Universitat Autònoma de Barcelona

Pathophysiological and structural underpinnings of frontotemporal lobar degeneration: a multimodal biomarker study

Ignacio Illán Gala

Doctoral Thesis

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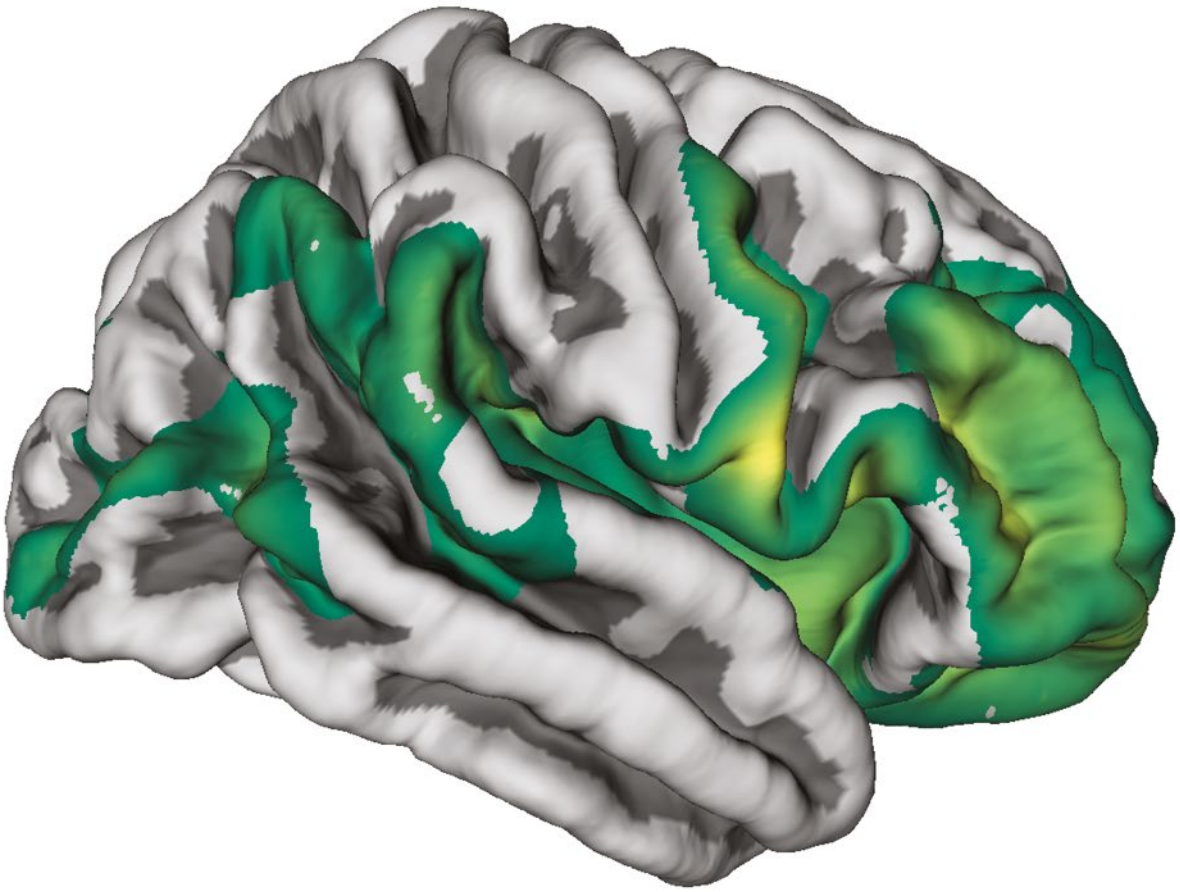

Universitat Autònoma de Barcelona
Programa de Doctorat en Neurociències
Institut de Neurociències
January 2019

Cover figure: Correlation of cortical mean diffusivity with the CSF levels of sAPPβ in bvFTD. The lateral view of the right hemisphere is displayed. Only clusters that survived familywise error at $p<0.05$ are shown. Analyses were adjusted for age, sex after a harminozation step. The full figure can be found in the chapter 6 **(Figure 5)**

A Santiago

Mi a no es tu a. Mi a es lúgubre y sabia. Tu a es una nota de luz en tu paladar, en el paladar claro del mundo. Qué juego de luces y sombras. A veces el idioma se cierne sobre ti y me asusto. A veces echas tú sobre él un desconcierto alegre de juego. Qué miedo, qué alegría, qué susto, qué tristeza, verte aprender las letras.

Francisco Umbral. Mortal y Rosa

DOCTORAL THESIS

Pathophysiological and structural underpinnings of frontotemporal lobar degeneration: a multimodal biomarker study

Ignacio Illán Gala

Dr. Alberto Lleó Bisa, Tutor and Thesis Director
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Doctoral Thesis
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Certificate of Direction

Dr. Alberto Lleó Bisa, who obtained his PhD in Medicine in the Universitat de Barcelona, Head of the Memory Unit in the Neurology Department in Hospital de la Santa Creu i Sant Pau and aggregate professor in the Universitat Autònoma de Barcelona and Dr. Juan Fortea Ormaechea, who obtained his PhD in Medicine in the Universitat de Barcelona, specialist in Neurology at Hospital de Sant Pau and medical director of the Alzheimer – Down Syndrome Unit

Certify:
That the work "Pathophysiological and structural underpinnings of frontotemporal lobar degeneration: a multimodal biomarker study", presented by Ignacio Illán Gala to qualify for Doctor in Neurosciences for the Universitat Autònoma de Barcelona has been done under our direction and meets all the requirements to be presented and defended in the presence of the corresponding Thesis Committee.

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About the author

Ignacio Illán-Gala obtained his Medical Degree at the Autonomous University of Barcelona (Barcelona, Spain) and did his internship in clinical Neurology at La Paz University Hospital (Madrid, Spain). The last year of his training as a Neurologist, he was granted by the Spanish Society of Neurology to join the behavioral neurology program of the Memory and Aging Center (San Francisco, USA). This experience committed him to focus his career in the study of the patients suffering from frontotemporal dementia. At the end of his internship, he joined the Memory Unit of the Santa Creu i Sant Pau, a leading behavioral neurology team where translational research is implemented by a multidisciplinary and highly-talented group of scientists including biologist, psychologists, engineers, nurses and neurologists. Currently, he is involved in the local Frontotemporal Lobar Degeneration (FTLD) clinical cohort and the multicentric Catalan FTD cohort (CATFI). In 2018, he was granted with the competitive Río Hortega grant, completed a master in neuropsychology at the Universitat oberta de Catalunya and he was selected as an Atlantic Fellow of the Global Brain Health Institute, at University of California San Francisco.

Abstract

Frontotemporal lobar degeneration (FTLD), is a heterogenic pathological construct encompassing multiple neuropathological conditions primarily affecting the frontal and temporal lobes. Although multiple clinical syndromes predict the neuropathological diagnosis of FTLD, clinical-patholocial correlations are far from being perfect. Cerebrospinal and imaging biomarkers represent powerful tools for the study of FTLD pathophysiology and improve the diagnostic accuracy of FTLD and its differentiation from other diseases.

This thesis aims to improve our understanding of the pathophysiological and structural underpinnings of FTLD-related neurodegeneration through a multimodal biomarker approach combining: (i) clinical markers of disease progression (i.e. CDR-FTLD, ALFRS), (ii) CSF biomarkers related to different pathophysiological aspects of FTLD (APP-derived peptides, YKL-40 and NfL) and (iii) the MRI study of cortical macrostructure (cortical thickness) and microstructure (cortical mean diffusivity) in FTLD—related syndromes (FTLD-S).

We provide novel insights into the pathophysiology of FTLD by showing that: (i)CSF levels of sAPPß, YKL-40 and NFL alone or in combination had a good diagnostic accuracy to discriminate FTLD-S from healthy controls and patients with Alzheimer's disease (ii) In the absence of Alzheimer's disease pathophysiology, APP-derived peptides related to the so-called amyloidogenic pathway (sAPPß, $Aß_{1-42}$, $Aß_{1-40}$, $Aß_{1-38}$) are globally reduced in FTLD-S and correlate with cortical macrostructure (cortical thickness); importantly this pattern of APP-derived differed from the observed in Alzheimer's disease were a selective decrease in the CSF levels of $Aß_{1-42}$ was observed; (iii) YKL-40, a biomarker related to astroglial activity, is increased in FTLD-S and their levels may be useful for the prediction of disease progression in the ALS-FTD continuum; and (iv) cortical mean diffusivity, a novel imaging biomarker is more sensitive that cortical thickness for the study of the earliest FTLD-related neurodegeneration. These findings add to our current understanding of FTLD pathophysiology and open new doors towards precision medicine approaches in FTLD-S.

Abbreviations

Aβ = β-amyloid
AD = Alzheimer disease
aFTLD-U = atypical frontotemporal lobar degeneration with ubiquitin-positive inclusions
AGD = argirophylic gran disease;
ALS = amyotrophic lateral sclerosis - frontotemporal dementia
ALSci-bi = amyotrophic lateral sclerosis with cognitive or behavioral impairment
ALSFRS-R = revised ALS Functional Rating Scale
ALS-FTD = amyotrophic lateral sclerosis – frontotemporal dementia
ALSni = amyotrophic lateral sclerosis without cognitive or behavioral impairment
AUC = area under the curve
BIBD = Basophilic inclusion body disease;
bvFTD = behavioral variant of frontotemporal dementia
CATFI = catalan frontotemporal dementia initiative
CBS = corticobasal syndrome
CBD = Corticobasal degeneration
CDR = Clinical Dementia Rating
CERAD = Consortium to Establish a Registry for Alzheimer's Disease
CI = confidence interval
CN = cognitively normal control
CTh = cortical thickness
DN = Dystrophic neurites;
DTI = diffusor tensor imaging
DWI = diffusion weighted imaging
ECAS = Edinburgh Cognitive and Behavioral ALS Screen
EWS = Ewing's sarcoma;
FTD = frontotemporal dementia
FTLD = frontotemporal lobar degeneration
FTLD-S = frontotemporal lobar degeneration-related syndromes
FTLS-UPS = FTLD-ubiquitin proteasome system
FTLDNI = frontotemporal lobar degeneration neuroimaging initiative
FUS = Fused in sarcoma;
GCI = Glial cytoplasmic inclusions;
GGT = globular glial tauopathy
GM = grey matter
GRN = granulin precursor gene
HSP = hospital de sant Pau
HCB = hospital clínic de Barcelona
HR = hazard ratio
IHC = Immunohistochemistry;
MAPT = microtubule- associated tau protein
MD = mean diffusivity
MMSE = Mini-Mental State Examination
MND = motor neuron disease
MPRAGE = magnetization-prepared rapid gradient echo
NCI = Neuronal cytoplasmic inclusions;
nfaPPA = nonfluent agrammatic primary progressive aphasia
NfL = neurofilament light chain
NIFID = Neuronal intermediate filament inclusion disease;
NII = Neuronal intranuclear inclusions;
PSP = progressive supranuclear palsy
p-tau = phosphorilated tau
sAPPβ = soluble β fragment of amyloid precursor protein
SPIN = Sant Pau Initiative on Neurodegeneration
svPPA = semantic variant of primary progressive aphasia
TAF15 = TAT-binding protein-associated factor 15;
TDP-43 = TAR DNA binding protein

TN = Topography of neurodegeneration;
t-tau = total tau
UCSF = university of California san Francisco
VBM = voxel-based morphometry
VCP = valosin containing protein
WM = white matter

Acknowledgements

This thesis summarizes more than three years of passionate work at the memory unit and the Alzheimer's laboratory of the Hospital de la Santa Creu i Sant Pau. In this privileged environment I have had the pleasure of working with experts in different disciplines and above all excellent people and friends. Many of the ideas, results and analyzes included in this thesis emerge from these interactions. I feel therefore, that this thesis is not only mine, but of all the members of the unit that have made essential contributions. Without their talent, work, generosity, coffees and laughter, this thesis would not have been possible. I thank you all.

Before taking the leap and deciding to embark on this adventure, I was educated and trained at the French high school in Alicante, the medical faculty of the Sant Pau hospital in Barcelona, my Neurology residency at the La Paz university hospital in Madrid and my internship at the Memory and Aging Center in San Francisco, all of which were fundamental experiences. There are so many people who in one way or another have helped me along the way that enumerating them all would be impossible. In particular, I would like to thank Alberto Lleó for the trust he placed in me. His constant support and guidance, has allowed me the time to phenotype in detail almost three hundred related syndromes of frontotemporal lobar degeneration, something for which, I will be eternally grateful. I would also like to extend my thanks to Juan Fortea for his valuable teaching, ambition, determination and a valuable source of inspiration and knowledge and to Professor Rafael Blesa, for inspiring me at medical school (even though he may not remember it) and pushing the Memory Unit to its current level of excellence. To Bruce Miller, I thank you for believing in me when I was a Neurology resident, and for pushing me to devote the rest of my life to the study of human cognition and behavior. I thank our patients whose generosity and trust makes all our sacrifices worthwile. Special mention goes to Víctor Montal, Daniel Alcolea, and my in-laws Ana and José María whose generosity made this thesis possible.

To start at the beginning, I am eternally grateful to my parents, Elena and Rafael, for their love and support and for having encouraged me to devote myself to something that I am passionate about, no matter how absurd it might seem. Perhaps if my mother had not agreed to buy me that book (the interpretation of dreams by Sigmund Freud), I would never have studied medicine and would not have discovered my passion. Now that I am a father, I value more the sacrifices you have made. Thank you. I thank my brother Rafael, for helping me make the best decision I ever made, my grandfather Santiago, for transmitting the beauty of printed words and for his unconditional love and my grandmothers, Elena and Mercedes, and grandfather, José Luis, for their extraordinary wisdom and humanity. And of course I thank my son, Santiago, and my wife, Laura, for reminding me every day what is really important and making everything worthwhile.

List of articles included in this thesis

1st Work: D. Alcolea, E. Vilaplana, M. Suárez-Calvet, **I. Illán-Gala**, R. Blesa, J. Clarimon, A. Lladó, R. Sánchez-Valle, J.L. Molinuevo, G. García-Ribas, Y. Compta, M.J. Martí, G. Piñol-Ripoll, G. Amer-Ferrer, A. Noguera, A. García-Martín, J. Fortea and A. Lleo, "**CSF sAPPβ, YKL-40, and neurofilament light in frontotemporal lobar degeneration**," Neurology. 2017 Jul 11;89(2):178-188. doi: 10.1212/WNL.0000000000004088. Epub 2017 Jun 7.

Impact Factor 2017 = 7.609; Journal Category: Clinical Neurology; Quartile in category: first quartile

2nd Work: I. Illán-Gala, D. Alcolea, V. Montal, O Dols-Icardo, L. Muñoz, N. de Luna, J. Turón-Sans, E. Cortés-Vicente, M.-B. Sánchez-Saudinós, A. Subirana, I. Sala, R. Blesa, J. Clarimón, J. Fortea, R. Rojas-García and A. Lleó, "**CSF sAPPβ, YKL-40, and NfL along the ALS-FTD spectrum**," Neurology. 2018 Oct 23;91(17):e1619-e1628. doi: 10.1212/WNL.0000000000006383. Epub 2018 Oct 5.

Impact Factor 2018 = 7.609; Journal Category: Clinical Neurology; Quartile in category: first quartile

3rd Work: I. Illán-Gala, V. Montal, S. Borrego-Écija, E. Vilaplana, J. Pegueroles, D. Alcolea, M.-B. Sánchez-Saudinós, J. Clarimón, J. Turón-Sans, N. Bargalló, S. González-Ortiz, H.J. Rosen, M.L. Gorno-Tempini, B.L. Miller, A. Lladó, R. Rojas-García, R. Blesa, R. Sánchez-Valle, A. Lleó and J. Fortea, "**Cortical microstructure in the behavioral variant of frontotemporal dementia: looking beyond atrophy**," Brain, accepted Dec 21

Impact Factor 2018 = 10.84; Journal Category: Clinical Neurology; Quartile in category: first quartile

4th Work: I. Illán-Gala, J. Pegueroles, V. Montal, D. Alcolea, E. Vilaplana, F. Sampedro, E. Morenas-Rodríguez, M. Carmona-Iragui, A. Subirana, M.B. Sánchez-Saudinós, R. Rojas-García, H. Vanderstichele, R. Blesa, J. Clarimón, J. Fortea, A. Lleó, "**Low amyloid precursor protein-derived peptides reflect neurodegeneration in frontotemporal dementia**," In preparation for submission.

Impact Factor 2018 = XX; Journal Category: Clinical Neurology; Quartile in category: XX

Índex

Chapter 1

Outline and introduction

Outline

This thesis aims to improve our understanding of the pathophysiological and structural underpinnings of FTLD-related neurodegeneration through a multimodal biomarker approach combining: (i) clinical markers of disease progression (i.e. CDR-FTLD, ALFRS), (ii) CSF biomarkers related to different pathophysiological aspects of FTLD (APP-derived peptides, YKL-40 and NfL) and (iii) the MRI study of cortical macrostructure (cortical thickness) and microstructure (cortical mean diffusivity) in FTLD-S.

I have included a total of six papers, five of them already published in journals of first decile. The main body of this thesis is composed of four papers that will be presented in **chapters 3, 4, 5 and 6**, respectively. The first three papers (**chapters 3, 4 and 5**) have been already accepted for publication in *Neurology* (**chapters 3 and 4**) and *Brain* (**chapter 5**). The fourth paper (**chapter 6**) is in preparation for submission.

Chapter 1 sets the framework for this thesis by introducing the concept of FTLD and the widening spectrum of associated phenotypes. In particular, I discuss current limitations of clinical-pathological correlations in FTLD and highlight the importance of pathophysiological and structural biomarkers to increase diagnostic certainty of underlying FTLD in FTLD-S. In **chapter 2** I describe the hypotheses and objectives of this doctoral thesis.

In **chapter 3**, I assess the clinical utility of three CSF biomarkers (sAPPβ, YKL-40 and NfL) for the diagnosis of FTLD in a large multicenter cohort of FTLD-S and we also evaluate the structural correlates of these biomarkers with cortical macrostructure in FTLD-S. **Chapter 4** addresses the clinical utility of these three CSF biomarkers along the ALS-FTD clinical spectrum and evaluate the relationship between CSF biomarkers and both cortical macrostructure and survival.

In **chapter 5**, I investigated the value of a novel neuroimaging biomarker, cortical mean diffusivity for the diagnosis of the bvFTD in a large multicentric cohort. In **chapter 6**, I explore the relationship between APP-derived peptides related to the amyloidogenic pathway ($A\beta_{1\text{-}42}$, $A\beta_{1\text{-}40}$, Aβ1-38 and sAPPβ) and cortical macrostructure in FTLD-S compared to Alzheimer's disease and controls.

Lastly, in **chapter 7** I provide a general discussion integrating the main results presented in this thesis, putting them into context and emphasizing the novel insights into pathophysiological aspects of FTLD. In addition to the paper included in the main body of the thesis, I have included two papers related to relevant aspects of the discussion as annexes (**supplementary paper 1** published in *J Neurol Neurosurg Psychiatry* and **supplementary paper 2**, published in *JAMA Neurol*). Finally, I discuss on the future perspectives regarding the application of both CSF and imaging biomarkers for the study of FTLD.

In summary, this thesis utilizes a multimodal biomarker approach to investigate the potential role of APP-derived peptides, YKL-40 and NfL as pathophysiological markers of FTLD-related neurodegeneration and proposes a novel neuroimaging biomarker for the study of cortical microstructural changes that may be more sensitive than cortical thickness for the study of FTLD-related neurodegeneration. This thesis adds to our current understanding of FTLD pathophysiology and opens new doors towards precision medicine approaches in FTLD-S.

Introduction

The many faces of FTLD and its clinical presentations

Frontotemporal dementia

Overview of frontotemporal dementia and its relationship with frontotemporal lobar degeneration. Frontotemporal dementia (FTD) is the name given to a group of clinical syndromes characterized by prominent impairment of behavior or language caused by neurodegeneration of frontotemporal cortex and/or its subcortical connections. Frontotemporal lobar degeneration (FTLD), on the other hand, is a neuropathological term referring to a wide range of neuropathological conditions defined by varying patterns of neurodegenerative changes and neuronal and glial inclusion.

All the behavioral, language and motor phenotypes that predict a neuropathological diagnosis of FTLD will be referred to in this thesis as FTLD-related syndromes (FTLD-S). The label FTD will be reserved for the three classic phenotypes associated with FTLD: namely, the behavioral variant of frontotemporal dementia (bvFTD), the semantic variant of primary progressive aphasia (svPPA) and the nonfluent/agrammatic variant of primary progressive aphasia (nfaPPA) (Bang *et al.*, 2015; Karageorgiou and Miller, 2014). Each of these clinical syndromes will be introduced in the next sections.

Although most patients diagnosed of FTD may have underlying FTLD at autopsy this clinical-pathological correlation is far from perfect. Each pathological subtype of FTLD can manifest as several FTLD-S and, conversely, each FTLD-S can herald multiple FTLD subtypes as depicted in **Figure 1** (Bang *et al.*, 2015; Elahi and Miller, 2017; Irwin *et al.*, 2015). Of note, emergent data from large clinical-pathological cohorts of 4R-tauopathies (progressive supranuclear palsy [PSP] and corticobasal disease [CBD]) over the last decade has unveiled a significant overlap between these previously-considered pure motor disorders and some FTLD-S (Armstrong *et al.*, 2013; Höglinger *et al.*, 2017; Irwin, 2016; Kovacs, 2015). This new array of motor presentations also included the amyotrophic lateral sclerosis-frontotemporal dementia (ALS-FTD) continuum. This clinical continuum will also be introduced in the following sections.

In addition to this clinical and pathological heterogeneity, some FTLD-S may resemble atypical forms of other neurodegenerative disease such as Alzheimer's disease and Lewy Body Disease. Particularly, the behavioral/dysexecutive variant of Alzheimer's disease can mimic bvFTD (Ossenkoppele *et al.*, 2015) and Lewy body disease (which often presents with comorbid Alzheimer's disease) may mimic nfaPPA and CBS-PSPS, as shown in **Figure 1** (Day *et al.*, 2017; Kasanuki *et al.*, 2018; Lee *et al.*, 2011; Rogalski *et al.*, 2016). Of note, Alzheimer's disease and Lewy body disease can be found as a concurrent neuropathological finding in some FTLD cases (Lleo *et al.*, 2015; 2018; Takeda, 2018; Toledo *et al.*, 2012).

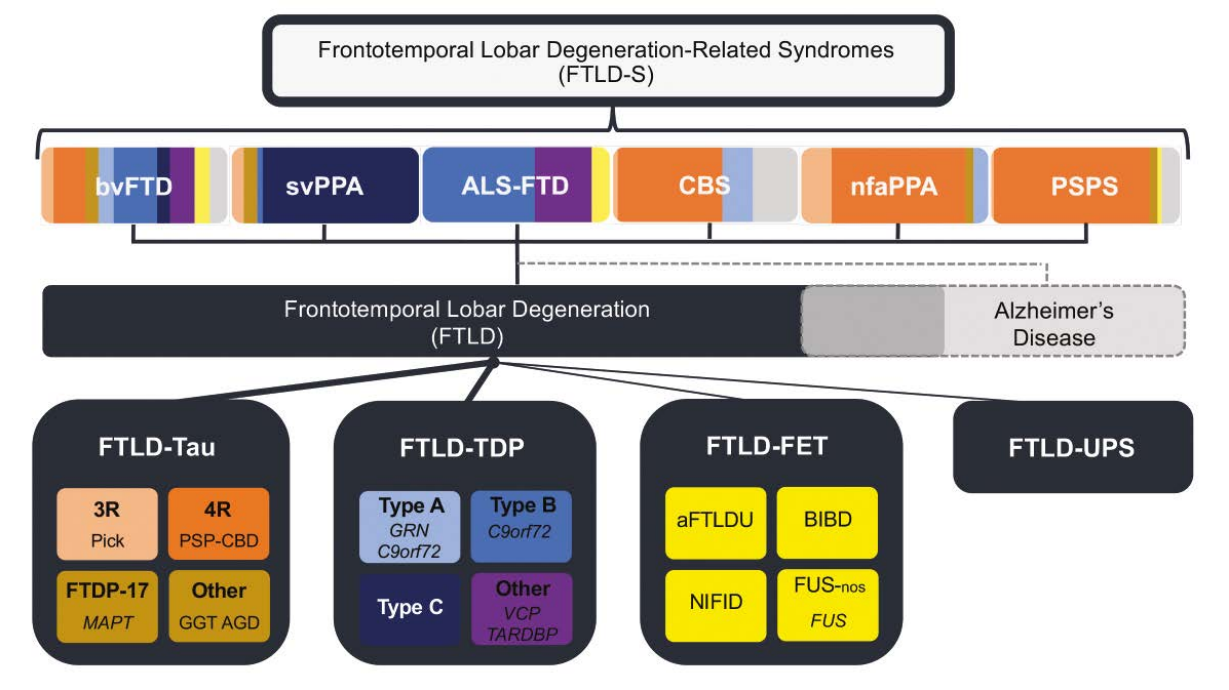

Figure 1. The complexity of clinical-pathological relationships along the FTLD spectrum. Abbreviations: ALS = amyotrophic lateral sclerosis - frontotemporal dementia; aFTLD-U = atypical frontotemporal lobar degeneration with ubiquitin-positive inclusions; AGD = argirophylic gran disease; BIBD = Basophilic inclusion body disease; bvFTD = behavioral variant of frontotemporal dementia; CBS = corticobasal syndrome; CBD = corticobasal degeneration; FTLD = frontotemporal lobar degeneration; FTLD-S = frontotemporal lobar degeneration-related syndromes; FUS = Fused in sarcoma; FUS-nos = Fused in sarcoma not otherwise specified; GGT = globular glial tauopathy; nfaPPA = nonfluent agrammatic primary progressive aphasia; NIFID = Neuronal intermediate filament inclusion disease; PSP = progressive supranuclear palsy; svPPA = semantic variant of primary progressive aphasia; TDP-43 = TAR DNA binding protein; VCP = valosin containing protein.

Frontotemporal dementia. The term of frontotemporal dementia (FTD) was introduced in 1994 by the Lund and Manchester groups to define patients with a suspected neurodegenerative disease manifesting with a progressive personality change (Brun *et al.*, 1994). Four years later, the concept of FTD was unified with two subtypes of PPA: the "semantic" and the "non-fluent" subtypes (Neary *et al.*, 1998). The concept of "semantic" dementia included patients displaying a combination of impaired word comprehension, "fluent aphasia" and to a lesser extent, impaired recognition of objects and faces (Hodges *et al.*, 1992; Mummery *et al.*, 1999; Snowden, 1999; Hodges, Patterson, 2007). However, it soon became evident that "fluent vs non-fuent" or "semantic vs non-fluent" schemes failed to reflect the complexity of syndromes related to the disintegration of the language network observed in patients with PPA (Mesulam, 2003). Those schemes were indeed unable to identify patients with underlying Alzheimer's disease at autopsy (Kertesz and Munoz, 2003; Galton et *al.*, 2000). A notable contribution to the study of PPA was made in 2004, when Maria Luisa Gorno-Tempini defined a distinct form of PPA that she named "logopenic progressive aphasia" (Gorno-Tempini *et al.*, 2004; Gorno-Tempini *et al.*, 2008). This new variant was characterized by the selective impairment of the phonological loop (i.e. phonological working memory) resulting in a profound disruption of repetition of long sentences and pseudowords, as well as naming and phonological errors with a relative sparing of semantic knowledge and syntactic abilities (Henry, Gorno-Tempini, 2010). The posterior pattern of atro-

phy and early pathological studies suggested that "logopenic progressive aphasia" (subsequently named "logopenic variant of primary progressive aphasia" [lgPPA] in current diagnostic criteria of PPA) might be primarily caused by Alzheimer's disease (Mesulam *et al.*, 2008; Rabinovici *et al.*, 2008). More recently, the concept of FTD has been redefined to encompass three clinical syndromes that have been shown to predict FTLD (Bang *et al.*, 2015; Montembeault *et al.*, 2018). Each of these three syndromes is characterized by distinct clinical features at disease onset. bvFTD is defined by a prominent personality change with progressive deterioration of social behavior and cognitive functions (Rascovsky *et al.*, 2011) while the svPPA and nfaPPA syndromes are characterized by a prominent impairment of language and/or speech (Gorno-Tempini *et al.*, 2011; Montembeault *et al.*, 2018).

The behavioral presentation of FTD. bvFTD is the most common FTD variant and is characterized by prominent behavioral abnormalities, including behavioral disinhibition, apathy, loss of empathy, stereotyped or compulsive behavior, hyperorality, and dietary changes (Bang *et al.*, 2015; Coyle-Gilchrist *et al.*, 2016). Current diagnostic criteria rely on the clinical features at presentation but may also consider the information provided by biomarkers to increase the diagnostic certainty (**Table 1**).

Accordingly, the diagnosis of possible bvFTD is based solely on the clinical syndrome and aims to identify patients at the mildest stages of disease, while the diagnosis of probable bvFTD attempts to classify patients with a high probability of underlying FTLD pathology and requires objective imaging changes (frontotemporal atrophy and/or cerebral hypometabolism). These criteria were developed by the FTDDC consortium in 2011 and accurately predict FTLD pathology, especially in the early onset cases (defined by a symptom onset before 65 years), where a focal pattern of frontal and/or temporal cerebral atrophy is frequently observed (Baborie *et al.*, 2011; 2012; Rascovsky *et al.*, 2011). The insidious behavioral changes of the bvFTD can be difficult to recognize as a neurodegenerative syndrome, and are often mistaken as midlife psychiatric disorders, especially in the absence of overt atrophy or hypometabolism in neuroimaging (Ducharme *et al.*, 2015; Gossink *et al.*, 2016; Lanata and Miller, 2016; Landqvist Waldö *et al.*, 2015; Piguet *et al.*, 2011; Shinagawa *et al.*, 2014; Woolley *et al.*, 2011). Notwithstanding, bvFTD is a heterogeneous syndrome. From a clinical perspective, the behavioral symptoms and a distinct cognitive profile with insensitivity to errors and relative sparing of visuoperceptual abilities dominate the clinical picture at disease onset (Piguet *et al.*, 2011; Ranasinghe *et al.*, 2016; Rascovsky and Grossman, 2013; Rascovsky *et al.*, 2007). There is a male predominance and the mean age at symptom onset is around 60 years, although a significant proportion of late-onset cases have been reported in European cohorts (Bang *et al.*, 2015; Coyle-Gilchrist *et al.*, 2016; O'Connor *et al.*, 2017; Ranasinghe *et al.*, 2016). The median survival is of 8.2–8.7 years from symptom onset, but disease course is highly heterogeneous and a subgroup of cases with a "slowly progressive variant" has also been reported (Davies *et al.*, 2006; Garcin *et al.*, 2009; Hodges *et al.*, 2003; Khan *et al.*, 2012; Mioshi *et al.*, 2010). Clinical and prognostic variability parallels the observed structural and neuropathological heterogeneity (Josephs, Hodges, *et al.*, 2011; Kim *et al.*, 2007; Perry *et al.*, 2017; Whitwell *et al.*, 2009). Recent studies from deeply phenotyped cohorts have identified distinct bvFTD subgroups characterized by different patterns of neurodegeneration, progression rates and neuropathological correlates (Josephs, Whitwell, *et al.*, 2011; Mioshi *et al.*, 2010; O'Connor *et al.*, 2017; Ranasinghe *et al.*, 2016; Whitwell *et al.*, 2009).

The language presentations of FTD. Primary progressive aphasia (PPA) refers to a group of focal neurodegenerative syndromes characterized by prominent language impairment at symptom

Neurodegenerative disease: the following symptom must be present to meet criteria for bvFTD

A. Shows progressive deterioration of behavior and/or cognition by observation or history (as provided by a knowledgeable informant).

II. Possible bvFTD: three of the following behavioral/cognitive symptoms (A–F) must be present to meet criteria. Ascertainment requires that symptoms be persistent or recurrent, rather than single or rare events.

A. Early* behavioral disinhibition [one of the following symptoms (A.1–A.3) must be present]:
- *A.1. Socially inappropriate behavior*
- *A.2. Loss of manners or decorum*
- *A.3. Impulsive, rash or careless actions*

B. Early* apathy or inertia [one of the following symptoms (B.1–B.2) must be present]: B.1. Apathy, B.2. Inertia

C. Early* loss of sympathy or empathy [one of the following symptoms (C.1–C.2) must be present]:
- *C.1. Diminished response to other people's needs and feelings*
- *C.2. Diminished social interest, interrelatedness or personal warmth*

D. Early* perseverative, stereotyped or compulsive/ritualistic behavior [one of the following symptoms (D.1–D.3) must be present]:
- *D.1. Simple repetitive movements*
- *D.2. Complex, compulsive or ritualistic behaviors*
- *D.3. Stereotypy of speech*

E. Hyperorality and dietary changes [one of the following symptoms (E.1–E.3) must be present]:
- *E.1. Altered food preferences*
- *E.2. Binge eating, increased consumption of alcohol or cigarettes*
- *E.3. Oral exploration or consumption of inedible objects*

F. Neuropsychological profile: executive/generation deficits with relative sparing of memory and visuospatial functions [all of the following symptoms (F.1–F.3) must be present]:
- *F.1. Deficits in executive tasks*
- *F.2. Relative sparing of episodic memory*
- *F.3. Relative sparing of visuospatial skills*

III. Probable bvFTD: All of the following symptoms (A–C) must be present to meet criteria.

A. Meets criteria for possible bvFTD

B. Exhibits significant functional decline (by caregiver report or as evidenced by Clinical Dementia Rating Scale or Functional Activities Questionnaire scores)

C. Imaging results consistent with bvFTD [one of the following (C.1–C.2) must be present]:
- *C.1. Frontal and/or anterior temporal atrophy on MRI or CT*
- *C.2. Frontal and/or anterior temporal hypoperfusion or hypometabolism on PET or SPECT*

IV. Behavioural variant FTD with definite FTLD Pathology: Criterion A and either criterion B or C must be present to meet criteria.

A. Meets criteria for possible or probable bvFTD

B. Histopathological evidence of FTLD on biopsy or at post-mortem

C. Presence of a known pathogenic mutation

V. Exclusionary criteria for bvFTD: Criteria A and B must be answered negatively for any bvFTD diagnosis. Criterion C can be positive for possible bvFTD but must be negative for probable bvFTD.

A. Pattern of deficits is better accounted for by other non-degenerative nervous system or medical disorders

B. Behavioral disturbance is better accounted for by a psychiatric diagnosis

C. Biomarkers strongly indicative of Alzheimer's disease or other neurodegenerative process

*: As a general guideline 'early' refers to symptom presentation within the first 3 years

Table 1. International consensus criteria for bvFTD. Adapted from Rascovsky *et al.*, 2011.

onset. Although other cognitive deficits may be present at diagnosis, language deficits must be the most salient clinical feature at disease onset (during the first two years of the disease) and the primary cause of functional impairment (Mesulam, 1982). The language presentations of FTD encompass two primary progressive aphasias : nonfluent agrammatic primary progressive aphasia (nfaPPA) and the semantic variant of primary progressive aphasia (svPPA)(Gorno-Tempini *et al.*, 2011). A third variant, named the logopenic variant of primary progressive aphasia (lgPPA), was also included in this consensus classification (Gorno-Tempini *et al.*, 2004). However, the lgPPA has been consistently related to Alzheimer's disease and consequently it will not be considered within the FTLD-S spectrum (Bergeron *et al.*, 2018; Spinelli *et al.*, 2017).

nfvPPA is an heterogeneous syndrome characterized by disgrammatism and/or motor speech deficits (i.e. apraxia of speech) with prominent left interior frontal and insular atrophy (Grossman, 2012; Josephs, 2006; Ogar *et al.*, 2007). Some authors have proposed the splitting of nfaPPA in two distinct syndromes: "progressive apraxia of speech" and "agrammatic aphasia". These authors argue that patients with isolated apraxia of speech show focal imaging abnormalities in premotor cortex, whereas patients with predominant agrammatism show more widespread involvement affecting premotor, prefrontal, temporal and parietal lobes, caudate and insula (Josephs *et al.*, 2012). However, more studies are needed to determine the potential for improving clinical-pathologic correlations in the nfaPPA variant. The median survival is 9.4–10.6 years and many patients develop motor syndromes during follow-up consistent with the diagnosis of CBS or PSPS (Armstrong *et al.*, 2013; Höglinger *et al.*, 2017; Irwin, 2016; Kovacs, 2015; Matías-Guiu *et al.*, 2014). From a neuropathological perspective, most patients are found to have a 4R tauopathy as illustrated in **Figure 1** (Bergeron *et al.*, 2018; Spinelli *et al.*, 2017).

Finally, svPPA is characterized by loss of object and word knowledge with strikingly focal anterior temporal atrophy (Collins *et al.*, 2017; Gorno-Tempini *et al.*, 2011; Grossman and Irwin, 2018; Mesulam *et al.*, 2014). Patients with svPPA have a primary impairment in semantic memory, therefore exceeding an isolated language dysfunction. Most of the evidence accrued over the last decades indicates that the language deficit in svPPA patients is multimodal in nature, which explains their difficulties in semantic tests involving different modalities of input such as language vision, sounds, smells and tactile sensation (Bozeat *et al.*, 2000; Luzzi *et al.*, 2007). This group is characterized by the longest survival (6.9–11.9 years) and most of the cases present FTLD-TDP type C at autopsy, as shown in **Figure 1** (Bergeron *et al.*, 2018; Spinelli *et al.*, 2017).

Motor syndromes related with FTLD

The diagnosis of FTD is predictive of a neuropathological diagnosis of FTLD in most cases (Elahi and Miller, 2017). However, this concept of FTD excludes clinical presentations of FTLD with a prominent motor impairment at symptom onset.

The ALS-FTD spectrum. Although the first observation of a clinical overlap between ALS and FTD dates from the beginning of the twentieth century (Braumühl 1932; Al-Chalabi *et al.*, 2016; Bak 2010; Brown and Al-Chalabi, 2017; Woolley and Strong, 2015), the study of the clinical and neuropathological overlap between ALS and FTD has been boosted in the last decade due to the emerging molecular evidence relating ALS and FTLD (Al-Chalabi *et al.*, 2012; Burrell *et al.*, 2016; Ng *et al.*, 2015; Swinnen and Robberecht, 2014).

From a clinical perspective, the development of specific diagnostic tools has allowed the in-depth study of the cognitive, language and behavioral changes in patients with ALS (Crockford *et al.*,

2018; Niven *et al.*, 2015). Recent data suggest that up to 50% of ALS patients show some form of cognitive impairment while up to 60% of the informants of ALS patients may report behavioral symptoms (Crockford *et al.*, 2018; Woolley and Strong, 2015). The concurrence of ALS and FTD phenotypes is also observed with a prevalence of full-blown FTD syndrome among ALS patients varying from 10-20% (Burrell *et al.*, 2016; Lillo *et al.*, 2012; Swinnen and Robberecht, 2014; Woolley and Strong, 2015). Moreover, ALS patients may present with language impairments that partially overlap with those observed in PPA (Ash *et al.*, 2014; 2015; Van Langenhove *et al.*, 2017), but with some distinctive features (Bak and Chandran, 2012; Grossman *et al.*, 2008).

From a neuropathological perspective there is also a significant overlap between ALS and FTLD as shown in **Figure 2**. Nearly 95% of ALS cases have TDP-43 pathology while a minority (<5%) have FUS pathology (Brettschneider *et al.*, 2013; Brettschneider, Arai, *et al.*, 2014; Brettschneider, Del Tredici, *et al.*, 2014). Indeed, ALS-TDP and FTLD-TDP are considered two extremes of a neuropathological continuum. At one extreme, ALS is characterized by neurodegeneration in motor cortex with variable impairment of frontal and temporal structures depending on the presence of cognitive and/or behavioral impairment (Whalhout *et al* 2015; Schuster *et al.*, 2014; Schuster *et al.*, 2013; Prudlo *et al.*, Neurology 2016). At the other extreme, FTLD is characterized by a prominent neurodegeneration of frontotemporal structures with neurodegenerative cores located at the anterior cingulate, insula, and anterior temporal pole and a variable neurodegenerative burden depending on disease stage and FTLD subtype (Seeley *et al.*, 2008; Seeley *et al.*, 2009; Seeley *et al.*, 2010; Nana *et al.*, 2018; Borroni, Cosseddu, *et al.*, 2015). Two distinct, but partially overlapping staging systems, have been proposed for TDP pathology in ALS and FTLD-TDP (Brettschneider *et al.*, 2013; Brettschneider, Arai, *et al.*, 2014; Brettschneider, Del Tredici, *et al.*, 2014).

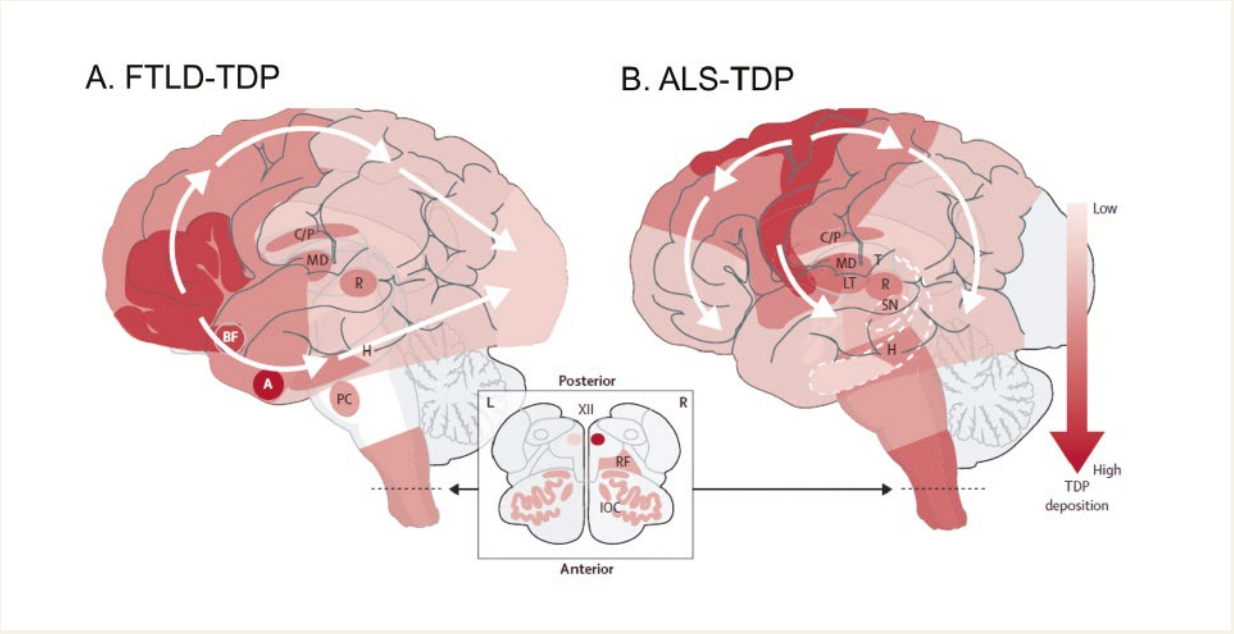

Figure 2. Neuropathological staging schemes in FTLD-TDP and ALS-TDP. Neuropathological staging schemes in: (A) FTLD-TDP (Braak et al., Nat Rev Neurol 2013) and (B) ALS-TDP (Brettschneider et al., Ann Neurol 2013): Adapted from Burrell et al., Lancet Neurol 2016). **Abbreviations:** FTLD-TDP = frontotemporal lobar degeneration with TAR DNA binding protein inclusions; ALS-TDP = amyotrophic lateral sclerosis with TAR DNA binding protein inclusions

Recent diagnostic criteria allow the classification of patients along the ALS-FTD continuum in three broad categories: (i) ALS without cognitive impairment; (ii) ALS with cognitive and/or behavioral impairment; and (iii) ALS-FTD for the patients with a full-blown FTD syndrome (Strong *et al.*, 2017). However, despite significant advances made in the last years further tools are needed for an objective staging of patients along the ALS-FTD continuum (Cykowski *et al.*, 2017; Henstridge *et al.*, 2017; Prudlo *et al.*, 2016).

The CBS-PSPS spectrum. PSP and CBD were originally described as two distinct atypical extrapyramidal syndromes (Gibb *et al.*, 1989; Steele *et al.*, 1964; 2014). However, recent literature refines these seminal descriptions by showing the existence of a substantial clinical, genetic and neuropathologic overlap between these two diseases (Burrell *et al.*, 2018; Irwin, 2016; Kobylecki *et al.*, 2015). From a neuropathological perspective, PSP and CBD are now included in the neuropathology spectrum of FTLD as 4R Tauopathies and genetic studies have shown common genetic risk factors between PSP and CBS, as well as between FTD and 4R tauopathies (Mackenzie *et al.*, 2009; 2010; Yokoyama *et al.*, 2017). From a clinical perspective, there is also a substantial clinical overlap between CBS and PSPS (**Table 2**). The new international diagnostic criteria for both CBS and PSPS reflect the clinical overlap between these clinical syndromes and FTD (Armstrong *et al.*, 2013; Höglinger *et al.*, 2017). In the case of PSP, "variant PSP syndromes" (i.e. nfaPPA and bvFTD) have been included in the recent movement disorder society criteria PSP criteria (Boxer *et al.*, 2017). The early identification of these phenotypes offers an opportunity to perform the diagnosis of PSP in non-motor phases, predating the canonical motor phase. Seemingly, in the case of CBS, a language and a behavioral phenotype (nfaPPA and fronto-spatial syndrome) have been included in the new CBD diagnostic criteria (Armstrong *et al.*, 2013). Overall, these new diagnostic criteria acknowledge that some FTLD-S (nfaPPA and bvFTD) may predate, occur simultaneously or follow the prototypical motor phenotypes that were recognized in the classical PSP and CBD diagnostic criteria (Litvan *et al.*, 1996; Riley *et al.*, 1990). Notwithstanding, despite significant advances it remains difficult to predict the specific pathology (i.e. PSP or CBD) in cases with suspected underlying 4R tauopathy (Caso *et al.*, 2014; Lleo *et al.*, 2018; Santos-Santos *et al.*, 2016). Thus, novel biomarkers are needed to identify the molecular underpinnings of FTLD-S and predict disease progression at the individual patient level (Elahi and Miller, 2017; Grossman, 2014; Meeter *et al.*, 2017; Tsai and Boxer, 2016).

Specificity of CBS-PSPS clinical features	CBD-related clinical features	PSP-related clinical features
Relatively-specific clinical features at symptom onset	Ideomotor / orobucal apraxia Myoclonus Dystonia Cortical signs / alien hand Visuospatial symptoms	Postural instability Supranuclear oculomotor palsy Freezing of gait
Unspecific clinical features at symptom onset	nfaPPA (including progressive apraxia of speech) Behavioral-disexecutive syndrome (bvFTD) Akinetic (atremoric) > rigid syndrome Macro square wave jerks, urinary incontinence, dysphagia	

Table 2. Overlap of clinical phenotypes within the CBS-PSPS clinical spectrum.

Epidemiology of FTLD-S

The exact prevalence of FTLD-S is unknown because these disorders are still frequently missed and misdiagnosed and thus, most approximations probably underestimate its true prevalence (Bang *et al.*, 2015). The prevalence of the core FTD syndromes (bvFTD, nfaPPA and svPPA) has been estimated to range from 15 to 22 per 100,000 people but it should be noted that until recently epidemiological studies have only considered a limited array of the FTLD-S (Coyle-Gilchrist *et al.*, 2016; Nilsson *et al.*, 2014). Keeping these limitations in mind, it is widely accepted that FTLD-S are a common cause of neurodegenerative dementia, especially among early-onset dementias (defined by a symptom onset less than 65 years) where it represents the second most common cause of dementia following Alzheimer's disease (Snowden *et al.*, 2011). Consequently, FTD is the second cause of dementia in the presenile age group (defined by an age at symptom onset less than 65 years) and accounts for 5–15% of all cases of dementia, with a prevalence of 3–26 per 100,000 people in the 45–65 age bracket (Vieira *et al.*, 2013). Although FTD has been classically described as an early-onset dementia, its incidence most prevalent in people aged between 60-69 years old and only 13% have an onset before the age of 50 (Knopman and R. O. Roberts, 2011; Neary *et al.*, 2005; Onyike and Diehl-Schmid, 2013; Ratnavalli *et al.*, 2002). Moreover, FTD might be more common than assumed among older adults because as this population rarely undergoes the type of investigation needed to establish a confident diagnosis *in vivo* and are not generally followed to autopsy (Marelli *et al.*, 2015; Piguet *et al.*, 2011). Indeed, 20 to 40% of the FTD cases may occur after 65 year of age and some of the FTLD-S (especially those with underlying FTLD-Tau) are an increasingly-recognized cause of late-onset dementia (Borroni *et al.*, 2008; Johnson *et al.*, 2005; Seo *et al.*, 2018; Shinagawa *et al.*, 2008; Ye *et al.*, 2015).

Frontotemporal lobar degeneration (FTLD)

Historical background. Frontotemporal Lobar Degeneration (FTLD) is a neuropathological umbrella term that encompasses distinct neuropathological entities sharing overlapping patterns of frontal and/or temporal gray atrophy and distinct neuroglial proteinaceous inclusions (Lashley *et al.*, 2015; Mackenzie and Neumann, 2016). Historically, the concept of FTLD has evolved over the last 130 years. The first descriptions of FTLD was made by Arnold Pick, one of the forefathers of modern cognitive neurology, between 1892 and 1906 (Pick 1982; Pick 1901; Pick 1904; Pick 1905; Pick 1906). Arnold Pick described a number of patients with progressive aphasia with circumscribed cerebral atrophy at autopsy (Derouesné, 2014). But these pioneer observations also included some patients with progressive personality change where he ultimately observed a focal atrophy of the frontal and/or temporal lobes. However, Arnold Pick was unable to determine the specific neuropathological signature of these seminal FTLD cases and two of these cases were since found to be atypical cases of Alzheimer's disease (Brion *et al.*, 1991). Ultimately, it was Alois Alzheimer who described the argyrophilic globular neuronal cytoplasmic inclusions that he named "Pick bodies". In the following decades, the heterogeneity of FTLD cases became apparent and the FTLD classification was progressively refined in the light of several major milestones (**Figure 3**). In the 1990s the tau isoform composition of sporadic tauopathies (PSP, CBD, Pick disease) was identified and in 2006, TDP-43 was found to be the main component of ubiquitin-positive FTLD cases without pick bodies (Rademakers *et al.*, 2012). Finally, the discovery of the FUS-positive inclusion in some FTLD cases and identification of *FUS* and *C9orf72* genes provided additional evidence supporting the overlap between FTLD and amyotrophic lateral sclerosis ALS (Liscic *et al.*, 2008; Rademakers *et al.*, 2012).

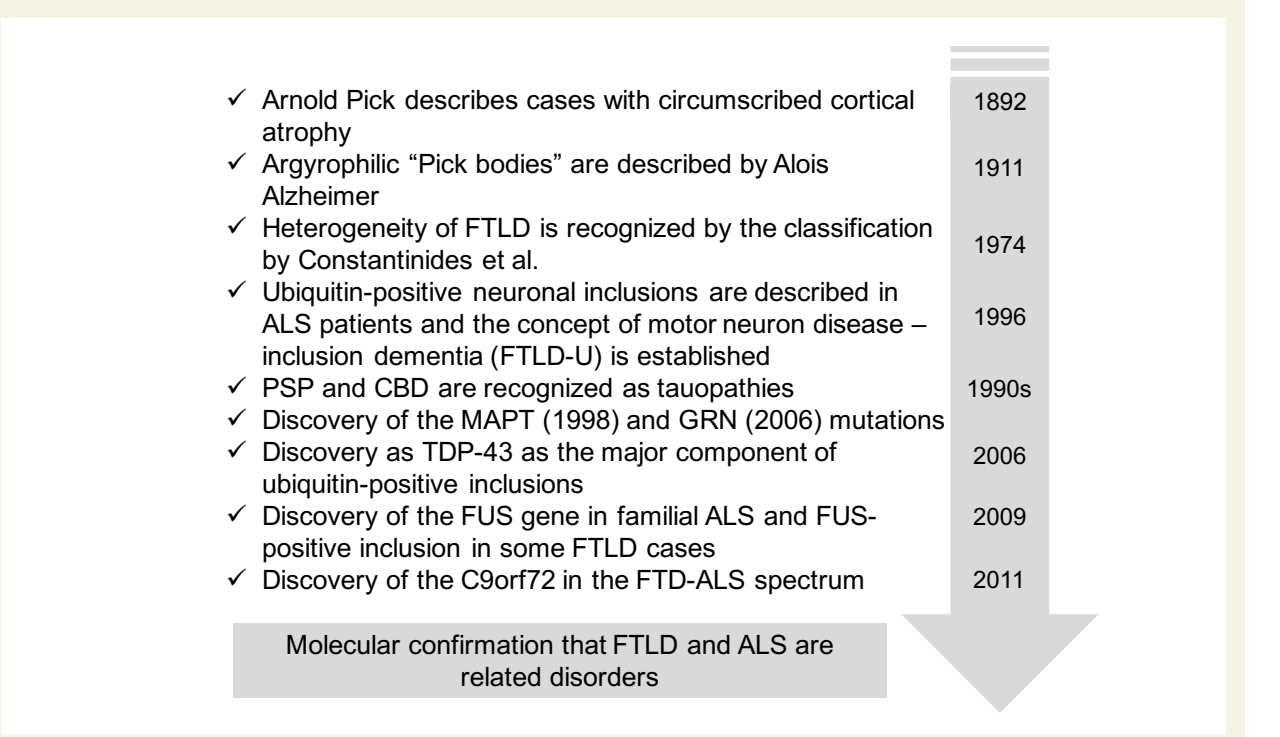

Figure 3. Major milestones of the evolution of the FTLD concept Adapted from Lashley *et al.*, Neuropathol Appl Neubiol 2015 and Rademakers *et al.*, Nat Rev Neurol 2012.

Neuropathological classification of FTLD. FTLD is characterized by prominent neurodegeneration of the frontal and temporal lobes and encompasses several neuropathological entities. Traditionally, FTLD was characterized by the presence and the morphology of abnormal intracellular protein accumulations demonstrated by classical histochemical techniques (Lashley *et al.*, 2015; Mackenzie and Neumann, 2016). Modern laboratory techniques, such as immunohistochemistry, have contributed to our understanding of the biochemical composition of these protein aggregates. These findings have allowed the refinement of FTLD classification according to the characterization of protein accumulations (Mackenzie and Neumann, 2016; Mackenzie *et al.*, 2009; 2010; 2011). The most recent classification scheme of FTLD recognizes four major subgroups (Lashley *et al.*, 2015; Mackenzie and Neumann, 2016; Rademakers *et al.*, 2012). Most FTLD cases (90–95%) are classified within the FTLD-tau or FTLD-TDP groups due to the abnormal accumulation of hyperphosphorylated tau or 43 kDa transactive response DNA-binding protein (TDP-43), respectively. The remaining 5–10% of cases are classified within the FTLD-FET (FET = Fused in sarcoma, Ewing's sarcoma, TATA-binding protein-associated factor 15) subgroup. Lastly, in a small proportion of FTLD cases the protein nature of the ubiquitin-p62-positive inclusions has not been identified. This group has been termed the FTLD-ubiquitin proteasome system (FTLD-UPS) group and is represented mostly by cases of affected individuals of a Danish pedigree due to mutation in the charged multivesicular body protein 2B (*CHMP2B*) gene (Skibinski et *al.*, 2005). Each of these FTLD groups can be further subclassified in different neuropathological entities (i.e. CBD and PSP) or FTLD subgroups. **Table 3** summarizes the current classification of FTLD and the neuropathological hallmarks of each of the FTLD subgroups.

FTLD group (aggregated protein/s)	Related gene/s	Topography of neurodegeneration and immunohistochemical (IHC) hallmarks for each FTLD subgroups
FTLD-Tau (Tau)	MAPT	**IHC reactivity: 3R > 4R** **Pick disease:** Topography of neurodegeneration = Frontotemporal; IHC = argyrophilic neuronal cytoplasmic inclusions called "Pick bodies" positive for 3R tau. **IHC reactivity: 4R > 3R** **PSP:** Topography of neurodegeneration = Subcortical degeneration > cortical degeneration; IHC = "globose" or "flame-shaped" neurofibrillary tangles in subcortical nuclei and tufted astrocytes positive for 4R tau. **CBD:** Topography of neurodegeneration = Peri-rolandic gray and white matter degeneration > subcortical degeneration; IHC = "astrocytic plaques" and ballooned achromatic neurons in the cerebral cortex (positive for 4R tau). **GGT:** Topography of neurodegeneration = Prominent white matter degeneration; IHC = Globular inclusions in oligodendrocytes and astrocytes (4R tau). **AGD:** Topography of neurodegeneration = Amygdala and fronto-temporal cortex; IHC = small argyrophilic dot-like spindle-shaped structures (grains) **IHC reactivity: 3R≈4R (variable)** **MAPT mutations:** Topography of neurodegeneration = frontotemporal structures but variable; IHC = no distinct features
FTLD-TDP (TDP-43)	C9orf72 PGRN VCP TARDBP SQSTM1 TBK1	**Type A:** Topography of neurodegeneration = Neocortex (layer II), subcortical nuclei and white matter; IHC = Neuronal cytoplasmic inclusions, neuronal intranuclear inclusions (PGRN mutations); **Type B:** Topography of neurodegeneration = Neocortex (superficial and deep layers), white matter, subcortical nuclei, medulla, and spinal cord; IHC = Neuronal cytoplasmic inclusions (but few dystrophic neurites and neuronal intranuclear inclusions) and glial cytoplasmic inclusions. **Type C:** Topography of neurodegeneration = Neocortex (superficial cortical layers); IHC = Long dystrophic neurites with few or no neuronal cytoplasmic inclusions (hippocampus), glial cytoplasmic inclusions and neuronal intranuclear inclusions. **Type D (only found in VCP mutations):** Topography of neurodegeneration = Neocortex and subcortical nuclei with sparing of the hippocampus, lower brainstem and cerebellum; IHC = Neuronal intranuclear inclusions and short dystrophic neurites with rare neuronal cytoplasmic inclusions.
FTLD-FET (FUS, EWS and TAF15)	FUS*	**aFTLD-U:** Topography of neurodegeneration = Frontal and temporal cortex, anterior striatum and hippocampal sclerosis; IHC = Neuronal cytoplasmic inclusions (for all FET proteins, ubiquitin and p62). **NIFID:** Topography of neurodegeneration = Cortex, subcortical nuclei, cerebellum, brainstem and spinal cord; IHC = Neuronal cytoplasmic inclusions/ Neuronal intranuclear inclusions reactive for both class IV neuronal IF and FET proteins. **BIBD:** Topography of neurodegeneration = Variable; IHC = Neural cytoplasmic basophilic inclusions and variable Neuronal cytoplasmic inclusions of FET proteins.
FTLD-UPS (Ubiquitin and p62)	CHMP2B	Topography of neurodegeneration = Variable; IHC = inmunoreactive to ubiquitin and p62 but negative for Tau, TDP-43, FET proteins

Table 3. Neuropathological classification of FTLD. *only related with familial ALS; **Abbreviations:** 3R/4R: three/four repeats; AGD: Argirophylic gran disease; GGT: Globular glial tauopathy; PSP: Progressive supranuclear palsy; CBD: Corticobasal degeneration; FTLD: Frontotemporal lobar degeneration; FUS: Fused in sarcoma; EWS: Ewing's sarcoma; TAF15: TAT-binding protein-associated factor 15; NIFID: Neuronal intermediate filament inclusion disease; BIBD: Basophilic inclusion body disease; IHC: Immunohistochemistry; aFTLD-U: Atypical frontotemporal lobar degeneration with ubiquitin-positive inclusions

Genetics of FTLD. FTLD is a highly heritable disorder with approximately 30-60% reporting a positive familiar history (Rohrer *et al.*, 2009; Wood *et al.*, 2013). Among cases with a positive familial history, 10-20% will harbor one causal mutation (Deleon and Miller, 2018). Notwithstanding, a genetic cause can still be found in about 6% of patients without a known family history of FTD (Elahi and Miller, 2017; Rademakers *et al.*, 2012). From a clinical perspective, patients presenting with the bvFTD and patients manifesting both ALS and FTD are most likely to be genetic while svPPA is most likely sporadic (Dols-Icardo *et al.*, 2018; Rohrer *et al.*, 2009; Seelaar *et al.*, 2009). Although genetic cases tend to be younger at disease onset than sporadic cases, each mutation is associated with a wide range of age at symptom onset. The three commonest genes associated with FTLD are *C9orf72*, *GRN* and *MAPT* (Ghetti *et al.*, 2015). The relative frequency and the main characteristics of FTLD-related mutations are summarized in **Supplementary Table 1**.

The study of the genetic architecture of FTLD has revealed important information about the FTLD pathophysiology and potential therapeutical targets (Ling *et al.*, 2013; Philips and Robberecht, 2011). Of note, recent studies investigating the genetic architecture of FTLD have linked FTLD with the amyloid precursor protein (APP), a protein that has been involved in Alzheimer's disease pathophysiology (Ferrari *et al.*, 2017).

Monitoring disease progression in FTLD-S

The diagnosis of mild FTLD-S. The spectrum of clinical syndromes associated with FTLD has expanded considerably in recent years (Hodges and Piguet, 2018). The new diagnostic criteria now allow the diagnosis of behavioral, language and motor phenotypes at mild disease stages where structural and metabolic imaging techniques are frequently uninformative (Boxer *et al.*, 2017; Chow *et al.*, 2008; Höglinger *et al.*, 2017; Josephs *et al.*, 2014; Mesulam *et al.*, 2012). Consequently, in the absence of specific pathophysiologic biomarkers the diagnosis of FTLD-S at milder stages has aroused new concerns (Kipps *et al.*, 2010). Particularly, some authors have emphasized the risk of false positive diagnostics in bvFTD cases lacking structural or metabolic changes in neuroimaging studies (Krudop *et al.*, 2017; Shinagawa *et al.*, 2016). Additionally, recent studies have shown that a subgroup of bvFTD cases without neuroimage changes may represent non-neurodegenerative cases (Devenney *et al.*, 2016; Gossink *et al.*, 2016). The term "phenocopies" has been proposed to label cases fulfilling the diagnostic criteria for possible bvFTD but failing to progress to frank dementia over time (Devenney *et al.*, 2018; Hornberger *et al.*, 2009; Kipps *et al.*, 2010). However, the existence of a "slowly-progressive" variant of bvFTD characterized by a slower cognitive decline and milder cortical atrophy has been consistently documented by several groups (Davies *et al.*, 2006; Khan *et al.*, 2012). In a recent longitudinal study, 70% of the patients classified as possible bvFTD at the first clinical encounter were reclassified as probable bvFTD during follow-up (at least three years of follow-up) (Devenney *et al.*, 2018). However, the remaining 30% failed to show clinical deterioration or structural abnormalities by neuroimaging during follow-up. In this study, a *C9orf72* mutation carrier was identified among the non-progressive group, a finding that had been previously reported (Devenney *et al.*, 2018; Gómez-Tortosa *et al.*, 2014; Khan *et al.*, 2012; Llamas-Velasco *et al.*, 2018). Thus, the true nature of patients with possible bvFTD remains controversial and additional biomarkers may contribute to either confirm or discard the neurodegenerative etiology in these cases (Vijverberg *et al.*, 2016; 2017). For example, in a particular patient classified as possible bvFTD at first evaluation due to the absence of significant cerebral atrophy on neuroimaging, the application of a new imaging biomarker able to detect subtle structural changes may support

the diagnosis of a neurodegenerative disease (Rascovsky and Grossman, 2013). Seemingly, other biomarkers of different modalities such as cerebrospinal fluid (CSF), may allow the exclusion of Alzheimer's disease pathophysiology as well as the identification of the molecular signature of the different FTLD subtypes.

Measuring disease severity in FTLD-S. In FTLD-S, disease progression ultimately leads to cognitive, behavioral, motor and functional deterioration (Mioshi *et al.*, 2007; Piguet *et al.*, 2011). Functional disability is an important tool for the estimation of disease severity, which in turn represents an indirect way of monitoring FTLD-related neurodegeneration. Clinical tools aiming at monitoring disease severity in FTLD-S need to consider specific aspects of the different phenotypes for an accurate staging of patients. In the last decade, significant advances have been made in our understanding of functional disability in FTLD-S (Garcin *et al.*, 2009; Mioshi and Hodges, 2009; Mioshi *et al.*, 2007; Rascovsky *et al.*, 2005). This has led to the development and validation of specific measures of disease severity and progression in FTLD-S: the FTLD-CDR (Knopman *et al.*, 2008) and the Frontotemporal Dementia Rating Scale (FRS) (Mioshi *et al.*, 2010). In addition, some specific forms of FTLD-S such as ALS and PSPS, due to the specific topography of the syndrome, have their own specific staging tools: the ALSFRS-R and the PSPRS, respectively (Cedarbaum *et al.*, 1999; Golbe and Ohman-Strickland, 2007). Although, the development of specific tools for assessing disease severity in FTLD-S represents a significant advance, these remain a proxy measure of FTLD-related neurodegeneration and often rely on subjective measures and informant-based questionnaires. In this sense, biomarkers could represent valuable tools for the objective assessment of disease severity in FTLD-S. Indeed, biomarkers may avoid reliance on subjective clinical features such as "emotional coldness" and may provide an objective quantification of the FTLD-related neurodegenerative burden. Specifically, biomarkers may improve our ability to predict disease progression at the individual patient level by identifying patients at higher risk of rapid progression (Swinnen and Robberecht, 2014). This may be particularly relevant for the design of clinical trials for syndromes characterized by a heterogeneous disease course such as the ALS-FTD spectrum (Westeneng *et al.*, 2018). Finally, biomarkers are objective measures that can improve both diagnostic accuracy and disease prognosis at the single subject level (Elahi *et al.*, 2017; Gaiani *et al.*, 2017; Steinacker *et al.*, 2017). These are key aspects for the design of clinical trials for FTLD-S (Tsai and Boxer, 2016).

Biomarkers for the study of FTLD

Biomarkers: definition and potential applications

A biomarker can be defined as an indicator of a physiological or pathological biological process that can be objectively measured *in vivo* (Lleo *et al.*, 2015). Biomarkers can be classified in different modalities depending on the technique on which they are based. For the study of neurodegenerative diseases, the main modalities are: (i) fluid biomarkers (i.e. blood or CSF); (ii) imaging biomarkers (i.e. structural imaging with MRI or FDG PET) and (iii) genetic biomarkers (i.e. *APOEε4*). Biomarkers can be used for four main purposes: (i) to guide clinical diagnosis by identifying key pathophysiological changes of a particular disease (so-called diagnostic markers), (ii) to estimate the risk or speed of progression of a particular disease (prognostic markers), (iii) to evaluate disease stage (staging markers), and (iv) to monitor progression or response to therapy (theragnostic markers). Thus, biomarkers may be important tools for the enrichment of both clinical studies and clinical trials. For example, the identification of patients at higher risk of progression may allow the equal distribution

of these "higher risk patients" across the placebo and treatment group. Ideally, biomarkers should be accurate, consistent, non-invasive and inexpensive (Illán-Gala *et al.*, 2018).

Cerebrospinal fluid biomarkers

CSF represents an invaluable tool for the study of neurodegenerative diseases due to its close relationship to the brain parenchyma and the relatively low concentration of proteins in comparison with other biofluids, such as blood (Zetterberg *et al.*, 2017). The CSF can be safely obtained in humans through a lumbar puncture (Alcolea, Martinez-Lage, *et al.*, 2014; Duits *et al.*, 2015; Engelborghs *et al.*, 2017). After performing this single procedure, the collection of CSF allows the study of multiple pathophysiological pathways and its storage for future analyses. The study of CSF has been recommended in the clinical setting for the exclusion of Alzheimer's disease in FTLD-S and it represents a relatively affordable option compared to other imaging studies such a positron emission tomography (PET) (Blennow et al., 2015; Simonsen *et al.*, 2017). Many biomarkers can be measured nowadays in CSF via enzyme-linked immunosorbent assay (ELISA). The most promising diagnostic and prognostic biomarkers in FTLD-S are presented below.

CSF biomarker for the differentiation of FTLD from Alzheimer's disease. The core CSF biomarkers for Alzheimer's disease are phospho-tau$_{181}$ (p-tau), total-tau (t-tau), and amyloid-β_{1-42} ($A\beta_{1-42}$). These peptides are related to two key pathophysiological features of Alzheimer's disease: the accumulation of hyperphosphorylated tau in neurofibrillary tangles (increased levels of t-Tau and p-tau) and extraneuronal Aβ deposition in plaques (decreased levels of $A\beta_{1-42}$) (Olsson *et al.*, 2016; Tapiola *et al.*, 2009). Additionally, the $A\beta_{1-42}$:$A\beta_{1-40}$ and $A\beta_{1-42}$:$A\beta_{1-38}$ ratios have shown a good agreement with the cerebral deposition of amyloid as assessed by PET (Janelidze *et al.*, 2016). These biomarkers have been comprehensively validated to exclude Alzheimer's disease in the diagnostic work-up of FTLD-S in pathology-confirmed cohorts (Bian *et al.*, 2008; Lleo *et al.*, 2018; Pijnenburg *et al.*, 2015; Tapiola *et al.*, 2009; Toledo *et al.*, 2012). For a detailed description of pathology-proven CSF studies in FTLD please refer to **Supplementary Table 1**. Core CSF biomarkers for Alzheimer's disease enable an accurate identification of patients with underlying Alzheimer's disease, both as a primary pathology and as a concurrent pathology, along the FTLD-S (Lleo *et al.*, 2018; Toledo *et al.*, 2012). Accordingly, current diagnostic criteria for the bvFTD, CBS and PSPS consider the use of CSF biomarkers to identify cases with a positive Alzheimer's disease biomarker profile in order to label those case with a lower degree of certainty (Armstrong *et al.*, 2013; Höglinger *et al.*, 2017; Rascovsky *et al.*, 2011).

Staging and prognostic CSF biomarkers in FTLD-S. Neurofilaments are the major constituent of the neuro-axonal cytoskeleton and play an important part in axonal transport and in the synapse (Yuan *et al.*, 2015). Neurofilament light chain (NfL) is the most abundant and soluble neurofilament subunit, and increased levels are thought to reflect axonal damage. Blood and CSF levels of NfL are increased in FTLD-S when compared to controls, and the clinical value of this protein lies in its correlation with disease severity and progression, survival, and cerebral atrophy (Landqvist Waldö *et al.*, 2013; Meeter *et al.*, 2016; 2018; Rohrer *et al.*, 2016; Scherling *et al.*, 2014; Skillbäck *et al.*, 2014; Wilke *et al.*, 2016). Levels of NfL are universally elevated in bvFTD, nfvPPA and svPPA, and are strongly increased in FTD with MND (Landqvist Waldö *et al.*, 2013; Meeter *et al.*, 2016; Pijnenburg *et al.*, 2015; Rohrer *et al.*, 2016; Scherling *et al.*, 2014). The CSF NfL levels seem to increase over time but further longitudinal data in FTLS-S is needed (Lu *et al.*, 2015; Rojas *et al.*, 2016).

Neuroinflammation biomarkers. Neuroinflammation plays an important role in FTLD and ALS pathophysiology (Heneka *et al.*, 2014; Philips and Robberecht, 2011). Accordingly, the CSF levels of chitinase-3-like protein 1 (also known as YKL-40), an inflammatory protein produced by astrocytes, were found to be elevated in FTD, but also in Alzheimer's disease, normal ageing and multiple sclerosis (Alcolea *et al.*, 2015; Comabella *et al.*, 2010; Craig-Schapiro *et al.*, 2010; Philips and Robberecht, 2011; Querol-Vilaseca *et al.*, 2017; Teunissen *et al.*, 2016). High levels of YKL-40 might be due to the activation of inflammatory pathways associated with neurodegeneration (Querol-Vilaseca *et al.*, 2017; Alcolea, Carmona-Iragui *et al.*, 2014; Alcolea, Vilaplana, *et al.*, 2015) and neuroinflammation has been highlighted as a key element of FTLD and ALS pathophysiology (Heneka *et al.*, 2014; Philips and Robberecht, 2011). Although YKL-40 lacks disease specificity, this *in vivo* marker could provide some information about the underlying pathology. However, evidence regarding the CSF levels of YKL-40 in the ALS-FTD continuum and its impact on disease progression is scarce (Bonneh-Barkay *et al.*, 2010). In the ALS-FTD continuum, microglial activation has been related to motor neuron death and faster disease progression (Boillee *et al.*, 2006; Frakes *et al.*, 2014; Yamanaka *et al.*, 2008). Thus, the combined study of a CSF biomarker of neuroinflammation, such as YKL-40, and clinical measures of disease progression in patients within the ALS-FTD continuum may allow the stratification of patients according to their expected progression rate. This information is critical for the design of clinical trials as it may permit the stratification of participants according to their expected disease progression rate at their inclusion in a clinical trial. Moreover, patients with higher inflammatory activity may benefit from specific anti-inflammatory treatment aiming at reducing progression rate while these same treatments may not be effective in patients without increased inflammatory activity.

APP-derived peptides. The amyloid precursor protein (APP) is a type I single-pass transmembrane protein with a large extracellular domain and a short cytoplasmic tail (**Figure 4**). APP undergoes very complex proteolytic processing, yielding biologically active fragments that may each have specific functions (Muller *et al.*, 2017). The cleavage of APP by β- and γ-secretase leads to the extracellular release of the amyloid β peptide (Aβ) and the N-terminal part of APP (sAPPβ) y the so-called 'amyloidogenic pathway'. APP can also be processed by α-secretase, which results in the release of soluble APPα (sAPPα) but not Aβ peptides in the so-called 'non-amyloidogenic pathway' (**Figure 4**). In normal conditions, sAPPα and sAPPβ constitute at least 50% of the total forms of APP in the brain. The remaining 50% of APP forms include Aβ peptides of variable lengths (Muller *et al.*, 2017). These include the full length $A\beta_{1-42}$ and other N-terminal and C-terminal truncated forms of Aβ, such as $A\beta_{1-15}$, $A\beta_{1-38}$, $A\beta_{1-40}$, among others, that can also be detected in human CSF (Ghidoni *et al.*, 2011; Nhan *et al.*, 2015; Portelius *et al.*, 2010; 2011). In Alzheimer's disease, the CSF levels of $A\beta_{1-42}$ are a reflection of cerebral amyloid deposition and are considered a core Alzheimer's disease biomarker (Zetterberg *et al.*, 2017; Jack *et al.*, 2016). However, numerous studies suggest that reduced neuronal/synaptic activity may also lead to less Aβ production in general (Cirrito *et al.*, 2005) and Aβ may also have an important role in synaptic function (Mucke and Selkoe, 2012). Interestingly, many FTLD cases have low CSF levels of $A\beta_{1-42}$ despite a negative amyloid PET and the nature of the discordance between CSF and imaging biomarkers of amyloid pathology in FTLD-S is unclear (for further details please refer to **Supplementary Table 1**) (Janelidze *et al.*, 2016; Leuzy *et al.*, 2016). Although some authors argue that coincident Alzheimer's disease pathology in FTLD cases may drive this observation, evidence from pathology-proven cohorts has shown decreased levels of $A\beta_{1-42}$ in both FTLD-tau and FTLD-TDP cases in the absence of

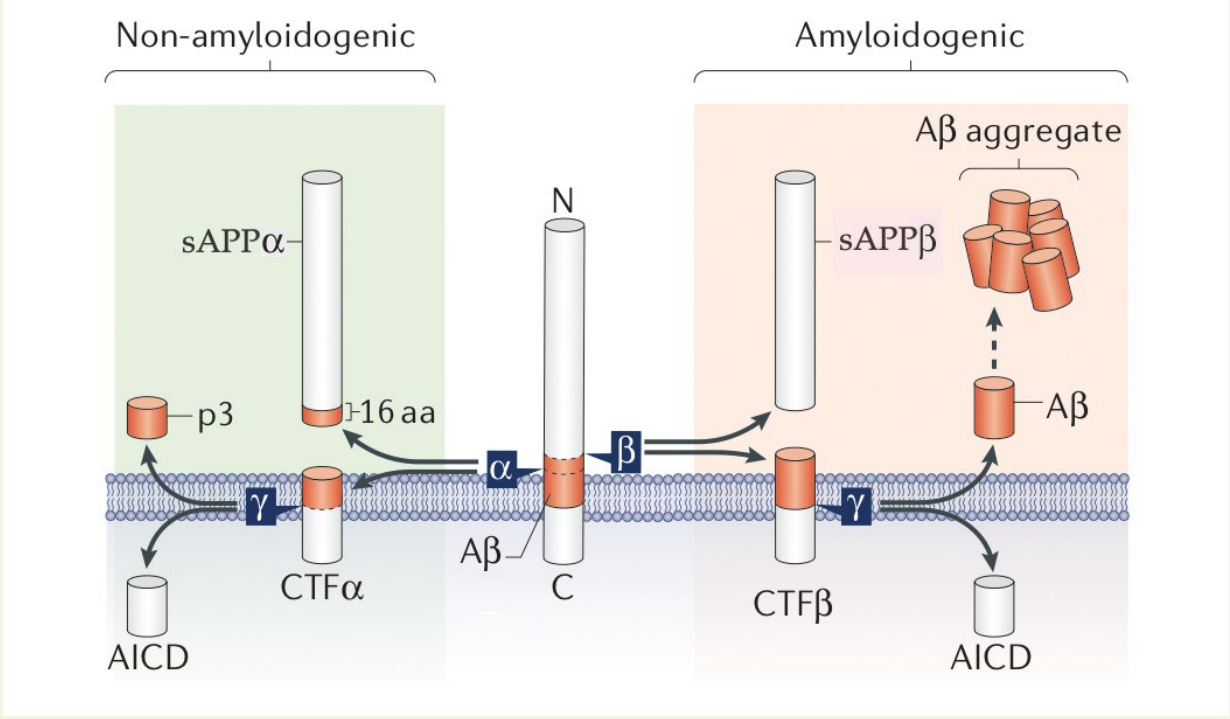

Figure 4. Schematic overview of canonical APP-processing pathways. Processing. Processing by α-secretase along the non-amyloidogenic pathway (green background) occurs in the amyloid-β (Aβ) region (shown in red), liberates sAPPα (α-secretase-generated APP ectodomain fragment) and generates p3. By contrast, processing along the amyloidogenic pathway (red background) generates Aβ (through β-secretase and γ-secretase cleavage) and liberates sAPPβ. An intracellular fragment (APP intracellular domain (AICD)) is released in both pathways. The positions of cleavage sites are indicated. Adapted from Muller *et al.*, Nat Rev Neurosci 2017.

significant comorbid Alzheimer's disease (Toledo *et al.*, 2012) and recent studies have provided new evidence linking FTLD and the CSF levels of APP-derived peptides (Paolicelli *et al.*, 2017; Bright *et al.*, 2015). Moreover, $A\beta_{1-42}$ levels were found to be decreased in up to 25% of patients with the *C9orf72* repeat expansion (Kämäläinen *et al.*, 2015; Wallon *et al.*, 2012).

Other APP-derived peptides have been reported as potential biomarkers in FTLD-S. FTLD-S. In some studies, $A\beta_{1-38}$ and $A\beta_{1-40}$ have been found to be decreased in CSF in FTLD syndromes when compared to controls (Bibl *et al.*, 2007; Gabelle *et al.*, 2011; Pijnenburg *et al.*, 2007; Struyfs *et al.*, 2015; Verwey *et al.*, 2010). However, some studies did not replicate this finding (Steinacker *et al.*, 2009; Verwey *et al.*, 2010). When compared to Alzheimer's disease, further discrepant results were reported (Bibl *et al.*, 2012; Struyfs *et al.*, 2015; Verwey *et al.*, 2010). Interestingly, one study reported a reduction in CSF levels of $A\beta_{1-38}$ and $A\beta_{1-40}$, but not $A\beta_{1-42}$ were reduced in *CHMP2B* mutation carriers compared to non-carriers (Rostgaard *et al.*, 2018).

As summarized in **Table 4**, lower levels of APP-derived peptides have been also linked to neurodegenerative and neuroinflammatory conditions other than Alzheimer's disease. In Lewy body disease, lower $A\beta_{1-42}$ have consistently found to predict faster disease progression of both cognitive and motor aspects of the disease (Lemstra *et al.*, 2017; Schrag *et al.*, 2017; Irwin *et al.*, 2017). However, in Lewy body disease cases it remains unclear whether lower CSF levels of $A\beta_{1-42}$ reflect concurrent Alzheimer's disease pathology, or alternatively, reflect other pathological changes, such as alpha-synuclein deposition (Coughlin *et al.*, 2018). Moreover, lower CSF

levels of $A\beta_{1-38}$ and $A\beta_{1-40}$ have been reported in multiple sclerosis (Augutis *et al.*, 2013; Pietroboni *et al.*, 2018) and bacterial meningitis (Krut *et al.*, 2013). Interestingly, other studies showed that central nervous system infections showed a universal decrease of APP-derived peptides (Spitzer *et al.*, 2018; Mattsson et *al.*, 2010). Additionally, reduced CSF levels of $A\beta_{1-42}$ have been reported in Creutzfeldt-Jakob Disease (CJD) (Dorey *et al.*, 2015; Kapaki *et al.*, 2001; Lattanzio *et al.*, 2017; Otto *et al.*, 2000; Van Everbroeck *et al.*, 2005; Varges *et al.*, 2011; Wiltfang *et al.*, 2003; Zanusso *et al.*, 2011), a disease characterized by accelerated synaptic damage. In particular, in a large study with neuropathological confirmation, CJD cases showed decrease levels of Aβ1–42 in CSF that were not related to the *APOE*ε4 genotype or postmortem cerebral amyloid plaque load (Lattanzio *et al.*, 2017).

Taken together, previous studies have shown variable reductions in APP-derived peptides in other diseases than Alzheimer's disease. Thus, because APP and APP-derived peptides have been related to synaptic and neuronal functioning, more studies are needed to clarify the relationship between neurodegenerative changes and APP-derived peptides in neurodegenerative disease other than Alzheimer's disease, such as FTLD.

Disease	$A\beta_{1-42}$	$A\beta_{1-38}$	$A\beta_{1-40}$	sAPPα	sAPPβ
Alzheimer's disease	↓	↑ or ↔	↑ or ↔	↑ or ↔	↔
FTLD-SΨ	↓	↓ or ↔	↓ or ↔	↓ or ↔	↓
Lewy Body disease (Parkinson's disease and Lewy body dementia)	↔ or ↓ * lower levels predict faster disease progression	↓	↑ / ↓ or ↔	↔	↔
Multiple Sclerosis	↔ or ↓ lower levels predict faster disease progression	↓	↓	↓	↓
Bacterial meningitis	NA	↓	↓	NA	NA
Lyme neuroborreliosis	↓	↓	↓	↓	↓
Creutzfeldt-Jakob disease	↓	NA	NA	NA	NA

Table 4. CSF levels of APP-derived peptides compared to healthy controls. ↑, ↓ and ↔ represents increased, decreased or unchanged CSF levels compared to healthy controls. The results of pathology-confirmed studies were prioritized when multiple studies were available in the literature; When conflicting results were reported in studies without pathology-confirmation, all results were considered; Ψ: for further details, please refer to **Supplementary Table 2**; *: $A\beta_{1-42}$ levels were decreased in pathology-confirmed Lewy body dementia cases and very subtle decreases have been reported in lewy body disease at the prodromal phase;

Macro- and Micro-Structural imaging

Neuroimaging is a valuable biomarker for the *in-vivo* study of cortical structure of FTLD-S. Although most imaging studies in neurodegenerative disease have traditionally focused on structural changes by assessing grey matter atrophy or hypometabolism, recent studies have examined microstructural properties of gray and white matter using MRI and diffusion tensor imaging (DTI). In the following sections I describe both the structural and microstructural changes identified with different imaging modalities along FTLD-S.

MRI principles and analysis. Magnetic Resonance Imaging (MRI) is a non-invasive technique that allows the study of several biologic tissue properties *in vivo.* MRI involves imaging of the proton, the positively charge particle of the hydrogen atom, and relies on the specific properties of the different tissues. MRI is a safe and well-tolerated neuroimaging technique that produces three dimensional detailed anatomical images without the use of damaging radiation (Roberts and Mikulis, 2007).

Different semi quantitative visual rating scales have been proposed for the diagnosis of FTLD-S in recent years (Ambikairajah *et al.*, 2014; Davies *et al.*, 2006; Harper *et al.*, 2016; Kipps *et al.*, 2007). However, the development of new image processing techniques has allowed the extraction of several measures in a semi-automated and observer independent way that have allowed the precise description of distinct patterns of neurodegeneration for each of the FTLD-S (Gordon *et al.*, 2016) and FTLD subtypes (Harper *et al.*, 2017; Josephs *et al.*, 2008; Lee *et al.*, 2011; Whitwell, Jack, *et al.*, 2010).

Several measures can be extracted from brain structural images with different specialized tools. One of them is voxel-based morphometry (VBM), which implies a voxel-wise comparison of local gray matter concentration (Ashburner and Friston, 2000). While VBM has proven useful in detecting different patterns of gray and white matter loss in FTLD-S (Rosen *et al.*, 2002; Zhang *et al.*, 2013), a voxel-based approach may fail to capture the subtle tissue-specific changes that take place at the cortical level. Another relevant measure that can be extracted from structural MRI is cortical thickness. This measure is usually extracted by means of software such as Freesurfer (Dale *et al.*, 1999; Fischl and Dale, 2000) and has demonstrated its capability to show subtle cortical changes in FTLD-S (O'Connor *et al.*, 2017) and even in preclinical mutation carriers. Although VBM has been extensively used in the literature probably facilitated by its easy-of-use, surface-based results (such as Freesurfer-based cortical thickness) are more accurate and reproducible at the single subject level (Clarkson *et al.*, 2011). Indeed, a surface-based approach solves some of the limitations and methodological concerns that have been previously reported using a voxel-based approach (Coalson *et al.*, 2018). Particularly, volume-based smoothing and registration substantially degrade cortical area localization compared with surface-based approaches. This is a key issue for mean diffusivity analyses since volume-based smoothing might cause the sampling of diffusivity values in a voxel with CSF, resulting in an error of x50 increases of diffusivity. However, surface-based registration may avoid these errors since optimal cortical would only select voxels located at the cortex (Coalson *et al.*, 2018).

Structural brain imaging in FTLD-S. As mentioned previously in this introduction, each FTLD-S, FTLD subtype (**Table 1**) and FTLD mutation (**Supplementary Table 1**) is characterized by a typical pattern of neurodegeneration. These patterns of gray matter loss can be detected by structural neuroimaging at a group level. However, at the single subject level the patterns of gray matter loss in FTLD-S are very heterogeneous and may not be evident at visual examination. In-

deed, one study showed that trained radiologists missed the different patterns of frontotemporal atrophy in up to 50% of bvFTD cases (Suárez *et al.*, 2009). Thus, the precise characterization of cerebral structural changes by neuroimaging techniques is needed.

Although some FTLD-S such as svPPA may display well-defined patterns of atrophy (Collins *et al.*, 2017), others may display variable patterns of gray matter neurodegeneration (Gordon *et al.*, 2016). bvFTD is associated with atrophy in the frontal and temporal lobes, the insula and the anterior cingulate cortex (Perry *et al.*, 2017; Schroeter *et al.*, 2014). However, cluster analyses of the cerebral structure in patients with the bvFTD have suggested the existence of distinct subgroups with diverging prognostic profiles (Ranasinghe *et al.*, 2016; Whitwell *et al.*, 2009).

Importantly, structural imaging can be applied in minimally symptomatic or preclinical genetic cases to investigate the earliest cortical structures that underwent neurodegeneration. In bvFTD, the study of mildly symptomatic cases allowed the identification of the frontal paralimbic cortices and insula as earliest cerebral regions that underwent neurodegeneration in this syndrome (Seeley *et al.*, 2008). In addition, structural neuroimaging may be useful for the characterization of the earliest cortical changes in preclinical mutation carriers (Cash *et al.*, 2018; Rohrer *et al.*, 2015; Whitwell *et al.*, 2012).

In summary, structural imaging provides a valuable tool for capturing neuroanatomical signatures of FTLD-S. The profiles commonly correspond well with the clinical presentation and may be useful for differentiating subtypes at the group, and in some cases individual, subject level. However, volumetric MRI changes may well be visible only after significant neuronal loss and the development of novel techniques that may be more sensitive to the earliest FTLD-related changes may be interesting. In the recent year, multiple diffusion-based studies have suggested that microstructural changes of the white matter may be more sensitive to the earliest FTLD-related changes than cortical gray matter loss.

Diffusion imaging principles. Diffusion weighted imaging is a type of MRI sequence designed to assess the mobility of water particles. In free space, water molecules would travel randomly in what we call a Brownian movement, a process characterized by Einstein in 1905. In the brain, however, the diffusion of water is restricted by biological tissues such as cell membranes, fibers and macromolecules (Le Bihan, 2003). The sum of contributions of these biological barriers limits the free movement of the water particles and determines the apparent diffusion coefficient.

Diffusion tensor imaging is a type of diffusion weighted imaging sequence that measures the diffusion in a number of different directions of the space. Using this multidimensional information of diffusion in each direction, each voxel can be represented with an ellipsoid representing the preferential direction of the diffusion of the water molecules. (**Figure 4 left**). This ellipsoid, in turn, can be represented mathematically by a tensor (**Figure 4 mid**). Typical diffusion tensor imaging measures are directly derived from the tensor, like the fractional anisotropy or the mean diffusivity. The fractional anisotropy represents the degree of directionality of the voxel. When the water diffusion is restricted to a certain direction the fractional anisotropy is high, as in the axons, where the water diffusion depends to the tract direction. On the contrary, in areas where there is no preferential diffusion direction (or isotropy), such as CSF, the fractional anisotropy is low. Another measure that can be extracted is mean diffusivity, which measures the total diffusion of the voxel, no matter the directionality. In free water, as in the CSF, diffusion is not restricted, the mean diffusivity is high. In locations where the water diffusion is determined by biological barriers, like inside the neurons and in

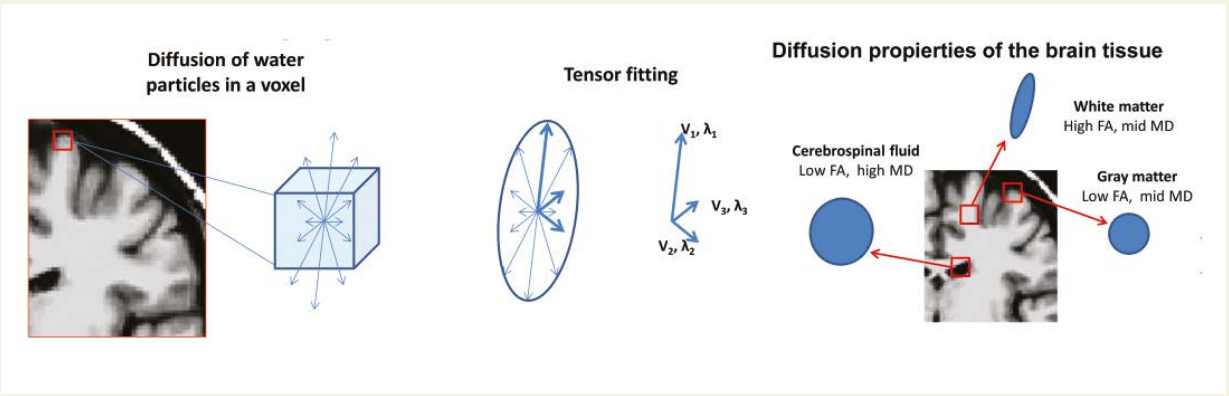

Figure 5. Diffusion Tensor Imaging Principles and diffusion properties of the different brain tissues. Left- Diffusion of water particles in each direction inside a determinate voxel. Mid-Tensor fitting. The diffusion information is used to estimate a mathematical object called tensor. Right. Diffusion properties of the different brain tissues. Figure courtesy of Eduard Vilaplana.

the interstitial space, the mean diffusivity is low (Weston *et al.*, 2015).

Thus, in the CSF mean diffusivity is high and fractional anisotropy is low, in the white matter mean diffusivity is intermediate and fractional anisotropy is high and in the gray matter mean diffusivity and fractional anisotropy are intermediate and low respectively (**Figure 4 right**). The main advantage of diffusion weighted imaging is that it is able to detect changes at the microstructural level (Weston *et al.*, 2015). In neurodegenerative diseases it is thought that the breakdown of biological barriers like myelin cell membranes or organelles would produce a measurable change in the diffusion properties of the tissue (Weston *et al.*, 2015). Thus, diffusion weighted imaging has proven to be a powerful tool to assess brain changes in multiple neurologic diseases (Agosta *et al.*, 2017). The potential contribution of diffusion weighted imaging to an early and more accurate diagnosis has received special attention in recent years. As previously explained, different measures can be extracted from diffusion tensor imaging analyses. Typically, diffusion studies have focused on the study of the white matter by means of diffusion tensor imaging probably due to the technical difficulties derived from the measurement of cortical mean diffusivity (i.e. fractional anisotropy and mean diffusivity measures) (Meeter *et al.*, 2017). Notwithstanding, cortical mean diffusivity may be a valuable biomarker for the study of the earliest cortical changes associated with FTLD.

Diffusion imaging and microstructure in FTLD-S. In the last decade, there has been a growing interest in diffusion weighted imaging as it has been hypothesized that subtle microstructural changes could precede macrostructural alterations (Alexander *et al.*, 2007). In FTLD-S, the white matter microstructural abnormalities as measured by DTI have been consistently found to have a more widespread distribution than gray matter volume loss (Steketee *et al.*, 2016). These observations have led some authors to suggest that white matter disruption may be more severe than gray matter damage in these patients (Agosta *et al.*, 2015). However, previous studies assessing grey matter microstructural changes in FTLD-related syndromes are limited, probably because of the technical difficulties of assessing microstructure at the cortical level (Whitwell, Avula, *et al.*, 2010). In contrast to the white matter, the most common metric used in the cortex is mean diffusivity, due to the absence of preferential diffusion direction (Fortea *et al.*, 2010; Weston *et al.*, 2015). Our group has recently reported following a surface-based approach, that cortical mean diffusivity may be a

sensitive biomarker for the study of the earliest cortical microstructural changes in Alzheimer's disease (Montal *et al.*, 2017). However, previous reports of gray matter alterations in FTLD-S are anecdotal (Whitwell, Avula, *et al.*, 2010). Thus, more research is required to understand the early cortical microstructural alterations in FTLD-S.

Finally, the combination of different biomarkers of the same modality (MRI for example) to asses cortical macro- and micro-structure may improve the potential for discrimination between different neurodegenerative diseases. Particularly, the combined study of cortical thickness and cortical mean diffusivity may provide novel insight into the topography of neurodegeneration in FTLD-S. This may be of particular interest in the case of milder stages of FTLD-S were cortical atrophy is not yet evident but other diffusion tensor imaging measures (such as cortical mean diffusivity) may be able to elicit microstructural changes at the cerebral cortex. Moreover, the eventual correlation of this cortical microstructural changes with established biomarkers of neurodegeneration in CSF (i.e. NfL) would further support its neurodegenerative origin. This approach has been proposed in previous studies. McMillan and collaborators showed that the combined MRI/diffusion tensor imaging study may be more accurate for the discrimination of FTLD and Alzheimer's disease (McMillan *et al.*, 2016) as well as different FTLD neuropathological subtypes (McMillan *et al.*, 2013). Overall, the combined macro- and micro-structural approach may be further enriching our ability to model FTLD-related neurodegeneration.

A multimodal biomarker approach for the study of FTLD-related neurodegeneration.

Because FTLD is a complex disease characterized by pathological and prognostic heterogeneity, a multimodal biomarker approach represents a powerful tool to investigate its pathophysiological underpinnings by combining the information provided by biomarkers ascribed to different aspects of FTLD. As illustrated in **Figure 6**, CSF biomarkers allow the evaluation of different pathophysiological aspects of FTLD, including: astroglial activity, synaptic activity, neuroaxonal damage and neurodegeneration. Some of these pathological mechanisms such as astroglial activity or neuroaxonal injury may important predictors of disease progression.

On the other hand, multimodal biomarker studies enable us to assess the relationship between specific pathophysiological aspects of FTLD and imaging correlates of FTLD-related neurodegeneration. For example, some studies have found lower levels of APP-derived peptides in FTLD-S but the reason for this reduction remains unknown. Mounting evidence supports the role of APP at the synapse under physiological conditions (Muller *et al.*, 2017). Because $A\beta_{1\text{-}42}$, $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}38}$ and sAPPβ are generated by proteolytic processing of APP, the finding of lower levels of APP-derived peptides in CSF may suggest that APP-derived peptides could be related to neurodegeneration in FTLD. Overall, the correlation between APP-derived peptides and cortical thickness in patients with FTLD-S would support the role of APP-derived peptides as neurodegeneration biomarkers in FTLD. Importantly, biomarkers can also be used to select FTLD-S without underlying Alzheimer's disease pathophysiology (Dubois *et al.*, 2014). By doing this, we would ascertain that the observed biomarker changes are not related to concurrent Alzheimer's disease pathology.

Another clinical scenario where biomarkers are needed to increase diagnostic certainty of underlying FTLD is FTLD-S lacking neuroimaging signs of neurodegeneration on visual inspection. In these cases, advanced diffusion tensor imaging measures such as cortical mean diffusivity may be able to elicit microstructural changes at

the cerebral cortex. The eventual correlation of cortical microstructural changes with CSF biomarkers related to neuroaxonal injury (i.e. NfL) or its correlation with clinical measures of disease progression (i.e. FTLD-CDR) would further support the role of this advanced imaging biomarker as a neurodegeneration biomarker.

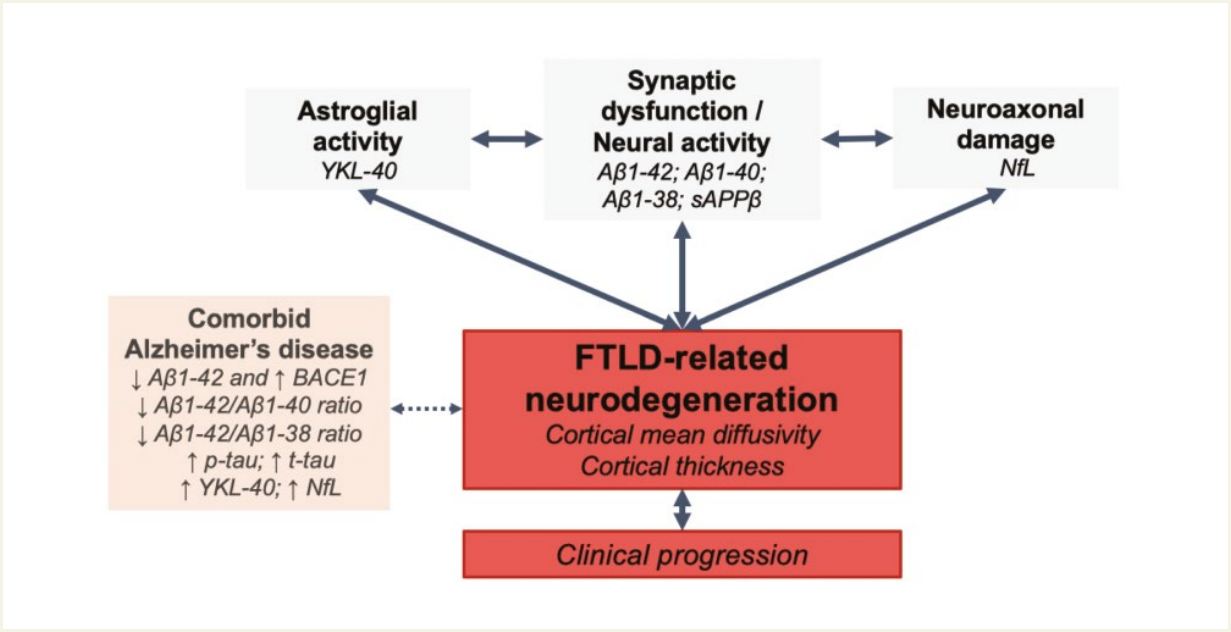

Figure 5. Multimodal biomarker approach for the study of frontotemporal lobar degeneration pathophysiology. Data on biomarkers is shown in italics. The arrows reflect hypothetical relationships, not direct causal links between pathological mechanisms and neurodegeneration.

Chapter 2

Hypotheses and objectives

Hypotheses

General hypotheses

The pathological heterogeneity of FTLD-S warrants the identification of biomarkers to improve diagnosis accuracy, disease progression and prognosis. FTLD-associated neurodegeneration may produce measurable changes in the CSF levels of APP-derived peptides (such as sAPPβ, reflecting neuronal/synaptic loss), Neurofilament light (reflecting neuroaxonal injury) and YKL-40 (reflecting astroglial activity), as well as changes in cortical macro- and micro-structure. The understanding of these changes could be relevant for the improvement of diagnostic accuracy, the prediction of disease course and our understanding of FTLD pathophysiology.

Specific hypotheses:

1. The CSF levels of sAPPβ, YKL-40 and NfL may be valuable biomarkers for the diagnosis of FTLD.
2. The CSF levels of sAPPβ, YKL-40 and NfL may reflect frontotemporal neurodegeneration and may be useful for staging and prognostic purposes of patients with FTLD-S.
3. Diffusion magnetic resonance imaging capture changes in the cortical microstructure through changes in cortical mean diffusivity. Particularly, cortical mean diffusivity may capture the earliest neurodegeneration-related changes in the cortex of patients with bvFTD even in the absence of cortical atrophy.
4. In the absence of Alzheimer's disease pathophysiology, the CSF levels of APP-derived peptides (sAPPβ, $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-38}$) may reflect neurodegenerative changes in FTLD-S.

Objectives

The specific objectives of this thesis are:

1. To study the clinical utility of the CSF levels of sAPPβ, YKL-40 and NfL for the diagnosis of FTLD-S and evaluate their correlation with cortical brain macrostructure.
2. To study the CSF levels of sAPPβ, YKL-40 and NfL along the ALS-FTD spectrum and evaluate their correlation with clinical measures, disease progression and cortical brain macrostructure.
3. To assess the cortical brain microstructural changes in the bvFTD and its relationship with cortical macrostructure.
4. To study the correlation between the CSF levels of the APP-derived peptides ($A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-38}$ and sAPPβ) and the cerebral macrostructure in FTLD-S without Alzheimer's disease pathophysiology, Alzheimer's disease and cognitively-healthy controls without Alzheimer's disease pathophysiology.

Chapter 3

Cerebrospinal fluid sAPPß, YKL-40 and neurofilament light in frontotemporal lobar degeneration

Paper published in
Neurology
(July 11th 2017)

Title:

Cerebrospinal fluid sAPPβ, YKL-40 and neurofilament light in frontotemporal lobar degeneration

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Abstract

Objective: We analyzed the clinical utility of three cerebrospinal fluid (CSF) biomarkers and their structural imaging correlates in a large cohort of patients with different dementia and parkinsonian syndromes within the spectrum of frontotemporal lobar degeneration (FTLD).

Methods: We analyzed three CSF biomarkers (YKL-40, sAPPβ, NFL) and core Alzheimer's disease (AD) biomarkers ($A\beta_{1-42}$, T-Tau, P-Tau) in patients with FTLD-related clinical syndromes (n=159): behavioral variant of frontotemporal dementia (n=68), non-fluent (n=23) and semantic (n=19) variants of primary progressive aphasia, progressive supranuclear palsy (n=28), and corticobasal syndrome (n=21). We also included patients with Alzheimer's disease (AD; n=72) and cognitively normal controls (CN, n=76). We compared cross-sectional biomarker levels between groups, studied their correlation with cortical thickness and evaluated their potential diagnostic utility.

Results: Patients with FTLD-related syndromes had lower levels of sAPPβ than CN and AD patients. The levels of sAPPβ showed a strong correlation with cortical structural changes in frontal and cingulate areas. NFL and YKL-40 levels were high in both FTLD and AD groups compared to controls. In the ROC analysis, the ratios sAPPβ/YKL-40 and NFL/sAPPβ had areas under the curve of 0.91 and 0.96, respectively, distinguishing FTLD patients from CN, and of 0.84 and 0.85 distinguishing patients with FTLD from patients with AD.

Conclusions: The combination of sAPPβ with YKL-40 and with NFL in CSF could be useful to increase the certainty of the diagnosis of FTLD-related syndromes in clinical practice.

Introduction

Frontotemporal lobar degeneration (FTLD) is the proposed neuropathological umbrella term to describe the spectrum of frontotemporal dementia syndromes and tauopathic parkinsonisms. Its clinical manifestations are predominantly personality changes and language alterations. Frontotemporal dementia can occasionally associate with motor symptoms (Bang *et al.*, 2015). From the neuropathological perspective, almost all FTLD cases can be classified in one of three subtypes according to the protein that aggregates in the CNS: TDP-43, and accounting for ~50% of cases (FTLD-TDP); Tau, found in ~40-45% (FTLD-Tau); and FUS, present in less than 10% of cases (FTLD-FUS) (Bang *et al.*, 2015; Irwin *et al.*, 2015).

The diagnosis of FTLD can be challenging. The correspondence between clinical syndrome and underlying neuropathology is usually unpredictable (Irwin *et al.*, 2015; Josephs *et al.*, 2011), and the phenotype often overlaps with that of Alzheimer's disease (AD). Furthermore, symptoms can be mild and/or slowly progressive, and complementary examinations can be uninformative, which hampers the diagnosis and the design of specific protein-targeted therapeutic strategies in FTLD.

Several cerebrospinal fluid (CSF) biomarkers have been studied to achieve a more accurate diagnosis of FTLD. Tau and TDP-43 can be detected in CSF, but their measurement is not sufficiently sensitive or specific for clinical routine use (Feneberg *et al.*, 2014; Irwin *et al.*, 2015; Oeckl *et al.*, 2015; Steinacker *et al.*, 2009; Suárez-Calvet *et al.*, 2014). Core CSF biomarkers for AD are not sufficiently adequate to diagnose FTLD either (Irwin *et al.*, 2015). We analyzed the clinical utility of the soluble β fragment of amyloid precursor protein (sAPPβ, a marker of APP processing), the marker of inflammation YKL-40 (also known as chitinase-3 like 1 protein),

and neurofilament light (NFL) in CSF and their structural imaging correlates in a large cohort of patients with FTLD-related clinical syndromes.

Materials and methods

Study participants and classification

We analyzed CSF samples from 307 participants recruited in 5 centers in Spain: Hospital de la Santa Creu i Sant Pau, Barcelona (HSP, n=173); Hospital Clínic, Barcelona (HC, n=73); Hospital Ramón y Cajal, Madrid (HRC, n=24), Hospital Santa Maria, Lleida (HSM, n=19), and Hospital Universitari Son Espases, Palma de Mallorca (HSE, n=18). One hundred and two of these participants were previously reported elsewhere (Alcolea *et al.*, 2014). Participants were classified in one of the following clinical groups according to internationally accepted diagnostic criteria: possible or probable behavioral variant of frontotemporal dementia (bvFTD) (Rascovsky *et al.*, 2011); semantic variant of primary progressive aphasia (svPPA) (Gorno-Tempini *et al.*, 2011); non-fluent variant of primary progressive aphasia (nfvPPA) (Gorno-Tempini *et al.*, 2011); cortico-basal syndrome (CBS) (Armstrong *et al.*, 2013); progressive supranuclear palsy (PSP) (Litvan *et al.*, 1996); AD dementia with evidence of the pathophysiological process (AD) (McKhann *et al.*, 2011); and cognitively normal controls (CN) (Alcolea, Martínez-Lage, *et al.*, 2015).

We also conducted a subgroup analysis in 149 participants who had a high level of certainty in their diagnosis and in which there was sufficient evidence to reliably predict the underlying proteinopathy (**Figure e-1**). This subset included patients with FTLD-related syndromes that either had an FTLD-associated pathogenic mutation (n=11), neuropathological confirmation (PSP=1 and CBD=4), or concomitant motor neuron disease (n=5). These groups are known to be strongly associated with a specific FTLD neuropathology (FTLD-TDP or FTLD-Tau). We included in this group patients with AD, since all had a CSF profile indicative of the AD pathophysiological process defined as abnormal amyloid-β_{1-42} ($A\beta_{1-42}$), total Tau (T-Tau) and phosphorylated Tau (P-Tau) levels (Alcolea, Martínez-Lage, *et al.*, 2015). We also selected CN that had normal values of $A\beta_{1-42}$, T-Tau and P-Tau (CN-CSF).

Standard Protocol Approvals, Registrations, and Patient Consents

All participants gave written informed consent to participate. The local ethics committee at each center approved the study.

CSF analysis

Eligibility to participate in the study included the availability of CSF in all participants. Core AD biomarkers ($A\beta_{1-42}$, T-Tau and P-Tau) were analyzed at each center using commercially available ELISA kits (Innotest™ β-Amyloid$_{1-42}$, Innotest™ hTAU Ag, and Innotest™ Phospho-Tau$_{181P}$; Fujirebio-Europe, Gent, Belgium) following previously reported methods (Alcolea *et al.*, 2014; Alcolea, Martínez-Lage, *et al.*, 2015). sAPPβ, YKL-40 and NFL levels were analyzed at HSP using commercially available ELISA kits (Human sAPPβ-w highly sensitive, IBL, Gunma, Japan; MicroVue™, Quidel, San Diego, CA, USA; and NF-light®, UmanDiagnostics, Umeå, Sweden, respectively) and following manufacturer's instructions. The analyses of YKL-40 and NFL were performed in a subset of 289 and 249 participants, respectively (**Figure e-1**).

Genetic analysis

Genetic testing was performed in patients with a familial history of dementia, and mutations were found in *C9orf72* (n=5), *GRN* (n=4), *VCP* (n=1) or *MAPT* (n=1). *APOE* was genotyped in 181 participants, using previously described methods (Guardia-Laguarta *et al.*, 2010).

Structural imaging acquisition and pre-processing

Although all participants had undergone routine brain imaging as part of the clinical evaluation, a subset of participants (n=115) from HSP (n=87) and HC (n=28) had an MRI protocol suitable for quantitative analysis. T1-weighted structural MRI was acquired on a 3T scanner Philips Achieva 3.0T X-series (HSP) and Siemens Trio (HC). Cortical reconstruction of the structural images was performed at HSP as previously described (Alcolea, Vilaplana, *et al.*, 2015).

Statistical analysis

We used R statistical software (v.3.1.3.) for all analyses. Non-normally distributed variables were log-transformed to achieve a normal distribution for further bivariate and multivariate analyses. To minimize the influence of possible outliers and heterogeneity of variances, we used robust linear models followed by weighted least squares analysis of covariance (ANCOVA), including age and centre as covariates when necessary. We assessed the diagnostic utility of CSF biomarkers using receiver operating characteristic (ROC) curves as implemented in "pROC" package for R. Lastly, we built classification tree models as implemented in "rpart" package for R. Further details about the statistical analysis are provided in **Supplemental Methods**.

Correlation analyses between cortical thickness and CSF biomarkers were performed using linear modelling of the thickness maps as implemented in FreeSurfer software package, version 5.1 (http://surfer.nmr.mgh.harvard.edu) following previously described methods (Alcolea, Vilaplana, *et al.*, 2015).

Primary research question / classification of evidence

Our primary research question was to determine whether the use of CSF levels of sAPPβ, YKL-40 and NFL or their ratios can distinguish patients with FTLD-related syndromes from patients with AD and from CN. This study provides Class III evidence that CSF levels of sAPPβ, YKL-40 and NFL are useful to identify patients with FTLD-related syndromes.

Results

Demographics, clinical data and core AD biomarker levels

Table 1 shows demographics, clinical data and biomarker levels in all groups. There were no differences in sex. Age was significantly different between groups and showed a significant correlation with $A\beta_{1\text{-}42}$, T-Tau, P-Tau, YKL-40 and NFL. Therefore, we included age as a covariate in the analyses of these biomarkers. MMSE scores did not differ between symptomatic groups. Disease duration at the time of CSF collection was slightly longer in patients with bvFTD than in patients with nfvPPA and AD. The only difference in core AD biomarkers among the FTLD-related clinical syndromes were lower levels of $A\beta_{1\text{-}42}$ in the group of PSP patients compared to the group of svAPP (Figure e-2).

Low levels of sAPPß and high levels of YKL-40 and NFL in CSF in different FTLD-related clinical syndromes

As shown in Figure 1, levels of sAPPβ in CSF were significantly lower in all FTLD-related clinical syndromes than in CN and AD. There were no differences in sAPPβ levels between subgroups of the FTLD-related clinical syndromes. In the analysis of participants with predictable underlying proteinopathy, sAPPβ levels were significantly

Clinical cohort (n=307)								
	bvFTD	**nfvPPA**	**svPPA**	**CBS**	**PSP**	**AD**	**CN**	**p-value**
n	68	23	19	21	28	72	76	
Age (years)	64.8 (9.7)	67.7 (7.0)	67.4 (9.94)	72.6 (6.9)	67.9 (6.9)	70.8 (7.8)	60.2 (8.3)	<0.001[1]
Sex (% female)	39.7%	47.8%	47.4%	57.1%	50.0%	61.1%	59.2%	0.196
MMSE	21.4 (7.6)	23.7 (5.4)	22.2 (5.5)	23.3 (6.7)	22.2 (5.2)	21.6 (4.6)	29.0 (1.1)	<0.001[2]
Disease duration (years)	4.6 (3.0)	2.3 (1.2)	3.4 (2.1)	4.6 (2.5)	4.1 (2.8)	3.3 (2.2)	–	0.003[3]
$A\beta_{1-42}$ (pg/ml)	653.0 (270.7)	637.7 (168.5)	764.5 (247.6)	480.1 (165.3)	452.2 (198.6)	346.3 (105.2)	728.0 (191.6)	<0.001[4]
T-Tau (pg/ml)	273.8 (151.5)	376.2 (222.9)	315.4 (158.3)	279.5 (108.0)	214.9 (131.6)	844.9 (370.5)	243.6 (259.5)	<0.001[4]
P-Tau (pg/ml)	43.5 (20.8)	60.8 (29.7)	47.8 (18.2)	43.4 (13.3)	35.0 (23.9)	106.0 (34.5)	44.3 (24.3)	<0.001[4]
sAPPβ (ng/ml)	546.6 (243.3)	639.6 (296.5)	596.9 (233.8)	556.4 (226.9)	543.7 (246.0)	1015.5 (346.7)	998.8 (429.0)	<0.001[4]
YKL40 (ng/ml)*	253.6 (69.1)	287.5 (59.0)	287.1 (49.9)	280.6 (60.4)	253.3 (61.3)	280.3 (47.6)	199.8 (50.3)	<0.001[4]
NFL (ng/ml)*	2174.4 (2394.9)	2042.4 (1617.3)	2394.4 (1388.1)	2264.3 (1216.5)	1469.8 (656.9)	1051.8 (395.4)	461.3 (220.2)	<0.001[4]
sAPPβ/YKL-40 ratio*	2.4 (1.2)	2.3 (1.0)	1.9 (0.9)	2.0 (0.8)	2.4 (0.7)	3.7 (1.3)	5.2 (2.0)	<0.001[4]
NFL/sAPPβ ratio*	5.2 (7.1)	2.9 (2.5)	3.7 (2.7)	4.3 (2.6)	3.4 (2.6)	1.1 (0.8)	0.5 (0.4)	<0.001[4]

Increased diagnostic certainty (n=149)					
	FTLD-TDP	**FTLD-Tau**	**AD-amyloid**	**CN-CSF**	**p-value**
n	15	6	72	56	
Age (years)	61.4 (6.5)	71.3 (8.6)	70.8 (7.8)	58.7 (8.0)	<0.001[5]
Sex (% female)	53.3%	33.3%	61.1%	60.1%	0.565
MMSE	24.1 (5.6)	n.a.	21.6 (4.6)	29.2 (1.0)	<0.001[6]
Disease duration (years)	3.9 (3.4)	5.0 (2.7)	3.3 (2.2)	–	0.244
$A\beta_{1-42}$ (pg/ml)	594.2 (236.4)	416.4 (167.8)	346.3 (105.2)	780.4 (145.7)	<0.001[4]
T-Tau (pg/ml)	269.0 (100.2)	276.8 (117.4)	844.9 (370.5)	194.0 (64.1)	<0.001[4]
P-Tau (pg/ml)	37.3 (8.9)	38.4 (18.0)	106.0 (34.5)	39.2 (10.7)	<0.001[4]
NFL (pg/ml)	2626.2 (1609.3)	3973.6 (4251.1)	1051.8 (395.4)	447.6 (207.4)	<0.001[4]
sAPPβ (ng/ml)	562.1 (196.5)	508.3 (339.6)	1015.5 (346.7)	972.8 (383.8)	<0.001[4]
YKL40 (ng/ml)	266.2 (63.8)	277.3 (45.1)	280.3 (47.6)	195.9 (43.0)	<0.001[4]
sAPPβ/YKL-40 ratio	2.2 (0.7)	2.1 (1.4)	3.7 (1.3)	5.1 (1.9)	<0.001[4]
NFL/sAPPβ ratio	4.9 (3.6)	10.7 (12.6)	1.1 (0.8)	0.5 (0.3)	<0.001[4]

[1]ANOVA, $F_{(6,298)}$=12.86. TukeyHSD post-hoc (p<0.05): bvFTD compared to CBS and AD. CN compared to bvFTD, nfvPPA, svPPA, CBS, PSP and AD
[2]ANOVA, $F_{(6,239)}$=15.92. TukeyHSD post-hoc (p<0.05): CN compared to bvFTD, nfvPPA, svPPA, CBS, PSP and AD.
[3]ANOVA, $F_{(5,213)}$=3.71. TukeyHSD post-hoc (p<0.05): bvFTD compared to nfvPPA and AD. CBS compared to nfvPPA.
[4]Robust linear model followed by weighted least squares ANCOVA. TukeyHSD post-hoc comparisons are detailed in Figures 1, 2 and e2.
[5]ANOVA, $F_{(3,145)}$=27.98. TukeyHSD post-hoc (p<0.05): FTLD-TDP compared to FTLD-Tau and AD. CN-CSF compared to FTLD-Tau and AD
[6]ANOVA, $F_{(2,126)}$=59.33. TukeyHSD post-hoc (p<0.05): CN-CSF compared to FTLD-TDP and AD (MMSE information was not available for the FTLD-Tau group).
* The analyses of YKL-40 and NFL were performed in a subset of 289 and 249 participants, respectively.

Table 1. Demographics and CSF biomarker results in the diagnostic groups. Unless otherwise specified, results are presented as means (standard deviation). P values were obtained by ANOVA (age, MMSE, disease duration), Chi-squared (sex) and ANCOVA (the other variables). For biomarker analysis we applied a robust linear model followed by weighted least squares ANCOVA. **Abbreviations:** bvFTD = behavioral-variant frontotemporal dementia; nfvPPA = non-fluent variant primary progressive aphasia; svPPA = semantic-variant primary progressive aphasia; CBS = corticobasal syndrome; PSP = progressive supranuclear palsy; AD = Alzheimer's disease; CN = cognitively normal controls; FTLD-TDP = frontotemporal lobar degeneration-TDP; FTLD-Tau = frontotemporal lobar degeneration-Tau; CN-CSF = cognitively normal controls with negative CSF biomarkers for AD; MMSE = mini-mental state examination.

lower in both FTLD-Tau and FTLD-TDP groups compared to CN-CSF and AD groups.

In contrast, YKL-40 levels were higher in all symptomatic groups (including AD) than in CN. The differences in YKL-40 levels between the groups of PSP and CN were not statistically significant (p=0.40). As shown in Figure 1, in the subset of patients with increased diagnostic certainty, age-adjusted YKL-40 levels were higher in the groups of FTLD-TDP and AD compared to CN-CSF.

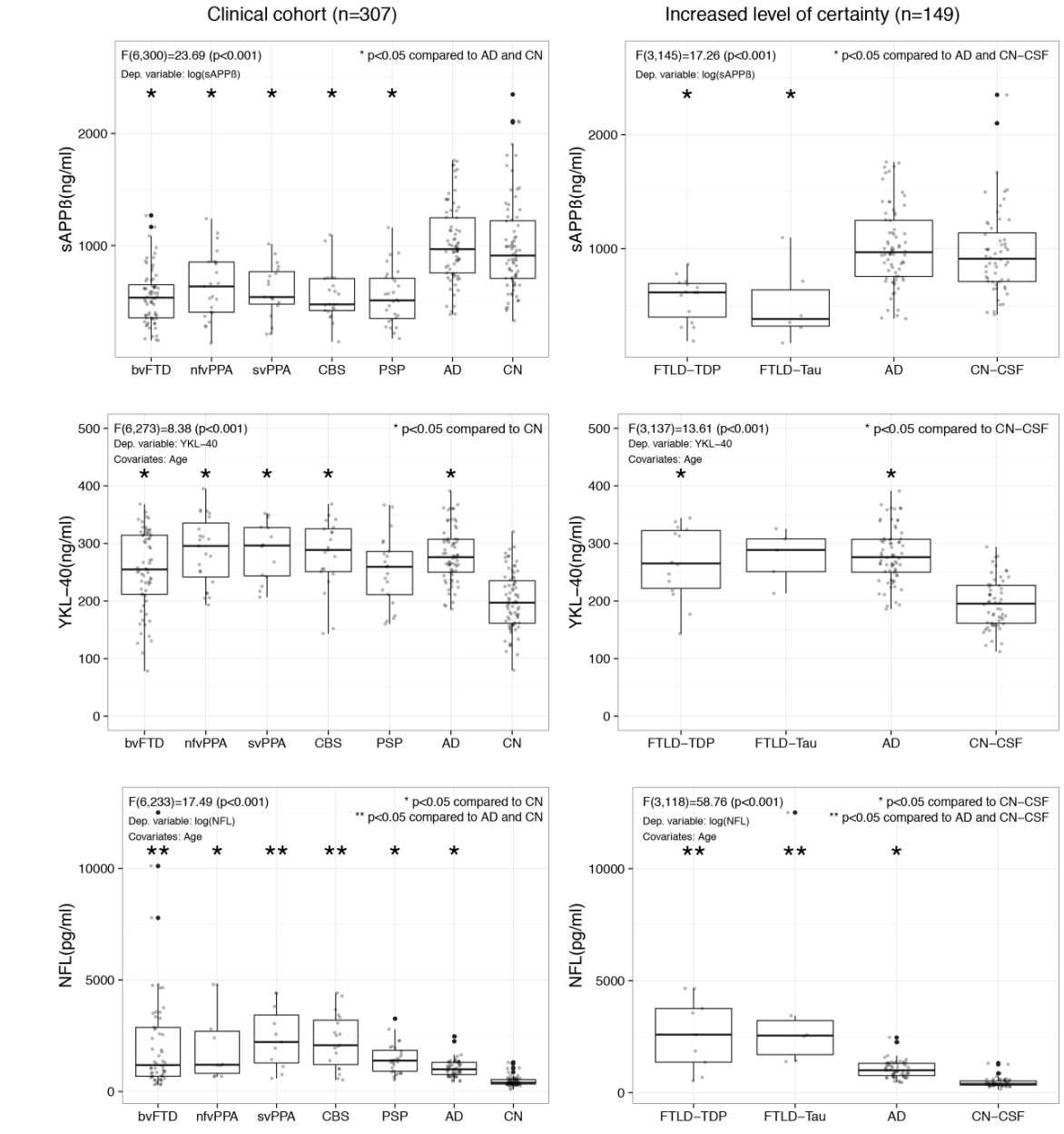

Figure 1. Levels of sAPPß, YKL-40 and NFL in CSF across diagnostic groups. Abbreviations: bvFTD = behavioral-variant frontotemporal dementia; nfvPPA = non-fluent variant primary progressive aphasia; svPPA = semantic-variant primary progressive aphasia; CBS = corticobasal syndrome; PSP = progressive supranuclear palsy; AD = Alzheimer's disease; CN = cognitively normal controls. FTLD-TDP = frontotemporal lobar degeneration-TDP; FTLD-Tau = frontotemporal lobar degeneration-Tau; CN-CSF = cognitively normal controls with negative CSF biomarkers for AD. Only statistically significant differences are displayed (ANCOVA and post-hoc TukeyHSD). All results were adjusted for age, and correction for multiple comparisons was applied.

NFL levels were higher in all symptomatic groups compared to CN, and in most FTLD-related syndromes compared to AD. In the subset with increased certainty, NFL levels were higher in FTLD-TDP and FTLD-Tau compared to AD, and in the three symptomatic groups compared to CN-CSF.

As shown in Figure 2, all FTLD-related clinical syndromes had lower sAPPβ/YKL-40 ratio and higher NFL/sAPPβ ratio than AD and CN groups. In the subset with increased diagnostic certainty, both FTLD-Tau and FTLD-TDP groups had a lower sAPPβ/YKL-40 ratio and a higher NFL/sAPPβ ratio than the AD and the CN-CSF groups.

Relationship of CSF biomarkers with clinical and genetic variables in the FTLD-related clinical syndromes

We analyzed the correlation between each of the six CSF biomarkers and clinical variables within the group of the FTLD-related clinical syndromes. Age showed a mild direct correlation with T-Tau (r=0.19, p=0.02), P-Tau (r=0.23, p<0.01), sAPPβ (r=0.16, p=0.05) and YKL-40 (r=0.27, p<0.01), an inverse correlation with $A\beta_{1-42}$ (r=-0.22, p<0.01), and no significant correlation with NFL (p=0.80). MMSE score only showed a significant correlation with NFL (r=-0.26, p=0.01). There was no correlation between any of the biomarkers studied and disease dura-

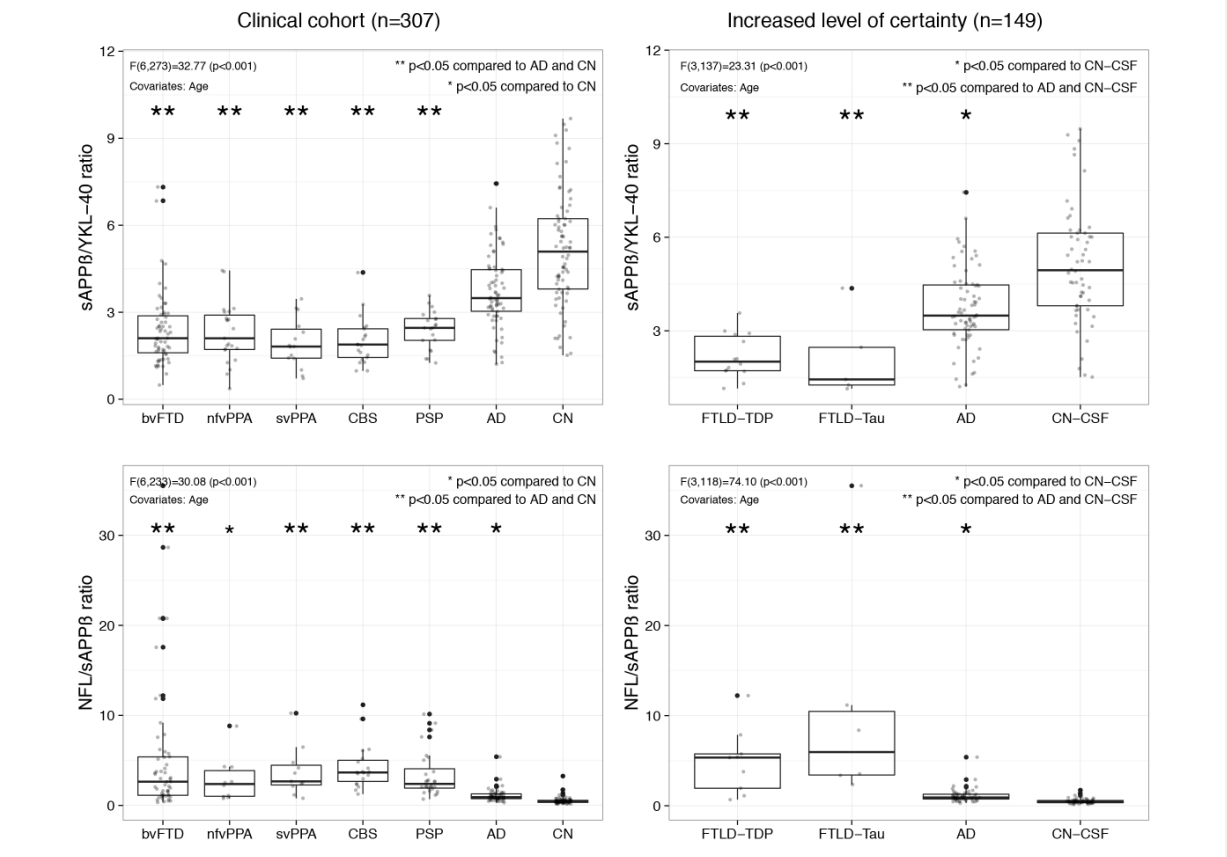

Figure 2. Levels of sAPPß/YKL and NFL/sAPPß ratios across diagnostic groups. **Abbreviations:** bvFTD = behavioral-variant frontotemporal dementia; nfvPPA = non-fluent variant primary progressive aphasia; svPPA = semantic-variant primary progressive aphasia; CBS = corticobasal syndrome; PSP = progressive supranuclear palsy; AD = Alzheimer's disease; CN = cognitively normal controls. FTLD-TDP = frontotemporal lobar degeneration-TDP; FTLD-Tau = frontotemporal lobar degeneration-Tau; CN-CSF = cognitively normal controls with negative CSF biomarkers for AD. Only statistically significant differences are displayed (ANCOVA and post-hoc TukeyHSD). All results were adjusted for age, and correction for multiple comparisons was applied.

tion at the time of CSF collection. There were no differences in the levels of any CSF biomarker between *APOEe4* carriers and non-carriers.

Diagnostic value of CSF sAPPß, YKL-40 and NFL in FTLD

We compared the diagnostic utility of sAPPβ, YKL-40 and NFL in CSF in the evaluation of FTLD-related syndromes. As shown in Figure 3A, in the clinical cohort, sAPPβ, YKL-40 and NFL showed a good accuracy to distinguish FTLD-related syndromes from controls, with an area under the curve (AUC) of 0.82 (95%CI 0.77-0.88), 0.79 (95%CI 0.73-0.85) and 0.93 (95%CI 0.90-0.97), respectively. The combination of biomarkers showed a better overall accuracy than single biomarkers. The sAPPβ/YKL-40 ratio had an AUC of 0.91 (95%CI 0.87-0.95), which was significantly higher than that for sAPPβ (p<0.001) and for YKL-40 (p<0.001). The NFL/sAPPβ ratio had an AUC of 0.96 (95%CI 0.94-0.99), higher than that for sAPPβ (p<0.001) and for NFL (p<0.05). For core AD biomarkers, $A\beta_{1-42}$, T-Tau and P-Tau, the individual AUC to distinguish FTLD patients from CN were below 0.70 (data not shown). The ratios T-Tau/$A\beta_{1-42}$ and P-Tau/$A\beta_{1-42}$ yielded AUC values of 0.76 (95%CI 0.69-0.83) and 0.68 (95%CI 0.61-0.76), respectively. These values were significantly lower than those yielded by the sAPPβ/YKL-40 ratio and the NFL/sAPPβ ratio (p<0.001). As shown in Figure 3B,

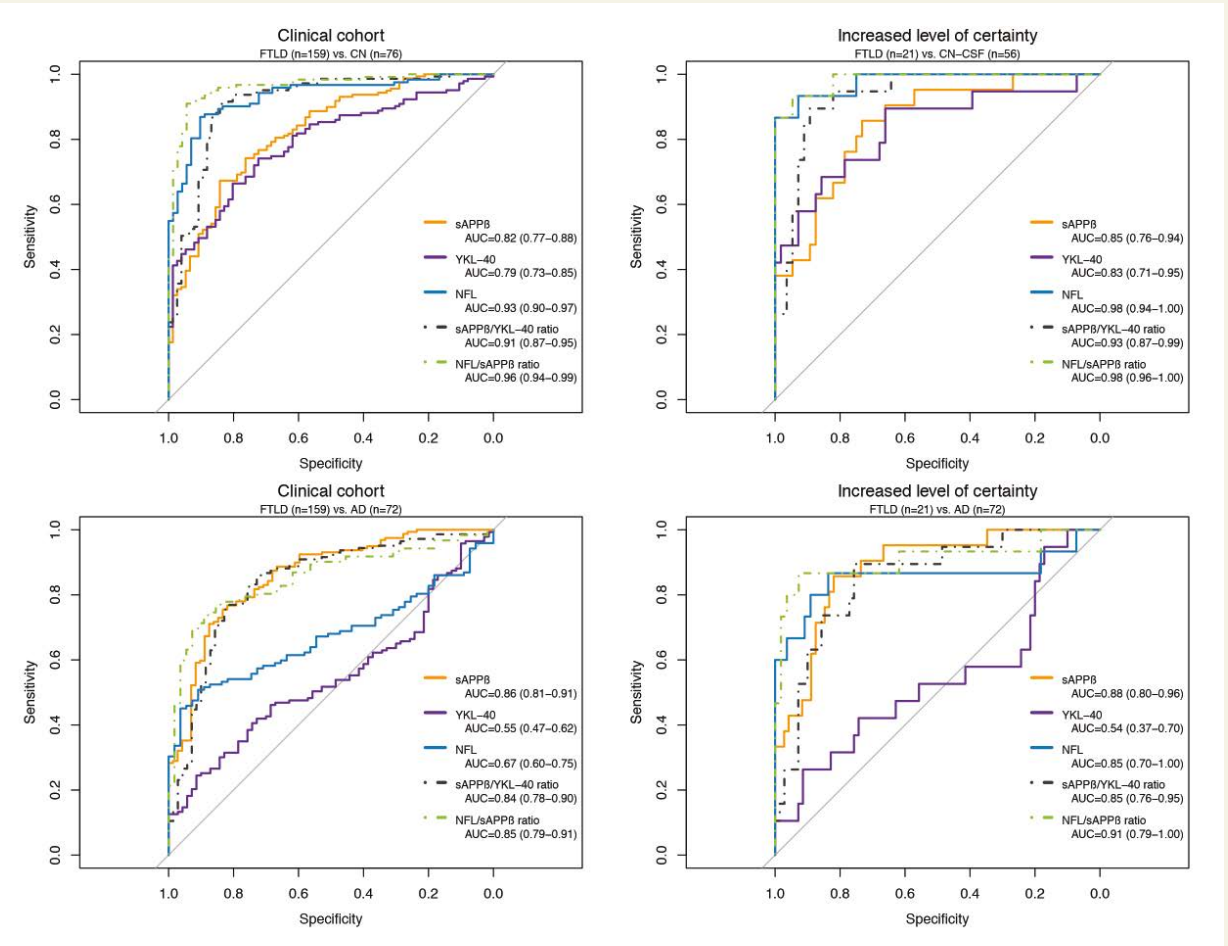

Figure 3. Analysis of CSF biomarkers' diagnostic utility through receiver operating characteristic (ROC) curves. Abbreviations: FTLD = frontotemporal lobar degeneration; CN = cognitively normal controls; CN-CSF = cognitively normal controls with negative CSF biomarkers for AD; AD = Alzheimer's disease; AUC = area under the curve. Values are expressed as AUC (CI 95%).

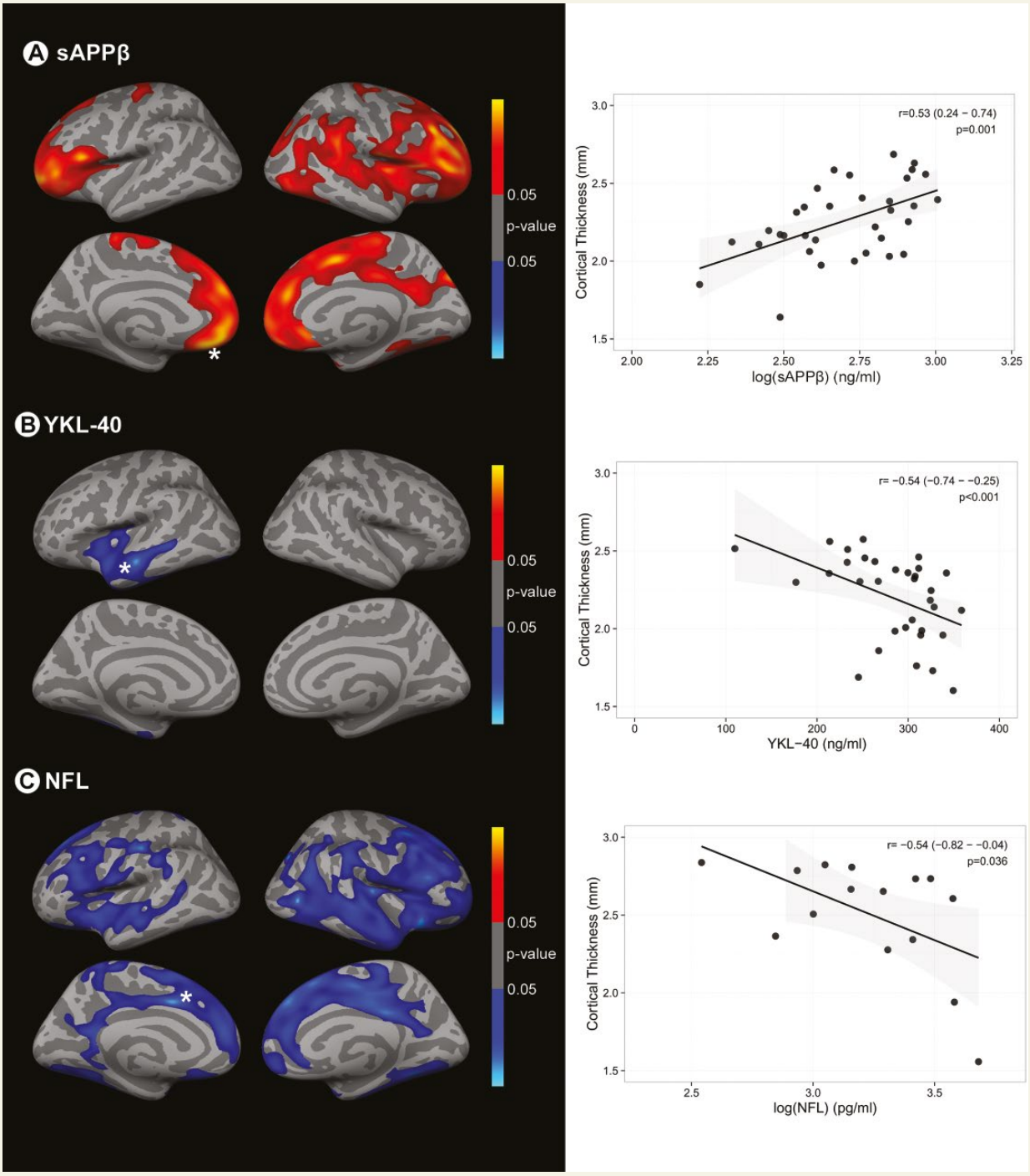

Figure 4. Relationship of cortical thickness with levels of sAPPß (A), YKL-40 (B) and NFL (C) in CSF in the group of FTLD-related syndromes (n=34). All analyses were adjusted by age and centre. Only regions that survived family-wise error correction (FWE p<0.05) are presented. The scatterplots show the relationship between each CSF biomarker and cortical thickness at the maximum significant vertex (asterisks). The same analysis was performed in the groups of Alzheimer's disease and cognitively normal controls and showed no significant correlations between cortical thickness and CSF levels of sAPPß or YKL-40. NFL levels correlated with cortical thickness in a small cluster of the left lateral temporal lobe in the Alzheimer's disease group. There was no correlation between cortical thickness and NFL levels in cognitively normal controls.

the results were similar in the subset of participants with increased diagnostic certainty. Table e-1 shows the best-fit cut-off values for sAPPβ, YKL-40, NFL, and their ratios in our sample.

To distinguish FTLD patients from AD patients, the AUC for the sAPPβ/YKL-40 ratio and for the NFL/sAPPβ ratio were 0.84 (95%CI 0.78-0.90) and 0.85 (95%CI 0.79-0.91), respectively, in the clinical cohort (Figure 3C), and 0.85 (95%CI 0.76-0.95) and 0.91 (95%CI 0.79-1.00) in the subset with increased level of certainty, respectively (Figure 3D).

In the classification tree analysis (Figure e-3), the optimal model to classify our participants in one of the three clinical groups (FTD, AD and CN) was built upon a combination of NFL and sAPPβ (resubstitution error=22.4%, cross-validation error=27.3%). In the subset with increased level of diagnostic certainty, the levels of NFL alone were sufficient to classify correctly 85.2% of the participants (resubstitution error 14.8%, cross-validation error 17.4%).

Imaging correlates of CSF sAPPß, YKL-40 and NFL in FTLD

To investigate the correlation of sAPPβ, YKL-40 and NFL levels with brain structure, we selected a subset of 34 participants with FTLD-related syndromes, 33 with AD and 48 CN-CSF who had structural MRI suitable for quantitative analysis. Table e-2 shows the demographics and clinical characteristics of these groups. As seen in Figure 4, in the group of FTLD-related syndromes we found a strong direct correlation between sAPPβ levels and cortical thickness, mainly in frontal and cingulate areas. YKL-40 levels correlated inversely with cortical thickness in lateral and inferior temporal areas. NFL levels correlated with cortical thickness in frontal, temporal and parietal areas. Similar results were obtained when patients scanned at HSP and patients scanned at HC were analysed independently. In the AD group, a lateral temporal cluster showed a significant correlation with NFL levels (data not shown). No other significant correlations were found between cortical thickness and sAPPβ, YKL-40 or NFL levels in the CN-CSF or in the AD groups.

Discussion

We found that sAPPβ, YKL-40 and NFL in CSF had a good diagnostic accuracy to discriminate FTLD patients from controls. Levels of sAPPβ were consistently reduced across the different FTLD-related clinical syndromes compared to AD patients and to CN, and levels of the inflammatory marker YKL-40 and of NFL were elevated in symptomatic groups (including AD) compared to CN. In the structural imaging analysis, sAPPβ levels correlated strongly with cortical thickness in patients with FTLD-related syndromes, mainly in frontal and cingulate areas; YKL-40 levels correlated with brain structure in lateral and inferior temporal areas; and NFL levels had a widespread correlation in frontal, temporal and parietal areas.

Markers of the APP processing in CSF have been investigated previously in FTLD. Some studies found a decrease in $A\beta_{1-38}$ and $A\beta_{1-40}$ levels in the CSF of patients with FTLD compared to AD patients and controls (Bibl *et al.*, 2007; Gabelle *et al.*, 2011; Verwey *et al.*, 2010). Moreover, previous studies described a decrease in levels of sAPPβ in the CSF of FTLD patients (Alcolea *et al.*, 2014; Alexopoulos *et al.*, 2012; Gabelle *et al.*, 2011). However, other authors did not find these differences (Magdalinou *et al.*, 2015) and results to date are inconsistent, likely due to the fact that data were obtained in small single center cohorts and that different assay platforms have been used for the analyses. In this study, we gathered a large collection of CSF samples from FTLD patients and confirmed that levels of sAPPβ were low in all FTLD-related syndromes. The cause underly-

ing this decrease remains unclear at this point. Because the sAPPβ fragment is generated by proteolytic processing from APP and APP is highly expressed in neurons in frontal and perisylvian areas, (Ferrari *et al.*, 2016) we hypothesize that low levels in CSF could reflect the pronounced neuronal loss and cortical atrophy in FTLD. This hypothesis is supported by the strong correlation between sAPPβ and cortical thickness in FTLD-vulnerable areas, a finding of key importance since atrophy in these regions is a characteristic trait that has been incorporated into diagnostic criteria (Rascovsky *et al.*, 2011). A possible explanation for the lack of decrease found in AD patients could be a higher expression of APP in FTLD-vulnerable areas than in AD-vulnerable areas (middle-temporal, parietal) (Ferrari *et al.*, 2016). Moreover, a reduction in sAPPβ in AD could be compensated by β-secretase overexpression or by a defective clearance system in the AD brain (Fukumoto *et al.*, 2002; Holsinger *et al.*, 2002; Mawuenyega *et al.*, 2010; Pera *et al.*, 2012; Yang *et al.*, 2003). In any case, regardless of the underlying mechanism, our data confirm that this APP-derived metabolite is reduced in CSF in FTLD and indicate that its levels correlate with the characteristic imaging traits of the disease.

Neuroinflammation is a well-known pathophysiological component in AD and other neurodegenerative diseases (Wyss-Coray and Mucke, 2002). In FTLD, a variety of inflammatory markers have been reported to be elevated in the CSF, although the clinical implications of these findings are unclear (Hu *et al.*, 2010; Oeckl *et al.*, 2015). Previous studies have found that YKL-40 levels are increased in symptomatic FTLD (Alcolea *et al.*, 2014; Craig-Schapiro *et al.*, 2010; Teunissen *et al.*, 2016) and in symptomatic and asymptomatic stages of AD compared to CN (Alcolea *et al.*, 2014; Alcolea, Martínez-Lage, *et al.*, 2015; Janelidze *et al.*, 2016). In the present study, we expand this finding and report that this increase is commonly found in various clinical, pathological and genetic subtypes of FTLD. The levels of YKL-40 in CSF in these patients might therefore reflect glial activation, a common feature of neurodegenerative diseases in general and of different FTLD-syndromes in particular. These findings are in agreement with a recent study by Teunissen et al. (Teunissen *et al.*, 2016) and support the notion that neuroinflammation is a common phenomenon in FTLD, and that it can be detected through CSF biomarkers. It will be of interest to confirm whether this reaction also occurs in the preclinical stages of the disease, as occurs in AD (Alcolea, Martínez-Lage, *et al.*, 2015).

NFL is an axonal cytoskeletal constituent essential for axonal growth (Meeter *et al.*, 2016). An increase in CSF levels of NFL has been described in different neurological conditions and also in healthy aging as a reflection of neuronal/axonal damage (Landqvist Waldö *et al.*, 2013; Meeter *et al.*, 2016; Scherling *et al.*, 2014). In FTLD, the levels of NFL in CSF correlate with the severity and progression of the disease (Meeter *et al.*, 2016; Scherling *et al.*, 2014). In line with these findings, we found increased levels in all symptomatic groups compared to CN and also in FTLD-related clinical syndromes compared to AD. Additionally, NFL was the only marker that showed a significant correlation with clinical measures (MMSE).

Taking advantage of the large sample size in our study, we assessed the clinical utility of sAPPβ, YKL-40 and NFL in CSF. As reported previously, we found that core AD biomarkers are not useful to distinguish patients with FTLD from CN (Grossman, 2010; Irwin *et al.*, 2013). In contrast, the sAPPβ/YKL-40 ratio, NFL alone, and the NFL/sAPPβ ratio correctly classified 91%, 93% and 96% of these participants, respectively. The AUCs were higher in the subset of participants with an increased diagnostic certainty. We believe that the combination of sAPPβ with YKL-40 or with NFL could be of interest in certain clin-

ical settings to distinguish patients with FTLD from those with disorders with similar clinical phenotypes, such as behavioral disturbances found in psychiatric disorders and other FTLD phenocopies. These biomarkers might also be useful to interrogate the neuropathological substrate in some clinical scenarios. For instance, in patients with CBS, a CSF signature of AD (low $A\beta_{1-42}$ and high T-Tau and P-Tau) in the presence of normal sAPPβ/YKL-40 or NFL/sAPPβ ratios might point towards an underlying AD, whereas the opposite situation might indicate FTLD-Tau. In contrast, a profile consisting of abnormal core AD biomarkers and abnormal sAPPβ/YKL-40 or NFL/sAPPβ ratios could indicate AD and FTLD co-pathology.

It is worth stressing that none of the biomarkers studied or their combinations were specific of FTLD-Tau or FTLD-TDP pathologies and cannot therefore be used to discriminate between the main pathologies underlying FTLD. Other reliable and proteinopathy-specific biomarkers are thus still needed to accurately distinguish between FTLD-Tau, FTLD-TDP and FTLD-FUS.

The main strength of this study is the large sample size, which allowed us to compare several clinical, pathological and genetic variants of the FTLD spectrum. Another strength is that the analysis of levels of sAPPβ, YKL-40 and NFL in CSF was centralized in one laboratory. The main limitation of the study is that in most cases, diagnosis was made using clinical criteria and misdiagnosis could therefore have occurred. To overcome this limitation, however, we used clinical and genetic information and established biomarkers to increase the certainty of the diagnoses and reliably predict the underlying proteinopathy when neuropathological confirmation was not available.

Our findings could be useful to increase the certainty of the diagnosis in clinical practice and, eventually, to select potential candidates for clinical trials with treatments directed to both FTLD-Tau and FTLD-TDP subtypes. These are key aspects to accelerate the development of effective treatments for patients with FTLD.

Acknowledgements

We would like to thank all the participants who agreed to take part in this study, Laia Muñoz and Carolyn Newey for technical and editorial assistance, respectively, and all the members of the clinical teams involved in selection and assessment of participants.

Study funding

This work was supported by CIBERNED (SIGNAL study) and Instituto de Salud Carlos III (PI11/03035-BIOMARKAPD-Joint Programming on Neurodegenerative Diseases, PI11/02425 and PI14/01126 to Dr. J. Fortea, PI13/01532 to Dr. R. Blesa, PI15/00026 to Dr. J. Clarimón, PI14/01561 to Dr. A. Lleó, and PI14/00282 to Dr. A. Lladó), jointly funded by Fondo Europeo de Desarrollo Regional (FEDER), Unión Europea, "Una manera de hacer Europa". This work was also supported in part by Generalitat de Catalunya (2014SGR-0235). Dr. D. Alcolea received funding from Instituto de Salud Carlos III (Contrato post-FSE "Río Hortega" CI12/00143). I. Illán-Gala is supported by an i-PFIS grant (IF15/00060) from the FIS, Instituto de Salud Carlos III. This work has also received support from "Marató TV3" grants 531/U/2014 to Dr. J. Fortea, 20142610 to Dr. A. Lleó, 117/U/2014 to Dr. R. Sánchez-Valle, Consolider-Ingenio to Dr. J.L. Molinuevo.

Supplementary material

Supplementary methods

We assessed normality of the variables using the Kolmogorov-Smirnov test and homogeneity of variances using Levene's test. We used the Chi-square test to assess differences in sex and analysis of variance (ANOVA) for age, MMSE score and disease duration. We used robust linear models followed by weighted least squares analysis of covariance (ANCOVA) to analyze differences in biomarker levels, including age and centre as covariates when necessary. All p-values in this analysis were corrected for multiple comparisons using Tukey's "Honest Significant Differences" post-hoc test.

Given a statistical power of 0.80, a general linear model analysis with 6 or less degrees of freedom in the numerator and 250 or more degrees of freedom in the denominator allows the detection of an effect size (f^2) of 0.05 (alpha=0.05).

For receiver operating characteristic (ROC) analyses, areas under the curve (AUC) were computed with the trapezoidal rule, and confidence intervals were estimated with DeLong's method. Two-sided tests (DeLong) were used to compare ROC curves. Best-fit cut-off values were obtained in the subset of participants with an increased level of certainty in their diagnosis setting the sensitivity level at 85%.

For the classification tree models, cross-validated estimates of risk were computed, and trees were subsequently pruned according to the best-fit complexity parameter and the lowest estimate of risk.

In the cortical thickness analysis, we applied a Gaussian kernel of 15mm full-width at half maximum. Age, sex and centre were included as covariates. An initial vertex-wise threshold was set at p=0.05 to find clusters. We tested Monte Carlo simulation with 10000 repeats in Qdec (family-wise error [FWE], $p<0.05$).

Supplementary tables

FTLD vs. CN		Increased diagnostic certainty 21 FTLD vs. 56 CN-CSF		Clinical Cohort 159 FTLD vs. 76 CN	
	Best-fit cut-off	Sensitivity	Specificity	Sensitivity	Specificity
sAPPβ	**718ng/ml**	86%	82%	76%	72%
YKL-40	**212ng/ml**	89%	66%	80%	62%
NFL	**1336pg/ml**	87%	100%	54%	100%
sAPPβ/YKL-40 ratio	**2.98**	85%	89%	80%	87%
NFL/sAPPβ ratio	**1.83**	87%	100%	70%	99%
FTLD vs. AD		**Increased diagnostic certainty** 21 FTLD vs. 72 AD		**Clinical Cohort** 159 FTLD vs. 72 AD	
sAPPβ	**718ng/ml**	86%	82%	77%	82%
YKL-40	**329ng/ml**	89%	19%	85%	19%
NFL	**1361pg/ml**	87%	84%	53%	84%
sAPPβ/YKL-40 ratio	**3.01**	89%	76%	82%	76%
NFL/sAPPβ ratio	**1.91**	87%	93%	70%	93%

Table e-1. Cut-off values for sAPPß, YKL-40 and NFL to discriminate between FTLD and cognitively normal controls and between FTLD and Alzheimer's disease. Best-fit cut-off values were obtained for sAPPß, YKL-40, the sAPPß/YKL-40 ratio and NFL/sAPPß ratio in the subset of participants with an increased level of certainty in their diagnosis (21 FTLD, 72 AD and 56 CN). Specificity was optimized for a sensitivity level of at least 85%. The two columns on the right display the sensitivity and specificity of these cut-off values when they were applied to the clinical cohort. Abbreviations: FTLD = frontotemporal lobar degeneration; CN = cognitively normal control; CN-CSF = cognitively normal controls with negative CSF biomarkers for Alzheimer's disease; AD = Alzheimer's disease.

Participants with structural MRI				
	FTLD (n=34)	**AD (n=33)**	**CN-CSF (n=48)**	**p**
Age (years)	65.5 (9.8)	70.8 (7.3)	57.8 (7.5)	<0.001[2,3]
Sex (% female)	46.7%	65.2%	64.6%	0.238
MMSE score	23.3 (6.2)	23.1 (3.3)	29.2 (1.0)	<0.001[2,3]
Disease duration (years)	2.8 (1.5)	3.3 (1.7)	-	0.923
$A\beta_{1-42}$ (pg/ml)	685.91 (236.06)	384.65 (94.34)	774.94 (137.6)	<0.001[1,3]
T-Tau(pg/ml)	334.42 (217.05)	839.15 (410.03)	189.29 (62.46)	<0.001[1,2,3]
P-Tau (pg/ml)	51.23 (24.11)	102.15 (31.42)	38.98 (10.82)	<0.001[1,3]
sAPPβ(ng/ml)	521.85 (227.09)	1058.8 (342.98)	965.36 (345.78)	<0.001[1,2]
YKL-40 (ng/ml)	274.38 (62.93)	282.27 (52.27)	194.25 (42.05)	<0.001[2,3]
NFL (pg/ml)	2244.14 (1389.78)	882.46 (298.28)	442.2 (220.61)	<0.001[2,3]

[1]TukeyHSD post-hoc (p<0.05): FTLD compared to AD
[2]TukeyHSD post-hoc (p<0.05): FTLD compared to CN-CSF
[3]TukeyHSD post-hoc (p<0.05): AD compared to CN-CSF

Table e-2. Demographics and CSF biomarker results in the subset of participants with structural MRI available for quantitative analysis. We included 34 participants with one of the following diagnoses: behavioural-variant frontotemporal dementia (n=8), non-fluent variant primary progressive aphasia (n=11), semantic-variant primary progressive aphasia (n=10), corticobasal syndrome (n=3) or progressive supranuclear palsy (n=2). We also included patients with AD dementia and evidence of the pathophysiological process (n=33) and a group of cognitively normal participants with negative CSF biomarkers for AD (n=48). Unless otherwise specified, results are presented as means (standard deviation). P values were obtained by ANOVA (age, MMSE, disease duration), Chi-Squared (sex) and ANCOVA (rest of the variables). For biomarker analysis we applied a robust linear model followed by weighted least squares ANCOVA. Abbreviations: FTLD = Frontotemporal lobar degeneration; AD = Alzheimer's disease; CN-CSF = cognitively normal controls with negative CSF biomarkers for AD. MMSE = mini-mental state examination.

Supplementary figures

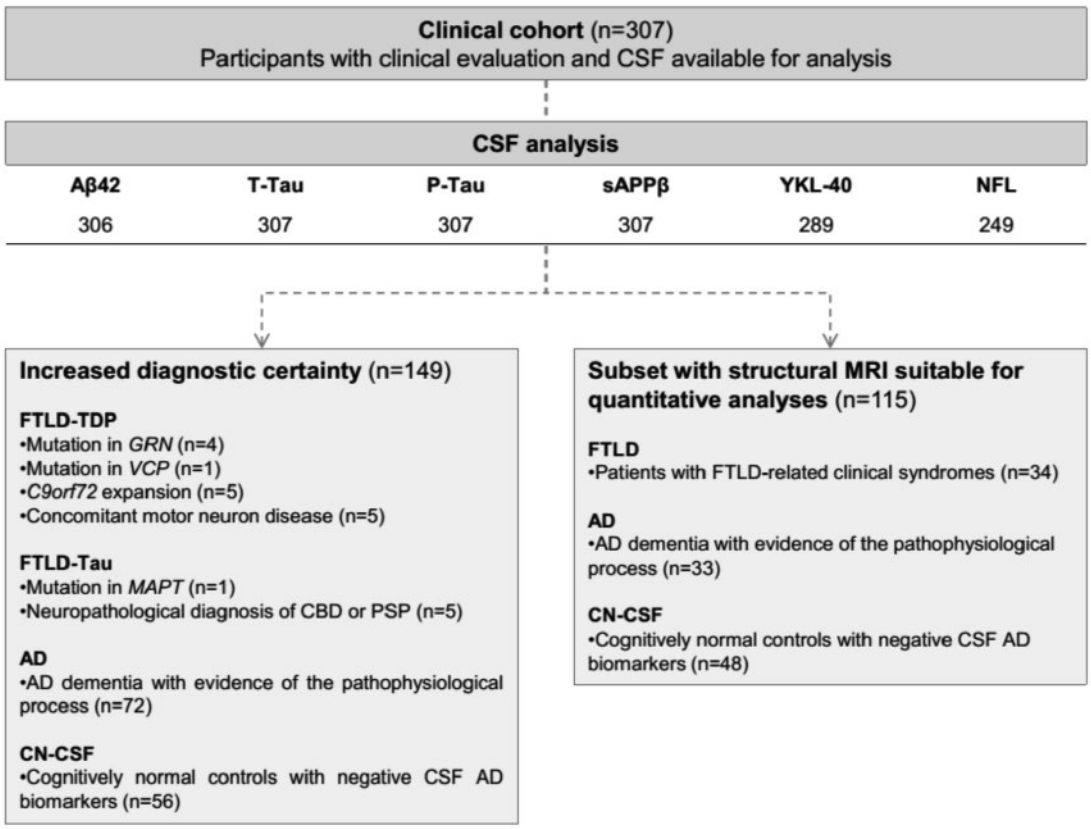

Figure e-1. Flow of participants. Abbreviations: FTLD-Tau = frontotemporal lobar degeneration-Tau; FTLD-TDP = frontotemporal lobar degeneration-TDP; AD = Alzheimer's disease; CN-CSF = cognitively normal controls with

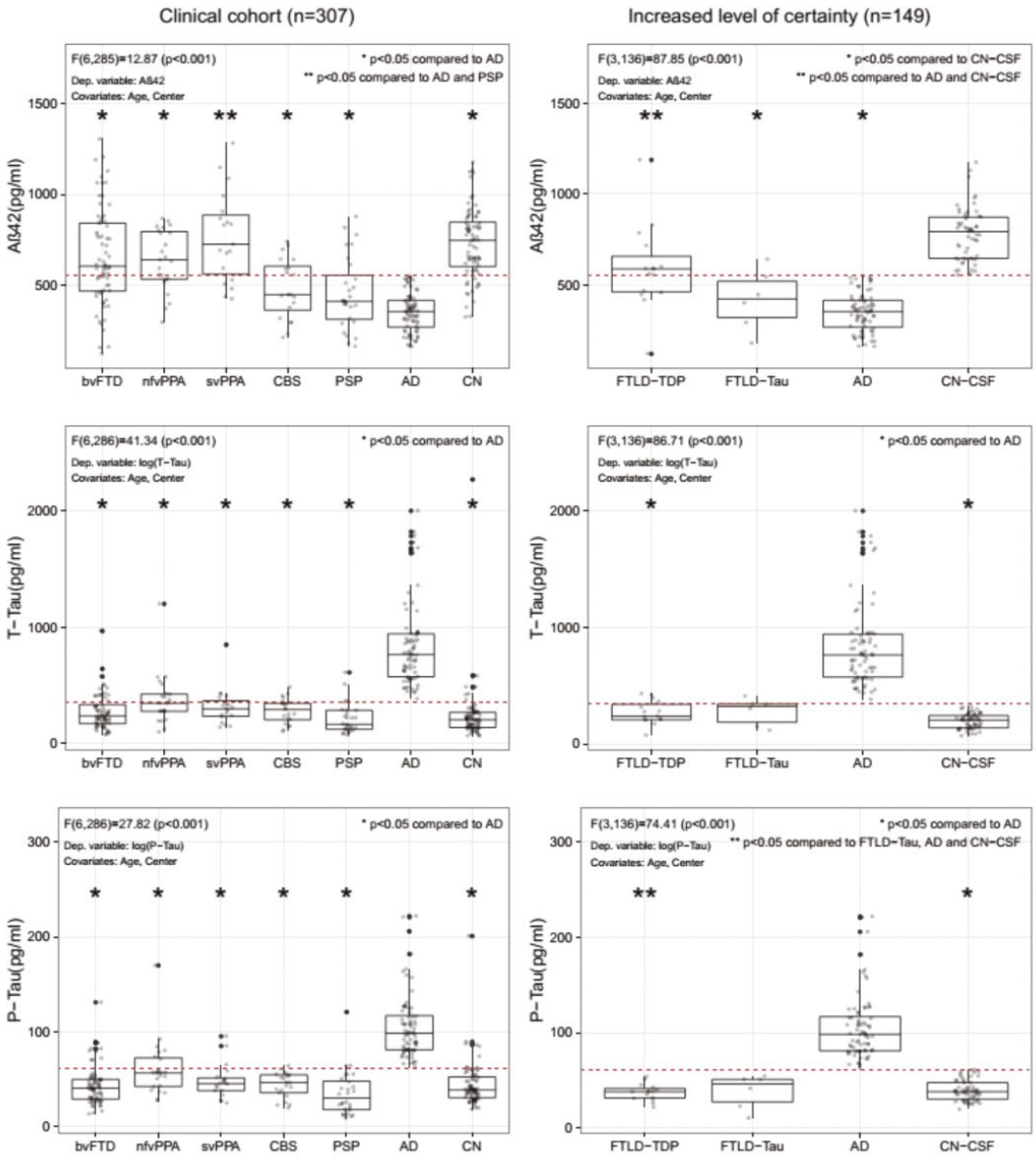

Figure e-2. Core AD CSF biomarkers across diagnostic groups. Abbreviations: bvFTD = behavioural-variant frontotemporal dementia; nfvPPA = non-fluent variant primary progressive aphasia; svPPA = semantic-variant primary progressive aphasia; CBS = corticobasal syndrome; PSP = progressive supranuclear palsy; AD = Alzheimer's disease; CN = cognitively normal controls. FTLD-TDP = frontotemporal lobar degeneration-TDP; FTLD-Tau = frontotemporal lobar degeneration-Tau; CN-CSF = cognitively normal controls with negative CSF biomarkers for AD. Only statistically significant differences are displayed (ANCOVA and post-hoc TukeyHSD). All results were adjusted for age and center, and correction for multiple comparisons was applied. Dashed lines indicate the cut-off values used in this study ($A\beta_{42}$: 550pg/ml; T-Tau: 350pg/ml, P-Tau: 61pg/ml)*.*Alcolea D, Martínez-Lage P, Sánchez-Juan P, et al. Amyloid precursor protein metabolism and inflammation markers in preclinical Alzheimer disease. Neurology. 2015;85:626–633.

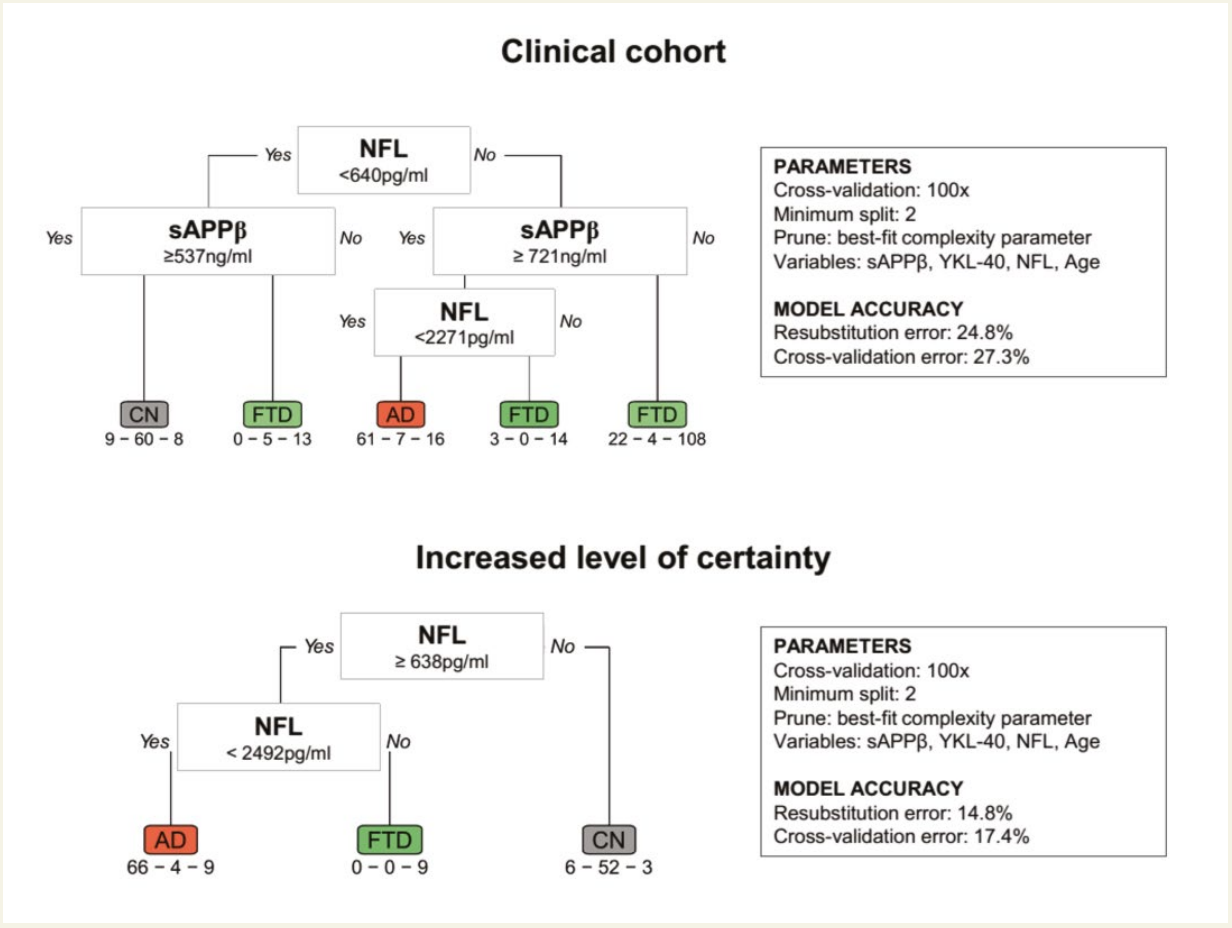

Figure e-3. Classification tree models for the diagnosis of FTD and AD using sAPPβ, YKL-40, NFL and Age. CN: Cognitively normal controls; FTD: Frontotemporal dementia; AD: Alzheimer's disease.

Chapter 4

CSF sAPPß, YKL-40, and NFL along the ALS-FTD spectrum

Paper published in
Neurology
(October 23th 2017)

Title:

CSF sAPPβ, YKL-40, and NfL along the ALS-FTD spectrum

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Abstract

Objective: To investigate the clinical utility of 3 cerebrospinal fluid (CSF) biomarkers along the clinical spectrum of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD).

Methods: We analyzed 3 CSF biomarkers: the soluble ß-fragment of amyloid precursor protein or sAPPß, YKL-40 and Neurofilament light (NfL) in FTD (n=86), ALS (n=38) and a group of age-matched cognitively normal controls (n=49). FTD participants with a CSF profile of Alzheimer's disease were excluded. We compared cross-sectional biomarker levels between groups, studied their correlation with cognitive and functional scales (global cognitive z-score, FTLD-CDR, revised ALS Functional Rating Scale and ALS progression rate), survival and cortical thickness.

Results: 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 and the sAPPß/YKL-40 ratio correlated with the disease severity. In the whole ALS-FTD spectrum, NfL levels and the NfL:sAPPß ratio correlated with global cognitive performance ($r=-0.41$, $p<0.001$ and $r=-0.44$, $p<0.001$, respectively). In the ALS group, YKL-40 correlated with disease progression rate ($r=0.51$, $p=0.001$) and was independently associated with a shorter survival. In both FTD and ALS groups, the sAPPß/YKL-40 ratio showed a positive correlation with cortical thickness in frontotemporal regions.

Conclusions: sAPPß, YKL-40 and NfL could represent valuable tools for the staging and prognosis of patients within the ALS-FTD clinical spectrum.

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive paralytic disorder, defined by motor neuron degeneration (Brown and Al-Chalabi, 2017). However, patients with ALS may also display a continuum of cognitive and behavioral changes and up to 20% of ALS patients can be also diagnosed of some of the frontotemporal dementia (FTD)-related syndromes (Ng *et al.*, 2015; Woolley and Strong, 2015).

ALS and FTD share a common pathological hallmark that consists of the presence of TAR DNA binding protein (TDP-43) inclusions in the brain (Al-Chalabi *et al.*, 2012; Mackenzie and Neumann, 2016; Neumann *et al.*, 2006). Nearly 95% of ALS and 50% of FTD cases show partially overlapping patterns of TDP-43 inclusions across frontotemporal structures (Al-Chalabi et al., 2012; Mackenzie and Neumann, 2016; Neumann et al., 2006). In addition, neuropathologic and genetic studies have suggested that neuroinflammation may play a central role in the pathophysiology of ALS and FTD (Brettschneider *et al.*, 2013; 2014; Dols-Icardo *et al.*, 2018; Radford *et al.*, 2015).

Cerebrospinal (CSF) biomarkers may provide important insights into this clinical and pathological continuum by tracking different aspects of the pathophysiology. The axonal marker neurofilament light chain (NfL) is increased in CSF in ALS and FTD, reflects disease severity and correlates with brain atrophy (Menke *et al.*, 2015; Scherling *et al.*, 2014). We previously showed that levels of the soluble β fragment of amyloid precursor protein (sAPPβ) are decreased in CSF in FTD and correlate with frontotemporal neurodegeneration (Alcolea et al., 2017). In addition, YKL-40 (also known as Chitinase-3-like 1 protein or CHI3L1), a marker of astrocytic activity, is increased in CSF in FTD (Alcolea et al., 2014) but reports in the ALS-FTD continuum

are limited (Bonneh-Barkay *et al.*, 2010; Thompson *et al.*, 2018).

We aimed to investigate the CSF levels of sAPPß, YKL-40 and NfL in the entire ALS-FTD spectrum, and evaluate their correlation with clinical measures, disease progression and cortical thickness.

Methods

Study participants and classification

We analyzed CSF samples of 181 participants of the Sant Pau Initiative on Neurodegeneration (SPIN cohort: *https://santpaumemoryunit.com/our-research/spin-cohort/*). Patients with FTD were evaluated at the Memory Unit and were classified in one of the following clinical groups according to current diagnostic criteria: behavioral variant of frontotemporal dementia (bvFTD) (Rascovsky *et al.*, 2011), semantic variant of primary progressive aphasia (svPPA)(Gorno-Tempini *et al.*, 2011), non-fluent agrammatic primary progressive aphasia (nfaPPA), progressive supranuclear palsy (PSP) and corticobasal syndrome (CBS) (Armstrong *et al.*, 2013; Höglinger *et al.*, 2017). Patients with FTD and pathophysiological evidence of Alzheimer's disease ([AD], as defined by a t-tau/A$ß_{1-42}$ ratio > 0.52)(Alcolea *et al.*, 2014) were excluded according to the current diagnostic criteria (Armstrong *et al.*, 2013). During follow-up, patients with FTD were actively screened for signs or symptoms suggestive of motor neuron involvement and were referred to the Motor Neuron Disease (MND) clinic for further clinical and electrophysiological evaluation. Patients with FTD with confirmed motor neuron involvement were included in the ALS group.

Patients with ALS were prospectively recruited from the MND clinic at the Hospital de Sant Pau. Patients included in the study fulfilled El Escorial revised criteria for probable, probable laboratory-supported or definite ALS (Brooks *et al.*, 2000). All patients underwent a cognitive and behavioral screening that included a separate interview with a reliable informant and the administration of the Edinburgh Cognitive and behavioral ALS Screen (ECAS) (Niven *et al.*, 2015). ALS patients were classified according to previously reported criteria in one of the following groups: ALS without cognitive or behavioral impairment (ALSni), ALS with cognitive or behavioral impairment (ALSci-bi) and ALS-FTD (Strong *et al.*, 2017). For the main group comparisons, ALS-FTD participants were included in the ALS group.

Finally, a group of age-matched cognitively normal controls was randomly-selected from the SPIN cohort (Alcolea *et al.*, 2017; Sala *et al.*, 2017). Eighty-one (47%) participants have been previously reported elsewhere.(Alcolea *et al.*, 2017)

Sample composition

We included 86 patients with FTD, 38 with ALS and 49 cognitively normal controls. Among FTD patients, we included 46 cases of bvFTD, 8 patients with svPPA, 12 with nfaPPA and 20 within the PSP-CBS spectrum (Armstrong *et al.*, 2013; Höglinger *et al.*, 2017). The ALS group included 10 ALSni, 14 ALSci, 3 ALSci-bi and 11 ALS-FTD cases.

CSF analysis

Availability of CSF was required for the inclusion in the study. All biomarkers were analyzed at the Sant Pau Memory Unit Laboratory with commercially available ELISA kits of sAPPß, YKL-40 and NfL (human sAPPß-w, highly sensitive, IBL, Gunma, Japan; MicroVue, Quidel, San Diego, CA; NF-light, UmanDiagnostics, Umea, Sweden, respectively) following previously reported methods and manufacturer's instructions (Alcolea *et al.*, 2014; 2015; 2017).

Disease-staging and cognitive measures

In patients with FTD, we obtained the modified frontotemporal lobar degeneration clinical dementia rating (FTLD-CDR) as previously described (Knopman *et al.*, 2008). In patients with ALS, we obtained the revised ALS functional rating scale (ALSFRS-R) at the time of CSF sampling, and then calculated the ALS progression rate by dividing its value by the time from disease onset to CSF sampling, as previously described (Labra *et al.*, 2016). We defined disease onset as the time when the first symptom was observed (cognitive/behavioral or motor) according to the information provided by the patient or the informants. A total of 150 (83%) participants underwent a complete neuropsychological evaluation within 6 months of CSF sampling, using a previously described protocol (Sala *et al.*, 2017). In the FTD group, we z-transformed the raw values of neuropsychological measures using means and standard deviations of the group of age- and sex-matched controls selected for this study (all with a CDR sum of boxes of 0). A global cognitive score was calculated by averaging the following scores: CERAD list recall, CERAD list recognition, Boston Naming Test, Semantic fluency, Phonologic fluency, reverse digit span, Trail Making Test part A and B and the number location subtest of the Visual Object and Space Perception battery. In the ALS group we z-transformed the raw values of the ECAS total score using means and standard deviations of the control group selected for this study.

Genetic analysis and neuropathological study

All patients were screened for the *C9orf72* expansion. Additionally, patients with familial history of neurodegenerative diseases or psychiatric illness were screened for other known causal genes of FTLD (*MAPT*, *GRN*) and ALS (*TBK1, VCP, TARDBP*). Mutations were found in *C9orf72* (n = 5), *GRN* (n=1), *VCP* (n=1), *TARDBP* (n=1). Four participants in the ALS group (10.5%) and two in the FTD group (2.3%) had autopsy confirmation of motor neuron disease and FTLD, respectively.

Image acquisition, processing and analysis

A structural MRI was available for quantification in a subsample of 82 patients. Sixty-five participants were scanned on a 3T Philips Achieve using a T1-weighted MPRAGE protocol with a repetition time of 8.1 milliseconds, echo time of 3.7 milliseconds, 160 slices and voxel size of 0.94x0.94x1mm. Seventeen participants were scanned on a different 3T Philips Achieva using a T1-weighted MPRAGE protocol with a repetition time of 6.74 milliseconds, echo time of 3.14 milliseconds, 140 slices and a voxel size of 0.9x0.9x1.2 mm. Briefly, surface-based cortical reconstruction was performed using FreeSurfer v5.1 software package (http://surfer.nmr.mgh.harvard.edu) as previously reported (Dale *et al.*, 1999; Montal *et al.*, 2017). After a slice-by-slice visual inspection of the pial and white matter surface segmentation, 12 participants were excluded due to processing errors, leading to a final sample of 70 participants (21 ALS and 49 FTD). In this sample, a vertex-wise general lineal model (as implemented in FreeSurfer v5.1) was used to assess the correlation between CSF biomarkers and cortical thickness for each group independently (ALS and FTD). Specifically, for each surface vertex, a general lineal model was computed using cortical thickness as dependent variable and CSF values as independent variable. All these analyses were covariated by age, sex and magnetic resonance equipment. To control for false positives, a Monte-Carlo simulation with 10000 repeats as implemented in FreeSurfer (FWE < 0.05) was tested.

Statistical analysis

Differences between groups were assessed using ANOVA or t-test for continuous variables and the Chi-square for dichotomous or categorical data. Biomarker raw values not following a normal distribution were log-transformed to achieve a normal distribution. We calculated correlations between CSF biomarkers levels using Pearson's coefficient. For these analyses, we considered normally-distributed log-transformed values of CSF biomarkers when these did not follow a normal distribution. In the ALS group, we performed Cox regression analysis incorporating age at diagnosis, ALSFRS-R score, sex and onset site (spinal vs bulbar) as prognostic covariates. We also performed a univariate survival analysis by means of the Breslow test due to the high short term mortality in the ALS group. For the NfL survival analysis, participants were dichotomized in two groups according to the median NfL levels. For the YKL-40 survival analysis, participants were dichotomized according to the optimal YKL-40 cut-off (best Youden J index) for the differentiation between ALS patients and controls (AUC= 0.709 [95% CI: 0.595-0.824], p=0.001; cut-off=262). Statistical significance for all tests was set at 5% (α= 0.05) and all statistical tests were two-sided. All analyses were performed using SPSS 24 (Armonk, NY: IBM Corp.).

Standard protocol approvals, registrations, and patient consent

The study was approved by the local ethics committee and was conducted in accordance with the Declaration of Helsinki. All participants gave their written informed consent to participate in the study.

Data availability

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Primary research question/ classification of the evidence

Our primary research question was to determine whether the CSF levels of sAPPβ, YKL-40, and NfL, or their ratios relate to clinical measures of cognitive impairment, disease progression rate and frontotemporal cortical thickness within the ALS-FTD continuum. This study provides Class III evidence that CSF levels of sAPPß, YKL- 40, and NfL are useful to assess frontotemporal neurodegeneration and the progression rate in the ALS-FTD continuum.

Results

Demographics and clinical data

Table 1 shows the demographics, clinical data and biomarker levels in the FTD, ALS and control groups. There were no differences in age, sex or disease duration at CSF sampling between groups. Age at CSF sampling showed a mild correlation with YKL-40 levels (r=0.32, p<0.001) in the entire cohort. Disease duration at the time of CSF sampling correlated inversely with sAPPß and NfL levels (r=-0.22, p=0.02 and r=-0.24, p=0.01, respectively). As expected, the FTD group had worse cognitive performance than the ALS group and both FTD and ALS groups were more cognitively impaired than the control group (H(2)=57.2, p<0.001).

Different sAPPß levels in the FTD and ALS group

CSF levels of sAPPß differed between groups (figure 1). The FTD and ALS groups showed lower sAPPß levels than controls. The FTD group had lower sAPPß levels than the ALS group, and this difference remained significant after excluding patients with ALS-FTD from the ALS group. However, the differences in the sAPPβ levels between the ALS and control group were

	FTD	ALS	Controls
n (% of total sample)	86 (49.7)	38 (22)	49 (28.3)
Age at disease onset, years	64.2 (15.2)	66.6 (11.1)	–
Age at CSF sampling, years	67.8 (14.6)	69.2 (12.1)	66.3 (10.3)
Time from symptom onset to CSF sampling, months	3.7 (3.4)	1.8 (2.6)	–
Women, n (%)	32 (37.2)	20 (43.5)	23 (46.9)
Cognitive z-score	-2.4 (2.1)[b]	-1.6 (1.9)[a]	–
Single CSF biomarkers			
sAPPß, ng/mL	480 (294)[bc]	581.1 (373.4)[ac]	795.7(687.2)[ab]
YKL-40, ng/mL	266.7 ± 78.2[c]	287.2 ± 90.3[c]	224.4 ± 57.9[ab]
NfL, pg/mL	1250 (1609)[bc]	3082 (2280)[ac]	520 (249)[ab]
Biomarker ratios			
sAPPß:YKL-40	1.9 (1)[c]	2.24 (1.4)[c]	4.1 (3.6)[ab]
NfL:sAPPß	2.61 (4.3)[bc]	5.32 (6)[ac]	0.56 (0.7)[ab]
NfL:YKL-40	5.02 (4.9)[bc]	11.78 (8.5)[ac]	2.27 (1.2)[ab]

Table 1. Clinical and CSF data of the participants. Quantitative variables are shown as mean ± standard deviation, for continuous variables following a normal distribution and as median (interquartile range) for quantitative variables that were not normally distributed. Categorical variable are described with the number of participants and the relative frequency (%); a: different from FTD ($p<0.05$); b: different from ALS ($p<0.05$); c: different from controls ($p<0.05$); ns: no significant differences between groups ($p>0.05$). Abbreviations: FTD = Frontotemporal Dementia; ALS = Amyotrophic Lateral Sclerosis; CSF = Cerebrospinal Fluid.

no longer significant when ALS-FTD patients were excluded from the ALS group (post-hoc: DMS, p=0.066, 95% CI of the mean difference of log(sAPPß): -0.44 to 0.01).

Higher levels of YKL-40 and NfL in patients with ALS

As shown in table 1 and figure 1, YKL-40 levels were higher and the sAPPß:YKL-40 ratio was lower in the FTD and ALS groups compared to controls, but no differences were noted between the FTD and ALS groups. As previously reported, CSF NfL levels were different in the three groups: the highest NfL levels were observed in the ALS group, followed by the FTD group and the control group. Similar differences were observed between groups for the NfL:sAPPß and NfL:YKL-40 ratios (table 1).

Imaging correlates of sAPPß, YKL-40 and the sAPPß:YKL-40 ratio

We next studied a subset of 70 participants with structural MRI suitable for quantitative analyses. As shown in figure 3, there was a direct correlation of the sAPPß:YKL-40 ratio with the cortical thickness in the ALS and FTD groups. In ALS, this correlation was found in the superior temporal areas bilaterally, and in the lateral frontal regions in the left hemisphere. In FTD, this correlation was found as a bilateral widespread significant map in both temporal and frontal regions and the precuneus.

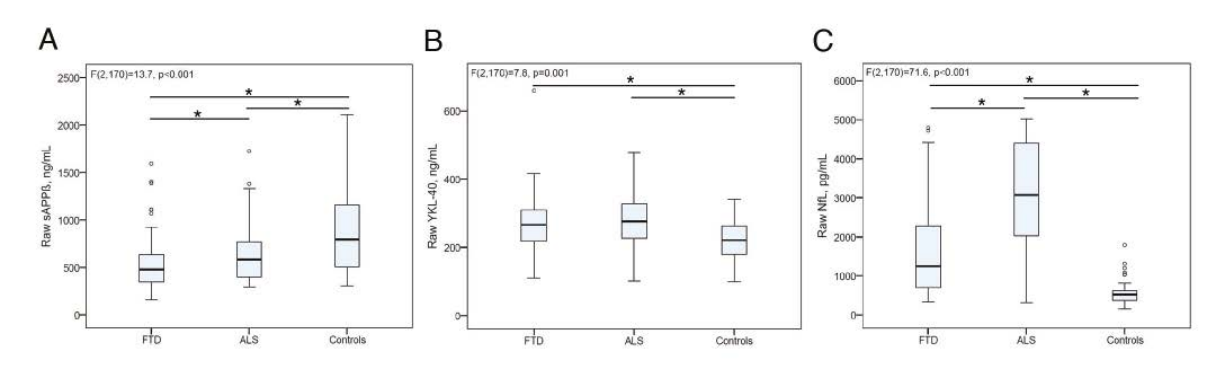

Figure 1. Group differences between cerebrospinal fluid biomarkers. Group differences between CSF biomarker raw levels of: A) sAPPß, B) YKL-40, C) NfL; *: p<0.05 for post-hoc: DMS, ANOVA performed with Log-transformed values in variables not following a normal distribution (NfL and sAPPß). **Abbreviations:** FTD = Frontotemporal Dementia; ALS = Amyotrophic Lateral Sclerosis; CSF, = Cerebrospinal Fluid.

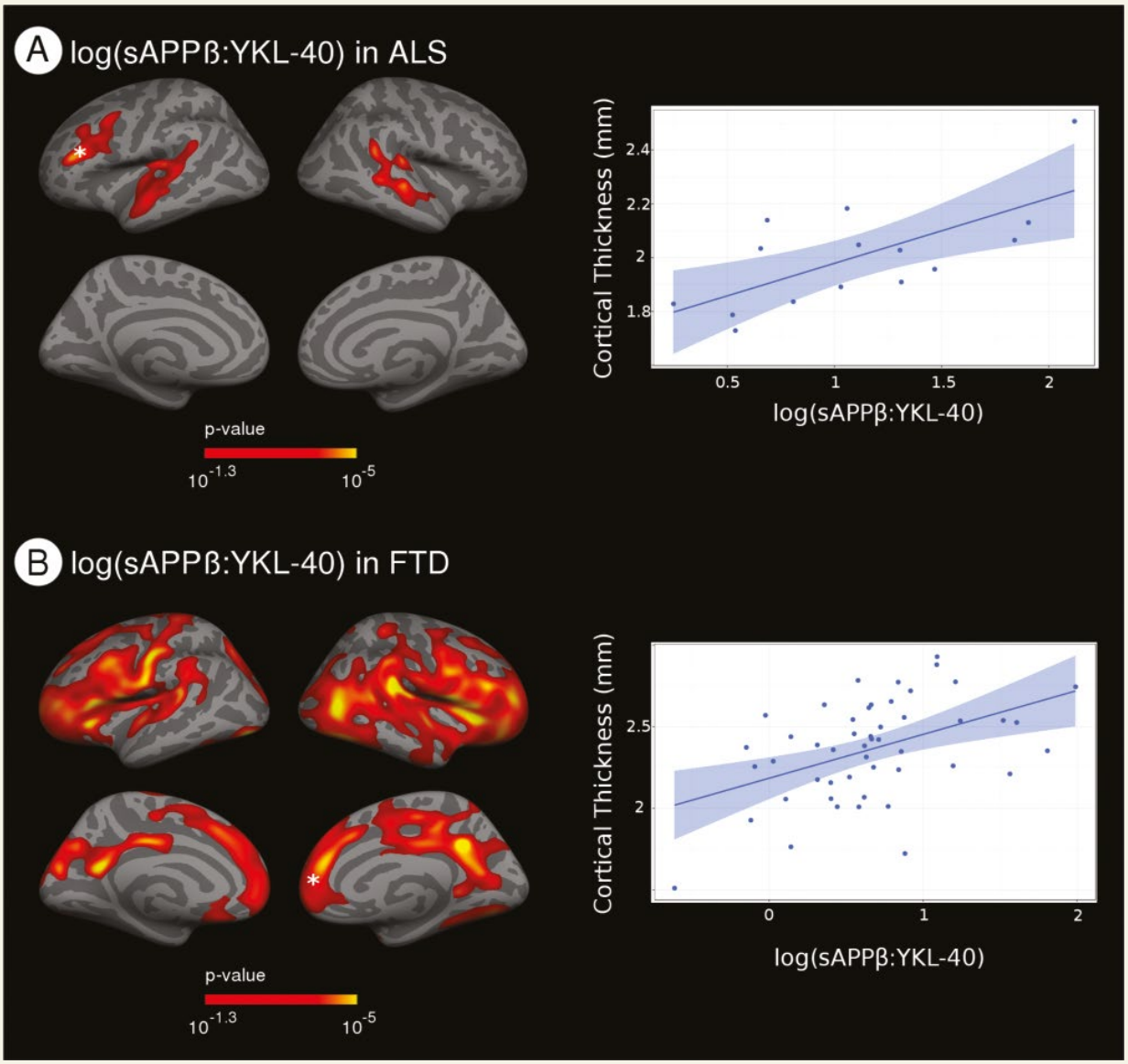

Figure 3. Relationship of cortical thickness with the sAPPß:YKL-40 ratio in the ALS (n=21) and FTD groups (n=49). A) Relationship of cortical thickness with the sAPPß:YKL-40 ratio levels in the ALS group (n=21, including 6 ALS-FTD patients); B) Relationship of cortical thickness with the sAPPß:YKL-40 ratio levels in the FTD group (n=49, without ALS-FTD patients). Red colored regions represent a direct correlation. For illustrative purposes, a scatterplot shows the individual log(sAPPß:YKL-40) and the value of cortical thickness in the corresponding cortical region (marked with a white asterisk).

CSF biomarkers across the ALS subgroups

No differences in age, sex or disease duration at CSF sampling were noted between the ALS subgroups based on cognitive or behavioral symptoms (data available from Dryad [table 3] https://doi.org10.5061/dryad.59sm77d). As expected, the ALSci-bi group showed a lower performance in the ECAS total score than the ALSni group, reflecting lower cognitive performance. No differences in CSF biomarkers were found neither between the different clinical subgroups of ALS patients nor between ALS patients with and without FTD as shown in the data available from Dryad (tables 3-4) https://doi.org10.5061/dryad.59sm77d.

Relationships between CSF biomarkers and cognitive measures

In the whole sample, the global cognitive performance as measured by the cognitive z-score showed the highest correlation with NfL levels and the NfL:sAPPß ratio (r=-0.41, p<0.001 and r=-0.44, p<0.001, respectively), with lower correlations for levels of sAPPß and YKL-40 (r=0.27, p=0.001 and r=-0.25, p=0.002, respectively). However, when we restricted the analysis to the ALS group no correlations were found between CSF biomarkers and the cognitive z-score.

Relationships between CSF biomarkers, disease severity and progression rate in FTD and ALS

In patients with FTD, the FTLD-CDR score showed the highest correlation with the sAPPß:YKL-40 ratio (r=-0.42, p=0.001, figure 2A). The FTLD-CDR score also correlated with sAPPß and NfL levels (r=-0.38, p=0.002, r=0.39, p=0.002, respectively) but not with YKL-40 (r=0.12, p=0.35). In the ALS group, none of the biomarkers correlated with disease severity, as measured by the ALSFRS-R. However, levels of YKL-40 and to a lesser extent levels of NfL correlated with the ALS progression rate (r=0.51, p=0.001, and r=0.39, p=0.02, respectively figure 2B).

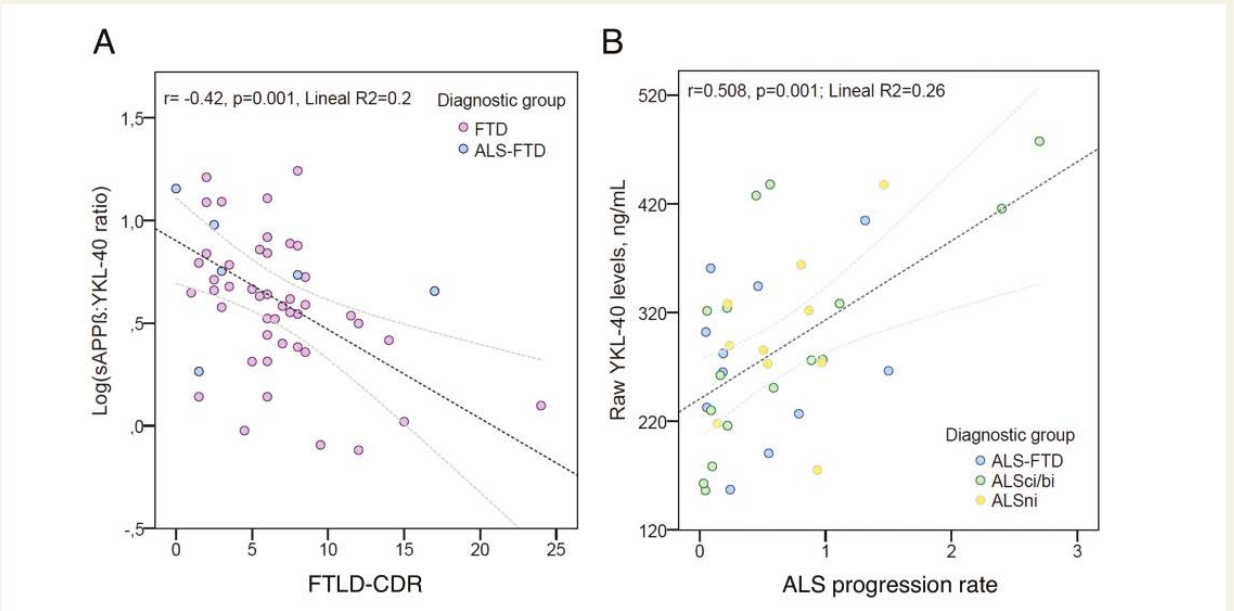

Figure 2. Relationships between cerebrospinal fluid biomarkers and measures of disease severity and progression in ALS and FTD. A) Correlation between sAPPß:YKL-40 ratio (log-transformed) and FTLD-CDR score in the FTD group (including the ALS-FTD patients with available FTLD-CDR scores, n=6); B) correlation between the raw YKL-40 levels and the ALS progression rate in the ALS group (including the ALS-FTD subgroup).

NfL and YKL-40 CSF levels are associated with a shorter survival in ALS

We finally investigated the relationship between NfL and YKL-40 and survival in the ALS group (mean follow-up of 11,6 months from the baseline assessment [SD=20.4], number of deaths: 18 [47,4%]). As shown in table 2, higher baseline levels of both NfL and YKL-40 were associated with a shorter survival in patients with ALS (for NfL, HR=1.0004, p=0.003; for YKL-40 HR=1.012, p=0.005). When we introduced both NfL and YKL-40 levels in the model, only YKL-40 levels remained significant (HR=1.009, p=0.048). As shown in figure 4, Kaplan-Meier survival curves showed different survival curves of ALS patients when stratified by NfL and YKL-40 levels (Breslow test, NfL p=0.036, YKL-40, p=0.045).

	Hazard ratio	95% Confidence Interval	p value
Model 1 (-2LL*=76.2, p=0.006)			
Age at disease onset	0.970	0.908-1.035	0.356
Gender (male)	0.857	0.229-3.204	0.819
ALFRS-R at CSF sampling	0.991	0.931-1.054	0.767
Bulbar onset	0.457	0.114-1.822	0.267
YKL-40	1.012	1.004-1.020	0.005
Model 2 (-2LL*=78.2, p=0.004)			
Age at disease onset	1.009	0.939-1.083	0.807
Gender (male)	1.178	0.288-4.829	0.820
ALFRS-R at CSF sampling	1.005	0.946-1.067	0.875
Bulbar onset	0.338	0.079-1.439	0.142
NfL	1.00036	1.000122-1.000598	0.003
Model 3 (-2LL*=73.7, p=0.001)			
Age at disease onset	0.981	0.911-1.056	0.613
Gender (male)	1.207	0.283-5.150	0.799
ALFRS-R at CSF sampling	1.007	0.944-1.074	0.835
Bulbar onset	0.364	0.086-1.541	0.170
YKL-40	1.009	1.000-1.018	0.048
NfL	1.000219	0.999950-1.000488	0.111

Table 2. Cox proportional hazards survival models in patients with ALS. Cox proportional hazards survival models were performed in the ALS group (n=38). We set three different models. We first introduced in the three models age at disease onset, ALSFRS-R at CSF sampling and site of onset (bulbar vs spinal) as independent variables and survival time as the dependent variable (mean follow-up of 11.6 months, with 18 deaths [47.4%] at the end of follow-up time). In model 1 we added the CSF levels of YKL-40 as independent variable. In model 2 we added the CSF levels of NfL as independent variable. Finally, in model 3 we added both the CSF levels of YKL-40 and NfL as independent variables. Key: -2LL: Log-Likelihood ratio (lower values indicate a better model fit).

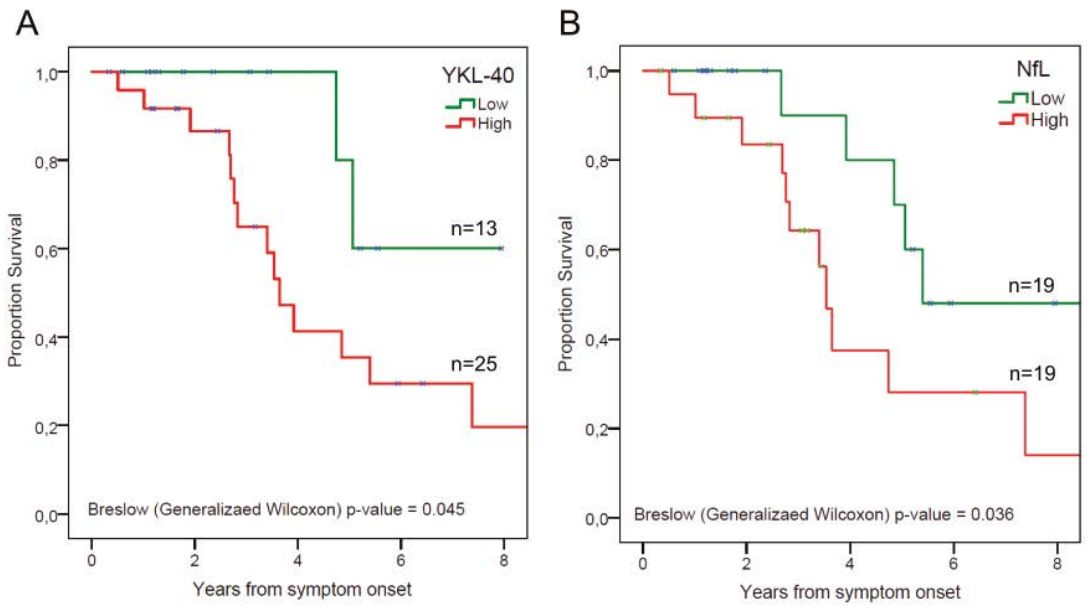

Figure 4. Survival analysis in ALS patients, stratified by YKL-40 and NfL CSF levels. Survival analysis in ALS patients (n=38), stratified by YKL-40 (A) and NfL CSF levels (B). For the YKL-40 survival analysis, participants were dichotomized according to the optimal YKL-40 cut-off (best Youden J index) for the differentiation between ALS patients and controls (AUC= 0.709 [95% CI: 0.595-0.824], p=0.001; cut-off=262). For the NfL survival analysis, participants were dichotomized in two groups according to the median NfL levels. p-values of Breslow (Generalized Wilcoxon) test are listed.

Discussion

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 describe that CSF levels of YKL-40 correlate with progression rate and survival in ALS.

Neuroinflammation is a relevant pathophysiological component in ALS and other neurodegenerative diseases (Heneka *et al.*, 2015; Philips and Robberecht, 2011; Querol-Vilaseca *et al.*, 2017). In ALS, microglial activation has shown to directly contribute to neuronal death (Frakes *et al.*, 2014). Biomarkers that track neuroinflammatory activity, such as YKL-40, may be a valuable tool for monitoring the inflammatory response during the disease course. YKL-40 (also known in the literature as chitinase-3-like 1 protein) has been found to be expressed by astrocytes in human brain tissue in healthy controls and in different neurodegenerative diseases (Querol-Vilaseca *et al.*, 2017). Interestingly, CSF levels of YKL-40 correlate with disease progression in multiple sclerosis (Comabella *et al.*, 2010). We show that YKL-40 levels in CSF are increased in ALS. Two previous reports have investigated CSF YKL-40 levels in patients with ALS (Bonneh-Barkay *et al.*, 2010; Thompson *et al.*, 2018). In one of these studies, YKL-40 and two other chitinases were found differentially abundant between ALS patients and controls (Thompson *et al.*, 2018). Notably all chitinases levels correlated with disease progression rate. Our results confirm the increase of YKL-40 in ALS and its correlation with progression rate. Conversely, the authors of this recent work found and association between CHIT1 but not YKL-40 level and survival, while we found that YKL-40 was indeed associated with a shorter survival (Thompson et al., 2018).

It is possible that methodological differences in the sample composition (i.e. inclusion of patients with progressive lateral sclerosis and progressive muscular atrophy) and the assay used may explain the observed differences. Taken together, these results reinforce previous evidence underlying the close relation between neuroinflammation and progression rate in ALS (Boillee *et al.*, 2006; Yamanaka *et al.*, 2008).

We also report lower CSF levels of sAPPß in ALS when compared to age-matched controls. We previously reported a reduction of CSF sAPPß levels across multiple FTLD-related syndromes levels in a large multicentre study (Alcolea *et al.*, 2017). These results have been recently replicated in an autopsy-confirmed FTLD cohort (Alcolea *et al.*, 2018). In those previous studies, the correlation of sAPPß levels with cortical thickness in frontotemporal areas led us to speculate that sAPPß levels may reflect neuronal loss in frontotemporal areas where the amyloid precursor protein is predominantly expressed (Ferrari *et al.*, 2017). We hypothesize that the observed intermediate levels of sAPPß in ALS patients could be related to lesser pathological burden in frontotemporal areas of ALS patients when compared to FTD patients. This may explain why the observed differences between the CSF levels of sAPPβ in the ALS group compared to the control group were no longer significant when ALS-FTD patients were excluded from the ALS group. Our imaging findings support this hypothesis as well, as we found a direct correlation of the sAPPß:YKL-40 ratio with cortical thickness in frontotemporal regions in the ALS and FTD groups.

We also measured the CSF levels of NfL, an established biomarker of neurodegeneration in the ALS-FTD continuum. NfL is an axonal cytoskeletal constituent essential for axonal growth (Scherling *et al.*, 2014). NfL levels have been found elevated in the CSF and serum of ALS patients as well as in other brain disorders such as AD and traumatic brain injury (Mattsson *et al.*, 2016; Neselius *et al.*, 2014). Consistent with the previous evidence supporting the NfL in CSF as a diagnostic biomarker in ALS, we found clear differences in the CSF levels of NfL of patients and controls. NfL levels separated patients from controls in a much cleaner manner than sAPPß and YKL-40. In addition to its role in diagnosis, NfL levels have also been related to ALS progression (Gaiani *et al.*, 2017). We found that NfL and YKL-40 levels at diagnosis predicted a shorter survival in ALS patients after adjusting for other established prognostic factors. Moreover, when we introduced both biomarkers in the Cox proportional hazards survival model, only YKL-40 levels remained significant. However, caution is needed in drawing firm conclusions around an independent role of YKL-40 levels and further studies are needed to ascertain the specific contribution of NfL and YKL-40 CSF levels to survival in ALS. Our results confirm previous results with NfL and further suggest that YKL-40 may be a useful addition in the prognostic evaluation of ALS (Oeckl *et al.*, 2016; Steinacker *et al.*, 2016).

The main strengths of this study are the prospective design and the detailed cognitive and behavioral evaluation of all patients. We applied a prospective deep phenotyping protocol that allowed us to investigate in detail correlations between CSF biomarkers and cognitive performance, disease severity and progression rate in the patients within the ALS-FTD continuum. This study has also some limitations. Although we found significant differences in the CSF levels of sAPPß and YKL-40 between patients and controls, we observed a considerable overlap between groups. Although this overlap may limit its value as diagnostic markers when used in isolation, its combination with other established biomarkers such as NfL may add significant prognostic information. Finally, the study lacks pathological confirmation of the diagnosis in most cases and misdiagnosis could have occurred. However, misdiagnosis in ALS is rare and FTD patients with evidence

of AD pathophysiology in their CSF biomarker profile were excluded to avoid the inclusion of patients with atypical variants of AD that may have been misdiagnosed as FTD.

This study supports the role of neuroinflammation in the ALS pathophysiology and progression. Further longitudinal studies should investigate the effect of sAPPß and YKL-40 (alone or combined with NfL) on the progression rate and prognosis. These are key aspects to accelerate the development of effective disease-modifying treatments for patients with ALS.

Acknowledgments

The authors thank the patients and their relatives for their support for this study.

Study funding

This study was supported by the Fondo de Investigaciones Sanitario (FIS), Instituto de Salud Carlos III (PI15/01618 to R.R., PI14/1561 and PI17/01896 to A.L), jointly funded by Fondo Europeo de Desarrollo Regional, Unión Europea, Una manera de hacer Europa. This work was also supported by Departament de Salut de la Generalitat de Catalunya, Pla Estratègic de Recerca i Innovació en Salut (SLT002/16/00408 to A.L), Fundació La Marató de TV3 (201437.10 to R.R); I. Illán-Gala is supported by an i-PFIS grant (IF15/00060) from the FIS, Instituto de Salud Carlos III and the Rio Hortega grant (CM17/00074) from "Acción Estratégica en

Supplementary material

	ALSni	ALSci-bi	ALS-FTD
n (%)	10 (26)	17 (45)	11 (29)
Age at onset, years	60.6 (17.1)	67.2 (9.2)	69.2 (13.4)
Women, n (%)	3 (27.3)	11 (64.7)	3 (27.3)
Time from symptom onset to CSF sampling, years	1.2 (1.9)	2.3 (3.5)	2 (7)
Bulbar onset, n (%)	2 (20)	4 (23.5)	1 (9.1)
Cognitive and functional evaluation			
ECAS total score†	110 (19.5)[b]	86 (15.5)[a]	93.5 (31)
ALSFRS-R	37 (12)	38 (14.5)	34 (14)
ALS progression rate	0.67 (0.71)	0.45 (0.84)	0.24 (0.7)
Single CSF biomarkers			
CSF sAPPß, ng/mL	585.8 (465.5)	584.5 (438.1)	577.8 (241.6)
CSF YKL-40, ng/mL	296.6 ± 73.2	284.8 ± 108.7	275.7 ± 74.2
CSF NfL, pg/mL	3691 (1561)	3070 (2357)	2229 (2741)

Table e-1. Comparison of patient characteristics and CSF biomarkers between ALS subgroups. Quantitative variable are shown as mean ± standard deviation for continuous variables following a normal distribution and as median (interquartile range) for quantitative variables that were not normally distributed. Categorical variable are described with the number of subjects and the relative frequency (%); a: different from FTD ($p<0.05$); b: different from ALS ($p<0.05$); c: different from controls ($p<0.05$); ns: no significant differences between groups; †: Available in 27/38 (71%) cases. **Abbreviations:** ALSni = Amyotrophic Lateral Sclerosis without cognitive or behavioral impairment; ALSci-bi = Amyotrophic Lateral Sclerosis with Behavioral and/or cognitive impairment; ALS-FTD = Amyotrophic Lateral Sclerosis with Frontotemporal Dementia; ALSFRS = Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; ECAS = Edinburgh Cognitive and behavioral ALS Screen; CSF = Cerebrospinal Fluid.

	ALS	ALS-FTD
N (total = 46)	27 (71)	11 (29)
Age at onset, years	63.5 (8.6)^{ns}	69.2 (13.4)^{ns}
Women, n (%)	15 (55.6)^{ns}	3 (27.3)^{ns}
Time from symptom onset to CSF sampling, years	1.62 (2.1)^{ns}	2 (7)^{ns}
Bulbar onset, n (%)	6 (22.2)^{ns}	1 (9.1)^{ns}
Cognitive and functional evaluation		
ECAS total score	89 (23)^{ns}	93.5 (31)^{ns}
Cognitive z-score	-1.83 ± 1.5^{ns}	-2.05 ± 2.4^{ns}
ALSFRS-R	38 (13)^{ns}	34 (14)^{ns}
ALS progression rate	0.54 (0.77)^{ns}	0.24 (0.70)^{ns}
Single CSF biomarkers		
CSF sAPPß, ng/mL	584.5 (427.1)^{ns}	577.8 (241.6)^{ns}
CSF YKL-40, ng/mL	289.2 ± 95.7^{ns}	275.7 ± 74.2^{ns}
CSF NfL, pg/mL	3648 (2043)^{ns}	2229 (2741)^{ns}

Table e-2 Comparison of patient characteristics and CSF biomarkers between ALS subgroups. Quantitative variable are shown as mean ± standard deviation for continuous variables following a normal distribution and as median (interquartile range) for quantitative variables that were not normally distributed. Categorical variable are described with the number of subjects and the relative frequency (%); a: different from FTD; b: different from ALS; c: different from controls; ns: no significant differences between groups. **Abbreviations** = ALSni, Amyotrophic Lateral Sclerosis without cognitive or behavioral impairment; ALSci-bi = Amyotrophic Lateral Sclerosis with Behavioral and/or cognitive impairment; ALS-FTD = Amyotrophic Lateral Sclerosis with Frontotemporal Dementia; ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; ECAS = Edinburgh Cognitive and behavioral ALS Screen; CSF = Cerebrospinal Fluid.

Chapter 5

Cortical microstructure in the behavioral variant of frontotemporal dementia: looking beyond atrophy

Paper accepted for publication in
Brain
(december 21st 2018)

Title:

CSF sAPPβ, YKL-40, and NfL along the ALS-FTD spectrum

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Abstract

Cortical mean diffusivity has been proposed as a novel biomarker for the study of the cortical microstructure in Alzheimer´s disease. In this multicenter study, we aimed to assess the cortical microstructural changes in the behavioral variant of frontotemporal dementia (bvFTD); and to correlate cortical mean diffusivity with clinical measures of disease severity and CSF biomarkers (neurofilament light and the soluble fraction beta of the amyloid precursor protein). We included 148 participants with a three-Tesla MRI and appropriate structural and diffusion weighted imaging sequences: 70 bvFTD patients and 78 age-matched cognitively healthy controls. The modified frontotemporal lobar degeneration clinical dementia rating was obtained as a measure of disease severity. A subset of patients also underwent a lumbar puncture for CSF biomarker analysis. Two independent raters blind to the clinical data determined the presence of significant frontotemporal atrophy to dichotomize the participants into possible or probable bvFTD. Cortical thickness and cortical mean diffusivity were computed using a surface-based approach. We compared cortical thickness and cortical mean diffusivity between bvFTD (both using the whole sample and probable and possible bvFTD subgroups) and controls. Then we computed the Cohen's d effect size for both cortical thickness and cortical mean diffusivity. We also performed correlation analyses with the modified frontotemporal lobar degeneration clinical dementia rating score and CSF neuronal biomarkers. The cortical mean diffusivity maps, in the whole cohort and in the probable bvFTD subgroup, showed widespread areas with increased cortical mean diffusivity that partially overlapped with cortical thickness, but further expanded to other bvFTD-related regions. In the possible bvFTD subgroup, we found increased cortical mean diffusivity in frontotemporal regions, but only minimal loss of cortical thickness. The effect sizes of cortical mean diffusivity were notably higher than the effect sizes of cortical thickness in the areas that are typically involved in bvFTD. In the whole bvFTD group, both cortical mean diffusivity and cortical thickness correlated with measures of disease severity and CSF biomarkers. However, the areas of correlation with cortical mean diffusivity were more extensive. In the possible bvFTD subgroup, only cortical mean diffusivity correlated with the modified frontotemporal lobar degeneration clinical dementia rating. Our data suggest that cortical mean diffusivity could be a sensitive biomarker for the study of the neurodegeneration-related microstructural changes in bvFTD. Further longitudinal studies should determine the diagnostic and prognostic utility of this novel neuroimaging biomarker.

Abbreviations

bvFTD = behavioral variant of frontotemporal dementia; CATFI = catalan frontotemporal dementia initiative; CSF = cerebrospinal fluid; FTD-ALS = frontotemporal dementia-amyotrophic lateral sclerosis; FTLD = frontotemporal lobar degeneration; FTLD-CDR = frontotemporal lobar degeneration clinical dementia rating; FTLDNI = frontotemporal lobar degeneration neuroimaging initiative; FTLD-Tau = tau subtype of frontotemporal lobar degeneration; FTLD-TDP = transactive response DNA-binding protein 43 kDa subtype of frontotemporal lobar degeneration; HSP = hospital de sant Pau; HCB = hospital clínic de Barcelona; MRI = magnetic resonance imaging; NfL = neurofilament light; PSP-CBD: progressive supranuclear palsy-corticobasal degeneration; sAPPβ = soluble fragment-beta of the Amyloid Precursor Protein; UCSF = university of California san Francisco.

Introduction

Frontotemporal lobar degeneration (FTLD) is a neuropathological construct encompassing multiple neurodegenerative diseases sharing partially overlapping patterns of frontal and/or temporal grey matter neurodegeneration (Bang *et al.*, 2015). The behavioral variant of frontotemporal dementia (bvFTD) is a common clinical presentation of FTLD (Seo *et al.*, 2018). Clinically, bvFTD is characterized by progressive personality changes followed by social, cognitive and functional deterioration (Ranasinghe *et al.*, 2016). With the exception of genetically determined cases, the diagnosis of bvFTD relies on the clinical and neuroimaging features (Rascovsky *et al.*, 2011; Wood *et al.*, 2013). The refinement of the diagnostic criteria proposed by the frontotemporal dementia consortium has been an important step forward to improve the diagnosis of the bvFTD. Furthermore, these criteria have shown a good diagnostic value in pathology-confirmed cases (Balasa *et al.*, 2015; Chare *et al.*, 2014; Perry *et al.*, 2017; Rascovsky *et al.*, 2011; Seo *et al.*, 2018). In the frontotemporal dementia consortium criteria, the presence of frontal and/or temporal atrophy increases the diagnostic certainty once the clinical criteria for possible bvFTD are met. However, a number of patients are still misdiagnosed with other neurodegenerative and non-neurodegenerative diseases (Bang *et al.*, 2015). Several factors, such as the absence of prominent cortical atrophy in up to a third of the patients (Ranasinghe *et al.*, 2016; Rascovsky *et al.*, 2011), may contribute to misdiagnosis. Conversely, possible bvFTD may include both neurodegenerative cases in early phases of the disease and non-neurodegenerative phenocopies (Gossink *et al.*, 2016; Khan *et al.*, 2012). Thus, the development of novel biomarkers able to increase the diagnostic certainty of FTLD is essential (Binney *et al.*, 2017; Downey *et al.*, 2015; Lam *et al.*, 2013; Meeter *et al.*, 2017). These are key aspects for the detection of patients with FTLD-related syndromes, especially at the earliest phase in clinical practice and for the selection of candidates to trials with protein-specific targeted therapies that may be more effective in earlier stages (Elahi and Miller, 2017).

Most neuroimaging studies in bvFTD have been focused on the cortical macrostructure with different metrics (grey matter density in voxel-based morphometry studies or cortical thickness in surface-based analyses) (Elahi *et al.*, 2017; Mahoney, Simpson, *et al.*, 2014; Meeter *et al.*, 2017) or white matter microstructural properties (namely diffusion tensor imaging metrics such as, fractional anisotropy). However, diffusion tensor imaging can also be used to measure the magnitude of diffusivity (mean diffusivity), in the cerebral cortex (Weston *et al.*, 2015; Montal *et al.*, 2017). Higher cortical mean diffusivity values reflect microstructural disorganization and disruption of cellular membranes, and have been proposed as a sensitive biomarker which might antedate macroscopic cortical changes (Weston *et al.*, 2015). However, only a single small study has assessed mean diffusivity changes in frontotemporal dementia (Whitwell *et al.*, 2010). In that previous study no clear differences were found between gray matter density and gray matter mean diffusivity, as assessed on a voxel-based approach. However, the voxel-based approach may fail to capture the subtle tissue-specific changes that take place at the cortical level (Weston et al., 2015).

In bvFTD, there are no validated pathophysiologic biomarkers to reflect the underlying pathology, with the exception of pathogenic mutations that predict specific FTLD subtypes. However, CSF biomarkers may also contribute to our understanding of FTLD pathophysiology (Lleo *et al.*, 2018; Meeter *et al.*, 2017). Particularly, the CSF levels of neurofilament light (NfL) (an axonal cytoskeletal constituent essential for axonal growth) have shown to be a useful neurodegeneration biomarker in FTLD-related syndromes (Menke *et al.*, 2015; Scherling *et al.*, 2014). In addition to

NfL, we have recently shown that the levels of the soluble fragment-beta of the Amyloid Precursor Protein (sAPPβ) (Alcolea *et al.*, 2017) may be useful to track neurodegeneration in frontotemporal structures in frontotemporal dementia (Alcolea *et al.*, 2017; Illán-Gala I *et al.*, 2018).

In this multicentre study, we aimed to assess the cortical mean diffusivity changes in a large multicenter cohort of bvFTD patients, and to correlate these changes with clinical measures of disease severity (FTLD-CDR) and CSF biomarkers (NfL and sAPPβ). We hypothesized that cortical mean diffusivity may be more sensitive than cortical thickness to detect the cortical changes associated with bvFTD.

Material and Methods

Study participants

Participants were recruited in three different centers from two collaborative studies: The Catalan Frontotemporal Dementia Initiative (CATFI) and the Frontotemporal Lobar Degeneration Neuroimaging Initiative (FTLDNI).

The CATFI is a multicenter study focused on the development of novel biomarkers and therapeutic interventions for patients suffering from frontotemporal dementia. The CATFI study includes patients from three centers (Hospital de Sant Pau [HSP], Hospital Clínic de Barcelona [HCB] and Hospital Arnau de Vilanova). The principal investigator of the CATFI study is Dr. Alberto Lleó. The primary goals of FTLDNI are to identify neuroimaging modalities and methods of analysis for tracking FTLD and to assess the value of imaging versus other biomarkers in diagnostic roles. The Principal Investigator of FTLDNI is Dr. Howard Rosen at the University of California, San Francisco (UCSF). For up-to-date information on participation and protocol, please visit: http://memory.ucsf.edu/research/studies/nifd.

The inclusion criteria in this study were: (i) diagnosis of possible or probable bvFTD according to the frontotemporal dementia consortium criteria (Rascovsky *et al.*, 2011); and (ii) 3T MRI study available for structural and cortical mean diffusivity analysis (see below for details). In both cohorts the diagnosis was made by neurologists with expertise in the evaluation the FTLD-related syndromes after an extensive neurological and neuropsychological evaluation. Moreover, patients were followed longitudinally at each center to ascertain if they presented a progressive clinical deterioration or developed a second FTLD-related syndrome (i.e. amyotrophic lateral sclerosis or a progressive supranuclear palsy phenotype). Because the diagnosis of bvFTD has been related to non-neurodegenerative conditions in some cases that do not show the typical clinical progression, we identified bvFTD patients with increased certainty of underlying FTLD when any of the following criteria were met: (i) clinical evidence of disease progression (clinical deterioration evidenced during follow-up or progression to a second phenotype related to FTLD); (ii) genetic confirmation of FTLD (identification of a pathogenic mutation); (iii) confirmation of FTLD in those patients with neuropathological evaluation.

Figure1 shows the flowchart of the sample composition. A total of 192 participants with appropriate 3T structural and diffusion-weighted MRI were considered for analysis. Of these, 44 (23%) participants were excluded due to quality control issues or processing errors. All the excluded cases were bvFTD patients.

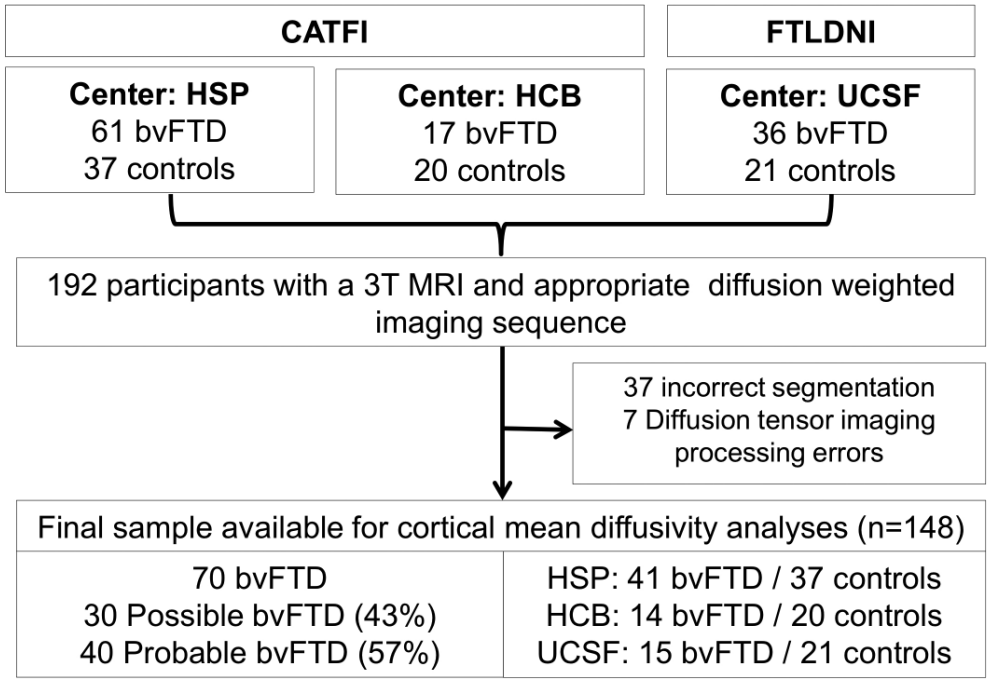

Figure 1. Flowchart of the sample composition. **Abbreviations:** bvFTD = behavioral variant of frontotemporal dementia; CATFI = catalan frontotemporal dementia initiative; FTLDNI = frontotemporal lobar degeneration neuroimaging initiative; HSP = hospital de sant Pau; HCB = hospital clínic de Barcelona; UCSF = university of California san Francisco.

Clinical measures of disease severity

The modified frontotemporal lobar degeneration clinical dementia rating (FTLD-CDR) was obtained as previously described, as a measure of disease severity the bvFTD (Knopman *et al.*, 2008). Higher scores in the FTLD-CDR reflect a higher disease severity.

Genetic studies

Patients were screened for genetic mutations known to cause autosomal dominant inheritance of frontotemporal dementia as previously reported (Illán-Gala I *et al.*, 2018; Perry *et al.*, 2017).

Pathological assessment

Neuropathological assessments were performed at the Barcelona brain bank (n=1) or at UCSF (n=5) following previously described procedures (Tartaglia *et al.*, 2010; Balasa *et al.*, 2015). Pathology-proven FTLD cases were classified in one of the major molecular subtypes (tau, TDP-43, FUS or unclassifiable).

MRI acquisition

MRIs (3 T) were acquired at three different sites. The acquisition parameters by center can be found in the Supplementary Material. All centers had a structural MPRAGE T1-weighted acquisition of approximately 1 X 1 X 1 mm isotropic resolution and an EPI diffusion-weighted acquisition of at least 2.7 X 2.7 X 2.7 mm isotropic resolution.

Possible/Probable classification according to MRI atrophy on visual inspection

In order to determine the presence of significant frontotemporal atrophy consistent with the diagnosis of probable bvFTD according to the frontotemporal dementia consortium criteria (Rascovsky *et al.*, 2011), all the MRIs from bvFTD participants analyzed in this study (n=114) were visually inspected by two independent raters blinded to the clinical data in order to determine the presence of significant frontotemporal atrophy to dichotomize the participants into possible bvFTD (bvFTD patients with a negative or conflicting atrophy rating) or probable bvFTD (bvFTD patients rated as positive atrophy by the two raters)(Rascovsky *et al.*, 2011).

CSF sampling and analysis

A subset of 32 CATFI patients had also cerebrospinal fluid (CSF) available. We measured the CSF levels of NfL and sAPPβ as previously described (Alcolea *et al.*, 2014; 2015; 2017). All biomarkers were analyzed at the Sant Pau Memory Unit Laboratory with commercially available ELISA kits (NF-light, Uman Diagnostics, Umea, Sweden; human sAPPß-w, highly sensitive, IBL, Gunma, Japan).

Cortical thickness processing

Cortical thickness reconstruction was performed with the Freesurfer package v5.1 (http://surfer.nmr.mhg.hardvard.edu) using a procedure that has been described in detail elsewhere (Fischl and Dale, 2000). All individual cortical reconstructions were visually inspected in a slice-by-slice basis to check for accuracy of the grey/white matter boundary segmentation. From the initial 114 bvFTD subjects with 3T MRI available from the three centers, 37 (32.5%) were excluded due to segmentation issues. Cognitively healthy controls scans did not require manual editing. Finally, each individual reconstructed brain was registered, and cortical thickness maps were morphed, to the *fsaverage* standard surface provided by Freesurfer, using a spherical registration, enabling an accurate inter-subject matching of cortical locations for the computation of further statistics. Prior to statistical analyses, we smoothed the cortical thickness maps using a Gaussian kernel with FWHM of 10mm as implemented in Freesurfer (Hagler *et al.*, 2006).

Cortical mean diffusivity processing

We used a previously described home-made surface-based approach to process cortical diffusion MRI (Montal *et al.*, 2017). Recent studies have shown the potential of surface-based methods to measure microstructural changes in neurodegenerative diseases (Montal *et al.*, 2017; Parker *et al.*, 2018) and the cortical architecture (Ganepola *et al.*, 2017). An important advantage of these methods is the mitigation of partial volume effects or kernel-sensitive CSF signal inclusion during the smoothing step (Coalson *et al.*, 2018). Briefly, diffusion weighted imaging data were first corrected for motion effects applying a rigid body transformation between the b=0 image and the diffusion-weighted acquisitions. Then, after removing non-brain tissue using the Brain Extraction Tool, diffusion tensors were fitted and mean diffusivity was computed using the FSL's dtifit command. We then computed the affine transformation between the skull-stripped b0 and the segmented T1-weighted volume using a boundary-based algorithm as implemented in Freesurfer's bbregister. This approach takes advantage of the accurate segmentation of the white matter surface and pial surface obtained during the Freesurfer's segmentation (cortical thickness processing section), to accurately register the b0 and the T1-weighted image, maximizing the intensity gradient across grey matter and white matter between both volumes. At this point, all the diffusion to T1 registrations were visually inspected to exclude those subjects with an erro-

neous co-registration. Then, the mean diffusivity volume for each individual was sampled at the midpoint of the cortical ribbon (half the distance along the normal vector between the white matter surface and the gray matter surface) and projected to each individual surface reconstruction obtained during the Freesurfer processing, to create a surface map of cortical mean diffusivity (using Freesurfer's mri_vol2surf command). Finally, individual cortical mean diffusivity maps were normalized to an average standard surface using a spherical registration, enabling an accurate inter-subject matching of cortical locations for the statistical analyses. Prior to statistical analyses, we applied a Gaussian kernel of 15 mm as implemented in Freesurfer, in order to obtain equivalent data effective smoothing between cortical thickness and cortical mean diffusivity (Bejanin *et al.*, 2018; La Joie *et al.*, 2012).

Cortical mean diffusivity harmonization between centers

Diffusion tensor imaging metrics are very sensitive to acquisition parameters (Zhu et *al.*, 2011). Thus, harmonization approaches are required to mitigate center-specific differences in multicenter studies. We applied a multi-center harmonization algorithm based on ComBat, in order to reduce center-specific differences in cortical mean diffusivity quantifications prior to any statistical analysis (Fortin *et al.*, 2017). Briefly, ComBat uses an empirical Bayes framework to estimate the additive (mean) and multiplicative (variance) contribution of each site, at each vertex, for a specific diffusion tensor imaging metric, and corrects these effects. Importantly, this approach allows the inclusion of biological information (such as clinical group, age or biomarkers), and it has been reported to preserve within-site biological variability, thereby increasing the statistical power.

Statistical methods

Group differences in the clinical and biomarker data were assessed using t-test or ANOVA for continuous variables, and Chi-squared tests were used for dichotomous or categorical data. Biomarker values not following a normal distribution were log-transformed. Statistical analyses were performed with the IBM SPSS Statistics 25 (IBM corp.) software. Statistical significance for all tests was set at 5% (α=0.05), and all statistical tests were two-sided.

We first performed group comparisons for cortical mean diffusivity and cortical thickness with a two-class general linear model, as implemented in Freesurfer, comparing bvFTD and the cognitively healthy controls groups. These analyses were repeated for each center independently. Moreover, as it has been reported that some possible bvFTD cases may represent either non-neurodegenerative cases or cases with a slowly progressive clinical course, we also compared the patterns of cortical thickness and cortical mean diffusivity in both the probable and possible subgroups. We then performed a vertexwise partial correlation analysis in the bvFTD group between the cortical mean diffusivity and cortical thickness and the log-transformed CSF sAPPβ and NfL values, in addition to the FTLD-CDR. Specifically, a general linear model was created in which cortical mean diffusivity or cortical thickness was included as the dependent variable, and CSF values and FTLD-CDR scores were independent variables. We included age, sex, and center as nuisance variables in the cortical thickness analysis. In mean diffusivity analysis, only age and sex were included since diffusion tensor imaging data were already harmonized between centers in a previous step. The correlation between both metrics and FTLD-CDR was also assessed segregating the bvFTD group into possible and probable. Only results that survived multiple comparisons (family wise error < 0.05) based on Monte Carlo simulation with 10,000 re-

peats as implemented in Freesurfer are presented. We used a very stringent threshold of α=0.001 for the group analyses and a threshold of α=0.05 for the correlation analyses. A full description of the multiple comparisons methodology can be found in the Supplementary material.

We computed the Cohen's d effect size metric for both cortical thickness and cortical mean diffusivity, in a vertex-wise basis, in order to obtain a topographical representation of the effect size for the group comparison between bvFTD patients and cognitively healthy controls. Effect size computation was restricted to cortical regions showing statistically significant differences between bvFTD and cognitively healthy controls for either cortical thickness or cortical mean diffusivity. We then computed the difference between the cortical thickness and cortical mean diffusivity effect size maps to obtain a topographical representation of the net effect size for each metric. For the figure projection and design, we used a freely available python library to overlay the results into the standard fsaverage surface (Pysurf: https://pysurfer.github.io).

Data availability

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Results

Demographics and sample composition

Table 1 shows the demographics, clinical and neuroimaging features of the participants in the study. Age at MRI and years of education was similar between the bvFTD and HC groups. There were more women in the cognitively healthy controls group than in the bvFTD group ($\chi^2(1)=23.090$; $p<0.001$). Age-at-symptom-onset, age at MRI, time from symptom onset to MRI, sex distribution, education, FTLD-CDR, and follow-up time were similar between the possible and probable bvFTD groups. However, the proportion of patients with an increased certainty of FTLD at the end of follow-up was higher in the probable bvFTD group than in the possible bvFTD group ($\chi^2(1)=8.089$; $p=0.004$). As shown in Figure 1, 44 out of 114 (38.6%) bvFTD participants were excluded because of segmentation or diffusion weighted imaging processing errors. The excluded patients had higher FTLD-CDR than the included bvFTD participants ($t(92)=2.041$; $p=0.044$; Supplementary Table 3).

Group comparison of cortical thickness and cortical mean diffusivity

We first compared cortical thickness and cortical mean diffusivity between bvFTD and cognitively healthy controls. As shown in Figure 2, the bvFTD group showed cortical thinning in the prefrontal cortex, the insula, the cingulate gyrus (anterior, dorsal and posterior), the orbitofrontal cortex, the anterior temporal pole, the lateral and medial temporal lobe, the angular gyrus and the precuneus. The cortical mean diffusivity map involved more regions, encompassing the whole of the frontal and temporal cortices, and extending to posterior regions such as the inferior parietal and occipital lobe. Thus, while cortical thickness and cortical mean diffusivity maps showed a partial overlap, cortical mean diffusivity changes extended beyond the areas of cortical thinning. Of note, we observed similar patterns of cortical thickness and cortical mean diffusivity changes when each cohort was analyzed separately (data not shown).

We found moderate to high effect sizes for cortical thickness in the prefrontal cortex, the insula, the anterior and posterior cingulate gyrus, the lateral and medial temporal lobe and the precuneus bilaterally (Figure 2-bottom). For cortical mean diffusivity, we obtained widespread maps of moderate to high effect sizes. The highest ef-

Characteristics	Possible bvFTD	Probable bvFTD	All bvFTD	Cognitively healthy controls
n (% of bvFTD)	30 (43)	40 (57)	70 (100)	78
Age at symptom onset, years	60.2 ± 11.4[ns]	57.9 ± 8.8[ns]	58.8 ± 10	–
Age at MRI, years	65.8 ± 10.9[ns]	62.4 ± 9.2[ns]	63.8 ± 10[ns]	62.3 ± 6.1[ns]
Time from onset to MRI, years	5.5 ± 4.2[ns]	4.5 ± 3.1[ns]	4.9 ± 3.6	–
Sex Male/Female, n	24/6[d]	27/13[d]	51/19[d]	26/52[c]
Education, years	12.5 ± 5.6[ns]	13 ± 5.4[ns]	12.7 ± 5.5[ns]	13.4 ± 4.3[ns]
FTLD-CDR†	6.4 ± 3.7[ns]	8.3 ± 4[ns]	7.5 ± 4	–
Follow-up time, years	1.7 ± 1.4[ns]	1.9 ± 2[ns]	1.8 ± 1.7	–
Last reported phenotype	24 bvFTD 1 bvFTD with progressive aphasia 2 FTD-ALS 3 PSP-CBD	27 bvFTD 4 bvFTD with progressive aphasia 7 FTD-ALS 2 PSP-CBD	51 bvFTD 5 bvFTD with progressive aphasia 9 FTD-ALS 5 PSP-CBD	
Increased certainty of underlying FTLD (% of cases)	21 (70)[b]	38 (95)[a]	59 (84.3)	–
Definitive bvFTD (% of cases)	7 (23.3)[ns] 4 *C9orf72*, 0 *GRN*, 1 *MAPT*, 0 *TARDBP* 2 FTLD-TDP (1 *C9orf72*), 1 FTLD-Tau	12 (30)[ns] 7 *C9orf72*, 2 *GRN*, 0 *MAPT*, 1 *TARDBP* 1 FTLD-TDP (1 *TARDBP*), 2 FTLD-Tau	19 (27.1) 11 *C9orf72*, 2 *GRN*, 1 *MAPT*, 1 *TARDBP* 3 FTLD-TDP (1 *C9orf72* and 1 TARDBP), 3 FTLD-Tau	–

Table 1. Demographics, clinical and neuroimaging features of the participants.

Demographics, clinical and neuroimaging features of the participants. Values reported are mean ± standard deviation.

a: Different from the possible bvFTD group ($p<0.05$)

b: Different from the probable bvFTD group ($p<0.05$)

c: Different from the all bvFTD group ($p<0.05$)

d: Different from the HC group ($p<0.05$)

ns: non-significant differences

†: available in 59 of the 70 (84.3%) bvFTD patients

Abbreviations: bvFTD = behavioral variant of frontotemporal dementia; FTD-ALS = frontotemporal dementia-amyotrophic lateral sclerosis; FTLD = frontotemporal lobar degeneration; FTLD-CDR = frontotemporal lobar degeneration clinical dementia rating; FTLD-Tau = tau subtype of frontotemporal lobar degeneration; FTLD-TDP = transactive response DNA-binding protein 43 kDa subtype of frontotemporal lobar degeneration; n = number; PSP-CBD: progressive supranuclear palsy-corticobasal degeneration.

fect sizes for cortical mean diffusivity were observed at the frontal and temporal cortex bilaterally. Importantly, the effect sizes of cortical mean diffusivity were higher than the effect sizes of cortical thickness in bvFTD-related areas such as the anterior and dorsal cingulate, the prefrontal dorsal cortex and the insula in both hemispheres. In these areas we observed moderate to high net effect sizes favoring cortical mean diffusivity.

Cortical thickness and cortical mean diffusivity in possible and probable bvFTD

We then assessed cortical thickness and cortical mean diffusivity separately in the possible and probable bvFTD subgroups (Figure 3). In the probable bvFTD group we observed extensive clusters of cortical thinning that included essentially the same regions typically involved in the bvFTD that were observed in the Figure 2. Similar to what we observed in the primary analyses,

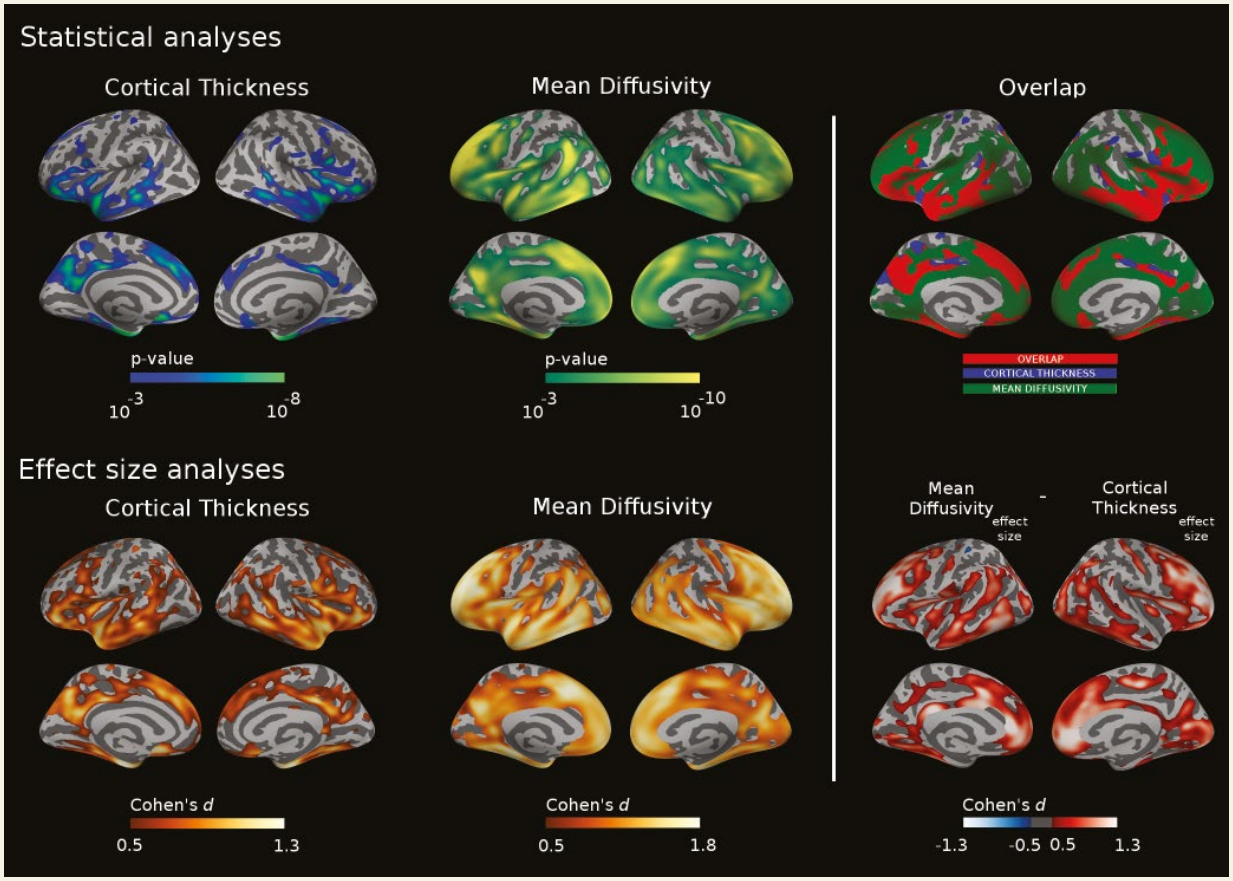

Figure 2. Group comparison of cortical thickness and cortical mean diffusivity between bvFTD and cognitively healthy controls. Top: Statistically significant results between all bvFTD and cognitively healthy controls for cortical thickness and cortical mean diffusivity. Regions in blue represent thinner cortex in the bvFTD group, whereas regions in green, represents higher cortical mean diffusivity in the bvFTD group. For illustration purposes, we included the overlapping map between both metrics (top-right). Cortical thickness analyses were adjusted for age, sex and center. Mean diffusivity analyses were adjusted for age and sex after a harmonization step. Only the clusters that survived familywise error correction $P<0.05$ are shown. Bottom: Medium to large effect sizes between the bvFTD and cognitively healthy controls for both cortical thickness and cortical mean diffusivity. The orange-gold colour represents higher effect size. In addition, the difference between both maps of effect size is displayed (bottom-right). The red-white colour represents gray matter areas where the cortical mean diffusivity has higher effect size than cortical thickness.

the cortical mean diffusivity changes were more widespread than the cortical thickness changes as shown in the overlap map of Figure 3 (Figure 3-top). We also observed moderate to high net effect sizes favoring cortical mean diffusivity in the rostral middle frontal, superior frontal, anterior cingulate, the insula and in more posterior regions (posterior temporal, precuneus and occipital lobe) (Figure 3-top). In the possible bvFTD subgroup, we observed small clusters of cortical thinning in the insula, and the medial temporal lobe in both hemispheres. Interestingly, we observed extensive cortical mean diffusivity increases in the dorsal and medial prefrontal cortex, as well as in the supplementary motor cortex and the frontal pole in both hemispheres (Figure 3-bottom). In the possible bvFTD group, we also observed moderate to high net effect sizes favoring cortical mean diffusivity in the rostral middle frontal and superior frontal cortex in both hemispheres (Figure 3-bottom).

Relationship between cortical thickness and cortical mean diffusivity with the FTLD-CDR

We next evaluated the capacity of cortical thickness and cortical mean diffusivity to reflect the disease severity in the bvFTD as measured by the FTLD-CDR scale. When pooling together all the bvFTD subjects, we observed an inverse correlation between FTLD-CDR scores and cortical thickness in small clusters in the inferior frontal gyrus, the anterior insula, the anterior temporal pole and the medial temporal lobe in both hemispheres and a correlation in the medial orbitofrontal cortex and in the precuneus in the left hemisphere. We observed larger clusters of

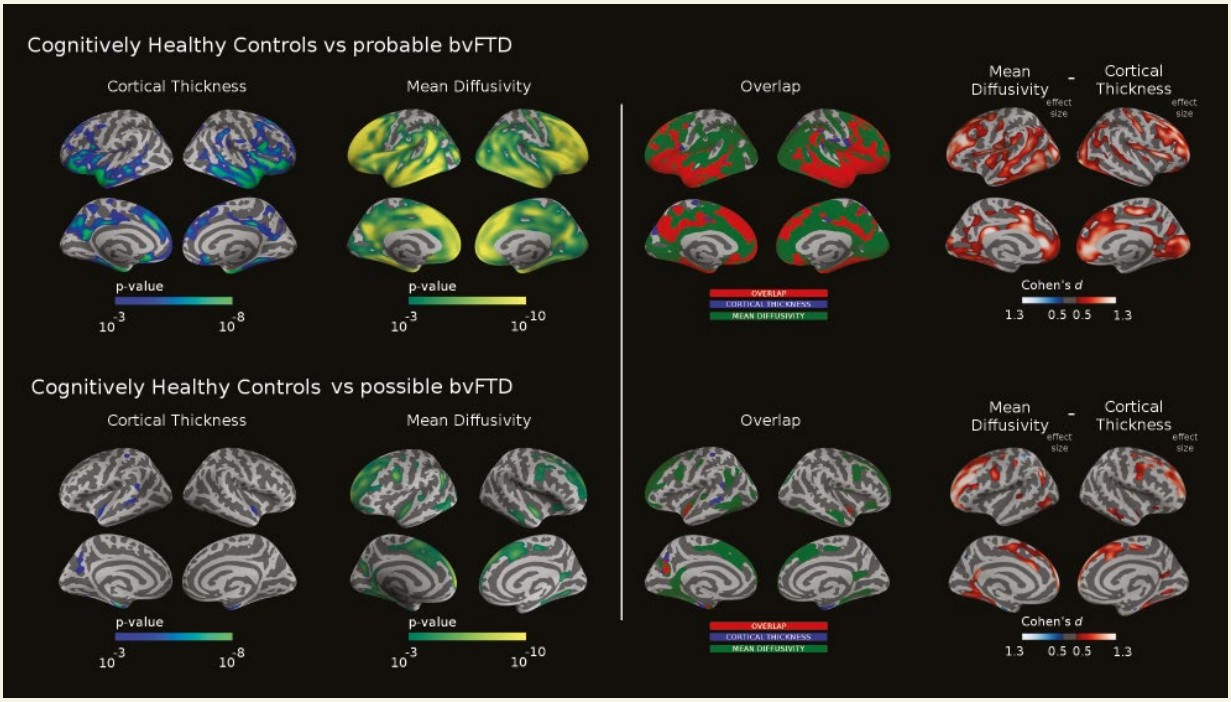

Figure 3. Group comparison of cortical thickness and cortical mean diffusivity between patients with possible and probable bvFTD and cognitively healthy controls. Cortical thickness and cortical mean diffusivity group comparisons between probable (top) and possible (bottom) bvFTD against cognitively healthy controls. We included the overlapping map (top and bottom) between both metrics. Cortical thickness analyses are adjusted by age, sex and center. Mean diffusivity analyses were adjusted by age and sex after a harmonization step. Only clusters that survived familywise error correction ($P<0.05$) are shown. For visualization purposes, different color codes were used for cortical thickness and cortical mean diffusivity. In addition, the net difference in effect size is displayed for probable bvFTD (top-right) and possible bvFTD (bottom-right). Red-white colour represents gray matter areas where the cortical mean diffusivity has higher effect size than cortical thickness.

significant positive correlations between cortical mean diffusivity and FTLD-CDR scores in both hemispheres (Figure 4-top). Similar results were found when restricting the analyses to the probable bvFTD group (Figure 4-middle). When restricting the analysis to the possible bvFTD, we did not find any correlation between cortical thickness and FTLD-CDR scores. However, cortical mean diffusivity was positively associated with FTLD-CDR scores in the anterior cingulate, frontal insula and lateral temporal in both hemispheres (Figure 4-bottom).

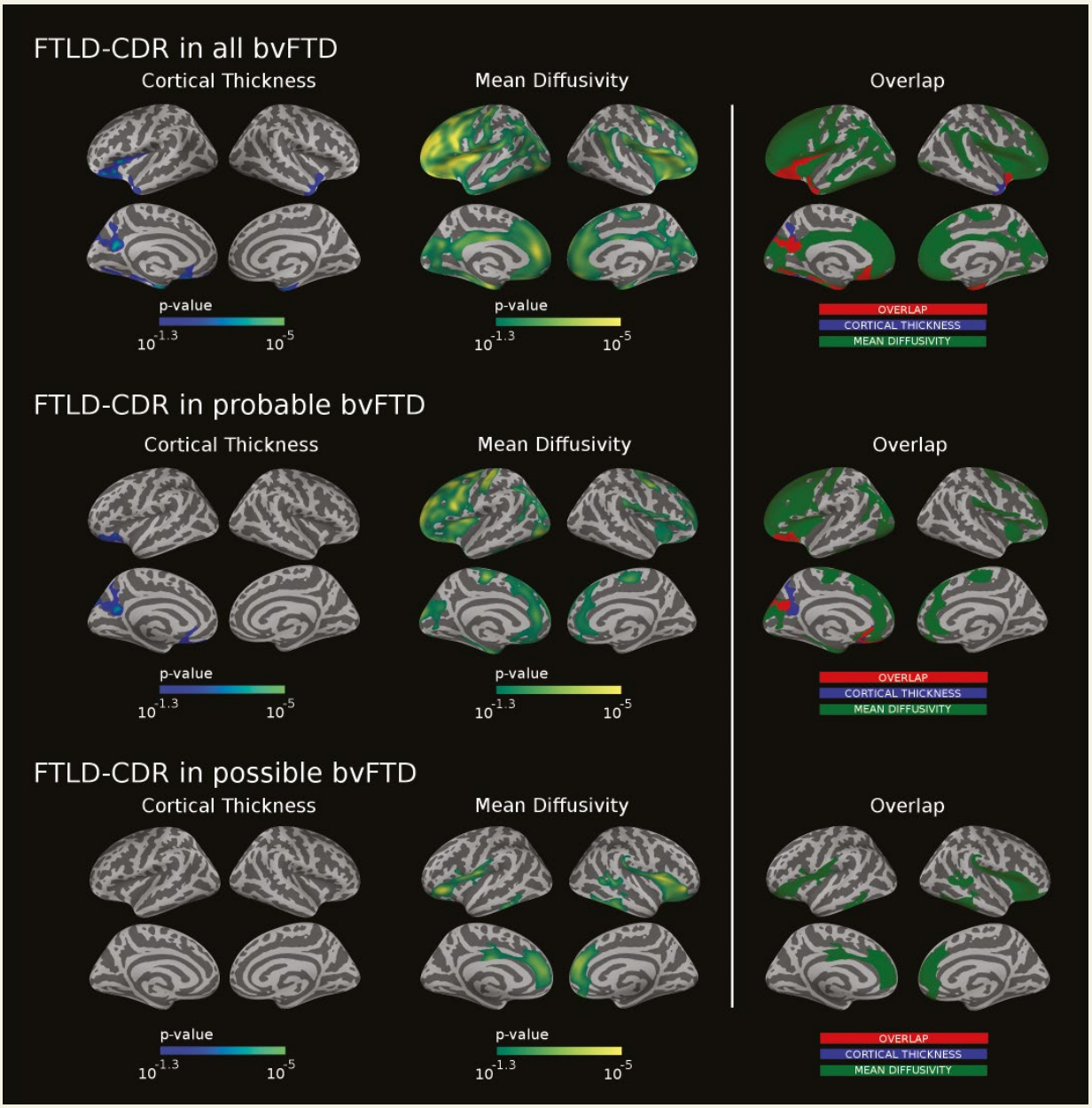

Figure 4. Relationship between cortical thickness and cortical mean diffusivity with the frontotemporal lobar degeneration clinical dementia rating score. Correlation of cortical mean diffusivity with the frontotemporal lobar degeneration clinical dementia rating score in the whole sample (top), probable bvFTD subgroup (middle) and possible bvFTD subgroup (bottom). Small regions of cortical thinning associated to higher FTLD-CDR scores (blue) were found in the probable subgroup, whereas extensive areas of increases of cortical mean diffusivity related to increases in FTLD-CDR scores (green) were found in both subgroups. Cortical thickness analyses were adjusted for age, sex and center. Mean diffusivity analyses were adjusted for age and sex after a harmonization step. The overlap between both maps is displayed on the right (top and bottom).

Correlation of cortical thickness and mean diffusivity changes with CSF biomarkers

We finally assessed the correlation of cortical thickness and cortical mean diffusivity with CSF NfL and sAPPβ levels. CSF NfL levels were negatively correlated with cortical thickness in dorsolateral and medial prefrontal areas of the frontal lobe. The correlation between CSF NfL levels and cortical mean diffusivity included those areas, but also areas in the temporal and parietal lobes (Figure 5-top). CSF sAPPβ levels were positively correlated with cortical thickness in regions of the prefrontal cortex, the insula, the temporo-parietal union and the lateral temporal cortex. The negative correlation between CSF sAPPβ levels and cortical mean diffusivity extended to more widespread frontal and temporal regions, as well as to posterior regions (Figure 5-bottom).

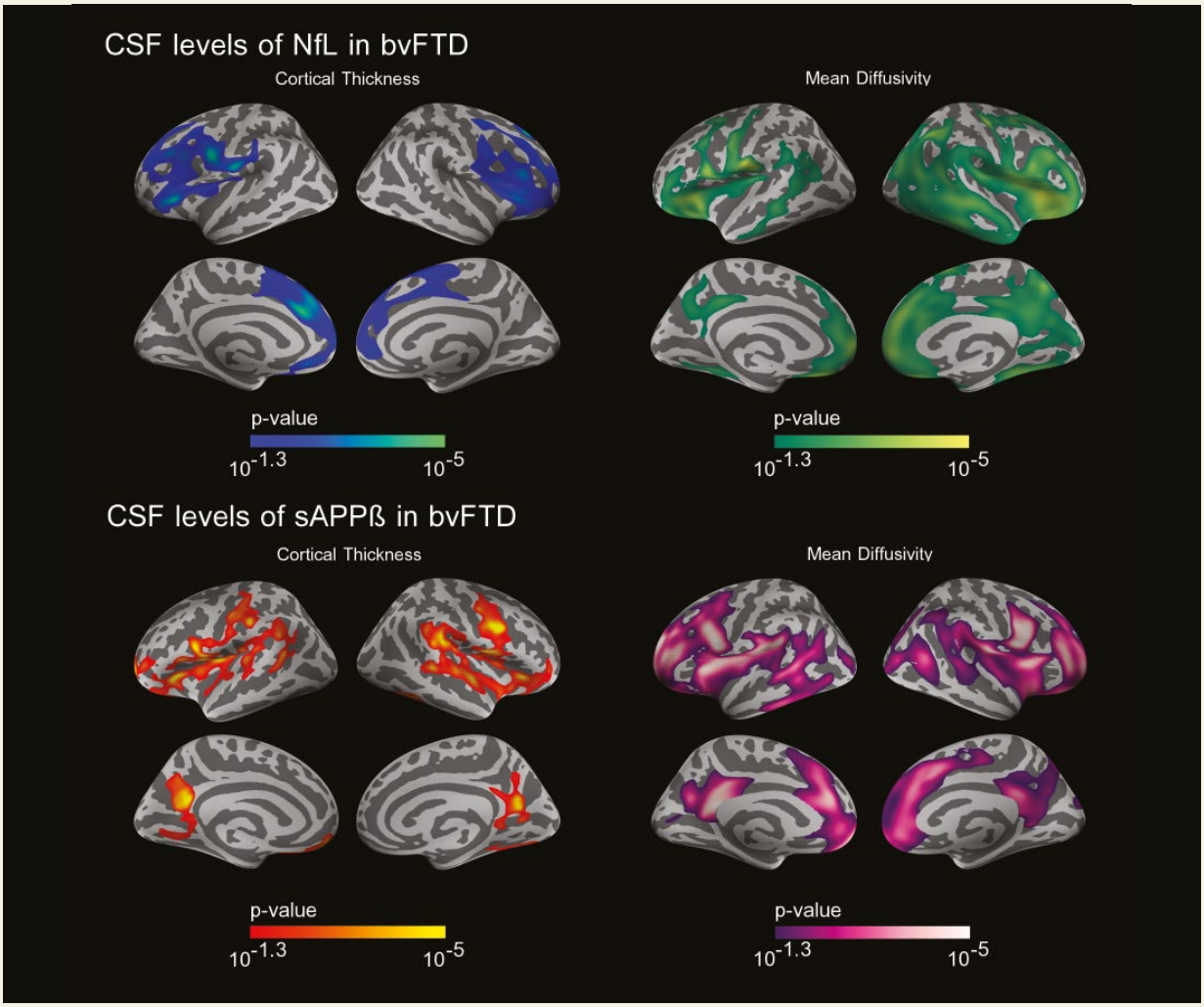

Figure 5. Correlation of cortical thickness and cortical mean diffusivity with CSF biomarkers. Relationship of cortical thickness and cortical mean diffusivity with the CSF levels of Neurofilament light (NfL) (top) and the CSF levels of the soluble fraction beta of the amyloid precursor protein (sAPPß) (bottom) in the subgroup of bvFTD participants with CSF sample available for analysis (n=32). As NfL and sAPPß values were not normally distributed, we used log-transformed values for these biomarkers NfL levels negatively correlated with cortical thickness (blue) and positively correlated with cortical mean diffusivity (green). sAPPß positively correlated with cortical thickness (red) and negative correlated with cortical mean diffusivity (purple). Cortical thickness analyses were adjusted for age, sex and center. Mean diffusivity analyses were adjusted for age and sex after a harmonization step. Only clusters that survived familywise error correction at P<0.05 are shown.

Discussion

In this study we investigated the value of cortical mean diffusivity as a biomarker in bvFTD in a large multicenter sample. We showed that altered cortical mean diffusivity not only coincided with areas that showed cortical thinning, but also involved other areas that typically become affected with disease progression (Binney *et al.*, 2017). Furthermore, we found cortical mean diffusivity was increased in patients classified as possible bvFTD that had only minimal cortical thinning. Clinical measures of disease severity (FTLD-CDR) and CSF neuronal biomarkers (CSF NfL and sAPPβ levels) showed a more widespread correlation with cortical mean diffusivity than with cortical thickness. Taken together, these findings suggest that cortical mean diffusivity might be more sensitive than cortical thickness to detect the earliest disease-related cortical changes in bvFTD.

Cortical mean diffusivity has been recently proposed as a sensitive biomarker for the detection of the earliest cortical changes in sporadic AD (Montal *et al.*, 2017; Weston *et al.*, 2015). We show, for the first time in bvFTD using a surface-based approach, that cortical mean diffusivity increases spread beyond the areas of cortical thinning in bvFTD, even in patients with possible bvFTD. Most previous studies using diffusion tensor imaging in bvFTD patients have focused on the white matter, probably because of the technical difficulties in the study of cortical microstructure (Papma *et al.*, 2017). We identified a single previous small study (with 16 bvFTD patients) assessing cortical diffusion tensor imaging in the bvFTD using a volume-based approach (Whitwell *et al.*, 2010). This study found overlapping patterns between atrophy and increases on cortical mean diffusivity. Our study builds on these results using a larger sample, a surface-based approach, and the inclusion of bvFTD patients at milder disease stages. Consequently we were able to show the added value of cortical mean diffusivity as a more sensitive biomarker in bvFTD over cortical thickness.

We found minimal cortical thinning when comparing possible bvFTD patients and controls. However, we observed extensive cortical mean diffusivity increases in regions known to be affected in bvFTD (Schroeter *et al.*, 2014; Brettschneider *et al.*, 2014; Irwin *et al.*, 2016). Moreover, we calculated effect size maps to quantify the impact of cortical thickness and cortical mean diffusivity for the differentiation of bvFTD patients from controls. Importantly, we obtained moderate to high net effect size favoring cortical mean diffusivity in critical bvFTD-related cortical regions such as the anterior cingulate, the prefrontal dorsal cortex and the insula. The suggestion that cortical mean diffusivity may be more sensitive than cortical thickness to detect the bvFTD cortical changes is further supported by our correlation analyses with the FTLD-CDR and CSF NfL and sAPPβ levels. Both the clinical measures of disease severity and the CSF biomarkers, they all showed a better correlation with cortical mean diffusivity than with cortical thickness. The FTLD-CDR has been validated as a tool for disease monitoring in clinical trials (Knopman *et al.*, 2008). Although the FTLD-CDR scores also correlated with cortical thickness in some small frontotemporal clusters, we found a substantially widespread correlation with cortical mean diffusivity. Moreover, when restricting the analyses in the possible bvFTD subgroup, only associations between cortical mean diffusivity and FTLD-CDR scores were found. This finding supports a possible role for cortical mean diffusivity as a candidate neuroimaging biomarker for disease staging.

To further evaluate the role of cortical mean diffusivity as a neurodegeneration biomarker, we investigated its correlation with CSF biomarkers in a subgroup of patients. NfL is one of the major constituents of the axonal cytoskeleton and plays an important role in axonal transport. The meas-

urement of NfL levels both in the CSF and in serum correlates with disease severity, progression and survival in multiple neurodegenerative diseases (Landqvist Waldö *et al.*, 2013; Meeter *et al.*, 2016; Pijnenburg *et al.*, 2015; Rohrer *et al.*, 2016; Scherling *et al.*, 2014; Wilke *et al.*, 2016). We also measured CSF sAPPβ levels, as we have previously shown that this biomarker correlates with frontotemporal neurodegeneration in FTLD-related syndromes (Alcolea D *et al.*, 2017; Illán-Gala I *et al.*, 2018). The association between cortical mean diffusivity and CSF values further reinforce the notion that cortical mean diffusivity changes reflect the underlying neurodegeneration.

Although we acknowledge that it is possible that some patients classified as possible bvFTD may not have underlying FTLD (Devenney *et al.*, 2016; Gossink *et al.*, 2015), recent studies in deep-phenotyped cohorts have shown that a significant proportion of bvFTD cases do not have frontotemporal atrophy and may be characterized by a slower disease course (Ranasinghe *et al.*, 2016; Rascovsky *et al.*, 2011). In the present study, 70% patients classified as possible bvFTD were found to have an increased certainty of underlying FTLD as suggested by follow-up, genetic and neuropathological information available. Indeed, longitudinal decline was observed in most possible bvFTD patients and psychiatric diagnoses were excluded by expert clinicians. Of note, four cases classified as possible bvFTD were found to have a *C9orf72* expansion, a finding that has been previously reported in different cohorts (Khan *et al.*, 2012; Gómez-Tortosa *et al.*, 2014; Llamas-Velasco *et al.*, 2018; Devenney *et al.*, 2018). Thus, we propose that the patients classified as possible bvFTD are at high risk of having underlying FTLD and that our cortical mean diffusivity results support that at least a proportion of possible bvFTD patients have a neurodegenerative disease. Cortical mean diffusivity may be a relevant tool for increasing the diagnostic certainty in these "slowly progressive" bvFTD without overt frontotemporal atrophy (Davies *et al.*, 2006; Khan *et al.*, 2012).

Taken together, our findings support the role of cortical mean diffusivity as a novel potential neurodegeneration biomarker in bvFTD. We hypothesize that cortical mean diffusivity may be a sensitive tool for the refinement and monitoring of the very earliest cortical changes genetically-determined FTLD (Rohrer *et al.*, 2015). Importantly, further longitudinal studies should explore the ability of cortical mean diffusivity to predict disease progression at the single-subject level. Additionally, our study is the first to report the potential added value of cortical diffusion tensor imaging changes over cortical thickness in bvFTD. Further studies could explore the added value of the combined study of white and grey matter diffusion tensor imaging changes to improve pathological predictions (Downey *et al.*, 2015; McMillan *et al.*, 2014). All the aforementioned points are key aspects for candidate selection in clinical trials once protein-specific targeted therapies become available (Elahi and Miller, 2017).

The main strengths of this study are the relatively large number of bvFTD participants at a mild to moderate disease stage, and the surface-based analyses using a previously validated technique. This surface-based approach solves some of the limitations and methodological concerns that have been previously reported when using a voxel-based approach (Coalson *et al.*, 2018). Moreover, we enriched our description of the cortical mean diffusivity in the bvFTD with established clinical measures of disease severity and CSF biomarkers. This study has also some limitations. First, we acknowledge that a substantial proportion of bvFTD cases (38.6%) were excluded due to segmentation or diffusion tensor imaging processing errors. Even though this is an inherent limitation of our surface-based approach, future improvements in T1 MRI acquisitions or the use of higher field MRIs, together with software

improvements will likely reduce the number of subjects excluded due to segmentation errors. Of note, we observed that the excluded patients belonged to the probable bvFTD group (77.3% of the excluded cases) and were at a more advanced disease stage, as measured by the FTLD-CDR. Notwithstanding, cortical mean diffusivity may still provide valuable topographical information regarding the earliest cortical microstructural changes in patients at very mild disease stages (for example, sporadic bvFTD cases without overt cortical atrophy or even genetic cases) were less segmentation errors are expected to occur. Second, it may be argued that there may be confounding results related to the different acquisition protocols across centers. However, the results presented in the current study were obtained after using a validated state-of-the-art algorithm to harmonize diffusion data between centers (Fortin *et al.*, 2017; Montal *et al.*, 2017). Moreover, results were similar when analyzing each center independently regardless of the use of different diffusion weighted imaging sequences. Third, although we provide cross-sectional evidence that cortical mean diffusivity changes may be a novel sensitive metric to reflect neurodegeneration, further longitudinal studies and using presymptomatic mutation carriers should confirm that cortical mean diffusivity changes antedate cortical atrophy in patients with bvFTD. Fourth, because most of the included bvFTD cases did not have neuropathological evaluation, misdiagnosis could have occurred, especially in the possible bvFTD group. However, a high proportion of cases were found to have an increased certainty of underlying frontotemporal lobar degeneration when considering the available clinical, genetic and neuropathological information. Finally, as neuropathological evaluation was not available in most cases we were not able to explore the precise pathological correlates of the observed cortical mean diffusivity changes.

In summary, this study supports the use of cortical mean diffusivity as a valuable novel biomarker for the cortical mapping of neurodegeneration-related microstructural changes in bvFTD. Further longitudinal studies in different populations including preclinical mutation carriers are needed to fully determine the diagnostic and prognostic utility of this biomarker, particularly at the earliest stages of the disease.

Acknowledgments

The authors thank the patients and their relatives for their support for this study. We thank Laia Muñoz for technical assistance and María Carmona-Iragui, Estrella Muñoz-Rodríguez, Roser Ribosa for their collaboration in the recruitment of patients for this study. We also thank Olivia Belbin for editorial assistance and Anna Karydas from UCSF for her assistance to get updated FTLDNI data.

Funding

The Catalan frontotemporal initiative (CATFI) is funded by the Health Department of the Government of Catalonia (grant PERIS SLT002/16/00408 to Alberto Lleó and Raquel Sánchez-Valle). The principal investigator of the CATFI study is Dr. Alberto Lleo. FTLDNI data collection and sharing for this project was funded by the Frontotemporal Lobar Degeneration Neuroimaging Initiative (National Institutes of Health Grant R01 AG032306). The study is coordinated through the University of California, San Francisco, Memory and Aging Center. FTLDNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California. This work was also supported by research grants from the Carlos III Institute of Health, Spain (grants PI11/02526, PI14/01126 and PI17/01019 to Juan Fortea, PI13/01532 and PI16/01825 to Rafael Blesa, PI15/01618 to Ricard Rojas-García, PI14/1561 and PI17/01896 to Alberto Lleó; AC14/00013 to Raquel Sánchez-

Valle) and the CIBERNED program (Program 1, Alzheimer Disease to Alberto Lleó and SIGNAL study, www.signalstudy.es), partly funded by Fondo Europeo de Desarrollo Regional (FEDER), Unión Europea, "Una manera de hacer Europa". This work has also been supported by a "Marató TV3" grant (20141210 to Juan Fortea, 044412 to Rafael Blesa, 20143710 to Ricard Rojas-García and 20143810 to Raquel Sánchez-Valle) and by Generalitat de Catalunya (2014SGR-0235 to Alberto Lleó, PERIS SLT006/17/125 to Daniel Alcolea and SLT006/17/00119 to Juan Fortea), and BBVA foundation (grant to A. Lleó) and a grant from the Fundació Bancaria La Caixa to Rafael Blesa. I. Illán-Gala is supported by the i-PFIS grant (IF15/00060) from the FIS, Instituto de Salud Carlos III and the Rio Hortega grant (CM17/00074) from "Acción estratégica en Salud 2013-2016" and the European Social Fund. Dr. Sergi Borrego-Écija is the recipient of Emili Letang post-residency research grant from Hospital Clínic de Barcelona

Chapter 6

Low amyloid precursor protein-derived peptides reflect neurodegeneration in frontotemporal dementia

Paper in preparation for submission

Title:

Low amyloid precursor protein-derived peptides reflect neurodegeneration in frontotemporal dementia

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Abstract

Objective: To explore the relationship between APP-derived peptides related to the amyloidogenic pathway and cortical thickness in frontotemporal lobar degeneration-related syndromes (FTLD-S) compared to Alzheimer's disease (AD) and healthy controls (HC).

Methods: We included 177 participants from the Sant Pau Initiative on Neurodegeneration (SPIN cohort) with CSF available: 46 patients with AD, 77 with FTLD-S and 54 HC. We measured the CSF levels of $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-38}$ and sAPPβ using ELISA. We correlated the CSF levels with measures of cognitive impairment, disease severity and cortical thickness in a subset of patients with brain MRI available.

Results: CSF levels of all APP-derived peptides were reduced in the FTLD-S group when compared to the HC group. The CSF levels of $A\beta_{1-40}$, $A\beta_{1-38}$ and sAPPβ were lower in the FTLD-S group than in the AD and HC groups. In the FTLD-S group, phonological fluency correlated with $A\beta_{1-42}$, $A\beta_{1-38}$ and sAPPβ levels (r=0.365, *p*=0.003; r=0.304, p=0.016; r=0.264, p=0.038; respectively). The CSF levels of $A\beta_{1-42}$ $A\beta_{1-40}$, $A\beta_{1-38}$ and sAPPβ showed a positive correlation with cortical thickness in the FTLD-S group in frontotemporal regions but not in the HC or AD groups.

Interpretation: APP-derived peptides in CSF can reflect frontotemporal neurodegeneration in FTLD-S.

Introduction

Frontotemporal Lobar Degeneration (FTLD) is a neuropathological umbrella term encompassing multiple proteinopathies with common patterns of neurodegeneration in frontotemporal regions. In contrast to Alzheimer's disease (AD), there are no specific pathophysiological biomarkers for FTLD and current diagnostic criteria rely on the identification of particular syndromes and patterns neurodegeneration on neuroimaging (Elahi and Miller, 2017). Cerebrospinal fluid (CSF) biomarkers have been studied in neurodegenerative disease as a way to track different pathophysiological processes in the central nervous system (Molinuevo *et al.*, 2018). Levels of core AD biomarkers, amyloid β1-42 ($A\beta_{1-42}$), total tau (t-tau), and phosphorylated tau (p-tau), can be useful in FTLD-related syndromes (FTLD-S) to exclude AD (Olsson *et al.*, 2016; Toledo *et al.*, 2012). Specifically, low $A\beta_{1-42}$ levels in CSF has been considered a biomarker of cerebral amyloidosis (Jack *et al.*, 2016; 2018) and therefore its presence in patients with FTLD-S is usually interpreted as a sign of either atypical AD mimicking FTLD or comorbid AD pathology in FTLD cases (Lleo *et al.*, 2018).

However, several studies in patients with FTLD have shown substantial disagreement between CSF levels of $A\beta_{1-42}$ and amyloid positron emission tomography (Janelidze *et al.*, 2016; Leuzy *et al.*, 2016). In addition, previous studies have also reported lower CSF levels of $A\beta_{1-42}$ in pathology-proven FTLD cases without comorbid AD (Toledo *et al.*, 2012) as well as in genetically-confirmed FTLD (Kämäläinen *et al.*, 2015; Wallon *et al.*, 2012) suggesting that in FTLD, $A\beta_{1-42}$ levels could be affected by mechanisms independent of AD pathology.

Aβ peptides are generated by proteolytic cleavage from the Amyloid Precursor Protein (APP) by sequential action of β- and γ-secretases (Ghidoni *et al.*, 2011; Müller *et al.*, 2017). In addition to $A\beta_{1-42}$, the proteolytic process of APP also generates other shorter Aβ species, such as Aβ 40 and 38, which can be used as markers of APP metabolism (Struyfs *et al.*, 2015). We and others have reported that different Aβ peptides are reduced in FTLD-related syndromes (Bibl *et al.*, 2007; Gabelle *et al.*, 2011; Lleo and Saura, 2011; Lleo

et al., 2015; Pijnenburg *et al.*, 2007; Verwey *et al.*, 2010). Another APP-derived peptide generated from APP by BACE1 is the soluble β fraction of APP (sAPPβ) (Ghidoni *et al.*, 2011; Müller *et al.*, 2017). sAPPβ levels are also reduced in CSF in FTLD-related syndromes and correlate with cortical thickness in frontotemporal regions (Alcolea *et al.*, 2014; Alexopoulos *et al.*, 2012; Bibl *et al.*, 2007; Illán-Gala, Alcolea, *et al.*, 2018; Perneczky *et al.*, 2011).

However, it remains unknown if $A\beta_{1-42}$ and other Aβ peptides generated in the amyloidogenic APP pathway are related to neurodegeneration in FTLD-S or AD (Ossenkoppele *et al.*, 2015). We hypothesized that, similarly to what we have observed for sAPPβ, the decrease in the CSF levels of $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-38}$ may reflect non-AD neurodegeneration in FTLD. The identification of variable patterns of APP-derived peptides and their relationship with neurodegeneration across neurodegenerative diseases is important since it may contribute to the *in-vivo* identification of FTLD and its differentiation from atypical AD and other non-neurodegenerative diseases. In this study we explore the relationship between APP-derived peptides related to the amyloidogenic pathway (i.e. $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-38}$ and sAPPβ) and cortical cortical thickness in FTLD-S compared to AD and healthy controls (HC).

Material and Methods

Study participants and classification

We included 177 participants from the Sant Pau Initiative on Neurodegeneration (SPIN cohort: https://santpaumemoryunit.com/our-research/spin-cohort/): 46 patients with AD (24 at the mild cognitive impairment stage and 22 at the mild dementia stage), 77 with FTLD-S (51 bvFTD, 13 non-fluent or semantic variant of PPA and 13 within the PSP-CBD spectrum) and 54 healthy controls (HC). Patients with AD and FTLD-S were diagnosed at the Memory Unit according to current diagnostic criteria. The diagnosis of AD and FTLD-S was made by neurologists of the Memory Unit after an extensive neurological and neuropsychological evaluation using current diagnostic criteria (Armstrong *et al.*, 2013; Dubois *et al.*, 2014; Gorno-Tempini *et al.*, 2011; Höglinger *et al.*, 2017; Rascovsky *et al.*, 2011). Further details on the clinical cohort and protocol can be found elsewhere (Alcolea, SPIN paper in preparation). All AD patients had low levels of $A\beta_{1-42}$ and elevated levels of t-tau or p-tau in CSF according to our published cut-offs (Alcolea *et al.*, 2015). To avoid the inclusion of non-AD participants with comorbid AD we excluded FTLD-S and HC cases with a CSF biomarker profile suggestive of AD (low $A\beta_{1-42}$ levels and increased t-tau or p-tau levels according to the local validated thresholds) (Alcolea *et al.*, 2015).

Clinical measures of general cognitive impairment and disease severity

We obtained the Mini-mental State Examination (MMSE)(34) the phonological fluency (words beginning with letter P) (Peña-Casanova *et al.*, 2009) and the delayed total score of the Free and Cued Selective Reminding Test (FCSRT). (Peña-Casanova *et al.*, 2009) We selected these specific cognitive tests from our neuropsychological battery (Sala *et al.*, 2017) to include a global measure of cognitive impairment (MMSE), one measure related to frontal cerebral structures (phonological fluency) and middle temporal structures (FCSRT delayed total score). We also included the clinical dementia rating the sum of boxes (CDR-SOB) score of in all participants and the modified frontotemporal lobar degeneration clinical dementia rating (FTLD-CDR) as a measure of global disease severity in the FTLD-S group (Knopman *et al.*, 2008).

CSF sampling and analyses

All biomarkers were analyzed at the Sant Pau Memory Unit Laboratory with commercially available ELISA kits of $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-38}$ and sAPPß (Lumipulse; EUROIMMUN; EUROIMMUN; IBL, respectively) following previously reported methods and manufacturer's instructions (Alcolea *et al.*, 2014; 2015; 2017).

Image acquisition, processing and analysis

A subset of 86 participants had 3T MRI available for quantitative neuroimaging analyses. Of these, 79 participants were scanned on a 3T Philips Achieve using a T1-weighted MPRAGE protocol with a repetition time of 8.1 milliseconds, echo time of 3.7 milliseconds, 160 slices and voxel size of 0.94x0.94x1mm and 7 participants were scanned on a different 3T Philips Achieva using a T1-weighted MPRAGE protocol with a repetition time of 6.74 milliseconds, echo time of 3.14 milliseconds, 140 slices and a voxel size of 0.9x0.9x1.2 mm. Briefly, surface-based cortical reconstruction was performed using FreeSurfer v5.1 software package (http://surfer.nmr.mgh.harvard.edu) as previously reported (Montal *et al.*, 2017). In this sample, a vertex-wise general linear model (as implemented in FreeSurfer v5.1) was used to assess the correlation between CSF biomarkers and cortical thickness for each group independently. Specifically, for each surface vertex, a general linear model was computed using cortical thickness as dependent variable and CSF values as independent variable. All these analyses were covariated by age, sex and magnetic resonance equipment. To control for false positives, a Monte-Carlo simulation with 10000 repeats as implemented in FreeSurfer (FWE < 0.05) was tested.

Genetic studies

APOE was genotyped according to previously described methods (Calero *et al.*, 2009).

Statistical methods

We assessed normality of the variables by means of the Kolmogorov-Smirnov test. Variables not following a normal distribution were log-transformed for further bivariate and multivariate analyses ($A\beta_{1-42}$, sAPPβ, $A\beta_{1-42}/A\beta_{1-40}$ and $A\beta_{1-42}/A\beta_{1-40}$ ratios). Group differences in baseline characteristics were assessed using t-test, ANOVA or Kruskal-Wallis test for continuous variables, and χ^2 for categorical data. We calculated Spearman's correlation coefficient with bootstrapping-based 95% confidence intervals (bias corrected and accelerated for 1000 samples). For the analyses of group differences in CSF biomarkers between groups we applied analyses of covariance (ANCOVA) including age at CSF and *APOE*ε4 as covariates for the study of differences in CSF biomarkers between groups. All *p* values were corrected for multiple comparisons, all statistical tests were two-sided and statistical significance was set at 5% (α=0.05). Statistical analyses were performed with the IBM SPSS Statistics 25 (IBM corp.) software.

Standard protocol approvals, registrations, and patient consent

The study was approved by the local ethics committee and was conducted in accordance with the Declaration of Helsinki. All participants gave their written informed consent to participate in the study.

Results

Demographics and *APOE* of participants

As shown in table 1, there were no differences in age at symptom onset, age at CSF sampling, sex and MMSE score between the AD and FTLD-S groups. The control group was younger at CSF sampling than the AD and FTLD-S groups ($F(2,174)=7.905$, $p=0.001$). As expected, *APOE*ε4 carriers were overrrepresented in the AD group than in the FTLD-S and control groups ($\chi^2(2)=11.417$, $p=0.003$) but the frequency was similar in the FTLD-S and control groups (23% and 20%, respectively $p>0.05$).

Levels of APP-derived peptides are reduced in FTLD-S

All the CSF levels of all APP-derived peptides measured in this study were reduced in the FTLD-S group when compared to the HC group (Fig 1, A-D). The CSF levels of $A\beta_{1-42}$ were significantly different between groups even after accounting for age at CSF sampling and the presence of *APOE*ε4 ($F(2,170)=55.844$, $p<0.001$). As expected, the lowest levels of $A\beta_{1-42}$ were observed in the AD group (Table 1). However, $A\beta_{1-42}$ levels were also decreased in FTLD-S when compared to HC ($t(123.05)=3.8$, $p<0.001$, $r=0.340$)(Fig 1), although still within the normal range according our amyloid-PET validated cut-offs (Alcolea D, BioRX 2018).

The CSF levels of $A\beta_{1-40}$, $A\beta_{1-38}$ and sAPPβ also differed between groups after accounting for age at CSF sampling and the presence of *APOE*ε4 ($F(2,170)=16.519$, $p<0.001$; $F(2,170)=55.844$, $p<0.001$; $F(2,170)=55.844$, $p<0.001$; respectively). The CSF levels of $A\beta_{1-40}$, $A\beta_{1-38}$ and sAPPβ were lower in the FTLD-S group than in the AD and HC groups (Fig 1, B-D). As shown in Table S2, we found that CSF levels of $A\beta_{1-38}$ had the biggest effect size for the differentiation of FTLD-S from controls (partial $\eta^2=0.185$) after accounting for age at CSF sampling and *APOE*ε4.

As expected, the $A\beta_{1-42}/A\beta_{1-40}$ and $A\beta_{1-42}/A\beta_{1-38}$ ratios differed between groups (Fig 1, E-F). The $A\beta_{1-42}/A\beta_{1-40}$ and the $A\beta_{1-42}/A\beta_{1-38}$ ratios were lower in the AD group than in the FTLD-S and HC groups ($t(102.036)=19.941$, $p<0.001$ and $t(67.664)=15.324$, $p<0.001$, respectively). Moreover, the $A\beta_{1-42}/A\beta_{1-38}$ ratio was higher in the FTLD-S group when compared to HC group ($t(99.916)=3.066$, $p=0.003$) but the effect size was small ($r=0.288$).

Effect sizes of the observed differences

We studied the effect sizes for the observed differences in the CSF levels of APP-derived taking into account covariates such as age at CSF sampling and *APOE*ε4. As shown in Table S2, we found that CSF levels of $A\beta_{1-38}$ had the biggest effect size for the differentiation of FTLD-S from controls (partial $\eta^2=0.185$) after accounting for age at CSF sampling and *APOE*ε4. As expected, the effect size for the differentiation of FTLD-S from AD was bigger for $A\beta_{1-42}$ than for $A\beta_{1-40}$ or $A\beta_{1-38}$ (partial $\eta^2=0.261$ vs $\eta^2=0.113$ and $\eta^2=0.096$, respectively). In addition, the CSF levels of sAPPβ showed a moderate effect sizes for the differentiation of FTLD-S from HC and AD even after accounting for age at CSF sampling and *APOE*ε4 (partial $\eta^2=0.113$ and $\eta^2=0.118$, respectively).

The relationship between the different APP-derived peptides varies between groups

We explored the relationships between the different APP-derived peptides in each clinical group. As shown in Table S1, the CSF levels of sAPPβ showed moderate correlations with $A\beta_{1-42}$, $A\beta_{1-40}$ and $A\beta_{1-38}$ ($r=0.535$ to $r=0.661$) in FTLD-S and control groups but not in the AD group, were only mild correlations were observed ($r=0.308$ and $r=350$, respectively). Moreover, sAPPβ lev-

Demographic, clinical and genetic characteristics	AD	Probable bvFTD	All bvFTD	Cognitively healthy controls
Age at CSF	71.8 ± 7[c]	71.0 ± 9[c]	66.3 ± 6[a,b]	F(2,174)=7.905 *p*=0.001
Age at clinical onset	68.5 ± 8	66.3 ± 9	–	t(120)=1.317 *p*=0.190
Male, n (%)	17 (37)	44 (57)	29 (54)	$\chi^2(2)$=4.949 *p*=0.084
MMSE score	23.5 ± 5[c]	24.2 ± 5[c]	28.8 ± 1a,b	H(2)=62.183 *p*<0.001
FCSRT delayed total, /16	6.3 ± 4[b,c]	8.7 ± 5[a,c]	15.3 ± 1[a,b]	H(2)=90.360 *p*<0.001
Phonological fluency	9.2 ± 5[c]	7.1 ± 5[c]	15.4 ± 5[a,b]	H(2)=56.627 *p*<0.001
CDR-SOB	3.6 ± 3[c]	4.6 ± 4[c]	0 ± 0[a,b]	H(2)=102.672 *p*<0.001
FTLD-CDR *(only in FTLD-S)*	NA	6.3 ± 6	NA	
APOEε4, n (%)	22 (48)[b,c]	17 (23)[a]	11 (20)[a]	$\chi^2(2)$=11.417 *p*=0.003
CSF biomarkers				ANCOVA[§]
$A\beta_{1-42}$, pg/mL	841 ± 194[b,c]	1542 ± 637[a,c]	1958 ± 627 [a,b]	F(2,170)=55.844 p<0.001 partial η^2=0.396
Aβ1-40, pg/mL	7903 ± 2401[b]	5643 ± 2516[a,c]	7557 ± 1943[b]	F(2,170)=16.519 p<0.001 partial η^2=0.163
$A\beta_{1-38}$, pg/mL	2425 ± 740[b]	1747 ± 767[a,c]	2548 ± 756[b]	F(2,170)=21.724 p<0.001 partial η^2=0.204
sAPPβ, ng/mL	771 ± 351[b]	515 ± 282[a,c]	776 ± 387[b]	F(2,170)=16.48 p<0.001 partial η^2=0.162
$A\beta_{1-42}/A\beta_{1-40}$ ratio	0.11 ± 0.02 [b,c]	0.29 ± 0.07[a]	0.27 ± 0.07[a]	F(2,170)=106.362 p<0.001 partial η^2=0.556
$A\beta_{1-42}/A\beta_{1-38}$ ratio	0.36 ± 0.09[b,c]	0.90 ± 0.18[a,c]	0.80 ± 0.21[a,b]	F(2,170)=126.230 p<0.001 partial η^2=0.598

Table 1. Demographics, clinical and CSF biomarker data. Demographics, clinical and CSF biomarker data. Values reported are mean ± standard deviation. *p* values were obtained by comparing the groups AD, FTLD-S and HC. Post-hoc comparisons are detailed in Fig 1. §: ANCOVA adjusted for age at CSF sampling and *APOE*ε4; a: Different than the AD group (p<0.05); b: Different than the FTLD-S group (p<0.05); c: Different than the control group (*p*<0.05). **Abbreviations:** AD = Alzheimer's disease; Aß = Amyloid ß; CDR = Clinical dementia rating; FTLD-S = Frontotemporal lobar degeneration-related syndromes; HC = healthy controls; sAPPß = soluble ß fragment of amyloid precursor protein.

els were not correlated with $A\beta_{1-38}$ levels in the AD group (r=0.272, p=0.067). Finally, as shown in Table S1 Aβ peptides ($A\beta_{1-42}$, $A\beta_{1-40}$ and $A\beta_{1-38}$) showed moderate to high correlations with each other in all clinical groups (r=0.505 to r=0.900).

Correlation between APP-derived peptides and measures of cognitive and disease severity

When analyzing the whole sample, CDR-SOB correlated with $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-38}$ and sAPPβ (r=-0.298 [-0.416; -0.180], r=-0.205 [-0.340; -0.061], r=-0.335 [-0.433; -0.224], r=-0.246 [-0.352; -0.113], respectively, all $p<0.05$). Moreover, MMSE and FCSRT delayed total scores correlated with $A\beta_{1-42}$ (r=0.263 [0.108; 0.394,

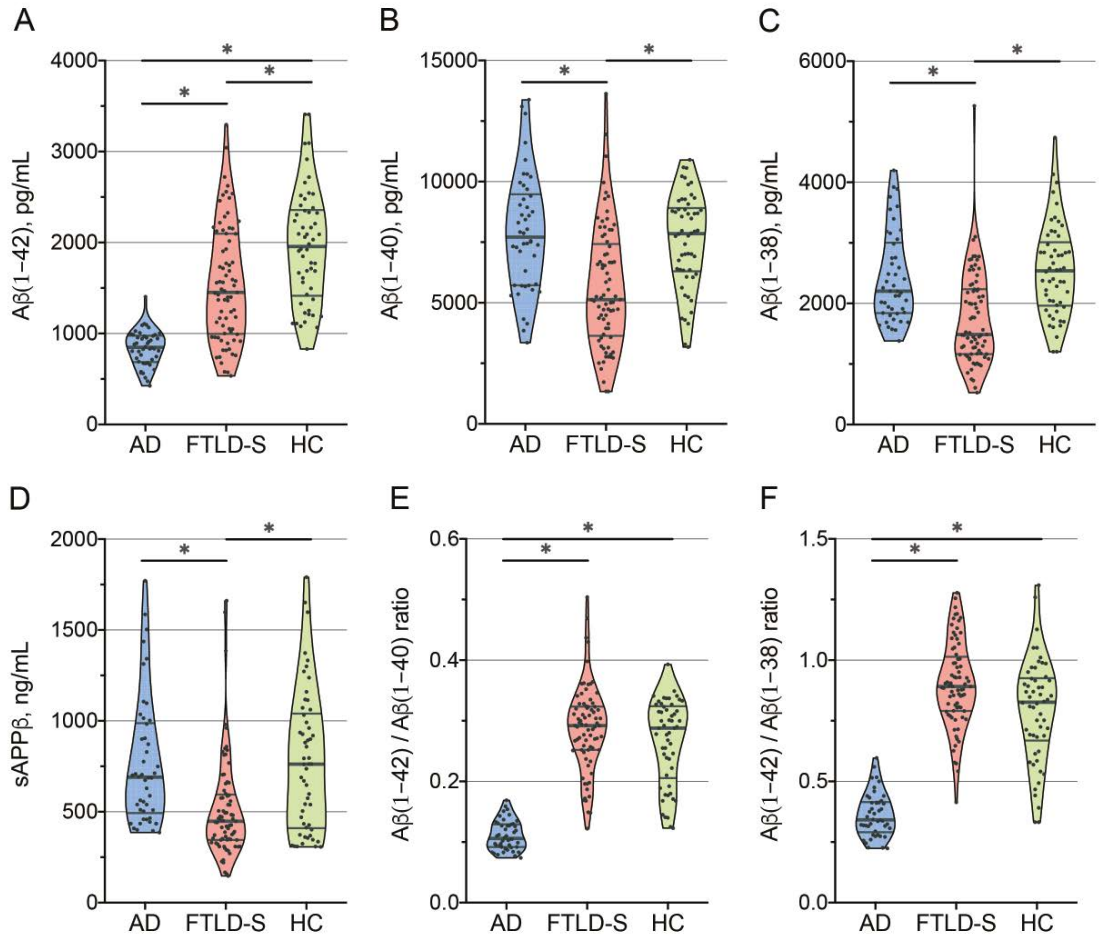

Figure 1. Levels of APP-derived peptides in CSF in the different groups. CSF levels of (A) Aß1-42, (B) Aß1-40, (C) Aß1-38, (D) sAPPß, (E) Aß1-42/Aß1-40 ratio and (F) Aß1-42/Aß1-38 ratio across groups. Only statistically significant differences are displayed (Bonferroni's post-hoc test; *: p<0.001). We applied correction for multiple comparisons. Abbreviations: AD = Alzheimer's disease; Aß = Amyloid ß; FTLD-S = Frontotemporal lobar degeneration-related syndromes; HC = healthy controls; sAPPß = soluble ß fragment of amyloid precursor protein.

p=0.001]; r=0.384 [0.250; 0.499, *p*<0.001], respectively). When we restricted the analysis to the FTLD-S group, only phonological fluency showed a mild correlation with $A\beta_{1-42}$, $A\beta_{1-38}$ and sAPPβ levels (r=0.365 [0.143; 0.573], *p*=0.003; r=0.304 [0.083; 0.540], p=0.016, r=0.264 [0.015; 0.485], p=0.038, respectively). Conversely, in the AD and controls groups we did not find significant correlations between CSF biomarkers and measures of cognitive or disease severity.

Correlation between APP-derived peptides and cortical macrostructure in FTLD-S

Finally, we investigated the relationship between APP-derived peptides in CSF and the markers of neurodegeneration measured with structural MRI. As shown in Fig 2**,** in the FTLD-S group the CSF levels of $A\beta_{1-42}$ showed a positive correlation with cortical thickness (namely, lower CSF values of $A\beta_{1-42}$ reflected thinner cortex) in

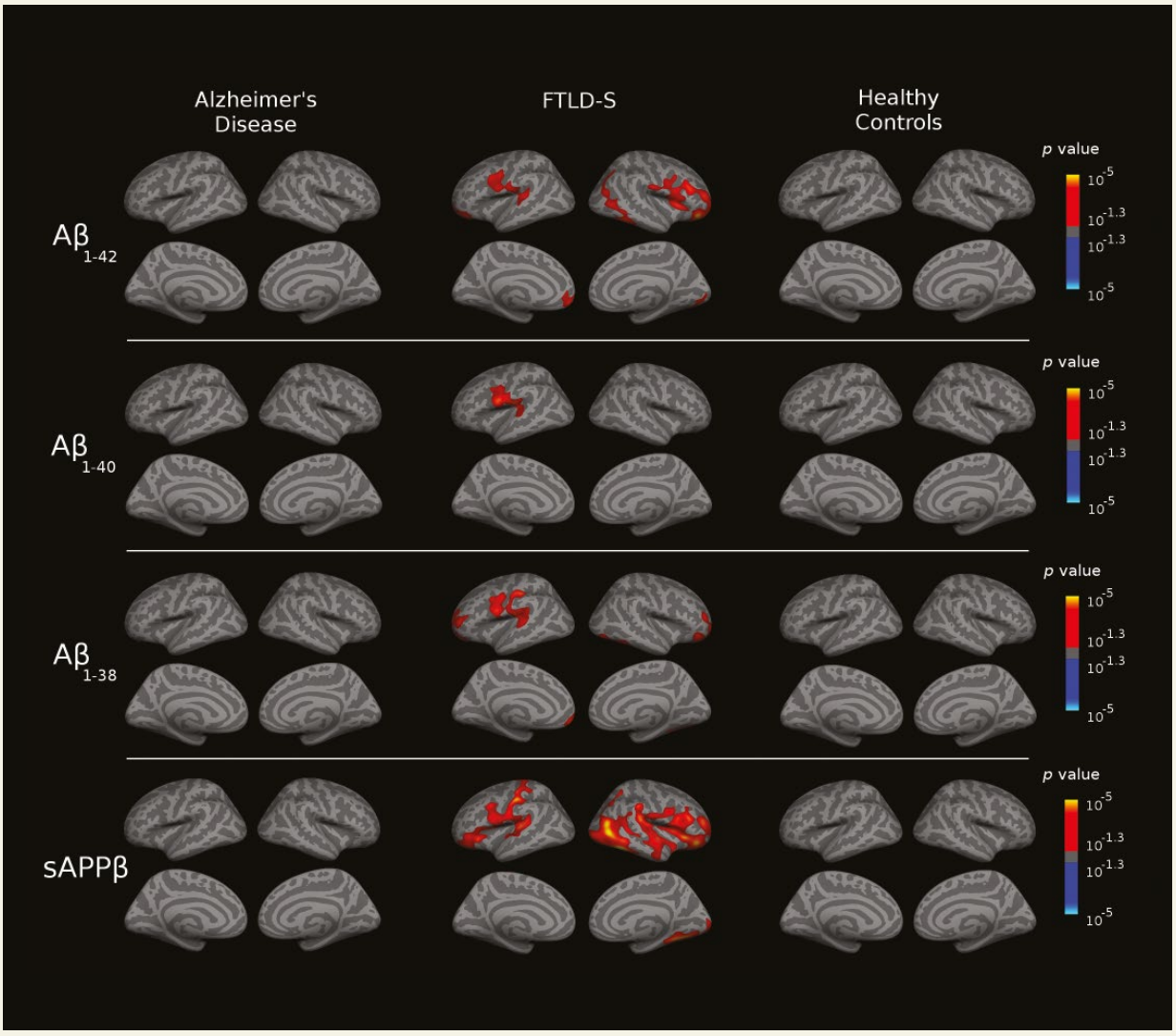

Figure 2. Structural CSF-MRI correlations. Relationship between cortical thickness and APP-derived peptides in AD (n=26, left column), FTLD-S (n=37, middle column) and HC (n=23, right column). Colored region represent significant correlations between cortical thickness and the CSF levels of the appropriate APP-derived peptide. Only clusters that survived familywise error correction are shown (*p*<0.05). All analyses were adjusted for age, sex and magnetic resonance equipment. Positive correlations are red-orange colored.

the prefrontal cortex, anterior cingulate, middle frontal, frontal pole and the insula. Conversely, the CSF levels of $A\beta_{1-42}$ were not related to cortical thickness in the AD or the HC group. Similarly, the CSF levels of $A\beta_{1-40}$ and $A\beta_{1-38}$ showed a positive correlation with cortical thickness in frontal and to a lower extent in superior temporal areas in the FTLD-S group but not in the AD group (Fig 2). In the FTLD-S group, the CSF levels of sAPPβ showed a positive correlation with cortical thickness in prefrontal cortex, anterior cingulate, middle frontal, frontal pole, the insula but also more posterior regions such as the precuneus and lateral temporal (right hemisphere) (Fig 2). Of note, neither $A\beta_{1-42}/A\beta_{1-40}$ nor $A\beta_{1-42}/A\beta_{1-38}$ ratios correlated with cortical thickness in the FTLD-S or the AD groups (data not shown).

Discussion

In this study we confirm a global decrease in the CSF levels of $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-38}$ and sAPPβ in FTLD-S. Importantly, we describe for the first time in FTLD-S a direct correlation between cortical thickness and four APP-derived peptides related to the amyloidogenic processing pathway of APP. These findings suggest that the CSF levels of these APP-derived peptides may be a reflect of neurodegeneration in FTLD-related syndromes. In previous works, we hypothesized that low CSF levels of sAPPβ in FTLD-S were likely due to frontotemporal degeneration because APP is predominantly expressed in neurons in frontal regions (Alcolea *et al.*, 2017; Ferrari *et al.*, 2017; Grothe *et al.*, 2018). Importantly, low levels of sAPPβ were previously confirmed in pathology-confirmed series of FTLD, indicating that this profile is linked to FTLD and not to AD (Alcolea *et al.*, 2018). Other studies investigating the CSF levels of APP-derived peptides in FTLD-S have revealed inconsistent results, likely due to differences in inclusion criteria, sample size and the lack of AD biomarkers in some to exclude atypical AD (Gloeckner *et al.*, 2008; Janelidze *et al.*, 2016; Steinacker *et al.*, 2009; Verwey *et al.*, 2010). In this study we found that, in addition to sAPPβ, four Aβ peptides are reduced in CSF in FTLD-S.

Our findings suggest an amyloid-independent relationship between FTLD-related neurodegeneration and APP-derived peptides. These findings have important implications in the field of CSF biomarkers as $A\beta_{1-42}$ peptide is considered an indirect biomarker of cerebral amyloidosis in current research frameworks (Illán-Gala, Pegueroles, *et al.*, 2018; Jack *et al.*, 2018). Our data suggest that $A\beta_{1-42}$ in CSF may have a dual role as a biomarker: on one hand, $A\beta_{1-42}$ may be an AD state biomarker but on the other, $A\beta_{1-42}$ can track neurodegeneration in FTLD and possibly in other non-AD dementias. This study also explains the improved agreement between amyloid PET and CSF measures observed with the $A\beta_{1-42}/A\beta_{1-40}$ ratio in non AD-dementias (Janelidze *et al.*, 2016; Leuzy *et al.*, 2016). Because both $A\beta_{1-40}$ and $A\beta_{1-38}$ are also related to neurodegenerative changes in FTLD-S, the ratios can correct appropriately CSF levels of $A\beta_{1-42}$ for the differentiation of AD from FTLD (Janelidze *et al.*, 2016; Leuzy *et al.*, 2016). Our data also support the findings of a recent study in FTLD-S showing that the CSF levels of $A\beta_{1-42}$ correlated with gray matter density and were comparable to neurofilament light as a predictor of longitudinal MRI changes (Ljubenkov *et al.*, 2018).

Our results also help to improve our understanding of some unexpected findings in non-AD conditions. Previous studies have suggested that APP-derived peptides in the CSF may be altered in neurological conditions, such as Multiple Sclerosis, independently of AD pathology (Augutis *et al.*, 2013). Moreover, a substantial disagreement between the burden of amyloid pathology in post-mortem studies and CSF levels of $A\beta_{1-42}$ has been also described in other diseases characterized by prominent gray matter neurodegeneration such as Creutzfeldt Jakob disease (Lattanzio *et al.*, 2017). Taken together, these previous find-

ings support that central nervous system non-AD neurodegeneration may influence the CSF levels of Aβ peptides. The notion that CSF levels of APP-derived peptides may be also altered in diseases other than AD argues against a specific role of $A\beta_{1-42}$ levels in CSF as a measure of cerebral amyloidosis and opens the possibility that it reflects synaptic or neuronal loss in a variety of neurological conditions. In addition, the data clearly supports the use of the ratio $A\beta_{1-42/40}$ as a specific biomarker of brain amyloidosis in AD (Illán-Gala et al., 2018).

We found a direct correlation between the CSF levels of Aβ peptides and frontotemporal neurodegeneration in FTLD-S but not in AD. We have previously reported the correlation of sAPPβ with cortical thickness in FTLD-S and in the amyotrophic lateral sclerosis-frontotemporal continuum (Alcolea *et al.*, 2017; Illán-Gala, Alcolea, *et al.*, 2018). In this study we expand previous findings by showing that $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-38}$ also correlate with cortical thickness in FTLD-S. Several factors may help to explain this observation. We speculate that the topography of neurodegeneration may influence APP-derived peptides as APP itself is highly expressed in neurons in frontotemporal regions under physiological conditions (Ferrari *et al.*, 2017). Thus, prominent neuronal loss in FTLD-S may cause a global decrease in APP processing leading to the observed decrease in APP-derived peptides. This could help to understand the observation of low levels of APP-derived peptides in other conditions that also affect frontotemporal regions, such as adult chronic hydrocephalus (Miyajima *et al.*, 2012). Moreover, recent data-driven studies have observed that the subgroup of AD characterized by diffuse atrophy (including frontal regions) has the lowest levels of $A\beta_{1-42}$ (Kate *et al.*, 2018). Whether the CSF levels of $A\beta_{1-42}$ may also reflect neurodegeneration in frontotemporal regions in addition to cerebral amyloid deposition in some AD cases with diffuse neurodegeneration remains to be elucidated. However, it is likely that the heterogeneity of AD patients in most studies, including ours, and the fact that levels of $A\beta_{1-42}$ are mainly driven by brain amyloid pathology difficult the possibility to test if there is a correlation between APP-derived products and cortical thickness in AD.

In this work, we did not find significant correlations between APP-derived peptides and cortical thickness in the AD group. In AD, APP-derived peptides may be influenced by disease-specific pathophysiological events. We and others have shown that β-site amyloid precursor protein-cleaving enzyme (BACE) activity is increased in sporadic AD (Fukumoto *et al.*, 2002; Pera *et al.*, 2012) and this may lead to the increase in APP-derived peptides. This in turn may lead to the pseudonormalization of reduced APP-derived peptides reflecting AD-related neurodegeneration. The sole exception may be $A\beta_{1-42}$, that has been hypothesized to remain low because of its sequestration in amyloid plaques in the brain (DeMattos *et al.*, 2002). This multifactorial modification of APP-derived peptides in AD may explain why we failed to find a significant correlation of $A\beta_{1-42}$, $A\beta_{1-40}$ and $A\beta_{1-38}$ peptides in the AD group in our study.

However, previous studies in Alzheimer's disease have found that CSF levels of $A\beta_{1-42}$ correlate with gray matter density in cross-sectional studies and longitudinal MRI structural changes in longitudinal studies (Ljubenkov *et al.*, 2018; Ossenkoppele *et al.*, 2015).

The main strengths of this study are the inclusion of well-characterized participants that allowed us to identify the structural correlates and patterns of APP-derived peptides in FTLD-S and AD. This study has also some limitations. First, this study lacks neuropathological confirmation and misdiagnosed could have occurred. However, in all participants the diagnosis of underlying FTLD and AD was supported by CSF biomarkers and imaging studies. Second, we relied on cross-sectional data and further studies

should evaluate the implications of APP-derived peptides changes for longitudinal biomarker trajectories and clinical outcomes of FTLD-related neurodegeneration. Finally, although we used CSF to exclude patients with FTLD-S and a CSF biomarker profile of AD we cannot exclude entirely that some of the FTLD-S may have some concurrent AD pathological changes at autopsy and therefore we could not account for comorbid AD pathology.

In summary, we showed that FTLD-S show a CSF biomarker profile consisting of a global reduction of APP-derived peptides from the amyloidogenic pathway and that this reduction correlated with a neurodegeneration marker. This pattern of APP-derived peptides should be taken into account in patients with FTLD-S. Further studies should delve into the mechanisms underlying the observed decrease in APP-derived peptides in FTLD-S.

Acknowledgments

This work was supported by research grants from the Carlos III Institute of Health, Spain (grants PI14/01126 and PI17/01019 to Juan Fortea, grants and PI13/01532 and PI16/01825 to Rafael Blesa, grants PI14/1561 to Alberto Lleó) and the CIBERNED program (Program 1, Alzheimer Disease to Alberto Lleó and SIGNAL study, www.signalstudy.es), partly funded by Fondo Europeo de Desarrollo Regional (FEDER), Unión Europea, "Una manera de hacer Europa". This work has also been supported by a "Marató TV3" grant (20141210 to Juan Fortea and 044412 to Rafael Blesa) and by Generalitat de Catalunya (2014SGR-0235) and a grant from the Fundació Bancaria La Caixa to Rafael Blesa. I. Illán-Gala is supported by the i-PFIS grant (IF15/00060) from the FIS, Instituto de Salud Carlos III and the Rio Hortega grant (CM17/00074) from "Acción estratégica en Salud 2013-2016" and the European Social Fund.

We acknowledge all the participants in this study and all the collaborators of the SPIN cohort. We also acknowledge Soraya Torres and Laia Muñoz for technical assistance. We thank EUROIMMUN for the delivery of the $A\beta_{1-38}$ and $A\beta_{1-40}$ ELISA assays for this study.

Funding

This study was supported by the Fondo de Investigaciones Sanitario (FIS), Instituto de Salud Carlos III (PI15/01618 to R.R., PI14/1561 and PI17/01896 to A.L), jointly funded by Fondo Europeo de Desarrollo Regional, Unión Europea, Una manera de hacer Europa. This work was also supported by Departament de Salut de la Generalitat de Catalunya, Pla Estratègic de Recerca i Innovació en Salut (SLT002/16/00408 to A.L), Fundació La Marató de TV3 (201437.10 to R.R); I. Illán-Gala is supported by an i-PFIS grant (IF15/00060) from the FIS, Instituto de Salud Carlos III and the Rio Hortega grant (CM17/00074) from "Acción Estratégica en Salud 2013-2016" and the European Social Fund.

Supplementary material

A) AD	Aβ(1-42)	Aβ(1-40)	Aβ(1-38)
sAPPβ	0.308 (0.115-0.487)*	0.350 (0.113-0.563)*	0.272 (-0.006-0.553)
Aβ(1-42)	–	**0.704 (0.499-0.822)***	**0.583 (0.377-0.741)***
Aβ(1-40)	–	–	**0.796 (0.677-0.877)***
B) FTLD-S	Aβ(1-42)	Aβ(1-40)	Aβ(1-38)
sAPPβ	**0.640 (0.429-0.802)***	**0.596 (0.420-0.742)***	**0.661 (0.477-0.795)***
Aβ(1-42)	–	**0.824 (0.732-0.911)***	**0.847 (0.773-0.910)***
Aβ(1-40)	–	–	**0.852 (0.784-0.925)***
C) HC	Aβ(1-42)	Aβ(1-40)	Aβ(1-38)
sAPPβ	585.8 (465.5)	**0.535 (0.317-0.699)***	**0.638 (0.419-0.784)***
Aβ(1-42)	–	**0.505 (0.289-0.713)***	**0.522 (0.242-0.767)***
Aβ(1-40)	–	–	**0.900 (0.840-0.947)***

Table S1. Correlation between APP-derived peptides along clinical groups. Correlation between Aß(1-42), Aß(1-40) and Aß(1-38) and sAPPß in A) AD group, B) FTLD-S group and C) HC group. Results are shown as pearson correlation coefficient (95% confidence interval). 95% confidence intervals were calculated by means of bias-corrected accelerated bootstrapping (1000 samples). Statistically significant correlations ($p<0.05$) are marked in an asterisk (*). Moderate-to-high correlations ($r>0.5$) are marked in bold.

Statistical analysis	Aβ(1-42)	Aβ(1-40)	Aβ(1-38)	Aβ(1-38)
Effect size for the differences between FTLD-S and HC (ANOVA)	r=0.340 r^2=0.116	r=0.368 r^2=0.135	**r=0.412** r^2=0.170	r=0.412 r^2=0.170
Effect size for the differences between FTLD-S and AD (ANOVA)	**r=0.631** r^2=0.399	r=0.332 r^2=0.110	r=0.342 r^2=0.117	r=0.458 r^2=0.209
Effect size for the differences between FTLD-S and HC (ANCOVA)§	r=0.295 partial η^2=0.088	r=0.344 partial η^2=0.118	**r=0.430** partial η^2=0.185	r=0.337 partial η^2=0.113
Effect size for the differences between FTLD-S and AD (ANCOVA)§	**r=0.511** partial η^2=0.261	r=0.337 partial η^2=0.113	r=0.309 partial η^2=0.096	r=0.343 partial η^2=0.118

Table S2: Effect size for the differences between APP-derived peptides between. Correlation between Aß(1-42), Aß(1-40) and Aß(1-38) and sAPPß in A) AD group, B) FTLD-S group and C) HC group. Results are shown as pearson correlation coefficient (95% confidence interval). 95% confidence intervals were calculated by means of bias-corrected accelerated bootstrapping (1000 samples). Statistically significant correlations ($p<0.05$) are marked in an asterisk (*). Moderate-to-high correlations ($r>0.5$) are marked in bold.

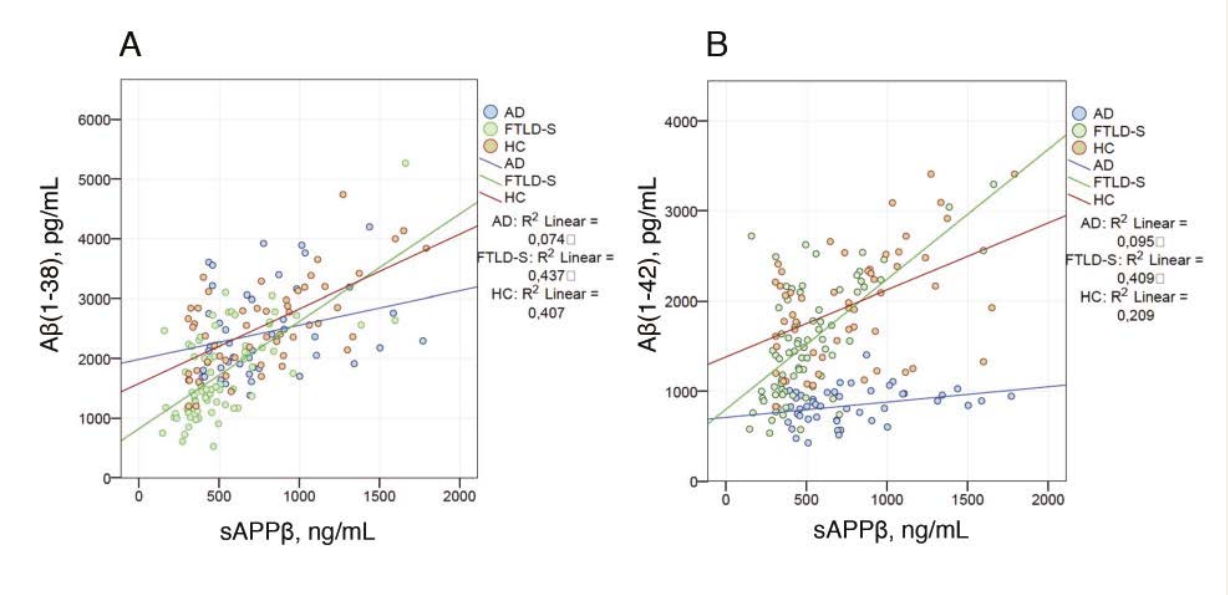

Figure S1. Correlation between sAPPß and Aß(1-38)(A) and Aß(1-42) (B).

Chapter 7

Discussion

In this doctoral thesis we investigated the pathophysiological underpinnings of FTLD-related neurodegeneration and its macro- and micro-structural neuroimaging correlates through a multimodal biomarker approach combining clinical measures, CSF and neuroimaging biomarkers (Figure 1). We provide novel insights into the pathophysiology of FTLD by showing that: (i) APP-derived peptides related to the amyloidogenic pathway (sAPPβ, $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-38}$) are globally reduced in FTLD-S and correlate with cortical thickness; (ii) YKL-40, a biomarker related to astroglial activity, is increased in FTLD-S and may be useful for the prediction of disease progression in the ALS-FTD continuum; and (iii) cortical mean diffusivity is more sensitive than cortical thickness for the study of the earliest FTLD-related neurodegeneration. These findings add to our current understanding of FTLD pathophysiology and open new doors towards precision medicine approaches for FTLD-S.

The potential role of APP in FTLD pathophysiology

The major focus of research related to APP and APP-derived peptides has been the characterization of Alzheimer's disease pathophysiology (Scheltens *et al.*, 2016). Indeed, autosomal dominant forms of Alzheimer's disease caused by *APP*, *PSEN1* or *PSEN2* mutations are characterized by altered APP processing leading to a relative or absolute increase in $A\beta_{1-42}$ production and/or enhanced aggregation (Pera *et al.*, 2013). However, in sporadic cases other factors such as altered balance between production and clearance of Aβ may be involved in disease pathophysiology (Tarasoff-Conway *et al.*, 2015).

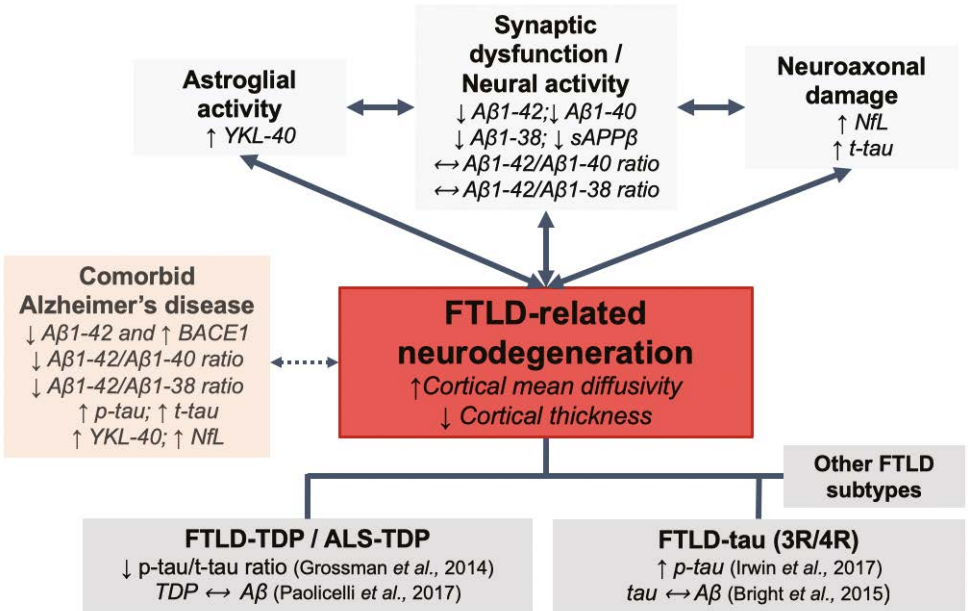

Figure 1: Pathophysiological mechanisms and associated biomarkers implicated in FTLD-related neurodegeneration. Data on biomarkers are shown in italics. Arrows reflect hypothetical relationships, not direct causal links between pathological mechanisms and FTLD-related neurodegeneration.

However, several recent papers have linked APP-derived peptides with FTLD pathophysiology. First, in a recent study, TDP depletion was related to increased uptake of extraneuronal Aβ and synaptic damage by microglia (Paolicelli *et al.*, 2017). Interestingly, the authors of this work also reported a lower frequency of Alzheimer's disease pathology in human brain samples of patients with ALS-TDP when compared to age-matched controls (Paolicelli *et al.*, 2017). These observations suggest that the loss of TDP function may be related to the observed microglia-driven reduction of APP-derived peptides. Second, in a study investigating the genetic architecture of FTLD, the *APP* gene was related to *C9orf72* (an FTLD-TDP causing gene) by means of a novel approach called "weighted protein-protein interaction network analyses". This co-expression genetic analysis suggested a pathophysiological link between *APP* and *C9orf72* genes (Ferrari *et al.*, 2017). Third, several studies have related extraneuronal levels of Aβ with increased FTLD-tau pathology (Bright *et al.*, 2015; Gotz *et al.*, 2001). Particularly, the injection of transgenic FTLD-tau mice with aggregated Aβ was related to increase FTLD-tau burden suggesting an upstream role for Aβ oligomerization in initiating FTLD-tau pathology (Gotz *et al.*, 2001). Consistent with these findings, another recent study found that in FTLD-tau cases, higher CSF levels of $A\beta_{1\text{-}42}$ were linked to faster disease progression (Ljubenkov *et al.*, 2018) while lower CSF levels of $A\beta_{1\text{-}42}$ were related to faster disease progression in the FTLD-TDP subgroup. Taken together, these studies indicate that APP-derived peptides are implicated in the pathophysiology of FTLD.

The relationship between APP, synapses and neuronal function

The finding of reduced levels of APP-derived peptides in FTLD-S may be counterintuitive for some authors as the accumulation of Aβ peptide in extracellular plaques is a core neuropathological hallmark of Alzheimer's disease. However, in addition to the well-established role in Alzheimer's disease pathogenesis, APP may also play an important role in the aging brain. Recent studies have shown that APP is located at the presynaptic active zone where it interacts with a number of synaptic proteins (**Figure 2**) (Lassek *et al.*, 2013; 2015). Indeed, APP and APP-derived peptides may also have important physiological functions at the synapse in healthy individuals (Muller *et al.*, 2017). Many of these functions remain largely unexplored, and include: (i) trans-synaptic adhesion (Wang *et al.*, 2009); (ii) signaling functions involving brichos domain-containing 1 and 2 (BRIC1 and BRIC2) that may reduce beta- alpha- and gamma-secretase cleavage of APP (Matsuda *et al.*, 2005); and (iii) synaptic transmission (Muller *et al.*, 2017). Importantly, the expression and synaptic localization of APP has been related to learning deficits of mouse mutants, supporting the role of these proteins in synaptic plasticity, learning and memory (Muller *et al.*, 2017).

Further links between neuronal activity and synaptic function of APP-derived peptides come from studies showing that Aβ levels in CSF have been shown to fluctuate over time in the same individuals under physiological conditions (Bateman *et al.*, Neurology 2007). These fluctuations have been related to (i) the activity-dependent release of dynamic production of Aβ *in-vivo* following full-length APP endocytosis from the presynaptic terminal (Cirrito *et al.*, Neuron 2008); (ii) metabolism of Aβ peptides by microglia, which has been related in turn to synapse loss; (iii) clearance of Aβ peptides from the interstitial fluid through the paravascular clearance pathways (Kress *et al.*, 2014); (iv) reduction of Aβ production during sleep related to decreased cerebral activity (Lucey *et al.*, 2018). Additionally, neuronal activity has been related to local Aβ deposition, supporting the link between neuronal activity and local Aβ production and deposition (Bero *et al.*, 2011).

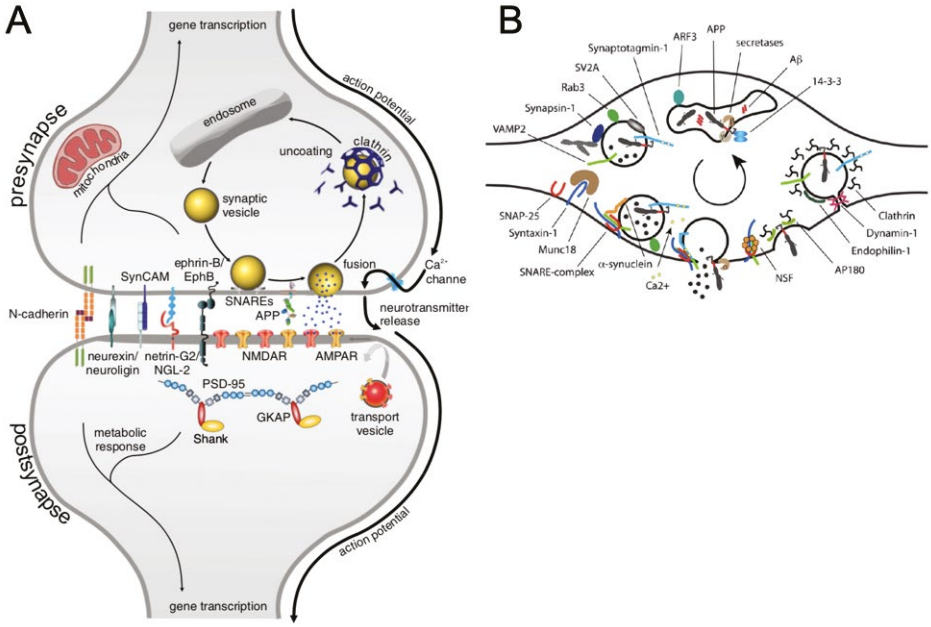

Figure 2: Interactions of APP with synaptic proteins. A) General scheme of the relationship between APP and other relevant presynaptic and postsynaptic proteins at the synaptic junction; adapted from Lassek *et al.*, 2015. B) Detailed diagram of APP and related proteins along the synaptic vesicle cycle.

Taken together, previous evidence suggests that APP-derived peptides could be related to the prominent neuronal loss observed in FTLD and that APP may play an important role at the synapse. This leads to the possibility that APP levels in CSF could also be a biomarker of synaptic impairment. Previous studies assessing the role of APP-derived peptides in FTLD-S CSF were scarce, and usually tested a restricted number of APP-derived peptides in small samples of patients with FTLD-S (Steinacker et *al.*, 2009). In this thesis we investigated the correlation between CSF levels of sAPPβ and other APP-derived peptides related to the amyloidogenic pathway of APP processing with cortical thickness as a measure of neuronal loss.

APP-derived peptides and NfL levels reflect FTLD-related neurodegeneration

Previous studies have highlighted the role of NfL levels in CSF as a potential biomarker of neurodegeneration (Khalil *et al.*, 2018). Conversely, interpretation of the significance of CSF sAPPβ levels has been controversial due to the conflicting results reported. Of note, in a previous study by Steinacker and collaborators, the CSF levels of sAPPβ correlated with disease progression in ALS (Steinacker *et al.*, 2011). In this work, the authors speculated that sAPPβ may protect neurons from proteasomal stress (Copanaki *et al.*, 2010) and that its decrease in CSF could reflect progressive neuronal loss or dysfunction in neurodegenerative diseases (Sennvik *et al.*, 2000). In

chapter 3, **chapter 5** and **chapter 6**, we showed that lower sAPPβ levels were related to cortical thinning in FTLD-S. Importantly, we also observed significant correlations between sAPPβ levels, NfL levels, and cortical thickness. Because NfL levels have been shown to reflect neuroaxonal injury in several neurodegenerative and non-neurodegenerative conditions, our findings suggest that sAPPβ levels may reflect FTLD-related neurodegeneration (Khalil *et al.*, 2018).

In **chapter 4** we found that the sAPPβ/YKL-40 ratio showed a direct correlation with cortical thickness (lower values predicted lower cortical thickness) in prefrontal and lateral temporal regions. It is worth mentioning that sAPPβ and NfL also correlated with cortical thickness when considered in isolation. However, these results did not survive correction for multiple comparisons, potentially due to the relatively small size of the neuroimaging subgroup in this study. Overall, our results support the role of sAPPβ as a neurodegeneration biomarker in FTLD-S and also add to the mounting evidence suggesting that ALS and FTD reflect the two extremes of a same neurodegenerative continuum. These results demonstrate the importance of APP-derived biomarkers and inflammation, but also show the potential of such biomarkers for diagnostic and prognostic purposes. Further studies should determine whether CSF biomarkers can improve patient classification within the ALS-FTD continuum and identify patients at higher risk of cognitive or behavioral impairment during the disease course. This is an important issue since recent studies have shown that disease progression is associated with increasing cognitive and behavioral impairment (Crockford, Newton, Lonergan, Chiwera, *et al.*, 2018) and that cognitive and behavioral impairment impact caregiver distress and decision-taking during disease course (Lillo, Mioshi, *et al.*, 2012; Olney *et al.*, 2005).

In **chapter 6** we showed a global decrease in APP-derived peptides in FTLD-S in contrast with the selective decrease of $A\beta_{1-42}$ levels in AD. Our data suggest that the CSF levels of $A\beta_{1-42}$ may have a dual role as a biomarker: on the one hand, $A\beta_{1-42}$ may be an AD state biomarker, but on the other, $A\beta_{1-42}$ may also track neurodegeneration in FTLD. Interestingly, when reviewing the literature, we found previous reports of decreased $A\beta_{1-42}$ levels in pathology proven FTLD without comorbid AD when compared to healthy age-matched controls. However, the authors of these previous reports did not delve into these results, probably because the observation of low $A\beta_{1-42}$ is a finding suggestive of underlying AD pathophysiology for many influential authors (Jack *et al.*, 2016). Moreover, our results may help to explain previous reports of high disagreement rates between CSF levels of $A\beta_{1-42}$ and amyloid positron emission tomography in FTLD-S (Janelidze *et al.*, 2016; Leuzy *et al.*, 2016). Because both $A\beta_{1-40}$ and $A\beta_{1-38}$ levels are also affected by FTLD-related neurodegeneration, $A\beta_{1-42}$/ $A\beta_{1-40}$ and $\beta 1\text{-}42/A\beta_{1-38}$ ratios could be used to differentiat AD from FTLD (Janelidze *et al.*, 2016; Leuzy *et al.*, 2016, Lewczuk *et al.*, 2017). Interestingly, a recent study in FTLD-S showed that the CSF levels of $A\beta_{1-42}$ correlated with gray matter density and were comparable to NfL CSF levels as a predictor of longitudinal MRI changes (Ljubenkov *et al.*, 2018). Overall, these findings support the link between the CSF levels of $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-38}$ and sAPPβ and FTLD-related neurodegeneration.

Although recent studies have revealed a pathophysiological link between APP-derived peptides and FTLD (Paolicelli *et al.*, 2017), it remains unclear whether APP-derived peptides reflect general neurodegeneration or FTLD-specific pathophysiological events. Similar to sAPPβ, in **chapter 6**, we found a direct correlation between $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-38}$ and cortical thickness. However, we failed to find a significant a direct correlation between APP-derived peptides related to the amyloidogenic pathway and cortical thickness in the Alzheimer's disease group. We hypothesized that

in Alzheimer's disease, APP-derived peptides may also be affected by multiple disease-specific pathophysiological mechanisms. Supporting this hypothesis, our group has shown that β-site amyloid precursor protein-cleaving enzyme (BACE) activity is increased in sporadic Alzheimer's disease (Pera *et al.*, 2012; Fukumoto *et al.*, 2002). However, the CSF levels of $A\beta_{1-42}$ have been hypothesized to remain low in Alzheimer's disease because of its sequestration in amyloid plaques in the brain (Zetterberg *et al.*, 2017; DeMattos *et al.*, 2002). We hypothesize that these previous observations together with other factors such as the impairment of the clearance systems could explain the selective decrease in $A\beta_{1-42}$ levels in Alzheimer's disease (Tarasoff-Conway *et al.*, 2015). Thus, APP-derived peptides in Alzheimer's disease may be related to multiple pathophysiological dynamic processes. This combination of factors may explain why most previous studies have failed to find a significant correlation between the CSF levels of $A\beta_{1-42}$, $A\beta_{1-40}$ and $A\beta_{1-38}$ and neuroimaging measures of neurodegeneration in Alzheimer's disease. However, two notable exceptions to this last statement should be mentioned. First, Ossenkoppele *et al* studied the relationship between CSF core Alzheimer's disease biomarkers and neurodegenerative changes in different clinical subtypes of Alzheimer's disease (namely, early and late onset, logopenic variant of primary progressive aphasia and posterior cortical atrophy). They hypothesized that tau (t-tau and p-tau) markers would correlate with neurodegenerative changes. However, contrary to their hypothesis they found that the CSF levels of $A\beta_{1-42}$ correlated with gray matter density for each Alzheimer's disease subtype (Ossenkoppele, Mattsson, *et al.*, 2015). Importantly, the correlation was observed in cerebral areas were neurodegeneration is typically observed in each of the studied subtypes of Alzheimer's disease. Second, Kate Ten and collaborators followed a data-driven approach to define the different patterns of Alzheimer's disease-related neurodegeneration in in a large multicenter study (Kate Ten *et al.*, 2018). Interestingly, they found that the Alzheimer's disease subgroup was characterized by a diffuse pattern of atrophy and was also the group distinguished by the lowest $A\beta_{1-42}$ levels. Interestingly, similar results have been observed in other neurodegenerative diseases. In a recent study aiming to define the subtypes of Parkinson's disease, the "diffuse malignant" subtype was characterized by greater atrophy in MRI, lower CSF levels of $A\beta_{1-42}$ and faster disease progression (Fereshtehnejad *et al.*, 2017). Similarly, patients with Lewy body dementia and lower CSF $A\beta_{1-42}$ levels (but not higher levels of t-tau of p-tau) have consistently showed a faster disease progression (Walker *et al.*, 2015). Although these finding may reflect concurrent Alzheimer's pathology in some cases (Toledo et *al.*, 2012) it may also indicate more synaptic damage. Taken together, these studies suggest that the observed heterogeneity of neurodegeneration in Alzheimer's disease and other neurodegenerative disease may be hiding a hypothetical relationship between $A\beta_{1-42}$ and neurodegeneration. Overall, future studies should evaluate whether APP-derived peptides reflect general neurodegeneration or, on the contrary, may reflect specific aspects of FTLD pathophysiology.

The relationship between astroglial activity and disease progression in the ALS-FTD continuum

In **chapter 4**, we showed that the CSF levels of YKL-40 were able to predict disease progression in ALS. This is a relevant issue since prognostic heterogeneity is one of the major challenges for the development of clinical trials in ALS (Brown and Al-Chalabi, 2017). Consistent with previous findings, we found that higher CSF NfL levels were also associated with faster disease progression (Steinacker *et al.*, 2016; Feneberg *et al.*, 2018). However, previous studies addressing the role of YKL-40 in ALS were scarce and failed to find significant differences between controls and ALS patients (Bonneh-Barkay *et al.*, 2010). The

results presented in **chapter 4** extended these previous results by showing that the CSF levels of YKL-40 predicted disease progression independently of NfL levels. Importantly, CSF levels of YKL-40 appeared to outperform NfL when predicting survival in the ALS-FTD continuum. Although our results were based on a relatively small sample, several papers have corroborated our findings since the publication of our paper. Of note, Thompson *et al.* reported that YKL-40 and two other macrophage-derived chitinases (chitotriosidase and chitinase-3-like protein 2) were increased in ALS when compared to both ALS mimics and healthy controls (Thompson *et al.*, 2018). They also performed a survival analysis, but contrary to our findings, they reported that only chitotriosidase was independently-associated with survival. Methodological differences in the sample composition (i.e. inclusion of patients with progressive lateral sclerosis and patients with ALS-FTD) might explain the observed differences. Another relevant work published after our paper was accepted also showed that CSF YKL-40 levels were increased in ALS when compared to healthy controls and that CSF YKL-40 levels correlated with progression (Andrés-Benito *et al.*, 2018). Interestingly they also? found increased YKL-40 mRNA levels in anterior horn of the spinal cord, which correlated with microglial markers (AIF1 and CD68).

Our results are in line with previous evidence linking neuroinflammation to ALS pathophysiology (Yamanaka *et al.*, 2008; Boillee *et al.*, 2006; Frakes *et al.*, 2014). Indeed, TDP-43 inclusions are not only found in neurons, but also in the cytoplasm of glial cells (Nishihira *et al.*, 2008). In particular, under stress conditions, microglia and astrocytes that express TDP-43 were shown to produce pro-inflammatory cytokines as well as neurotoxic mediators. Moreover, TDP-43 has been shown to interact with the nuclear factor-kappa B, a master regulator of several genes involved in the inflammatory response (Swarup *et al.*, 2011). Taken together, our results and previous evidence from human and animal studies reinforce the close relationship between neuroinflammation and progression rate in the ALS-FTD continuum (Yamanaka *et al.*, 2008; Boillee *et al.*, 2006). These are key aspects that should be taken into account when designing clinical trials for disease-modifying treatments in ALS. Indeed, novel immunomodulation drugs, such as fingolimod, hold promise as new potential disease-modifying drugs for ALS and are being tested in ongoing clinical trials (Berry *et al.*, 2017; Potenza *et al.*, 2016).

Implications for research in early symptomatic stages of FTLD-S

The first descriptions of FTLD-S were made by talented neurologists that were pioneers in the characterization of novel neurodegenerative syndromes (Derouesné, 2014; Steele *et al.*, 1964; 2014). These first descriptions were usually made at advanced disease stages and usually emphasized end-stage features of the disease (Steele *et al.*, 1964). Recently, many neurodegenerative syndromes have been further characterized in clinico-pathological cohorts and deep-phenotyped cohorts. Consequently, new diagnostic criteria enable the diagnosis of FTLD-S at milder disease stages and now also consider information from pathophysiological and topographical biomarkers (Rascovsky *et al.*, 2011; Gorno-Tempini *et al.*, 2011; Armstrong *et al.*, 2013; Mesulam *et al.*, 2014; Hoglinger *et al.*, 2017).

Despite of these advances, many neurologists are not familiar with the diagnosis of FTLD-S and the diagnosis of these patients is usually delayed. Dramatically, many patients with FTLD-S are still misdiagnosed with psychiatric conditions before receiving the diagnosis of neurodegenerative disease. This is particularly striking in the case of women with bvFTD, who are more frequently misdiagnosed with psychiatric disease than men (Woolley *et al.*, 2011; and unpublished data from the SPIN cohort). Similarly, misdiagnosis

is frequent in other neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, especially when the diagnosis is not supported by pathophysiological biomarkers (Scheltens *et al.,* 2016; Rizzo *et al.,* 2016). Of note, the same patient may receive a different diagnosis depending on the area of expertise of the treating physician. For example, a patient with nfaPPA and a mild rigid-acinetic syndrome evaluated by a movement disorders expert may receive the diagnosis of mild Parkinson disease for many years until the emergence of a canonical PSP- or CBD-related syndrome, when the diagnosis will be ultimately changed to probable PSP or CBD. This example illustrates the need for a common approach to the study of FTLD-S, and the importance of FTLD-related biomarkers to support the diagnosis of FTLD at mild disease stages. In the case of bvFTD, some authors have reported that a proportion of possible bvFTD cases may not have underlying FTLD, while others defend that possible bvFTD cases represent early stage FTLD (Borroni, Cosseddu, *et al.,* 2015). In **chapter 5** we included a wide range of bvFTD cases recruited in different centers, most of whom had an increased degree of certainty of underlying FTLD. Indeed, a significant proportion of possible bvFTD cases progressed to PSP-CBD phenotypes or motor neuron disease. This finding highlights the importance of taking into account the earliest cognitive and behavioral aspects of neurodegenerative diseases as a way of advancing the diagnosis to the mild symptomatic stages, where novel treatments may be more effective (Tsai and Boxer, 2016). However, even if cognitive and behavioral aspects are meticulously compiled, clinical phenotypes may not be sufficient to predict FTLD diagnosis *in-vivo*. In the largest clinical-pathological sample of bvFTD half of the pathology-proven PSP cases did not display the clinical cardinal elements of PSP syndrome during disease course (namely, oculomotor palsy and prominent postural instability) (Perry *et al.,* 2017). These relevant findings from deeply-phenotyped cohorts highlight the limitations of clinical characterization for the prediction of pathological diagnoses (Meeter *et al.,* 2017; Boxer *et al.,* 2017). Further refinement of diagnostic criteria will be needed to improve the accuracy, reproducibility and consistency of clinical diagnoses by incorporating disease-specific biomarkers that may be both sensitive and specific to detect FTLD-related neurodegeneration at the earliest disease stages. This in turn may contribute to an early diagnosis of FTLD and may have the potential to transform current clinical practice in behavioral and cognitive neurology.

But even when mild FTLD-S are evaluated in multidisciplinary clinics by expert clinicians, their classification may still be problematic. In our experience, many patients that undergo the deep phenotyping protocol in the FTLD-S unit at our center are unclassifiable according to the current diagnostic criteria or they meet criteria for more than 1 syndrome. Most of these cases usually present with prominent behavioral features emerging two to three years before consultation. They also display motor symptoms and signs that do not dominate the clinical picture at the time of diagnosis. Importantly, many informants would only report significant personality changes during separate interviews conducted following the so-called "biographical narrative approach" (Miller *et al.,* 2015). Until then, many informants may be unaware that the observed behavioral changes may be related to a neurodegenerative disease. When trying to apply the new diagnostic criteria, some of these cases could be classified either as bvFTD, "frontal variant of PSP" or even "fronto-spatial" CBS. Thus, to improve the consistency of clinical classifications across cohorts, patients presenting with cognitive, behavioral, language and motor complaints in whom Alzheimer's disease and Lewy body disease have been appropriately ruled out, should all be evaluated in multidisciplinary FTLD-S clinics following standardized protocols (Grossman and Irwin, 2016). This would avoid systematic biases related to patient referral to either cognitive neurology or movement disorders clinics (Josephs *et al.,* 2008; Lee at al., 2011; Re-

spondek *et al.*, 2014). Importantly, the clinical evaluation of FTLD-S should always include separate structured interviews with reliable informants and detailed assessment of social cognition by experienced behavioral neurologists because most patients have anosognosia (Lansdall *et al.*, 2017, 2018; Henry *et al.*, 2016).

Figure 3 illustrates the potential use of biomarkers to advance the diagnosis of FTLD in earlier clinical stages when future targeted therapies may be more effective. The information provided by biomarkers at diagnosis could also be taken into account for the prediction of disease progression and the selection of candidates for clinical trials. On the one hand, pathophysiological biomarkers measuring key aspects of the neurodegenerative process (i.e. neuroinflammation or FTLD subtype) could help in the characterization of underlying neurodegenerative process. This information may be crucial for candidate selection for future trials targeting specific aspect of FTLD pathophysiology (i.e. anti-inflammatory or anti-tau drugs). On the other hand, the development of novel imaging biomarkers more sensitive than conventional imaging biomarkers may increase our sensitivity for the detection of FTLD-related neurodegenerative changes by lowering the detection threshold of imaging techniques. This, in turn, may allow the diagnosis at earliest clinical stages.

APP-derived peptides, YKL-40 and NfL as potential diagnostic biomarkers in FTLD-S

In chapter 3 we confirmed increased NfL and YKL-40 levels in FTLD-S compared to Alzheimer's disease and controls. However, we extended previous findings by showing that the CSF levels of sAPPβ were decreased in the FTLD-S and showed a similar capacity for the differentiation

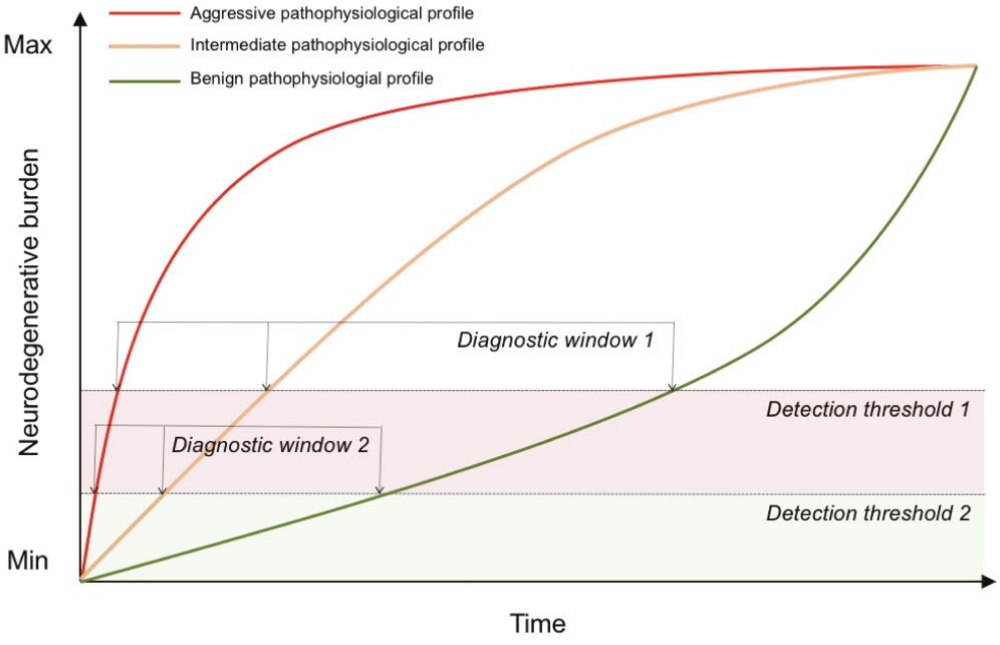

Figure 3: Hypothetical contribution of biomarker-based phenotyping for advancing FTLD detection and improving the prediction of disease progression.

of FTLD-S from controls than NfL in a large multicentric study.

In **chapter 4**, we found the highest levels of NfL in the ALS group reflecting the massive neuroaxonal injury in this group when compared to other neurodegenerative diseases (Khalil et al., 2018). On the other hand, we found intermediate levels of sAPPβ in the ALS group compared to the FTD and control groups, and increased levels of YKL-40 levels in both the ALS and the FTD group. Interestingly, sAPPβ and YKL-40 correlated with cognitive impairment in the ALS-FTD continuum, and YKL-40 correlated with measures of progression rate, supporting the potential of these biomarkers to track disease progression. We only found one previous study evaluating the CSF levels of sAPPβ in ALS. Consistent with our findings, Steinacker and collaborators reported that the CSF levels of sAPPβ predicted disease progression in ALS (Steinacker *et al.*, 2011).

Following the publication of the papers presented in **chapter 3** and **chapter 4**, we conducted a follow-up study to replicate our results in a unique cohort of pathology-proven FTLD cases with antemortem CSF available (**Annex 5; Supplementary paper 1**). This dataset allowed us to explore the role of sAPPβ and YKL-40 to discriminate between FTLD-tau and FTLD-TDP. In this study, we confirmed that CSF levels of sAPPβ were reduced and that those of YKL-40 were increased in both FTLD-TDP and FTLD-tau neuropathological subtypes of FTLD (Alcolea *et al.*, 2018). Unfortunately, none of the studied biomarkers (sAPPβ and YKL-40) allowed the accurate differentiation of FTLD subtypes. However, the group of FTLD-tau (without concurrent Alzheimer's disease) showed higher levels of YKL-40 than pure FTLD-TDP, and YKL-40 levels correlated with pathological tau burden. These results highlight the intensity of astroglial activity in FTLD-tau (Kovacs *et al.*, 2015; 2017; Querol-Vilaseca *et al.*, 2017) and also the importance of considering concurrent Alzheimer's disease pathology when interpreting CSF biomarker results (Toledo *et al.*, 2012; Lleo *et al.*, 2018).

Taken together, our results suggest that sAPPβ levels, as well as the sAPPβ/NfL and sAPPβ/YKL ratios may be useful biomarkers to increase the diagnostic certainty of underlying FTLD in certain clinical settings. Although most patients classified as possible bvFTD will eventually develop the typical frontotemporal atrophy during follow-up, some cases will remain stable over time or will be reclassified as psychiatric phenocopies (Devenney *et al.*, 2016). In these cases, CSF biomarkers may contribute to the early identification of FTLD mimics. Recently, other authors have explored the potential value of CSF biomarkers to distinguish FTLD-S from non-neurodegenerative phenocopies. Particularly, Vijverberg *et al.*, reported that the combination of NfL, the p-tau/tau ratio, and YKL40 discriminated well between probable/definite bvFTD and primary psychiatric disorders (Vijverberg *et al.*, 2017). However, Vijverberg *et al.*, did not assess the value of the CSF levels of sAPPβ or other APP-derived peptides. In the light of the results presented in **chapter 6**, future works in similar clinical scenarios are warranted to evaluate the role sAPPβ and other APP-derived peptides, alone or in combination with NfL, YKL-40 or other biomarkers to distinguish between bvFTD cases with increased certainty of underlying FTLD and other FTLD mimics.

Limitations

Several limitations should be kept in mind when interpreting the results of this thesis. First, most of the participants with FTLD-S or Alzheimer's disease were not pathology-confirmed and thus, misdiagnosis could have occurred. However, all participants were deeply-phenotyped following an extensive clinical protocol and Alzheimer's disease pathophysiological process was excluded by CSF biomarkers in FTLD-S to avoid misdiagnosis or cases with comorbid pathology.

Moreover, we included a significant proportion of FTLD-S cases with an increased certainty of underlying FTLD (namely, pathology-confirmed cases, carriers of FTLD-related mutations or cases with available follow-up in whom a second FTLD-S was observed). Second, because most patients did not have a pathological confirmation of FTLD or Alzheimer's disease, we were unable to establish the clinical-pathological correlates of the studied CSF and imaging biomarkers. We could not explore the differences in CSF and imaging profile of between FTLD subtypes. However, some information can be found in the **supplementary paper 2** where we compared the CSF biomarker profile of FTLD-TDP and FTLD-tau subtypes. Third, we did not evaluate the prognostic value of the CSF levels of sAPPβ, YKL and NfL in all FTLD-S. However, we were able to evaluate the prognostic utility of these CSF biomarkers in the ALS-FTD continuum, a population characterized by a fast disease progression and a short survival. Finally, we did not delve into the potential added value of the combination of CSF and the topography of neurodegeneration for the differentiation of FTLD-S, Alzheimer's disease and healthy controls.

Future directions

In this thesis we showed that CSF and neuroimaging biomarkers could provide important insights for the characterization of FTLD-related neurodegeneration. However, only neuroimaging biomarkers allow the study of the topography of neurodegeneration. Taking into account the topography of neurodegeneration may be important for the *in-vivo* differentiation of FTLD from other neurodegenerative and non-neurodegenerative conditions. Particularly, we envision that the study of cerebral microstructure with cortical mean diffusivity could be further enriched by its combination with a second measure of subcortical microstructure. This would enable the computation on a surface-based approach of a cortical/subcortical ratio of mean diffusivity at each vertex. By doing this we may gain important insights into the microstructural architecture of FTLD-related neurodegeneration. This is a relevant issue since some FTLD subtypes are characterized by a prominent neurodegeneration of subcortical white matter (Irwin *et al.*, 2017).

Neurodegenerative diseases are characterized by neuronal dysfunction along specific brain networks (Seeley *et al.*, 2009) and neuroimaging biomarkers have been used to identify network-specific patterns of neurodegeneration associated with FTLD and other neurodegenerative diseases (Ranasinghe *et al.*, 2016; Niethammer *et al.*, 2014; Meles *et al.*, 2017; Teune *et al.*, 2013). Importantly, multivariate methods, such as spatial covariance mapping allow the quantification of a particular pattern of neurodegeneration (i.e. network expression) at the single subject level (Niethammer *et al.*, 2012). Interestingly, the combination of the CSF biomarker profile and network expression quantification in a given individual could be used to derive a probabilistic-derived biomarker-based classification system of FTLD-S according to the expected underlying neurodegenerative disease (Schindlbeck *et al.*, 2018). These multimodal algorithm-based classifications remain to be defined and validated in future studies and may certainly contribute to overcome the limitations of clinical phenotyping for the prediction of underlying neurodegenerative diseases of FTLD-S (Tang *et al.*, 2010; Schindlbeck *et al.*, 2018).

The combination of CSF and neuroimaging biomarkers could improve our ability to predict disease progression in a particular individual. For example, in ALS the presence of primary motor neuron involvement could be operationalized by means of the study of cortical mean diffusivity at the motor cortex. By doing this, we would overcome the subjectivity of eliciting first motor neuron signs in ALS patients with concurrent secondary motor neuron signs. This would enable an objective quantification of primary mo-

tor neuron impairment in ALS patients and may contribute in turn to the refinement of current classification systems in ALS (Al-Chalabi *et al.*, 2016). Moreover, the combination of markers of astroglial activity (i.e. YKL-40) with markers of cortical microstructure (i.e. cortical mean diffusivity) may also contribute to refine diagnostic classification (Al-Chalabi et al., 2016) and improve diagnostic accuracy.

In this thesis we studied several biomarkers in CSF but recently, the development of novel technologies such as single-molecule array (SiMoA) has enabled the detection of multiple biomarkers in blood, including NfL and APP-derived peptides. These novel techniques open a window of opportunity to study multiple pathophysiological biomarkers in plasma. Of note, plasma biomarkers present a number of advantages over CSF biomarkers. Importantly, the study of plasma biomarkers does not require a lumbar puncture and is minimally invasive (i.e. allowing repeated sampling for disease monitoring). Recent studies using these novel techniques suggest that $A\beta_{1-42}$ could be a useful screening biomarker for Alzheimer's disease in plasma (Molinuevo *et al.*, 2018). Because we showed a specific profile of APP-derived peptides, we hypothesized that the study of sAPPβ levels and $A\beta_{1-42}/A\beta_{1-40}$ and $A\beta_{1-42}/A\beta_{1-38}$ ratios could be of special interest for the differentiation of FTLD-S from Alzheimer's disease. Further studies should explore the ability of plasma biomarkers to differentiate between FTLD and Alzheimer's disease or healthy controls.

We hypothesized that APP-derived peptides may have an important role in FTLD pathophysiology. Because APP processing has been linked to neuronal and synaptic activity, it would be interesting to further investigate the relationship between neuropathological hallmarks of FTLD (i.e. TDP-43 or tau inclusion), APP-derived peptides and synapse markers in different FTLD subtypes. Our group has experience in novel techniques allowing the study of different proteins at the synapse level in post-mortem human brains (Colom-Cadena *et al.*, 2017) as well as in CSF (Lleo *et al.*, 2019). Particularly, array-tomography may enable the visualization of APP-derived peptides, and the study of their relationship with synapses and the pathological hallmarks of FTLD. Such observations could eventually reinforce the hypothetical role of APP-derived peptides as synaptic biomarkers and their relationship with FTLD pathophysiology. This would be an important observation since recent studies have suggested that in the absence of AD pathophysiology, $A\beta_{1-42}$ levels may be related to disease progression in some FTLD-subtypes (Ljubenkov *et al.*, 2018). It would be important to investigate whether other synaptic proteins also are related to disease progression in FTLD as well. Thus, future studies in pathology proven cohorts should characterize the specific role APP-related peptides at the synapse and their relationship with FTLD-subtypes.

In recent years, the study of large multicentric cohorts of FTLD-related mutation carriers at preclinical or minimally-symptomatic stages of the disease has provided great insight into the FTLD early disease process (Rohrer *et al.*, 2013). Emerging data from these studies suggests that mutation carriers may present cognitive and cerebral structural changes up to 10 years before the emergence of full-blown FTLD-S, while subtle cognitive changes may be evident up to 5 years before disease onset (Cash *et al.*, 2018; Popuri *et al.*, 2018; Rohrer *et al.*, 2015). These findings indicate that, similar to what it has been observed in other neurodegenerative diseases, such as Alzheimer's disease or Huntington disease, the disease process in FTLD precedes the clinical onset of canonical phenotypes (Ross *et al.*, 2014; Scheltens *et al.*, 2016). Novel biomarkers may aid to advance the diagnosis of FTLD to mildly symptomatic or preclinical stages similar to what has been done in Alzheimer's disease (Jack *et al.*, 2018) and this in turn, may allow the definition the phenotypes associated with prodromal

FTLD. Some of the results presented in this thesis may contribute to achieving this goal. In **chapter 5** we proposed that cortical mean diffusivity could be a useful biomarker to detect the earliest cortical changes in FTLD, and future studies should explore its value to detect and monitor FTLD-related changes in preclinical mutation carriers. When conducting these studies, a number of interesting questions would arise. First, would cortical mean diffusivity changes predate cortical thinning in genetic FTLD? Second, because each mutation is related to a single FTLD neuropathological subtype, the study of earliest neuroimaging changes in FTLD-related mutation carriers may shed light to identify specific structural signatures of FTLD subtypes (Rohrer *et al.,* 2015). For example, some FTLD-subtypes may be characterized by prominent subcortical white matter neurodegeneration (Kovacs et *al.,* 2017) while others may target specific gray matter regions (Nana *et al.,* 2018). Third, because genetic and sporadic FTLD cases have shown to diverge in CSF biomarker profiles, it would be interesting to compare the structural correlates of sporadic and genetic forms of FTLD-tau and FTLD-TDP cases (for further information please refer to **supplementary paper 1**)(Lleo *et al.,* 2018). Finally, because we proposed that APP-derived peptides may be useful biomarkers in FTLD-S, future studies should also explore its role for the detection of the earliest clinical and structural changes in asymptomatic or minimally symptomatic mutation carriers.

Another unsolved issue is the lack of specific biomarkers for the different neuropathological subtypes of FTLD, which are needed for the selection of patients in drug trials targeting specific proteinopathies (Tsai and Boxer, 2016). Although significant advances have been made in recent years (Oeckl *et al.*, 2015; 2016; Teunissen *et al.,* 2016; Del Campo *et al.,* 2018), more research is needed to validate novel pathway-specific biomarkers allowing the *in-vivo* distinction of FTLD subtypes. Recently, we had the opportunity to collaborate with the University of Pennsylvania to assess whether core Alzheimer's disease CSF biomarkers could be used to select FTLD neuropathological subtypes (Lleo *et al.,* 2018). In this work we proposed a diagnostic algorithm that used different cut-off of cerebrospinal fluid phosphorylated tau/$A\beta_{1\text{-}42}$ ratio and phosphorylated tau to first exclude Alzheimer disease cases and then in a second step to discriminate between pure FTLD-TDP and FTLD-tau by means of the phosphorylated tau levels (for further details, please refer to **supplementary paper 1**). Other studies have reported that low levels of both p-tau and the p-tau:t-tau ratio may differentiate FTLD-TDP from FTLD-tau (Borroni *et al.*, 2015; Grossman *et al.*, 2014; Hu *et al.*, 2013; Meeter *et al.*, 2018). These findings agree with the finding that p-tau levels correlate with FTLD-Tau in studies of quantitative neuropathology (Irwin *et al.*, 2017). However, a large longitudinal study involving hundreds of PSP patients reported that lower CSF levels of p-tau predicted faster disease progression (Rojas *et al.*, 2018). This is an interesting finding since we have found a high correlation between the CSF levels of p-tau (and not t-tau) and APP-derived peptides in FTLD-S (unpublished results from the SPIN cohort). On the other hand, this is a controversial issue as some authors suggest that the capacity of the p-tau:t-tau ratio to differentiate FTLD-TDP from FTLD-tau seems to be driven by the higher levels of t-tau in patients with motor neuron disease and not by lower levels of p-tau in FTLD-TDP (Kuiperij *et al.*, 2017; Meeter *et al.*, 2018; Pijnenburg *et al.*, 2015). However, p-tau levels have been found to correlate with the neuropathological burden of FTLD-tau in pathology-confirmed studies (Irwin *et al.,* 2017). Overall, these findings may suggest common pathophysiological mechanisms between tau phosphorylation and APP processing and may challenge the view of p-tau as a specific marker of neurofibrillary tangles in Alzheimer's disease (Illán-Gala *et al.,* 2018).

Chapter 8

Conclusions

1 CSF levels of sAPPβ, YKL-40 and NFL, alone and in combination have good diagnostic accuracy to discriminate FTLD-S from healthy controls and AD dementia subjects. In addition, these levels correlate with cortical thickness in patients with FTLD-S.

2 In the ALS-FTD continuum CSF levels of YKL-40 are increased and levels of sAPPβ are decreased compared to healthy controls. Furthermore, in ALS CSF levels of YKL-40 predict disease progression and survival. These markers can be useful for the clinical diagnosis, staging and prognosis of patients within the ALS-FTD continuum.

3 Cortical mean diffusivity is more sensitive than cortical thickness for the detection of the earliest bvFTD-related cortical changes and correlate with rates of disease severity showing promise as surrogate biomarker of underlying neurodegeneration in bvFTD.

4 The CSF levels of $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-38}$ and sAPPβ are globally decreased in FTLD-S compared to healthy controls and correlate with cortical thickness in FTLD-S but not in Alzheimer's disease or healthy controls. These biomarkers could reflect neuronal and synaptic loss in FTLD.

Chapter 9

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Chapter 10

Annexes

Annex 1: Key definitions and abbreviations

Key term (abbreviation)	Definition
Frontotemporal lobar degeneration (FTLD)	The pathological term for a group of neurodegenerative disorders affecting the frontal and/or temporal lobes accompanied by protein inclusions (such as Tau, TDP 43 or FUS).
Frontotemporal lobar degeneration-related syndromes (FTLD-S)	This term refers to all the clinical syndromes predicting the neuropathological diagnosis of FTLD. Namely, the behavioral variant of frontotemporal dementia (bvFTD), the semantic variant of primary progressive aphasia (svPPA), the nonfluent/agrammatic variant of primary progressive aphasia, the amyotrophic lateral sclerosis-frontotemporal dementia continuum (ALS-FTD), and the syndromes associated with 4R tauopathies (progressive supranuclear palsy and corticobasal syndromes).
Frontotemporal dementia (FTD)	Clinical umbrella term that encompasses three distinct clinical syndromes: the behavioral variant of frontotemporal dementia (bvFTD), the nonfuent/agrammatic variant of Primary Progressive Aphasia (nfaPPA) and the semantic variant of Primary Progressive Aphasia (svPPA). These syndromes predict the neuropathological diagnosis of FTLD and are encompassed within the label of FTLD-S.
Primary progressive aphasia (PPA)	A clinical syndrome defined by Mesulam in 1982 (Mesulam et al., Ann Neurol 1982) characterized by a predominant language impairment and relative preservation of cognitive and behavioral functions. The last consensus criteria (Gorno-Tempini et al., Neurology 2011) defined three PPA subtypes: two variants predicting FTLD (nfaPPA and svPPA) and a third variant that is mainly related to Alzheimer's disease (the logopenic variant of PPA).

Annex 2: Supplementary table 1

Gene (Chr.) → protein encoded	Mechanism of neurodegeneration	Frequency among familial cases	Frequent clinical presentations	Symmetrical, orbitofrontal, medial and dorsolateral frontal, followed by temporal lobes, parietal and occipital lobes, cerebellum, posterior thalamus.
C9orf72 (9p21.2) → unknown	Endosomal trafficking dysregulation; RNA-foci formation and impaired transcription processing; dipeptide repeat protein toxicity	13-50%	bvFTD ALS-FTD CBS-PSPS Late-onset psychosis	Symmetric frontal, anterior cingulate cortex, insular, anterior, and medial temporal lobe
MAPT (17q21.1) → Microtubule-associated tau protein	Impaired microtubule stabilisation and/or increased propensity of tau self-aggregation	5-20%	bvFTD CBS-PSPS	Symmetric frontal, anterior cingulate cortex, insular, anterior, and medial temporal lobe
GRN (17q21.32) →Progranulin	Impaired neurotrophic function, promotion of inflammation, impaired lysosomal-mediated protein degradation	5-20%	bvFTD PPA CBS-PSPS	Asymmetrical, anterior temporal, temporo-parietal, frontal, anterior cingulate cortex, insular
TBK1 (12q.14.2) → Tank-binding kinase 1	Impaired endosomal-lysosomal and autophagic protein degradation pathway	3%	bvFTD ALS-FTD	Asymmetric atrophy in various locations (frontotemporal, parietal, cerebellum, medial temporal)
TARDBP (1p36.22) →TDP-43	Impaired RNA-binding, transcription, translation, alternative splicing, RNA transport and stabilization	Rare	ALS ALS-FTD	Symmetrical frontal, temporal pole atrophy
FUS (16p11.2) →FUS	Impaired RNA-binding, transcription, translation, alternative splicing, RNA transport and stabilisation	Rare	ALS ALS-FTD	Frontal and temporal atrophy with striking striatal atrophy
VCP (9p13.3) → Valosin-containing protein	Impaired ubiquitin-proteasome mediated protein degradation and autophagy	Rare	bvFTD ALS-FTD IBM-Paget	Frontal, temporal, and parietal lobes, especially prefrontal cortex and superior temporal gyrus; hippocampus, caudate nucleus, amygdala
CHMP2B (3p11.2) →Chromatin- modifying protein 2B	Impaired endosomal-lysosomal and autophagic protein degradation pathway	Rare	bvFTD ALS-FTD	Generalised cortical atrophy, mostly severe in frontal and temporal cortices
SQSTM1 (5q35.3) → p62	Impaired autophagy and apoptosis	1-3%	bvFTD ALS-FTD	Asymmetric frontotemporal atrophy
UBQLN2 (Xp11.21) → Ubiquilin 2	Impaired ubiquitination	2%	vFTD ALS-FTD CBS-PSPS	Bilateral frontotemporal atrophy
TREM2 (6p.21.1) → Triggering receptor expressed on myeloid cells	Impaired phagocytosis and immune response	Rare	bvFTD svPPA	White matter changes and thinning of corpus callosum
CHCHD10 (22q.11.23) → Coiled-coil-helix-coiled-coil-helix domain-containing protein 10	Unknown	Rare	bvFTD ALS-FTD	Bilateral frontotemporal atrophy

Annex 3: Supplementary table 2

Study (First author; journal; year of publication)	Number of autopsy-confirmed cases by neuropathological group	Aβ 1-42	NfL	t-Tau	p-Tau$_{181}$
Green; Neurosci Lett; 1999	17 AD; 27 FTLD	NA	NA	AD>FTLD>HC	NA
Clark; Arch Neurol; 2003	74 AD*; 10 FTLD; (73) HC	AD < FTLD, HC	NA	AD > FTLD > HC	NA
Grossman; Ann Neurol; 2005	17 AD; 12 (73) FTLD; (13) HC	AD < FTLD, HC	NA	AD > FTLD, HC	AD > FTLD, HC
Bian; Neurology; 2008	19 AD; 19 (11) FTLD; 0 HC	AD < FTLD, CN	NA	AD > FTLD, HC	NA
Engelborghs; Neurobiol Aging; 2008	73 AD*; 2 FTLD; (100) HC	NA	NA	NA	NA
Koopman; Neurochem Int; 2009	95 AD; 10 FTLD‡; 0 HC	AD < FTLD	NA	AD > FTLD	AD > FTLD
Tapiola; Arch Neurol; 2009	83 AD; 9 FTLD‡	NA	NA	NA	NA
Brunnstrom; Alzheimer Dement; 2010‡	8 AD*; 12 FTLD	NA	NA	AD > FTLD	NA
Irwin; Ann Neurol; 2012	41 AD*; 20 FTLD[1]	NA	NA	AD > FTLD	NA
Toledo; Acta Neuropathol. 2012‡	71 AD; 29 FTLD‡; (66) HC	AD < FTLD < HC	NA	AD > FTLD > HC	AD > FTLD > HC
Schoonenboom; Neurology; 2012	6 (512) AD; 6 (180) FTLD; (275) SCM	AD < FTLD, SMC	NA	AD>FTLD>SMC	AD>FTLD,SMC
Landqvist; BMC Neurol; 2013	(20) AD; 10 (34) FTD; (26) HC	AD < FTLD	HC<AD<FTLD	AD>FTLD	AD>FTLD
Irwin; Ann Neurol 2017	26 AD; 80 FTLD[2]	AD < FTLD	NA	AD > FTLD	AD > FTLD
Goossens; Alzheimers Res Ther 2018	(45) AD; 16 (46) FTLD[3]; (20) HC	AD < FTLD, HC	HC<AD<FTLD	AD >FTLD, HC	AD >FTLD, HC
Paterson; Alzheimers Res Ther 2018**	(156) AD; (45) bvFTD**;	AD < bvFTD < HC	HC<AD<bvFTD	AD >bvFTD, HC	AD >bvFTD, HC
Lleó; JAMA Neurol; 2018	89 AD; 40 FTLD; 14 FTLD-AD	AD < FTLD	NA	AD > FTLD	AD > FTLD
Del Campo; Ann Clin Transl Neurol 2018	(47) AD; 28 (114) FTLD; (88) HC	AD < FTLD < HC	HC<AD<FTLD	AD > FTLD	AD > FTLD

Table S1. Comparative studies of core AD CSF biomarkers and NfL in autopsy-confirmed cohorts for the differentiation between FTLD, AD and Controls. () denotes cases without neuropathological diagnosis (including mutations); *: AD contains cases with comorbid Lewy body or vascular disease; **: The neuropathological diagnosis of FTLD was confirmed in only 10 cases of the total sample (no details) and only separate analyses were provided for different FTLD-S (AD-like CSF profile in PNFA suggest misdiagnosis); ‡: no data on genetic testing. 1: 1 MAPT and 3 GRN cases; 2: 12 C9orf72; 8 GRN, 3 MAPT; 3: 19 C9orf72; 9 GRN, 0 MAPT; **Abbreviations:** AD = Alzheimer 's disease; AUC = area Under the Curve (Receiver Operating Characteristics analysis); FTLD = frontotemporal lobar degeneration; HC = healthy control; NA = not assessed; SMC = subjective memory complaints;

Annex 4: Supplementary table 3

Study (First author; journal; year of publication)	Sample composition	Aβ 1-42	Aβ 40	Aβ 37	Aβ 38	sAPPβ	sAPPα
Sjogren; J Neural Transm; 2000	17 FTD; 60 AD; 32 HC	AD<FTD<HC	NA	NA	NA	NA	NA
Clark; Arch Neurol; 2003	74 AD*; 10 FTLD; (73) HC	AD < FTLD, HC	NA	NA	NA	NA	NA
Grossman; Ann Neurol; 2005	17 AD; 12 (73) FTLD; (13) HC	AD < FTLD, HC	NA	NA	NA	NA	NA
Bibl; J Neural Transm; 2007	30 FTD; 30 AD; 30 HC	AD<FTD<HC	NA	NA	AD<FTD<HC	NA	NA
Bibl; Mol Psychiatry 2007	36 FTD; 71 AD; 71 HC	AD<FTD<HC	(FTD=HC)<AD	NA	FTD<(AD=HC)	NA	NA
Bian; Neurology; 2008	19 AD; 19 (11) FTLD; 0 HC	AD < FTLD, CN	NA	NA	NA	NA	NA
Bibl; Proteomics Clin Appl 2008	30 FTD; 40 AD; 30 HC	AD<FTD<HC	FTD<HC	NA	FTD<HC	NA	NA
Steinacker; J Neural Transm 2009	12 FTD; 21 ALS-FTD; 13 HC	FTD<HC	FTD=ALS-FTD=HC	NA	FTD=ALS-FTD=HC	ALS-FTD<HC	FTD<ALS
Pijnenburg; J Neurol Neurosurg Psychiatry 2007	21 FTD; 39 AD; 30 HC	AD<(FTD=HC)	FTD<(AD=HC)	NA	NA	NA	NA
Gloeckner; J Alzheimers Dis 2008	10 FTD; 23 AD; 19 HC	(AD=FTD)<HC	AD<(FTD=HC)	NA	NA	NA	NA
Koopman; Neurochem Int; 2009	95 AD; 10 FTLD‡; 0 HC	AD < FTLD	NA	NA	NA	NA	NA
Verwey; J Inmunol Methods 2009	14 FTD; 20 AD; 27 HC	NA	FTD<(AD=HC)	NA	NA	NA	NA
Gabelle; Brain Res 2010	26 DFT; 21 AD	NA	NA	NA	NA	FTD<AD	FTD=AD
Verwey; J Alzheimers Dis 2010	55 FTD; 50 AD; 40 HC	AD<(FTD=HC)	FTD<HC, FTD=AD	NA	NA	NA	NA
Gabelle; J Alzheimers Dis 2011	34 FTD; 52 AD; 42HC	AD<FTD<HC	FTD<(AD=HC)	NA	FTD<AD<HC	FTD<AD	FTD=AD
Bibl; Dement Geriatr Cogn Disord; 2011	25 FTD, 25 AD; 20 HC	AD<FTD<HC	FTD<(AD=HC)	NA	FTD<(AD=HC)	NA	NA
Perneczky; Neurology 2011	16 FTD; 21 AD	FTD=AD	NA	NA	NA	FTD<AD	FTD<AD

Schoonenboom; Neurology; 2012	6 (512) AD; 6 (180) FTLD; (275) SCM	AD < FTLD, SMC	NA	NA	NA	NA	NA
Bibl; J Neural Transm; 2012	17 FTD; 22 AD; 20 HC	AD<FTD<HC	FTD<(AD=HC)	NA	FTD<(AD=HC)	NA	NA
Alexopoulos; J Alzheimers Dis 2012	17 FTD; 61 AD	AD<FTD	NA	NA	NA	FTD<AD	NA
Landqvist; BMC Neurol; 2013	(20) AD; 10 (34) FTD; (26) HC	AD < FTLD	NA	NA	NA	NA	NA
Alcolea; J Alzheimers Dis 2014	22 FTD; 59 AD; 24 HC	FTD=AD<HC	NA	NA	NA	FTD<HC=AD*	NA
Toledo; Acta Neuropathol. 2012‡	71 AD; 29 FTLD‡; (66) HC	AD < FTLD < HC	NA	NA	NA	NA	NA
Magdalinou; J Neurol Neurosurg Psychiatry 2015	16 FTD; 26 AD; 30 HC	AD<(HC=FTD)	NA	NA	NA	FTD=AD=HC	FTD=AD=HC
Struyfs; J Alzheimers Dis 2015	17 FTD; 50 AD; 50 HC	AD<FTD<HC	FTD<AD, HC	FTD<AD, HC	FTD<AD, HC	NA	NA
van Waalwijk van Doorn;J Neurochem; 2016	20 FTD; 20 AD; 20 SMC	NA	NA	NA	NA	FTLD-S=AD	FTLD-S=AD
Janelidze; Ann Cin Transl Neurol 2016	33 FTD; 110 AD; 53 HC	AD<HC=FTD	AD<FTD<HC	NA	AD=FTD<HC	NA	NA
Rostgaard; Neurology 2018	16 CHMPB2 mutation carrires (MC) and 14 non-carrirers (NC); *NOTE: 6 of the MC were symptomatic*	MC=NC	MC<NC	NA	MC<NC	NA	NA
Goossens; Alzheimers Res Ther 2018	(45) AD; 16 (46) FTLD[3]; (20) HC	AD < FTLD, HC	NA	NA	NA	NA	NA
Paterson; Alzheimers Res Ther 2018**	(156) AD; (45) bvFTD**; 30 HC	AD < bvFTD < HC	NA	NA	NA	bvFTD<AD<HC	bvFTD<AD<HC
Lleó; JAMA Neurol; 2018	89 AD; 40 FTLD; 14 FTLD-AD	AD < FTLD	NA	NA	NA	NA	NA
Alcolea; J Neurol Neurosurg Psychiatry; 2018	97 AD; 49 FTLD; 77 HC	NA	NA	NA	NA	FTLD<AD, HC	NA

Table S2. Comparative studies of APP-derived CSF biomarkers in FTLD-S. *: lower beta secretase activity in the FTD group, compared to the HC and AD groups; **: The neuropathological diagnosis of FTLD was confirmed in only 10 cases of the total sample (no details) and only separate analyses were provided for different FTLD-S (AD-like CSF profile in PNFA suggest misdiagnosis); Abbreviations: AD = Alzheimer 's disease; bvFTD = behavioral variant of frontotemporal dementia; FTD = Frontotemporal dementia; FTLD = frontotemporal lobar degeneration; HC = healthy control; NA = not Assessed; SMC = subjective memory complaints;

Annex 5: Supplementary paper 1

Paper accepted for publication in
JAMA Neurol
(June 1st 2018)

Title:

A two-step cerebrospinal algorithm for the selection of Frontotemporal Lobar Degeneration subtypes

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Abstract

Importance: Cerebrospinal fluid (CSF) core Alzheimer's disease (AD) biomarkers have shown an excellent capacity for the *in vivo* detection of AD. Previous studies have shown that CSF levels of phosphorylated tau (p-tau) also correlate with tau pathology in frontotemporal lobar degeneration (FTLD) after accounting for AD co-pathology.

Objective: To develop an algorithm based on core AD CSF measures to exclude cases with AD pathology and then differentiate between FTLD-tau and FTLD-TDP.

Design, setting and participants: A case-control study at an academic medical center. Participants were selected from a database of 1796 subjects included between 1992 and 2016 with different neurodegenerative diseases with available CSF. Three patient cohorts were included: A cohort of sporadic, autopsy-confirmed FTLD and AD (n=143), a cohort of frontotemporal degeneration (FTD) with TDP- or tau-associated mutations (n=60); and a living cohort of patients with syndromes highly predictive of FTLD (Progressive supranuclear palsy (PSP) and FTD-Amyotrophic Lateral Sclerosis (FTD-ALS), n=62).

Main outcomes and measures: CSF values of amyloid-β ($A\beta_{1-42}$), total tau (t-tau) and p-tau obtained using the INNO-BIA AlzBio3 (xMAP; Luminex) assay or INNOTEST® enzyme-linked immunosorbent assay transformed using a previously validated algorithm. Sensitivities and specificities for differentiating AD from FTLD groups were calculated.

Results: This autopsy cohort included FTLD-tau (n = 27; mean [SD] age at onset, 60.8 [9.7] years), FTLD-TDP (n = 13; mean [SD] age at onset, 62.4 [8.5] years), AD (n = 89, mean [SD] age at onset, 66.5 [9.7] years); and mixed FTLD-AD (n = 14, mean [SD] age at onset, 70.6 [8.5] years). The p-tau/$A\beta_{1-42}$ ratio showed an excellent diagnostic accuracy to exclude AD cases in the autopsy cohort with single neurodegenerative pathologies (AUC 0.978 95% CI 0.956-0.999). CSF p-tau levels showed a good AUC (0.87, 95% CI 0.73-1.00) for discriminating pure FTLD-TDP from pure FTLD-Tau. The application of an algorithm using cut-points of CSF p-tau/$A\beta_{1-42}$ ratio and p-tau allowed a good discrimination of pure FTLD-TDP cases from the remaining FTLD-Tau and mixed FTLD cases. The diagnostic value of this algorithm was confirmed in an independent cohort of living patients with PSP and FTD-ALS (AUC 0.9, 95% CI 0.81-0.99). However, the algorithm was less useful in FTD cases carrying a pathogenic mutation (AUC 0.58, 95% CI 0.38-0.77) due to elevated p-tau levels in *C9orf72* carriers.

Conclusions and Relevance: AD CSF core biomarkers can be used with high specificity for the in vivo identification of patients with pure FTLD-TDP and FTLD-tau when accounting for comorbid AD and genetic status.

Introduction

Frontotemporal lobar degeneration (FTLD) is a neuropathological umbrella term coined to describe a group of neurodegenerative disorders with prominent frontal and temporal lobe atrophy presenting with a wide spectrum of behavioural, language and motor disturbances. Most FTLD cases can be classified in two main subtypes according to the protein that aggregates in the CNS: FTLD-TDP (~50 %), which is associated with aggregates composed of transactive response DNA-binding protein of approximately 43kDa (also known as TDP-43), and FTLD-Tau (~45%), which is associated with aggregates containing the microtubule-associated protein tau.[1] Although the majority of cases are considered sporadic, up to 25% of patients may have a pathogenic mutation, mainly in the *MAPT*, *GRN* and *C9orf72* genes.[2] While genetic testing may enable

a definite diagnosis in mutation carriers, the *in vivo* diagnosis of the majority of FTLD cases with sporadic disease is challenging because there is no reliable correspondence between the clinical syndrome and the underlying neuropathology.[3]

Cerebrospinal fluid (CSF) markers have been studied in neurodegenerative diseases as a way to track different pathophysiological processes in the CNS. In Alzheimer's disease (AD), levels of β-amyloid ($A\beta_{1-42}$), total-tau (t-tau) and phosphorylated tau (p-tau), also named core AD biomarkers, have shown excellent diagnostic accuracy for the detection of AD at the prodromal and dementia stages.[4] Core AD biomarkers are also useful in FTLD-related syndromes to exclude AD.[5-7] In addition, core AD biomarkers could also be used to distinguish the different neuropathological subtypes of FTLD. In particular, previous studies have shown that low levels of p-tau or the ratio of p-tau/t-tau could be useful biomarkers for TDP-43 proteinopathies.[7-9] Further, p-tau is more specific for tau pathology, as t-tau also reflects non-specific neuronal and axonal damage.[10,11] Importantly, we have recently described an independent association of ante mortem CSF p-tau levels with post mortem cerebral tau pathology in a large series of autopsy-confirmed FTLD, suggesting that low p-tau is a specific marker for TDP-43 proteinopathies.[12]

It is clear that specific markers for FTLD-TDP and FTLD-Tau are needed and some promising advances have been made.[13] Unfortunately, many biomarker studies of FTLD-related syndromes may be confounded by co-occurring secondary AD pathology[7]. It is possible that this secondary AD pathology confounds measurement of CSF analytes, with consequences for clinical trial outcomes that include CSF measurement of tau. In addition, most studies have grouped FTD patients with and without pathogenic mutations assuming that they all have a similar CSF biomarker profile. The present study aimed to develop a two-step algorithm where we first exclude cases with significant AD pathology and then use CSF tau analytes to differentiate between sporadic FTLD-Tau and FTLD-TDP. This algorithm was tested in three different cohorts of FTD patients: a sporadic autopsy cohort, a genetic cohort and a living cohort with syndromes highly predictive of FTLD-Tau and FTLD-TDP.

Methods

Patients

Participants were selected from a database of 1796 subjects with different neurodegenerative diseases with available CSF included during May 1992 to April 2016 at the Center for Neurodegenerative Disease Research (CNDR) at the University of Pennsylvania.

Autopsy cohort: we included data from patients with ante mortem CSF and a neuropathological diagnosis of AD or FTLD who were followed longitudinally at the Frontotemporal Degeneration Center (FTDC) or Alzheimer Disease Core Center (ADCC) to autopsy establishment of their underlying neuropathology in the Center for Neurodegenerative Disease Research (CNDR) at the University of Pennsylvania.[14] A total of 143 sporadic cases were included: 89 pure AD cases, 40 cases of FTLD (27 FTLD-Tau, 13 FTLD-TDP), and 14 cases with AD plus FTLD (10 with FTLD-Tau and 4 with FTLD-TDP, collectively known as FTLD-AD). All FTLD cases were screened for the three most common mutations (*MAPT*, *GRN*, and *C9orf72*) as previously described.[2] Cases presenting as motor neuron disease, Lewy body dementia and those with concurrent FTLD-Tau and FTLD-TDP (3 cases) were excluded.

Genetic cohort: we included a group of 60 FTD patients with pathogenic mutations and CSF available for analysis. This group was composed of 33 patients with mutations in *C9orf72*, 13 in

GRN, 4 in *TARDBP* and 10 in *MAPT* genes.
Replication sporadic cohort: we included a group of 62 living patients with clinical phenotypes that are highly predictive of FTLD-Tau and FTLD-TDP: 39 patients with progressive supranuclear palsy (PSP) and 23 patients with amyotrophic lateral sclerosis associated with FTD (ALS-Mild Cognitive Impairment and FTD-ALS) diagnosed according to current diagnostic criteria.[15,16] All individuals participated in a written informed consent procedure with their caregivers, when appropriate, that was approved by the institutional review board at the University of Pennsylvania.
A subset of these patient samples has been previously published.[5-7,12,14]

Biofluid collection and analysis

CSF samples were obtained as described previously.[5,14] We obtained data from $A\beta_{1-42}$, t-tau and p-tau levels previously analyzed using the ELISA assay (INNOTEST®, Fujirebio-Europe, Belgium) or the Luminex xMAP platform (INNO-BIA AlzBio3TM, for research use-only reagents, Fujirebio-Europe, Belgium) at the CNDR (ELISA) and the Biomarker Core (xMAP) of the AD Neuroimaging Initiative (ADNI) at the University of Pennsylvania.[17-19] CSF values from ELISA were transformed to xMAP values using the validated formulas[5] CSF biomarker measures for $A\beta_{1-42}$ and p-tau/$A\beta_{1-42}$ ratio in the autopsy cohort were available for 123/143 (86%), a valid p-tau result was available for 122/143 (85.3%) and t-tau/Aβ1-42 for all cases.

Neuropathological analysis

Autopsy was performed as previously described.[3] Microscopic diagnosis was made by experienced neuropathologists (E.B.L. and J.Q.T.) using current neuropathological diagnostic criteria.[20-24] Cases were divided into those with one neuropathological diagnosis and those with multiple diagnoses using Braak and CERAD stages of AD pathology.[20,21] Concurrent pathologies were registered as previously described.[7] In FTLD-Tau patients, sections of the hippocampus were stained with Thioflavin-S, as described,[25] to distinguish co-morbid AD neurofibrillary tangle pathology from primary FTLD tauopathy. We used pathological criteria of "low AD" to define secondary comorbid AD (either AD Braak tau stage ≥ B2 or AD Braak tau stage B1 and CERAD ≥ C2) in FTLD cases.[23] We used the term pure FTLD for cases with a primary neuropathological diagnosis of FTLD and no comorbid AD and FTLD-AD for cases with FTLD and concomitant AD as in previous studies.[7]

Statistical analysis

Variables were examined for normality and one-way ANOVA or Kruskal-Wallis test were performed across the groups as appropriate. For group-wise comparisons and regression models we used natural log (ln) transformation to obtain normally-distributed CSF variables for analysis. Because the autopsy and validation cohorts differed in disease duration and age at which CSF samples are obtained, and because these factors may influence CSF analyte levels, we performed a logistic regression analysis for p-tau that included age and disease duration as covariates. These logistic regressions were completed in the autopsy cohort and the probabilities then were entered into receiver operating characteristic curves (ROC). We calculated the optimal cutoff that was used to assess sensitivity and specificity and then applied this logistic regression model to the independent validation cohort.
Statistical significance for all tests was set at $P < .05$. All analyses were performed using SPSS 20.0 (Armonk, NY: IBM Corp.) or STATA 12.0 (College Station, Texas; STATA Corp).

Results

Demographic, clinical and biomarker data of the autopsy cohort

Demographic, clinical and neuropathological characteristics of the autopsy patient sample are summarized in Table 1. The FTLD-AD group had a later age at onset when compared to that of FTLD-Tau. The age at death was higher in the AD group than in the FTLD-Tau and FTLD-TDP groups. Age at CSF sampling was higher in the FTLD-AD group than in FTLD-Tau and FTLD-TDP. *APOE*ε4 allele was overrepresented in the AD group when compared to the other groups.

Raw CSF analyte values for each neuropathological group are shown in Figure 1. CSF $A\beta_{1-42}$ levels were lower in both the pure AD and FTLD-AD groups compared with the FTLD-tau group (Table 1). CSF t-tau levels were higher in the pure AD group than in FTLD-tau. Finally, CSF p-tau levels were higher in the pure AD group than in pure FTLD groups. The FTLD-AD group showed intermediate values for tau analytes between pure AD and pure FTLD, highlighting the effect of comorbid AD on CSF biomarkers.[7] As reported in previous studies,[7-9] CSF p-tau levels were lower in FLTD-TDP than in FTLD-tau ($H(2) = 7.43$, $P = .006$). t-tau/$A\beta_{1-42}$ and p-tau/$A\beta_{1-42}$ ratios also showed clear differences among groups (Table 1).

		Pure cases		Mixed cases
	AD n=89	FTLD-Tau n=27	FTLD-TDP n=13	FTLD-AD n=14
Age at onset, y (SD)	66.5 (9.7)	60.8(8.9)[d]	62.4(8.5)	70.6(8.5)[b]
Age at death, y (SD)	75.9(10.1)[bc]	68.4(8.8)[ad]	68.4(9)[a]	79.2(11.3)[b]
Age at CSF, y (SD)	70.2 (9.5)	64.2(9.4)[d]	65.3(7.6)[d]	74.5(10.1)[bc]
Time from onset to CSF, y (SD)	3.72 (2.4)	3.41(2)	2.92(1.7)	3.9(3.1)
Men, n (%)	50 (56.8)	15 (65.2)	4 (33.3)	8 (57.1)
*APOE*ε4 positive, n (%)	56 (63.6)[a]	4 (17.4)[a]	4 (33.3)	5 (37.5)
CSF Aβ1-42 (SD), n=123	137.8(50.6)[b]	244.1(46.1)[ad]	216.8(63.3)[d]	148.7(29.7)[bc]
CSF t-tau (SD), n=143	120.3(88)[b]	48(22.7)[a]	54.1(39.1)	65.6(46.1)
CSF p-tau (SD), n=139	41.1(30.3)[bc]	11.9(3.8)[acd]	7.9(5.4)[abd]	17.7(8.6)[bc]
CSF t-tau/ $A\beta_{1-42}$ (SD), n=143	1.01(0.91)[bcd]	0.20(0.12)[a]	0.26(0.2)[a]	0.44(0.29)[a]
CSF p-tau/ $A\beta_{1-42}$ (SD), n=122	0.34(0.26)[bcd]	0.05(0.02)[ad]	0.04(0.03)[ad]	0.14(0.06)[abc]

Table 1. Demographic and CSF biomarker data of subjects of the sporadic autopsy cohort by neuropathological group. [a] P<.05 compared to AD; [b] P<.05 compared to FTLD-Tau; [c] P<.05 compared to FTLD-TDP; [d]: P<.05 compared to AD-FTLD. Abbreviations: Standard deviation.

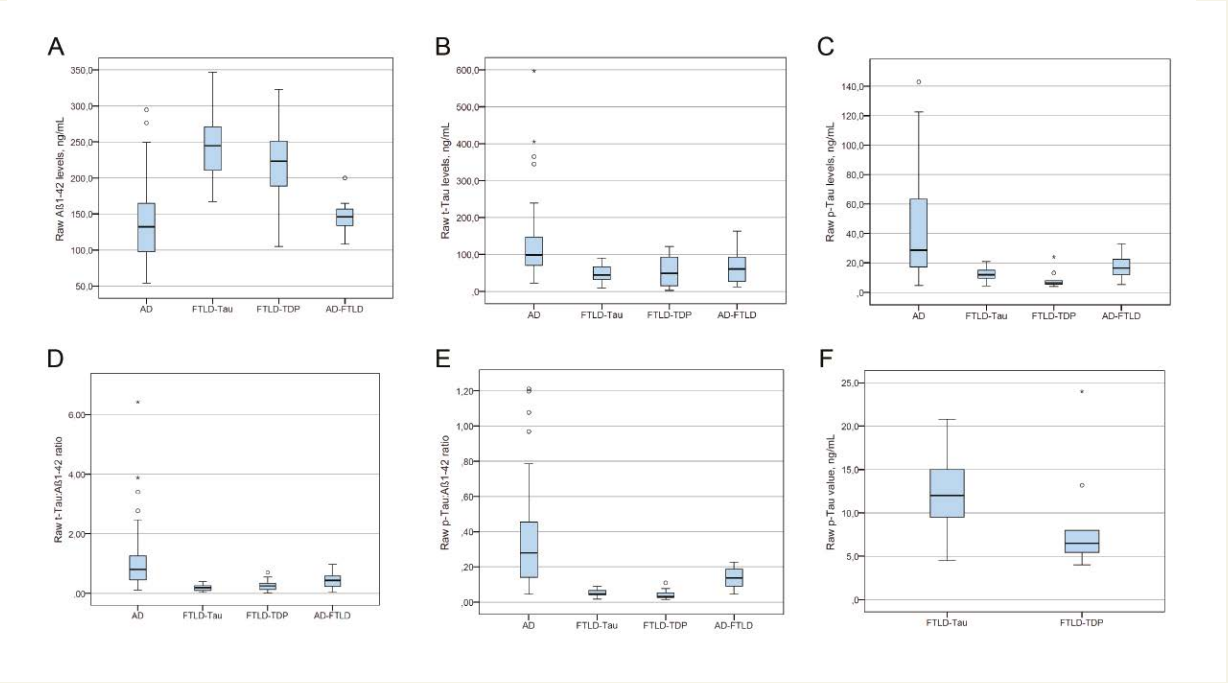

Figure 3: Raw CSF levels of CSF Aß$_{1-42}$, t-tau, t-tau/Aß$_{1-42}$ and p-tau/Aß$_{1-42}$ in the autopsy cohort. A-E) Raw CSF levels of CSF Aß$_{1-42}$, t-tau, p-tau, t-tau/Aß$_{1-42}$ and p-tau/Aß$_{1-42}$ across neuropathological groups. F) Detailed comparison of p-tau values between FTLD-Tau and FTLDP-TDP.

Because of the AD-like CSF profile in the FTLD-AD cohort, we developed a two-stage process for the biofluid-based diagnosis of FTLD spectrum disorders. First, we established cut points of CSF analytes for each form of pathology in the subset of patients with pure pathology. Then we applied these criteria to our entire cohort, which includes individuals with mixed FTLD-AD pathology, to develop a two-stage process for differentiating FTLD-Tau and FTLD-TDP in individuals with sporadic FTLD; specifically, the first stage excludes individuals with primary or secondary AD pathology, and the second stage distinguishes between FTLD-TDP and FTLD-tau in individuals less likely to have primary or secondary AD pathology.

Establishing a diagnostic algorithm based on AD biomarkers in patients with single neurodegenerative pathologies

We performed ROC analyses for the differentiation between pure AD and pure FTLD (both FTLD-Tau and FTLD-TDP) and results are shown in Figure 2A**.** The p-tau/Aβ$_{1-42}$ ratio showed the best area under the curve (0.978; 95% CI 0.956 to 0.999; $P < .001$) followed by the t-tau/Aβ$_{1-42}$ ratio (0.905; 95% CI 0.851 to 0.96; $P < .001$). A p-tau/Aβ$_{1-42}$ ratio cut-off of 0.09 achieved a 91.3% sensitivity (95% CI 82.8% to 96.4%) and 96.8% specificity (95% CI 83.3% to 99.9%) with a likelihood ratio of 28.3.

We next investigated the capacity of CSF p-tau levels to distinguish between pure sporadic FTLD-Tau and FTLD-TDP cases. We performed ROC analysis accounting for the differences in age and time from diagnosis at CSF sampling

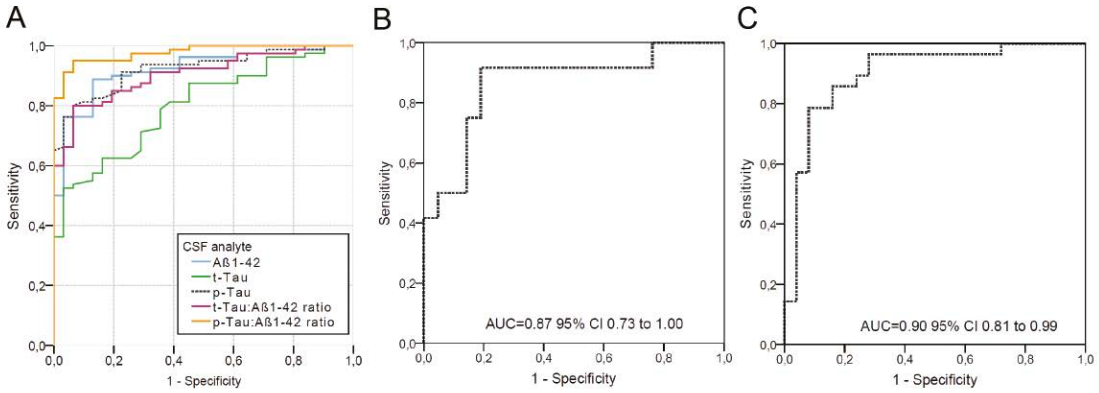

Figure 2. Receiver Operating Characteristic Curves. A) Sensitivity and specificity of CSF /$Aß_{1-42}$, t-tau, p-tau, t-tau//$Aß_{1-42}$ and p-tau//$Aß_{1-42}$ in pure AD relative to pure FTLD in the autopsy cohort. B) Sensitivity and specificity of CSF p-tau levels in FTLD-Tau relative to FTLD-TDP in the autopsy cohort after excluding comorbid AD (at neuropathological evaluation); C) Sensitivity and specificity of CSF p-tau in the validation cohort after excluding comorbid AD (using p-tau//$Aß_{1-42}$).

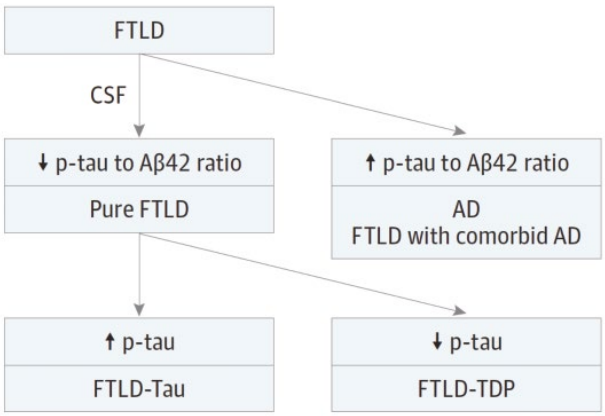

Figure 3. CSF algorithm. A two-stage algorithm for the identification of FTLD. In a first step cases with AD pathology are excluded by means of the application of p-tau/$Aß_{1-42}$ ratio and subsequently cases with FTLD-Tau and FTLD-TDP are separated by means of p-tau cut-off in the subgroups of patients a non-AD CSF biomarker profile.).

and levels of p-tau showed a good capacity to discriminate between pure FTLD-Tau and FTLD-TDP with an area under the curve of 0.87 (95% CI 0.73-1.00 Figure 2B). ROC analysis using raw values are shown in the eFigure 1 in the Supplement. However, when we included all FTLD cases including FTLD-AD the area under the curve dropped to 0.69 (CI 95% 0.51-0.87) indicating that comorbid AD confounds the diagnostic value of p-tau. The optimal probabilistic cut-off for p-tau after adjusting for age at CSF and time from diagnosis to CSF achieved 81% sensitivity (95% CI 74% to 88%) and 92% specificity (95% CI 85% to 99%) for the differentiation between FTLD-Tau and FTLD-TDP. Therefore the best results were obtained when we applied a two-step algorithm based on the application of the p-tau/$Aβ_{1-42}$ ratio to exclude cases with any AD pathology (i.e. primary AD or mixed FTLD-AD) and then the p-tau to distinguish between FTLD-Tau and FTLD-TDP (Figure 3).

Performance of the classification algorithm in a genetic FTD cohort

We next applied this algorithm to a cohort of 60 FTD patients carrying pathogenic mutations in order to test the hypothesis that mutation status may influence CSF biomarker profile.[12] Most patients (50, 83,3%) in this cohort had TDP-associated mutations (*C9orf72*, *GRN*, *TARDBP* and *VCP*) while tau-associated mutations were less frequent (10, 16,6%). Demographic and clinical characteristics of this sample are summarized in the eTable 1 in the Supplement. There was no difference in p-tau levels between TDP- and tau-associated mutations (Mann-Whitney U=121, *P*=.53). After exclusion of patients with presumed AD pathology based on the p-tau/$A\beta_{1-42}$ ratio, the area under the curve for p-tau was 0.58 (95% CI 0.38-0.77) to discriminate between groups. These results indicate that the algorithm is not useful in cases with FTD carrying pathogenic mutations. These findings are in agreement with our previous observation of elevated p-tau levels in patients with the *C9orf72* expansion.[12]

Validation of the two-stage CSF algorithm in an independent cohort

To further confirm the performance of the algorithm in a clinically relevant scenario we applied the CSF algorithm in an independent living cohort of 69 patients with clinical syndromes highly predictive of FTLD-Tau and FTLD-TDP. We included 39 patients with a clinical diagnosis of PSP and 23 with FTD-ALS. Patients with FTD-ALS with *C9orf72* mutations (n=7) were excluded. Demographic and clinical characteristics of the validation sample are summarized in Table 2. Age at CSF was higher in the PSP group (Mann-Whitney U=696, *P*<.001) compared to the FTD-ALS group. p-tau levels were lower in the FTD-ALS group (Mann-Whitney U=611, *P*=.02, Table 2) compared to the PSP group. After the exclusion of subjects with p-tau/$A\beta_{1-42}$ > 0.09 (expected comorbid AD), p-tau CSF levels showed an AUC of 0.9 (95% CI 0.81-0.99, *P*<.001, Figure 2C) for age-adjusted p-tau values. The probabilistic cut-off calculated in the autopsy cohort had a sensitivity of 89% (95%CI 0.79-0.99%)

	FTD-ALS n=23	PSP n=39
Age at onset, y (SD)	55.1 (10.9)[b]	65 (7.7)[a]
Age at CSF, y (SD)	57.5 (11.3)[b]	68.4 (7.5)[a]
Time from onset to CSF, y (SD)	2.7 (2.4)[b]	3.7 (2.1)[a]
Male, n (%)	15 (38.5)[b]	17 (73.9)[a]
*APOE*ε4 positive, n (%)	4/12 (33.3)	8 (57.1)
% Expected comorbid AD*	2/20 (10)	2/20 (10)
CSF $A\beta_{1-42}$ (SD)	260.1(72.2)	254.7(89.8)
CSF t-tau (SD)	59.6(28.6)	47.7(19.3)
CSF p-tau (SD)	11.4(7.4)[b]	13.9(5.9)[a]
CSF t-tau/ $A\beta_{1-42}$ (SD)**	0.26(0.19)	0.21(0.11)
CSF p-tau/ $A\beta_{1-42}$ (SD)	0.05(0.05)[b]	0.06(0.04)[a]

Table 2. Demographic and CSF biomarker data of subjects of the sporadic living cohort. [a] *P*<.05 compared to FTD-ALS; [b] *P*<.05 compared to PSP; * p-Tau/$A\beta_{1-42}$ ≥ 0.09 **n= 61. Abbreviations: SD: Standard deviation, FTD-ALS: Frontotemporal dementia-Amyotrophic Lateral Sclerosis, PSP: Progressive Supranuclear Palsy.

and a specificity of 73% (95% CI 0.63- 0.83) for the detection of FTD-ALS.

Discussion

The main finding of this study is that a two-stage algorithm based on three frequently used CSF biomarkers can be applied to first exclude cases with AD pathology (as the primary or as a secondary neuropathological diagnosis) and to identify FTLD-Tau and FTLD-TDP subtypes of FTLD in a cohort of sporadic FTLD. This algorithm may be a valuable tool for the enrichment of clinical trials and research studies on FTLD that require the diagnosis of FTLD subtypes.

Accurate diagnosis of the underlying pathology in FTLD spectrum disorders is a crucial step in developing a strategy for disease-modifying treatments in these conditions. The current diagnosis of sporadic FTD is based on clinical criteria supported by the presence of anatomic markers (characteristic MRI atrophy or ^{18}Fluoro-D-glucose-positron emission tomography (PET) hypometabolism).[26,27] However, estimates of misdiagnosis suggest that up to 30% of patients with FTD receive another diagnosis, in particular AD, and that an equal number of AD cases are misdiagnosed as FTLD.[3] Although the recent development of tau PET tracers represents an opportunity for detecting some subtypes of FTD, its clinical utility remains uncertain.[28-30] Amyloid PET markers may be useful in distinguishing cases with or without AD pathology, but false-positive and false-negative findings often occur.[31] CSF offers the possibility of detecting different pathophysiological changes in the CNS. Core CSF AD biomarkers are the most investigated biochemical markers in FTLD and they have been mainly used for the identification of AD cases rather than as a confirmation of FTLD. Other markers, such as neurofilament light chain (NfL), have been investigated in FTLD. Levels of NfL are elevated in FTD and they correlate with disease progression.[32,33] However, NfL levels are also increased in AD, suggesting a lack of disease specificity. It is clear that novel and more specific markers of FTLD are needed and some promising findings have been reported.[13,34] Nonetheless, in this work we present evidence that traditional CSF biomarkers for AD can be successfully used to improve accurate selection of sporadic cases with FTLD.

We first applied the p-tau/$A\beta_{1-42}$ ratio to exclude cases with AD irrespective of the clinical phenotype. As previously published[5,7,18], both tau/$A\beta_{1-42}$ ratios performed better than single analytes for the prediction of AD pathology. This should be taken into account in future research criteria for both AD and FTLD syndromes since the use of independent $A\beta_{1-42}$ and tau cut-offs may influence the diagnostic accuracy of the proposed criteria, especially for atypical AD phenotypes (e.g. CBS and the behavioral variant of AD). The fact that the selected cut-off is based on a sample of "pure" AD cases has as a consequence that the identification of mixed FTLD-AD cases is indeterminate. Specifically, since a small degree of concomitant AD pathology has a marked effect on core CSF biomarkers,[7] cases with FTLD and comorbid AD may be excluded by the application of a strict cut-point calculated based on cases with single neuropathologic conditions. Although cases with both FTLD and AD may represent a minority of all FTLD cases (<20%)[7], concomitant AD pathology may interfere with treatments targeting FTLD-specific pathologies or may obscure clinical outcomes in a trial since this pathway may not be affected by the drug. Therefore, we believe that a classification algorithm for FTLD, such as the one proposed here, should aim at selecting cases with single neurodegenerative pathologies that are more likely to respond to therapies.

We next applied a p-tau cut-point, building on previous evidence that this protein could be a useful biomarker for FTLD-TDP.[7-9] Consistent

with these prior reports, we observed that sporadic cases with FTLD-TDP had lower p-tau levels in CSF than cases with FTLD-Tau. The more likely explanation of this finding is that p-tau in FTLD reflects more accurately pathologic tau, while t-tau also reflects non-specific neuronal and axonal damage.[10,35] This is supported by recent evidence showing that CSF p-tau levels are positively associated with cerebral tau burden in FTLD.[12] Therefore, the current data support the model that CSF p-tau levels in FTLD are lower in FTLD-TDP due to the lack of tau pathology. However, this difference can be obscured by the existence of comorbid AD pathology that may be observed in a minority of FTLD cases. It is important to focus on excluding co-occurring AD pathology since we and others have observed that AD co-pathology in forms of FTLD is much more common than co-occurring FTLD-tau and FTLD-TDP. The two-stage algorithm proposed in this study thus aims to identify cases with single neurodegenerative pathologies by first excluding cases with common dual pathologies such as co-occurring AD.

About 25% of clinical FTD cases are mutation carriers[2], and identification of the mutation can lead to a reliable prediction of the underlying histopathologic diagnosis. In this study we found that the proposed algorithm was less useful in FTD patients with pathogenic mutations. This is in agreement with our previous observation that the *C9orf72* expansion is associated with higher CSF p-tau levels.[12] These findings suggest that biomarker data and cut-offs cannot be equally applied to genetic and sporadic cases. This difference in biomarker profiles between genetic and sporadic disease has also been described in other neurodegenerative conditions such as AD.[36,37] In addition, the utility of a diagnostic algorithm is likely to be more clinically relevant in sporadic FTD when pathology cannot be inferred from the clinical syndrome.

The value of this algorithm was confirmed in an independent cohort of FTD patients with syndromes highly predictive of FTLD-Tau and FTLD-TDP. The value of p-tau in the living cohort showed a high sensitivity but modest specificity. It is important to mention that the phenotypes in the living cohort (PSP and FTD-ALS) and in the autopsy cohort differed, and it is possible than the existence of motor neuron disease or the specific topographical pattern of aggregation in 4-repeat tauopathies may influence p-tau levels.

The main strength of this study is the use of a large autopsy-confirmed cohort with detailed neuropathological data. This allowed us to establish a gold-standard reference for CSF biomarkers, and to consider concurrent pathologies known to impact CSF biomarker cutoffs.[7] Limitations should be considered when evaluating our findings. We did not obtain cross-validation in an independent autopsy cohort because a comparable pathology-proven dataset to replicate these findings is currently exceedingly rare. However, we replicated the ability of CSF p-tau for the discrimination of FTLD-Tau from FTLD-TDP after excluding subjects with expected comorbid AD in an independent living cohort. Further collaborative autopsy-proven studies are needed to refine and operationalize the proposed CSF algorithm. It is worth mentioning that we did not take into account the clinical phenotypes or imaging biomarkers (eg. amyloid or tau PET or MRI); however, our methods suggest that CSF is a lower cost alternative to PET imaging to exclude AD co-pathology in clinical FTD. Confidence in the diagnosis of FTLD-TDP may be improved, for example, if a low p-tau level is associated with clinical features of semantic variant primary progressive aphasia[38] Thus, it is likely that combinations of clinical features and CSF biomarkers can further improve diagnostic accuracy and multimodal assessments should be further studied in patients followed to autopsy.

In conclusion, we show that core AD CSF biomarkers can be used to improve specificity for

the *in vivo* identification of patients with sporadic FTLD-TDP and FTLD-Tau. This involves a two-stage algorithm that first excludes cases with likely AD pathology. We anticipate that this algorithm will be improved with the addition of novel pathway-specific biomarkers of FTLD that will undoubtedly increase the diagnostic accuracy in the FTLD-related syndromes.

Acknowledgments

Conflict of interest disclosure: No disclosures are reported.

Funding/Support: This study was supported by the National Institutes of Health (grants AG010124, AG032953, AG043503, NS088341, AG017586, NS053488, AG052943, and AG038490); the Wyncote Foundation; Newhouse Foundation; Arking Family Foundation; and the Health Research and Innovation Strategic Plan (PERIS) (grant SLT002/16/00408). I. Illán-Gala is supported by the i-PFIS grant (IF15/00060) from the FIS, Instituto de Salud Carlos III and the Rio Hortega Grant (CM17/00074), Instituto de Salud Carlos III.

Role of the Funder/Sponsor: The sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Access to Data and Data Analysis: Drs Lleó and Grossman had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Additional Contributions: We thank the patients and their families for participation in this research.

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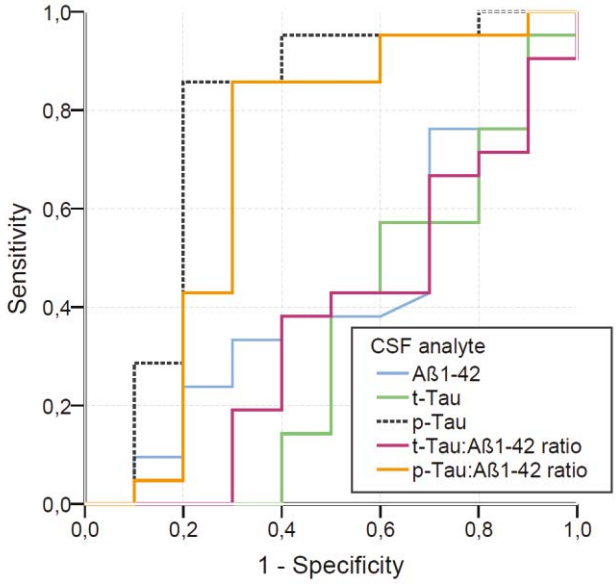
Supplementary material



eFigure 1. Sensitivity and specificity of CSF raw values of $A\beta_{1-42}$, t-tau, p-tau, t-tau/$A\beta_{1-42}$ and p-tau/$A\beta_{1-42}$ ratios in pure FTLD-Tau relative to pure FTLD-TDP. ROC analysis and levels of p-tau achieved the best results (AUC=0.78, 95% CI 0.56-0.99).

	Tau mutations n=10	TDP mutations n=50
Age at onset, y (SD)	10 *MAPT*	33 *C9orf72* 13 *GRN* 4 *TARDBP*
Age at onset, y (SD)	53.8 (4.6)b	57.9 (6.9)a
Age at CSF, y (SD)	57.3 (5.4)	60.1 (10.7)
Time from onset to CSF, y (SD)	3.6 (1.9)	3.4 (2.7)
Male, n (%)	4 (40)	29 (58)
*APOE*ε4 positive, n (%)	2/10 (20)	2/20 (10)
% Expected comorbid AD*	3 (30)	9 (18)

Supplement: eTable 1. Demographic and clinical characteristics of the genetic cohort. [a] *P*<.05 compared to Tau mutations; b P<.05 compared to TDP mutations. * p-Tau/$A\beta_{1-42}$ ≥ 0.09. Abbreviations: Standard deviation.

Annex 6: Supplementary paper 2

Paper accepted for publication in

J Neurol Neurosurg Psychiatry

(October 8th 2018)

Title:

Elevated YKL-40 and low sAPPβ/YKL-40 ratio in ante-mortem cerebrospinal fluid of patients with pathologically-confirmed FTLD

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Abstract

Background: The combination of high YKL-40 (a glial inflammatory marker) and low sAPPβ (a soluble fragment of amyloid precursor protein) in cerebrospinal fluid (CSF) has been associated with frontotemporal lobar degeneration (FTLD) in clinical series. We investigate these biomarkers in a neuropathologically-confirmed cohort of patients with FTLD.

Methods: CSF samples were selected from the Penn FTD Center (University of Pennsylvania). Participants were followed to autopsy and had a neuropathological diagnosis of FTLD-Tau (n=24), FTLD-TDP (n=25) or Alzheimer's disease (AD, n=97). We compared levels of YKL-40 and sAPPβ between groups and with cognitively normal controls (n=77), and assessed their diagnostic utility using receiver operating characteristic (ROC) curves. We also investigated the effect of AD co-pathology and the correlation between these CSF markers and tau burden at autopsy.

Results: Both FTLD groups had lower levels of sAPPβ, higher levels of YKL-40 and lower sAPPβ/YKL-40 ratio in CSF compared to controls. The group of pure FTLD-Tau (without AD co-pathology) showed higher levels of YKL-40 than AD and than pure FTLD-TDP. YKL-40 levels correlated with pathological tau burden. The sAPPβ/YKL-40 ratio had an area under the curve of 0.91 (95%CI 0.86-0.96) to distinguish FTLD subjects from controls, but lower values to distinguish FTLD from AD (AUC 0.70; 95%CI 0.61-0.79) and to discriminate FTLD-Tau from FTLD-TDP (AUC 0.67; 95%CI 0.51-0.82).

Conclusions: The sAPPβ/YKL-40 ratio may help distinguish patients with FTLD from those with neuropathological AD that show similar clinical phenotypes, and this may be due in part to elevated CSF YKL-40 levels in FTLD-Tau.

Introduction

Frontotemporal lobar degeneration (FTLD) is a pathological and genetically heterogeneous disorder that leads to neurodegeneration in frontal and temporal regions. Patients with FTLD can present different clinical syndromes typically affecting language and/or behavior. Patients with FTLD harbor either deposits of tau, TDP-43 or FUS,[1–3] but in most cases the specific underlying proteinopathy cannot be ascertained *in vivo*. Thus, patients with different clinical syndromes may show identical neuropathological findings, and in turn, a specific clinical syndrome can be the expression of more than one proteinopathy.[2] The challenges in predicting the underlying proteinopathy is an important limitation for achieving an accurate diagnosis and for the development of protein-specific therapeutic approaches. In addition, some patients with FTLD might present with subtle or very slowly progressive behavioral symptoms.[2,4] The development of biomarkers to distinguish these patients from others with psychiatric non-neurodegenerative conditions is a relevant area of research.

Cerebrospinal fluid (CSF) biomarkers provide an opportunity to measure changes *in vivo*that may reflect pathophysiological events in the brain. In Alzheimer's disease (AD), the use of CSF biomarkers has dramatically improved the accuracy of the diagnosis.[5,6] In FTLD, however, there is a lack of well-established diagnostic markers in CSF.[7] Core AD biomarkers ($A\beta_{1-42}$, t-tau and p-tau) are being used in FTLD-related syndromes to exclude AD[8] but specific markers of FTLD are also needed. Unfortunately, many biomarker studies in FTLD-related syndromes may be confounded by co-occurring secondary AD pathology and the impact of this concomitant pathology on biomarker levels is not usually assessed.[9] Neurofilament light levels have been shown to be increased in FTLD but also in AD, indicating lack of disease specificity. Levels in CSF of the astroglial marker of inflammation

YKL-40 are higher in different neurodegenerative diseases than in controls, without disease specificity.[10–12] Other studies have described low CSF levels of markers of the amyloid precursor protein (APP) processing in patients with FTLD compared to those of patients with AD and controls.[10,13–16] In previous studies, we found that the combination of sAPPβ (the soluble β fragment of APP) and YKL-40 was consistently associated with FTLD-related clinical syndromes in a clinical cohort.[10,16] In the present study, we investigate this biomarker profile in *ante-mortem* CSF samples obtained from a neuropathologically confirmed cohort of FTLD patients accounting for the presence of comorbid AD pathology.

Methods

CSF samples

A total of 223 *ante-mortem* CSF samples obtained between 1992 and 2015 were selected from the Penn FTD Center at the University of Pennsylvania (Philadelphia, USA).[17,18] Preanalytical processing details can be found elsewhere.[17] Subjects were followed to autopsy and had a neuropathological diagnosis of FTLD-Tau (n=24), FTLD-TDP (n=25) or AD (n=97) (Figure 1). We also analyzed CSF samples from 77 cognitively normal controls. All participants gave written informed consent to participate at the moment of CSF collection, and the Institutional Review Board at the University of Pennsylvania approved the study.

Neuropathological classification and quantification of tau burden

Neuropathological diagnosis was established following previously described methods and international published criteria.[18–22] Patients with a primary neuropathological diagnosis of Pick's disease, corticobasal degeneration, progressive supranuclear palsy, argyrophilic grain disease or non-classifiable non-AD tauopathies were classified as FTLD-Tau. Tau burden was measured digitally and in a validated parametric manner in gray and white matter of three different areas (mid-frontal cortex, angular gyrus, and anterior cingulate gyrus) as the percentage of area that contained tau deposits, as previously described.[18,20] A global measure of tau burden was obtained as the sum of these three values. Patients with FTLD with TDP-43 inclusions were classified as FTLD-TDP. According to the location and type of TDP-43 inclusions, these patients were subsequently classified as type A, B or C, following consensus criteria.[23]

A subset of patients with FTLD-Tau (n=20/24) and FTLD-TDP (n=18/25) had a neurofibrillary tangle score of B0 or B1 in the NIA-AA classification[24,25] and therefore had no evidence of significant AD co-pathology. This subset of patients was analyzed independently in order to examine the levels of CSF biomarkers in cases with pure FTLD pathology excluding the effects of comorbid AD pathology. All patients with a neuropathologic diagnosis of AD had scores of B2 or B3 in the NIA-AA classification.

CSF analysis

We analyzed CSF levels of sAPPβ and YKL-40 at Hospital Sant Pau using commercially available ELISA kits (Human sAPPβ-w highly sensitive, IBL, Gunma, Japan and MicroVueTM and Quidel, San Diego, CA, USA, respectively) and following previously reported methods.[10,16,26]

Statistical analysis

We assessed normality of the variables using the Kolmogorov-Smirnov test. sAPPβ and the ratio sAPPβ/YKL40 were log-transformed to achieve a normal distribution for further bivariate and multivariate analyses. We used the Chi-square test to assess differences in sex and analysis of

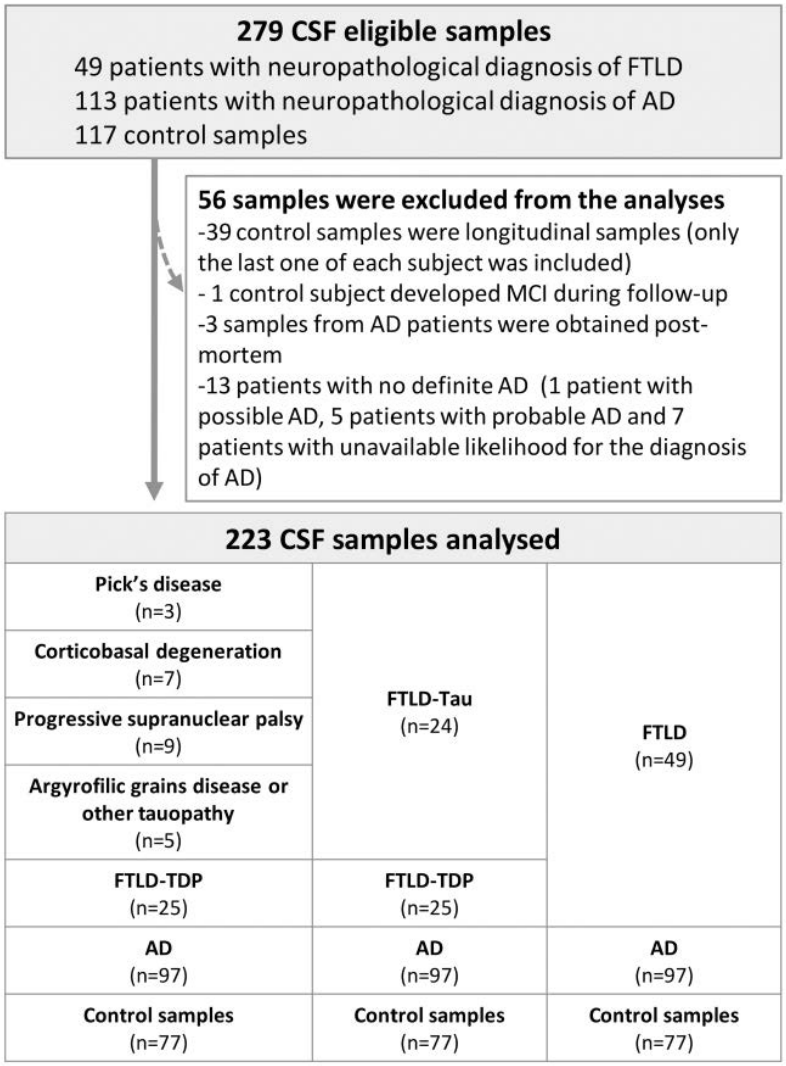

Figure 1. Flow-chart of participants and samples included in the study

variance (ANOVA) for age, education, age at death, time interval from symptoms onset to CSF collection and time interval from symptom onset to death. To minimize the influence of possible outliers and heterogeneity of variances, we used robust linear models followed by weighted least squares analysis of covariance (ANCOVA), including age and sex as covariates. All p-values were corrected for multiple comparisons using Tukey's "Honest Significant Differences" post-hoc test. We assessed the diagnostic utility of CSF biomarkers using receiver operating characteristic (ROC) curves. We used "MASS" and "pROC" packages from the R statistical software (v. 3.1.3) for statistical analyses.

Role of the funding sources

The study sponsors had no role in the design of the study, in the collection, analysis and interpretation of data, in the writing of the report, or in the decision to submit the paper for publication. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Demographics and clinical data

Table 1 shows demographic and clinical data of the subjects according to their final neuropathological diagnosis. The FTLD-Tau group had higher proportion of males (p=0.004). There were differences between groups in age at death, time interval from symptoms onset to death and time interval between CSF sampling and death. Therefore, sex and age at CSF collection were included as covariates in all biomarker statistical analysis.

In the FTLD group, there was no association between CSF biomarkers and time interval between onset and CSF sampling or between CSF sampling and death. In the AD group, we found small yet significant direct associations of sAPPβ levels and of the sAPPβ/YKL40 ratio with the time interval between onset and CSF sampling (adjusted R^2=0.05; p=0.06 and adjusted R^2=0.11; p=0.002, respectively) and with time from CSF sampling to death (adjusted R^2=0.17; p=0.013 and adjusted R^2=0.20; p=0.002, respectively).

FTLD patients have lower levels of sAPPß and higher levels of YKL-40 in CSF than controls

As displayed in Figure **2**, there were differences in levels of sAPPβ ($F_{(3,217)}$=6.73; p<0.001), YKL-40 ($F_{(3,217)}$=18.12; p<0.001) and the sAPPβ/YKL-

	FTLD-Tau	FTLD-TDP	AD	Control	p-value*
n	*24*	*25*	*97*	*77*	
Age at CSF collection (y)	66.9 (11.3)	66.4 (8.7)	71 (10.6)	68.2 (9)	57.9 (6.9)a
Female, No. (%)	6 (25)	13 (52)	45 (46)	50 (65)	0.004
Education (y)	15.6 (3.5)	15.1 (2.7)	14.8 (3.1)	16.2 (3.2)	0.05
Age at death (y)	71.4 (12.6)	70 (9.5)	76.6 (10.6)	N/A	0.008
Participants with no AD pathology (NIA-AA stage≤B1), No. (%)	20 (83)	18 (72)	0	N/A	0.003
Time interval onset-CSF (y)	3.8 (2.7)	3.7 (2.7)	4.3 (2.6)	N/A	0.521
Time interval CSF-death (y)	4.5 (3.6)	3.6 (3)	5.6 (3.6)	N/A	0.029
Total disease duration onset-death (y)	8.5 (4.6)	7.2 (3.3)	9.8 (4.1)	N/A	0.013
sAPPβ (ng/ml)	477.5 (120)	544.9 (239.4)	717.1 (441.6)	848.4 (381.9)	<0.001
YKL40 (ng/ml)	299.8 (69.1)	265.6 (48.5)	268.2 (77.2)	210.3 (55.1)	<0.001
onset-death (y)	1.67 (0.56)	2.07 (0.78)	2.77 (1.5)	4.19 (1.89)	<0.001

Table 1. Demographics, clinical, pathological and CSF data. Unless otherwise specified, results are expressed as mean (standard deviation). *p-values were obtained by comparing the groups of FTLD-Tau, FTLD-TDP,AD and Control.TukeyHSD post-hoc comparisons are detailed in Figure 2. **Abbreviations:** CBD: corticobasal degeneration; PSP: progressive supranuclear palsy; FTLD: frontotemporal lobar degeneration; AD: Alzheimer's disease; CSF: cerebrospinal fluid.

40 ratio ($F_{(3,217)}$=24.74; p<0.001) between groups. In the post-hoc analysis, each patient group (FTLD-Tau, FTLD-TDP and AD) showed lower levels of sAPPβ, higher levels of YKL-40 and lower sAPPβ/YKL-40 ratios compared to controls. The levels of YKL-40 in the group of FTLD-Tau were higher than those in AD, but no significant differences were found between the groups of FTLD-TDP and AD or between FTLD-Tau and FTLD-TDP. The sAPPβ/YKL-40 ratio was lower in both FTLD groups compared to controls and compared to the AD group. We found no differences in levels of sAPPβ, YKL-40 or their ratio between TDP subtypes or between tau subtypes (Table 2). We did not find differences in any biomarker between patients with mutations and patients without mutations (data not shown)

	FTLD-Tau					FTLD-TDP				
	Pick	CBD	PSP	Other tauopathy	p-value*	A	B	C	Non-specified	p value
n	3	7	9	5	NA	1	11	8	5	NA
Age at CSF collection (y)	58.3 (5.8)	57.6 (8.2)	73.8 (7.3)	72.6 (12.4)	0.01	73 (NA)	65.1 (8.7)	70.3 (9.8)	62 (4.2)	0.32
Sex (% female)	33.3%	42.9%	22.2%	0.0%	0.39	100.0%	54.6%	50.0%	50.0%	0.82
Education (y)	16.7 (2.3)	15.5 (3.1)	16.1 (2.9)	14 (5.4)	0.88	14 (NA)	14.4 (2.3)	16.5 (3.2)	16.5 (2.1)	0.43
Age at death (y)	63.3 (6.8)	60.1 (9)	76.7 (8.6)	82.4 (11.5)	<0.01	75 (NA)	68 (10.3)	74.4 (9.9)	63 (2.8)	0.32
% with no AD pathology (NIA-AA stage≤B1	100%	100%	78%	60%	0.25	100%	56%	100%	80%	0.11
Time interval onset-CSF (y)	4.3 (3.1)	2.5 (1)	4.3 (3.5)	4.2 (2.7)	0.48	1 (NA)	4.5 (3.3)	3.6 (1.8)	2 (1.4)	0.36
Time interval CSF-death (y)	5 (1.7)	2.6 (1.5)	2.9 (2.1)	9.8 (3.3)	0.01	2 (NA)	2.9 (2.9)	4.1 (2.2)	1 (1.4)	0.26
Total disease duration onset-death (y)	9.3 (4.7)	5.3 (1.4)	7.2 (4.7)	14 (1)	0.02	3 (NA)	7.4 (3.1)	7.8 (3)	3 (0)	0.19
sAPPβ (ng/ml)	518.3 (141.9)	452.2 (120.6)	487.4 (112.9)	470.7 (150.8)	0.86	415 (NA)	533.9 (179.9)	525.8 (121)	441.2 (24.6)	0.76
YKL40 (ng/ml)	269.3 (3.6)	314.6 (57.7)	314.4 (90.2)	271 (59.6)	0.24	280.3 (NA)	280.3 (48.6)	251.2 (48.7)	228.8 (54.9)	0.54
Ratio sAPPβ/ YKL-40	1.92 (0.51)	1.54 (0.71)	1.63 (0.55)	1.77 (0.49)	0.47	1.48 (NA)	1.9 (0.51)	2.21 (0.8)	2 (0.59)	0.69

Table 2. Demographics, clinical, pathological and CSF data in FTLD-Tau and FTLD-TDP subgroups. Unless otherwise specified, results are expressed as mean (standard deviation). Differences between subtypes were assessed by Kruskal-Wallis Rank Sum test for numeric quantitative variables and Chi-squared test for categorical variables.

Relationship of sAPPß and YKL-40 with tau protein aggregates in FTLD

In the FTLD group, YKL-40 levels in CSF (but not sAPPβ or the sAPPβ/YKL-40 ratio) showed a weak direct correlation with pathological tau burden in mid-frontal cortex (R^2= 0.18; p=0.02), angular gyrus (R^2=0.19; p=0.02), anterior cingulate gyrus (R^2= 0.15; p=0.05) and the global measure of tau burden (R^2=0.18; p=0.03, Supplementary Figure 1). These correlations were non-significant when FTLD-TDP and FTLD-Tau were analyzed separately.

Influence of AD co-pathology

To explore the effects of incidental AD co-pathology on biomarker results, we repeated the analysis in the subgroup of FTLD patients that had no significant AD pathology, defined by a neurofibrillary tangle score of B0 or B1 in the NIA-AA classification (Figure 2). Similarly to the results found in the whole sample, there were differences in levels of sAPPβ ($F_{(3,206)}$=6.17; p<0.001), YKL-40 ($F_{(3,206)}$=20.49; p<0.001) and the sAPPβ/YKL-40 ratio ($F_{(3,206)}$=22.38; p<0.001) between groups. The groups of pure FTLD-Tau and pure FTLD-TDP patients showed lower levels of sAPPβ compared to controls. The group of pure FTLD-Tau showed higher levels of YKL-40, not only compared to AD, but also compared to

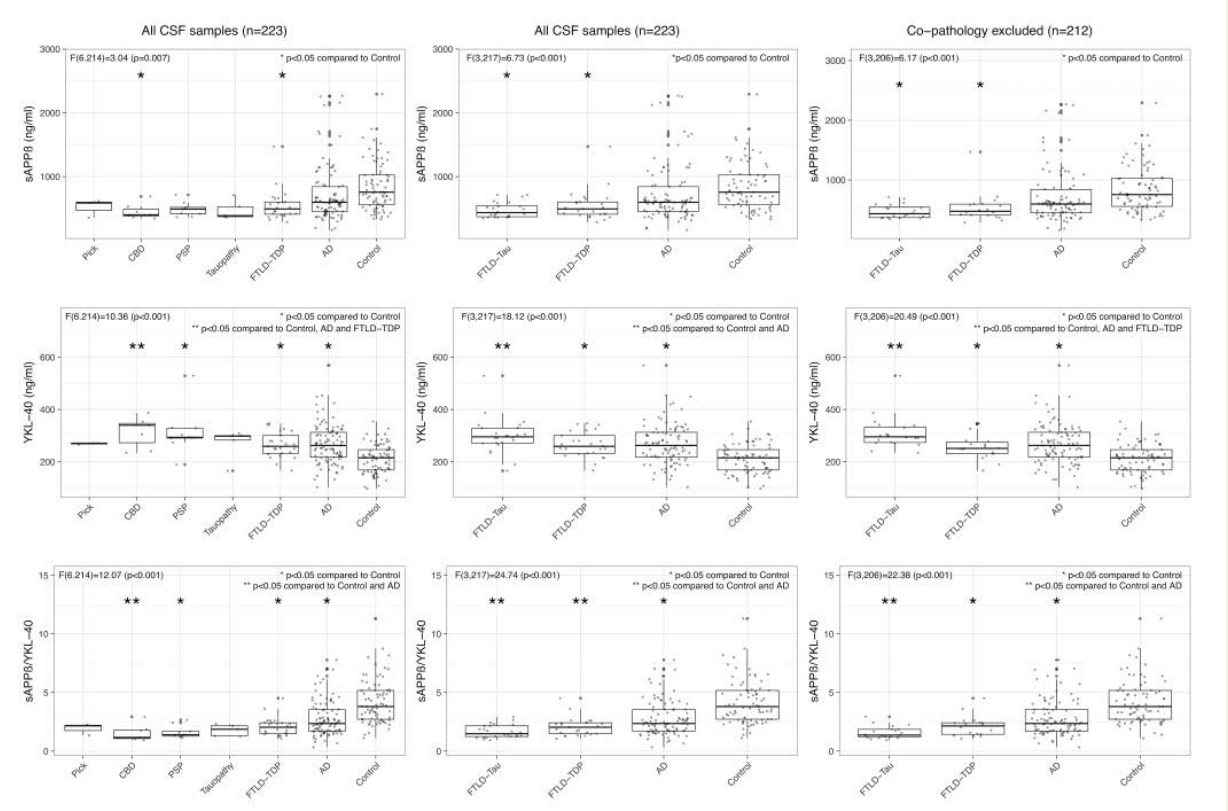

Figure 2. Levels of sAPPß, YKL-40 and the sAPPß/YKL-40 ratio in CSF across pathological diagnostic groups. CBD: corticobasal degeneration; PSP: progressive supranuclear palsy; Tauopathy: argyrophilic grain disease (n=4) and unclassifiable tauopathy (n=1); FTLD-TDP: frontotemporal lobar degeneration-TDP; AD: Alzheimer's disease; FTLD-Tau: frontotemporal lobar degeneration-Tau. Only statistically significant differences are displayed (ANCOVA and post-hoc TukeyHSD). All results were adjusted for age and sex, and correction for multiple comparisons was applied.

the group of pure FTLD-TDP. Both FTLD-Tau and FTLD-TDP groups had lower sAPPβ/YKL-40 ratios compared to controls, and in this subset only those with FTLD-Tau were significantly lower than those in the AD group.

Diagnostic value of CSF sAPPß and YKL-40 in FTLD

The ROC curve analyses are displayed in Figure 3. Both sAPPβ and YKL-40 had an area under the curve (AUC) above 0.80, and the sAPPβ/YKL-40 ratio, had an AUC of 0.91 (95% CI 0.86-0.96) to distinguish FTLD patients from controls. Similar results were found in the subgroup with no AD co-pathology (Table 3). An optimal cut-off point for sAPPβ/YKL-40 ratio of 2.45 had a sensitivity and specificity above 85% to discriminate FTLD from controls.

The overall diagnostic accuracy of the sAPPβ/YKL-40 ratio was lower to distinguish FTLD patients from AD patients (AUC 0.70; 95%CI 0.61-0.79) and to distinguish patients with FTLD-Tau from those with FTLD-TDP pathology (AUC 0.67; 95%CI 0.51-0.82). In the subgroup with no comorbid AD pathology, the sAPPβ/YKL-40 ratio allowed a correct discrimination between FTLD-Tau and FTLD-TDP in 71% of patients (95%CI 0.54-0.88). In this subgroup, the area under the curve of YKL-40 to distinguish FTLD-Tau patients from controls (AUC 0.91; 95%CI 0.85-0.97) was significantly higher compared to that to discriminate FTLD-TDP from controls (AUC 0.74; 95%CI 0.62-0.85) (Supplementary Figure 2).

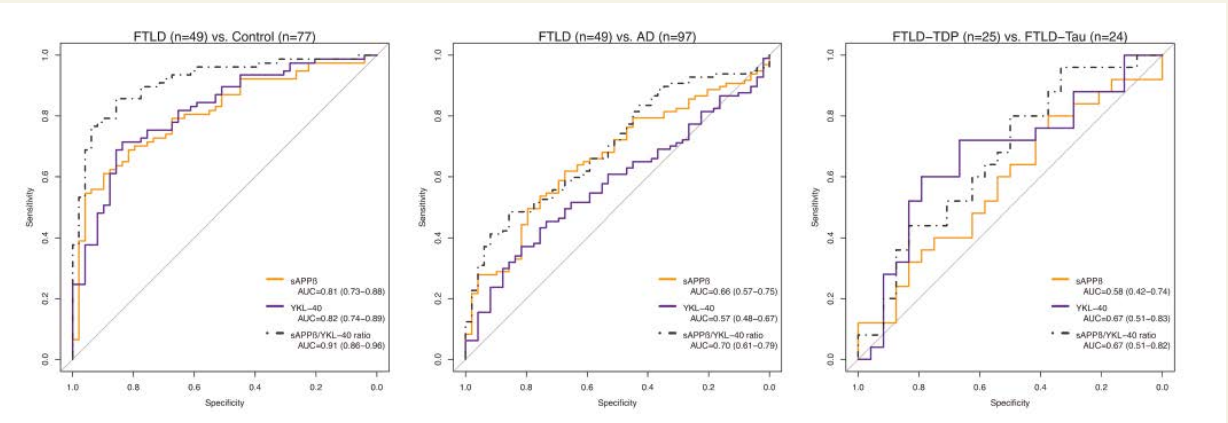

Figure 3. Receiver operating characteristic (ROC) curves for the analysis of CSF biomarkers' diagnostic utility. Abbreviations: FTLD: frontotemporal lobar degeneration; AD: Alzheimer's disease; AUC: area under the curve. All participants were included in these analyses. Values are expressed as AUC (CI 95%).

FTLD vs. CN								
	All participants 49 FTLD vs. 77 CN				**No co-pathology** 38 FTLD vs. 77 CN			
	AUC (95% CI)	Best-fit cut-off	Se.	Sp.	AUC (95% CI)	Best-fit cut-off	Se.	Sp.
sAPPβ	0.81 (0.73-0.88)	463ng/ml	86%	51%	0.82 (0.74-0.90)	463ng/ml	86%	53%
YKL-40	0.82 (0.74-0.89)	278ng/ml	86%	53%	0.83 (0.75-0.90)	278ng/ml	87%	50%
sAPPβ/YKL-40 ratio	0.91 (0.86-0.96)	2.45	86%	86%	0.91 (0.86-0.97)	2.45	86%	87%
FTLD vs. AD								
	All participants 49 FTLD vs. 97 AD				**No co-pathology** 38 FTLD vs. 97 AD			
	AUC (95% CI)	Best-fit cut-off	Se.	Sp.	AUC (95% CI)	Best-fit cut-off	Se.	Sp.
sAPPβ	0.66 (0.57-0.75)	393ng/ml	85%	27%	0.68 (0.58-0.77)	393ng/ml	85%	29%
YKL-40	0.57 (0.48-0.67)	336ng/ml	85%	16%	0.58 (0.49-0.68)	339ng/ml	87%	18%
sAPPβ/YKL-40 ratio	0.70 (0.61-0.79)	1.51	86%	39%	0.71 (0.62-0.81)	1.51	86%	55%
FTLD-Tau vs. FTLD-TDP								
	All participants 24 FTLD-Tau vs. 25 FTLD-TDP				**No co-pathology** 20 FTLD-Tau vs. 18 FTLD-TDP			
	AUC (95% CI)	Best-fit cut-off	Se.	Sp.	AUC (95% CI)	Best-fit cut-off	Se.	Sp.
sAPPβ	0.58 (0.42-0.74)	393ng/ml	85%	27%	0.58 (0.39-0.76)	364ng/ml	89%	20%
YKL-40	0.67 (0.51-0.83)	336ng/ml	85%	16%	0.79 (0.63-0.94)	328ng/ml	89%	30%
sAPPβ/YKL-40 ratio	0.67 (0.51-0.82)	1.51	86%	39%	0.71 (0.54-0.88)	1.28	89%	35%

Table 3. Cut-off values for sAPPß and YKL-40 to discriminate between FTLD and cognitively normal controls and between FTLD and Alzheimer's disease. Best-fit cut-off values were obtained for sAPPß, YKL-40 and the sAPPß/YKL-40. Specificity was optimized for a sensitivity level of at least 85%. **Abbreviations:** FTLD: frontotemporal lobar degeneration; CN: cognitively normal control; AD: Alzheimer's disease. AUC: area under the curve; Se: sensitivity; Sp: specificity.

Discussion

The main finding of this study is that pathologically-confirmed FTLD patients have higher levels of YKL-40 and lower levels of sAPPβ in CSF compared to controls. The combination of these biomarkers (sAPPβ/YKL-40 ratio) provides high diagnostic accuracy to distinguish patients with FTLD from controls. This appears to be due largely to the group with FTLD-Tau, where we found that CSF YKL-40 levels in patients without AD co-pathology are elevated compared to patients with FTLD-TDP and to those with AD, and that CSF YKL-40 levels correlate with tau burden in FTLD.

In our previous study of these CSF analytes in clinically diagnosed patients,[16] we did not detect differences between patients with high likelihood of FTLD-Tau and patients with high likelihood of FTLD-TDP. The present study examines these analytes in patients with neuropathological confirmation. It is important to note here that, in the pure FTLD group (after excluding patients with AD co-pathology), we found higher levels of YKL-40 in FTLD-Tau compared with FTLD-TDP. This reinforces the notion that coincident pathologies, comorbid AD in this case, have an impact on CSF biomarkers.[9,25,27] We also found that, in agreement with another study,[28] YKL-40 in FTLD-Tau is elevated compared to AD and controls.

Although YKL-40 lacks disease specificity, this marker could provide some *in vivo* information about the underlying pathology. High levels of YKL-40 might be due to the activation of inflammatory pathways associated to neurodegeneration.[11,29–31] Previous evidence supports this hypothesis. In human brain, YKL-40 immunoreactivity is detected in a subset of reactive astrocytes.[29] It is also worth mentioning that YKL-40 immunoreactivity correlates with tau deposits in different tauopathies.[29] FTLD-Tau is associated with significantly greater independent gray matter pathology in astrocytes and gray/white matter pathology oligodendrocytes.[32] In the present study, we expand these data by showing a relationship between levels of YKL-40 in CSF and FTLD-Tau pathology. We found a mild, yet significant correlation between levels of YKL-40 in CSF and the amount of regional and global tau pathology. The relationship between tau pathology and CSF YKL-40 is also supported by our observation that patients with FTLD-Tau without AD co-pathology had higher levels of YKL-40 in CSF than patients with FTLD-TDP and than patients with AD. Taken together, these findings support the idea that although the pathway mediated by YKL-40 is activated in different neurodegenerative conditions, it is particularly sensitive to tau aggregation.[29]

Likewise, low levels of sAPPβ may be informative. This could be the result of reduced overall APP processing or availability due to accelerated atrophy and neuronal loss in frontotemporal regions, which are characteristic of FTLD but also present to some extent in other neurodegenerative diseases such as advanced or atypical AD.[13,16]

Our previous study reported that the combination of CSF sAPPβ with YKL-40 in clinically-defined patients had a good diagnostic performance in a clinical setting to distinguish frontotemporal dementia from AD and cognitively normal controls.[16] The present study extends these findings to patients with neuropathological confirmation. We confirm differences in levels of sAPPβ, YKL-40 and the sAPP/YKL-40 ratio in CSF between FTLD patients and controls. However, diagnostic accuracy of the sAPPβ/YKL-40 ratio was lower to distinguish FTLD patients from AD patients (AUC 0.70) than in our previous study.[16]

The results of this study have clinical implications. Although the sAPPβ/YKL-40 ratio does not appear to be useful to distinguish TDP-43

from tau proteinopathies or to discriminate between patients with FTLD and AD, this marker could be useful in combination with AD biomarkers in patients with atypical or mild symptoms of frontotemporal dementia. For instance, patients with behavioural symptoms and normal AD biomarkers that have low sAPPβ/YKL-40 ratio in CSF would likely have FTLD pathology, whereas those with high sAPPβ/YKL-40 ratio would more likely correspond to psychiatric non-neurodegenerative conditions.[16]

We acknowledge that our study has some limitations. First, although CSF analytes in well-annotated autopsy cases of these uncommon conditions are rare, we were able to analyze only very small groups of patients. Second, the time between CSF acquisition and death (and therefore, neuropathological confirmation) is variable and reaches up to 10 years in some cases. This variability might underestimate the relationship between participants' CSF biochemical signature and their final neuropathological findings. Finally, our control participants lack neuropathological confirmation. However, complete clinical and neuropsychological evaluations were performed to exclude significant medical (and specifically neurological) conditions in these participants.

In summary, the results of this study provide pathological confirmation of a CSF biomarker profile found in patients with FTLD that consists of high levels of YKL-40, low levels of sAPPβ, and low sAPPβ/YKL-40 ratio. Although this profile is not specific of the underlying proteinopathy, the findings suggest that the inflammatory marker YKL-40 may be particularly associated with FTLD-Tau pathology, and these analytes could be clinically useful in particular clinical settings in combination with AD biomarkers.

Acknowledgements

We would like to thank all the participants who agreed to take part in this study and all the members of the clinical teams involved in their selection and assessment.

This work was supported by CIBERNED and Instituto de Salud Carlos III PI11/02425 and PI14/01126 to Dr. J. Fortea, PI13/01532 to Dr. R. Blesa, PI15/00026 to Dr. J. Clarimón, and PI14/01561 to Dr. A. Lleó), jointly funded by Fondo Europeo de Desarrollo Regional (FEDER), Unión Europea, "Una manera de hacer Europa". This work was also supported in part by Generalitat de Catalunya (2014SGR-0235), a PERIS grant (SLT002/16/00408), and "Marató TV3" grants 201412 10 to Dr. J. Fortea, and 201426 10 to Dr. A. Lleó. I. Illán-Gala was supported by an i-PFIS grant (IF15/00060) from the Fondo de Investigación en Salud, Instituto de Salud Carlos III.

This study was also supported by grants AG010124, AG032953, AG043503, NS088341, AG017586, NS053488, AG052943, AG038490 from the National Institute of Health; and the Wyncote Foundation, Dana Foundation, Brightfocus Foundation, Penn Institute on Aging, Newhouse Foundation and Arking Family Foundation.

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Supplementary material

Title:

Elevated YKL-40 and low sAPPβ/YKL-40 ratio in ante-mortem cerebrospinal fluid of patients with pathologically-confirmed FTLD

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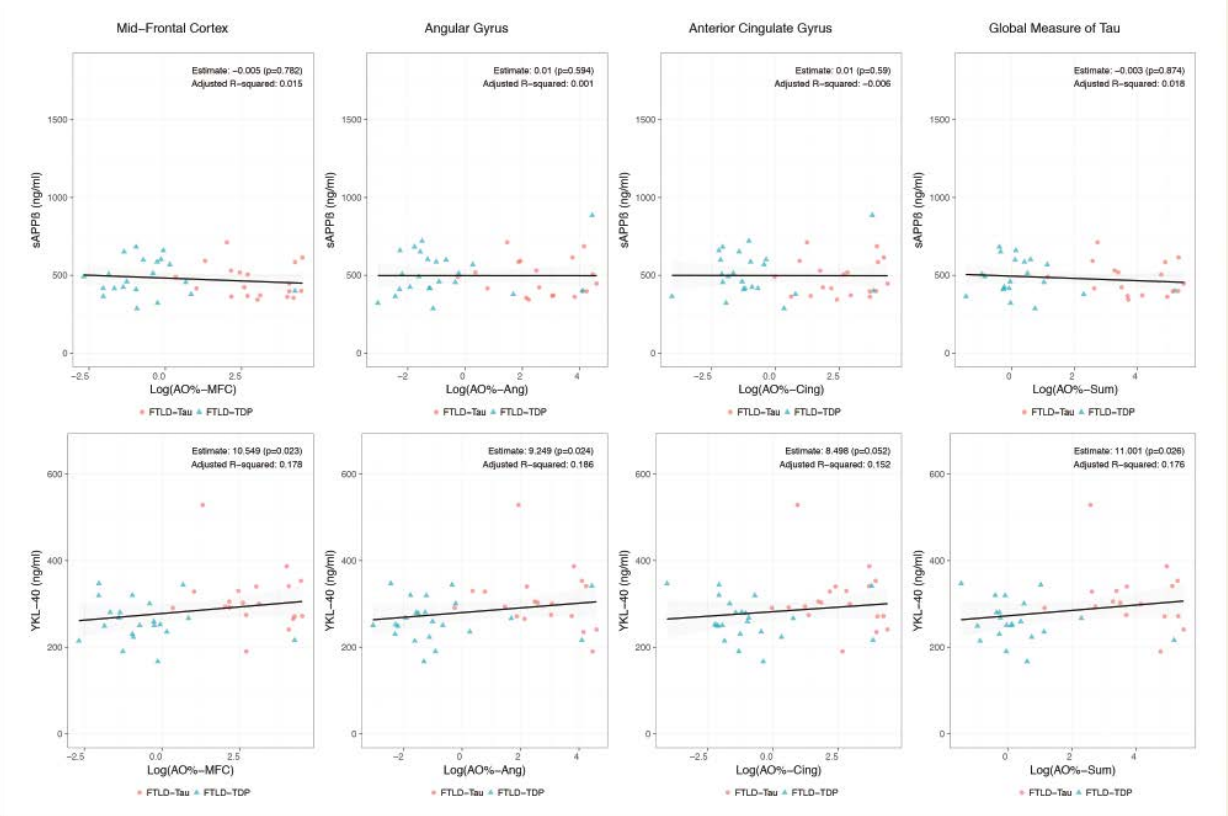
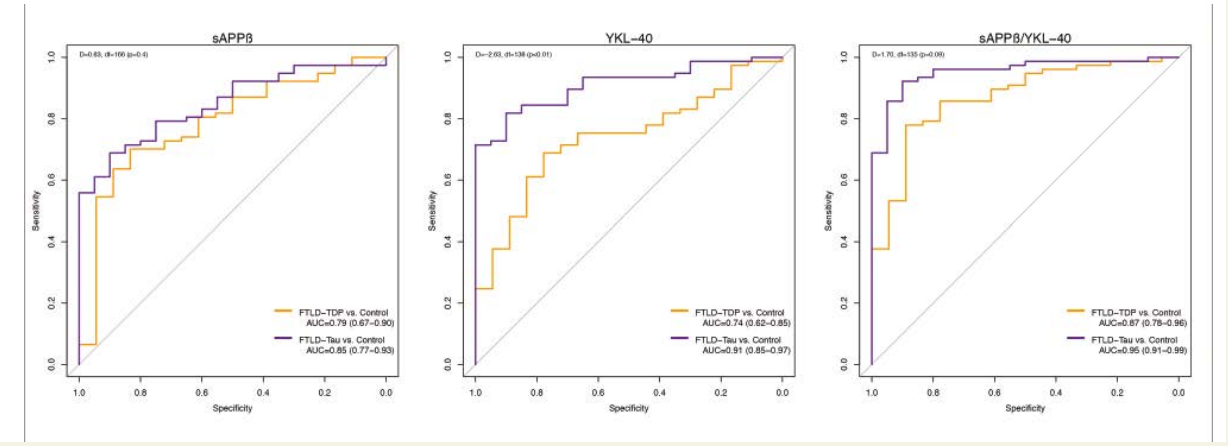


Supplementary Figure 1 - Association of sAPPβ and YKL-40 in CSF with cerebral tau burden. The x axis is expressed as the logarithm of percentage of area occupation (AO%) by tau deposits.



Supplementary Figure 2 - Receiver operating characteristic (ROC) curves for the analysis of CSF biomarkers' diagnostic utility to distinguish FTLD-Tau and FTLD-TDP patients from controls. FTLD: frontotemporal lobar degeneration; AUC: area under the curve. Only participants with no comorbid AD pathology were included in these analyses. Values are expressed as AUC (CI 95%).

Annex 7: Acknowledgments in mother language

Esta tesis es el resultado de más de tres años de apasionado trabajo en la unidad de memoria y el laboratorio de Alzheimer del Hospital de la Santa Creu i Sant Pau. En este entorno privilegiado he tenido el placer de trabajar con enormes profesionales, expertos en diferentes disciplinas y ante todo excelentes personas y amigos. Muchas de las ideas, resultados y análisis incluidos en esta tesis son consecuencia directa de estas interacciones. Siento por lo tanto que, esta tesis no es solo mía, sino de todos los integrantes de la unidad que han contribuido a que se hiciera realidad. Con su talento, trabajo, generosidad, cafés y risas la han hecho posible. Gracias a todos.

Antes de dar el salto y decidirme a emprender esta aventura mis experiencias en el liceo francés de Alicante, la facultad de medicina del hospital de Sant Pau, la propia residencia de Neurología en el hospital universitario la Paz y mi estancia en el Memory and Aging Center en San Francisco han sido sin duda experiencias fundamentales. Son tantas las personas que de un modo u otro me han ayudado en el camino, que enumerarlas a todas sería imposible. En particular, me gustaría agradecer especialmente a Alberto Lleó la confianza que depositó en mi. Gracias a su apoyo constante y a sus enseñanzas he podido aprender fenotipando al detalle a casi trescientos síndromes relacionados de la degeneración lobular frontotemporal sin restricciones de tiempo ni recursos. Te estaré siempre agradecido Alberto. A Juan Fortea por sus valiosas lecciones, su ambición, determinación y exigencia, que han sido fruto de motivación e inspiración constantes. Al profesor Rafael Blesa, por inspirarme también durante mi etapa de estudiante de medicina (aunque él no lo recuerde) y hacer posible que la unidad haya llegado al nivel de excelencia internacional en el que se encuentra actualmente. A Bruce Miller por creer en mí cuando tenía todo por aprender, incendiar mi curiosidad y empujarme a dedicar el resto de mi vida al estudio del comportamiento humano. A nuestros pacientes cuya generosidad y confianza da sentido a todos nuestros sacrificios. Mención especial a Víctor Montal, Daniel Alcolea, y mis suegros Ana y José María sin cuya generosidad esta tesis directamente no habría sido posible.

Remontándome más en el tiempo, no puedo dejar de estar eternamente agradecido a mis padres, Elena y Rafael, por habérmelo dado todo a cambio de nada y haberme animado siempre a dedicarme a lo que me gustara, por absurdo que pareciera. Quizás si mi madre no hubiera accedido a comprarme aquel ejemplar de la interpretación de los sueños de Freud, nunca hubiera estudiado medicina y no hubiera descubierto mi pasión. Ahora que soy padre valoro todavía más los sacrificios que habéis hecho. Gracias. A mi hermano Rafael por haberme ayudado a elegir el camino correcto. A mi abuelo Santiago, por transmitirme el valor de la palabra impresa y dejarme su amor incondicional. A mis abuelas Elena y Mercedes y a mi abuelo José Luis por su descomunal sabiduría y humanidad. Y por supuesto a mi hijo Santiago y a mi mujer Laura por recordarme cada día lo verdaderamente importante y hacer que todo valga la pena.

About the author

Ignacio Illán-Gala obtained his Medical Degree at the Autonomous University of Barcelona (Barcelona, Spain) and did his internship in clinical Neurology at La Paz University Hospital (Madrid, Spain). The last year of his training as a Neurologist, he was granted by the Spanish Society of Neurology to join the behavioral neurology program of the Memory and Aging Center (San Francisco, USA). This experience committed him to focus his career in the study of the patients suffering from frontotemporal dementia. At the end of his internship, he joined the Memory Unit of the Santa Creu i Sant Pau, a leading behavioral neurology team where translational research is implemented by a multidisciplinary and highly-talented group of scientists including biologist, psychologists, engineers, nurses and neurologists. Currently, he is involved in the local Frontotemporal Lobar Degeneration (FTLD) clinical cohort and the multicentric Catalan FTD cohort (CATFI). In 2018, he was granted with the competitive Río Hortega grant, completed a master in neuropsychology at the Universitat oberta de Catalunya and he was selected as an Atlantic Fellow of the Global Brain Health Institute, at University of California San Francisco.

Abstract

Frontotemporal lobar degeneration (FTLD), is a heterogenic pathological construct encompassing multiple neuropathological conditions primarily affecting the frontal and temporal lobes. Although multiple clinical syndromes predict the neuropathological diagnosis of FTLD, clinical-patholocial correlations are far from being perfect. Cerebrospinal and imaging biomarkers represent powerful tools for the study of FTLD pathophysiology and improve the diagnostic accuracy of FTLD and its differentiation from other diseases.

This thesis aims to improve our understanding of the pathophysiological and structural underpinnings of FTLD-related neurodegeneration through a multimodal biomarker approach combining: (i) clinical markers of disease progression (i.e. CDR-FTLD, ALFRS), (ii) CSF biomarkers related to different pathophysiological aspects of FTLD (APP-derived peptides, YKL-40 and NfL) and (iii) the MRI study of cortical macrostructure (cortical thickness) and microstructure (cortical mean diffusivity) in FTLD—related syndromes (FTLD-S).

We provide novel insights into the pathophysiology of FTLD by showing that: (i)CSF levels of sAPPß, YKL-40 and NFL alone or in combination had a good diagnostic accuracy to discriminate FTLD-S from healthy controls and patients with Alzheimer's disease (ii) In the absence of Alzheimer's disease pathophysiology, APP-derived peptides related to the so-called amyloidogenic pathway (sAPPß, $Aß_{1-42}$, $Aß_{1-40}$, $Aß_{1-38}$) are globally reduced in FTLD-S and correlate with cortical macrostructure (cortical thickness); importantly this pattern of APP-derived differed from the observed in Alzheimer's disease were a selective decrease in the CSF levels of $Aß_{1-42}$ was observed; (iii) YKL-40, a biomarker related to astroglial activity, is increased in FTLD-S and their levels may be useful for the prediction of disease progression in the ALS-FTD continuum; and (iv) cortical mean diffusivity, a novel imaging biomarker is more sensitive that cortical thickness for the study of the earliest FTLD-related neurodegeneration. These findings add to our current understanding of FTLD pathophysiology and open new doors towards precision medicine approaches in FTLD-S.

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