s.	ALScbi
ALS-FTD	0.007	0.01	n.s.	0.01

Site of onset	ALS-CN		
ALSbi	0.003.	ALSbi	
ALSci	n.s	0.04	ALSci
ALS-FTD	0.0001	n.s.	0.0003

Site of onset	AL-CN			
ALSbi	0.01	ALSbi		
ALSci	n.s.	n.s.	ALSci	
ALScbi	n.s.	n.s.	n.s.	ALScbi
ALS-FTD	0.0001	n.s.	0.01	0.0009

Education	ALS-CN		
ALSbi	n.s.	ALSbi	
ALSci	0.0001	0.03	ALSci
ALS-FTD	0.0001	n.s.	n.s

Education	AL-CN			
ALSbi	n.s.	ALSbi		
ALSci	0.0001	0.0001	ALSci	
ALScbi	n.s.	n.s.	n.s.	ALScbi
ALS-FTD	0.0002	0.002	n.s.	n.s.

Survival	ALS-CN		
ALSbi	n.s.	ALSbi	
ALSci	n.s.	n.s.	ALSci
ALS-FTD	0.0001	0.013	0.002

Survival	AL-CN			
ALSbi	0.001	ALSbi		
ALSci	0.001	0.001	ALSci	
ALScbi	0.01	0.01	n.s.	ALScbi
ALS-FTD	0.0001	0.0002	0.02	0.03

Patients with non-executive impairment The presence of non-executive impairment was searched for in the population-based cohort. A total of 11 patients showed an impairment in memory and visuospatial domains. Four of them had also an executive and/or behavioural impairment (2 ALSci and 2 ALScbi), while seven were classified as cognitively normal according to the ALSFTD-2. These non-executive impaired patients were slightly older (70.1 years, SD 8.3) and had more frequently a

bulbar onset (six cases, 54.5%). Finally, their median survival was similar to the ALScbi group (2.4 years, CI 1.8 to 3.1).

Discussion

Since 2009 cognitive impairment in ALS has been diagnosed according to the ALSFTD-1 criteria.[3] The revised ALSFTD-2 criteria, published in 2017, were deemed necessary due to the considerable improvement in the understanding of the cognitive profile of patient with ALS, in particular, but not exclusively, the recognition of the extent of the deficits in social cognition and language.[4] ALSFTD-2 criteria are more operational than the former ones and have the aim of delineating more homogenous cognitive groups. To evaluate how the new classification of cognitive impairment in ALS impacts on the characteristics of the cognitive subgroups and their distribution, we applied the ALSFTD-2 criteria to a large population-based cohort and to two series of patients seen in referral ALS centres. Overall, the revised criteria determined the reclassification of 14.6% of patients of the population-based cohort and of 18.0% of those of the referral cohort.

The change of classification of these patients was mainly due to three modifications of the ALSFTD-2 criteria compared with the previous ones: first, the increased emphasis for language impairment, which can be diagnosed in presence of isolated impaired verbal fluency (letter) or of two non-overlapping tests, in which language impairment is not solely explained by verbal fluency deficits; second, the greater emphasis on apathy, whose presence is sufficient to make a diagnosis of ALSbi; third, the inclusion in the criteria for ALS-FTD of loss of insight and/or psychotic symptoms. As a consequence of these changes in the classification criteria, patients who were previously categorised as ALS-CN were reclassified either as ALSci or as ALSbi. This change was particularly marked in the referral cohort (20 out of 148 patients, 13.5%) but was also present to a minor extent in the epidemiological-based cohort (14 out of 182 patients, 7.7%). Inter-rater agreement of the classification of cognitive impairment in ALS was very high (k value 0.91 in the population-based series and 0.87 in the referral cohort),13 indicating that the revised ALSFTD-2 criteria are highly reliable and that experienced professionals can accurately and consistently apply these criteria in the clinical setting. These observation holds also for each cognitive category, with a k statistics varying between 0.76 and 1, the complete concordance being observed for the diagnosis of ALS-FTD. We found that the reclassification of patients from ALSFTD-1 to ALSFTD-2 has a substantial impact on the characteristics of the groups of patients. In particular, the newly

proposed ALScbi group originates mainly from patients previously included in the ALSci group and is characterised by the oldest age at onset compared with all other groups and a survival intermediate between ALSci and ALS-FTD. Moreover, patients reclassified as ALScbi have an educational level higher than that of ALS-FTD and ALSci, but lower than that of ALS-CN and ALSbi. Patients with mixed cognitive and behavioural impairment but not meeting the criteria for FTD have been previously reported. A previous epidemiological-based study performed by our group found that 11 (6%) out of the 183 patients of the cohort had an impairment in one executive and/or one non-executive test associated with behavioural changes; these patients were labelled as ALS with non-classifiable cognitive impairment.[2] Similarly, another paper based on a clinical series found that 1 out of 23 patients with ALS showed both a cognitive and a behavioural impairment.[14] It remains to be clarified whether the ALScbi category represents a transitional stage to FTD similar to mild cognitive impairment in Alzheimer's disease. With both classification, patients categorised at different cognitive diagnoses showed several clinical differences. In particular, patients with more severe cognitive impairment (ALS-FTD and ALScbi) were older than patients with normal cognition and had a lower education. Similar findings have been reported in other clinical[1 2 15] and epidemiological studies.[16 17] The higher frequency of bulbar onset in patients with cognitive impairment has been also reported.[18 19] Finally, the marked predominance of females in the ALS-FTD group is likely related to their higher frequency of bulbar impairment. A relatively small percentage of patients with ALS in the population-based cohort showed an impairment in non-executive domains, mainly memory and visuospatial domains, in isolation (seven cases, 2.2%), or associated (four cases, 1.2%) to executive and behavioural impairment. The codification of these cases, who accounted for about 5% of cases in two previous population-based studies[1 2] remains uncertain. Similarly, a clinical-based series, on basis of a principal component analysis, showed that 24% of patients did not meet ALSFRS-1 criteria and were characterised by preeminent deficit in social cognition, language and episodic memory.[20] A recent study did not find any difference in the ALS-non-specific functions (memory, visuospatial) evaluated with the ECAS, across disease stages classified according to King's staging.[19] In the ALSFTD-2 original paper it has been suggested that non-executive impairment is rare in isolation and it occurs at a comparable rate in controls, making questionable the introduction of a specific category in the classification. An interesting observation of our study is that the cognitive classification of patients was quite different in the two cohorts. Besides the well-known differences of epidemiological and referral cohorts in ALS,[21 22] that is, younger age at onset, lower

number of bulbar onset patients and better survival in the referral cohort, we also found that referral cohort was characterised by a lower frequency of patients with ALS-FTD (3.9% vs 14.6% with the ALSFTD-2) and, correspondingly, a higher percentage of ALS-CN (62.4% vs 52.3%) ($p<0.0001$). This difference is likely to be related to the poorer propensity of patients with cognitive dysfunction and their caregiver to seek advice to referral ALS centres, but are usually followed by the local neurological departments. We have found that the revised classification of frontotemporal dementia in ALS causes a shift of some 15% of patients from their original category to a new one.

Most changes are due to the establishment of the novel category of ALScbi, which accounts for 10% of patients; this category is intermediate between ALSci and ALS-FTD in terms of prognosis and includes older and more educated patients. Additionally,~10% of cases who were previously classified as non-cognitively impaired were reclassified to the ALSci and, to lesser extent, to the ALSbi categories with the novel classification. Finally, some patients previously classified as ALSci were diagnosed as FTD with the revised classification. These latter modifications were due to the increased role attributed to the impairment in verbal fluency (letter) and social cognition in the diagnosis of cognitive impairment in ALS by the ALSFTD-2 criteria. It is possible that the higher sensitivity of ALSFTD-2 criteria compared with ALSFTD-1 leads to the inclusion of some false positive diagnoses of cognitive and/or behavioural impairment. For example, the relevance given to apathy in the diagnosis of ALSbi could indeed reduce the specificity of the criteria, considering the complexity of the theoretical construct of this particular behaviour[23] and of its neuroanatomical and cognitive substrates.[24] However, it should be noted that in a study based on ECAS, apathy was the most common behavioural symptoms detected in patients with ALS compared with patients with FTD, in whom disinhibition predominated.[25] Longitudinal studies evaluating the progression over time of such patients are necessary to rule out this possibility. However, despite this risk, we think that the higher sensitivity of ALSFTD-2 criteria for detecting early cognitive and behavioural signs entail several clinical advantages: first, they allow to identify and classify earlier the cognitive-behavioural impairment, also alerting caregivers for subtle modifications of cognition and/or behaviour; second, they have a better prognostic yield; third, they permit the clinician to timely discuss patients' directives on future therapies. Moreover, more sensitive diagnostic criteria for cognitive and behavioural impairment will improve the clinical and biological studies on the effects of cognitive damage in patients with ALS and, in perspective, will

be useful for detecting the early signs of cognitive impairment when specific treatment for FTD will be develop

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Brain Metabolic Correlates of Apathy in Amyotrophic Lateral Sclerosis: a ^{18}F-FDG-PET study

Canosa A, Vacchiano V, D'Ovidio F, Calvo A, Moglia C, Manera U, Vasta R, Liguori R, Arena V, Grassano M, Palumbo F, Peotta L, **Iazzolino B**, Pagani M, Chiò A.

Abstract

Objective. To evaluate brain metabolic correlates of apathy in ALS.

Methods. 165 ALS patients underwent 18F-FDG-PET and FrSBe. FrSBe provides "before" and "after" apathy subscores, referring to premorbid and morbid conditions. "After" apathy subscore and "before-after" gap were regressed against whole brain metabolism. Among patients with pathological "after" apathy subscore (i.e. ≥65), we compared patients with "before" apathy subscore ≥65 and <65, and patients with "before-after" gap <22 and ≥22.

Results. In the whole sample, the "after" apathy subscore negatively correlated with metabolism in dorsolateral prefrontal (DLPFC), dorsomedial prefrontal (DMPFC), ventrolateral prefrontal (VLPFC), premotor (PMC) and anterior cingulate (ACC) cortices, and insula bilaterally. A positive correlation was found in cerebellum and pons. The "before-after" gap negatively correlated with metabolism in bilateral DLPFC, DMPFC, and PMC, left VLPFC and ACC, and positively correlated with cerebellar and pontine clusters. Among patients with "after" apathy subscore ≥65, we found no difference between subjects with "before" apathy subscore ≥65 and <65. Patients with "before-after" gap ≥22, compared to patients with gap <22, showed relative hypometabolism in bilateral DLPFC and DMPFC, left ACC and PMC, and relative cerebellar and pontine hypermetabolism.

Conclusion. No studies on brain 18F-FDG-PET correlates of apathy have been performed in ALS. We found that FrSBe "after" apathy subscore correlated with metabolic changes in brain regions known as neuroanatomical correlates of apathy. Furthermore, our findings support the relevance of the gap between premorbid and morbid conditions to detect behavioural changes due to the neurodegenerative process underlying ALS.

Introduction

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease affecting upper and lower motor neurons. Death usually occurs within 2-5 years, mainly due to respiratory failure.[1] According to population-based studies ~50% of ALS patients show cognitive and/or behavioural impairment falling along the frontotemporal spectrum at diagnosis.[2,3] Apathy has been included among features characterizing behavioural dysfunction since the first diagnostic criteria for ALS-related frontotemporal syndromes.[4] Apathy has assumed a central role in the recently revised criteria, stating that the presence of apathy by itself allows a diagnosis of behavioural impairment associated with ALS (ALS-bi).[5] Apathy is shared among many neurological and psychiatric

disorders. It has been defined in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5),[6] as characterized by "diminished motivation and reduced goal-directed behaviour, accompanied by decreased emotional responsiveness." Diagnostic criteria for apathy have been revised in 2018:[7] the patient must present a quantitative reduction of goal-directed activity as compared to her/his previous level of functioning; symptoms must persist for at least four weeks, and affect at least two of the three apathy dimensions (behaviour/cognition; emotion; social interaction); apathy should lead to functional impairment, and should not be fully ascribable to other factors (e.g. effects of substances or major changes in the patient's environment).

In order to determine if behavioural symptoms of ALS patients represent a change due to the neurodegenerative process, premorbid status must be assessed. At the ALS Centre of Turin (Italy) the neuropsychological assessment of ALS patients includes the evaluation of behavioural dysfunction based on direct observation, patient's history, and the Frontal Systems Behavior Scale (FrSBe).[8] FrSBe evaluates 3 domains (apathy, disinhibition and executive dysfunction) and provides "before" and "after" ratings, referring respectively to premorbid condition and the time the scale is performed.

Being ^{18}F-2-fluoro-2-deoxy-D-glucose-PET (^{18}F-FDG-PET) a marker of neuronal integrity *in vivo,*[9] in this study we evaluated brain metabolic correlates, assessed through ^{18}F-FDG-PET, of the apathy subscore of FrSBe in an ALS series. Since we hypothesized that both the "after" apathy score and the change between "before" and "after" conditions could be relevant to characterize ALS-related behavioural dysfunction, we aimed at evaluating the relationship of both of them with brain metabolism.

Materials and Methods

Patients

A total of 165 patients diagnosed with definite, probable or probable laboratory-supported ALS according to El Escorial Revised Diagnostic Criteria[10] at the ALS Centre of Turin (Italy) in the period 2009-2015 were included. They were enrolled at diagnosis or, less frequently, during the first follow up visit (usually 2 months later). Patients with a history of neurological disorders affecting cognition (major stroke, severe head injuries, mental retardation), alcohol and drug dependence, psychiatric diseases (including mood disorders), or use of high-dose psychoactive medications were not enrolled, nor were patients whose native language was not Italian. Respiratory failure was excluded through clinical assessment, peripheral blood oxygen saturation, and, when

necessary, spirometry and arterial blood gases analysis, within 4 weeks before or after the enrolment. Patients underwent ^{18}F-FDG-PET and neuropsychological assessment including FrSBe. The whole test battery has been reported elsewhere.[3] Neuropsychological evaluation and ^{18}F-FDG-PET were performed within 1 month of each other.

^{18}F-FDG-PET acquisition

^{18}F-FDG-PET was performed according to published guidelines.[11] Patients fasted at least six hours before the exam. Blood glucose was <7.2 mmol/l in all cases before the procedure. After a 20-minute rest, about 185 MBq of ^{18}F-FDG was injected. The acquisition started 60 minutes after the injection. PET/CT scans were performed by a Discovery ST-E System (General Electric). Brain CT (thickness of 3.75 millimetres, 140 kVolt, 60-80 mAs) and PET scan (1 FOV of 30 transaxial centimetres) were sequentially acquired, the former being used for attenuation correction of PET data. PET images were reconstructed with 4 iterations and 28 subsets with an initial voxel size of 2.34x2.34x2.00 mm and data were collected in 128x128 matrices.

Behavioural assessment

FrSBe[8] is a 46-item scale, including a total score and three subscores: apathy (14 items), disinhibition (15 items) and executive dysfunction (17 items). Items are rated in a 5-point scale: 1 (almost never), 2 (seldom), 3 (sometimes), 4 (frequently), 5 (almost always). FrSBe contains "before" and "after" ratings, referring respectively to premorbid condition and the time the scale is performed (in our series at diagnosis). We used the Family version evaluated by a close relative, since reports from caregivers are of outstanding importance in light of the possible loss of insight of patients.[5] The higher is the score, the more severe is behavioural impairment. Scores ≥65 are interpreted as pathological according to the FrSBe manual for each section and the total score.[8] We considered the "after" apathy subscore as a measure of behavioural impairment at diagnosis. The "before-after" change was estimated in two different ways. The former was the gap between "before" and "after" apathy subscores, calculated as follows: "after" apathy subscore – "before" apathy subscore. The latter assessed the apathetic/non apathetic status based on the cut off of 65 points to evaluate eventual change of status between "before" and "after" conditions. So, we could subdivide apathetic patients (i.e. "after" apathy subscore ≥65) into two groups: subjects with premorbid score already in the pathological range (i.e. "before" apathy subscore ≥65) and subjects with premorbid score within the normal range (i.e. "before" apathy subscore <65). Both

methods were considered as possible proxies of behavioural changes due to the neurodegenerative process. In order to identify a possible cut-off to consider a "before-after" gap as significant, we examined a comparable neurological group as reference, as suggested by the manual of the scale.[8] We considered 517 incident ALS patients from the Piemonte and Valle d'Aosta Register for ALS,[12] who underwent a neuropsychological assessment, including FrSBe, at diagnosis, between 2009 and 2015. We excluded 22 patients, who displayed a negative gap between "before" and "after" conditions, possibly due to misinterpretation of the scale by the rater. We also excluded those patients who underwent PET (n=165, the present study sample). The median value of the gap resulted 12 (interquartile range 4-22). The threshold between the third and fourth quartile (i.e. 22) was hypothesized as a possible cut-off value to consider a "before-after" gap of the apathy subscore as significant.

Statistical analysis

Comparisons between means were made with the Student's t-test or analysis of variance; comparisons between categorical variables were made with the χ^2 test and Fisher's test when applicable.

SPM12 implemented in Matlab R2018b (MathWorks, Natick, MA, USA) was used for image normalization. A customized brain ^{18}F-FDG-PET template[13] was utilized for spatial normalization. Intensity normalization was performed using the 0.8 default SPM value of grey matter threshold and images were subsequently smoothed with a 10-mm filter and submitted to statistical analysis.

First, we aimed at evaluating the correlations between brain metabolism and both "after" apathy subscore and "before-after" gap of the apathy subscore of FrSBe, performing two multiple regression analyses in the whole sample (n=165). Subsequently, we focused on patients with the "after" apathy subscore ≥65, i.e. subjects showing scores considered as pathological at diagnosis (n=84), to evaluate whether a further characterization of such patients based on the "before-after" change was worthwhile. We divided such group into two subgroups to compare them: subjects showing a "before" apathy subscore ≥65 (i.e. already in the pathological range) *versus* patients displaying a "before" apathy subscore <65 (i.e. within the normal range). Then, we divided the same group of patients with the "after" apathy subscore ≥65 into the following two subgroups to compare them: patients showing a "before-after" gap <22 *versus* patients with a "before-after" gap ≥22. Comparisons were performed through the two-sample t-test model of SPM12.

In all analyses we did not include age, sex and education as covariates, since FrSBe scores were already corrected for these variables. Furthermore, we did not include a measure of global cognitive status (i.e. classification according to the diagnostic criteria for ALS-FTSD)[5] or executive dysfunction as covariates, since they resulted highly correlated with apathy subscores ($r=0.77$, $p<0.001$). On the other hand, we included the FrSBe "after" subscore related to disinhibition as covariate in all the analyses, since it resulted only marginally correlated with the "after" apathy subscore ($r=0.57$; $p<0.001$). Details about the pitfalls of including highly correlated variables as covariates in multiple regression models are reported elsewhere.[14]

For all the analyses the height threshold was set at $P<0.005_{uncorrected}$ ($P<0.05_{FWE\text{-}corrected}$ at cluster level) and only clusters containing >125 contiguous voxels were considered significant. Brodmann areas (BAs) were identified at a 0–2-mm range from the Talairach coordinates of the SPM output isocentres corrected by Talairach Client (http://www.talairach.org/index.html).

Protocol approvals

The study was approved by the ethical committee "Comitato Etico Interaziendale Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino". The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Patients signed a written informed consent.

Data availability statement

Data will be available upon request by interested researchers.

Results

Demographic and clinical data

We compared demographic and clinical data of patients who underwent ^{18}F-FDG-PET (n=165) to the reference population-based series (n=330). The comparison is summarized in Supplemental Table 1. No significant difference was found for sex distribution, education, and site of onset (bulbar/spinal). Otherwise, in patients who underwent ^{18}F-FDG-PET age resulted slightly lower and ALSFRS-R resulted slightly higher, probably due to the higher difficulty of elderly people and patient with worse disability in reaching the PET Centre. In the group of patients with the "after" apathy subscore ≥65, i.e. subjects showing scores considered as pathological at diagnosis (n=84), we compared demographic and clinical data of subjects showing a "before" apathy subscore ≥65

versus patients displaying a "before" apathy subscore <65, and subjects showing a before-after gap <22 *versus* subjects with a before-after gap ≥22. In both comparisons we did not find any difference in terms of sex distribution, site of onset (bulbar/spinal), age at assessment, education, and ALSFRS-R at assessment. Such data are summarized in Supplemental Table 2.

Supplemental Table 1. Comparison of the demographic and clinical data of the study sample with the reference population-based series.

	Reference population-based series	Study sample	Total	P-value
Sex				0.250
Female (%)	142 (43%)	80 (48%)	222 (45%)	
Male (%)	188 (57%)	85 (52%)	273 (55%)	
Age at assessment, mean (SD)	67.9 (10.26)	65.5 (10.75)	67.14 (0.47)	**0.015**
Education (years), mean (SD)	8.47 (4.01)	8.57 (3.98)	8.50 (3.99)	0.793
ALSFRS-R at assessment, mean (SD)	40.42 (0.30)	42.12 (0.35)	41.00 (0.24)	**0.001**
Site of onset				0.947
Bulbar (%)	121 (36.7%)	60 (36.4%)	181 (36.6%)	
Spinal (%)	209 (63.3%)	105 (63.6%)	314 (63.4%)	
Total	330	165	495	

SD: Standard Deviation. ALSFRS-R: Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised. Significant differences are reported in bold.

Supplemental Table 2. In the group of patients with the "after" apathy subscore ≥65, we compared demographic and clinical data of subjects showing a "before" apathy subscore ≥65 *versus* patients displaying a "before" apathy subscore <65, and subjects showing a before-after gap <22 *versus* subjects with a before-after gap ≥22.

	Patients with After Apathy Subscore ≥65, classified according to "Before" Apathy Subscore			Patients with After Apathy Subscore ≥65, classified according to Apathy "Before-After" Gap		
	"Before" Apathy Subscore <65	"Before" Apathy Subscore ≥65	P-value	"Before-After" Gap <22	"Before-After" Gap ≥22	P-value
Sex						
Male (%)	30 (51.7)	10 (38.5)	0.261	23 (52.3)	17 (42.5)	0.370
Female (%)	28 (48.3)	16 (61.5)		21 (47.7)	23 (57.5)	
Site of onset						

Bulbar (%)	22 (37.9)	9 (34.6)	0.771	14 (35.0)	17 (38.6)	0.730
Spinal (%)	36 (62.1)	17 (65.4)		26 (65.0)	27 (61.4)	
Age at assessment, mean (SD)	68.0 (9.7)	66.9 (8.8)	0.617	66.7 (10.0)	68.7 (8.7)	0.341
Education (years), mean (SD)	8.1 (4.2)	7.7 (3.4)	0.733	8.2 (4.0)	7.7 (3.9)	0.506
ALSFRS-R score at assessment, mean (SD)	37.8 (6.6)	38.5 (5.1)	0.648	38.1 (6.8)	37.9 (5.3)	0.833
Total (%)	58 (69.0)	26 (31.0)		44 (52.4)	40 (47.6)	

ALSFRS-R: Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised. SD: Standard Deviation.

^{18}F-FDG-PET data

Correlation between the "after" apathy subscore and brain metabolism in the whole sample (n=165)

The "after" apathy subscore negatively correlated with metabolism in dorsolateral prefrontal (DLPFC), dorsomedial prefrontal (DMPFC), ventrolateral prefrontal (VLPFC), premotor (PMC) and anterior cingulate (ACC) cortices, and insula bilaterally (Table 1, Figure 1A). A positive correlation was found in cerebellum and pons (Figure 2A).

Correlation between "before-after" gap and brain metabolism in the whole sample (n=165)

The "before-after" gap negatively correlated with metabolism in bilateral DLPFC and DMPFC, left VLPFC, left ACC, bilateral PMC (Table 2, Figure 1B), and positively correlated with clusters including cerebellum and pons (Figure 2B).

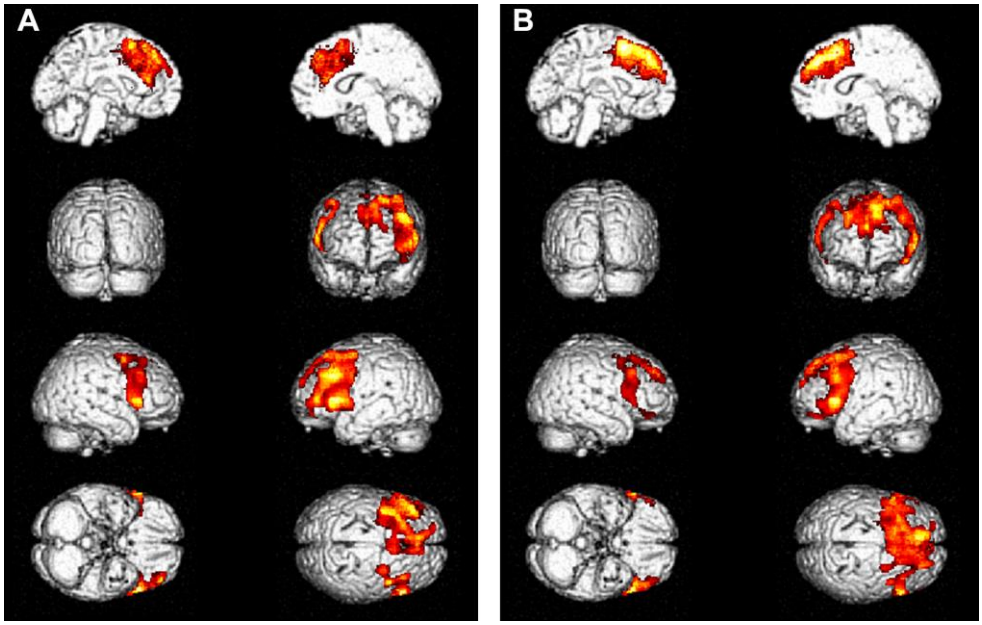

Figure 1. A. Clusters of negative correlation between FrsBe "after" apathy subscore and whole brain metabolism in the whole sample (n=165) are projected on brain surface. B. Clusters of negative correlation between FrsBe apathy "before-after" gap and whole brain metabolism in the whole sample (n=165) are projected on brain surface.

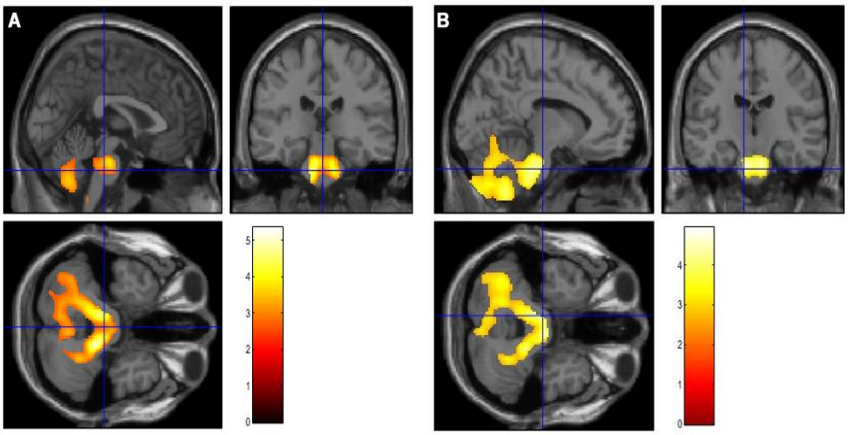

Figure 2. A. Clusters of positive correlation between FrsBe "after" apathy subscore and whole brain metabolism in the whole sample (n=165) are represented on a brain MRI template.

B. Clusters of positive correlation between FrsBe apathy "before-after" gap and whole brain metabolism in the whole sample (n=165) are represented on a brain MRI template.

Table 1: Clusters of negative correlation between FrsBe "after" apathy subscore and whole brain metabolism in the whole sample.

P (FWE-corr)	Cluster Extent	Z-score	Talairach Coordinates			Lobe	Cortical Region	BA
0.000	9655	4.42	-53	16	8	Frontal	Left Precentral Gyrus	44
		4.39	-44	19	29	Frontal	Left Middle Frontal Gyrus	9
		4.28	-38	35	31	Frontal	Left Superior Frontal Gyrus	9
		3.95	-18	28	52	Frontal	Left Superior Frontal Gyrus	6
		3.82	-40	2	35	Frontal	Left Precentral Gyrus	6
		3.81	-34	5	53	Frontal	Left Middle Frontal Gyrus	6
		3.74	-40	19	1	Sub-lobar	Left Insula	13
		3.73	10	26	21	Limbic	Right Anterior Cingulate	32
		3.67	-6	43	40	Frontal	Left Medial Frontal Gyrus	8
		3.52	8	14	53	Frontal	Right Superior Frontal Gyrus	6
		3.50	-6	34	17	Limbic	Left Anterior Cingulate	32
		3.45	6	39	33	Frontal	Right Medial Frontal Gyrus	9
0.006	2196	4.06	30	1	50	Frontal	Right Middle Frontal Gyrus	6
		3.81	55	27	28	Frontal	Right Middle Frontal Gyrus	46

		3.61	57	20	8	Frontal	Right Inferior Frontal Gyrus	45
		3.51	38	21	3	Sub-lobar	Right Insula	13
		3.32	38	29	45	Frontal	Right Middle Frontal Gyrus	8
		3.00	42	13	31	Frontal	Right Middle Frontal Gyrus	9
		2.91	42	25	36	Frontal	Right Precentral Gyrus	9
		2.78	40	20	51	Frontal	Right Superior Frontal Gyrus	8

Table 2. Clusters of negative correlation between FrsBe apathy "before-after" gap and brain metabolism in the whole sample.

P (FWE-corr)	Cluster Extent	Z-score	Talairach Coordinates			Lobe	Cortical Region	BA
0.000	11985	4.88	10	16	47	Frontal	Right Superior Frontal Gyrus	6
		4.87	-8	43	42	Frontal	Left Superior Frontal Gyrus	8
		4.39	-51	20	5	Frontal	Left Inferior Frontal Gyrus	45
		4.34	-20	6	49	Frontal	Left Medial Frontal Gyrus	6
		4.32	10	42	31	Frontal	Right Medial Frontal Gyrus	9
		4.31	-16	14	51	Frontal	Left Superior Frontal Gyrus	6
		4.06	-46	17	27	Frontal	Left Middle Frontal Gyrus	9
		3.97	-50	11	18	Frontal	Left Inferior Frontal Gyrus	44

		3.81	-6	12	44	Frontal	Left Medial Frontal Gyrus	32
		3.76	20	29	39	Frontal	Right Middle Frontal Gyrus	8
		3.72	-34	5	55	Frontal	Left Middle Frontal Gyrus	6
		3.72	-4	17	38	Limbic	Left Cingulate Gyrus	32
		3.52	55	25	26	Frontal	Right Middle Frontal Gyrus	46
		3.45	28	6	48	Frontal	Right Middle Frontal Gyrus	6
		3.38	51	21	32	Frontal	Right Middle Frontal Gyrus	9

Patients with the "after" apathy subscore ≥65 (n=84): "before" apathy subscore ≥65 (n=26) versus "before" apathy subscore <65 (n=58).

We found no difference between the two groups.

Patients with the "after" apathy subscore ≥65 (n=84): "before-after" gap <22 (n=44) versus "before-after" gap ≥22 (n=40)

In patients with "before-after" gap ≥22 as compared to patients with "before-after" gap <22 clusters of relative hypometabolism were found in bilateral DLPFC and DMPFC, left ACC, and left PMC (Table 3, Figure 3A), while clusters of relative hypermetabolism were found in cerebellum and pons (Figure 3B).

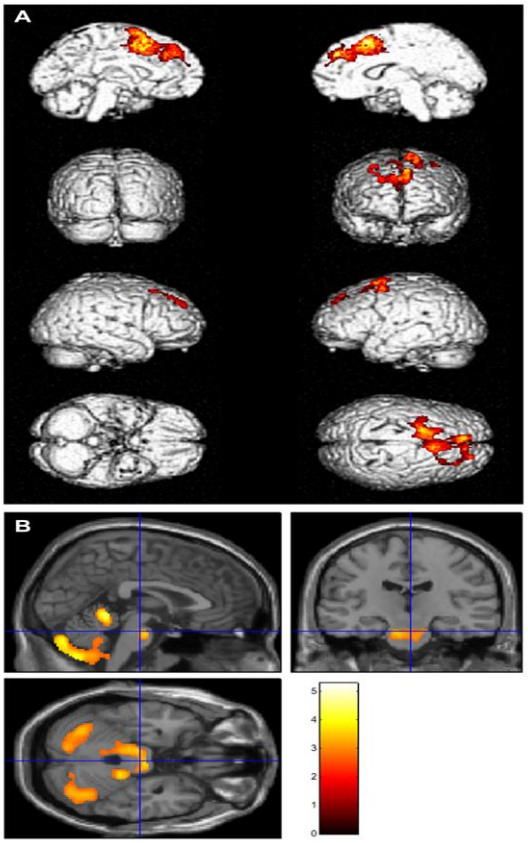

Figure 3. A. Clusters of relative hypometabolism in patients with FrsBe apathy "before-after" gap ≥22 as compared to patients with "before-after" gap <22 are projected on brain surface.
B. Clusters of relative hypermetabolism in patients with FrsBe apathy "before-after" gap ≥22 as compared to patients with "before-after" gap <22 are represented on a brain MRI template.

Table 3. Clusters of relative hypometabolism in patients with FrsBe apathy "before-after" gap ≥22 as compared to patients with "before-after" gap <22, in the sample of patients with FrsBe "after" apathy subscore ≥65.

P (FWE-corr)	Cluster Extent	Z-score	Talairach Coordinates			Lobe	Cortical Region	BA
0.001	3120	3.73	10	18	47	Frontal	Right Medial Frontal Gyrus	8
		3.49	-12	11	60	Frontal	Left Superior Frontal Gyrus	6
		3.33	-6	43	42	Frontal	Left Superior Frontal	8

							Gyrus	
		3.30	-6	8	44	Frontal	Left Medial Frontal Gyrus	32
		3.18	10	46	33	Frontal	Right Superior Frontal Gyrus	9
		3.11	-32	5	53	Frontal	Left Middle Frontal Gyrus	6
		2.92	4	35	30	Frontal	Right Medial Frontal Gyrus	9
		2.91	-2	38	31	Frontal	Left Medial Frontal Gyrus	9
		2.91	32	39	42	Frontal	Right Middle Frontal Gyrus	8
		2.88	28	26	50	Frontal	Right Superior Frontal Gyrus	8
		2.62	4	55	19	Frontal	Right Medial Frontal Gyrus	10

Discussion

To our knowledge, no other studies on brain ^{18}F-FDG-PET correlates of apathy have been performed in ALS patients. Furthermore, we aimed at evaluating the relationship between cerebral metabolism and behavioural changes, defined as the difference between "before" and "after" apathy subscores of the FrSBe scale. We found that the higher was the apathy subscore at diagnosis, the lower was the metabolism in brain regions known to be involved in apathy circuitry (DLPFC, DMPFC, VLPFC, PMC, ACC, and insula). Similarly, the metabolism of largely overlapping regions tended to decrease as the "before-after" gap increased, suggesting the possible metabolic correlates of behavioural changes due to the neurodegenerative process. Since motor impairment remains the core feature of ALS and bulbar onset is significantly associated with cognitive impairment, we ran further analyses to control for the possible impact of motor disability and site of onset on our results, adding the ALSFRS-R total score and spinal/bulbar onset as covariates in the multiple regression analyses. They provided substantially unchanged results (data not shown).

Many structural MRI studies of apathy in FTD have been conducted. In a Voxel-Based Morphometry (VBM) study including patients with behavioural variant FTD (bvFTD, n=48) and Primary Progressive Aphasia (n=14), FrSBe apathy subscore resulted significantly correlated with atrophy of the right DLPFC, with trends to significance in the left DLPFC, right ACC, right lateral orbitofrontal cortex (LOFC), right temporoparietal junction, and right putamen.[15] A VBM and Diffusion Tensor Imaging MRI study[16] evaluated the grey and white matter correlates of apathy across the three components of initiation, planning and motivation as measured by the Philadelphia Apathy Computerized Test, in a sample of 18 bvFTD patients. DLPFC atrophy was predominantly related to the cognitive component (planning) and to deficits in set-shifting, task setting and abstraction. ACC atrophy was linked to the initiation component deficit. PMC was found to play an important role in energization, and intentional movement planning. These data suggest that the components of apathy underlie partially distinct circuits.

A more recent study[17] applied Principal Component Analysis to identify clusters of behavioural changes based on the Frontal Behaviour Inventory subscores in 102 non-demented ALS patients. The apathetic profile resulted correlated with the thinning of bilateral orbitofrontal cortex.

Few ^{18}F-FDG-PET studies have been conducted to disclose the metabolic correlates of apathy in FTD. A study[18] compared 12 apathetic bvFTD patients, 6 disinhibited bvFTD patients, and 24 healthy controls (HC). Considering separately the two bvFTD subgroups in comparison with HC, the apathetic group showed a distinctive relative hypometabolism bilaterally in frontal medial cortex, frontal polar cortex, anterior orbitofrontal cortex, DLPFC, insula, and thalamus. The role of orbitofrontal cortex in apathetic manifestations has been supported also by a study[19] comparing two bvFTD subgroups, defined based on their apathy scores on the Neuropsychiatric Inventory: the apathetic group showed specific metabolic impairment in the orbitofrontal cortex, as compared to HC (a result not shared by the non-apathetic FTD patients).

A more recent study on the neural correlates of apathy in bvFTD and Alzheimer's Disease (AD)[20] evaluated the relationship between brain metabolism and apathy, employing ^{18}F-FDG-PET and the Lille Apathy Rating Scale. The authors included 42 bvFTD, 42 AD, and 30 HC. In bvFTD patients a distinct neuroanatomical correlate was found: apathy resulted to be associated with lower metabolism in the left lateral prefrontal, medial frontal/anterior cingulate, and orbitofrontal and anterior insular cortices.

A recent review focused on the neuroanatomical correlates of the components of apathy in FTD, assessed through MRI and ^{18}F-FDG-PET.[21] The authors suggested that DLPFC atrophy was mainly

related to the cognitive component (planning) and associated with deficits in set-shifting, task setting and abstraction. The impairment of the initiation component and the energization deficits were reported to be mainly related to neuronal loss in the dorsomedial frontal areas (ACC, middle cingulate cortex, medial superior frontal gyrus, supplementary motor area). The involvement of ventral prefrontal areas (subgenual ACC, medial and LOFC) was reported to be predominantly associated with the emotional/affective components (subjective motivation) and social cognition. The anterior insula could also have a role in the subjective motivation state across all components given its role in the perception of emotionally significant stimuli, integration of interoceptive inputs and close connections with prefrontal structures.

In our study we identified clusters of negative correlation between apathy subscores and glucose metabolism in regions including DLPFC, DMPFC, VLPFC, PMC, ACC, and insula, largely overlapping with cortical regions previously shown to be related to different apathy components in FTD.[21] Clusters of positive correlation included the cerebellum and the pons. Notably, cerebellar and brainstem metabolism tends to increase as ALS-related cognitive impairment worsens.[22] The cerebellum is known to be involved in cognitive and behavioural processes. Cerebellar damage can lead to the cerebellar cognitive affective syndrome (Schmahmann's syndrome).[23] Data from neuroimaging and neuromodulation/neurostimulation studies suggest that cerebellar compensatory reorganization might be involved in neurodegenerative diseases affecting cognition, e.g. Alzheimer's Disease and Frontotemporal Dementia.[24] Such compensatory cerebellar changes are expected to be more prominent as clinical cognitive and behavioural impairment become more severe.[25] One possibility to explain the finding of a positive correlation between cerebellar metabolism and both the "after" apathy score and the "before-after" gap is the involvement of the cerebellum in compensatory mechanisms. They might be prevalent in earlier stages and represent an adaptive mechanism to overcome frontal cognitive impairment, with effect dissipation over time. This point strengthens the view of ALS as a disease involving multiple neural systems and networks.

Clusters of negative and positive correlation between apathy subscores and brain metabolism were substantially overlapping for the "after" apathy subscore and the "before-after" gap. This finding underlines the importance of the "before-after" gap in the clinical use of the scale, since it could represent a proxy of the behavioural change due to the degenerative process. In agreement with the FrSBe manual,[8] we examined a comparable, reference, population-based series[12] to identify a possible cut-off of the gap to attribute a behavioural change to the neurodegenerative

process. We propose to consider the threshold between the third and fourth quartile as a possible cut-off value. The results of group comparisons support the hypothesis that the entity of the "before-after" gap might be more relevant than the change of category based on the cut-off value of 65 to attribute a behavioural change to the neurodegenerative process of ALS. Therefore, we suggest to consider the entity of the "before-after" gap along with the classification based on the cut-off value of 65 points in the clinical assessment of apathy through the FrSBe. However, we cannot exclude that the different sample sizes of the two groups in the comparison between apathetic patients with a "before" apathy subscore ≥65 (n=26) *versus* apathetic patients with "before" apathy subscore <65 (n=58), might have had a minimal effect on the results. Otherwise, in the comparison between apathetic patients with before-after gap <22 and apathetic patients with before-after gap ≥22 the two groups showed similar size (n=44 and n=40 respectively).

A possible limitation of our study is that MRI scans were not available for all subjects, not allowing partial volume effect correction for cortical atrophy. Nevertheless, studies employing voxel-based atrophy correction of resting glucose metabolism showed that metabolic measurements were relatively independent of brain atrophy.[26] A further possible limitation is that we did not characterize brain metabolic changes associated with different components of apathy.

In conclusion, to our knowledge no other studies on brain ^{18}F-FDG-PET correlates of apathy have been performed in ALS patients. We found that FrSBe "after" apathy subscore correlated with metabolic changes in brain regions known as neuroanatomical correlates of apathy. Furthermore, our data suggest the relevance of the gap between the premorbid and morbid conditions to detect behavioural changes attributable to the neurodegenerative process underlying ALS.

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Metabolic brain changes across different levels of cognitive impairment in ALS: a ^{18}F-FDG-PET study

Canosa A, Moglia C, Manera U, Vasta R, Torrieri MC, Arena V, D'Ovidio F, Palumbo F, Zucchetti JP, **Iazzolino B**, Peotta L, Calvo A, Pagani M, Chiò A.

Abstract

Objective To identify the metabolic changes related to the various levels of cognitive deficits in amyotrophic lateral sclerosis (ALS) using 18F-2-fluoro-2-deoxy-Dglucose positron emission tomography (18F-FDG-PET) imaging.

Methods 274 ALS patients underwent neuropsychological assessment and brain 18F-FDGPET at diagnosis. According to the criteria published in 2017, cognitive status was classified as ALS with normal cognition (ALS-Cn, n=132), ALS with behavioural impairment (ALS-Bi, n=66), ALS with cognitive impairment (ALS-Ci, n=30), ALS with cognitive and behavioural impairment (ALS-Cbi, n=26), ALS with frontotemporal dementia (ALS–FTD, n=20). We compared each group displaying some degree of cognitive and/or behavioural impairment to ALS-Cn patients, including age at PET, sex and ALS Functional Rating Scale-Revised as covariates. Results We identified frontal lobe relative hypometabolism in cognitively impaired patients that resulted more extensive and significant across the continuum from ALS-Ci, through ALS-Cbi, to ALS–FTD. ALS–FTD patients also showed cerebellar relative hypermetabolism. ALS-Bi patients did not show any difference compared with ALS-Cn.

Conclusions These data support the concept that patients with cognitive impairment have a more widespread neurodegenerative process compared with patients with a pure motor disease: the more severe the cognitive impairment, the more diffuse the metabolic changes. Otherwise, metabolic changes related to pure behavioural impairment need further characterisation.

Introduction

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurological disease affecting upper and lower motor neurons, leading to progressive weakness and muscle atrophy in patients. Death usually occurs within three years due to respiratory failure.[1] Clinical, genetic, and pathological data point to an overlap between ALS and another neurodegenerative disease, namely FTD.[2,3,4,5] These observations led to the formulation of the hypothesis that ALS-FTD forms a *continuum* with ALS and FTD representing the spectrum's extremes and a wide range of overlap in the center.[6] Population-based studies have reported that approximately 15% of ALS patients manifest a full-blown FTD, while about 35% display more subtle cognitive and behavioural deficits.[2,3] The

international criteria for diagnosing frontotemporal dysfunction in ALS were published in 2009 and revised in 2017.[7,8]

PET scanning using the ^{18}F-2-fluoro-2-deoxy-D-glucose ligand (^{18}F-FDG-PET) is a marker of neurodegeneration and neuronal injury *in vivo*.[9] We previously employed that powerful modality to assess the metabolic correlates of cognitive impairment in 170 ALS patients. Importantly, cognitive impairment in that study was classified according to the older 2009 criteria.[7] In that study, we demonstrated frontal lobe metabolic impairment reflecting the clinical and neuropathological *continuum* ranging from pure ALS, all the way through ALS with intermediate cognitive deficits, to ALS-FTD.[10]

In the current study, we update and refine our findings. Specifically, we evaluate the association of the metabolic changes assessed using ^{18}F-FDG-PET with the cognitive impairment levels defined by the revised 2017 criteria.[8] The newer ALS-FTD Spectrum classification introduced a novel category (ALS with Cognitive and Behavioural impairment, i.e., ALS-Cbi). The same 170 patients of our previous study [10] were included in the present study.

Materials and Methods

Patients

Patients were considered to be eligible for the study (n=274) if (i) they were diagnosed with definite, probable, and probable laboratory-supported ALS according to the El Escorial revised diagnostic criteria,[11] (ii) they had undergone brain ^{18}F-FDG-PET and neuropsychological assessment at the time of their diagnosis; and (iii) they had attended the ALS Centre of Turin in the period 2008-2015. Patients were enrolled at the time of diagnosis or during the first follow-up visit (2 months later). Neuropsychological evaluation and ^{18}F-FDG-PET were performed within one month of each other, and respiratory function was assessed for every subject within four weeks before or after neuropsychological evaluation and ^{18}F-FDG-PET imaging. None of the patients showed oxygen saturation <92% based on pulse oximetry at the time of their assessments. Patients were excluded from the study if (i) they had a history of neurologic disorders affecting cognition (major stroke, severe head injuries, mental retardation), (ii) they had a history of alcohol and drug dependence, severe mental illness, or use of high-dose psychoactive medications, or (iii) their native language was not Italian.

Neuropsychological Assessment and Cognitive Classification

The neuropsychological battery included:

- The Mini-Mental State Examination (MMSE)
- The letter and category fluency test
- The Frontal Assessment Battery (FAB)
- Digit Span Forward and Backward
- The Trail-Making Test (TMT) A and B
- The Rey Auditory Verbal Learning Test (RAVLT), immediate and delayed recall
- The Babcock Story Recall Test (BSRT), immediate and delayed recall
- The Rey-Osterrieth Complex Figure (ROCF), copy and delayed recall
- Raven's Colored Progressive Matrices (CPM47)

The neurobehavioral assessment included direct observation, patients' history, and the Frontal Systems Behaviour Scale (family form). Anxiety and depression were evaluated through the Hospital Anxiety and Depression Scale.[12] The raw data of the neuropsychological tests were adjusted for the subject age and years of education, according to normative Italian data. Adjusted scores were considered below the cut-off threshold (indicating deficit in cognitive performance) when they were below the 5th percentile from the Italian reference population's mean.

The cognitive status of the patients was classified according to diagnostic criteria published by Strong et al. in 2017,[8] into the following five categories:

- ALS with normal cognition (ALS-Cn, n=132);
- ALS with behavioural impairment (ALS-Bi, n=66);
- ALS with cognitive impairment (ALS-Ci, n=30);
- ALS with cognitive and behavioural impairment (ALS-Cbi, n=26)
- ALS with FTD (ALS-FTD, n=20).

^{18}F-FDG-PET acquisition

^{18}F-FDG-PET was performed according to published guidelines.[13] Patients fasted at least six hours before the exam. Blood glucose was <7.2 mmol/l in all cases before the procedure. After a 20-minute rest, about 185 MBq of ^{18}F-FDG was injected, and the acquisition started 60 minutes after the injection. The PET/CT scans were performed by a Discovery ST-E System (General Electric). The brain CT (thickness of 3.75 millimetres, 140 kVolt, 60-80 mAs) and the PET scans were sequentially

acquired, with the CT data used for attenuation correction of the PET data. The PET images were reconstructed with four iterations and 28 subsets with an initial voxel size of 2.34 x 2.34 x 2.00 mm, and the data were collected in 128×128 matrices.

Genetic Analysis

All patients underwent genetic analysis for the pathogenic repeat expansion of the *C9orf72* gene and protein-coding mutations in the *SOD1*, *TARDBP*, and *FUS* genes. All the coding exons and 50 bp of the flanking intron-exon boundaries of *SOD1*, exon 6 of *TARDBP*, and exons 14 and 15 of *FUS* were PCR amplified, sequenced using the BigDye Terminator v3.1 sequencing kit (Applied Biosystems Inc.), and run on an ABIPrism 3500 genetic analyzer. The majority of pathogenic variants are known to lie within these mutational hotspots. A repeat-primed PCR assay was used to screen for the presence of the GGGGCC hexanucleotide expansion in the first intron of *C9orf72*.

Statistical Analysis

The demographic and clinical features of the cognitive groups were compared using the Kruskal-Wallis and Chi-square test/Fisher's exact tests. Normality tests (Shapiro-Wilk test) were also performed. SPM12 implemented in Matlab R2018b (MathWorks, Natick, MA, USA) was used for image normalization. A customized brain ^{18}F-FDG-PET template[14] was utilized for spatial normalization. Intensity normalization was performed using the 0.8 default SPM value of grey matter threshold, and images were smoothed with a 10-mm filter before submission for statistical analysis.

To evaluate whether a global effect was present, we performed a full factorial analysis of all groups included in the design matrix as implemented in SPM12. The following were included as covariates in the analysis: age at the time of PET imaging, sex, and motor impairment (ALS Functional Rating Scale-Revised, i.e., ALSFRS-R). In situations where the hypothesis was confirmed, each group displaying cognitive or behavioural impairment (ALS-Bi, ALS-Ci, ALS-Cbi, and ALS-FTD) was compared with the ALS-Cn group. Comparisons among groups were performed using the *two-sample t-test* model of SPM12, including the age at the time of PET imaging, sex, and ALSFRS-R as covariates. The height threshold was set at $p<0.001$ ($p<0.05$ FWE-corrected at cluster level). If no cluster of significant difference was identified, a height threshold of $p<0.005$ ($p<0.05$ FWE-corrected at cluster level) was set to perform further exploratory analyses.

Protocol approvals

The study was approved by the ethical committee "Comitato Etico Interaziendale Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino". All of the patients signed written informed consent.

Data availability statement

The data is available to interested researchers upon request.

Results

Demographic and clinical data

The clinical data for the different cognitive groups are shown in Table 1 and Supplementary Table 1. We found no significant difference between the various cognitive groups based on their sex distribution, their site of onset (spinal *versus* bulbar), the frequency of genetic mutations, or the forced vital capacity at the time of the ^{18}F-FDG-PET imaging. There was a significant difference for age at the time of PET imaging, but its impact was minimal as age was included as a covariate in the analyses. Patients displaying worse cognition also had a worse level of motor disability, as measured by the ALSFRS-R total score, a higher progression rate expressed by the ΔALSFRS-R (points lost per month), and more advanced King's stages. The impact of motor impairment was controlled by including the ALSFRS-R total score as a covariate in the analyses. Supplementary Table 2 lists the results of the neuropsychological tests across the different groups.

Table 1. Descriptive summary statistics of clinical data for the different cognitive groups (ALS-Cn, ALS with normal cognition; ALS-Bi, ALS with behavioural impairment; ALS-Ci, ALS with cognitive impairment; ALS-Cbi, ALS with cognitive and behavioural impairment; ALS-FTD, ALS with frontotemporal dementia). *p<0.05 was considered as significant, using Kruskal-Wallis test and chi-square test as appropriate.

	ALS-Cn	**ALS-Bi**	**ALS-Ci**	**ALS-Cbi**	**ALS-FTD**	
	Median (IQR)	*Median (IQR)*	*Median (IQR)*	*Median (IQR)*	*Median (IQR)*	p*

Age at PET (years)	65.0 (55.6-72.5)	62.0 (57.0-69.4)	70.5 (61.0-75.2)	70.9 (64.4-76.1)	72.4 (64.4-76.3)	**p<0.001**
ALSFRS-R total score at PET	43 (38-45)	40 (35-44)	42 (39-44)	39 (35-43)	37.5 (31-41)	**p<0.001**
ΔALSFRS-R at PET (points lost/months)	0.43 (0.25-0.84)	0.66 (0.37-0.88)	0.48 (0.23-0.82)	0.67 (0.42-0.99)	0.71 (0.38-1.19)	**p=0.045**
FVC% at PET	94.8 (82.6-108.0)	104.6 (86.3-114.8)	93.6 (81.0-106.3)	81.7 (68.7-100.0)	93.7 (80.5-107.2)	p=0.155
	n (%)	*n (%)*	*n (%)*	*n (%)*	*n (%)*	
Sex						p=0.423
Female	57 (43.2)	36 (54.5)	13 (43.3)	9 (34.6)	10 (50.0)	
Male	75 (56.8)	30 (45.5)	17 (56.7)	17 (65.4)	10 (50.0)	
Site of onset						p=0.353
Bulbar onset	48 (36.4)	24 (36.4)	15 (50.0)	9 (34.6)	11 (55.0)	
Spinal onset	84 (63.6)	42 (63.6)	15 (50.0)	17 (65.4)	9 (45.0)	
King's staging system at PET						**p=0.016**
Stage 1	67 (50.8)	25 (37.9)	15 (50.0)	7 (26.9)	6 (30.0)	
Stage 2	39 (29.5)	22 (33.3)	13 (43.3)	8 (30.8)	4 (20.0)	
Stage 3	23 (17.4)	19 (28.8)	1 (3.3)	10 (38.5)	9 (45.0)	
Stage 4a/4b	3 (2.3)	0 (0.0)	1 (3.3)	1 (3.8)	1 (5.0)	
Genetic status						p=0.692
C9orf72	13 (9.8)	4 (6.1)	3 (10.0)	3 (11.5)	3 (15.0)	
SOD1	2 (1.5)	1 (1.5)	0 (0.0)	1 (3.8)	0 (0.0)	
TARDBP	4 (3.0)	2 (3.0)	1 (3.3)	1 (3.8)	0 (0.0)	
FUS	2 (1.5)	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	
Wild-type	111 (84.1)	58 (87.9)	26 (86.7)	21 (80.8)	17 (85.0)	
Total	132 (274)	66 (274)	30 (274)	26 (274)	20 (274)	

Significant differences are reported in bold.

** Kruskal-Wallis test and Chi-square test/Fisher's exact test were performed for continuous and discrete variables respectively* .IQR, Interquartile Range; PET, Positron Emission Tomography; ALSFRS-R, ALS Functional Rating Scale-Revised; FVC, Forced Vital Capacity.

Supplementary Table 1. Descriptive summary statistics of clinical data, group comparisons. Kruskal-Wallis and Mann-Whitney U test were used as appropriate. ALSFRS-R: ALS Functional Rating Scale-Revised. ALS-Cn: ALS with normal cognition. ALS-Bi: ALS with behavioural impairment. ALS-Ci: ALS with cognitive impairment. ALS-Cbi: ALS with cognitive and behavioural impairment. ALS-FTD: ALS with frontotemporal dementia. Significant p-values are reported in bold.

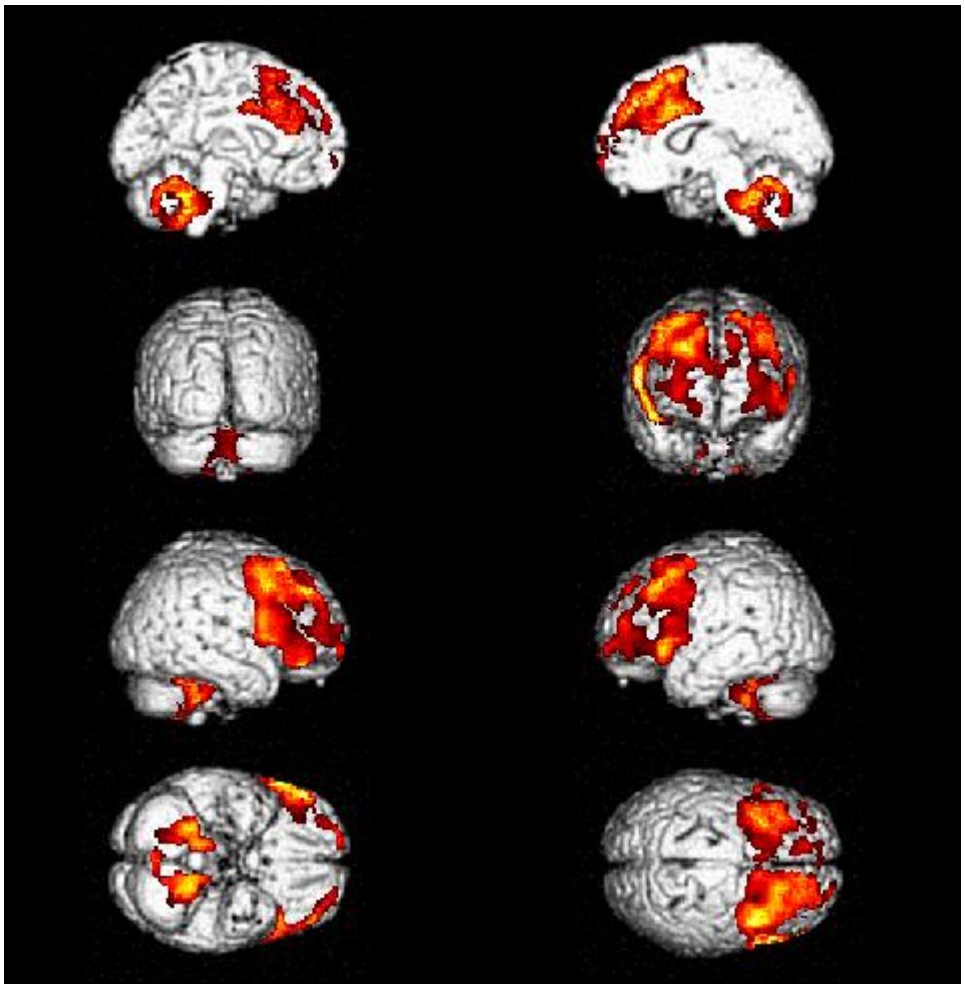
	Kruskal-Wallis (all groups)	**Mann-Whitney ALS-CN vs ALS-Bi**	**Mann-Whitney ALS-CN vs ALS-Ci**	**Mann-Whitney ALS-CN vs ALS-Cbi**	**Mann-Whitney ALS-CN vs ALS-FTD**
Age at PET (years)	**p < 0.001**	p = 0.285	p = 0.060	**p = 0.003**	**p = 0.012**
ALSFRS-R total score	**p < 0.001**	**p =0.006**	p = 0.347	**p = 0.006**	**p = 0.003**
ΔALSFRS-R (point per month)	**p = 0.045**	**p = 0.024**	p = 0.612	**p = 0.040**	p = 0.059
FVC%	p = 0.155	p = 0.152	p = 0.776	p = 0.155	p = 0.878

Supplementary Table 2. Adjusted score (age and years of education) for different neuropsychological tests, subdivided by cognitive categories (ALS with normal cognition, ALS-Cn; ALS with behavioural impairment, ALS-Bi; ALS with cognitive impairment, ALS-Ci; ALS with cognitive and behavioural impairment, ALS-Cbi; ALS with Frontotemporal Dementia, ALS-FTD). Mini-Mental State Examination (MMSE), letter and category fluency test, Frontal Assessment Battery (FAB), Digit Span Forward and Backward, Trail-Making Test (TMT) A and B, Rey Auditory Verbal Learning Test (RAVLT) immediate (ir) and delayed recall (dr), Babcock Story Recall Test (BSRT) immediate (ir) and delayed recall (dr), Rey-Osterrieth Complex Figure (ROCF) copy and delayed recall (dr), Raven's Colored Progressive Matrices (CPM47), Frontal Systems Behaviour Scale (FrSBe). # Kruskall-Wallis test. *Mann-Whitney U test. P-value < 0.05 were considered as significant and written in bold. Ns: not significant

Neuropsychological test	ALS-CN	ALS-Bi	ALS-Ci	ALS-Cbi	ALS-FTD	TOT	Kruskall-Wallis – all categories	ALS-CN vs ALS-Bi	ALS-CN vs ALS-Ci	ALS-CN vs ALS-Cbi	ALS-CN vs ALS-FTD	ALS-Bi vs ALS-Cbi	ALS-Ci vs ALS-Cbi	ALS-Cbi vs ALS-FTD
	Median (IQR)	*Median (IQR)*	*Median (IQR)*	*Median (IQR)*	*Median (IQR)*	*Median (IQR)*	*p#*	*p#*	*p**	*p**	*p**	*p**	*p**	*p**
MMSE	28.5 (27.4-30.0)	29.0 (27.6-30.0)	28.1 (26.5-30.0)	28.4 (26.6-30.0)	23.6 (21.1-26.7)	28.5 (27.0-30.0)	**<0.001**	ns	ns	ns	**<0.001**	**ns**	ns	**<0.001**
Letter fluency test	33.3 (25.3-39.7)	29.6 (25.5-33.9)	27.3 (19.4-31.2)	22.4 (14.9-33.9)	16.0 (13.6-22.4)	29.5 (22.9-36.8)	**<0.001**	ns	**0.001**	**<0.001**	**<0.001**	**0.001**	ns	ns
Category fluency test	20.5 (16.8-22.5)	19.8 (17.5-24.0)	19.8 (15.8-22.3)	21.0 (15.3-22.0)	13.8 (8.0-15.5)	20.0 (16.3-22.3)	**0.003**	ns	ns	ns	**<0.001**	ns	ns	**0.005**
FAB	16.3 (15.3-18.0)	16.5 (15.5-18.0)	13.3 (11.8-15.7)	13.8 (12.5-16.1)	10.8 (8.7-12.5)	15.8 (14.3-17.4)	**<0.001**	ns	**<0.001**	**<0.001**	**<0.001**	**<0.001**	ns	**0.009**
Digit Span Forward	5.5 (4.8-6.3)	5.5 (5.1-6.0)	5.2 (4.7-5.9)	5.4 (4.7-6.5)	4.8 (3.7-5.5)	5.5 (4.8-6.2)	ns	ns	ns	ns	**0.041**	ns	ns	ns
Digit Span Backward	4.1 (3.5-4.8)	4.1 (3.6-4.3)	3.8 (3.2-4.4)	4.2 (3.6-4.5)	3.1 (2.6-3.8)	4.0 (3.5-4.5)	**0.014**	ns	**0.032**	ns	**0.005**	ns	ns	**0.012**
TMT A	34.0 (22.0-44.0)	31.5 (21.0-46.0)	44.0 (27.0-78.0)	44.0 (29.0-64.0)	109.0 (76.0-153.0)	36.0 (22.0-51.0)	**<0.001**	ns	**0.014**	**0.015**	**<0.001**	ns	ns	**0.001**
TMT B	59.5 (33.0-86.0)	56.0 (29.0-99.0)	126.0 (56.0-243.0)	159.0 (65.5-303.0)	315.0 (283.0-403.0)	70.0 (35.0-133.0)	**<0.001**	ns	**0.001**	**<0.001**	**<0.001**	**0.002**	ns	**0.011**
TMT B-A	30.0 (2.0-58.0)	23.0 (9.0-69.0)	70.0 (32.0-192.0)	110.0 (15.0-227.5)	207.0 (152.0-224.0)	38.0 (8.0-85.0)	**<0.001**	ns	**0.001**	**0.001**	**<0.001**	**0.004**	ns	ns
RAVLT-ir	48.0 (39.3-54.4)	44.4 (37.6-51.0)	39.0 (34.3-48.8)	42.1 (42.1-42.1)	29.0 (17.6-38.2)	44.2 (36.8-51.8)	**0.018**	ns	ns	ns	**0.003**	ns	ns	ns
RAVLT-dr	10.6 (8.0-12.6)	9.5 (7.7-11.5)	7.6 (5.5-9.4)	7.8 (7.8-7.8)	4.4 (3.2-8.3)	9.2 (7.2-11.6)	**0.014**	ns	**0.016**	ns	**0.003**	ns	ns	ns
BSRT-ir	6.0 (5.4-6.9)	5.8 (4.8-6.7)	5.8 (4.8-7.0)	5.1 (4.5-6.3)	3.5 (1.3-4.7)	5.8 (4.8-6.9)	**0.017**	ns	ns	ns	**<0.001**	ns	ns	ns
BSRT-dr	6.5 (5.6-8.0)	6.9 (5.0-7.6)	5.4 (2.9-6.0)	5.0 (4.6-6.3)	3.8 (2.9-4.7)	6.3 (5.3-7.3)	**0.002**	ns	**0.009**	ns	**0.001**	ns	ns	ns
ROCFT-copy	34.0 (31.8-35.5)	33.9 (32.0-35.5)	30.1 (23.3-34.3)	31.5 (22.0-35.0)	24.8 (10.5-30.4)	33.3 (30.8-35.3)	**<0.001**	ns	**0.005**	**0.029**	**0.001**	**0.043**	ns	ns
ROCFT-dr	14.3 (12.0-18.5)	12.1 (9.5-18.3)	13.0 (8.5-18.0)	13.8 (7.5-16.3)	6.9 (0.0-8.8)	13.5 (9.8-18.0)	**0.001**	ns	ns	ns	**<0.001**	ns	ns	**0.007**
CPM47	30.6 (27.2-32.9)	30.1 (27.3-32.1)	27.3 (24.2-29.8)	24.6 (21.0-30.1)	19.8 (16.3-25.2)	29.5 (25.4-32.3)	**<0.001**	ns	**0.001**	**<0.001**	**<0.001**	**0.003**	ns	**0.016**
FrSBe	48 (43-53)	50 (50-66)	46 (43-49)	60 (46-65)	56 (46-73)	51 (44-61)	**<0.001**	**<0.001**	ns	**0.001**	**0.003**	ns	**0.001**	ns

^{18}F-FDG-PET data

Full Factorial Analysis (height threshold at p<0.001, p<0.05 FWE-corrected at cluster level). The full factorial design resulted in a significant main effect in large clusters, including frontal regions and the cerebellum (Supplementary Figure 1). We, therefore, calculated the *post-hoc* group comparisons.



Supplementary Figure 1. Results of the full factorial analysis in the whole sample. Glass brain rendering of the results of the full factorial analysis: clusters of significant main effect are projected on brain surface. The first row represents the view of the medial regions of the left hemisphere (on the left) and the right hemisphere (on the right). The second row represents the posterior view (on the left) and frontal view (on the right). The third row represents the right view (on the left) and the left view (on the right). The fourth row represents the view from below (on the left) and the view from above (on the right)*ALS-Cn vs ALS-FTD (height threshold at p<0.001, p<0.05 FWE-corrected at cluster level)*. The ALS-FTD group showed a large cluster of relative

hypometabolism that included the right cingulate gyrus, bilateral middle frontal gyri, the right precentral gyrus, bilateral superior frontal gyrus, and bilateral inferior frontal gyri (Figure 1A, Table 2). In the same group, the analysis showed a cluster of relative hypermetabolism, including the cerebellum (bilateral tonsils, the left dentate and the pyramids, and the right culmen) and the brachia pontis (Figure 2, Supplementary Table 3).

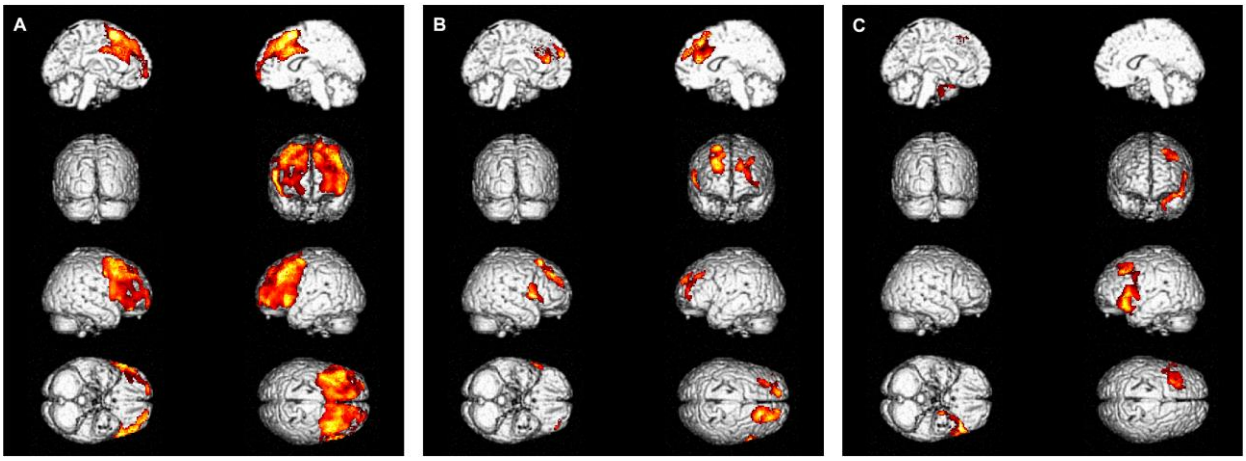


Figure 1. Clusters of relative hypometabolism of ALS-FTD, ALS-Cbi, and ALS-Ci *versus* ALS-Cn. Glass brain rendering of group comparisons: clusters of relative hypometabolism of ALS-FTD (A), ALS-Cbi (B), and ALS-Ci (C) as compared to ALS-Cn are projected on the brain surface. In each box, the first row represents the view of the medial regions of the left hemisphere (on the left) and the right hemisphere (on the right). The second row represents the posterior view (on the left) and the frontal view (on the right). The third row represents the right view (on the left) and the left view (on the right). The fourth row represents the view from below (on the left) and the view from above (on the right).

Table 2. Cluster of relative hypometabolism in ALS-FTD patients as compared to ALS-Cn subjects (height threshold at p<0.001, p<0.05 FWE-corrected at cluster level). BA: Brodmann Area.

P FWE-corrected	Cluster Extent	Z-score	Talairach Coordinates			Side	Cortical Region	BA
0.000	19853	5.56	10	21	30	Right	Cingulate Gyrus	32
		5.51	-30	18	47	Left	Middle Frontal Gyrus	8
		5.28	44	17	36	Right	Precentral Gyrus	9
		5.07	20	35	37	Right	Middle Frontal Gyrus	8
		5.03	-32	7	55	Left	Middle Frontal Gyrus	6
		5.01	-40	13	34	Left	Middle Frontal Gyrus	9
		4.94	22	24	50	Right	Superior Frontal Gyrus	8
		4.74	-38	17	-3	Left	Inferior Frontal Gyrus	47
		4.66	10	16	51	Right	Superior Frontal Gyrus	6
		4.66	55	12	14	Right	Inferior Frontal Gyrus	44
		4.57	-14	14	56	Left	Superior Frontal Gyrus	6
		4.52	-8	43	40	Left	Superior Frontal Gyrus	8
		4.47	-53	18	10	Left	Inferior Frontal Gyrus	45

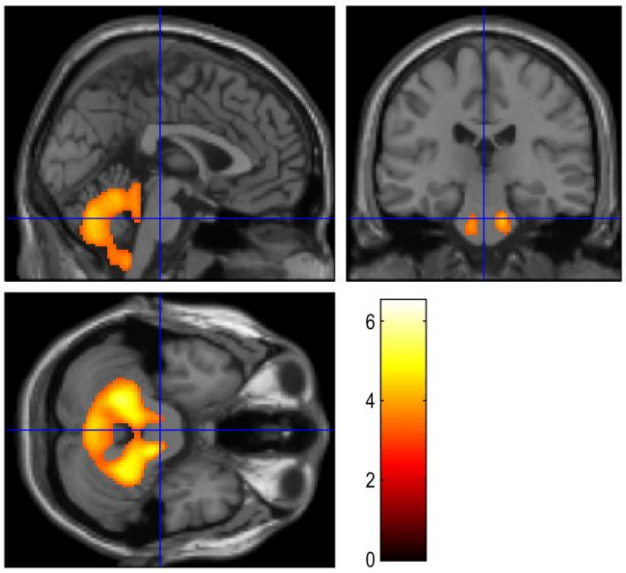


Figure 2. Cluster of relative hypermetabolism of ALS-FTD *versus* ALS-Cn. The cluster of relative hypermetabolism of ALS-FTD as compared to ALS-Cn is represented on a Magnetic Resonance Imaging template. Upper left box: sagittal section. Upper right box: coronal section. Lower left box: axial section.

Supplementary Table 3. Cluster of relative hypermetabolism in ALS-FTD patients as compared to ALS-Cn subjects (height threshold at p<0.001, p<0.05 FWE-corrected at cluster level). BA: Brodmann Area.

P FWE - corrected	Cluster Extent	Z-score	Talairach Coordinates			Side	Region
0.000	10246	6.09	-14	-45	-35	Left	Cerebellar Tonsil
		5.32	28	-43	-32	Right	Cerebellar Tonsil
		5.16	-20	-50	-24	Left	Dentate
		4.32	-4	-67	-24	Left	Pyramis
		4.05	2	-53	-12	Right	Culmen

ALS-Cn vs ALS-Cbi (height threshold at p<0.001, p<0.05 FWE-corrected at cluster level).

The ALS-Cbi group showed a cluster of relative hypometabolism in bilateral superior frontal gyri, bilateral middle frontal gyri, bilateral anterior cingulate gyri, the right cingulate gyrus, the right medial frontal gyrus, bilateral inferior frontal gyri, the right precentral gyrus, and the right insula

(Figure 1B, Table 3). No cluster of relative hypermetabolism was found in ALS-Cbi as compared to ALS-Cn.

Table 3. Clusters of relative hypometabolism in ALS-Cbi patients as compared to ALS-Cn subjects (height threshold at p<0.001, p<0.05 FWE-corrected at cluster level). BA: Brodmann Area.

P (FWE-corrected)	Cluster Extent	Z-score	Talairach Coordinates			Side	Cortical Region	BA
0.000	2156	4.64	24	44	31	Right	Superior Frontal Gyrus	9
		4.42	24	24	54	Right	Middle Frontal Gyrus	6
		4.35	20	35	37	Right	Middle Frontal Gyrus	8
		4.04	8	34	20	Right	Anterior Cingulate	32
		3.78	10	19	30	Right	Cingulate Gyrus	32
		3.55	12	39	44	Right	Superior Frontal Gyrus	8
		3.53	10	27	41	Right	Medial Frontal Gyrus	8
		3.48	-4	34	17	Left	Anterior Cingulate	32
		3.25	0	22	21	Left	Anterior Cingulate	24
0.009	751	4.43	-14	58	25	Left	Superior Frontal Gyrus	9
		3.71	-26	51	12	Left	Superior Frontal Gyrus	10
		3.54	-32	31	35	Left	Middle Frontal Gyrus	9
		3.41	-40	49	-1	Left	Inferior Frontal Gyrus	10
0.018	632	3.91	57	12	9	Right	Precentral Gyrus	44
		3.74	44	8	3	Right	Insula	13
		3.55	55	23	1	Right	Inferior Frontal Gyrus	45

ALS-Cn vs ALS-Ci (height threshold at p<0.005, p<0.05 FWE-corrected at cluster level).

Since we did not identify any significant difference setting the height threshold at p<0.001, we performed an exploratory analysis with the height threshold at p<0.005. The ALS-Ci group showed a cluster of relative hypometabolism, including the left superior, middle, and inferior frontal gyri, the superior temporal gyrus, and the uncus (Figure 1C, Table 4). No cluster of relative hypermetabolism was found in ALS-Ci as compared to ALS-Cn.

ALS-Bi versus ALS-Cn. No significant difference was found.

Table 4. Cluster of relative hypometabolism in ALS-Ci patients as compared to ALS-Cn subjects (height threshold at p<0.005, p<0.05 FWE-corrected at cluster level). BA: Brodmann Area.

P (FWE-corrected)	Cluster Extent	Z-score	Talairach Coordinates			Side	Cortical Region	BA
0.001	2793	4.27	-46	25	-11	Left	Inferior Frontal Gyrus	47
		3.67	-28	18	45	Left	Middle Frontal Gyrus	8
		3.52	-51	6	3	Left	Superior Temporal Gyrus	22
		3.44	-53	18	8	Left	Inferior Frontal Gyrus	45
		3.35	-22	28	47	Left	Superior Frontal Gyrus	8
		3.30	-22	-6	-35	Left	Uncus	36
		3.17	-50	11	-16	Left	Superior Temporal Gyrus	38
		2.99	-46	7	31	Left	Inferior Frontal Gyrus	9

Discussion

This study evaluated the association of the metabolic changes, assessed using ^{18}F-FDG-PET imaging, with different levels of cognitive impairment in ALS patients, defined by the revised 2017 criteria.[8] As compared to ALS-Cn, we found that frontal relative hypometabolism became more extensive and significant with increasing cognitive impairment, in the order ALS-Ci to ALS-Cbi, to ALS-FTD. Patients with ALS-FTD also showed cerebellar relative hypermetabolism as compared to

ALS-Cn. Our data show that the cognitive categories identified by the revised Strong criteria reflect a differential spread of the neurodegenerative process across the cortical regions involved in cognitive impairment.[10,15] Interestingly, patients with pure behavioural impairment did not show a metabolic difference compared to patients with normal cognitive function.

The natural course of cognitive function in ALS remains unclear. In part, this is because the longitudinal neuropsychological assessment of ALS patients is challenging due to the progressive deterioration of motor function and speech. Published studies[16–21] suggest a progressive deterioration of cognitive functions among those patients displaying cognitive deficits at diagnosis. In contrast, subjects with normal cognitive performance at the first examination seem to remain stable over time. These studies failed to re-examine patients with a rapidly worsening motor disability during the follow-up, with attrition rates of 75% after the first year. This is a significant limitation since cognitive impairment is known to be associated with a worse disease course.[2,3,16,22] A recent study with low attrition showed progression of cognitive or behavioural impairment in more than one-third of patients, including a proportion of patients with normal cognitive performance at baseline.[23]

As ^{18}F-FDG-PET imaging is an *in vivo* measure of neuronal integrity,[9] it offers an alternative means of assessing patients longitudinally. Therefore, it might be combined with neuropsychological testing to evaluate cognitive impairment in ALS over time, providing early information on the spreading of brain pathology along the disease course.[10] To date, longitudinal studies employing ^{18}F-FDG-PET in ALS are lacking,[24] with the exception of few case reports.[25,26] A study[27] aimed at identifying the pattern of progression over time of cerebral glucose metabolic changes in mild frontal variant FTD performing ^{18}F-FDG-PET found that the pathological changes spread from the frontal lobes to the parietal and temporal cortices. These data suggest that the metabolic changes that we have found in the present study may parallel brain metabolic changes of FTD over time. This hypothesis needs to be confirmed by longitudinal studies, as cognitive and behavioural symptoms in ALS might evolve in a different pattern compared with behavioural predominant FTD.[28]

In a recent cross-sectional study[29] we evaluated the relationship between patients' cognitive impairment, classified according to the recently published revised ALS-FTD Consensus Criteria,[8] and patients' motor impairment, assessed through the King's[30] and the MiTos[31] Staging Systems. Our findings suggested that motor and cognitive function worsen in parallel, supporting the hypothesis of a regional ordered sequence of ALS pathology.

Similarly, an MRI study found that frontotemporal cortex atrophy was highly proportional to the progression rate of ALS, as measured by the change of ALSFRS-R per month.[32] Our current study also found a higher degree of motor disability among patients with worse cognitive performance. These data are consistent with neuropathological studies showing the spread of phosphorylated TDP-43 (pTDP-43) proteinopathy throughout the illness. pTDP-43 appears to spread via axonal transport from the primary motor cortex to the prefrontal areas, suggesting that all ALS patients could be susceptible to developing frontal cognitive impairment over time.[33] Our data show that ^{18}F-FDG-PET detects the extent of the neurodegeneration across the cortical regions involved in cognition. Furthermore, hypometabolism seems to extend from brain regions adjacent to the primary motor cortex to anterior frontal areas, in a manner that parallels the clinical spectrum of ALS-Ci, ALS-Cbi, and ALS-FTD. We argue that ^{18}F-FDG-PET enriches the information provided by structural MRI, especially as cortical metabolism abnormalities may precede grey matter loss.[34]

In contrast to our previous paper,[10] we included the new category of ALS-Cbi. These patients showed metabolic changes that were intermediate between ALS-Ci and ALS-FTD groups. Additionally, we increased the number of ALS-Bi patients, but still did not find metabolic differences among this group compared to cognitively normal cases. This surprising finding suggests that the difference between ALS-Bi and ALS-Cn is under the threshold for detection, possibly due to the heterogeneity of behavioural impairment in ALS that ranges from disinhibition to apathy. Indeed, a recent study investigating the cortical changes related to behavioural impairment in ALS[35] reported that the different phenotypic profiles (i.e., disinhibited/hostile, dysexecutive and apathetic) correspond to a different pattern of cortical thinning assessed using brain MRI. Unfortunately, the size of the ALS-Bi group in our series did not allow further stratification.

Among the extensive neuropsychological assessment, only the Digit Span Forward test was normal across the different ALS groups. This reflects the fact that short-term memory is relatively spared in cognitive impairment related to the frontotemporal lobes. As expected, ALS-FTD patients showed significantly worse scores in all tests performed, while ALS-Bi showed an impairment only in the behavioural assessment, i.e., FrSBe. In the direct comparison between ALS-Ci and ALS-Cbi the only significant difference was found in the behavioural assessment (i.e., FrSBe). Along the spectrum of cognitive impairment including ALS-Cn, ALS-Ci, ALS-Cbi, and ALS-FTD, deficits of letter fluency, sustained and divided attention, set-shifting, and executive function (i.e., letter fluency test, TMT A, B, and B-A, FAB, and copy of the ROCFT) were found in all categories. The

deterioration of such functions seems to be the core of ALS-related cognitive impairment, even when it is less severe. The degree of their impairment might be related to the extending frontal cluster of relative hypometabolism that we found in our series.

We observed relative hypermetabolism of the cerebellum in ALS patients with FTD. The cerebellum is involved in both motor and extra-motor function, including cognition.[36] Lesions involving the posterior lobe of the cerebellum are associated with impairment of executive functions and behavioural changes. Such deficits are part of the "cerebellar cognitive affective syndrome", related to the disruption of the cerebellar modulation of neural circuitry linking prefrontal, posterior parietal, superior temporal, and limbic cortices with the cerebellum.[37] Also the dentate nucleus seems to be related to non-motor functions through its output to prefrontal cortex.[38,39] The cerebellum's role in ALS has been previously investigated with ^{18}F-FDG-PET studies reporting cerebellar hypermetabolism, perhaps related to astrocytosis or microglial activation.[10,40] Magnetic Resonance Imaging (MRI) studies have reported decreased gray matter volume (GMV) of the cerebellum in ALS.[41,42] A recent functional MRI study suggested the coexistence of neurodegenerative and adaptive changes.[43] Compiling these results into a coherent framework is challenging. Indeed, patients included in different studies might reside in different disease stages.[44] One possibility is that the compensatory changes are prevalent in earlier stages and represent an adaptive mechanism to overcome frontal cognitive impairment. This effect would dissipate over time.

Our study has limitations. First, we did not evaluate patients longitudinally to establish whether they will develop full-blown FTD over time. Second, we did not consider the possible role of cognitive reserve. This mechanism has been postulated for other diseases causing cognitive impairment, such as Alzheimer's Disease[45] and FTD.[46] Third, the small number of genetic mutation carriers in our series did not allow us to evaluate the possible impact of genetic characteristics on brain metabolism. Fourth, structural MRI scans were not available for all subjects, meaning that we could not correct for cortical atrophy. Nevertheless, previous studies have shown that metabolic measurements were relatively independent of brain atrophy.[47]

In summary, our data show that cognitive categories identified according to the revised Strong criteria reflect the spreading of the neurodegenerative process across cortical regions involved in cognition.[10,15] Our data pave the way for the use of ^{18}F-FDG-PET to study the natural history of cognition in ALS, enriching the information provided by the standard neuropsychological testing.

This has broad applicability in the clinical setting since cognitive impairment has a strong negative impact on ALS outcome.[2,48,49] In research, ^{18}F-FDG-PET imaging could enable the evaluation of the spread of brain pathology *in vivo*. Metabolic changes assessed through ^{18}F-FDG-PET may also be studied as surrogate markers of disease progression in the context of drug development and clinical trials.

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Decline of cognitive and behavioral functions in Amyotrophic Lateral Sclerosis: a longitudinal study

Bersano E, Sarnelli MF, Solara V, **Iazzolino B**, Peotta L, De Marchi F, Facchin A, Moglia C, Canosa A, Calvo A, Chiò A, Mazzini L.

Abstract

Background. A cognitive impairment, ranging from frontotemporal dementia (FTD) to milder forms of dysexecutive or behavioural dysfunction, is detected in 30-50% of patients affected by amyotrophic lateral sclerosis (ALS) at diagnosis. Such condition considerably influences the prognosis, and possibly impacts on the decision-making process with regards to end-of-life choices. The aim of our study is to examine the changes of cognitive and behavioural impairment in a large population of ALS from the time of diagnosis to a 6-month follow-up (IQR 5.5-9.0 months), and to examine to what extent the progression of cognitive impairment affects survival time and rate of disease progression.
Methods. We recruited 146 ALS patients classified according to revised criteria of ALS and FTD spectrum disorder. In a multidisciplinary setting, during 2 subsequent visits we examined clinical features with ALSFRS-r score, FVC% and BMI, and cognitive status with an extensive neuropsychological evaluation.
Results. At second examination, one-third of patients showed a worsening of cognitive impairment, namely 88% of ALSbi, 27% of ALSci, 40% of ALScbi, and, interestingly, also 24% of cognitive normal ALS developed a significant cognitive dysfunction. We find that those who changed their cognitive status presented a lower ALSFRS-r score at t1 and a shorter survival time compared to those who did not change, regardless of the type of cognitive impairment.
Conclusion. We show how cognitive disorders in ALS patients can not only be present at diagnosis, but also manifest during disease and influence the progression of motor deficit and the prognosis

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder for a long time considered a motor syndrome but with growing evidence supporting a multisystemic involvement [1]. 30-50% of ALS patients show cognitive impairment, ranging from frontotemporal dementia (FTD) to milder forms of executive or behavioural dysfunctions [2,3]. Recently, the revised diagnostic criteria of neuropsychological features in ALS supported the notion of a clinical-pathological continuum between ALS and frontotemporal lobar degeneration [4,5]. The impairment of cognitive functions is a relevant negative prognostic risk factor, independent from other known factors such as age, site of onset, diagnostic delay, disease severity, and respiratory function [6,7].

ALS tertiary Centres commonly investigate patient's neuropsychological function in the early stage of disease, and current guidelines on ALS clinical management recommend the neurologist to take

into account cognitive impairment for disease management [8]. Indeed, cognitive and behavioural symptoms deeply impact on ALS patient's psychological well-being and on the caregiver burden [9], possibly influencing the treatment adherence and the decision-making process with regards to end-of-life choices.

Recent researchers have suggested a strict correlation between cognitive performances and disease severity, measured with the ALS-functional rating scale revised (ALS-FRS-r) [10], and the clinical stage, evaluated with King's and MiToS Staging Systems [11,12,13]. Although it has been proposed to re-test patients for cognition every 6 months [14], it remains unclear whether the cognitive status of ALS patients worsens with disease progression.

The few published longitudinal studies have reported a stability of cognitive features in ALS patients [15,16,17], but they are limited by the sample size and the use of restricted cohorts. Instead, Elamin et al., in a large case-control study, describe the decline in cognition function in ALS patients who were cognitively impaired at baseline. Conversely, patients without deficit at diagnosis remained cognitively intact; though, the same authors suggest that these patients may show cognitive impairment on longer follow-up [10].

An issue, even more evident in longitudinal approach, is also the necessity to use manageable and common, but comprehensive, instruments for evaluating cognitive functions in ALS. The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) test is now recognized as a useful tool [18]; recent studies, however, noted a significant improvement at some ECAS scores during follow up, therefore proposing the implementation of ECAS alternate forms to evaluate patients longitudinally [19]. Behavioural disfunctions in ALS were specifically inspected with the ALS Cognitive-Behavioural Screen (ALS CBS) [20]and, in addition, with the Frontal Behavioral Inventory-ALS (FBI-ALS) [21], with evidence of a progression during disease associated with cognitive stability.

The low number of patients in previous studies, the difficulty in choosing a unique, in-depth and manageable battery of neuropsychological tests, and the disease that determines an increasing disability in a short time, are the main factors that make unclear whether a patient without cognitive impairment at time of ALS diagnosis could develop it during disease course. The aim of this study is to examine the changes of cognitive and behavioural status, applying the revised criteria of ALS- FTD spectrum disorder (ALS-FTSD) [4], from the time of the diagnosis across a 6 months follow-up in a large population of ALS patients attending two tertiary ALS Centres. Furthermore, the study examines the influence of the progression of cognitive impairment on survival and the rate of progression of the disease.

Methods

We recruited patients classified probable with laboratory supported, probable, or definite ALS according to the revised El Escorial criteria [22,23], occurred at the time of the diagnosis to the two tertiary ALS Centres of Piedmont region, Italy, during the period from 1st January 2008 to 1st May 2017, who agreed to undergo a neuropsychological follow up. Exclusion criteria included history of neurological disorders affecting cognition, alcohol or drugs addiction syndrome, and use of high-dose psychoactive medications. One hundred forty-six patients were recruited, with a mean of age of 62.6 years (SD = 11.9, range 30-85), being predominantly male (83 males and 63 females) and with spinal onset (105 spinal-onset and 41 bulbar-onset). The average number of education years was 9.8 (SD = 4.3, range 0-18 years) and, in keeping with previous studies [24], it was higher for patients without cognitive impairment (11.1 years, SD = 4.2, range 0-18 versus 6.9 years, SD = 3.0, range 3-13). All patients were screened for the presence of the GGGGCC hexanucleotide expansion in the first intron of *C9orf72*.

At each visit the disease severity was assessed with the ALSFRS-r score [25], pulmonary function tests to calculate the forced vital capacity (FVC %), and a nutritionist calculation of Body Mass Index (BMI).

The neuropsychological evaluation consisted in a clinical interview with the patient and the caregiver, and the administration of a battery of neuropsychological tests to inspect executive function, memory, visuospatial function, social cognition and language, at an estimated time of approximately two hours in an outpatient setting.

The neuropsychological battery was performed by a team of neuropsychologists specialised in ALS and dementia and included tests both in written and verbal form to best fit patients according to their different motor disabilities. We tested logic and deductive skills with Raven's Progressive Colored Matrices [26]; global cognition with Clock Drawing Test [27]; executive functions with Cognitive Estimates Test [28], Frontal Assessment Battery (FAB) [29], and Boston Naming Test [30]; memory with the Digit Span test [31] and the Short Story Test [32]; attention with the Trail Making A-B Test [33]; language with verbal fluency and comprehension with Phonemic (letters F, P, L) and Category Fluency Test [34]. We also submitted the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) [35] to patients diagnosed ALS after 2014.

We screened the presence of behavioural symptoms by submitting caregivers an adapted version of Neuropsychiatric Inventory (NPI) [36] or Frontal Systems Behaviour Scale [37].

As mood disorders can both impact on cognitive performance and influence the prognosis [38], anxiety and depression were inspected with an interview by a psychologist and assessed with the Hospital Anxiety and Depression Scale (HADS). The item "I feel slowed down" was discussed with patients in order to have him/her not to refer to physical disability [39].

Based on results from the above extensive neuropsychology and behavioural tests, patients were grouped according to revised criteria of ALS and FTD spectrum disorder (ALS-FTSD) [4] in the following categories: ALS with normal cognition (ALS-CN); ALS with behavioural impairment (ALSbi); ALS with cognitive impairment (ALSci); ALS with combined cognitive and behavioural impairment (ALScbi); ALS with frontotemporal dementia (ALS-FTD).

Patients with impairment in two non-executive domains and no deficits in executive function were excluded from follow up.

Classification was performed in blind by neuropsychologists working in the two Centers, with very high inter-rater agreement between blinded raters. In case of disagreement, a joint discussion was held until consensus was reached.

The same neuropsychological battery test was performed at baseline (t0) and after 6 to 8 months (t1). Patients with severe motor condition limiting the compliance to application of the tests were excluded from individual analyses but the cases were retained in the dataset.

Statistical methods

In order to test the hypotheses, different analyses were performed. For frequency comparison, a chi-square test was used. A GLM model (rmANOVA) was used for the analysis of the relationship between cognitive status change and disease progression assessed with ALSFRS-r score, FVC and BMI. When two groups or two sessions of test were compared, a Wilcoxon signed rank test or Wilcoxon rank sum test was applied. Survival was calculated from onset to death/tracheostomy or censoring date using Log-Rank test and Cox analysis. A p level <0.05 was considered statistically significant. Statistical analyses were performed using R statistical environment.

Standard protocol approvals, registrations, and patient consents

The study design was carried out in accordance with the guidelines given in the Declaration of Helsinki and was approved by the institutional Ethical Committees of the two ALS Centres. The database was managed according to the Italian law for the protection of privacy.

Results

Cognitive and behavioral functions at t0 and t1.

During the study period, 146 patients were recruited at the two ALS Centres in a multidisciplinary setting and monitored by expert neuropsychologists. The median time from diagnosis to the first neuropsychological assessment was 3 months (IQR 5 months). At baseline (t0), the patients were classified as: 101 ALS-CN; 8 ALSbi; 26 ALSci; 5 ALScbi; 6 with comorbid FTD (ALS-FTD).

The 6 ALS-FTD identified at t0 were not re-tested because, by definition, they remain ALS-FTD over time. Although we applied both written and verbal form tests depending on patients' disability, we had to exclude 6 patients from the subsequent analysis because resulted untestable due to severe motor impairment reducing the compliance to neuropsychological assessment.

The application of revised criteria of ALS-FTSD [4]in our cohort showed, in particular, the relevance of apathy in ALSbi and language dysfunction in ALSci, consistently with previous studies [40].

At second neuropsychological evaluation (t1), performed after a median time of 7 months (IQR 5.5-9.0 months), 45 patients (32%) worsened their cognitive performances, and no one showed improvements (Figure 1 and Table 1).

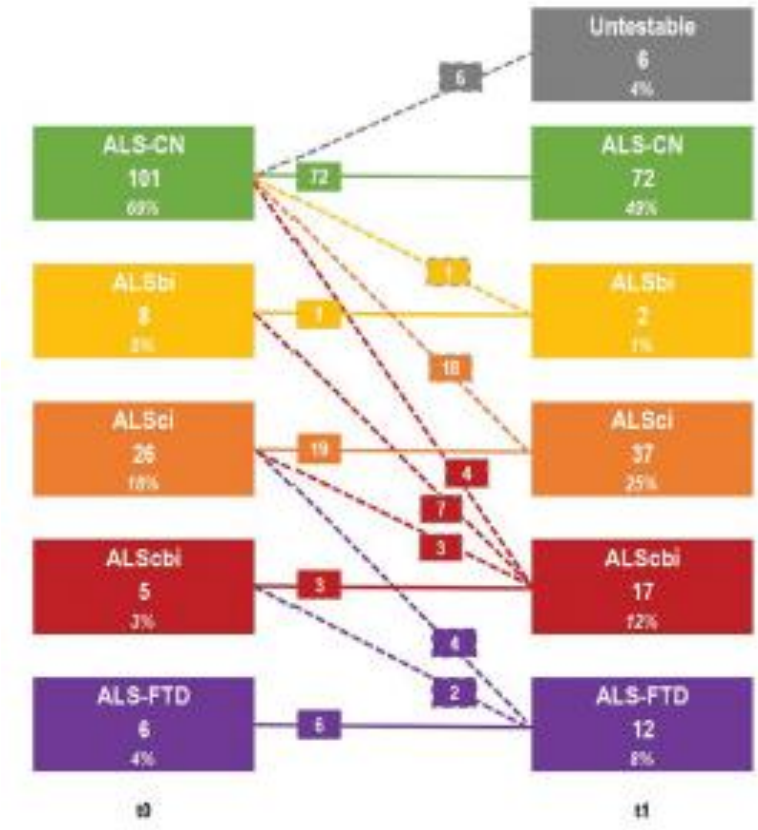


Figure 1: Patient's cognitive classification in two neuropsychological assessments at t0 and at t1 according to revised criteria of ALS and FTD spectrum disorder [4].

Table 1: Patient's neuropsychological classification at t0 and at t1.

		t1						
		Normal cognition	**ALSbi**	**ALSci**	**ALScbi**	**ALS-FTD**	**Untest.**	**TOT**
t0	**Normal cognition**	72	1	18	4	-	6	**101**
	ALSbi	-	1	-	7	-	-	**8**
	ALSci	-	-	19	3	4	-	**26**
	ALScbi	-	-	-	3	2	-	**5**
	ALS-FTD	-	-	-	-	6	-	**6**
	TOT	**72**	**2**	**37**	**17**	**12**	**6**	**146**

Table 2: Main characteristics of patients whose cognitively status changed and did not change

	Cognitively changed (45 pts)	**Cognitively unchanged (95 pts)**	***p-value***
Median Age at diagnosis of ALS	65.3 years old (SD=10.9)	60.8 years old (SD=12.3),	*W=1706.5 p=0.05*
Gender	Female: 18 Male: 27	Female:40 Male: 55	$\chi^2_{(1)}$*= 0.002 p=0.95*
Onset ALS	Bulbar: 16 Spinal: 29	Bulbar: 20 Spinal: 75	$\chi^2_{(1)}$*= 2.64 p= 0.10*
Median Education	8 years	11 years	*W=2700 p <0.05*
Median ALSFRS-r score	43	42.5	*W=1878.5, p=0.38*
Median FVC (%)	96.5	99.5	*W=1148, p=0.21*

Median BMI	24.6	24.5	*W=1668.5, p=0.87*
Survival	29 months	31,5 months	*([χ2(1)=4.0, p<0.05] z = 1.99, p<0.05*
Spinal-onset with bulbar sign at t1 **Spinal-onset without bulbar sign at t1**	5/11 (45.5%) 10/59 (16.9%)	6/11 (54.5%) 49/59 (89.1%)	*p <0.05*

Considering the patients who has impaired cognition at first evaluation, more than half of ALScbi and 35% of ALSci did not change at the follow up visit. Instead, those who worsened becoming ALS-FTD are respectively 40% (2 of 5) of ALScbi and 15% (4 of 26) of ALSci. Moreover, 7 ALSbi (87% of ALSbi at t0) became ALScbi, and 3 ALSci (11.5%) became ALScbi. Overall, ALSbi worsened more frequently than ALSci (χ^2= 0.0039, p< 0.05).

No patient with normal cognition at first evaluation developed ALS-FTD, and 71% (72 of 101 patients) did not change during the follow up. However, at the follow-up examination, 0.9% (1 patient) showed behavioural impairment (ALSbi), 17.8% (18 of 101) cognitive impairment (ALSci), and 3.9% (4 patients) showed both behavioural and cognitive impairment (ALScbi).

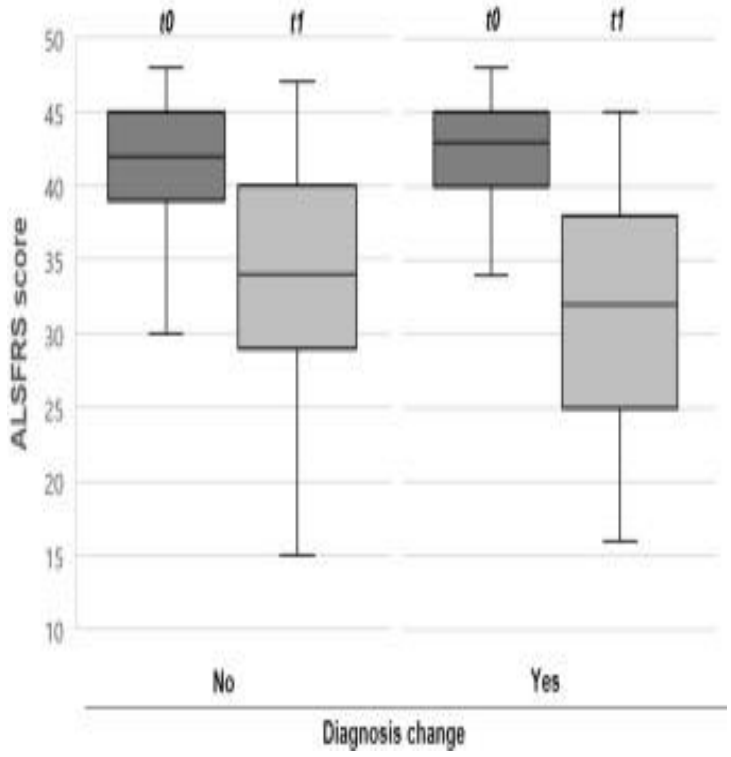
Interestingly, 11 out of the 70 spinal-onset patients developed bulbar signs at t1 evaluation, and 5 of these (45.5%) developed also cognitive impairment; whereas, of those patients not developing bulbar signs at t1 (n=59), only 10 subjects (16.9%) had cognitive decline (p< 0.05), indicating that the appearance of bulbar impairment increases the likelihood to be cognitive impaired.

Comparison between patients who changed cognitive status ("cognitively changed") vs who did not change ("cognitively unchanged").

The subsequent analysis was aimed at comparing the 45 subjects who changed their cognitive status from t0 to t1 ("cognitively changed"), and the 95 who did not change ("cognitively unchanged") both in the absence and in the presence of all types of cognitive impairment. The demographic features of the two groups, such as gender and bulbar onset are comparable, with the only exception of age (table 2) (W=1706.5, p=0.05).

The 19 patients carrying *C9orf72* hexanucleotide repeat expansion were not at higher risk to change neuropsychological assessment during follow up than other patients [$\chi^2_{(2)}$=1.424 p=0.5].

When considering the ALS-FRS-r score of the 138 patients who performed two neuropsychological assessments at t0 and t1, results from rmANOVA, show a significant difference between the cognitively changed and unchanged [$F_{(1,136)}$ =9.10 p<0.005 η^2_G =0.017]; indeed, the patients who presented a cognitive change had a lower ALS-FRS-r score at t1 compared to the cognitively unchanged at t1. Also the survival, calculated with Log-Rank Test [$\chi^2_{(1)}$=4.0, p<0.05] and Cox analysis (z=1.99, p<0.05), was significantly shorter in cognitively changed (Figure 2). Conversely, we observed a similar rate of decline of FVC % and of BMI between two groups. Furthermore, based on rmANOVA analysis, we observe a significant difference between the "cognitively changed" and "cognitively unchanged" in neuropsychological tests that investigate executive functions, in particular FAB [F(1,59) = 12.86 p<0.001 η2p=0.18], Trail Making Test B-A [F(1,50) = 13.47 p<0.001 η2p = 0.21, verbal fluency [F(1,63) = 8.85 p<0.005 η2p=0.12], and Raven's Progressive Coloured Matrices [F(1,96) = 7.97 p<0.01 η2p=0.08] (Supplementary Table and Supplementary Figure). At first evaluation, a mild-moderate depression is the most frequent behavioural symptom reported (85%); instead, at t1 we observe an increase in patients with apathy and, consequently, a higher NPI score [F(1,79)=15.5 p<0.0005 η2p=0.1].



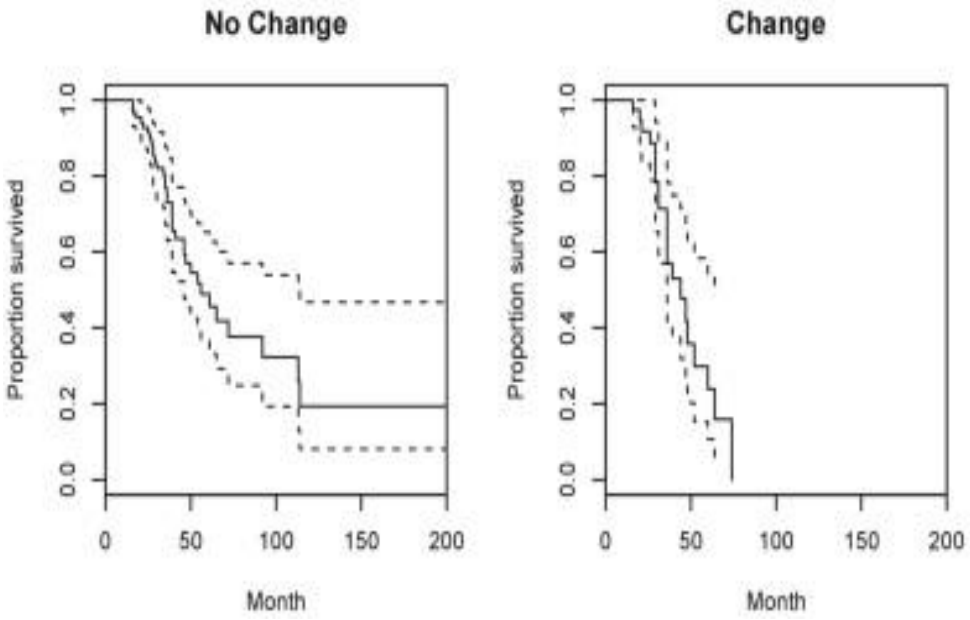


Figure 1: Median ALS-FRS-r score and Kaplan-Meier plot for survival

Discussion

We have observed that about one third of our longitudinal cohort of ALS patients showed a deterioration of cognitive impairment from the time of diagnosis to the second evaluation, and that this change correlates with the severity of the disease measured with the ALS-FRS-r scale. These finding are apparently in contrast with most of previously published longitudinal studies suggesting, instead, a stability of cognitive functions during the course of ALS This discrepancy is most likely due to the reduced sample size [15], and some methodological pitfalls in previous studies. In fact, in some patient analyzed by Schreiber there were a cognitive decline but it was noticeably more slowly compared to motor decline [16]; Kasper et al., instead, had to reclassify patients who had the cognitive impairment disappeared at subsequent control, assuming that the poor initial performance reflected an initial unfamiliarity with neuropsychological test [17]. Behavioural changes are, instead, consistent with previous studies [21].

We also find that gender, type of onset, BMI, FVC % and ALSFRS-r at diagnosis do not significantly differ between patient cognitively changed and unchanged at t1. Furthermore, in keeping with previous papers [41], we observed that the appearance of bulbar impairment as well as the older age of patients is more likely to modify cognitive performances during disease. Another prominent finding is that patients who had a worsening of their cognitive function during the disease have a faster motor progression and a shorter survival compared to the group of the cognitively unchanged. These results are consistent with the conclusions of the study by Elamin et al. [10], which

is, at present, the only published longitudinal study with an extensive sample size and a deep neuropsychological approach. Compared to such study, in our cohort we also find a significant share (24.2%) of ALS patients who were cognitively normal at t0 and developed cognitive impairment at t1. We believe that, in our study, the use of a large battery of neuropsychological tests and the application of the revised classification of cognitive and behavioral impairment in ALS allowed for a more reliable and earlier detection of cognitive status which resulted in greater sensitivity in detecting the changes over time [40].We can speculate that the progression of cognitive deficits in ALS can be due to the spreading of structural changes in the frontal cortex and the progressive accumulation of dysfunction, in accordance to prion-like propagation of the disease [42].

This study is not without limitations. We are aware that advanced neuroimaging techniques could have eased the clustering of patients according to the risk to develop cognitive impairment [43,44,45]. Also, a recent paper has shown that cognitive performance correlates with the stage of the disease and the structural disease pathology evidenced by changes in diffusion tension imaging (DTI) [46]. Alternative methods of cognitive testing, such as those with eye-tracker controlled or brain computer interface [47,48] could have been used to test patients excluded from analyses because untestable due to severe motor impairment. Last, future studies could focus on the better identification of which neuropsychological tests are more effective in predicting cognitive decline. Our study is the largest longitudinal analysis evaluating ALS with a comprehensive battery of neuropsychological tests and making use of the most recent classification, highlighting how cognitive disorders can not only be present at diagnosis, but also manifest during disease and influence the progression of motor deficit and the prognosis. These findings outline the importance of identifying early markers of cognitive change and informing patients and caregivers on the likeliness of developing cognitive dysfunction. As in other neurodegenerative diseases, particularly dementia and FTD, when dealing with ALS patients clinicians need to anticipate potential difficulties in communicating end-of-life decisions and focus on the impacted quality of life, in particular by ensuring the patient's treatment adherence and by mitigating the caregiver's burden.

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Cognitive impairment across ALS clinical stages in a population-based cohort.

Chiò A, Moglia C, Canosa A, Manera U, Vasta R, Brunetti M, Barberis M, Corrado L, D'Alfonso S, Bersano E, Sarnelli MF, Solara V, Zucchetti JP, Peotta L, **Iazzolino B**, Mazzini L, Mora G, Calvo A.

Abstract

Objective. To assess the association of the degree of severity of motor impairment to that of cognitive impairment in a large cohort of ALS patients.

Methods. This is a population-based cross-sectional study on ALS patients incident in Piemonte, Italy, between 2007 and 2015. Cognitive status was classified according to the revised ALS-FTD Consensus Criteria. The King's and the Milano Torino Staging (MiToS) systems were used for defining the severity of motor impairment.

Results. Of the 797 patients included in the study, 163 (20.5%) with ALS-FTD, 38 (4.8%) with cognitive and behavioral impairment (ALScbi), 132 (16.6%) with cognitive impairment (ALSci), 63 (7.9%) with with behavioral impairment (ALSbi), 16 (2.0%) with non-executive impairment, and 385 (48.2%) cognitively normal. According to King's staging, the frequency of cases with ALS-FTD progressively increased from 16.5% in stage 1 to 44.4% in stage 4; conversely the frequency of ALSci, ALSbi and ALScbi increased from King's stage 1 to King's stage 3 and decreased thereafter. A similar pattern was observed with the MiToS staging. ALS-FTD was more frequent in patients with bulbar involvement at time of cognitive testing. Patients with *C9ORF72* expansion (n=61) showed more severe cognitive impairment with increasing both King's and MiToS stages.

Conclusion. Our findings suggest that ALS motor and cognitive components may worsens in parallel, and that cognitive impairment becomes more pronounced when bulbar function is involved. Our data support the hypothesis that ALS pathology disseminates in a regional ordered sequence, through a cortico-efferent spreading model.

Introduction

Amyotrophic lateral sclerosis (ALS) can no longer be considered a disease limited to the motor system but rather a multisystem disorder that involves cognitive domains in at least half of all

cases.[1] Longitudinal studies point to a relative stability of the cognitive function in patients who are not impaired at first examination, and a progression of the impairment in patients already compromised, [2-7] although such studies are complicated by the progressive loss of speech and motor function in the hands that hinders the accuracy of neuropsychological testing and causes a high patients attrition rate further complicating our ability to evaluate the pattern of cognitive impairment in ALS patients over time.[2,3] A recent study performed on a cross-sectional clinical-based cohort of patients evaluated using the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) has shown that cognitive and behavioural deficits are more frequent and severe with advanced disease.[8]

The aim of our study was to assess the relationship between patients' cognitive impairment, classified according to the recently published revised ALS-FTD Consensus Criteria,[9] and patients' motor impairment, classified according to King's and MiToS ALS staging systems.[10,11] To do this, we used a population-based series of ALS patients, representing one of the largest and most complete cohorts evaluated to date.

Methods

Patients.

The study population consisted of the patients identified through the Piemonte and Valle d'Aosta register for ALS (PARALS) incident in the 2007-2015 period. The PARALS is a prospective epidemiological register established in 1995, whose characteristics have been published previously.[12] Patients with history of neurological disorders affecting cognition (major stroke, severe head injuries, mental retardation), alcohol- and drug-dependence, severe mental illness and use of high-dose psychoactive medications were tested but their data were excluded from the analysis. Incident patients who were not of Italian native Italian speakers were assessed using an unstructured interview and therefore were excluded from the analysis. Patients whose cognitive testing was performed >12 months after diagnosis were excluded.

Neuropsychological evaluation.

ALS patients underwent a battery of neuropsychological tests encompassing executive function, memory, visuospatial function, social cognition and language, selected according to the Diagnostic Criteria for the Behavioural variant of Frontotemporal Dementia[13] and the ALS-FTD Consensus Criteria.[9] All patients underwent the following neuropsychological battery: Mini Mental State

Examination (MMSE); Wisconsin Card Sorting Test (WCST); Trail Making Test A and B (TMT A-B); Digit Span Forward and Backward; Letter and Category fluency test; Boston Naming Test (BNT); Rey Auditory Verbal Learning Test (RAVLT); Babcock Story Recall Test (BSRT); Rey-Osterrieth Complex Figure Test (ROCF); Raven's Coloured Progressive Matrices (CPM47); Frontal Assessment Battery (FAB). The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) was added to the battery in 2014 when it became available. Raw data of each neuropsychological test were adjusted for age and years of education according to the Italian normative.

Neurobehavioral dysfunction was determined both by direct observation by the neuropsychologist and by patient's history,[13] and with the Frontal Systems Behavior Scale (FrSBe) using the Family-form evaluated by a close relative/caregiver (scores: normal ≤59, borderline 60-64; pathological ≥65).[14] If a subject had scores reflecting an abnormality of frontal systems in both the premorbid and the post-illness evaluations, the patient was considered pathological only if there was an increase of ≥10 points in the T-score between the two forms.[15] Anxiety and depression were assessed using the Hospital Anxiety and Depression Scale (HADS); the item "I feel slowed down" was discussed with the patients in order to have them not to refer to physical disability.[16]

The battery was administered following the same sequence in order to avoid the possible differential interference of the answers of one test over the others. The administration of the battery required about one and half hour,[17] and was performed in the morning. If the subject felt too tired, a further session was scheduled to complete the battery within two weeks of the first session. Oxygen saturation at the time of the neuropsychological testing was measured with a pulse oximeter, and none of the patients displayed evidence of hypoxemia (oxygen saturation <92 mm Hg) based on this evaluation.

Cognitive categorization.

Patients' cognitive status was classified according to the revised ALS-FTD Consensus Criteria.[9] According to these criteria, patients may be classified into four main categories: (a) ALS patients with a FTD syndrome (ALS-FTD); (b) ALS patients with behavioral impairment (ALSbi); (c) ALS patients with cognitive impairment (ALSci); and (d) ALS patients with combined cognitive and behavioral impairment (ALScbi), which includes patients who fulfill criteria for both ALSci and ALSbi. In addition, we designated patients with isolated non-executive impairment in the domains of memory and visuospatial function as ALSnex.[9,15,18] Patients who did not fit into these categories were classified as cognitively normal (ALS-CN).

All patients were classified in a blind fashion by two experts in ALS neuropsychology. When there was disagreement, the case was discussed until a final diagnosis was agreed. The concordance rate was over 90% for all diagnoses.[17]

ALS Staging.

The King's staging is based on the spreading of motor symptoms in three different body regions (bulbar, upper limbs, and lower limbs), and on the use of non-invasive ventilation (NIV) and enteral nutrition. The five stages of the King's staging system are: 1, one region involved; 2, two regions involved; 3 three regions involved; 4A, patient needs gastrostomy; 4B, patient needs non-invasive ventilation.[10] The stage can be derived from the direct observation of the patients and also from the ALSFRS-R scale.[19]

In contrast to the region-based King's system, the Milano Torino Staging System (MiToS) is aimed at determining the main milestones of patients' disability, based on the loss of four principal functions (communication, swallowing, ambulation, and breathing).[11] Each of these represents a domain of the staging. The score, which ranges from 0 to 4, is given by the sum of the number of lost domains, with 0 representing no domain lost, and 4 the loss of all four domains. MiToS can be directly calculated from the ALSFRS-R scale.

ALSFRS-E decline.

Disease severity was assessed with the ALSFRS-R decline, calculated as the mean monthly number of points lost from onset to time of neuropsychological assessment:

$$[(48\text{-ALSFRS-R}_{\text{time of assessment}})/48]/\text{time from onset to diagnosis (in months)}.$$

Genetics.

Genetic assessment was performed in 749 cases (94.3%). All the coding exons and 50bp of the flanking intron-exon boundaries of *SOD1*, of exon 6 of *TARDBP*, and of exons 14 and 15 of *FUS* were PCR amplified, sequenced using the Big-Dye Terminator v3.1 sequencing kit (Applied Biosystems Inc.), and run on an ABIPrism 3130 genetic analyzer. These exons were selected as the vast majority of known pathogenic variants are known to lie within these mutational hotspots (Chia et al., 2017) A repeat-primed PCR assay was used to screen for the presence of the GGGGCC hexanucleotide expansion in the first intron of *C9orf72*.[20]

Statistical methods.

Comparisons between means were made with Student's t-test or analysis of variance (ANOVA). Comparisons between categorical variables were made with χ^2 test. A p level <0.05 was considered significant. Statistical analyses were carried out using the SPSS 25.0 statistical package (SPSS, Chicago, IL, USA).

Standard Protocol Approvals, Registrations, and Patient Consents.

The study was approved by the Ethical Committees of the two ALS centers involved in the study. All patients provided written informed consent before enrollment. The databases were anonymized according to the Italian law for the protection of privacy.

Data Availability Statement.

Data will be available upon request by interested researchers.

Results

Out of the 1,311 ALS patients incident in Piemonte and Valle d'Aosta in the 2007-2015 period, 797 (60.8%) have been included in the study. A flow chart of the sequence of participants' selection is reported in Figure 1. Non-included patients were older and more clinically impaired than those who underwent the examination (Table 1). The mean number of education years of the enrolled patients is similar to that of the age and sex-matched Piemonte and Valle d'Aosta population at the 2011 census (8.6 SD 4.0 years vs. 8.9 SD 4.1 years) (http://www.ruparpiemonte.it/infostat/). The median time from diagnosis to neuropsychological testing was 51 days (IQR 22-131).

Of the 797 patients who were included in the analysis, 163 (20.5%) were diagnosed as ALS-FTD, 38 (4.8%) as ALScbi, 132 (16.6%) as ALSci, 63 (7.9%) as ALSbi, and 385 (48.2%) were cognitively normal (ALS-CN). Isolated non-executive impairment (ALSnex) was detected in 16 cases (2%), while in 6 patients non-executive impairment was associated with executive impairment (ALSci).

The demographic and clinical characteristics of patients at the various stages according to the King's and the MiToS classification systems are reported in Tables 2 and 3. Mean age at onset and at time of neuropsychological testing was higher and mean number of years of education was lower in patients with more advanced disease. Interestingly, the mean time of testing since disease onset was similar in all stages.

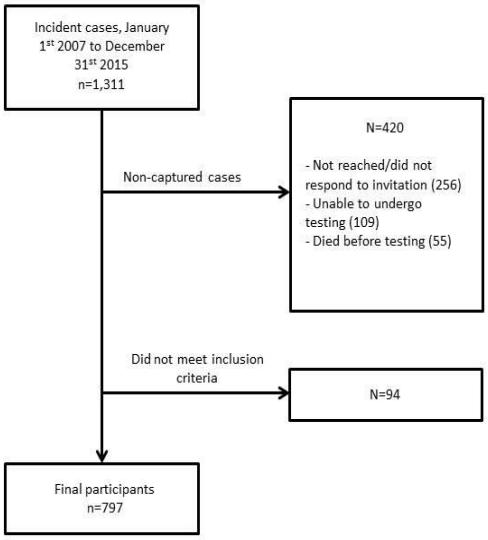

Figure 1. Flow chart reporting the enrollment of cases

Moreover, frequency of bulbar symptoms at time of testing was higher as patients' stage reflected more advanced disease. Of the 797 patients, at time of cognitive testing 11 (1.4%) had a severe depression (HADS score 15-21), 57 (7.2%) a moderate depression (HADS score 11-14), 108 (13.6%) a mild depression (HADS score 8-10) and 619 (77.7%) no depression. There was no correlation between the level of depression and Strong classification.

Table 1. Demographic and clinical characteristics of included and non-included patients

	Included patients (n=797)	Non-included patients (n=514)	p
Female (%)	362 (45.4%)	250 (48.6%)	0.14
Bulbar onset (%)	265 (33.2%)	184 (35.8%)	0.19
Age at onset, yrs (SD)	65.5 (10.5)	68.7 (11.4)	0.0001
Diagnostic delay, mos (SD)	11.6 (10.6)	11. (11.3)	0.59
Education, yrs. (SD)	8.6 (4.0)	7.9 (3.6)	0.01
Mean ALSFRS-R score (SD)	41.3 (5.7)	39.0 (7.4)	0.0001
Mean ALSFRS-R points lost/month	0.96 (1.36)	1.26 (1.56)	0.0001

Table 2. Demographic and clinical variables according to King's stage

	Stage 1 (n=407)	Stage 2 (n=219)	Stage 3 (n=135)	Stage 4 (n=36)	**P value**
Age at testing, yrs. (SD)	65.3 (9.1)	66.2 (10.0)	70.2 (9.8)	72.2 (10.1)	0.0001
Time of testing since onset, mos. (SD)	13.7 (9.8)	14.9 (10.3)	15.5 (10.2)	14.8 (11.3)	0.626
Time of testing, since diagnosis, mos. (SD)	3.0 (0.4)	3.1 (0.4)	3.0 (0.4)	2.8 (0.3)	0.867
Female (%)	179 (44.0%)	94 (42.9%)	73 (54.1%)	16 (44.4%)	0.17
Education, yrs. (SD)	9.13 (4.28)	8.44 (3.78)	7.57 (3.53)	7.31 (3.02)	0.0001
Marital status (unmarried, divorced, and widow-widower/married)	19/81	17/83	30/70	23/77	0.13
Age at onset, yrs. (SD)	64.1 (10.4)	65.0 (10.2)	68.8 (10.8)	71.9 (6.7)	0.0001
Diagnostic delay, mos. (SD)	10.8 (9.1)	11.7 (11.9)	12.5 (11.2)	12.0 (11.4)	0.08
Site of onset (Bulbar, %)	140 (34.4%)	64 (29.2%)	48 (35.6%)	15 (41.7%)	0.351
Bulbar symptoms at time of testing (%)	145 (35.6%)	104 (47.9%)	135 (100%)	26 (72.2%)	0.0001
Riluzole use (%)	380 (94.1%)	209 (96.3%)	124 (91.9%)	29 (80.5%)	0.01
Mean ALSFRS-R score (SD)	44.7 (2.0)	40.4 (3.8)	35.6 (5.4)	28.3 (7.6)	0.0001
Mean ALSFRS-R points lost/month	0.51 (0.57)	1.02 (0.85)	1.67 (2.01)	3.00 (3.09)	0.0001
FVC% of predicted *	93.3 (23.5)	88.0 (22.5)	80.0 (28.2)	67.0 (24.2)	0.0001
C9ORF72 expanded (%) **	30/386 (7.8%)	18/212 (8.5%)	10/124 (8.1%)	3/29 (10.7%)	0.231

* missing in 66 patients (King's 1, 26; King's 2, 16; King's 3, 20; King's 4, 4)

** 46 patients were not tested (King's 1, 21; King's 2, 7; King's 3, 11; King's 4, 7). The frequency of *C9ORF72* is calculated on patients who underwent genetic testing.

Table 3. Demographic and clinical variables according to MiToS stage

	Stage 0 (n=584)	Stage 1 (n=189)	Stage 2 (n=24)	P value
Age at testing, yrs. (SD)	66.0 (10.7)	68.2 (11.4)	74.5 (10.9)	0.0001
Time of testing since onset, mos. (SD)	14.3 (8.6)	15.9 (10.4)	15.7 (13.1)	0.436
Time of testing, since diagnosis, mos. (SD)	3.0 (3.2)	3.8 (3.6)	3.3 (3. 0)	0.732
Female (%)	257 (44.0%)	90 (48.6%)	15 (62.5%)	0.13
Education, yrs. (SD)	8.91 (4.17)	7.84 (3.49)	6.79 (2.54)	0.0001
Marital status (unmarried, divorced, and widow-widower/married)	20/80	21/79	37/63	0.009
Age at onset, yrs. (SD)	64.8 (10.5)	66.8 (10.1)	73.4 (7.8)	0.0001
Diagnostic delay, mos. (SD)	10.4 (8.4)	12.2 (10.6)	12.4 (10.2)	0.0001
Site of onset (Bulbar, %)	230 (39.4%)	26 (13.9%)	11 (45.8%)	0.0001
Bulbar symptoms at time of testing	309 (52.9%)	81 (42.8%)	20 (83.4%)	0.0001
Riluzole use	549 (94.2%)	178 (95.2%)	15 (62.5%)	0.0001
Mean ALSFRS-R score (SD)	43.5 (3.1)	36.5 (5.1)	23.8 (5.6)	0.0001
Mean ALSFRS-R points lost/month	0.68 (0.72)	1.46 (1.77)	3.83 (3.49)	0.0001
FVC% of predicted *	90.6 (24.6)	83.5 (24.6)	68.1 (28.0)	0.0001
C9ORF72 expanded (%) **	47 (8.4%)	14 (8.0%)	0	0.376

* missing in 66 patients (MiToS 0, 38; MiToS 1, 23; MiToS 2, 5)

** 46 patients were not tested (MiToS 0, 27; MiToS 1, 14; MiToS 2, 5). The frequency of C9ORF72 is calculated on patients who underwent genetic testing.

Cognitive classification and King's stages.

According to King's staging, the frequency of cases with ALS-FTD was progressively higher going from stage 1 (16.5% of cases) to stage 4 (44.4% of cases). The frequency of ALSci, ALSbi and ALScbi increased from King's stage 1 to King's stage 3 and was lower in stage 4 (Figure 2A). Conversely,

the frequencies of ALSnex did not modify across the stages (King's stage 1, 1.7%; King's stage 2, 3.2%; King's stage 3, 0.7%; King's stage 4, 2.8%).

Cognitive classification and MiToS stages.

A similar pattern was observed when the MiToS staging classification was applied. The frequency of ALS-FTD was progressively higher going from stage 0 (20.2% of cases) to stage 2 (50.0% of cases). The frequency of intermediate cognitive impairment (ALScbi, ALSci and ALSbi) increased from stage 0 to stage 1 and decreased thereafter (Figure 2B). No patients were tested in MiToS stages 3 and 4. The frequencies of ALSnex did not modify across the stages (MiToS stage 0, 2.2%; MiToS stage 1, 1.1%; MiToS stage 2, 4.2%). Overall, cognitive impairment was more frequent in patients with more advanced disease based on both the King's and MiToS staging systems: over 60% of patients manifested mild or severe cognitive impairment in more severe stages (King's stage 4, 63.9%, MiToS stage 2, 70.8%), compared to 45.2% (King's state 1) and 51.4% (MiToS stage 0) in the early stages.

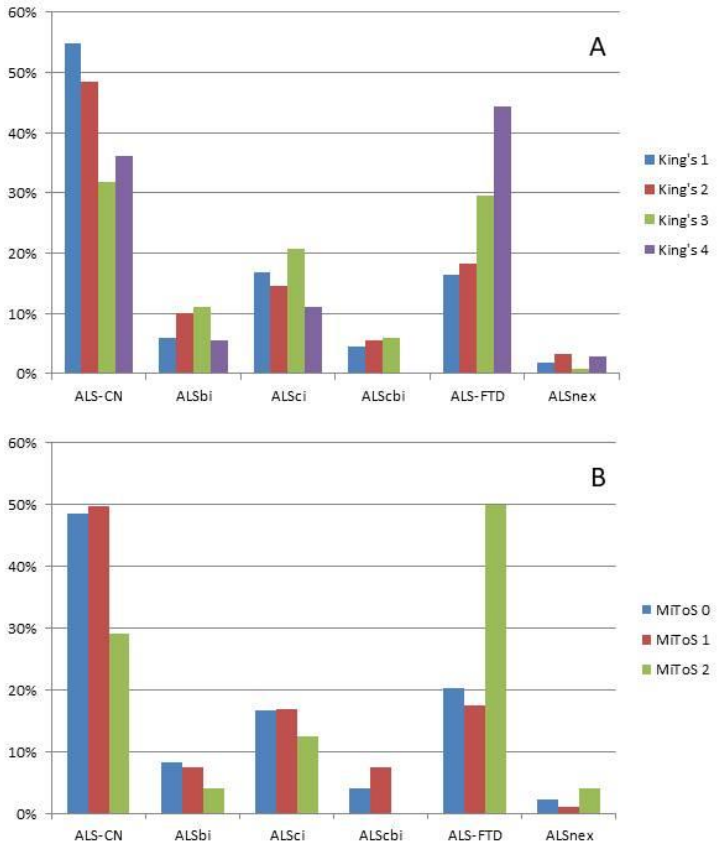

Figure 2. **A.** Cognitive classification and King's stage. Number of patients: King's stage 1, 407; King's stage 2, 219; King's stage 3, 135; King's stage 4, 36.

B. Cognitive classification and MiToS stage. Number of patients: MiToS stage 1, 584; MiToS stage 2, 189; MiToS stage 3, 24. ALS-CN, ALS cognitively normal; ALSbi, ALS patients with behavioral impairment; ALSci, ALS patients with cognitive impairment; ALScbi, ALS patients with combined cognitive and behavioral impairment; ALS-FTD, ALS patients with a FTD syndrome; ALSnex, ALS patients with isolated non-executive impairment.

Cognitive impairment and bulbar involvement.

ALS-FTD was more frequent in patients with bulbar onset (Figure 3, A-D) and in those with bulbar involvement at time of cognitive testing (Figure 4, A-D). This pattern was observed with both classification systems accounting for 70% of patients both in King's stage 4 and in MiToS stage 2. The corresponding percentage of cognitively normal (ALS-CN) patients decreased from about 40% in King's stages 1 and 2 and MiToS stage 0 to approximately 20% in King's stage 4 and MiToS stage 2. This difference was even more marked in ALS cases who did not show bulbar symptoms at time of cognitive evaluation: in fact, about 70% of this type of patient had a preserved cognition at all King's and MiToS stages.

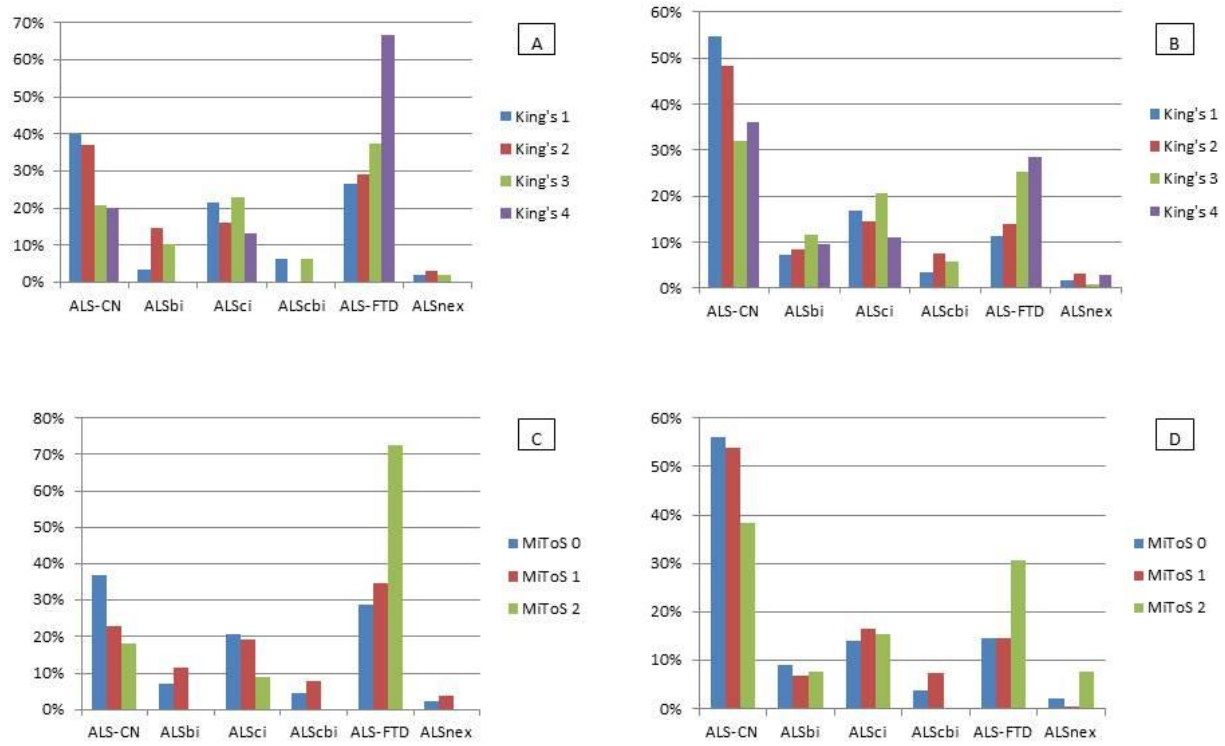

Figure 3. Cognitive classification and ALS patients staging according to the site of onset.

A. King's staging, bulbar onset.

B. King's staging, spinal onset.

C. MiToS staging, bulbar onset.

D. MiToS staging, spinal onset. ALS-CN, ALS cognitively normal; ALSbi, ALS patients with behavioral impairment; ALSci, ALS patients with cognitive impairment; ALScbi, ALS patients with combined cognitive and behavioral impairment; ALS-FTD, ALS patients with a FTD syndrome; ALSnex, ALS patients with isolated non-executive impairment.

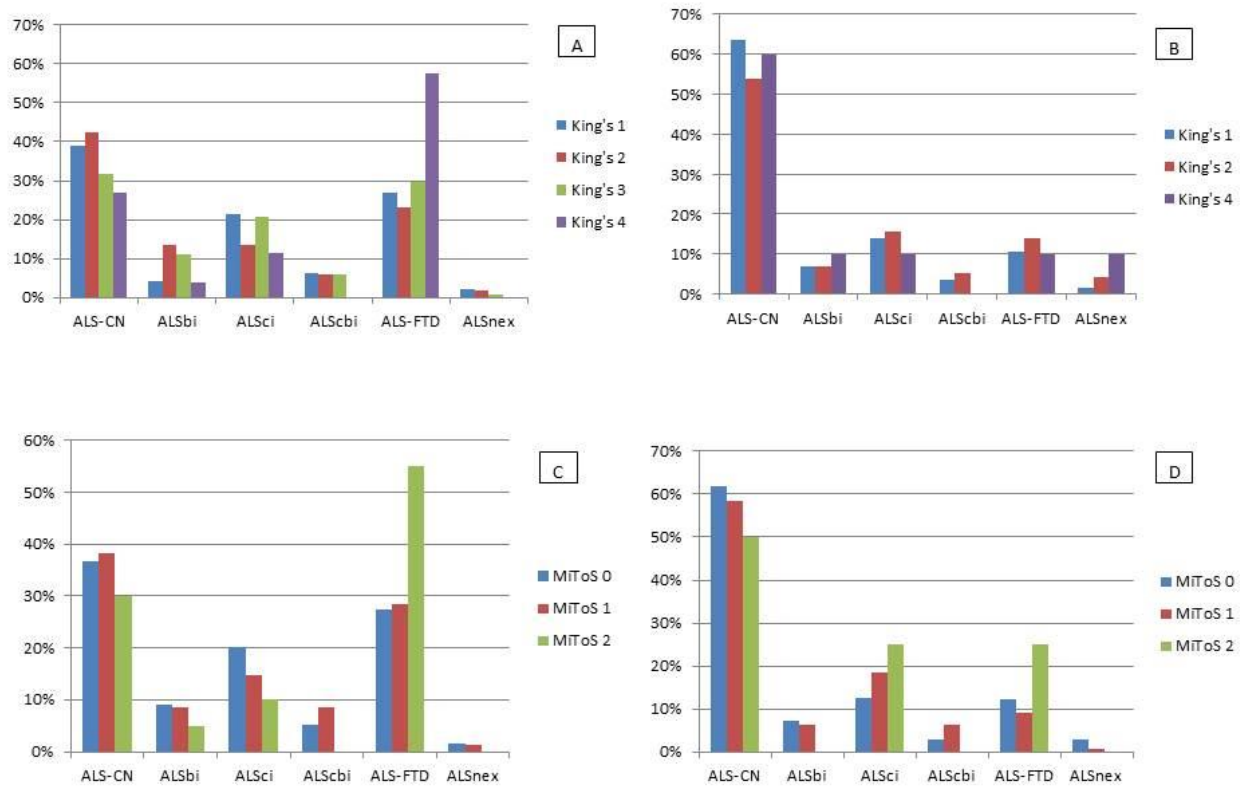

Figure 4. Cognitive classification and ALS patients staging according to the presence of bulbar symptoms at time of cognitive testing.

A. King' staging, bulbar sign present.

B. King's staging, no bulbar signs. There are no patients in King's stage 3 in the group of subjects without bulbar signs at time of cognitive testing, in accordance of the method of classification.

C. MiToS staging, bulbar sign present.

D. MiToS staging, no bulbar signs. ALS-CN, ALS cognitively normal; ALSbi, ALS patients with behavioral impairment; ALSci, ALS patients with cognitive impairment; ALScbi, ALS patients with

combined cognitive and behavioral impairment; ALS-FTD, ALS patients with a FTD syndrome; ALSnex, ALS patients with isolated non-executive impairment.

Cognitive impairment, sex and stage.

Women were more likely to be cognitively impaired than men at each stage of the King's and MiToS classification system (data not shown). However, this difference disappeared when patients were subdivided according to their site of onset, indicating that the observed differences in cognitive status across genders was mostly due to the higher frequency of bulbar onset among women. In our cohort, 43.6% of women and 25.1% of men presented with bulbar-onset disease, and 60.8% of women and 43.6% of men had bulbar signs at time of cognitive evaluation.

C9orf72 expansion and stage.

Genetic testing has been performed in 751 cases (94.2% of study population). Of these, 61 (8.1%) carried a pathological *C9orf72* expansion. These patients showed more severe cognitive impairment with increasing both King's and MiToS stages (data not shown). The number of patients with other mutations (*SOD1,* 15; *TARDBP,* 13; *FUS*, 3) was too low to allow for meaningful analyses.

Discussion

In our large population-based study we found that cognitive impairment was more frequent among patients in the advanced stages of ALS based on two classification systems compared to earlier stages of the disease. Indeed, nearly two thirds of cases had some evidence of cognitive impairment in more advanced stages. In contrast to previous publications,[2-7] our data point to a correlation between the severity of motor impairment and the severity of cognitive deficits, and suggest that cognitive function may not remain stable in ALS patients. Importantly, our data indicate that the presence of bulbar signs is strongly associated with more severe cognitive impairment at all stages of disease. Furthermore, cognitive impairment was more severe in patients at worse stage of their disease both in patients with and without the *C9orf72* expansion (known to be associated with FTD).

Our study add novel findings to two previous cross-sectional studies assessing the correlation between ALS stages and cognition. A small clinical-based cohort study, which excluded patients with FTD and classified patients according to the 2009 ALS-FTD Consensus Criteria,[21] reported that

patients' cognition was more impaired with the worsening of King's stage.[22] A larger multicenter cross-sectional study, which compared the domains of ECAS and King's staging but not classified patients according to the revised ALS-FTD Consensus Criteria, found that ALS-specific cognitive deficits and behavioral impairment were more frequent in advanced disease stages.[8]

Previously published longitudinal studies on ALS cognition purported that patients with normal cognition at first examination remained stable over time.[2-7] However, these studies suffered severe attrition rates over the course of their follow-up (only 50% of patients were re-tested at six months and less than 25% at one year). Cognitive impairment is known to be associated to a more rapid decline of motor function in ALS.[2,14,18,23] We believe that these studies failed to detect progression in cognitive impairment due to the selective loss to follow-up among the very patients most likely to manifest cognitive decline. Our population-based study allowed for a more accurate assessment of the relationship of these important clinical parameters, but its cross-sectional design does not allow to clarify this important issue.

The mean delay between symptoms onset and the diagnostic interview was similar in patients at all King's and MiToS stages, suggesting that the cognitive categorization was related to the rate of motor decline. From a pathological perspective, our findings logically support the notion that patients manifesting more advanced stages of disease at the time of diagnosis have experienced a faster spread of lesions involving both the motor cortex and the prefrontal and temporal cognitive cortices compared to patients at lower stages.[24-26] Moreover, our data suggest that the rate of lesions spreading is different within subgroups of patients, but that, within each subgroup, this spread rate is the same within motor and cognitive cortices.

In general, the emergence of bulbar symptoms in ALS patients is associated with a more severe impairment of cognition.[8,15,27,28] We postulate that this association is related to connections between the prefrontal cognitive cortex and the cortical areas controlling facial and speech muscles which could favor the dissemination of TDP43 lesions from motor to cognitive area, or vice versa.[24,25,29] Supporting this hypothesis is the observation emerging from follow-up studies that patients with FTD that subsequently develop ALS typically present with bulbar weaknesss.[30,31]

At present, the ALS research community makes a distinction between the milder forms of cognitive impairment (ALSbi, ALSci, and ALScbi) and florid FTD dementia, maintaining that they represent separate processes. Supporting this notion is that milder forms of cognitive impairment are rarely seen among FTD patients. Our data does not support this arbitrary distinction and instead strongly point to the milder forms being closely related to FTD. Notably, the occurrence of

intermediate cognitive impairment was less frequent among our patients in more advanced stages of disease, while florid FTD was more frequent. The presence of motor impairment brings ALS patients to the attention of neurologists, who then have the opportunity to diagnose milder forms of cognitive impairment among this patient population. In contrast, these more subtle forms of cognitive impairment are frequently overlooked in FTD patients as they are not typically severe enough to bring that individual to medical attention. Our observations are consistent with the notion that intermediate forms of cognitive impairment should be considered the equivalent of mild cognitive impairment in Alzheimer's disease.[32]

The demographics of patients in each King's and MiToS stages are significantly different in a predictable template. First, mean age at onset was significantly higher from less severe to more severe King's and MiToS stages. This is expected since more severe stages include patients with more rapidly declining motor function, who are likely to be older. Likewise, the increased frequency of cognitive impairment observed among patients in King's stage 4 and MiToS Stage 2 parallels the findings of epidemiological studies, which have shown that FTD incidence increases with age.[33,34] Second, the mean number of years of education significantly decreases with the increase of motor and cognitive severity. A lower level of education compared to cognitively normal controls has been reported in patients with Alzheimer's disease[35] and in patients with FTD.[36] Education is thought to create a reserve capacity that allows some people to better endure brain damage, an effect that could also apply to the cognitive performance of ALS.

As expected in our series the presence of a *C9orf72* expansion was related to a more severe cognitive impairment at each stage of both staging systems.[37] The cognitive picture of *C9orf72* patients is also characterized by the lower frequency of ALScbi and ALSbi, perhaps as patients are more likely to have progressed to florid FTD, although the relatively reduced number of patients with *C9orf72* expansion in this series does not allow us to draw definitive conclusions.

We found that non-ALS related cognitive domains had almost the same frequency at each King's and MiToS stage, supporting the notion that non-executive impairment is rare in isolation among ALS patients. Our data challenge the introduction of this category in the classification.[8,9] However, it has been reported that memory deficits in ALS are distinctly different from those observed in amnestic mild cognitive impairment (aMCI) and can be explained only to some extent in the context of comorbid executive dysfunction, indicating qualitative differences in temporal lobe dysfunction between ALS and aMCI patients.[38] Therefore further research in this area of cognition is warranted.

King's and MiToS staging systems clearly reflect the degree of motor impairment experienced by patients. However, both these rating systems do not incorporate the severity of cognitive impairment, which can now be considered a central component of the ALS syndrome. Such cognitive impairment has a profound negative impact on patients' survival,[15,18] ability to adapt to life-prolonging interventions,[39] quality of life,[40] and decision-making capacity.[41,42] It also negatively affects caregivers' burden and quality of life.[44,44] Based on these observations and our own data, we maintain that there is an urgent need to integrate cognitive measures into ALS clinical rating scales.

The cross-sectional design of our study may have limited our findings. Nevertheless, patients were tested early after diagnosis and the cognitive impairment at that time point likely reflects the rapidity of lesion spreading within non-motor cortical areas of the brain. A second limitation of our study is that 40% of incident patients were not enrolled, primarily because they were not reached or did not respond to the invitation or because they were too disable to be tested. Non-enrolled patients were older, had a lower education level than those who were included in the study, and had also a more severe disease. Alternative methods of cognitive testing in severely disabled patients, such as eye-tracker controlled cognitive batteries and brain-computer interfaces potentially represent important improvements in our ability to assess cognitive status among the ALS population.[45,46] Third, the assumptions related to more severe stages (King's 4 and MiToS 2) should be considered with caution due to the small number of patients tested in these stages.

This study has several strengths. First, it is population-based and includes incident patients tested for cognition shortly after the diagnosis. Second, it comprises the largest population of patients to be tested to date. Third, it is based on a large battery of tests evaluating the main domains of cognitive impairment, including those who are classified as non-ALS specific. Fourth, it compares the King's and MiToS staging to the revised ALS-FTD Consensus Criteria,[9] allowing us to assess the association of the degree of severity of motor impairment to that of cognitive impairment. Fifth, it includes the genetics of ~95% of patients.

Our findings have significant implications in clarifying the dynamics of ALS progression, suggesting that its motor and cognitive components may worsen in parallel, and that cognitive worsening is more pronounced in subjects with bulbar involvement. Our data support the hypothesis that ALS pathology disseminates in a regional ordered sequence, through a cortico-efferent spreading model.[21,26] Moreover, our data showed that intermediate cognitive categories may represent a transitional condition between normal cognition and FTD. In that regard, ALS represents an unique

opportunity to study the initial clinical and pathological stages of FTD. Further research on functional connectivity between motor and cognitive areas of the brain will be important to delineate the dynamics of the spreading of pathology in ALS.

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The Interplay Among Education, Brain Metabolism, and Cognitive Impairment Suggests a Role of Cognitive Reserve in Amyotrophic Lateral Sclerosis

Canosa A, Palumbo F, **Iazzolino B**, Peotta L, Di Pede F, Manera U, Vasta R, Grassano M, Solero L, Arena V, Moglia C, Calvo A, Chiò A, Pagani M.

Ab s t r a c t

We tested the Cognitive Reserve (CR) hypothesis in Amyotrophic Lateral Sclerosis (ALS), enrolling 111 patients, using education as CR proxy, 18F-FDG-PET to assess brain damage, and ECAS to measure cognition. Education was regressed out against brain metabolism, including age, sex, spinal/bulbar onset, ALSFRS-R, and ECAS as covariates. Clusters showing a significant correlation were used as seed regions in an interregional correlation analysis (IRCA) in the ALS group and in 40 controls. In the ALS group, we found a negative correlation between brain metabolism and education in the right anterior cingulate and bilateral medial frontal gyrus. In the IRCA in the ALS group, the medial frontal cluster metabolism positively correlated with that of frontotemporal regions (right > left), bilateral caudate nuclei, and right insula, and negatively correlated with that of corticospinal tracts, cerebellum, and pons. In controls, the IRCA showed significant positive correlations in the same regions but less extended. Our results agree with the CR hypothesis. The negative correlation between the medial frontal cluster and the cerebellum found only in ALS patients might reflect cerebellar compensation.

Introduction

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease affecting upper and lower motor neurons. Patients show progressive muscle weakness and wasting involving bulbar and spinal regions, usually leading to death within 2-5 years after the onset due to respiratory failure (van Es et al., 2017). Approximately 15% of ALS patients display a full-blown frontotemporal dementia (FTD), while ~35% have more subtle cognitive alterations involving executive and non-executive domains [26,35].

Reserve mechanisms have been hypothesized to explain what makes people with certain lifestyle resilient against aging or diseases causing cognitive impairment. The Cognitive Reserve (CR) hypothesis was introduced by the neuropathologist Robert Katzmann to account for the possible discrepancy between neuropathological findings in Alzheimer's Disease (AD) and the severity of cognitive symptoms shortly before death [20] .

The concept of CR has evolved over the last decades towards an active, dynamic model referring to the adaptability of cognitive processes of the adult brain that helps to cope with brain damage in order to minimize symptomatology [28] .Two further constructs have been proposed to account for resilience mechanisms: Brain Reserve (BR) and Brain Maintenance (BM). BR has been conceived as a fixed, passive construct, referring to the neurobiological capital (numbers of neurons, synapses, etc.) at any point in time, that allows some people to better cope with brain aging and pathology than others before clinical or cognitive changes emerge. BM has been defined as the reduced development over time of age-related brain changes and pathology based on genetics or lifestyle. BR and BM are fundamentally related notions. They might be considered as two facets of the same concept, viewed at different timescales[48] .CR, BR, and BM likely interplay to outline individual vulnerability to cognitive decline.

CR may be modulated by lifetime exposures, including education, occupation, physical exercise, leisure activities, or social engagement. Research exploring CR requires the inclusion of three components: brain status (i.e. brain pathology), cognitive performance measures, and a proxy of reserve (either a sociobehavioural proxy, i.e. an index of lifetime exposure/premorbid ability, or a functional brain measure)[48].

Individuals with higher CR seem to tolerate a larger amount of brain pathology so the point at which cognitive functions begin to be affected will be later than in those with lower CR [47, 55].This initially protective phase is followed by more rapid decompensation once the protection is overwhelmed[3]. A recent, large, cohort study including neuropathological data underlines that high lifespan CR is associated with a reduction in dementia risk, even in the presence of high brain pathologies [57] .

The advent of neuroimaging techniques has provided *in vivo* support for the CR hypothesis by showing that individuals with presumably greater CR (e.g. higher educational or occupational levels) can tolerate more severe pathological burden, as assessed through structural or functional neuroimaging, at similar levels of cognitive function[32,53].

Several studies employing neuroimaging have suggested a role of CR in AD and Mild Cognitive Impairment (MCI) due to AD [2,14,15,28] , FTD [45,46] ,subcortical vascular dementia[18, 60], and multiple sclerosis[49] .However, CR has never been studied in ALS.

Our aim was to test the CR hypothesis in an ALS cohort. We used years of education as CR proxy and brain ^{18}F-2-fluoro-2-deoxy-D-glucose-Positron Emission Tomography (^{18}F-FDG-PET) as tool to assess brain lesion load. We hypothesized that CR might be underpinned by a higher metabolic

connectivity. In order to test this hypothesis we employed the Interregional Correlation Analysis (IRCA), since it has been proven to be useful in the assessment of metabolic connectivity in neurodegenerative diseases [29].

Materials and Methods

Participants

We included 111 ALS patients diagnosed at the ALS Centre of Turin ("Rita Levi Montalcini" Department of Neuroscience, University of Turin) between 2016 and 2018. Diagnosis was made according to the revised El Escorial diagnostic criteria for definite, probable and probable laboratory supported ALS [5] (Brooks et al., 2000). Patients were recruited at the time of diagnosis or less frequently during the first follow up visit (usually 2 months later). Respiratory function was assessed through clinical evaluation, peripheral blood oxygen saturation, and, when necessary, spirometry and arterial blood gases analysis, within 4 weeks before or after the enrolment to exclude respiratory failure. DNA was available for genetic analysis for 106 out of 111 subjects.

We included in the analyses 40 Healthy Controls (HC). We considered eligible as controls subjects referred to the PET Centre for suspected lung cancer (i) with no oncologic disease detected, (ii) with brain PET scan reported as normal by the nuclear medicine physician, (iii) without history of neurological disorders, and (iiii) with normal neurological examination. No neuropsychological assessment was performed.

Genetic analysis

All the coding exons and 50 bp of the flanking intron-exon boundaries of *SOD1*, of exon 6 of *TARDBP*, and of exons 14 and 15 of *FUS* have been PCR amplified, sequenced using the BigDye Terminator v3.1 sequencing kit (Applied Biosystems Inc.), and run on an ABIPrism 3500 genetic analyzer. These exons were selected as the vast majority of known pathogenic variants are known to lie within these mutational hotspots. A repeat-primed PCR assay was used to screen for the presence of the GGGGCC hexanucleotide expansion in the first intron of *C9orf72*.

Assessment of cognitive performance

A multi-domain screening tool, the Edinburgh Cognitive and Behavioural Amyotrophic Lateral Sclerosis Screen (ECAS), was used as measure of cognitive functioning [31,36],using the validated

Italian version [38] .ECAS is designed specifically to assess cognitive performances in ALS patients, accommodating for both limb- and bulbar-related motor disability, as it can be administered in a verbal or written form. ECAS has been designed not only to determine the presence of cognitive impairment but also to quantify the severity and the nature of the deficits. It includes tasks assessing different cognitive domains, grouped in ALS specific cognitive measures (Language, Verbal Fluency, Executive Functions) and non-ALS specific measures (Verbal Memory and Visuospatial Abilities). It also includes a brief carer interview to check for the presence of behavioural changes based on FTD diagnostic criteria. It provides two subscores (ALS specific and non-ALS specific) and a total score (ALS total). The latter was used in our study as measure of the overall cognitive functioning.

Cognitive reserve proxy

We considered education as proxy of CR. It was rated considering the number of completed years of schooling, adding the possible years of apprenticeship only when formal education was present. Patients' education history was confirmed by their caregivers.

^{18}F-FDG-PET acquisition

^{18}F-FDG-PET was performed according to published guidelines [54] .Patients fasted at least six hours before the exam. Blood glucose was <7.2 mmol/l in all cases before the procedure. After a 20-minute rest, about 185 MBq of ^{18}F-FDG was injected. The acquisition started 60 minutes after the injection. PET/CT scans were performed on a Discovery ST-E System (General Electric). Brain CT (slice thickness of 3.75 millimetres, 140 kV, 60-80 mAs) and PET scan were sequentially acquired, the former being used for attenuation correction of PET data. The PET images were reconstructed with 4 iterations and 28 subsets with an initial voxel size of 2.34 x 2.34 x 2.00 mm and data were collected in 128×128 matrices. In the patient group a whole-body scan was performed setting head-first. In the control group a separate brain scan was performed after the whole-body one with a time difference of 15 minutes. The ^{18}F-FDG-PET acquisition procedure was performed in the same environmental conditions in patients and controls.

Statistical analysis

SPM12 implemented in Matlab R2018b (MathWorks, Natick, MA, USA) was used for image spatial normalization to a customized brain ^{18}F-FDG-PET template [10]. Intensity normalization was

performed using the 0.8 default SPM value of grey matter threshold and images were subsequently smoothed with a 10-mm filter and submitted to statistical analysis. In our ALS sample (n =111) education (years) was regressed out against whole brain metabolism. The SPM12 Multiple Regression routine was implemented with age at PET, sex, site of onset (spinal/bulbar), ALS Functional Rating Scale Revised (ALSFRS-R) total score at PET, and ECAS total score at PET as covariates. The height threshold was set at $P<0.005_{uncorrected}$ ($P<0.05_{FWE\text{-}corrected}$ at cluster level) and only clusters containing >125 contiguous voxels were considered significant. Since the presence of the hexanucleotide repeat expansion of *C9orf72* can impact on brain metabolism[9], we performed a sensitivity analysis with the same procedure on the patients for whom the genetic analysis resulted negative for *C9orf72, SOD1, TARDBP*, and *FUS* mutations.

Metabolic clusters showing a significant negative or positive correlation with education were then used as *seed regions* in a multiple regression analysis in the same sample to identify cerebral regions whose metabolism was positively or negatively correlated with that of the seed clusters (i.e interregional correlation analysis, IRCA)[29] .Since our hypothesis was that cognitive reserve might be underpinned by a higher metabolic connectivity as assessed through the IRCA, as comparison we performed the IRCA using the same seed regions in a control group (n=40), including age at PET and sex as covariates. In the IRCA the height threshold was set at $P<0.001_{uncorrected}$ ($P<0.05_{FWE\text{-}corrected}$ at cluster level) and only clusters containing >125 contiguous voxels were considered significant.

Brodmann areas (BAs) were identified at a 0–2-mm range from the Talairach coordinates of the SPM output isocentres corrected by Talairach Client (http://www.talairach.org/index.html).

Protocol approvals

The study was approved by the ethical committee “Comitato Etico Interaziendale Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino”. Patients signed a written informed consent. They did not receive any remuneration for participation.

Data availability statement

Data will be available upon request by interested researchers.

Results

Participants characteristics

The demographic and clinical characteristics of ALS patients included in the study are reported in Table 1. The control group (n=40) included 29 males and 11 females, with a median age at PET of 66.5 years (Interquartile Range 55.0-72.0). As compared to ALS patients, age at PET and sex distribution of controls did not result significantly different (p=0.54 and p=0.80 respectively).

Table 1. Demographic and clinical characteristics of the ALS patients included in the study. PET: Positron Emission Tomography. ALSFRS-R: Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised. ECAS: Edinburgh Cognitive and Behavioural Amyotrophic Lateral Sclerosis Screen. IQR: Interquartile Range.

ALS patients (n=111)	
Median Age at PET, years (IQR)	63.5 (54.9-69.8)
Sex	
Male (%)	63 (56.8)
Female (%)	48 (43.2)
Total (%)	111 (100)
Onset	
Bulbar (%)	35 (31.5)
Spinal (%)	76 (68.5)
Total (%)	111 (100)
Median Education, years (IQR)	11 (8-13)

Median ALSFRS-R total score (IQR)	40 (36-43)
Median ECAS total score (IQR)	104 (94.5-112.5)
Genetic status	
C9orf72 (%)	9 (8.1)
C9orf72+TARDBP (%)	1 (0.9)
TARDBP (%)	3 (2.7)
SOD1 (%)	2 (1.8)
FUS (%)	0 (0)
Missing (%)	5 (4.5)
Wild type (%)	91 (82)
Total (%)	111 (100)

Relationship between brain metabolism and education

In the ALS sample we found a negative correlation between brain metabolism and education in a cluster including right anterior cingulate cortex and bilateral medial frontal gyrus (Table 2, Figure 1 - see Supplementary Figure 1 for the graph). No clusters of positive correlation were found. The sensitivity analysis, including only the 91 patients whose genetic screening was available and resulted normal, showed substantially unchanged findings (data not shown). The medial frontal cluster was used as *seed region* for the following IRCA.

Table 2. Cluster of negative correlation between brain metabolism and education in the ALS group. BA: Brodmann Area.

P (FWE-corrected)	Cluster Extent	Z-score	Talairach Coordinates			Side	Cortical Region	BA
			x	y	z			
0.005	1798	3.66	4	21	28	Right	Cingulate Gyrus	32
		3.55	2	36	26	Right	Anterior Cingulate	32
		3.22	4	2	44	Right	Cingulate Gyrus	24
		3.14	-2	49	40	Left	Medial Frontal Gyrus	8
		2.98	6	-5	57	Right	Medial Frontal Gyrus	6
		2.62	4	47	14	Right	Medial Frontal Gyrus	9

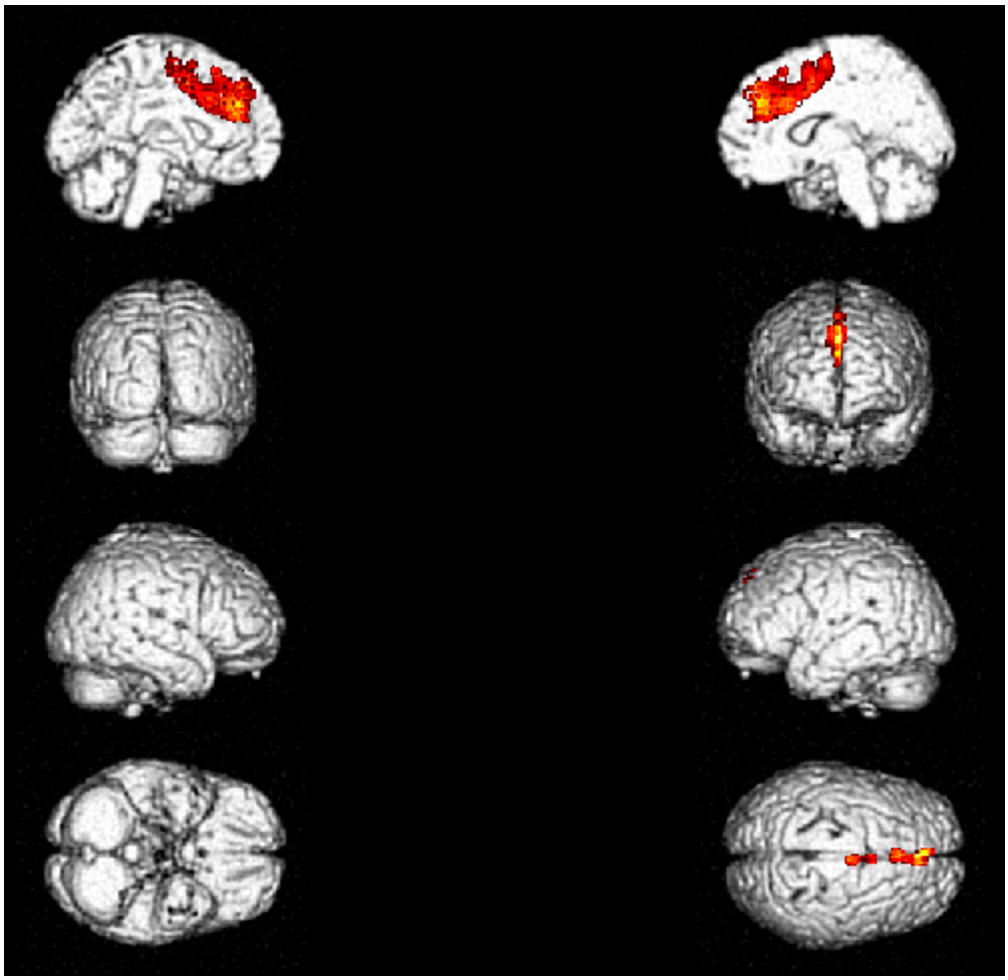
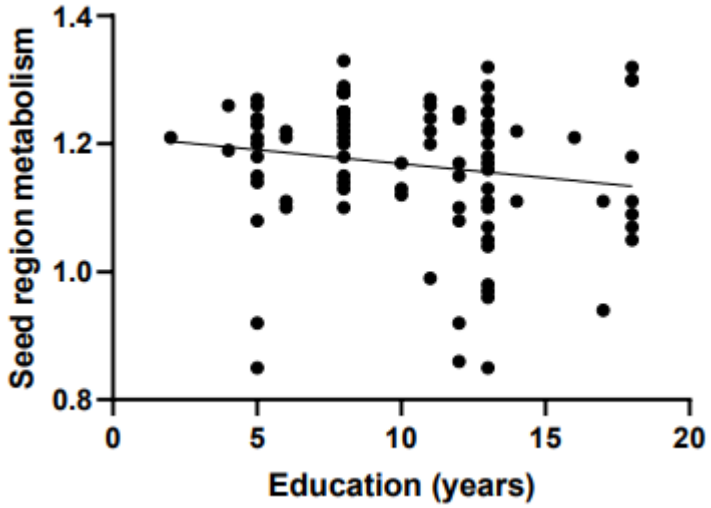


Figure 1. Glass brain rendering of multiple regression of education (years) against whole brain metabolism in the ALS group. The clusters showing a statistically significant negative correlation are projected on brain surface.



Supplementary Figure 1. Graph of the multiple regression of education against whole brain metabolism (negative correlation) in the ALS sample.

IRCA

The IRCA in the ALS sample showed that the metabolism of the medial frontal cluster positively correlated with that of a large cluster including frontotemporal regions, with right prevalence, bilateral caudate nuclei, and right insula (Table 3, Figure 2 - see Supplementary Figure 2 for the graph). We found a negative correlation with the metabolism of the corticospinal tracts, the cerebellum, and the pons (Figure 3 - see Supplementary Figure 3 for the graph).

Table 3. Results of the IRCA in the ALS group: regions whose metabolism resulted positively correlated with that of the medial frontal cluster of interest. IRCA: Interregional Correlation Analysis; BA: Brodmann Area.

P (FWE-corrected)	Cluster Extent	Z-score	Talairach Coordinates			Side	Cortical Region	BA
			x	y	z			
0.000	32633	6.55	2	31	37	Right	Medial Frontal Gyrus	8
		6.52	-42	9	-14	Left	Superior Temporal Gyrus	38
		5.94	-8	10	5	Left	Caudate (Head)	
		5.88	30	22	49	Right	Superior Frontal Gyrus	8
		5.71	14	10	9	Right	Caudate (Body)	
		5.62	-53	13	18	Left	Inferior Frontal Gyrus	45
		5.62	12	12	3	Right	Caudate (Head)	
		5.54	46	11	-17	Right	Superior Temporal Gyrus	38
		5.31	26	11	57	Right	Middle Frontal Gyrus	6
		5.22	6	55	-23	Right	Superior Frontal Gyrus	11
		5.16	2	-34	50	Right	Paracentral Lobule	5
		4.99	42	19	-11	Right	Inferior Frontal Gyrus	47
		4.96	42	16	1	Right	Insula	13
		4.92	2	13	-14	Right	Subcallosal Gyrus	25

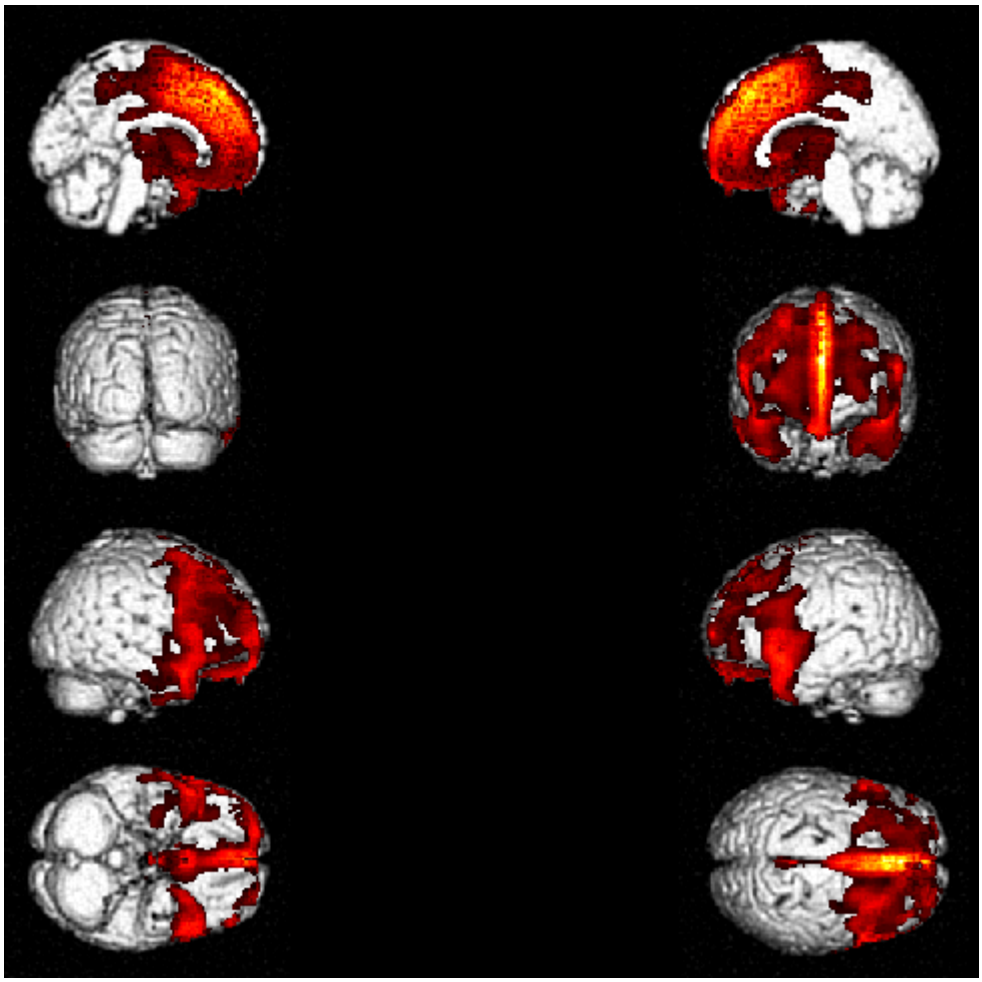
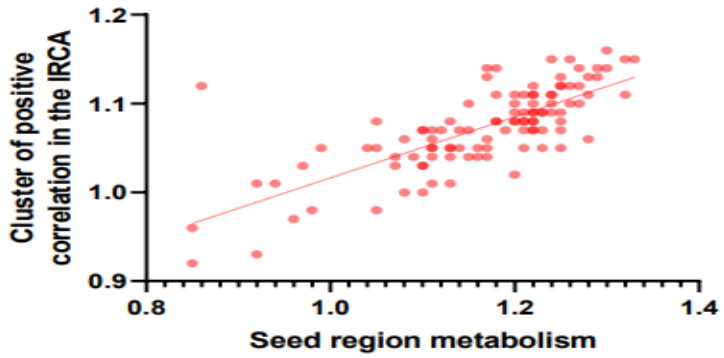


Figure 2. Glass brain rendering of the results of the Interregional Correlation Analysis (IRCA) in the ALS sample: clusters of positive correlation with the metabolism of the medial frontal cluster of interest are projected on brain surface.



Supplementary Figure 2. Graph of the Interregional Correlation Analysis (IRCA) in the ALS sample (positive correlation).

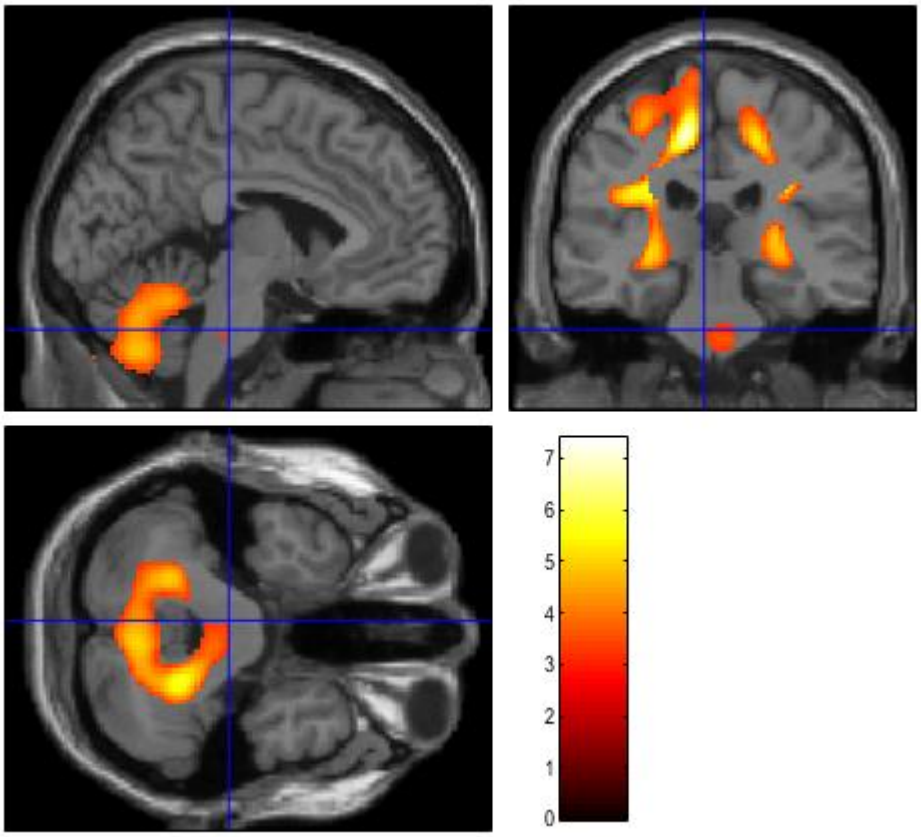
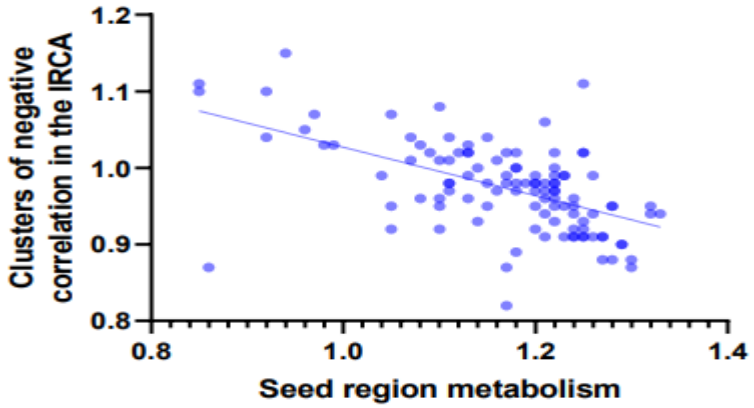
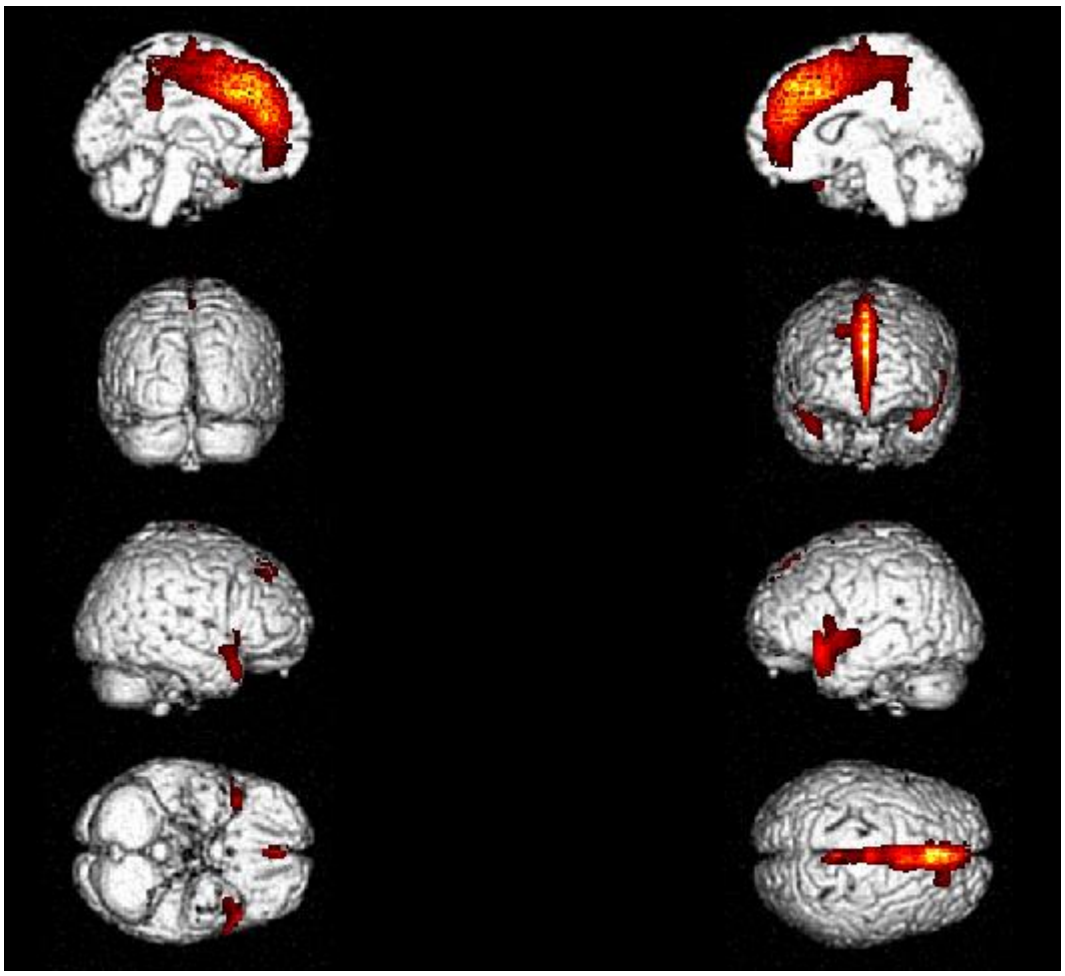


Figure 3. Results of the Interregional Correlation Analysis (IRCA) in the ALS sample: clusters of negative correlation with the metabolism of the medial frontal cluster of interest are represented on a Magnetic Resonance Imaging template.



Supplementary Figure 3. Graph of the Interregional Correlation Analysis (IRCA) in the ALS sample (negative correlation).

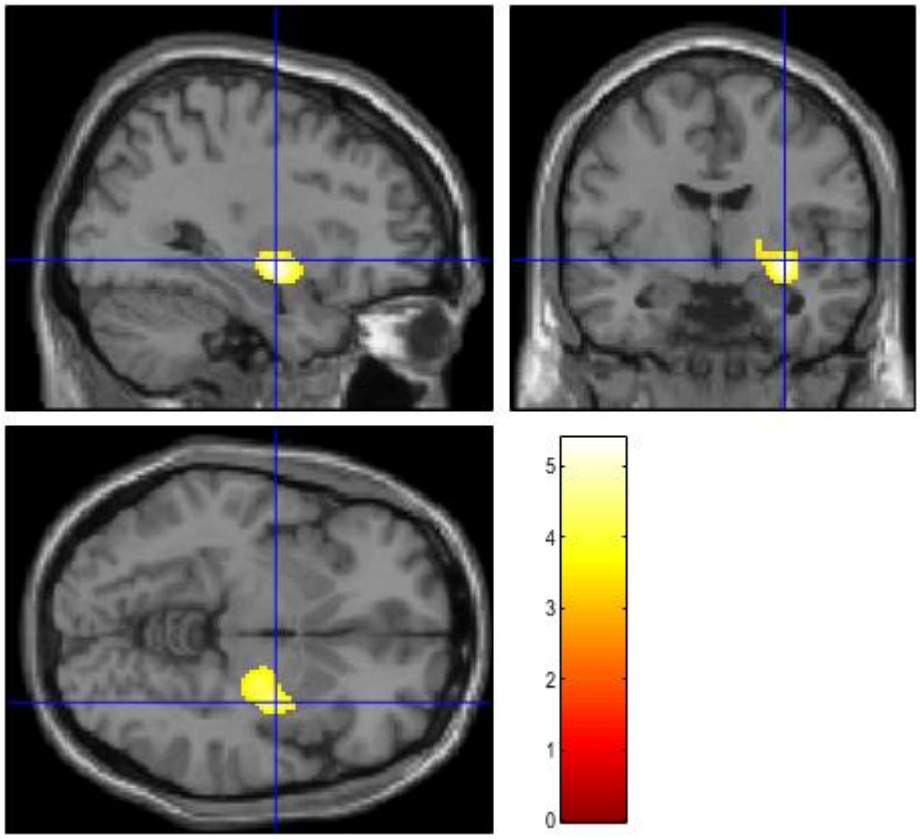
In the control group the IRCA showed that the metabolism of the medial frontal cluster positively correlated with regions partially overlapping with those found in ALS patients, but less extended (see Supplementary Figure 4 and Supplementary Table 1), and negatively correlated with the right claustrum and putamen (See Supplementary Figure 5 and Supplementary Table 2).



Supplementary Figure 4. Glass brain rendering of the results of the Interregional Correlation Analysis (IRCA) in the control group: clusters of positive correlation with the metabolism of the medial frontal cluster of interest are projected on brain surface.

Supplementary Table 1. Results of the IRCA in the control group: regions whose metabolism resulted positively correlated with that of the medial frontal cluster of interest. IRCA: Interregional Correlation Analysis; BA: Brodmann Area.

P (FWE-corrected)	Cluster Extent	Z-score	Talairach Coordinates			Side	Cortical Region	BA
			x	y	z			
0.000	7854	Inf	0	31	35	Left	Medial Frontal Gyrus	6
		7.79	2	40	18	Right	Anterior Cingulate	32
		4.65	4	-28	55	Right	Paracentral Lobule	6
0.000	1203	5.13	-40	17	-9	Left	Inferior Frontal Gyrus	47
		4.40	-44	-6	-3	Left	Insula	13
		4.02	-36	11	-21	Left	Superior Temporal Gyrus	38
0.043	368	3.87	40	16	-24	Right	Superior Temporal Gyrus	38
		3.66	50	9	-11	Right	Superior Temporal Gyrus	38
		3.25	50	19	-1	Right	Inferior Frontal Gyrus	47



Supplementary Figure 5. Results of the Interregional Correlation Analysis (IRCA) in the control group: clusters of negative correlation with the metabolism of the medial frontal cluster of interest are represented on a Magnetic Resonance Imaging template.

Supplementary Table 2. Results of the IRCA in the control group: regions whose metabolism resulted negatively correlated with that of the medial frontal cluster of interest. IRCA: Interregional Correlation Analysis; BA: Brodmann Area.

P (FWE-corrected)	Cluster Extent	Z-score	Talairach Coordinates			Side	Cortical Region
			x	y	z		
0.004	663	4.57	32	-6	-6	Right	Claustrum
		4.29	24	-15	10	Right	Putamen

Discussion

In the present study we evaluated the interplay among education, brain metabolism, and cognitive impairment in 111 ALS patients to verify the applicability of the CR hypothesis to cognitive impairment associated with ALS. We found a negative correlation between education and brain metabolism in a cluster including regions typically affected in FTD [19].Results were independent from age, sex, site of onset, level of motor disability, and, more importantly, from the level of cognitive impairment. A sensitivity analysis including only the cases with negative genetic screening confirmed the above-mentioned results, excluding the possible impact of genetic mutations on our results. This statistical design, already adopted in previous papers[14,15,33], evaluates the correlation between education and regional cerebral metabolic rate of glucose (rCMRglc) after eliminating the impact of possible confounders on rCMRglc variance. The inclusion of a measure of cognitive performance (i.e. ECAS) allowed the correction for the level of cognitive decline. Our findings are in agreement with the CR hypothesis, since in our series higher education is associated with higher pathological burden, as assessed through rCMRglc. Our data are in keeping with those observed for different diseases associated with cognitive impairment, such as AD[14,15,24,47] ,preclinical AD (cognitively healthy subjects with low CSF levels of $A\beta_{1-42}$)[12], amnestic MCI converters to AD[14,29], FTD[4,32,37,45] , multiple sclerosis [49],and subcortical vascular dementia[18, 60] . The cluster with inverse relationship between education and metabolism that we found in medial frontal cortex partially overlaps with regions reported by different studies about CR in behavioural

variant-FTD[4,32,39] .It includes regions involved in different cognitive and behavioral processes affected by frontotemporal cognitive impairment, such as set shifting [13] ,social cognition [1] , and apathy[11] .To evaluate a possible influence of genetic mutations on our results, we repeated the first analysis in the subgroup of 91 subjects for whom genetic analysis was available and resulted negative for mutations in major ALS-related genes. The findings did not change. Nevertheless, the assessment of the CR hypothesis in ALS patients carrying genetic mutations requires a targeted study, since reserve mechanisms seem to play a role also in presence of unfavorable genetic characteristics [15,39,40,41].

In the first step of our analysis on ALS patients we did not identify any cluster of positive correlation between brain metabolism and education, which could represent the correlate of compensation mechanisms. We performed an IRCA to assess metabolic connectivity of the medial frontal cluster that we found in the first step analysis, in order to identify possible networks underpinning CR mechanisms.

In the IRCA the metabolism of the medial frontal cluster resulted positively correlated with that of bilateral (right>left) frontotemporal regions and caudate nuclei, and right insula. This is in line with the finding that medial frontal cortex, as well as dorsolateral frontal cortex, temporal cortex, and insula, show typical hypometabolism in cognitive decline of the frontotemporal type [19]. Also caudate nucleus may be hypometabolic in FTD[27] .It seems to be part of the neurocircuitry of facial emotion identification[23] and apathy [11] ,and its damage may be an early finding in FTD [44].Otherwise, the metabolism of the medial frontal cluster showed an inverse correlation with that of the cerebellum, the corticospinal tracts and the pons. This finding is in line with our previous data showing that an increasing gradient of metabolism in clusters including the cerebellum, the corticospinal tracts and the brainstem parallels the decreasing metabolic gradient in frontal regions that we observe as the cognitive impairment worsens along the ALS-FTD *continuum* [6]. Taken together, the results of the IRCA show that the metabolism of the medial frontal cluster is correlated with metabolic changes that are associated with cognitive impairment related to ALS. In order to evaluate whether some of the findings of the IRCA could reflect reserve mechanisms we performed the IRCA using the same seed regions in a control group as comparison. Differently from controls, in the ALS group the metabolism of the medial frontal cluster showed correlations with areas typically involved in ALS neurodegeneration, as expected (i.e. positive correlation with larger frontotemporal clusters, and a negative correlation with the corticospinal tracts). Noteworthy, we also found an inverse correlation between the metabolism of the medial frontal

cluster and cerebellar metabolism only in the ALS group and not in the control group, which might be due to the recruitment of reserve networks. Indeed, the cerebellum is known to be involved in cognitive and behavioural processes. Cerebellar damage can lead to the cerebellar cognitive affective syndrome (Schmahmann's syndrome) .Data from neuroimaging and neuromodulation/neurostimulation studies suggest that cerebellar compensatory reorganization might be involved in neurodegenerative diseases affecting cognition, e.g. Alzheimer's Disease and Frontotemporal Dementia[16] .Such compensatory cerebellar changes are expected to be more prominent as clinical cognitive and behavioural impairment become more severe [25] .Therefore, the negative correlation between the medial frontal cluster and the cerebellum that we found in the IRCA might reflect cerebellar involvement in compensation to frontal cognitive damage.

Our study has some limitations. First, we did not include in the analysis proxies of CR other than education. Indeed, occupation, leisure activities, and social engagement have been reported to positively modulate CR [14,15,45,46,47,48, 50,56, 59] .Nevertheless, education has been robustly proven to be a valid, independent proxy of CR in cognitive disorders[12,14,18,29,33,60]). Second, we estimated the level of cognitive impairment through a single tool, i.e. ECAS. Nevertheless, ECAS has several advantages: it has been designed specifically to assess cognitive performance in ALS patients and to quantify the severity and the nature of the deficits; it encompasses multiple ALS specific (Language, Verbal Fluency, Executive Functions) and non-ALS specific cognitive domains (Verbal Memory and Visuospatial Abilities); it also includes a brief carer interview to check for the presence of behavioral changes based on FTD diagnostic criteria; it has high sensitivity and specificity to impairment characteristic of ALS; it accommodates for both limb- and bulbar-related motor disability, as it can be administered in a spoken or written form; it is suitable for use in a clinical setting, since it is not time consuming (15-20 minutes) and can be administered by healthcare professionals other than neuropsychologists, such as doctors or nurse specialists [31,36];it is widely used and has been validated in different languages [22, 31,36,38,43, 51] Ye et al., 2016). Third, MRI scans were not available for all subjects. Therefore, we could not evaluate whether the analysis of gray matter changes in our study sample would have shown similar results. Indeed, we could not perform partial volume effect (PVE) correction for cortical atrophy. Notably, studies employing voxel-based atrophy correction of resting glucose metabolism showed that metabolic measurements were relatively independent of brain atrophy [17] .Nevertheless, we acknowledge that the lacking of PVE correction is a limitation of the study.

Conclusion

To our knowledge, this is the first neuroimaging study providing data in favor of a role of CR in the field of cognitive impairment associated with ALS. Based on our findings, we suggest that ALS patients with higher education level, which is a proxy of CR, can cope better with brain pathology. The study of reserve mechanisms is of outstanding importance because it might pave the way towards prevention strategies and rehabilitation protocols based on cognitive stimulation [34].Furthermore, the study of the underlying molecular mechanisms might suggest potential targets for enviromimetics drugs, namely therapeutic agents aiming to mimic and/or enhance the effects of environmental stimulation[30] .Nevertheless, our understanding of the strengths and weaknesses of the different experimental models has to improve before we are able to achieve a successful translational research in this field [34].The challenge of tackling cognitive impairment associated with ALS must be pursued, since neurobehavioral dysfunction has a negative effect on ALS outcome [7], and a negative impact on caregivers' burden and quality of life [8], even more than physical disability[21] .

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Differential neuropsychological profile of ALS patients with and without *C9orf72* mutation

Iazzolino B, Peotta L, Zucchetti JP, Canosa A, Manera U, Vasta R, Grassano M, Palumbo F, Brunetti M, Barberis M, Sbaiz L, Moglia C, Calvo A, Chiò A.

Abstract

Objective. To identify the different neuropsychological profiles of patients with (ALSC9+) and without (ALSC9-) C9orf72 expansion.

Methods. The study population includes 741 ALS patients who were consecutively diagnosed at the Turin ALS expert center in the period 2010-2018 and who underwent both cognitive/behavioral and genetic testing. Patients' neuropsychological patterns were compared (a) at the same degree of cognitive and behavioral deficit according to the revised ALS-FTD Consensus Criteria; and (b) at the same level of motor impairment according to the King's staging system.

Results. ALSC9+ patients had significant lower scores in tests exploring executive functions, and verbal memory both when classified as cognitively normal and when diagnosed in the intermediate cognitive categories. Considering the clinical perspective, ALSC9+ patients showed significantly lower scores compared to ALSC9- patients at King's stage 1 and 3 in almost all the examined neuropsychological domains, while at King's stage 2 ALSC9+ patients were more severely affected only in the verbal memory domain. Behavioral function was comparably impaired in the two cohorts, while anxiety and depression were extremely rare in ALSC9+ patients.

Conclusions. ALSC9+ patients show a different neuropsychological profile compared to ALSC9- ones, being more impaired in executive functions and verbal memory domains at all King's stages. Verbal memory emerged as a particularly vulnerable function in ALSC9+, with worse performances even when patients were still classified as cognitively normal. Both anxiety and depression were comparatively much less frequent in ALSC9+ patients, pointing to a possible impairment of their emotional insight.

Introduction

Amyotrophic lateral sclerosis (ALS) is a multisystem neurodegenerative disease involving motor and cognitive functions, due to the progressive degeneration of neurons of motor and frontal cortices and of bulbar and spinal motor nuclei. Six to 10 percent of ALS patients carry a GGGGCC pathological expansion in the first intron of the *C9orf72* gene.[1] About 15% of ALS patients at diagnosis show a cognitive impairment than can be classified as frontotemporal dementia (ALS-FTD), while another 35% has an intermediate level of cognitive and behavioural impairment.[2,3] Intermediate impairment has been recently classified as isolate executive dysfunction (ALSci); isolated behavioural impairment (ALSbi); and cognitive and behavioral dysfunction who fulfil criteria for both ALSci and ALSbi (ALScbi).[4] The remaining patients are cognitively normal (ALS-CN).

Previous papers have indicated that patients carrying *C9orf72* pathological expansion are more likely to present a cognitive and behavioral impairment than those who are not mutated.[5-7] However, very few is known about the specific cognitive domains that are differentially involved in patients with (ALSC9+) or without (ALSC9-) *C9orf72* expansion.[8]

The aim of this study was to determine whether ALS patients with *C9orf72* expansion showed a different impairment of cognitive and behavioural domains compared to patients without *C9orf72* expansion (a) at the same degree of cognitive and behavioral deficit, classified according to the revised ALS-FTD Consensus Criteria;[4] and (b) at the same level of motor impairment, classified according to King's staging.[9]

Methods

The study population includes all ALS patients who were consecutively seen at the Turin ALS expert center in the period 2010-2018 and who underwent both cognitive/behavioral and genetic testing. Patients were diagnosed according to El Escorial revised criteria.[10]All patients were evaluated with the ALS Functional Rating Scale revised (ALSFRS-R) at time of cognitive testing.

Patients with history of neurological disorders affecting cognition (major stroke, severe head injuries, mental retardation), alcohol-dependence and drug-dependence, severe mental illness, and use of high-dose psychoactive medications were tested but not included in data analysis. Patients who were not of Italian mother tongue were assessed only through an unstructured interview and therefore were excluded from the analysis. A total of 129 population-based controls were also tested with the same battery.

Neuropsychological battery*.*

ALS patients underwent a battery of neuropsychological tests encompassing executive function, memory, visuospatial function, and language, selected according to the Diagnostic Criteria for the Behavioural variant of Frontotemporal Dementia,[11] and ALS-FTD Consensus Criteria.[4] Patients underwent the following neuropsychological battery: Letter Fluency test (FAS); Category Fluency Test (CAT); Frontal Assessment Battery (FAB); Trail Making Test (TMT) A, B and B-A; Rey-Osterrieth Complex Figure Test (ROCF), immediate (IR) and differed recall (DR); Rey Auditory Verbal Learning Test (RAVL), immediate (IR) and differed recall (DR); Babcock Story Recall Test (BSRT), immediate (IR) and differed recall (DR); Digit Span Forward and Backward; Raven's Colored Progressive Matrices (CPM47); Mini Mental State Examination (MMSE). The raw scores of all tests were age-,

sex-, and education-corrected using the more recent Italian normative (for reference see Supplementary Table 1).

In selected cases, according to the judgment of the neuropsychologist, supplementary tasks were administered for a comprehensive evaluation of language; the following tests were used: semantic systems tests (7 and 8) of the Battery for the Analysis of Aphasic Deficits and the Token test. However, since these test were not performed in all patients, they results are not reported.

Neurobehavioral dysfunction was determined with the Frontal Systems Behaviour Scale (FrSBe), using the Family-form evaluated by a close relative/caregiver (scores: normal ≤59, borderline 60-64; pathological ≥65). For the purpose of this study, we considered the change in points for each of the 3 domains of FrSBe (apathy, disinhibition, executive) from the before disease to the disease scores. If a subject had scores reflecting a frontal systems abnormality both in the premorbid and in the post-illness forms, he/she was considered pathological only if there was an increase of ≥10 points at the T-score between the two forms.[3]

Anxiety and depression were assessed with the Hospital Anxiety and Depression Scale (HADS); the item "I feel slowed down" was discussed with patients in order to have him/her not to refer to physical disability.[3]

Domain classification.

Tests were classified according the main neuropsychological domain they assess (Table 1).[4,12-14] A domain was considered impaired if at least one of the tests exploring that domain had a score under the normative cut-off, with the exception of executive functions and verbal memory, which were considered impaired if at least two not overlapping tests had a score under the cut-off.[4]

Table 1. Cognitive domains explored

Domains	**Tests**
Executive functions	Letter Fluency test (FAS)
	Category Fluency Test (CAT)
	Trail Making Test B-A (TMT B-A)

	Frontal Assessment Battery (FAB)
Verbal memory	Rey Auditory Verbal Learning Test , Immediate Recall (RAVL-IR)
	Rey Auditory Verbal Learning Test, delayed Recall (RAVL-DR)
	Babcock Story Recall Test , Immediate Recall (BSRT-IR)
	Babcock Story Recall Test, Delayed Recall (BSRT-DR)
Visual Memory	Rey-Osterrieth Complex Figure Test, differed recall (ROCF-DR)
Visuoconstructive abilities	Rey-Osterrieth Complex Figure Test, Immediate Recall (ROCF-IR)
	Clock Drawing Test (Clock)
Attention/working memory	Digit Span Forward (FW)
	Digit Span Backward (BW)
Psychomotor speed	Trail Making Test A (TMT A)
Fluid intelligence	Raven's Colored Progressive Matrices (CPM47)
Cognitive flexibility	Trail Making Test B (TMT B)
Non-ALS	Mini Mental State Examination (MMSE)

Classification of cognitive phenotypes.

Patients were classified according to the consensus criteria for the diagnosis of frontotemporal cognitive and behavioral syndromes in ALS.[4] We considered five categories: ALS-CN, ALSci, ALSbi, ALScbi and ALS co-morbid with frontotemporal dementia (ALS-FTD). For the aims of this study, intermediate cognitive categories (ALSbi, ALSci and ALScbi) were merged.

ALS Staging.

Patients' motor impairment was classified according to the King's staging system. King's staging is based on the spreading of motor symptoms in three different body regions (bulbar, upper limbs, and lower limbs), and on the use of non-invasive ventilation (NIV) and enteral nutrition.[9] The five stages of the King's staging are: 1, one region involved; 2, two regions involved; 3 three regions involved; 4A, patient needs gastrostomy; 4B, patient needs non-invasive ventilation. The stage can be derived from the direct observation of the patients and also from the ALSFRS-R scale.[15] For the purpose of this study, we considered only stages 1 to 3, since stages 4A and 4B are not anatomical but functional and thus do not necessarily correspond to the spreading of the anatomical lesions.

Genetic testing.

All the coding exons and 50 bp of the flanking intron-exon boundaries of SOD1, of exon 6 of *TARDBP*, and of exons 14 and 15 of *FUS* have been PCR amplified, sequenced using the Big-Dye Terminator v3.1 sequencing kit (Applied Biosystems Inc.), and run on an ABIPrism 3130 genetic analyzer. These exons were selected as the vast majority of known pathogenic variants are known to lie within these mutational hotspots. A repeat-primed PCR assay was used to screen for the presence of the GGGGCC hexanucleotide expansion in the first intron of *C9orf72*. A cut-off of ≥30 repeats was considered pathological.[16]

Statistical analysis.

Comparisons were performed on age-, sex-, and education-corrected scores. Since all cognitive tests had not a normal distribution, the Mann-Whitney U test was used for comparisons. Data were analyzed in subsequent steps. First, we compared the results of cognitive tests of ALSC9+ vs ALSC9- patients for each cognitive and behavioral level, merging the intermediate cognitive categories (ALS-CN; ALSci/ALSbi/ALScbi; ALS-FTD). Second, we compared the results of cognitive tests of ALSC9+ vs ALSC9- patients for each King's stage, independently from their level of

cognitive impairment. Third, we grouped the tests according to their domain and compared the number of ALSC9+ vs. ALSC9- cases who showed an impairment in each domain.

Two-tailed p-values are reported; Holmes correction for multiple testing was used. Effect sizes were also calculated (Supplementary Table 2).[17] All analyses were performed with SPSS 26.0 statistical package (SPSS, Chicago, IL, USA).

Ethical considerations.

The study was approved by the Ethical Committee of the ALS center (Comitato Etico Azienda Ospedaliero-Universitaria Città della Salute e della Scienza, Torino). All patients provided written informed consent before enrollment. The databases were anonymized according to the Italian law for the protection of privacy.

Data Availability Statement.

Data will be available upon request by interested researchers.

Results

In the period January 1st, 2010 to December 31st, 2018, a total of 853 ALS patients underwent neuropsychological examination. Of these, 28 were not tested for genetic mutations and are therefore excluded from the present study. Also, 24 patients with *TARDBP* mutations, 12 with *SOD1* mutations, and 5 with *FUS* mutations were excluded. Of the remaining 784 patients, 68 carried a *C9orf72* expansion (ALSC9+) and 716 (ALSC9-) did not carry any mutation of the four examined genes. All analyses were performed excluding 43 patients at King's stages 4A and 4B, who were all ALSC9-. Therefore, the final study population included 68 ALSC9+ patients and 673 ALSC9- patients.

Patients and controls did not differ for the main demographic variables (Supplementary Table 3). A comparison of the cognitive tests results in the whole series of patients (ALSC9+ and ALSC9-) and controls is reported in the Supplementary Table 4. In addition, a comparison of controls with ALS-CN patients with and without *C9orf72* expansion is reported in Supplementary Table 5.

Differences between ALSC9+ and ALSC9- patients according to their degree of cognitive and behavioral impairment

The clinical and demographic characteristics of ALSC9+ and ALSC9- patients according to the level of cognitive and behavioral impairment are reported in Table 2. ALSC9+ patients were younger than ALSC9- at all levels of cognitive impairment but did not show any other significant difference. The median time from diagnosis to cognitive testing was always lower than 3 months. Both the median upper limbs ALSFRS-R score (items 4 + 5) and the median bulbar score ALSFRS-R score (items 1 + 2 + 3) at each level of cognitive impairment were similar between ALSC9+ and ALSC9- patients.

Table 2. Clinical and demographic characteristics of ALS patients with (ALSC9+) and without (ALSC9-) *C9orf72* expansion according to the degree of cognitive impairment

	ALS-CN			**ALSbi/ALSci/ALScbi**			**ALS-FTD**		
	ALSC9+ (n=30)	**ALSC9- (n=400)**	***p***	**ALSC9+ (n=24)**	**ALSC9- (n=225)**	***p***	**ALSC9+ (n=14)**	**ALSC9- (n=48)**	*p*
Median age at onset (IQR), years	55.4 (50.4-62.4)	63.8 (55.2-70.7)	*0.0001*	58.9 (50.4-66.2)	67.7 (60.3-74.6)	*0.001*	62.5 (59.8-71.0)	73.8 (69.2-77.9)	*0.001*
Median age at test (IQR), years	55.8 (51.3-62.7)	65.5 (56.7-72.3)	*0.0001*	61.3 (50.9-68.2)	69.4 (62.7-76.0)	*0.0001*	65.5 (61.0-71.9)	75.7 (70.4-79.9)	*0.001*
Median time from	1.9 (1.2-	2.6 (1.6-	*0.171*	2.9 (1.8-	2.6 (1.5-	*0.484*	1.7 (1.0-	2.3 (1.7-	*0.378*

diagnosis to test (IQR), months	3.8)	5.0)		5.1)	4.9)		4.9)	4.9)	
Median education (IQR), years	8 (8-13)	8 (8-13)	*0.577*	8 (5-12)	8 (5-13)	*0.984*	5 (5-8)	5 (5-8)	*0.436*
Sex (female) (%)	17 (56.7%)	165 (41.2%)	*0.145*	10 (41.7%)	99 (44.0%)	*0.998*	8 (57.1%)	24 (50.0%)	*0.868*
Onset (bulbar) (%)	9 (30.0%)	106 (26.5%)	*0.838*	14 (58.3%)	73 (32.4%)	*0.02*	7 (46.7%)	33 (68.6%)	*0.331*
Bulbar signs at time of test (%)	15 (50.0%)	199 (49.8%)	*0.979*	16 (66.7%)	143 (63.6%)	*0.937*	11 (78.6%)	39 (81.2%)	*0.823*
Median ALSFRS-R score at time of test (IQR)	42 (40-45)	42 (38-45)	*0.482*	40 (38-42)	40 (36-43)	*0.542*	37 (29-41)	38 (30-42)	*0.443*
Median	7 (5-8)	7 (6-8)	*0.685*	6 (5-8)	6 (5-8)	*0.578*	6 (5-8)	6 (5-8)	*0.850*

ALSFRS-R upper limbs score (items 4 + 5)									
Median ALSFRS-R bulbar score (items 1 + 2 + 3)	11 (10-12)	11 (9-12)	*0.832*	10 (8-12)	10 (9-12)	*0.781*	9 (7-11)	9 (6-11)	*0.628*
Median ALSFRS-R decline /month (IQR)	0.57 (0.36-0.81)	0.46 (0.24-0.84)	*0.336*	0.66 (0.34-0.99)	0.56 (0.30-0.95)	*0.03*	0.78 (0.47-1.28)	0.64 (0.34-1.15)	*0.01*
Median FVC% at test (IQR)	95 (86-107)	97 (84-108)	*0.729*	96 (84-107)	94 (78-108)	*0.536*	84 (57-102)	87 (54-101)	*0.947*

The median scores of each test according to genetic status and degree of cognitive impairment are reported in Table 3. Among ALS-CN patients, ALSC9+ had significantly lower scores in tests exploring executive functions (FAS, CAT, TMT B-A) and verbal memory (RAVL-IR, RAVL–DR). Among patients with intermediate cognitive impairment, ALSC9+ patients had significantly lower scores in

tests exploring executive functions (FAS, CAT), attention and working memory (Digit Span FW) and verbal memory (RAVL-IR, RAVL-DR). Finally, there were no differences between ALSC9+ and ALSC9- patients classified as ALS-FTD.

Table 3. Median values (interquartile range) of age-, and education-corrected scores of cognitive tests in ALS patient with (ALSC9+) and without (ALSC9-) *C9orf72* expansion, according to level of cognitive impairment. P values are calculated with Mann-Whitney U test

	ALS-CN			**ALSci/ALSbi/ALScbi**			**ALS-FTD**		
	ALSC9+ N=30	**ALSC9- N=400**	**P**	**ALSC9+ N=24**	**ALSC9- N=225**	**p**	**ALSC9+ N=14**	**ALSC9- N=48**	p
MMSE	28.6 (27.3-30.0) N=30	28.5 (27.3-30.0) N=391	0.969	26.6 (26.0-27.9) N=23	27.3 (26.0-29.3) N=238	0.117	22.7 (20.9-26.4) N=12	23.0 (20.7-25.7) N=48	0.695
FAS	30.1 (25.1-33.3) N=29	32.5 (26.2-38.7) N=393	**0.042**	17.3 (12.0-29.6) N=23	25.4 (18.4-32.0) N=221	**0.03**	17.9 (12.3-24.9) N=11	17.6 (14.6-22.4) N=39	0.752
CAT	17.8 (14.8-20.5) N=27	20.3 (17.0-23.0) N=387	**0.044**	14.8 (12.5-18.0) N=23	18.0 (14.7-21.0) N=217	**0.026**	9.2 (9.0-13.0) N=10	13.3 (11.3-14.7) N=34	0.058
FAB	16.1 (14.4-16.7) N=29	15.9 (14.8-17.4) N=346	0.474	13.5 (11.5-14.7) N=21	13.5 (12.0-15.2) N=223	0.473	11.9 (9.5-12.5) N=11	9.8 (8.2-11.9) N=48	0.190
Digit Span FW	5.6 (4.9-6.2) N=28	5.8 (5.0-6.4) N=352	0.179	4.8 (4.5-5.1) N=22	5.5 (5.0-6.0) N=212	**0.001**	4.6 (4.3-5.0) N=11	4.8 (4.2-5.7) N=45	0.416

Digit Span BW	4.0 (3.6-5.0) N=19	4.0 (3.6-4.7) N=298	0.969	3.6 (2.9-3.9) N=21	3.7 (3.1-4.3) N=193	0.109	3.5 (3.3-3.8) N=9	2.9 (2.7-3.7) N=34	0.173
TMT A	32 (26-43) N=28	33 (22-44) N=359	0.832	43 (31-77) N=22	46 (33-73) N=215	0.996	78 (54-108) N=10	103 (65-153) N=44	0.102
TMT B	77 (45-115) N=28	60 (34-91) N=359	0.066	183 (136-259) N=21	129 (63-263) N=212	0.201	295 (193-315) N=10	334 (278-288) N=40	0.111
TMT B-A	39 (20-81) N=28	27 (7-56) N=359	**0.039**	126 (81-155) N=21	79 (31-168) N=212	0.188	196 (156-217) N=10	188 (142244) N=40	0.946
Clock *	5 (4-5) N=30	5 (4-5) N=335	0.815	4 (3-5) N=20	4 (3-5) N=203	0.838	3 (2-3) N=8	3 (2-3) N=48	0.46
RAVL-IR	38.7 (31.7-42.3) N=15	43.3 (37.3-51.1) N=210	**0.001**	29.3 (25.3-37.0) N=14	37.2 (32.0-44.3) N=134	**0.017**	23.6 (21.0-40.0) N=6	30.2 (25.0-36.0) N=21	0.976
RAVL-DR	7.7 (5.2-8.9) N=15	9.0 (7.2-111.5) N=210	**0.002**	5.2 (4.7-7.4) N=14	7.7 (6.1-9.6) N=134	**0.047**	4.0 (1.8-12.1) N=6	4.6 (3.2-7.6) N=21	0.574
BSRT-IR	5.5 (4.6-6.8) N=16	6.3 (4.8-7.1) N=227	0.442	5.3 (3.5-6.2) N=14	5.6 (4.5-6.7) N=129	0.281	4.7 (4.7-5.1) N=5	3.5 (1.6-5.7) N=22	0.537
BSRT-DR	6.6 (5.0-7.4) N=15	7.0 (5.5-8.0) N=227	0.354	6.2 (4.7-7.4) N=13	6.0 (4.7-7.4) N=127	0.225	3.5 (2.9-5.9) N=5	3.5 (2.0-4.7) N=22	0.871
ROCF-IR	31.1 (30.3-	32.5 (30.5-	0.407	29.8 (23.5-	29.8 (23.3-	0.975	14.7 (11.5-	16.2 (7.0-	0.470

	34.3) N=20	34.7) N=292		33.3) N=18	33.0) N=183		27.2) N=8	25.3) N=35	
ROCF-DR	12.7 (8.5-13.7) N=20	12.8 (9.7-17.4) N=292	0.313	9.3 (6.1-11.5) N=18	10.3 (6.8-14.0) N=183	0.476	8.5 (3.8-9.5) N=8	8.0 (3.0-9.8) N=33	0.758
CPM47	29.8 (26.8-32.0) N=29	30.3 (27.3-32.5) N=377	0.180	24.2 (19.1-29.5) N=23	26.2 (21.5-29.5) N=223	0.292	18.5 (15.0-27.3) N=11	20.0 (17.2-26.5) N=48	0.965
HADS-A	6 (4-8) N=25	7 (5-10) N=317	0.232	6 (5-9) N=19	7 (5-10) N=191	0.394	5 (3-7) N=9	6 (2-11) N=28	0.550
HADS-D	4 (2-6) N=25	5 (2-7) N=317	0.212	4 (1-7) N=19	5 (3-8) N=191	0.835	6 (4-9) N=9	6 (3-9) N=28	0.842

Differences between ALSC9+ and ALSC9- patients according to King's stage

In Table 4 the clinical and demographic characteristics of ALSC9+ and ALSC9- patients according to King's stage are reported. As expected, ALSC9+ were more frequently cognitively impaired than ALSC9- according to Strong's classification, with 38 (55.9%) vs 271 (40.6%) ($p<0.001$) cases respectively. ALSC9+ were younger than ALSC9- ($p<0.001$) but did no differ for other clinical and demographic characteristics. In particular, the median time from diagnosis to neuropsychological testing was less than 3 months at all King's stages. With increasing King's stage, the median monthly decline of ALSFRS-R from onset to the time of cognitive evaluation significantly increased but was always lower in ALSC9- patients than in ALSC9+ ones. Both the median upper limbs ALSFRS-R score and the median bulbar score ALSFRS-R score at each King's stage were similar between ALSC9+ and ALSC9- patients.

Table 4. Clinical and demographic of ALS patients with (ALSC9+) and without (ALSC9-) *C9orf72* expansion according to King's stage.

	King's 1			**King's 2**			**King's 3**		
	ALSC9+ (n=34)	**ALSC9- (n=287)**	***p***	**ALSC9+ (n=19)**	**ALSC9- (n=195)**	***p***	**ALSC9+ (n=15)**	**ALSC9- (n=191)**	*p*
Median age at onset (IQR), years	56.4 (50.5-65.9)	65.7 (57.6-72.3)	*0.001*	58.9 (48.8-65.9)	66.1 (57.7-72.8)	*0.007*	60.4 (54.6-64.9)	67.8 (57.7-75.7)	*0.04*
Median age at test (IQR), years	57.4 (51.7-67.3)	67.0 (60.0-73.5)	*0.001*	60.4 (49.6-66.5)	67.7 (58.9-73.7)	*0.006*	61.0 (55.2-67.6)	69.2 (59.6-76.5)	*0.023*
Median time from diagnosis to test (IQR), months	2.7 (1.1-4.5)	2.3 (1.4-4.2)	*0.956*	2.4 (1.6-4.5)	2.8 (1.7-4.5)	*0.714*	2.3 (1.2-3.5)	2.7 (1.7-4.6)	*0.117*
Median education (IQR), years	8 (8-12)	8 (5-13)	*0.234*	8 (5-13)	8 (5-13)	*0.905*	8 (5-13)	8 (5-13)	*0.649*
Sex (female) (%)	16 (47.1%)	114 (40.1%)	*0.438*	9 (47.4%)	89 (45.9%)	*0.901*	10 (66.7%)	85 (44.2%)	*0.165*
Onset (bulbar) (%)	12 (35.3%)	102 (35.6%)	*0.977*	8 (42.1%)	43 (22.2%)	*0.05*	10 (66.7%)	67 (35.3%)	*0.016*

Bulbar signs at time of test (%)	15 (44.1%)	110 (38.4%)	*0.639*	12 (63.2%)	84 (43.3%)	*0.097*	15 (100%)	191 (100%)	*1.00*
Median ALSFRS-R score at time of test (IQR)	44 (42-46)	45 (42-46)	*0.762*	40 (37-42)	40 (37-42)	*0.887*	35 (30-41	35 (30-38)	*0.506*
Median ALSFRS-R upper limbs score (items 4 + 5)	8 (7-8)	8 (7-8)	*0.397*	7 (5-7)	6 (5-7)	*0.663*	6 (4-6)	5 (4-6)	*0.534*
Median ALSFRS-R bulbar score (items 1 + 2 + 3)	12 (10-12)	12 (10-12)	*0.869*	10 (8-12)	12 (10-12)	*0.039*	8 (7-10)	9 (8-10)	*0.369*
Median ALSFRS-R decline /month (IQR)	0.35 (0.18-0.57)	0.50 (0.18-0.50)	*0.513*	0.68 (0.40-.95)	0.61 (0.42-1.00)	*0.886*	0.94 (0.54-2.61)	0.92 (0.55-1.48)	*0.235*
Median	96 (86-	99 (86-	*0.665*	96 (87-	96 (82-	*0.752*	81 (58-	88 (71-	*0.133*

FVC% at test (IQR)	107)	110)		108)	110)		97)	103)	

The median scores of each performed tests according to genetic status and King's stage are reported in Table 5. At King's stage 1 ALSC9+ were more severely affected than ALSC9- in test exploring executive function (FAS, CAT, FAB, TMT B-A), verbal memory (RAVL-IR, RAVL-DL), attention and working memory (Digit span FW and BW), cognitive flexibility (TMT B) and in MMSE. At King's stage 2, ALSC9+ were more severely affected than ALSC9- only in one test exploring verbal memory (BSRT-IR). At King's stage 3, ALSC9+ were more severely affected in tests exploring executive functions (FAS, CAT, TMT B-A), verbal memory (RAVL-IR), attention and working memory (Digit span FW) and cognitive flexibility (TMT B).

Table 5. Median values (interquartile range) of age-, sex- and education-corrected scores of cognitive tests in ALS patient with (ALSC9+) and without (ALSC9-) C9orf72 expansion, according to King's stages. P values are calculated with Mann-Whitney U test 672

	King's 1			**King's 2**			**King's 3**		
	ALSC9+ N=34	ALSC9- N=287	p	ALSC9+ N=19	ALSC9- N=195	p	ALSC9+ N=15	ALSC9- N=191	p
MMSE	27.3 (26.4-28.6) N=32	28.3 (27.0-30.0) N=283	**0.013**	27.9 (26.9-30.0) N=18	28.0 (26.7-30.0) N=191	0.869	27.0 (23.9-28.3) N=15	27.3 (26.0-29.0) N=189	0.322
FAS	25.3 (17.9-31.8) N=31	31.4 (23.9-38.0) N=283	**0.001**	19.5 (13.2-24.3) N=18	28.9 (22.6-35.0) N=187	0.468	14.6 (11.1-24.9) N=14	26.4 (20.6-33.4) N=180	**0.002**
CAT	15.0 (13.0-18.0) N=30	19.5 (16.0-22.3) N=279	**0.001**	16.3 (12.3-20.7) N=16	19.8 (16.3-22.0) N=185	0.189	9.5 (9.0-14.8) N=14	18.0 (14.5-21.5) N=174	**0.001**

FAB	14.1 (12.2-15.5) N=32	15.3 (14.0-16.9) N=241	**0.011**	14.9 (13.3-16.1) N=16	15.3 (13.4-16.7) N=170	0.563	13.5 (11.7-15.0) N=13	14.5 (12.5-16.2) N=171	0.422
Digit Span FW	5.0 (4.8-5.8) N=30	5.8 (5.0-6.4) N=269	**0.002**	5.0 (4.3-5.8) N=16	5.5 (5.0-6.3) N=169	0.102	4.6 (3.9-5.3) N=15	5.5 (5.0-6.0) N=157	**0.002**
Digit Span BW	3.8 (3.0-4.0) N=23	4.0 (3.5-4.6) N=228	**0.037**	3.6 (3.3-3.9) N=13	3.9 (3.4-4.5) N=144	0.125	3.6 (3.3-4.2) N=13	3.8 (3.2-4.3) N=138	0.918
TMT A	37 (30-53) N=30	34 (25-50) N=267	0.444	41 (29-58) N=15	36 (23-54) N=177	0.856	69 (43-108) N=15	45 (31-73) N=158	0.386
TMT B	89 (58-174) N=29	62 (37-116) N=263	**0.015**	90 (66-165) N=15	80 (38-150) N=173	0.298	220 (102-303) N=15	102 (64-214) N=156	**0.023**
TMT B-A	53 (22-126) N=28	30 (8-72) N=263	**0.01**	61 (20-122) N=15	41 (13-102) N=173	0.286	155 (74-206) N=15	63 (28-141) N=155	**0.002**
Clock *	5 (3-5) N=30	5 (3.5-5) N=242	0.42	5 (4-5) N=15	5 (4-5) N=164	0.97	3.5 (3-5) N=13	4 (3-5) N=165	0.46
RAVL-IR	29.3 (26.4-38.9) N=16	40.6 (34.0-48.5) N=147	**0.001**	37 (33.5-44.2) N=11	42.5 (34.5-49.5) N=106	0.124	32.7 (23.6-40.0) N=9	39.6 (34.0-45.1) N=104	**0.046**
RAVL-DR	5.2 (5-6.6) N=16	8.6 (6.4-10.6) N=147	**0.0001**	8.3 (5.0-9.4) N=11	8.6 (6.6-10.8) N=106	0.510	6.2 (4.7-9.3) N=9	8.0 (6.1-10.2) N=104	0.215
BSRT-IR	5.5 (4.6-6.3) N=17	5.7 (4.5-6.9) N=152	0.623	5.0 (4.5-6.2) N=10	6.5 (5.1-7.1) N=107	**0.025**	4.7 (3.6-5.4) N=9	5.6 (4.4-6.8) N=112	0.189
BSRT-DR	6.1 (5.0-6.8) N=15	6.3 (5.1-7.7) N=144	0.332	6.0 (4.3-7.4) N=10	7.1 (5.7-8.0) N=105	0.241	4.4 (3.5-6.9) N=9	6.5 (4.7-7.7) N=111	0.116

ROCF-IR	30.7 (18.3-32.8) N=21	31.8 (29.8-34.5) N=227	**0.05**	33.2 (30.2-34.2) N=13	31.2 (28.5-34.0) N=140	0.346	27.5 (17.7-30.0) N=12	30.8 (25.2-33.5) N=131	0.122
ROCF-DR	9.0 (6.1-12.7) N=21	11.6 (7.8-16.8) N=226	0.079	12.7 (9.5-14.2) N=13	11.8 (9-15.5) N=140	0.786	8.5 (5.5-10.5) N=12	11.5 (8.0-15.0) N=131	0.076
CPM47	27.5 (24.2-31.8) N=30	29.3 (25.6-32.3) N=276	0.218	27.3 (25.3-29.5) N=18	28.8 (24.3-31.9) N=183	0.351	21.8 (18.5-31.0) N=15	27.8 (23.6-30.5) N=173	0.124
HADS-A	6 (4-10) N=26	7 (5-10) N=231	0.580	5 (5-8) N=15	7 (5-10) N=151	0.132	7 (3-9) N=11	7 (5-10) N=139	0.162
HADS-D	3 (2-6) N=26	4 (2-7) N=231	0.189	5 (2-7) N=15	5 (3-8) N=151	0.643	4 (4-7) N=11	6 (4-8) N=139	0.526

Frequency of impaired cognitive and behavioural domains in ALSC9+ vs. ALSC9- according to King's stage

In general, ALSC9+ patients had more frequently than ALSC9- at least one test under the normative cut-off in all domains across King's stages (Figure 1). However, this difference was significant only for the executive functions (p=0.004), visual memory (p=0.002), and verbal memory (p=0.03) at King's stage 1; visual memory (p=0.003) at King's stage 2; and for fluid intelligence (p=0.035), verbal memory (p=0.01) attention/working memory (p=0.031), and cognitive flexibility (p=0.04) at King's stage 3. Anxiety and depression were more frequent in ALSC9- in all King's stages. Behavioural impairment was identified with similar frequency at each stage in both cohorts. The most affected behavioral domain in both ALSC9+ and ALSC9- was apathy and its frequency significantly increased from King's stage 1 to King's stage 3 (Figure 2).

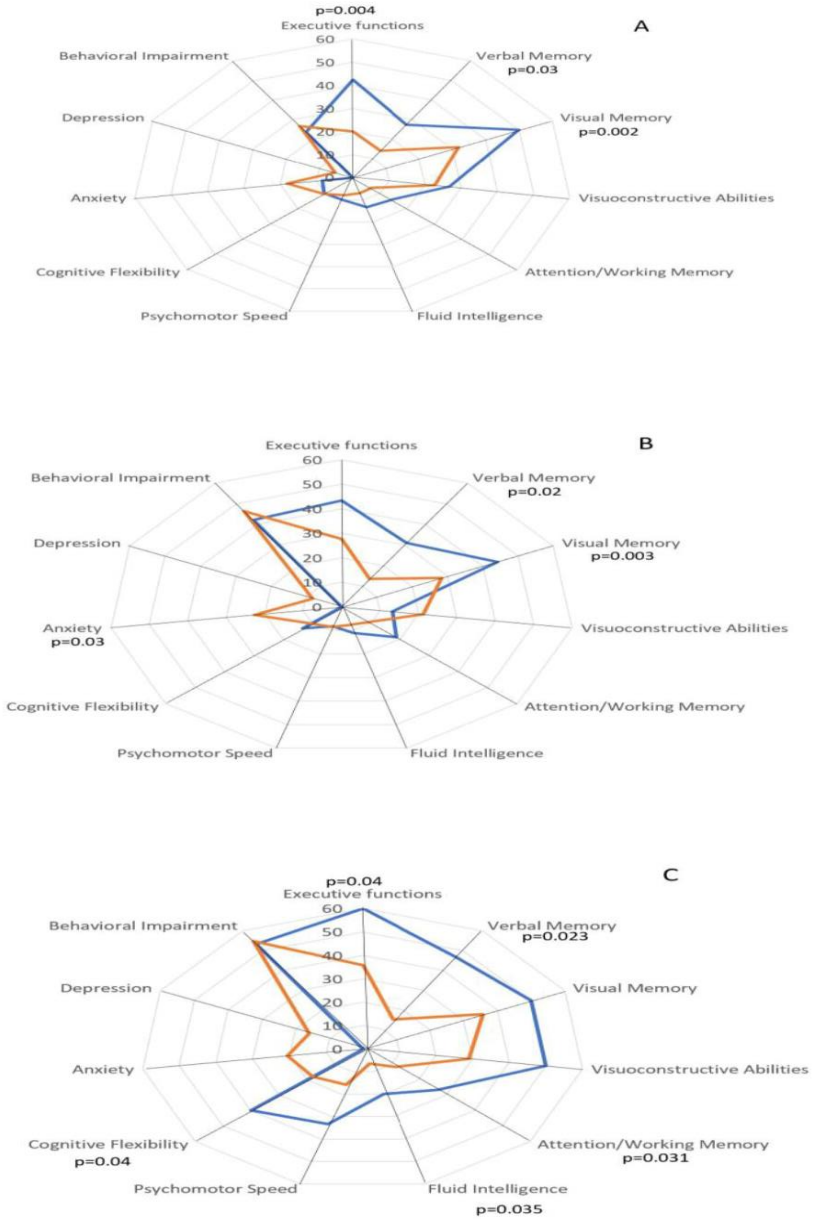


Figure 1. Frequency of impaired cognitive domains in ALSC9+ vs ALSC9- according to King's stage. ALSC9+, blue; ALSC9-, red. A, King's state 1; B, King's stage 2; C, King's stage 3.

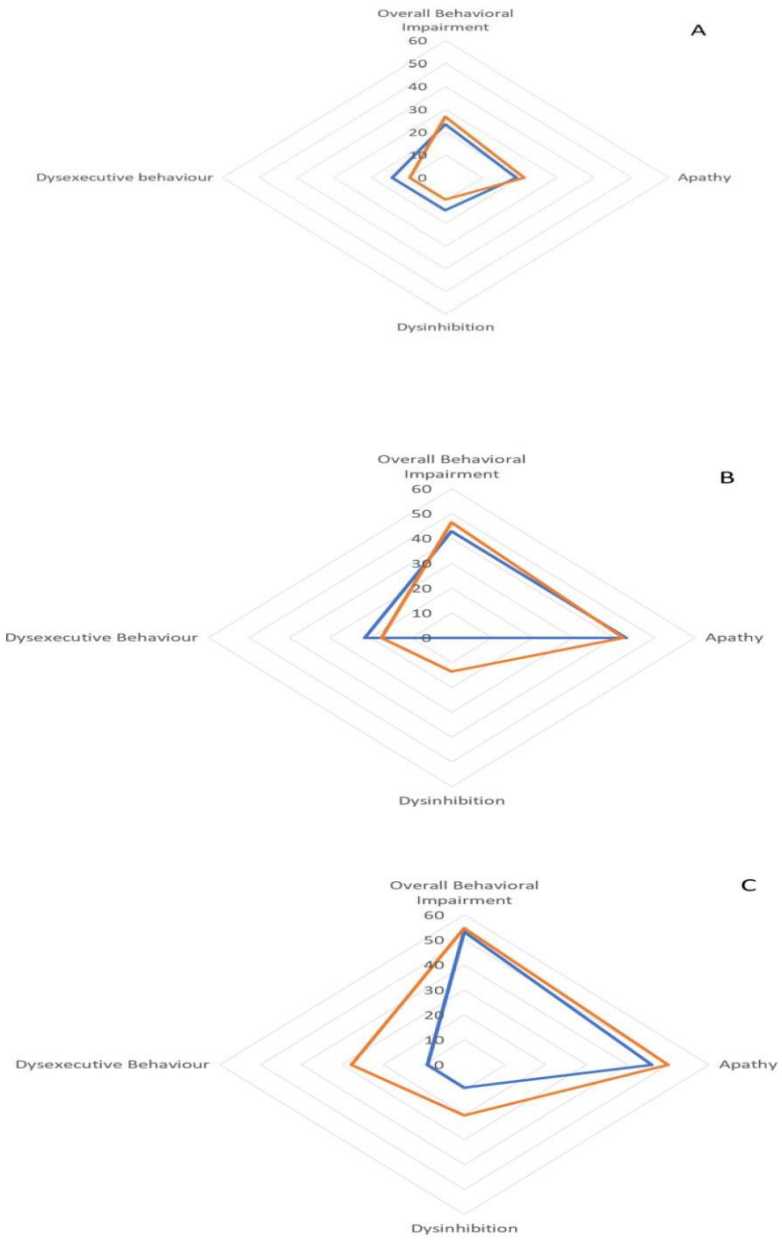


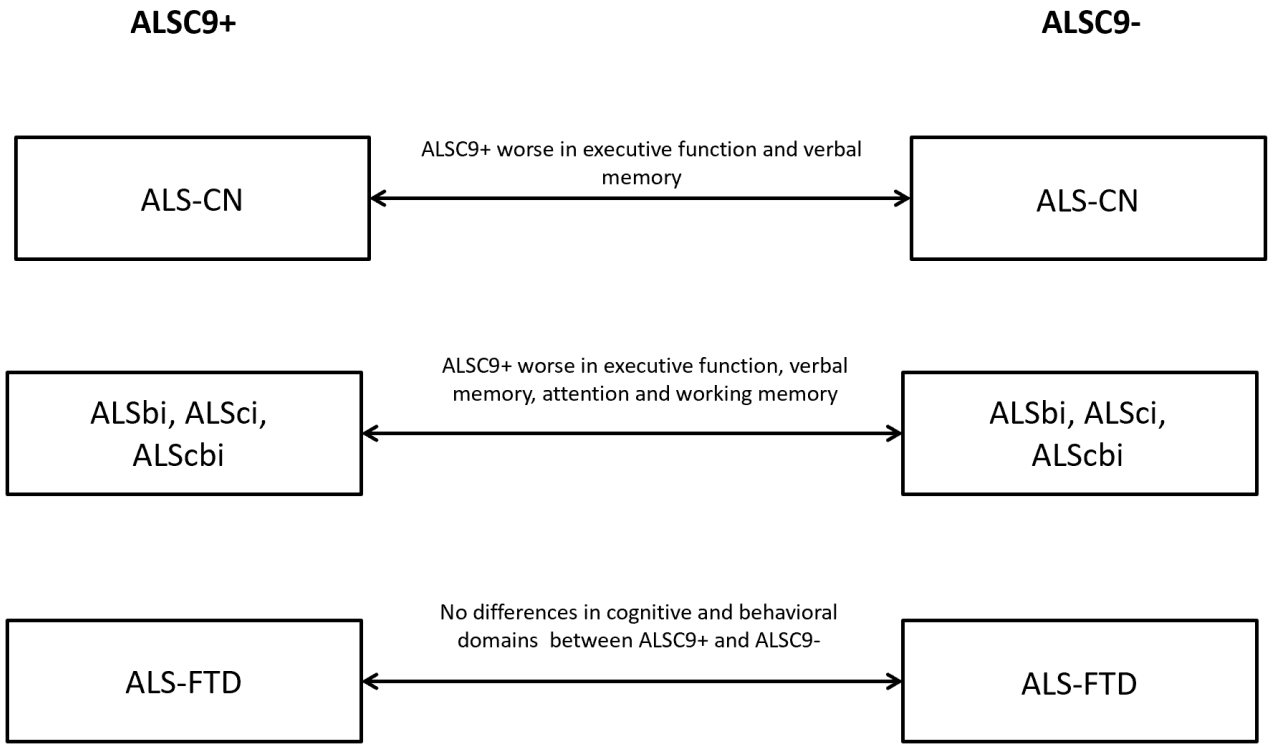
Figure 2. Frequency of impaired behavioral domains evaluated with FrSBe in ALSC9+ vs ALSC9- according to King's stage. ALSC9+, blue; ALSC9-, red. A, King's state 1; B, King's stage 2; C, King's stage 3

Discussion

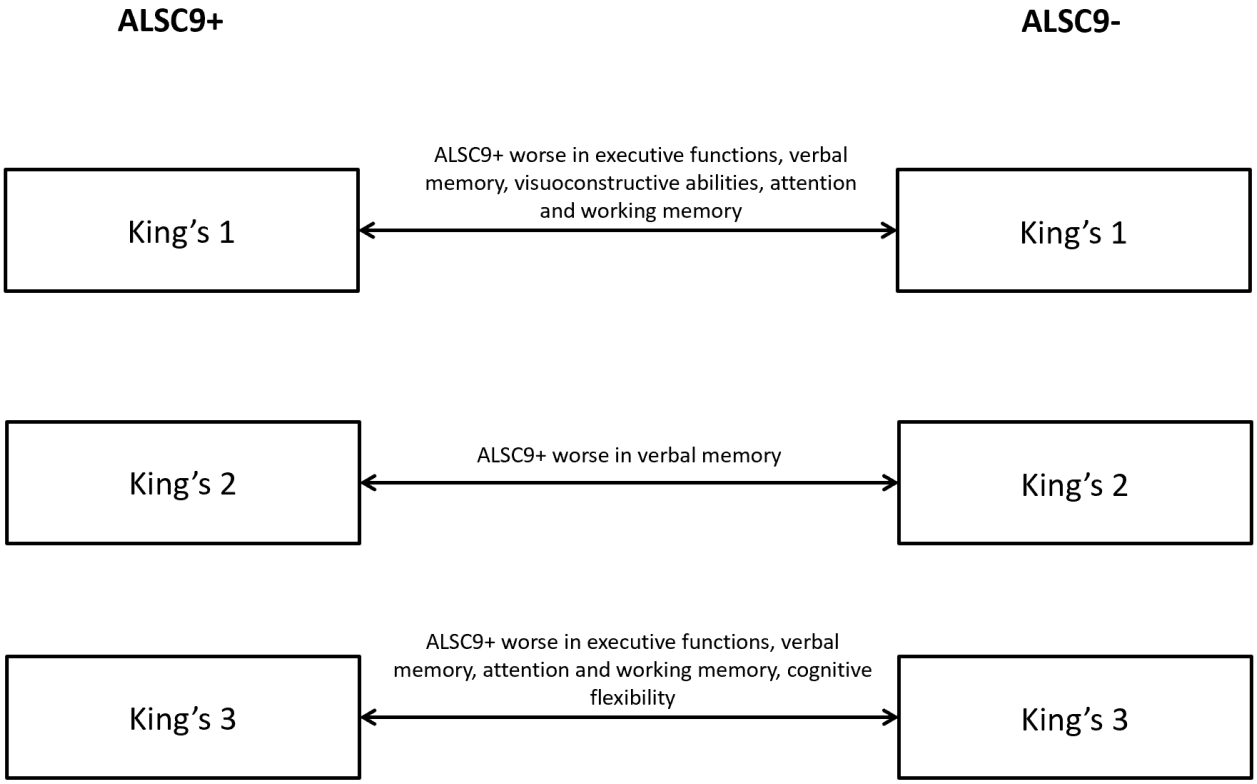
In this study we have compared the cognitive performances of a large cohort of ALS patients with *C9orf72* expansion to those of patients without genetic mutations using a comprehensive battery of cognitive and behavioural tests. In order to identify the specific features of cognitive and

behavioral profile of *C9orf72* patients we conducted two parallel analyses: first, we compared ALSC9+ and ALSC9- patients across different levels of cognitive impairment according to Strong's classification.[4] Second, we compared ALSC9+ and ALSC9- patients across different levels of motor impairment, according King's staging system.[9] A graphic summary of findings is reported in Supplementary Figures 1 and 2.

Supplementary Figure 1. Graphic representation of the cognitive differences between ALSC9+ and ALSC9- from a the cognitive/behavioral Strong's classification perspective.



Supplementary Figure 2. Graphic representation of the cognitive differences between ALSC9+ and ALSC9- from the motor impairment (King's staging) perspective.



From the perspective of the cognitive/behavioral classification, in patients who were classified as cognitively normal (ALS-CN), ALSC9+ patients had significant worse scores in the domains of executive function, and verbal memory, although no test scored under the normative cut-off. Interestingly, ALSC9+ patients who were classified as cognitively normal had significant poorer performances compared to controls in tests assessing several domains, including executive function, attention/working memory, verbal memory, visual memory and visuoconstructive abilities. It is also notable that ALSC9+ patients had a worse cognitive performance despite being more than seven years younger than ALSC9-. We have recently demonstrated that age is a strong determinant of the onset of cognitive dysfunction in ALS,[18] but the present finding suggests that the occurrence of *C9orf72* mutation influences cognition independently from patients' age.

Among patients classified in the intermediate cognitive categories (ALSci, ALSbi, and ALScbi), ALSC9+ performed worse at tests assessing executive functions, verbal memory, and attention and working memory. Lastly, no significant differences between ALSC9+ and ALSC9- patients in cognitive performances were found at the level of ALS-FTD, suggesting that at this stage of full-

blown dementia cognitive profiles are similar. We did not find any difference in behavioral domains impairment across the different levels of cognitive/behavioral classification. Overall, the feature that mostly differentiates ALSC9+ and ALSC9- across different degrees of cognitive/behavioral impairment is the worst performance of ALSC9+ at verbal memory tasks, associated to a more severe impairment in tests exploring the executive functions and visual memory.

When considering the clinical perspective, we have found that ALSC9+ patients showed significantly lower scores compared to ALSC9- patients at King's stage 1 and 3 in tests assessing most of the examined domains (executive function, attention and working memory, visual memory, verbal memory, cognitive flexibility, and visuoconstructive abilities but only for King's stage 1), whereas at King's stage 2 ALSC9+ patients were more severely affected only for only one test related to the verbal memory domain (BRST-IR). Behavioral function was similarly impaired in the two cohorts, with an increase of frequency of behavioral manifestations in more severe King's stages; the most commonly involved behavioral domain was apathy, in keeping with the concept that apathy is a key feature in ALS.[4,19] Finally, in all King's stages both anxiety and depression were more frequent in ALSC9- patients, but this difference did not reach a statistical significance.

A novel observation of our study is that verbal memory is a key cognitive feature in ALSC9+. There are no studies specifically assessing verbal memory in ALS patients with *C9orf72* expansion; two small studies performed on *C9orf72* mutated patients with pure FTD reported that the impairment of verbal and/or visual episodic memory was second in frequency only to executive dysfunction.[20,21] In our series, ALSC9+ patients have a significant worse performance than ALSC9- ones in verbal memory tests in King's stage 1 and, interestingly enough, at the ALS-CN level, thus representing a subtle pre-clinical feature of these patients. In non-mutated ALS patients verbal memory has been reported to be occasionally impaired, in particular in tests assessing immediate recall.[2,3,12,13,22,23]

A functional relationship between verbal memory and executive dysfunction has been proposed. For instance, a negative influence of executive function impairment on verbal memory has been hypothesized in Parkinson's disease[24] and amnestic mild cognitive impairment (MCI).[25] A recent paper found a correlation between immediate recall tasks and executive functions, comparing performances at episodic memory tests between ALS patients and amnestic and non-amnestic MCI patients.[23] Finally, canonical correlation analyses in 212 subjects seen for neuropsychological evaluation with different disorders indicated that the two cognitive domains shared 55-60% of

variance.[26] Similarly, in our study, the failure in verbal memory tests may be at least partially explained by executive function deficits, as poor performances in both memory and executive tasks are frequently, even not always, found associated in patients.

Another observation in our study is the very low occurrence of anxiety and depression in ALSC9+ patients, although a significant difference has been only detected for anxiety in King's stage 2. Several studies have highlighted that both depression and anxiety are relatively infrequent in ALS patients,[27-31] but there are no studies specifically devoted to assessing *C9orf72* mutated subjects. It is likely that the lower frequency of anxiety and depression in ALSC9+ patients is related to their reduced insight.[32] However, in the present study, emotional insight has not been specifically addressed. Studies on emotional processing in a *C9orf72* patients will help to better clarify if the emotional response is different in these subjects.

In our cohort we found that already at the ALS-CN level, i.e. when no formal cognitive impairment is present, ALSC9+ patients show a significant worse performance compared to ALSC9- in several neuropsychological tests assessing the executive function and verbal memory domains (FAS, CAT, TMT B-A, RAVL-IR, and RAVL-DR). This could imply in ALSC9+ patients a 'cognitive' presymptomatic/subclinical condition characterized by lower performances at specific cognitive tasks when motor symptoms are already present. In keeping with our observation, it has been reported that subtle cognitive, structural, and microstructural changes can be detected early in *C9orf72* presymptomatic carriers.[32-34]

This study is not without limitations. First, the cross-sectional design may limit our conclusions related to the clinical perspective. Nevertheless, patients were tested early after diagnosis and the cognitive impairment at that time point likely reflects the rapidity of lesion spreading within non-motor cortical areas of the brain.[35] Second, this study does not include *C9orf72* patients with pure FTD, limiting the possibility to generalize our findings to this population.[36] Third, the performance in some neuropsychological tests used in this study may be negatively impacted by patients' motor or bulbar impairment (namely FAS, CAT, and TMT) and may have influenced the cognitive classification. However, in our cohort bulbar and upper limb ALSFRS-R scores of ALSC9+ and ALSC9- patients were similar across all King's stages and all levels of cognitive/behavioral impairment, suggesting that the differences we have found in test scores cannot be ascribed solely to motor performances impairment. Fourth, in the study we could not assess social cognition, because we have included specific tests in our battery only from 2017 and therefore we had these data for relatively few patients. Social cognition is an emerging area in ALS[12,37] and its evaluation in

C9orf72 patients is undoubtedly relevant. Fifth, in the present cohort we have assessed only a subgroup of patient for language impairment, according to the judgement of the neuropsychologist. Therefore, we could not include these tests in the present study. More recently, we have added to the general battery specific tests for language.

Our data suggest that ALSC9+ patients show a different neuropsychological profile compared to ALSC9- patients. They are more impaired in cognitive functioning than ALSC9- patients, especially in the domains of executive functions, visual memory, and verbal memory, at all King's stages, and even when they are still classified as cognitively normal (i.e., when they have no neuropsychological tests under the normative cut-off). Notably, verbal memory emerged as a particularly vulnerable function in ALSC9+ patients. The involvement of behavioral function, in particular apathy, was similar in ALSC9+ and ALSC9- patients. Finally, according to our data, it is conceivable that a subclinical cognitive impairment is already present in early motor stages in some ALSC9+ patients. Longitudinal studies are necessary to clarify whether this subclinical cognitive impairment in ALSC9+ patients tends to progress over time to a clinically overt cognitive impairment.

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IV. Conclusions

The main scope of this project was to evaluate whether cognitive function deteriorates over time in ALS. In this context, we studied the possible influence of different factors on cognition, including disease staging, genetics, and education.

Moreover, in 2017 the international criteria for the diagnosis of frontotemporal spectrum disorders associated with ALS have been updated (Strong et al, 2017)[33]. Therefore, we analysed the differences between the previous and the current classification criteria and we performed a study focused on apathy, that has assumed a central role in the revised criteria to characterize behavioural impairment.

According to our data, the revised criteria lead to changes in cognitive classification as compared to the previous criteria. Most of changes are due to the introduction of the novel category of ALScbi, which accounts for approximately 10% of patients.

Based on the central role of apathy in the revised criteria, in a further study we evaluated the brain metabolic correlates of apathy in ALS patients. We used ^{18}F-2-fluoro-2-deoxy-D-glucose positron emission tomography (^{18}F-FDG-PET) to study brain metabolic changes, and Frontal Systems Behaviour Scale (FrSBe) as behavioural assessment. This scale provides "before" and "after" apathy subscores, referring to premorbid and morbid conditions. This is a relevant aspect, since the diagnostic criteria underline the relevance of the change as compared to the premorbid condition in the assessment of behavioural function in ALS. We found that apathy FrSBe "after" subscore correlated with metabolic changes in brain regions known as neuroanatomical correlates of apathy. Similar results were obtained considering the difference between the “before” and the “after” score, supporting the relevance of the gap between premorbid and morbid conditions to detect behavioural changes due to the neurodegenerative process underlying ALS.

In a previous study performed in 2015, we reported that ^{18}F-FDG-PET can enrich the information provided by neuropsychological testing of ALS patients, showing brain metabolic changes associated with the different degrees of cognitive impairment (Canosa et al , 2016)[7]. We employed a similar method to identify metabolic changes related to the various levels of cognitive deficits, classified according to revised criteria (2017). We identified frontal lobe relative hypometabolism in cognitively impaired patients that resulted more extensive and significant across the *continuum* from ALS-Ci, through ALS-Cbi, to ALS-FTD. ALS-FTD patients also showed cerebellar relative hypermetabolism. ALS-Bi patients did not show any difference compared with ALS-Cn. These data support the hypothesis that patients with cognitive impairment have a more widespread

neurodegenerative process compared with patients with a pure motor disease: the more severe the cognitive impairment, the more diffuse the metabolic changes. These results show that cognitive categories identified according to the revised Strong criteria reflect the spreading of the neurodegenerative process across cortical regions involved in cognition.

Moreover, we evaluated the trend of cognitive impairment over time in patients with ALS, since published data on this issue were relatively conflicting.

Therefore, we analysed cognitive and behavioural function in an ALS series at diagnosis and after 6-months. At the latter examination, one-third of patients showed a worsening of cognitive performance as compared to the diagnosis timepoint, namely 88% of patients classified as ALSbi at diagnosis, 27% of ALSci, 40% of ALScbi, and 24% of cognitively normal subjects. We found that patients showing deterioration over time displayed a lower ALSFRS-r score at the first examination and a shorter survival compared to those who did not show cognitive deterioration over time. Strikingly, we showed that cognitive disorders in ALS patients can develope along the disease course also in patients showing normal cognitive function at diagnosis.

We focused a further study on the association between the severity of motor impairment and that of cognitive impairment. A cross-sectional population-based cohort of incident ALS cases were classified according to the revised ALS-FTD Consensus Criteria. The King's Staging System and the Milano-Torino Staging System (MiToS) were used to rate the severity of motor impairment. Our findings suggest that ALS motor and cognitive impairments may worsen in parallel, and that cognitive impairment becomes more pronounced when bulbar function is involved. Our data support the hypothesis that ALS pathology disseminates in a regional ordered sequence, through a cortico-efferent spreading model, confirming the hypothesis that motor and cognitive impairment are two different aspects of the same underlying pathologic process.

In the context of the study of the influence of environmental factors on cognitive impairment, we explored the role of Cognitive Reserve. We considered education as reserve proxy, and we employed ^{18}F-FDG-PET to assess the level of brain damage and ECAS to measure the severity of cognitive impairment. In our sample education level was negatively correlated with brain metabolism in medial frontal regions, independently of the degree of cognitive impairment, in agreement with the Cognitive Reserve hypothesis that postulates that higher education allows to cope better with brain pathology. Our results potentially pave the way toward prevention strategies and rehabilitation protocols based on cognitive stimulation.

Moreover, also genetic determinants of cognitive phenotype have been explored. *C9orf72* expansion is known to increase the risk of FTD in association with ALS. We analysed the different neuropsychological profiles of ALS patients with (ALSC9+) or without *C9orf72* mutation (ALSC9-). ALSC9+ patients showed a different neuropsychological profile compared to ALSC9- patients, characterized by more severe deficit of executive functions and verbal memory domain. Verbal memory resulted to be more vulnerable in ALSC9+ as compared to ALSC9-, even among patients classified as cognitively normal.

Taken together, our studies have addressed different issues in the field of cognitive impairment in ALS, including the influence of genetic and environmental factors, the neuroimaging correlates, and the natural course over time. Nevertheless, many aspects need further investigation, including the study of social cognition and of the determinants of patients' vulnerability to cognitive deterioration. The study of cognitive impairment associated with ALS is of outstanding importance since cognitive and behavioural changes have a deep impact on patients' prognosis and on caregivers' burden.

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