BIOMARKERS PROFILE IN DIFFERENT MOTOR NEURON DISEASE PHENOTYPES

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1. Abstract of the project

Background/Main objectives

Amyotrophic lateral sclerosis (ALS) has long been considered a uniform disease with a characteristic clinical presentation. However, there is increasing evidence of clinical, prognostic, neuropathological, and genetic heterogeneity of the disease. Recent advances have demonstrated an overlap between ALS and frontotemporal dementia (FTD) suggesting that they represent part of a clinicopathological spectrum. Cortical atrophy has been associated with neuropsychiatric and cognitive changes, and patients with features of executive impairment progress more rapidly. However little is known about the frequency of these changes in the different phenotypes of motor neuron disease and their effect on the prognosis of the disease.

The main objective of the study is to comprehensively characterize the biomarker profile in patients with ALS stratified by clinical phenotypes, in comparison with controls and patients with FTD, to analyze their utility in the definition of different variants and in prediction of survival.

Methodology

Patients with motor neuron disease will be classified by clinical phenotype into three different categories, according to the presence of upper or lower motor neuron signs or both. All patients will undergo complete longitudinal neuropsychological and cognitive assessments. MRI 3D structural sequences will be acquired and analyzed using the Freesurfer software. Aß 1-42, t-tau, p-tau levels, YKL-40 levels and BACE activity will be quantified in CSF.

The clinical pattern and biomarker profile will be compared across patients with different clinical phenotypes, with patients with FTD and healthy controls.

Expected results

The characterization of the biomarker profile should allow us to assess the clinical utility to establish a precise characterization of patients and identify factors linked to survival variability.

Hypothesis and description of the project objectives

Frontotemporal dementia (FTD) and motor neuron disease (MND) patients share clinical and pathological features. Distinguishing clinical features in MND has implications for pathophysiology and prognosis. Neuroimaging and CSF biomarkers will help to classify clinical phenotypes more accurately and will help to predict prognosis

Specific aims

To study differences in biomarker expression among patients with ALS and other related motor neuron disorders, and among patients with ALS, ALS-FTD and FTD patients.

To characterize the biomarker profile among patients with amyotrophic lateral sclerosis (ALS) and other related motor neuron disorders, and in comparison to patients with frontotemporal dementia (FTD) and healthy controls (HC) through:

. An analysis of cortical thickness (Cth) maps across different phenotype variants of MND, FTD patients and HC.
. An analysis of the CSF biomarkers (core AD biomarkers, YKL-40 and TDP-43) in the different groups.
. A correlation analysis of the Cth at baseline by groups with:
- Cognitive and behavioral assessments.
- CSF and plasma biomarkers

To analyze the concordance of cortical changes and CSF biomarkers with clinical and pathological patterns.

To assess the clinical and biomarker changes (CTh maps and CSF biomarkers) during follow-up in the different groups.

To determine whether clinical features and biomarker profile at baseline (clinical group, MRI and CSF biomarkers) are associated with prognosis and survival.
To analyze the relationship of clinical phenotypes with the postmortem neuropathological pattern.

2. Obtained results

Recruitment of patients and samples

The estimated total number of patients to be included in the study was 50. The subjects of the study have been selected among patients with a confirmed diagnosis of motor neuron disease (MND), attended at the MND Clinic of the Neuromuscular Diseases Unit.

Patients included in the study have been classified into three different groups according to the clinical phenotype, defined by neurological examination and electrophysiological studies. ALS patients (classified as probable or definite ALS according to the Awaji and El Escorial Criteria revised), patients with progressive muscle atrophy (PMA) and primary lateral sclerosis (PLS). Patients have been further classified into ALS with FTD, ALS with cognitive impairment or behavioral symptoms (ALS Ci/Bi), and those without. Patients with ALS-FTD must fulfil criteria for both ALS and Rascosvky criteria for the behavioral variant of FTD (Rascosvky, 2011).

The final number of the sample is 111 patients with MND who have signed informed consent for inclusion in the study. In this group, 12 of them are classified as ALS-FTD, 7 with PLS and 5 with PMA. We have collected samples of plasma, serum and DNA of all the subjects included in the study. We have carried out 72 cognitive assessments, 42 neuroimaging studies with MR and obtained CSF samples of 45 patients. Additionally we have collected samples of brain tissue of 14 cases. In addition, information and samples from the group of patients with FTD and healthy controls were available. Patients with FTD included in the studies come from a pre-selected Cohort of the Memory Unit that meets criteria of the behavioral variant and with available CSF samples, MRI data, DNA and plasma, as well as clinical information and cognitive assessments.

Processing and analysis of clinical data, samples of CSF and MR images

Study 1

This work **"CSF sAPPβ, YKL-40, and NfL along the ALS-FTD spectrum"** has been published in the journal **Neurology**.

Composition of the sample: 38 ALS patients classified according to diagnostic criteria: 11 ALS-FTD; 17 ALS Ci/Bi and 10 ALS without cognitive or behavioral impairment

(Strong 2016). Average age = 66.6; 43.5% women; 86 FTD verified according to diagnostic criteria: 46 bvFTD, 12 nfPPA, 8 svPPA (Rascovsky 2011, Gorno-Tempini 2011). Average age = 66.6; 29% women; 49 healthy controls without cognitive alterations (SPIN cohort). Average age = 64.2; 37.2% women.

A determination and analysis of the levels of 3 biomarkers -sAPPβ, YKL-40 and NfL- were performed in the CSF of patients with FTD, ALS and a group of control subjects. We compared cross-sectional biomarker levels between groups, studied their correlation with cognitive and functional scales (cognitive measures and the ratio of progression) and with measurement of cortical thickness.

We found increased levels of YKL-40 and decreased levels of sAPPß in both FTD and ALS groups, compared to controls. The lowest sAPPß levels and sAPPß/YKL-40 ratio were found in the FTD group. In FTD, sAPPß, YKL-40 and the sAPPß/YKL-40 ratio correlated with the disease severity. In the whole ALS-FTD spectrum, NfL and sAPPß correlated with global cognitive performance ($r=0.48$, $p<0.001$ and $r=0.36$, $p<0.001$, respectively). In the ALS group, YKL-40 correlated with disease progression rate ($r=0.51$, $p=0.001$) and the sAPPß/YKL-40 ratio showed a positive correlation with cortical thickness in frontotemporal regions.

In this study, we report decreased levels of sAPPß and increased levels of YKL-40 in the ALS-FTD clinical spectrum. Importantly, the ratio of sAPPß: YKL-40 correlated with cortical atrophy in frontotemporal regions in ALS and FTD. Finally, we also report that CSF levels of YKL-40 correlate with progression rate in ALS. In short, the study concludes that sAPPß, YKL-40 and NfL could represent valuable tools for the staging and prognostic stratification of patients within the ALS-FTD clinical spectrum, given the correlation with the degree of cortical atrophy and the degree of progression of the disease. The study shows that the levels of these proteins in CSF are useful for evaluating the process of neurodegeneration in frontotemporal regions and progression of these diseases. Additionally, the results underline the role of inflammation in the pathogenesis of the disease.

Study 2

The aim of the study was to evaluate the utility of cortical mean diffusivity (MD) to assess the cortical microstructural changes in the behavioral variant of the FTD

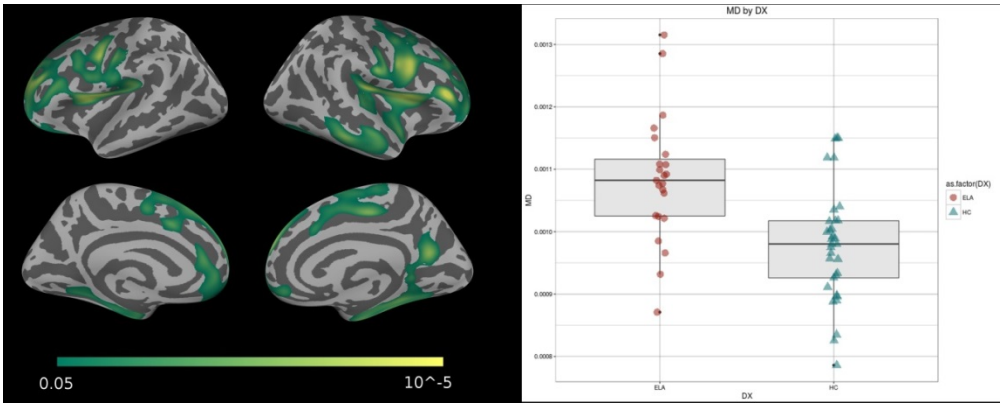
(bvFTD) and to correlate cortical MD with clinical measures of the disease and biomarkers in CSF(NfL and sAPPß). Composition of the sample: 70 patients with bvFTD and 78 healthy age-matched controls, including studies of neuroimage and CSF samples from nine patients ALS-FTD.

The MD maps, throughout the whole cohort and in the probable bvFTD group, showed widespread cortical areas with a increased MD that partially overlapped with the cortical thickness, but further expanded into other bvFTD-related regions. In the possible bvFTD group we found increased cortical MD in frontotemporal regions (especially in the dorsolateral and medial prefrontal cortex of both hemispheres), but only minimal cortical thickness loss. The size of the effect of cortical MD was significantly higher than the cortical thickness effect sizes in the areas normally involved in patients with bvFTD.

Both the mean diffusivity and the cortical thickness correlate with the severity measurements of the disease and the CSF biomarkers. However, the areas of correlation with MD were more extensive. The results suggest that cortical MD could be a sensitive biomarker for the study of microstructural changes related to neurodegeneration in bvFTD and ALS, especially at early stages (**Cortical microstructure in the behavioral variant of frontotemporal demon**: **looking beyond atrophy.** *Brain*, 2019, in press**).**

Study 3, *in progress*
Total size of the sample (2/2018) = 126 patients CSF available; MRI available of 82 patients. 38 ALS patients classified according to diagnostic criteria: 11 ALS-FTD; 17 ELA Ci /Bi and 10 ALS without cognitive or behavioral impairment (Strong 2016). Average age = 66.6; 43.5% women; 49 healthy controls without cognitive alterations (SPIN cohort). Average age = 64.2; 37.2% women.

The study aim is to assess changes in cortical microstructure and MD in ALS, in comparison with the bvFTD, as well as the correlation of the neuroimaging changes with clinical measures of severity and biomarkers in the CSF (NfL and sAPPß).



Preliminary analysis, the comparative analysis of MD between patients with ALS and controls shows changes in the cortical microstructure. Green areas show a significant increase in diffusivity in ALS and ALS-FTD patients with respect to controls that translate cortical microstructural changes.

Analysis of the clinical data of the cohort

Clinical, demographic and evolution data have been registered in a specific database for the study of their correlations with MRI and CSF findings. The precise phenotypic characterization of the patients included in the project, especially the subgroup of cases with concomitant ALS-FTD, allowed us a comparative analysis of the clinical pattern and the evolution of this subgroup with other forms of MND. The comparison has shown distinctive features in relation to survival and prognosis. We have identified a distinctive pattern of clinical presentation that could represent a specific phenotype of the disease with implications for diagnosis and therapeutic management. These observations have been the subject of the publication "**Distinct clinical features and outcome in motor neuron disease associated with behavioral variant frontotemporal dementia**" in the journal *Dement Geriatr Cogn Disorder,* 2018. The MND-FTD patients frequently displayed a distinctive motor pattern characterized by weakness and atrophy in distal upper limb muscles accompanied by severe swallowing problems, which are a frequent cause of serious complications with a potential life threat. The recognition of these characteristics may help to define specific subgroups of patients, which is important with regard to clinical management, outcome, nosological definition and research.

Additionally, the characterization of this subgroup of patients and the systematic collection of DNA samples has allowed their inclusion in an exome sequencing study in

collaboration with other groups. The study identifies probable pathogenic genetic variants in up to 20% of cases with ALS-FTD not carrying C9orf72 hexanucleotide repeat expansion (**Analysis of known amyotrophic lateral sclerosis and frontotemporal demon genes reveal to substantial genetic burden in patients manifesting both diseases not carrying the C9orf72 expansion mutation.** *J Neurol Neurosurg Psychiatry,* 2018). It is notable that a very significant proportion of cases of carriers of potential pathogenic mutations did not have family history of ALS or FTD. These data can help to define specific subgroups of patients, and also can determine clinical management and decision-making in clinical practice. Our data suggests that high-performance sequencing approaches in uniform series of patients with ALS and concomitant FTD might be helpful to disentangle the genetic of these two devastating disorders.

The review of the diagnostic process has also allowed a retrospective analysis of the differential diagnosis of the different forms of MND presentation. The work **"Early diagnosis of lateral amyotrophic sclerosis mimic syndromes: Pros and cons of current clinical diagnosis criteria"** has been published in the journal *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration,* 2017. This work provides valuable information regarding the application of the diagnostic criteria of the disease. This information is especially valuable in the health care area, given that this is a disease without a specific diagnostic test. In collaboration with another group, the registry has helped to carry out an epidemiological study with interesting results (**Amyotrophic lateral sclerosis: A higher than expected incidence in people over 80 years of age.** *Amyotroph Lateral Scler Frontotemporal Degener, 2016).* First of all, it is the second population-based epidemiological study in our country that confirms incidence and prevalence data similar to those of other studies in Europe. In addition the results of the study suggests that the age-specific incidence rate of ALS increases with age through the oldest age groups suggesting an age-risk effect to develop the disease that can support the notion that neurodegeneration of the motor system may be part of the aging process. Additionally, it places the focus on an especially fragile age group to which the disease is supposed to have been traditionally barely considered.
The inclusion in the project of neuropathological studies has been decisive for the description and precise characterization of very rare ALS cases due to accumulation of the fused in sarcoma protein (FUS) (**Does ALS-FUS without FUS mutation represent ALS-FET? Report of three cases.** *Neuropathol Appl Neurobiol,* 2018). In

this study we describe the clinical-pathological phenotype of 3 cases with FUS protein aggregates and the characterization of immunoreactivity for other FET- FUS / EWS / TAF15 family proteins - [Ewing's sarcoma EWS and the associated TATA associated protein factor 15 (TAF15)] RNA binding proteins that transport between the cytoplasm and nucleus assisted by protein Transportin 1 (Trn1). In a similar way to FTD cases with FUS aggregates, and contrary to ALS cases with mutations in *FUS,* the inclusion bodies for patients with ALS-FUS were immunoreactive for TAF15 and Trn1 and did not present pathogenic mutations in the gene which codifies for FUS. This study suggests the possibility that these cases may represent a distinctive group of the ALS-FUS spectrum with implications for the pathogenesis of the disease. Studies of tissue samples have also contributed to a collaborative assessment focused on systematic screening of aggregates ubiquitin/P62 neuropathological phenotype cases with C9orf72 hexanucleotide repeat expansion **(Systematic Screening of Ubiquitin / p62 Aggregates in Cerebellar Cortex Expands the Neuropathological Expansion of the phenotype C9orf72 Mutation.** *J Neuropathol Exp Neurol*, 2018).

3. Relevance and possible clinical implications

The main objective of the project was to comprehensively characterize the biomarker profile in patients with ALS, stratified by clinical phenotypes, in comparison with controls and patients with FTD, in order to analyze the usefulness of the biomarkers studied in the definition of the different variants and in prediction of survival.
The results of our studies provide valuable information with practical applicability as tools for the identification, stratification and prognosis of patients, as well as useful information for the knowledge of pathophysiological processes and progression of the disease. The results obtained also provide diagnostic tools, which should allow a more accurate nosological classification of the patients based on the clinical characteristics and information for decision-making in the clinical practice of patients with MND.
A detailed phenotypic, neuropathological and genetic characterization should help to define specific clinical subgroups of patients, important for routine clinical assistance, but also for stratification and evaluation for clinical trials, definition of prognosis, epidemiological and molecular research. The recognition of specific patterns may be relevant for a proper nosology of the disease and can improve the diagnostic process

and our understanding of the heterogeneity of the disease, from a clinical, but also genetic and neuropathological point of view.

The results of CSF studies have demonstrated the usefulness for stratification and prognosis of patients in the clinical spectrum ALS-FTD given the correlation with the degree of cortical atrophy and the rate of disease progression. Additionally, the results underline the role of inflammation in the pathogenesis of the disease.
In relation to the neuroimaging biomarkers for ALS, our results demonstrate the value of MR and specifically the techniques to study the mean diffusivity to detect microstructural alterations that allows to identify early cortical changes and possibly, in the future, a better categorization of the patients and understanding of the pathophysiological processes of the disease.

We believe that the characterization of biomarkers through different motor phenotypes, using a standardized approach to evaluation, may have an important impact on the global knowledge of the disease, identification of clinical subtypes and their related characteristics and may also help to improve our knowledge of the evolution of the disease. Its importance is capital in a population in which, due to the heterogeneity of the clinical phenotype, the diagnosis can be delayed and the prognosis is not well determined. All these factors may help to understand the pathophysiological basis of the disease and the factors that determine the progression to allow better selection of patients in homogenous groups for trials, as well as a better way to monitor the progression in clinical practice and drug trials.

The project has helped the collection of information and samples that have given rise to different publications and should allow the development of future studies.

4. Publications

CSF sAPPβ, YKL-40, and NfL along the ALS-FTD spectrum. Illán-Gala I, Alcolea D, Montal V, Dols-Icardo O, Muñoz L, de Luna N, Turón-Sans J, Cortés-Vicente E, Sánchez-Saudinós MB, Subirana A, Sala I, Blesa R, Clarimón J, Fortea J, Rojas-García R and Lleó A. *Neurology.* 2018 Oct 5. pii: 10.1212/WNL.0000000000006383. doi: 10.1212/WNL.0000000000006383. PMID: 30291183, *Impact Factor* ***8.055***

Cortical microstructure in the behavioral variant of frontotemporal dementia: looking beyond atrophy. Illán-Gala I, Montal V, Borrego-Écija S, Vilaplana E, Pegueroles J, Alcolea D, Mª Sánchez-Saudinós MB, Clarimón J, Turón-Sans J, Bargalló N, González-Ortiz S, Rosen HJ, Gorno-Tempini ML, Miller BL., Lladó A, Rojas-García R, Blesa R, Sánchez-Valle R, Lleó A and Fortea J on behalf of the Catalan Frontotemporal Dementia Initiative (CATFI) and the Frontotemporal Lobar Degeneration Neuroimaging Initiative (FTLDNI). *Brain*, in press, *Impact Factor* ***11.199***

Decreased circulating ErbB4 ectodomain fragments as a read-out of impaired signaling function in amyotrophic lateral sclerosis. Lopez-Font I, Sogorb-Esteve A, Javier-Torrent M, Brinkmalm G, Herrando-Grabulosa M, García-Lareu B, Turon-Sans J, Rojas-García R, Lleó A, Saura CA, Zetterberg H, Blennow K, Bosch A, Navarro X, Sáez-Valero J. *Neurobiol Dis.* 2018 Dec 27;124:428-438. doi: 10.1016/j.nbd.2018.12.021. PMID: 30594809, *Impact Factor* ***5.227***

Does ALS-FUS without FUS mutation represent ALS-FET? Report of three cases. Borrego-Écija S, Cortés-Vicente E, Cervera-Carles L, Clarimón J, Gámez J, Batlle J, Ricken G, Molina-Porcel L, Aldecoa I, Sánchez-Valle R, Rojas-García R, Gelpi E. *Neuropathol Appl Neurobiol.* 2018 Oct 29. doi: 10.1111/nan.12527. PMID: 30375034, *Impact Factor* ***6.059***

Analysis of known amyotrophic lateral sclerosis and frontotemporal dementia genes reveals a substantial genetic burden in patients manifesting both diseases not carrying the C9orf72 expansion mutation. Dols-Icardo O, García-Redondo A, Rojas-Garcia R, Borrego-Hernández D, Illán-Gala I, Muñoz-Blanco JL Rábano A, Cervera-Carles L, Juárez-Rufián A, de Luna Salva N, Galán L, Cortes-Vicente E, Fortea J, Blesa R, Grau-Rivera O, Lleó A, Esteban-Pérez J, Gelpi E, Clarimón J. *J Neurol Neurosurg Psychiatry.* 2018 Feb;89(2):162-168. doi: 10.1136/jnnp-2017-316820. Epub 2017 Sep 9. PMID: 28889094, *Impact Factor* ***7.144***

Distinct Clinical Features and Outcomes in Motor Neuron Disease Associated with Behavioural Variant Frontotemporal Dementia. Cortés-Vicente E, Turon-Sans J, Gelpi E, Clarimón J, Borrego-Écija S, Dols-Icardo O, Illán-Gala I, Lleó A, Illa I, Blesa R, Al-Chalabi A, Rojas-García R. *Dement Geriatr Cogn Disord.* 2018;45(3-4):220-

231. doi: 10.1159/000488528. Epub 2018 Jun 8. PMID: 29886477, *Impact Factor* ***2.886***

Systematic Screening of Ubiquitin/p62 Aggregates in Cerebellar Cortex Expands the Neuropathological Phenotype of the C9orf72 Expansion Mutation. Ramos-Campoy O, Ávila-Polo R, Grau-Rivera O, Antonell A, Clarimón J, Rojas-García R, Charif S, Santiago-Valera V, Hernandez I, Aguilar M, Almenar C, Lopez-Villegas D, Bajo L, Pastor P, Van der Zee J, Lladó A, Sanchez-Valle R, Gelpi E. *J Neuropathol Exp Neurol.* 2018 Aug 1;77(8):703-709. doi: 10.1093/jnen/nly047. PMID: 29889265, *Impact Factor* ***4.056***

Early diagnosis of amyotrophic lateral sclerosis mimic syndromes: pros and cons of current clinical diagnostic criteria. Cortés-Vicente E, Pradas J, Marín-Lahoz J, De Luna N, Clarimón J, Turon-Sans J, Gelpí E, Díaz-Manera J, Illa I, Rojas-Garcia R. *Amyotroph Lateral Scler Frontotemporal Degener.* 2017 Aug;18(5-6):333-340. doi: 10.1080/21678421.2017.1316408. Epub 2017 Apr 25. PMID: 28440098, *Impact Factor* ***2.982***

Amyotrophic lateral sclerosis: A higher than expected incidence in people over 80 years of age. Aragones JM, Altimiras J, Roura-Poch P, Homs E, Bajo L, Povedano M, Cortres-Vicente E, Illa I, Al-Chalabi A, Rojas-Garcia R. Amyotroph Lateral Scler Frontotemporal Degener. 2016 Oct - Nov;17(7-8):522-527. Epub 2016 May 25, *Impact Factor* ***2.982***