UNIVERSITAT DE BARCELONA

Determinantes genéticos del fenotipo clínico y neuropatológico en el espectro demencia frontotemporal – esclerosis lateral amiotrófica

Sergi Borrego Écija

DETERMINANTES GENÉTICOS DEL FENOTIPO CLÍNICO Y NEUROPATOLÓGICO EN EL ESPECTRO DEMENCIA FRONTOTEMPORAL - ESCLEROSIS LATERAL AMIOTRÓFICA

Memoria de tesis doctoral presentada por

Sergi Borrego Écija

para optar al grado de doctor por la Universidad de Barcelona

Dirigida por:

Raquel Sánchez del Valle Díaz

Ellen Gelpi Mantius

Programa de Doctorat Medicina i Recerca Translacional

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Informe de los directores de la tesis:

La Dra. Raquel Sánchez del Valle Díaz, doctora en Medicina por la Universidad de Barcelona y la Dra. Ellen Gelpi Mantius, doctora en Medicina por la Universidad de Barcelona,

Certifican:

Que la memoria titulada "Determinantes genéticos del fenotipo clínico y neuropatológico en el espectro demencia frontotemporal – esclerosis lateral amiotrófica", presentada por Sergi Borrego Écija, ha estado realizada bajo nuestra dirección y consideramos que reúne las condiciones necesarias para ser defendida ante el Tribunal correspondiente para optar al grado de Doctor por la Universidad de Barcelona.

Firmado,

Dra. Raquel Sánchez del Valle Díaz

Unidad de Alzheimer y
otros trastornos cognitivos
Servicio de Neurología
Hospital Clínic de Barcelona

Banco de Tejidos Neurológicos
Hospital Clínic de Barcelona – IDIBAPS

Division of Neuropathology and
Neurochemistry
Department of Neurology
Medical University of Vienna

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Ellen Gelpi Mantius

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I.

Listado de abreviaturas

3R: 3 repeticiones

4R: 4 repeticiones

AGD: enfermedad por gránulos argirófilos (argyrophilic grain disease)

APNF: afasia progresiva no fluente

APP: afasia primaria progresiva

APPvl: afasia primaria progresiva variante logopénica

APPvnf: afasia primaria progresiva variante no fluente o agramatical

APPvs: afasia primaria progresiva variante semántica

C9orf72: cromosoma 9 open reading frame 72

CHMP2B: Charged multivesicular body protein 2B

DCB: degeneración corticobasal

DFT: demencia frontotemporal

DFTP-17: demencia frontotemporal con parkinsonismo ligada a cromosoma 17

DFTvc: demencia frontotemporal variante conductual

DLFT: degeneración lobular frontotemporal

DLFT-U: degeneración lobular frontotemporal tau negativa, ubiquitina positiva

DS: demencia semántica

GGT: taupatía globular glial (globular glial tauopathy)

ELA: esclerosis lateral amiotrófica

EWS: Ewing's Sarcoma

FUS: fused in sarcoma

GRN: granulina

MAPT: microtubule-associated protein tau

OPTN: optineurina

PiD: enfermedad de Pick (Pick's disease)

PSP: parálisis supranuclear progresiva

SCB: síndrome corticobasal

S-PSP: síndrome de parálisis supranuclar progresiva

SQTSM1: Sequestosome 1

TAF15: TATA-binding protein associated factor 15

TARDBP: Transactive response DNA-binding protein

TBK1: TANK-binding kinase 1

TDP-43: transactive response DNA binding protein 43KDa

TMEM106B: Transmembrane protein 106B

Trnp1: Transportin 1

VCP: Valosin Containing Protein

II.

Introducción

2.1. Epidemiología de la degeneración lobular frontotemporal

La degeneración lobular frontotemporal (DLFT) es una enfermedad neurodegenerativa clínica, neuropatológica y genéticamente muy heterogénea. Las distintas entidades que se engloban bajo el término DLFT tienen en común la selectiva neurodegeneración de los lóbulos frontales y temporales, lo que conlleva un progresivo deterioro de la personalidad, el comportamiento social y/o el lenguaje del paciente.

La prevalencia estimada de las DLFT es de 10,84/100.000 personas y su incidencia de 1,61 /100.000 personas/año (Ratnavalli *et al.*, 2002; Coyle-Gilchrist *et al.*, 2016). La edad de inicio suele ser sobre la sexta década de la vida, siendo la segunda causa de demencia neurodegenerativa por debajo de los 65 años, después de la enfermedad de Alzheimer (Figura 1) (Garre-Olmo *et al.*, 2010).

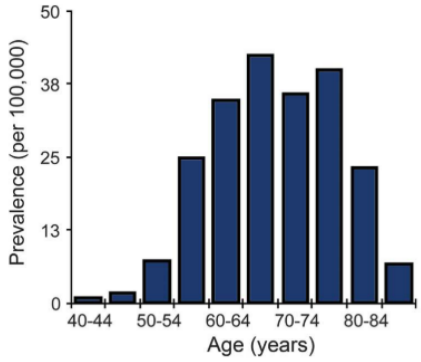


Figura 1: Prevalencia de la DFT por edad.
Adaptado de Coyle-Gilchrist et al 2016.

La DLFT conlleva una importante invalidez, así como una reducción dramática de la esperanza de vida, pues los individuos que sufren esta patología presentan una supervivencia media de unos 9 años desde el inicio de los síntomas. Todo ello conlleva que, pese a su relativamente baja prevalencia, sea una enfermedad con una enorme carga social y económica. Estudios recientes calculan unos costes de 108.700€ por paciente y año (43.500€ de costes directos y 65.200€ de costes indirectos) (Galvin *et al.*, 2017).

2.2. Aproximación histórica a la degeneración lobular frontotemporal

En 1892, Arnold Pick describió el caso de un hombre de 71 años con un progresivo deterioro cognitivo caracterizado por una severa alteración del lenguaje o afasia cuyo estudio post mortem mostraba una marcada atrofia en el lóbulo temporal izquierdo (Pick, 1892). En los años siguientes Pick describió varios casos más, todos ellos caracterizados por afasia y atrofia cerebral circunscrita a los lóbulos frontales y temporales en el estudio anatomopatológico (Pick, 1904; Pick *et al.*, 1995).

Años más tarde, en 1911, Alois Alzheimer describió algunas de las características neuropatológicas propias de la enfermedad: la atrofia cerebral focal, la presencia de inclusiones citoplasmáticas argirófilas (cuerpos de Pick) y las neuronas abalonadas (células de Pick) (Alzheimer, 1911) (Figura 2). En 1922 se propuso el nombre de enfermedad de Pick para referirse a esta nueva entidad (Gans, 1922).

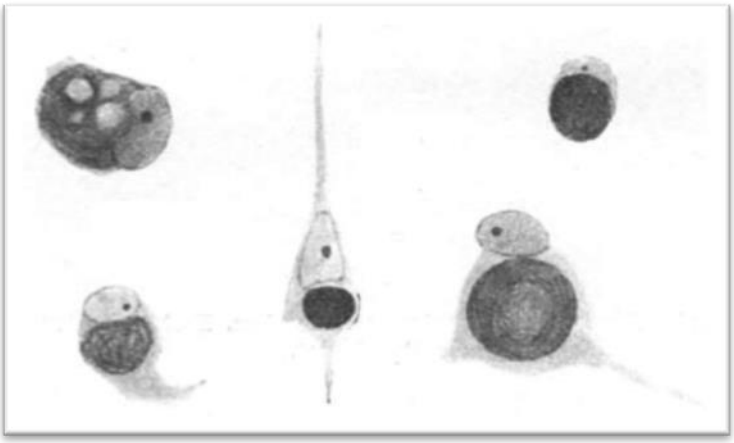


Figura 2: Ilustración de los cuerpos de Pick por Alois Alzheimer
(*Z Gesamte Neurol Psychiatr* 1911; 4: 356-85)

A pesar de estas certeras y precoces descripciones de la enfermedad, el avance científico en la DLFT quedó prácticamente estancado durante décadas. No fue hasta la década de los 80 cuando se volvieron a realizar avances relevantes en la investigación de esta enfermedad. En esta década, los grupos de Lund y Manchester describieron extensas series clínico-patológicas de pacientes con demencia en las que se halló que una proporción relevante de pacientes sufría una atrofia circunscrita a los lóbulos frontales (Neary *et al.*, 1986; Brun, 1987; Gustafson, 1987). Aunque con importantes similitudes neuropatológicas, solo una pequeña parte de éstos presentaban las características distintivas de la enfermedad de Pick (cuerpos y células de Pick). Estos trabajos fueron de vital importancia para la descripción de los aspectos clínicos asociados a la degeneración de los lóbulos frontales. En 1994 los grupos de Lund y Manchester propusieron el nombre de demencia frontotemporal (DFT) para esta entidad (Neary *et al.*, 1994).

Paralelamente, el doctor Marcel Mesulam describió en 1982 una serie de 6 pacientes con una historia de trastorno progresivo del lenguaje en ausencia de otros signos de demencia (Mesulam, 1982). A partir de este trabajo, en los años siguientes se describieron multitud de pacientes con las mismas características, los cuales pasaron a ser denominados bajo el diagnóstico sindrómico de afasia primaria

progresiva (APP). Sin embargo, pronto se hizo claramente manifiesto que el término APP se utilizaba para describir pacientes con características diferentes, por lo que se distinguieron dos síndromes en función del tipo de problema del lenguaje: la APP variante no fluente (APPvnf) y la APP variante semántica (APPvs). El primero de estos síndromes se caracterizaba por un habla no fluente y dificultosa, con apraxia verbal y agramatismo. En el segundo en cambio, los pacientes mostraban un lenguaje fluente pero vacío de contenido dada la pérdida gradual de memoria semántica (Davies *et al.*, 2005; Kertesz *et al.*, 2010; Grossman, 2012). Posteriormente se añadió un tercer síndrome, la APP variante logopénica (APPvl), caracterizado por un lenguaje logopénico, con problemas en la evocación de palabras en la denominación y en el lenguaje espontáneo (Gorno-Tempini *et al.*, 2004, 2008). No obstante, hoy en día este síndrome no se suele considerar dentro del grupo de las DLFT, dado que su sustrato neuropatológico más habitual es el de la enfermedad de Alzheimer.

2.3. Síndromes clínicos de la DLFT

Pronto se evidenció que la distinción entre los síndromes clínicos de DFTvc, APPvnf y APPvs era una simplificación pues a menudo los pacientes con DFTvc presentaban problemas de lenguaje y los pacientes con APPvnf o APPvs problemas de conducta. En este sentido, en 1998 se desarrollaron unos criterios diagnósticos que bajo el nombre de DLFT englobaban los tres síndromes clínicos (Neary *et al.*, 1998). En 2011 se actualizaron estos criterios diagnósticos en los que actualmente siguen vigentes (Gorno-Tempini *et al.*, 2011; Rascovsky *et al.*, 2011).

2.3.1. Demencia frontotemporal variante conductual

La DFTvc es la forma de presentación más frecuente de las DFT. Las manifestaciones clínicas de este síndrome comportan cambios progresivos en la personalidad, la cognición social y las funciones ejecutivas. Estos síntomas son debidos a la neurodegeneración de los lóbulos frontales y temporales del cerebro, pudiendo encontrarse atrofia en estas regiones en las pruebas de neuroimagen. La tabla 1 muestra los actuales criterios diagnósticos de este síndrome (Rascovsky *et al.*, 2011).

I. Deterioro progresivo conductual y/o cognitivo objetivado por observación o anamnesis
II. DFTvc posible: Tres de los siguientes síntomas (A-F) deben estar presentes:
A. Desinhibición en fases iniciales. Uno de los siguientes debe estar presente:
A.1.Comportamiento social inapropiado
A.2. Pérdida de maneras y decoro
A.3. Impulsividad, actuaciones sin meditar consecuencias
B. Apatía o inercia en fases iniciales. Uno de los siguientes debe estar presente:
B.1. Apatía
B.2. Inercia
C. Pérdida de empatía en fases iniciales. Uno de los siguientes debe estar presente:
C.1. Menor respuesta a necesidad y sentimientos de otras personas
C.2. Menor interés social, interrelaciones y amabilidad
D. Comportamientos perseverativos, esterotípicos o compulsivos/ritualísticos. Uno de los siguientes debe estar presente:
D.1. Movimientos simples repetitivos
D.2. Comportamientos reflejos compulsivos o ritualísticos
D.3. Habla esterotipada
E. Hiperoralidad y cambios en la dieta. Uno de los siguientes debe estar presente:
E.1. Cambios en la preferencia de alimentos
E.2. Ingesta compulsiva, incremento del consumo de enol o tabaco
E.3. Exploración oral o consumo de objetos no comestibles
F. Perfil neuropsicológico: déficits disejecutivos con relativa preservación de la memoria y funciones visuoespaciales. Todos los siguientes deben estar presentes:
F.1. Déficits en tareas ejecutivas
F.2. Relativa preservación de la memoria episódica
F.3. Relativa preservación de las habilidades visuoespaciales
III. DFTvc probable: Todos los siguientes (A-C) deben estar presentes:
A. Cumple criterios de DFTvc posible
B. Presenta declive funcional significativo (evidenciado por cuidador o escalas funcionales)
C. Neuroimagen consistente con el diagnóstico. Uno de los siguientes debe estar presente:
C.1. Atrofia frontal y/o temporal anterior en RMN o TC
C.2. Hipoperfusión o hipometabolismo frontal y/o temporal anterior en SPECT o PET
IV. DFTvc definitiva con patología de DLFT. El criterio A y los criterios B o C deben estar presentes.
A. Cumple criterios de DFTvc posible o probable
B. Evidencia hispopatológica de DLFT
C. Presencia de una mutación patogénica
V. Criterios de exclusión: Los criterios A y B no se deben cumplir en cualquier diagnósitco de DFTvc. El criterio C puede ser positivo para DFTvc posible, pero ha de ser negativo para DFTvc probable:
A. El patrón de déficits se explica mejor por otros trastornos médicos o neurológicos
B. La alteración conductual se explica mejor por un diagnóstico psiquiátrico
C. Biomarcadores altamente sugestivos de enfermedad de Alzheimer u otros procesos neurodegenerativos.

Tabla 1: criterios diagnósticos de Rascovsky et. al. 2011 para demencia frontotemporal variante conductual

2.3.2. Afasias primarias progresivas

Las APP engloban un grupo de síndromes de naturaleza neurodegenerativa que se caracterizan por el deterioro progresivo de las capacidades lingüísticas. Los actuales criterios diagnósticos diferencian entre

tres formas de APP en función de los dominios lingüísticos afectados (Gorno-Tempini *et al.*, 2011). La tabla 2 muestra los criterios clínicos de cada uno de estos síndromes.

Criterios diagnósticos para el diagnóstico de APP			
Criterios de inclusión	1. El síntoma clínico principal es el problema para el lenguaje. 2. Estos déficits son el principal motivo que afecta a las actividades de la vida diaria. 3. La afasia es el síntoma prominente en la presentación y las fases iniciales de la enfermedad.		
Criterios de exclusión	1. Los déficits están mejor explicados por otra enfermedad médica o neurológica. 2. Los problemas cognitivos están mejor explicados por un diagnóstico psiquiátrico. 3. Afectación mnésica o visuoperceptiva relevante al inicio de la enfermedad. 4. Alteración conductual relevante al inicio de la enfermedad		
Criterios diagnósticos de las variantes de APP			
	APP vnf	**APP vs**	**APP vl**
Criterios mayores	Uno de los dos siguientes: 1. Agramatismo 2. Apraxia del habla	Dos de los siguientes: 1. Afectación de la denominación por confrontación visual 2. Afectación de la comprensión de palabras aisladas	Dos de los siguientes: 1. Dificultad para evocar palabras aisladas en la conversación espontánea y en la denominación 2. Alteración de la repetición de frases largas
Criterios menores	Dos de los tres siguientes: 1. Afectación de la comprensión de frases sintácticamente complejas 2. Comprensión de palabras aisladas respetada 3. Reconocimiento de objetos respetado	Tres de los cuatro siguientes: 1. Reconocimiento de objetos alterado, particularmente de aquellos poco familiares 2. Dislexia o digrafía de superficie 3. Repetición conservada 4. Ausencia de agramatismo y de apraxia del habla	Tres de los cuatro siguientes: 1. Errores fonológicos en la conversación espontánea y en la denominación 2. Comprensión de palabras aisladas y reconocimiento de objetos respetados 3. Ausencia de agramatismo 4. Ausencia de apraxia del habla
Criterios de apoyo por imagen	Atrofia predominante en la región frontal posterior-insular izquierda en RM. Hipoperfusión o hipometabolismo en la región frontal posterior-insular izquierda en el SPECT o PET	Atrofia predominante en la región temporal anterior izquierda en RM. Hipoperfusión o hipometabolismo en la región temporal anterior izquierda en el SPECT o PET	Atrofia predominante en la región perisilviana posterio-parietal izquierda en RM. Hipoperfusión o hipometabolismo en la región perisilviana posterio-parietal izquierda en el SPECT o PET

Tabla 2: Criterios diagnósticos de ML Gorno – Tempini et al 2011 para afasia primaria progresiva y sus variantes

Cuando se cumplen los criterios clínicos de APP, pero no se cumplen los de ninguno de los subtipos o se cumplen los de varios de ellos, se clasifica como APP indeterminada.

Varios estudios de correlación clínicopatológica muestran que dos de estos síndromes, la APPvs y la APPvnf, se corresponden, en la mayoría de los casos, a formas de DLFT. Sin embargo, la APP variante

logopénica (APPvl) suele asociarse con una neuropatología de enfermedad de Alzheimer, por lo que es considerada una forma de presentación atípica esta enfermedad (Grossman, 2010; Spinelli *et al.*, 2017).

2.3.3. Síntomas motores: parálisis supranuclear progresiva, síndrome corticobasal y enfermedad de motoneurona

Además de los síndromes cognitivos clínicos clásicos, los pacientes con DLFT pueden presentar síntomas motores como parkinsonismo o enfermedad de motoneurona ya sea durante la evolución, de forma añadida al síndrome principal, o como presentación clínica.

El parkinsonismo puede aparecer en aproximadamente un 22% de los pacientes con DLFT (Padovani *et al.*, 2007) presentándose en la mayoría de los casos como un síndrome de parálisis supranuclear progresiva (S-PSP) o un síndrome corticobasal (SCB) (Kertesz *et al.*, 2000). Cabe destacar que los últimos criterios diagnósticos tanto del S-PSP como del SCB ya consideran las manifestaciones cognitivas frontales y de lenguaje como parte de los síndromes clínicos (Armstrong *et al.*, 2013; Höglinger *et al.*, 2017). El solapamiento entre el S-PSP, el SCB y la DLFT no sólo es clínico, sino también genético y neuropatológico. En este sentido, mutaciones en los genes de *microtubule-associated protein tau (MAPT)* y *progranulina* (*GRN)*, causantes de demencia frontotemporal con parkinsonismo ligada al cromosoma 17 (DFTP-17), pueden producir tanto los síndromes clínicos de DFT como S-PSP o SCB (Boeve and Hutton, 2008). Además, el depósito anómalo de proteína tau en sus isoformas de 4 repeticiones en el cerebro es el substrato neuropatológico de la mayoría de S-PSP y SCB, así como también de aproximadamente la mitad de los casos de DLFT (Forrest *et al.*, 2019).

Por otro lado, la esclerosis lateral amiotrófica (ELA) es un trastorno neurodegenerativo que causa principalmente la muerte de las neuronas motoras del cerebro y la médula espinal, provocando debilidad de los músculos inervados por éstas (Al-Chalabi *et al.*, 2016). Desde hace décadas existen trabajos que correlacionan la ELA con la DLFT (Hudson, 1981; Neary *et al.*, 1990; Burrell *et al.*, 2011). En los últimos años, multitud de descubrimientos genéticos y neuropatológicos han enfatizado la relación entre estas dos entidades clínicas. En 2006, Neumann et. al. descubrieron que la proteína TAR DNA binding protein 43kDA (TDP-43) era la proteína patológicamente agregada en la mayoría de los casos de

DLFT no tau y en la gran mayoría de los casos de ELA (Neumann *et al.*, 2006). Años más tarde, se descubrió que otra proteína, la proteína FUS (Fused in Sarcoma), también podía encontrarse patológicamente agregada en una minoría de casos de DLFT y ELA (Neumann et al. 2009). Desde el punto de vista genético, en los últimos años se han descubierto multitud de genes implicados en la fisiopatología de ambas entidades. El más frecuente de ellos está localizado en el cromosoma 9 y consiste en una expansión intrónica del hexaplete GGGGCC de la región *open reading frame 72* (*C9orf72*), y es la principal causa genética tanto de DLFT como de ELA (DeJesus-Hernandez *et al.*, 2011; Renton *et al.*, 2011). Otros genes, como *TANK-binding kinase 1* (*TBK1)*, *Transactive response DNA-binding protein* (*TARDBP)*, *Valosin Containing Protein* (*VCP)* o *Fused in Sarcoma* (*FUS)* también se han descrito en la fisiopatología de ambas entidades (Weishaupt *et al.*, 2016; Greaves and Rohrer, 2019). Gracias a estos descubrimientos, las entidades clínicas de la DLFT y la ELA son actualmente consideradas como dos extremos de un continuo de la misma enfermedad (Strong *et al.*, 2017).

En resumen, aunque inicialmente fueron descritas como trastornos de la conducta o del lenguaje, hoy sabemos que las DLFT pueden presentar una variedad de síntomas y signos mucho más amplia. Los trabajos observacionales de las últimas décadas han hecho evidente que existe, además, un importante solapamiento clínico, patológico y genético con otros síndromes clásicos como la ELA, el S-PSP y el SCB.

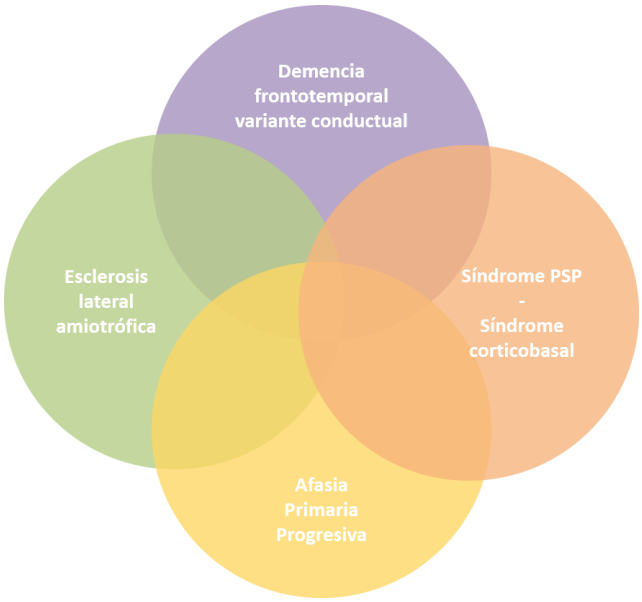


Figura 3: Solapamiento entre los síndromes de demencia frontotemporal, esclerosis lateral amiotrófica y parkinsonismos atípicos.

2.4. Neuropatología: degeneración lobular frontotemporal

Los cambios neuropatológicos propios de la DLFT consisten en una marcada pérdida neuronal y gliosis cortical que predomina en los lóbulos frontales y temporales del cerebro. No obstante, otras áreas cerebrales como la corteza parietal, los ganglios de la base o el tronco del encéfalo, también se pueden encontrar afectadas. Típicamente se observa una mayor afectación de capas corticales superficiales que da lugar a una microvacuolización del tejido o espongiosis laminar superficial en las áreas afectadas.

Como otras enfermedades neurodegenerativas, las DLFT se caracterizan además por el depósito patológico de proteínas en las neuronas y células gliales del sistema nervioso que forman distintos tipos de inclusiones intracelulares y que permiten clasificar las DLFT en función de la proteína patológicamente agregada en: a) DLFT con depósito de proteína tau (DLFT-tau); b) DLFT con depósito de proteína TDP-43 (DLFT-TDP); c) DLFT con depósito de la familia de proteínas FET (DLFT-FET), que incluye las proteínas Fus in Sarcoma (FUS) , Ewing Sarcoma (EWS) y TATA-binding protein-associated factor 15 de la familia (TAF15) o d) DLFT con proteínas todavía no identificadas y con inclusiones reactivas únicamente para elementos del sistema ubiquitina proteasoma (DLFT-UPS) (Figura 4).

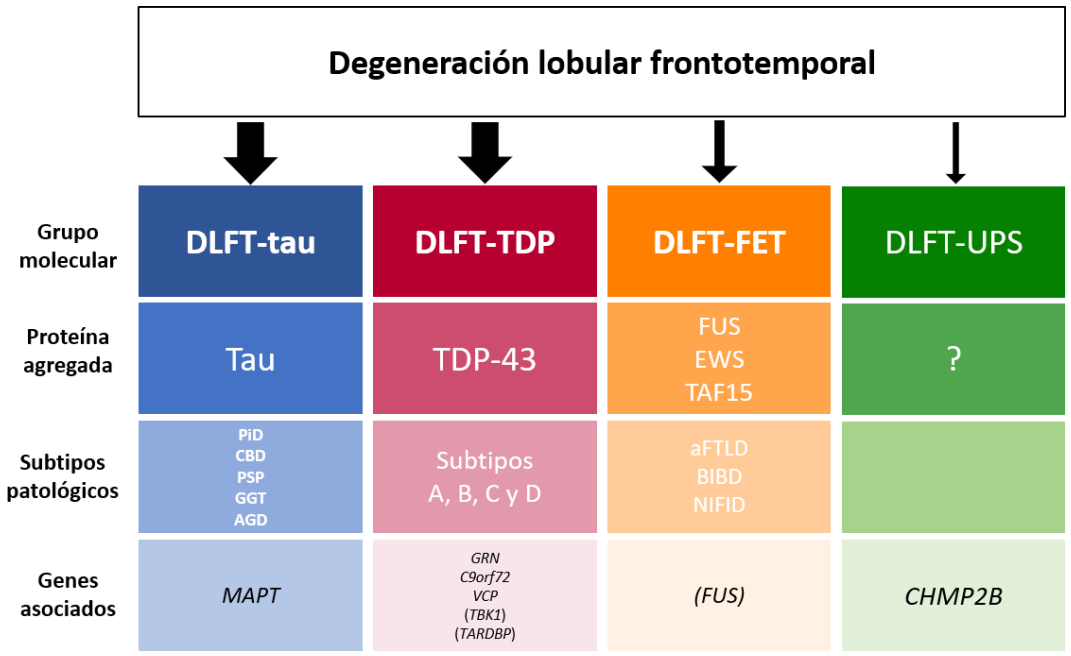


Figura 4: Clasificación molecular de la DLFT. Prácticamente todos los casos de DLFT se caracterizan por depósitos anormales de proteínas, pudiendo ser clasificados en DLFT-tau, DLFT-TDP y DLFT-FET. En los escasos casos de DLFT-UPS se aprecian inclusiones sólo reactivas para proteínas del sistema ubiquitina proteasoma (UPS por sus siglas en inglés). En función de la morfología y distribución de los depósitos, cada subtipo molecular se subclasifica en subtipos patológicos. Por último, mutaciones en determinados genes se asocian a subtipos moleculares diferentes de DLFT. Imagen extraída y adaptada de M Neumann y IAR Mackenzie, 2019.

2.4.1. Degeneración lobular frontotemporal asociada a depósitos de proteína tau (DLFT-tau)

La proteína tau es una proteína microtubular cuya principal función es la estabilización de los microtúbulos de los axones neuronales, aunque recientemente se considera también responsable de proteger las regiones dinámicas de los microtúbulos para que puedan elongarse y permanecer en movimiento continuo (Spillantini and Goedert, 2013; Qiang *et al.*, 2018). Es codificada por el gen *MAPT* en el cromosoma 17. En el cerebro humano existen seis isoformas de proteína tau en función del splicing alternativo de los exones 2, 3 y 10. Estructuralmente estas seis isoformas difieren en el número de residuos amino-terminales (entre 0 y 2 dependiendo de la codificación de los exones 2 y 3) y en el número de repeticiones del dominio de unión a microtúbulos (3 o 4 repeticiones - 3R y 4R - en función de la codificación del exón 10) (Figura 5).

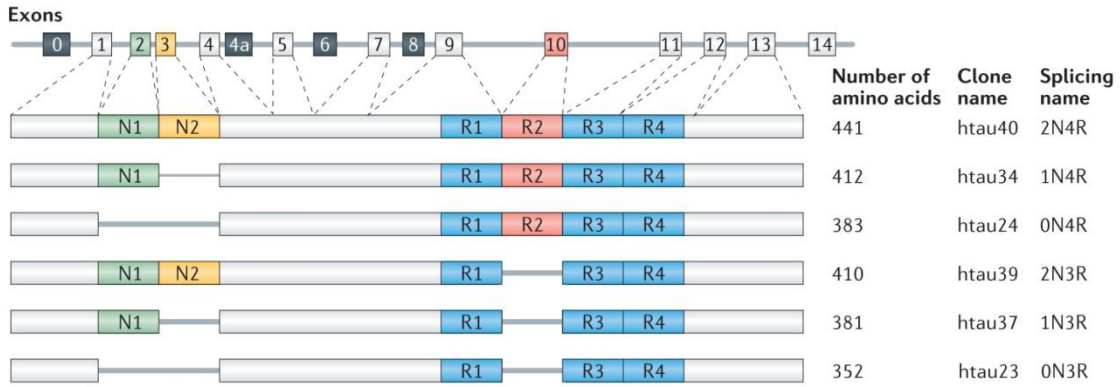


Figura 5: El gen *MAPT* humano y las seis isoformas de proteína tau en el cerebro humano resultantes del splicing alternativo. Adaptado de Y. Wang and E Mandelkow, 2015.

Las DLFT-tau se subclasifican en varios subtipos neuropatológicos en función de: a) tipo de isoforma tau agregada (3R, 4R o ambas) y b) distribución, tipo y morfología de las inclusiones neuronales y sobre todo astrogliales (Figura 6). En función de estos aspectos la DLFT-tau se subdivide en taupatías 3R, representadas principalmente por la enfermedad de Pick (PiD), y en taupatías 4R, que incluyen principalmente la degeneración corticobasal (DCB), la parálisis supranuclear progresiva (PSP), la enfermedad de gránulos argirófilos (AGD por sus siglas en inglés) y la taupatía globular glial (GGT por sus siglas en inglés).

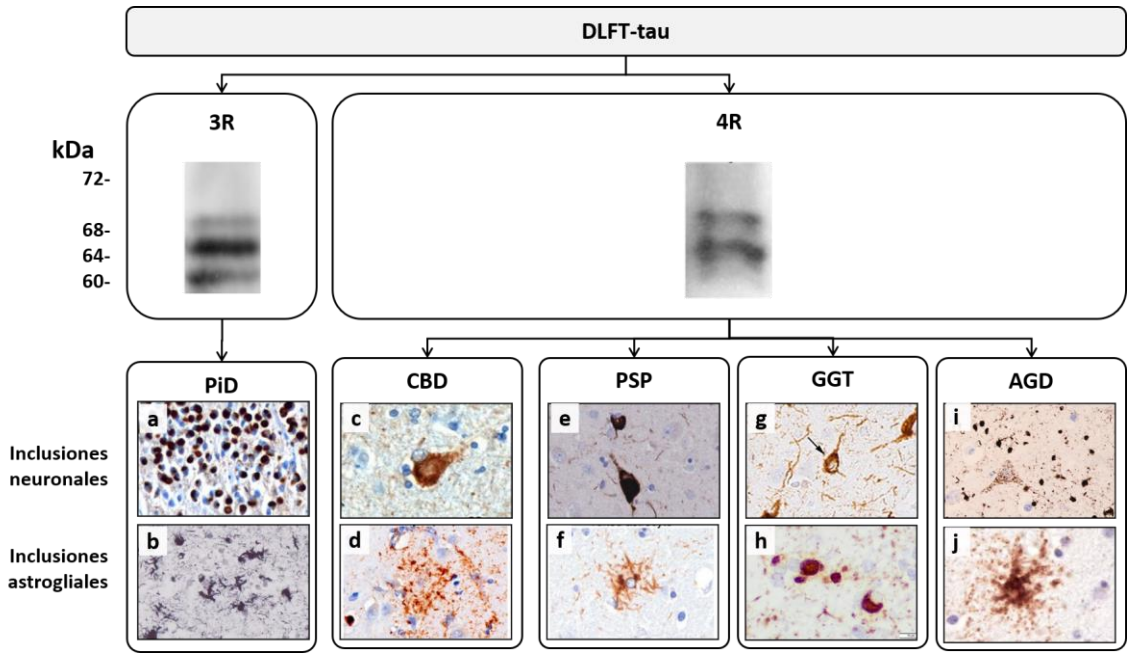


Figura 6: Clasificación neuropatológica de la DLFT-tau. En función de la isoforma de proteína tau acumulada (con tres o cuatro dominios de unión a microtúbulos) las DLFT-tau pueden subclasificarse en DLFT-tau de tres repeticiones (3R) o DLFT-tau de cuatro repeticiones (4R). La forma más frecuente de DLFT-tau de 3R es la enfermedad de Pick (PiD). En función de las características morfológicas de las inclusiones neuronales y astrogliales, las DLFT-tau de 4R pueden clasificarse en degeneración corticobasal (DCB), parálisis supranuclear progresiva (PSP), Taupatía globular glial (GGT) o enfermedad de gránulos argirófilos (AGD).

Enfermedad de Pick (PiD)

La enfermedad de Pick se caracteriza macroscópicamente por una muy severa atrofia frontal y temporal típicamente descrita como en "filo de cuchillo" (Yokota *et al.*, 2009). Histopatológicamente se aprecian inclusiones citoplasmáticas neuronales redondas y grandes, conocidas como cuerpos de Pick, compuestas por proteína tau 3R (Arai T *et al.*, 2001; Dickson, 2001). También se pueden observar inclusiones astrocitarias y pequeñas oligodendrogliales. Adicionalmente se presentan también neuronas abalonadas en la corteza cerebral, conocidas como neuronas de Pick (Kovacs, 2015). Clínicamente estos pacientes debutan con DFTvc y, menos a menudo, en las formas de APPvs y APPvnf (Hodges *et al.*, 2004; Davies *et al.*, 2005).

Parálisis supranuclear progresiva (PSP)

La PSP se caracteriza por la atrofia del núcleo subtalámico y globo pálido, del tegmento mesencefálico con despigmentación de la sustancia negra y del núcleo dentado del cerebelo. Desde el punto de vista microscópico se observa la presencia de ovillos neurofibrilares sobre todo en estructuras subcorticales, particularmente en núcleo subtalámico, globo pálido, sustancia negra, locus coeruleus, rafe y núcleos pontinos (Hauw *et al.*, 1994; Forrest *et al.*, 2019). Estos ovillos neurofibrilares suelen presentar una forma globosa. Además de estas inclusiones neuronales, son típicas las inclusiones astrogliales en penacho, que son esenciales para realizar el diagnóstico de la PSP. También son frecuentes las inclusiones oligodendrogliales en forma de coma (coiled bodies). Todas estas inclusiones son inmunorreactivas para proteína tau 4R (Buée and Delacourte, 1999; Dickson *et al.*, 2007).

En contra de lo que el nombre podría sugerir, estos hallazgos neuropatológicos no siempre se correlacionan con el síndrome clínico también conocido como S-PSP. La sintomatología depende de la distribución de la patología y comprende los síndromes DFTvc, APPvnf, S-PSP y SCB (Williams and Lees, 2009).

Degeneración corticobasal (DCB)

Los hallazgos macroscópicos característicos de la DCB muestran importante atrofia cortical asimétrica, frecuente atrofia del núcleo caudado, adelgazamiento del cuerpo calloso, dilatación ventricular y despigmentación de la sustancia negra. Desde el punto de vista histológico destacan inclusiones neuronales (con abundantes pretangles y abundantes procesos celulares, sobre todo en sustancia blanca), placas astrocíticas e inclusiones citoplasmáticas oligodendrogliales (coiled bodies / inclusiones helicoidales) argirófilas (bien visibles en la técnica de impregnación de plata según Gallyas) y tau positivas. Como en la PSP, estos depósitos de proteína tau están formados predominantemente por isoformas de 4R (Dickson *et al.*, 2002; Kovacs, 2015).

La correlación clínica de esta patología corresponde en algunos casos, pero no en todos, a un SCB. Otras presentaciones clínicas, como la DFTvc, la APPvnf o el S-PSP, no son infrecuentes (Josephs *et al.*, 2011).

Enfermedad por gránulos argirófilos (AGD por sus siglas en inglés)

Los hallazgos característicos de la AGD incluyen la presencia de gránulos argirófilos inmunoreactivos para tau 4R en estructuras temporo-mediales (Ferrer *et al.*, 2008). También suelen observarse neuronas abalonadas en amígdala, abundantes preovillos neuronales, astrocitos ramificados y cuerpos enrollados (inclusiones helicoidales) oligodendrogliales.

Se considera que la traducción clínica de la AGD es una demencia de inicio tardío. Sin embargo, no existe ninguna manifestación clínica específica que permita el diagnóstico en vida de la AGD, por lo que su diagnóstico sólo se puede realizar en el análisis post-mórtem (Saito *et al.*, 2004; Ikeda, 2008).

Taupatía globular glial (GGT por sus siglas en inglés)

La GGT es una taupatía recientemente descrita caracterizada por la presencia de inclusiones globulares oligodendrogliales Gallyas y tau 4R positivas, e inclusiones astrogliales positivas para tau 4R pero negativas en tinciones Gallyas (Ferrer *et al.*, 2014; Kovacs, 2015). Los criterios de consenso de la enfermedad establecen tres subtipos diferenciados por la localización y tipo de inclusiones y por la presentación clínica: el subtipo I se presenta predominantemente con afectación frontotemporal. En el subtipo II la patología se encuentra delimitada a la corteza motora y al tracto corticoespinal. Por último, en el subtipo III existiría tanto afectación frontotemporal como de la vía corticoespinal (Ahmed *et al.*, 2013).

Inicialmente la GGT fue considerada una enfermedad esporádica. No obstante, recientemente se ha descrito que diversas mutaciones en el gen *MAPT* pueden causar patología en forma de GGT (Tacik et al. 2017; Tacik et al. 2015; Zarranz et al. 2005).

Taupatía con inclusiones esferoidales hipocampales 4R

Recientemente se han descrito casos neuropatológicos de una nueva taupatía caracterizada por la presencia de inclusiones esferoidales en las neuronas del hipocampo (Miki *et al.*, 2009; Kovacs *et al.*, 2016). Estas inclusiones presentan una similitud morfológica con los cuerpos de Pick, pero se diferencian

en que presentan inmunoreactividad para tau 4R y no para tau 3R. En la mayoría de los casos, estas inclusiones se acompañan de esclerosis hipocampal y copatología de PSP. Aunque con muy pocos casos descritos, clínicamente los pacientes presentaron un cuadro de parkinsonismo y deterioro cognitivo.

2.4.2. Degeneración lobular frontotemporal y esclerosis lateral amiotrófica por depósito de TDP-43

La DLFT-tau representa aproximadamente el 40% de los casos de DLFT. Para designar los casos restantes de DLFT con inclusiones tau negativas - ubiquitina positivas se aplicó inicialmente el término DLFT-U. En 2006 dos trabajos independientes describieron tres patrones anatomopatológicos distintos de patología de DLFT-U en base a la distribución cortical, la morfología y la proporción relativa de inclusiones citoplasmáticas neuronales y neuritas distróficas en la tinción inmunohistoquímica para ubiquitina (Mackenzie *et al.*, 2006*a*; Sampathu *et al.*, 2006). Aunque con una nomenclatura diferente, los patrones descritos en estos dos trabajos eran prácticamente idénticos, lo cual reforzó notablemente la validez de las descripciones. Posteriormente se añadió un cuarto subtipo, correspondiente al patrón único de DLFT asociado a mutaciones en *VCP* y que clínicamente provoca miopatía por cuerpos de inclusión, enfermedad de Paget y demencia frontotemporal (Forman *et al.*, 2006). En 2011 se publicó el actual documento de consenso que agrupa y unifica los cuatro subgrupos (A, B, C y D) (Mackenzie *et al.*, 2011).

Paralelamente, la proteína TDP-43 fue identificada en 2006 como la proteína patológicamente agregada en la gran mayoría de casos de ELA y de DLFT-U (Neumann *et al.*, 2006). Este descubrimiento puso de manifiesto que estas dos entidades son probablemente dos extremos del espectro clínico-patológico de una misma enfermedad. En 2007 se aprobó un nuevo consenso en el que se denominó al subgrupo de DLFT con acumulación de TDP-43 como DLFT-TDP (Cairns *et al.*, 2007). En la mayoría de las series neuropatológicas, DLFT-TDP es el subgrupo molecular más frecuente de la DLFT, correspondiendo aproximadamente al 50% de los casos de DLFT. En los pacientes con diagnóstico de ELA esta proporción es incluso mayor, siendo TDP-43 la proteína patológicamente agregada hasta en un 95% de los casos (Arai *et al.*, 2006).

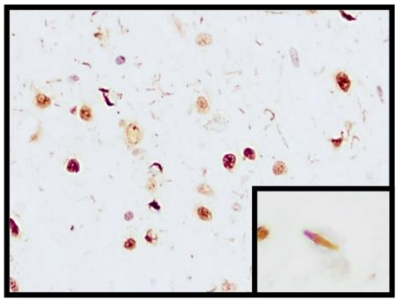
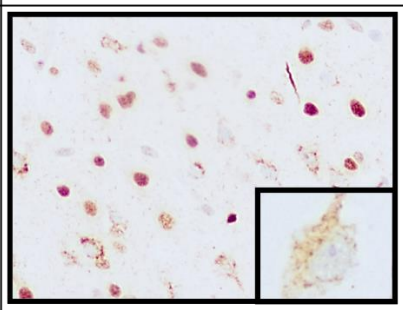
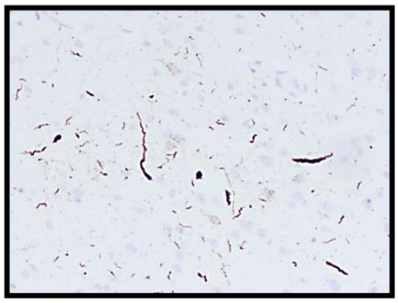
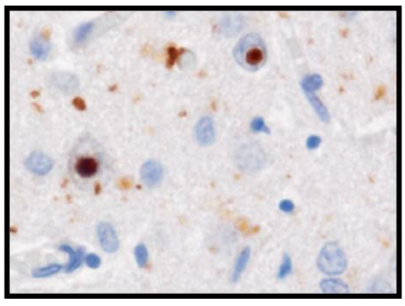
		Neuropatología	Fenotipo clínico	Mutaciones
Tipo A		• Inclusiones citoplasmáticas neuronales compactas • Neuritas distróficas • Inclusiones intranucleares neuronales lentiformes • Predominio II capa cortical	DFTvc APPvnf SCB	*GRN*
Tipo B		• Inclusiones citoplasmáticas neuronales granulares • Escasas neuritas distróficas cortas • Todas las capas corticales	DFTvc DFT-ELA	*C9orf72*
Tipo C		• Neuritas distróficas largas • Escasas inclusiones citoplasmáticas neuronales	PPAsv DFTvc	
Tipo D		• Neuritas distróficas cortas • Abundantes inclusiones intranucleares neuronales • Escasas inclusiones citoplasmáticas neuronales	IBMPFD familiar	*VCP*

Figura 7: Clasificación neuropatológica de las DLFT-TDP adaptado de Mackenzie and Neumann 2017.

Recientemente se ha propuesto una modificación de la clasificación neuropatológica para las DLFT-TDP basada en la inmunohistoquímica para TDP-43 en lugar de la de ubiquitina, puesto que ésta permite una mejor valoración morfológica de las inclusiones patológicas (Mackenzie and Neumann, 2017). Esta clasificación propone 4 subtipos en base a la morfología y distribución de las inclusiones TDP-43 en las distintas capas de la corteza cerebral. El subtipo A se caracteriza por inclusiones neuronales citoplasmáticas compactas, neuritas distróficas cortas e inclusiones intranucleares lentiformes de

predominio en la capa II cerebral. El subtipo B se caracteriza por la presencia de inclusiones neuronales citoplasmáticas granulares con presencia de escasas neuritas distróficas en todas las capas cerebrales. El subtipo C se caracteriza típicamente por neuritas distróficas alargadas con escasas inclusiones neuronales en todas las capas cerebrales. Por último, el subtipo D se caracteriza por la presencia de abundantes inclusiones neuronales intranucleares (Figura 7).

2.4.3. Degeneración lobular frontotemporal y esclerosis lateral amiotrófica por depósito de FUS

En 2009, dos estudios independientes identificaron que mutaciones en el gen *Fused in Sarcoma* (*FUS*) en el cromosoma 16 eran la causa de algunos casos de ELA familiar (Kwiatkowski *et al.*, 2009; Vance *et al.*, 2009). Los casos con esta mutación presentaban depósito patológico de proteína FUS pero no de TDP-43 en la neuropatología, por lo que pasaron a clasificarse como ELA-FUS.

La proteína FUS, codificada por el gen del mismo nombre, pertenece a la familia de proteínas FET, que es un acrónimo de FUS, EWS (Erwing sarcoma) y TAF15. Estas tres proteínas, inicialmente implicadas en la oncogénesis tumoral, tienen, al igual que la proteína TDP-43, una función de unión y procesamiento del RNA y guardan una importante similitud fisiopatológica con TDP-43 (Law *et al.*, 2006).

El descubrimiento de la implicación de FUS en la ELA motivó la investigación de su relación con la DLFT. De este modo se descubrió que FUS era el marcador patológico en la mayoría de casos de DLFT negativas para tau y TDP43, incluyendo las entidades conocidas como DLFT-U atípica, *neuronal intermediate filament inclusion disease* (NIFID) y *basophilic inclusion body disease* (BIBD) (Munoz *et al.*, 2009, Neumann *et al.*, 2009*a*, *b*; Urwin *et al.*, 2010; MacKenzie *et al.*, 2011).

El descubrimiento del nuevo subgrupo molecular de las DLFT-FUS proporcionó nueva evidencia en torno a que la DLFT y la ELA son entidades estrechamente relacionadas. No obstante, en los últimos años se han descrito diferencias tanto genéticas como moleculares entre la DLFT-FUS y la ELA-FUS. Desde el punto de vista genético, la mayoría de los casos de ELA-FUS descritos en la bibliografía son causados por mutaciones en el gen *FUS*, mientras que la mayoría de los casos de DLFT-FUS son esporádicos. Desde el punto de vista molecular, Neumann et al evidenciaron en varios estudios que mientras que los casos de ELA-FUS solo mostraban acumulación patológica de la proteína FUS, los casos de DLFT-FUS presentaban también co-acumulación del resto de proteínas FET (EWS y TAF15), así como de su transportador

nuclear: transportin 1(Trnp1) (Neumann *et al.*, 2011, 2012). Por este motivo, se ha propuesto renombrar estos casos de DLFT como DLFT-FET, manteniendo el término de ELA-FUS para los casos asociados a mutación de FUS. Para los autores de estos estudios, estas evidencias ponen de manifiesto que existen mecanismos fisiopatológicos diferentes entre la ELA-FUS y la DLFT-FET (Figura 8) (Rademakers *et al.*, 2012). No obstante, recientemente otros grupos han reportado casos aislados de DLFT por mutación en FUS y casos de ELA-FUS esporádicos (acumulando estos últimos el resto de proteínas FET), lo cual deja abierto el debate de si estas entidades comparten mecanismos fisiopatológicos (Broustal *et al.*, 2010; Van Langenhove *et al.*, 2010; Yan *et al.*, 2010; Fujita *et al.*, 2011; Matsuoka *et al.*, 2011; Huey *et al.*, 2012; Takeuchi *et al.*, 2013).

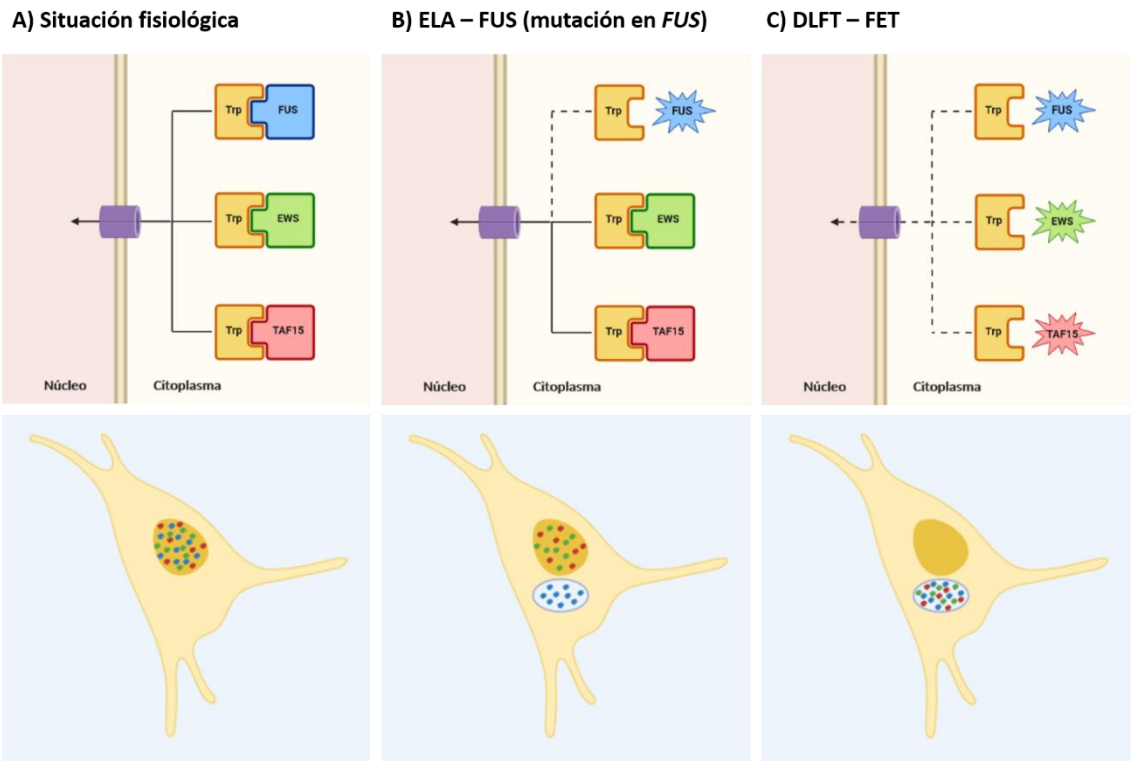


Figura 8: Mecanismos fisiopatológicos propuestos para la ELA-FUS y DLFT-FET. La familia de proteínas FET (FUS, EWS, TAF15) se unen al transportador Transportin 1 (Trp) que media el transporte de estas proteínas al interior del núcleo celular donde se encuentran de forma fisiológica (A). Los casos de ELA-FUS producidos por mutaciones en el gen *FUS*, impiden la interacción de la proteína resultante impidiendo su unión a Trp y su entrada al núcleo, provocando una inclusión citoplasmática de FUS, pero no de EWS ni de TAF15 (B). Los casos de DLFT-FET, muestran coacumulación de las tres proteínas FET en las inclusiones citoplasmáticas, lo que sugiere una alteración en las tres proteínas o en Trp (C).

2.5. Genética de la DLFT y la ELA

La DLFT abarca un grupo de enfermedades con una importante carga genética. Hasta un 30 – 50 % de los casos presentan antecedentes familiares de demencia, parkinsonismo y/o enfermedad de motoneurona (Rohrer *et al.*, 2009). En alrededor de un 15 – 20% de los casos, esta heredabilidad viene explicada por la presencia de una mutación patogénica que causa la herencia de la enfermedad en un patrón autosómico dominante. La mayoría de estos casos familiares son debidos a mutaciones en tres genes: *MAPT*, *GRN* y *c9orf72*. En los últimos años, un gran número de otros genes se han asociado a DLFT genéticamente determinada: *TBK1*, *FUS*, *TARDBP*, *VCP*, *Charged multivesicular body protein 2B (CHMP2B)*, Sequestosome 1 (*SQSTM1)* u *Optineurin (OPTN)*. Algunos de estos genes (*C9orf72*, *FUS*, *TBK1*, *TARDBP*, *VCP, OPTN*) pueden ser también causa de ELA familiar.

El diagnóstico genético de las DLFT y la ELA es importante desde diversos puntos de vista. En primer lugar, el diagnóstico de una mutación causante de herencia autosómica dominante permite el acceso a un adecuado consejo genético a sus familiares directos. El conocimiento de la existencia de la mutación subyacente permite también predecir más adecuadamente el pronóstico tanto de los sujetos sintomáticos como de los portadores asintomáticos. Por ejemplo, los sujetos portadores de mutaciones en *GRN* presentan una menor supervivencia que los sujetos sin esta mutación, mientras que en los sujetos portadores de mutaciones en *MAPT* la edad de presentación muestra una importante correlación con la edad de presentación familiar (Moore *et al.*, 2019). Una buena caracterización de los portadores de mutaciones patogénicas (ya sean sujetos sintomáticos o portadores asintomáticos de la mutación) permitirá establecer biomarcadores de inicio y pronóstico de la enfermedad, herramientas imprescindibles para la evaluación de resultados terapéuticos en futuros ensayos clínicos.

Por otro lado, el conocimiento de una mutación subyacente es, junto al diagnóstico histológico, la única forma de determinar con total fiabilidad la neuropatología subyacente (tau, TDP-43 o FUS) a la enfermedad. La enorme heterogeneidad clínica de las DLFT y la ELA hace improbable que todos los pacientes puedan beneficiarse de un mismo tratamiento. Es de esperar que los futuros tratamientos modificadores de la enfermedad vayan dirigidos a las diferentes causas moleculares causentes de la enfermedad. A día de hoy, ya existen ensayos clínicos con terapias biológicas dirigidas a algunas de estas vías alteradas. Un adecuado conocimiento de las relaciones genotipo-fenotipo puede ayudar a la futura

estratificación de estos tratamientos: la identificación de una característica fenotípica específica a un determinado diagnóstico molecular o genético puede permitir la selección de los pacientes con mayor probabilidad de beneficiarse de tratamientos dirigidos a una diana terapéutica. Así pues, dado que el diagnóstico genético conlleva también el diagnóstico patológico subyacente, este abre una ventana de oportunidad a los tratamientos dirigidos a cambiar el curso de la enfermedad abordando estos mecanismos fisiopatológicos (Panza *et al.*, 2020).

Microtubule-associated protein tau (MAPT)

En 1998 se describieron por primera vez mutaciones en el gen *MAPT* en miembros de familias afectas de DLFT (Foster *et al.*, 1997; Hutton *et al.*, 1998; Poorkaj *et al.*, 1998; Spillantini *et al.*, 1998). Como se ha descrito anteriormente, el gen *MAPT,* codifica la proteína de unión a microtúbulos tau. En función del splicing alternativo del gen, existen 6 isoformas de tau que, según el número de dominios de unión a microtúbulos, se clasifican en isoformas de 3 o 4 repeticiones (Figura 5).

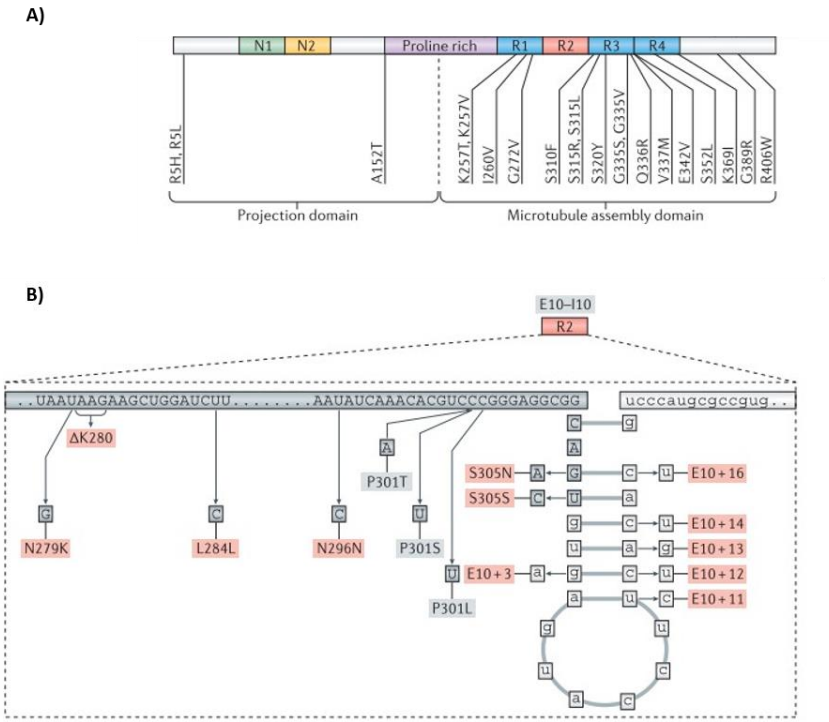


Figura 9: Representación de algunas de las mutaciones descritas en los exones e intrones del gen MAPT. A) La proteína Tau puede ser subdividida en dos dominios, el dominio amino terminal y el dominio de unión a microtúbulos. La mayoría de las mutaciones patogénicas se encuentran en este último dominio y se postula que afectan a la unión de la proteína con los microtúbulos. B) Mutaciones descritas en el exon e intrón 10 de MAPT. Las mutaciones sombreadas en cuadrado rojo se postulan que causan patogenicidad por la alteración en el splicing.

Adaptado de Y. Wang and E Mandelkow, 2015

En el momento actual se conocen más de 60 mutaciones patogénicas en el gen *MAPT* (Ghetti *et al.*, 2015; Moore *et al.*, 2019). Las mutaciones P301L, IVS10+16C>T, R406W y N279K engloban más del 50% de los casos descritos. La mayoría de las mutaciones en *MAPT* se producen dentro de la región de unión de tau al microtúbulo. Estas mutaciones causarían patogenicidad por alteración de la unión a microtúbulos. Otras mutaciones (especialmente las situadas en la secuencia intrónica y en el exón 10) causan patogenicidad por alteración del splicing alternativo del gen, lo que produciría un aumento patológico de la isoforma tau de 4 repeticiones.

El fenotipo clínico más frecuentemente asociado a mutaciones en *MAPT* es la DFTvc. También se han descrito presentaciones en forma de APP, tanto en forma de variante semántica como de variante no fluente/agramatical. Así mismo, no es infrecuente la presentación en forma de parkinsonismo atípico, ya sea cumpliendo criterios diagnósticos de PSP o de SCB. La presentación en forma de enfermedad de motoneurona, en cambio, es excepcional (Moore *et al.*, 2019). Una reciente revisión sugiere que las distintas mutaciones en *MAPT* pueden asociarse a fenotipos diferentes. Así, mientras las mutaciones P301L y R406W se asocian predominantemente al fenotipo de DFTvc, las mutaciones N279K se asociarían más frecuentemente a un fenotipo parkinsoniano (Moore *et al.*, 2019).

La edad media de inicio de la sintomatología es de 49.5 años (desviación estándar 10 años) con una duración media de la enfermedad de 9.3 años (desviación estándar 6.4 años). No obstante, al igual que el fenotipo clínico, la edad de presentación y la duración de la enfermedad pueden variar sustancialmente entre las distintas mutaciones del gen. Pacientes con la mutación R406W en el exón 13, por ejemplo, parecen presentar una edad de inicio significativamente más tardía (edad media de presentación 55.4 años) y de curso más lentamente progresivo (duración media de la enfermedad 16.9 años) (Moore *et al.*, 2019).

Las mutaciones en el gen *MAPT* causan DLFT con depósitos patológicos de proteína tau (DLFT-tau). No obstante, pueden existir variaciones importantes entre las distintas mutaciones e incluso entre individuos con la misma mutación (Tacik *et al.*, 2016), a pesar de compartir características comunes típicas de cada mutación.

En los actuales criterios de consenso neuropatológicos los casos de DLFT-tau son considerados un subtipo independiente (Cairns *et al.*, 2007). No obstante, recientemente Forrest et. al. han sugerido que los fenotipos neuropatológicos de las diferentes mutaciones en *MAPT* guardan importantes similitudes con los fenotipos esporádicos (PiD, PSP, DCB y GGT) y que la localización de la mutación seria determinante en dicho fenotipo neuropatológico (Forrest *et al.*, 2018). En este sentido, proponen una nueva clasificación neuropatológica de las DLFT-tau basada únicamente en el fenotipo del estudio neuropatológico, independientemente de la presencia o no de mutación en *MAPT*. La consideración de las DLFT-tau por mutaciones de *MAPT* como homólogos genéticos de las DLFT-tau esporádicas podría tener implicaciones importantes en el conocimiento molecular de estas enfermedades.

***Granulina* (*GRN*)**

En 2006 se identificó que mutaciones en *GRN,* gen también localizado en el cromosoma 17, son causa de DLFT genéticamente determinada (Baker *et al.*, 2006; Cruts *et al.*, 2006; Gass *et al.*, 2006). Este gen codifica la proteína progranulina, un factor de crecimiento con múltiples funciones. Hoy en día se conocen unas 130 mutaciones en *GRN* (Moore *et al.*, 2019). La mayoría de ellas provocan patogenicidad por pérdida de función del gen, causando así una haploinsuficiencia (Cruts and Van Broeckhoven, 2008).

Neuropatológicamente, las mutaciones en *GRN* causan DLFT-TDP del subtipo A presentando múltiples inclusiones neuronales citoplasmáticas e intranucleares (Mackenzie *et al.*, 2006*b*, 2011; Josephs *et al.*, 2007). Estas inclusiones son inmunoreactivas para TDP-43, pero no para progranulina, apoyando así el mecanismo fisiopatológico de haploinsuficiencia.

El mecanismo común de haploinsuficiencia explica que las diferentes mutaciones en *GRN* compartan un fenotipo clínico y patológico homogéneo. Típicamente, provocan una marcada atrofia fronto-temporo-parietal asimétrica (Whitwell *et al.*, 2009). La forma de presentación clínica más frecuente en las mutaciones de *GRN* es la DFTvc. Cuando la atrofia se presenta de forma más marcada en el hemisferio izquierdo, no es rara la forma de presentación en forma de APP, siendo ésta característicamente una forma mixta entre APP logopénica y APP no fluente (Rohrer *et al.*, 2010*a*). Así mismo, es relativamente frecuente su presentación en forma de SCB. La presentación en forma de enfermedad de motoneurona es excepcional (Moore *et al.*, 2019).

Los estudios de neuroimagen en pacientes con DLFT debida a mutaciones en *GRN* confirman el patrón de atrofia fronto-temporo-parietal con marcada asimetría (Beck *et al.*, 2008; Whitwell *et al.*, 2009). Varios estudios de neuroimagen realizados en portadores presintomáticos muestran resultados diversos. La mayoría de estos estudios no encuentra diferencias significativas entre la neuroimagen de portadores presintomáticos y sujetos controles (Borroni *et al.*, 2008, 2012; Moreno *et al.*, 2013; Caroppo *et al.*, 2015; Cash *et al.*, 2018; Olm *et al.*, 2018; Panman *et al.*, 2019), mientras que otros muestran diferencias estructurales hasta 15 años antes del inicio de los síntomas (Pievani *et al.*, 2014; Rohrer *et al.*, 2015).

La edad media de inicio de los síntomas es de 68.8 años (desviación estándar 9.8 años), pero existe una gran variabilidad en la edad de inicio entre individuos, incluso entre portadores de una misma mutación o entre miembros de una misma familia. Típicamente, la enfermedad sigue un curso rápido, causando una gran invalidez a los pocos años del inicio de la sintomatología y la muerte en 7.1 años de media (desviación estándar 3.9 años). No parecen existir diferencias en la edad de inicio y la duración de la enfermedad entre las distintas mutaciones en *GRN* (Moore *et al.*, 2019).

Expansión en *chromosome 9 open reading frame 72* (*C9orf72*)

En 2011, dos grupos de investigación descubrieron de forma paralela que la expansión de repeticiones del hexaplete GGGGCC en el intrón 1 del gen *cromosome 9 open reading frame 72 (C9orf72)* podía ser causa de DLFT y ELA genéticamente predeterminada (DeJesus-Hernandez *et al.*, 2011; Renton *et al.*, 2011). La población normal presenta entre 2 y 10 repeticiones de dicho hexaplete, considerándose patológica la presencia de más de 30 repeticiones. Varios estudios sitúan la expansión de *C9orf72* como la causa más frecuente de DLFT y de ELA genética en Europa y América del Norte, siendo rara en Asia u oriente Medio (Gijselinck *et al.*, 2012; Majounie *et al.*, 2012).

Las repeticiones de GGGGCC son bidireccionalmente transcritas en RNA repetitivo que, pese a encontrarse en una región no codificante del genoma, son traducidas a cinco tipos deferentes de repeticiones de dipéptidos (Mori *et al.*, 2013). Se postulan varios mecanismos fisiopatológicos en el desarrollo de la enfermedad, entre ellos, la pérdida de función de la proteína C9orf72, una ganancia de

función aberrante de esta proteína o una ganancia de función aberrante por parte de los dipéptidos (Balendra and Isaacs, 2018).

Tanto en su presentación en forma de DLFT como en su presentación en ELA, la expansión de *C9orf72* se asocia, neuropatológicamente, además de las inclusiones por dipéptidos, a depósitos de proteína TDP-43 (Mahoney *et al.*, 2012; Snowden *et al.*, 2012). La distribución de TDP-43 es la que determinará el fenotipo DLFT y/o ELA. En los casos con DLFT, el patrón morfológico de TDP-43 puede corresponder al tipo A o B de Mackenzie y Neumann.

Los fenotipos más frecuentes de presentación de la expansión en *C9orf72* son la DFTvc, la ELA o la combinación de ambas en forma de DFT-ELA (Snowden et al. 2012). Aunque menos frecuente, no es inhabitual la presentación en forma de APP (Moore *et al.*, 2019). La edad media de presentación de la enfermedad es de 58.4 años (desviación estándar 9.8 años). La duración media de la enfermedad hasta la muerte de 6.4 años (desviación estándar 4.8 años), pero presenta una gran variabilidad en función del síndrome clínico, siendo notablemente menor en los casos de inicio con enfermedad de motoneurona (2.9 años de media). Hoy en día, no existe evidencia de que el número de repeticiones de la expansión en *C9orf72* conlleve implicaciones clínicas , ni en la forma de presentación ni en la edad de inicio.

***Fused in Sarcoma* (*FUS*)**

Mutaciones en el gen *FUS,* localizado en el cromosoma 16, son causa de aproximadamente un 4% de las ELAs familiares (Kwiatkowski *et al.*, 2009; Vance *et al.*, 2009). Neuropatológicamente, estos casos presentan inclusiones patológicas de proteína FUS relativamente restringidas al sistema motor.

De forma mucho menos frecuente, también se han descrito mutaciones en *FUS* con presentación en forma de DLFT, pero se carece de confirmación neuropatológica de ningún caso (Van Langenhove *et al.*, 2010).

***TANK-binding kinase 1* (*TBK1*)**

En 2015 se identificaron por primera vez casos de ELA y DLFT causados por mutaciones en el gen *TBK1* (Cirulli *et al.*, 2015; Pottier *et al.*, 2015; Freischmidt *et al.*, 2017). En una serie de 482 pacientes con DLFT

o DFT-ELA y de 147 ELAS se encontraron mutaciones patogénicas en *TBK1* en un 1,1% de los casos de DLFT, 4.5% de los casos de DFT-ELA y en un 3,4% de las ELAS (Gijselinck *et al.*, 2015). La gran mayoría de las mutaciones en *TBK1* producen patogenicidad por pérdida de función de la proteína transcrita. La neuropatología subyacente muestra inclusiones patológicas de TDP-43, existiendo descripciones de DLFT-TDP de los subtipos A y B (Dols-Icardo *et al.*, 2018; Weinreich *et al.*, 2019).

Transactive response DNA-binding protein (TARDBP)

TARDBP es el gen que codifica la proteína TDP-43. El descubrimiento de mutaciones patogénicas en este gen se produjo a raíz de la identificación de TDP-43 como la proteína patológicamente agregada en la neuropatología de la mayoría de los casos de ELA y DLFT (Sreedharan *et al.*, 2008). No obstante, las mutaciones en *TARDBP* son raras y solo explican aproximadamente un 5% de los casos de ELA familiar, siendo su presentación en forma de DLFT excepcional (Borroni *et al.*, 2009; Gelpi *et al.*, 2014). Los pocos casos descritos con estudio neuropatológico parecen tener escasas inclusiones de TDP-43.

***Valosin Containing Protein* (*VCP*)**

Mutaciones en el gen *VCP* en el cromosoma 9 producen un síndrome familiar caracterizado por DLFT, miositis por cuerpo de inclusión y enfermedad de Paget (Watts *et al.*, 2004). Aproximadamente el 90% de los pacientes con mutación en *VCP* presenta miopatía, un 50% enfermedad de Paget y solo un 30% sufre DLFT. Dada esta penetrancia incompleta de los síntomas, es posible encontrar portadores con un fenotipo clásico de DFT. La neuropatología subyacente es una DLFT subtipo D (Forman *et al.*, 2006).

Charged multivesicular body protein 2B (CHMP2B)

Mutaciones en el gen *CHMP2B*, en el cromosoma 3.p11.2, producen DLFT con inclusiones immunopositivas para ubiquitina pero negativas para las proteínas tau, TDP-43 y FUS, por lo que se describen como DLFT-UPS (Skibinski *et al.*, 2005). Hoy en día se han descrito solo 4 mutaciones en 5 familias, siendo la DFTvc la forma de presentación más frecuente.

***Sequestosome 1* (*SQTSM1*)**

Mutaciones en el gen *SQSTM1*, el gen que codifica sequestosoma 1, la proteína p62 de unión a ubiquitina, se identificó inicialmente como causa de enfermedad de Paget (Laurin *et al.*, 2002). No obstante, en los últimos años se ha asociado también formas familiares de ELA y DLFT (Fecto *et al.*, 2011; Elisa *et al.*, 2012; Le Ber *et al.*, 2013). Los estudios neuropatológicos han descrito el depósito de TDP-43 en los casos de esta mutación, sin que se disponga todavía de la suficiente información como para agruparlos en un subtipo determinado.

***Optineurina* (*OPTN*)**

En 2010 se identificaron mutaciones en el gen *optineurina* (*OPTN*) como causa de ELA (Maruyama *et al.*, 2010). Posteriormente se evidenció que mutaciones en este gen también podían causar DLFT (Pottier *et al.*, 2015). Existen muy escasas descripciones de la anatomía patológica de pacientes con mutaciones en *OPTN*, que se asociaria al depósito anómalo de proteína TDP-43.

Transmembrane protein 106B (TMEM106B)

En 2010, un estudio de asociación del genoma completo (*genome-wide association study* or *GWAS)* en 515 pacientes con diagnóstico neuropatológico de DLFT-TDP halló una asociación significativa con un poliformismo de nucleótido único (*single nucleotid polymorfism* o *SNP*) en el gen *TMEM106B* en el cromosoma 7 (Van Deerlin *et al.*, 2010). Los hallazgos de este estudio sugieren que la presencia del haplotipo protector en *TMEM106B* protegería frente a la aparición de DLFT-TDP. Esta asociación con *TMEM106B* es especialmente fuerte en aquellos sujetos portadores de mutaciones en *GRN* donde se ha sugerido que, mediante una herencia autosómica recesiva, la presencia en homozigosis del alelo protector de *TMEM106B* protegería frente a la aparición de DLFT en los sujetos portadores de mutaciones en *GRN* (Finch *et al.*, 2011; Nicholson and Rademakers, 2016; Pottier *et al.*, 2018).

2.6. Correlaciones entre genotipo y fenotipos clínicos y neuropatológicos

El hecho que una forma de DLFT y/o ELA sea genéticamente determinada tiene una implicación relevante no sólo en el paciente, sino también en los familiares directos que se convierten en sujetos a riesgo de desarrollar la enfermedad en un futuro y/o transmitirla a su descendencia. Por este motivo, es

de vital importancia que las causas genéticas subyacentes se diagnostiquen de forma certera y lo más precozmente posible. Pese a la enorme heterogeneidad clínica, neuropatológica y genética en las DLFT, múltiples trabajos muestran que es posible encontrar correlaciones entre cada uno de estos aspectos de la enfermedad. La figura 10 representa algunas de estas correlaciones. Éstas, junto a la identificación de características fenotípicas específicas para determinadas mutaciones o genes mutados puede ayudar a los clínicos a determinar cuándo realizar el diagnóstico genético y a acotar los genes a estudiar en primer lugar. En el tiempo de las terapias biológicas en el que ya nos encontramos, el conocimiento de estas correlaciones genotipo-fenotipo puede también ayudar a seleccionar correctamente aquellos pacientes candidatos a ensayos clínicos terapéuticos para dianas fisiopatológicas específicas. Así mismo, la identificación de características fenotípicas específicas de ciertas mutaciones puede ayudar a desarrollar biomarcadores que permitan una mejor y más precoz identificación de los pacientes portadores de mutaciones, así como monitorizar los efectos terapéuticos de posibles ensayos clínicos.

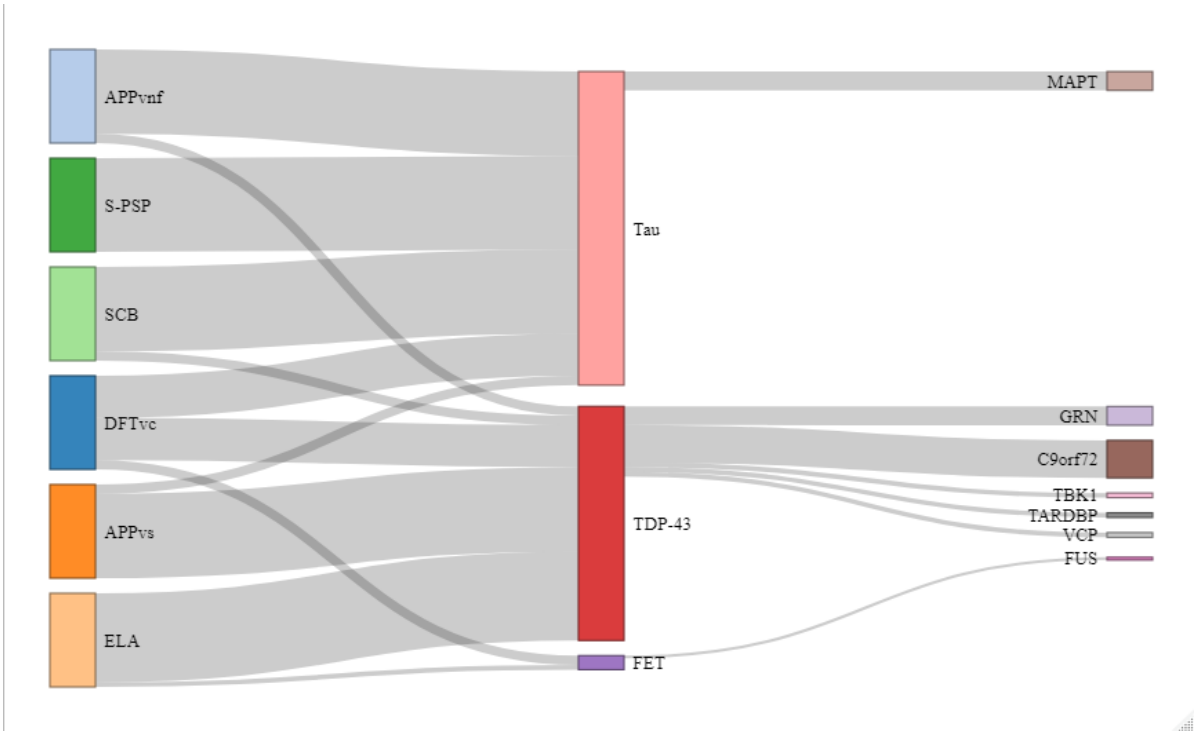


Figura 10: Representación de las correlaciones entre fenotipo clínico (izquierda), fenotipo neuropatológico (centro) y genotipo de las DLFT y la ELA (derecha).

2.7. Resumen

En resumen, la DLFT y la ELA son un grupo de enfermedades clínica, neuropatológica y genéticamente muy heterogéneas. Esta enorme heterogeneidad conlleva importantes dificultades en el diagnóstico y tratamiento de esta enfermedad. Los posibles futuros tratamientos modificadores de la enfermedad tendrán que ir dirigidos específicamente a los mecanismos fisiopatológicos subyacentes a cada uno de los distintos subtipos moleculares de DFT y/o ELA. Es por ello que, la identificación en vida de cada uno de estos subtipos moleculares es de vital importancia. A día de hoy, esto solo es posible en aquellos casos con una mutación patogénica demostrada. En este sentido, las DFT y la ELA genéticamente predeterminada son un importante modelo de enfermedad. El estudio de sujetos portadores de mutaciones abre la puerta a la identificación de biomarcadores clínicos, bioquímicos y de imagen específicos para cada grupo molecular. Además, la identificación de portadores presintomáticos de mutaciones patogénicas permite estudiar los primeros cambios fisiopatológicos propios de la enfermedad.

La presente tesis doctoral pretende estudiar y describir las relaciones genotipo – fenotipo en la DLFT y la ELA. El estudio de los fenotipos clínicos, de neuroimagen y neuropatológicos de la DLFT y la ELA y su relación con los diferentes determinantes genéticos causantes de la enfermedad puede ser de gran utilidad para conocer mejor los mecanismos fisiopatológicos subyacentes a estas enfermedades. Así mismo, conocer las correlaciones genotipo – fenotipo, permitirá mejorar el diagnóstico de estas enfermedades y dirigir adecuadamente el asesoramiento genético a los afectos y sus familiares.

III.

Hipótesis

1. Las diferencias fisiopatológicas de las diferentes mutaciones en el *MAPT* producirían diferentes fenotipos clínicos y neuropatológicos de degeneración lobular frontotemporal.
    1.1. Los pacientes con degeneración lobular frontotemporal genéticamente predeterminada por la mutación p.P301L en *MAPT* compartirían un fenotipo clínico y neuropatológico común. La agrupación espacial de casos de la mutación P301L en la comarca del Baix Llobregat sugeriría la presencia de un evento fundador común en dicha comarca.
    1.2. Los pacientes con degeneración lobular frontotemporal genéticamente predeterminada por la mutación p.P397S en *MAPT*, presentan un fenotipo clínico común entre sí, pero diferente al de los portadores de la mutación p.P301L.

2. Los sujetos en riesgo de desarrollar una degeneración lobular frontotemporal genéticamente determinada por mutaciones en *GRN* presentarían pérdida neuronal previa al inicio de síntomas que se reflejaría en cambios en el grosor cortical en fases presintomáticas
    2.1. El genotipo de *TMEM106B* podría influir en el grosor cortical de los portadores presintomáticos de *GRN*.

3. La aparición de deterioro cognitivo en la esclerosis lateral amiotrófica estaría determinada por factores genéticos y patológicos.

4. La ELA con depósito de proteína FUS pero sin mutación en el gen *FUS* presentaría coacumulación de las proteínas TAF15 y Trnp1 pudiendo ser la ausencia de este codepósito un posible biomarcador indicativo de mutaciones en *FUS*.

5. El proceso de cribado genético en las degeneraciones lobulares frontotemporales es complejo y requeriría un abordaje multidimensional para establecer las mejores estrategias para la identificación de mutaciones.

IV.

Objetivos

1. Describir y comparar el fenotipo en pacientes con demencia frontotemporal genéticamente predeterminada por distintas mutaciones en el gen *MAPT*:
    1.1. Describir el fenotipo clínico y neuropatológico en pacientes con demencia frontotemporal causada por la mutación p.P301L en el gen *MAPT.* Investigar el origen de esta mutación en esta cohorte con agregación espacial en la comarca del Baix Llobregat.
    1.2. Describir el fenotipo clínico y neuropatológico en pacientes con demencia frontotemporal causada por la mutación p.P397S en el gen de *MAPT* y compararlo con los de los pacientes portadores de la mutación p.P301L.

2. Investigar los cambios de grosor cortical en sujetos presintomáticos portadores de mutaciones en el gen *GRN* y su relación con la edad y la distancia estimada de inicio de los síntomas.
    2.1. Evaluar la influencia del genotipo de *TMEM106B* sobre el grosor cortical en portadores presintomáticos en *GRN*.

3. Identificar las características genéticas y neuropatológicas que determinan el deterioro cognitivo en una cohorte neuropatológica de esclerosis lateral amiotrófica.

4. Analizar la distribución de proteínas TAF15 y Trnp1 en las inclusiones patológicas de pacientes con esclerosis lateral amiotrófica asociada a depósitos de proteína FUS y su relación con la presencia o ausencia de mutaciones en el gen *FUS*.

5. Explorar diferentes estrategias de cribado genético en pacientes con diagnóstico neuropatológico de degeneración lobular frontotemporal.

VI.

Resultados

Trabajo 1

Frontotemporal Dementia Caused by the P301L mutation in the *MAPT* gene: clinicopathological features of 13 cases from the same geographical origin in Barcelona, Spain

S Borrego-Écija, J Morgado, L Palencia-Madrid, O Grau-Rivera, R Reñé, I Hernández, C Almenar, M Balasa, A Antonell, JL Molinuevo, A Lladó, M Martínez de Pancorbo, E Gelpi, R Sánchez-Valle

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Original Research Article

Frontotemporal Dementia Caused by the P301L Mutation in the *MAPT* Gene: Clinicopathological Features of 13 Cases from the Same Geographical Origin in Barcelona, Spain

Sergi Borrego-Écija[a] Joana Morgado[a, g] Leire Palencia-Madrid[b] Oriol Grau-Rivera[c] Ramón Reñé[d] Isabel Hernández[e] Consuelo Almenar[f] Mircea Balasa[a, h] Anna Antonell[a] José Luis Molinuevo[a] Albert Lladó[a] Marian Martínez de Pancorbo[b] Ellen Gelpi[c] Raquel Sánchez-Valle[a, c]

[a]Alzheimer's Disease and Other Cognitive Disorders Unit, Neurology Department, Hospital Clínic-Institut d'Investigacions Biomediques August Pi i Sunyer (IDIBAPS), Barcelona, [b]BIOMICs Research Group, Lascaray Research Center, University of the Basque Country, Vitoria-Gasteiz, [c]Neurological Tissue Bank of the Biobanc-Hospital Clínic-IDIBAPS, Barcelona, [d]Neurology Department, Hospital Universitari de Bellvitge, Hospitalet de Llobregat, [e]Memory Clinic of Fundació ACE, Institut Català de Neurociències Aplicades, Barcelona, and [f]Hospital Benito Menni CASM Germanes Hospitalaries, Sant Boi de Llobregat, Barcelona, Spain; [g]Neurology Department, Centro Hospitalar Lisboa Central, Lisboa, Portugal; [h]Global Brain Health Institute, Trinity College Dublin, Dublin, Ireland

Keywords
Frontotemporal lobar degeneration · FTLD-tau · FTDP-17 · *MAPT* · P301L · Globular glial tauopathy · Tauopathies

Abstract
Background/Aims: We identified and studied 13 patients carrying the P301L mutation in the *MAPT* gene from the same area (Baix Llobregat County) in Barcelona, Spain. ***Methods:*** The demographic and clinical features were reviewed retrospectively. Detailed neuropathological characterization was obtained in 9 subjects. To investigate the origin of the P301L mutation in these families, 20 single nucleotide polymorphisms (SNPs) in the *MAPT* gene were analyzed. ***Results:*** The mean age at disease onset was 51 years and the mean disease duration was 7 years. The most common initial symptoms were behavioral changes (54%), followed by language disturbances (31%) and memory loss (15%). 46% developed parkinsonism. Neuropathology showed an extensive neuronal and glial 4-repeat (4R) tauopathy with "mini-Pick"-like bodies in the dentate gyrus as the characteristic underlying pathology in all cases. In 1 subject, additional 4R globular glial inclusions were observed. All the mutation carriers showed the

Raquel Sanchez-Valle
Alzheimer's Disease and Other Cognitive Disorders Unit
Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi I Sunyer
Villarroel, 170, ES–08036 Barcelona (Spain)
E-Mail rsanchez@clinic.ub.es

same haplotype for the SNPs analyzed, suggesting a common ancestor. ***Conclusion:*** These findings suggest a relative homogeneous clinicopathological phenotype in P301L *MAPT* mutation carriers in our series. This phenotype might help in the differential diagnosis from other tauopathies and be a morphological hint for genetic testing. The haplotype analysis results suggest a founder effect of the P301L mutation in this area.

Introduction

The term "frontotemporal dementia and parkinsonism linked to chromosome 17" (FTDP-17) was adopted in 1996 to describe the clinical phenotype observed in 13 families [1]. In 1998, mutations in the microtubule-associated protein tau gene (*MAPT*) in chromosome 17 were described in some of these families [2]. To date, nearly 50 pathogenic *MAPT* mutations have been reported [3], accounting for 10–23% of familial cases of frontotemporal lobar degeneration (FTLD) with tau pathology (FTLD-tau) [4]. Clinically, FTLD related to *MAPT* mutations may affect behavior, language, memory, and executive functions. Parkinsonism has also been reported. *MAPT* mutations are associated with abnormal deposition of hyperphosphorylated tau (pTau) in neurons with or without glial involvement. These deposits consist of 3-repeat and/or 4-repeat (4R) classes of tau isoforms. Significant variability has been described concerning the clinical and pathological phenotype depending on the *MAPT* mutation and even among individuals carrying the same mutation [5–13].

The main objective of this study was to describe the demographics, clinical and neuropathological characteristics of the 13 patients with FTLD associated with the P301L mutation in the *MAPT* gene identified in our center. We analyzed the haplotype of 20 single nucleotide polymorphisms (SNPs) located around the P301L mutation in available family members to evaluate a possible founder effect of this mutation in our area.

Methods

Participants

We reviewed the database of our genetic counseling program for familial dementias (PICOGEN) [14] to identify the patients carrying a *MAPT* mutation and related family members. The study was approved by the Hospital Clinic of Barcelona Ethics Committee and all subjects gave written informed consent for the study.

Genetic Analysis

Direct sequencing of exons 1 and 9–13 of the *MAPT* gene was performed. *MAPT* H1/H2 haplotype was determined with the SNP rs1800547 and the *APOE* genotype with two genotyping assays (rs429358 and rs7412), using TaqMan genotyping technologies (Life Technologies, Carlsbad, CA, USA). Twenty tag SNPs located around the P301L mutation in the *MAPT* gene (rs63751273) encompassing a region of more than 50 kb were selected with a minimum allele frequency >0.2 (rs754593, rs2435206, rs3785885, rs2435207, rs2435211, rs8079215, rs2435200, rs2471738, rs8067056, rs4792897, rs733966, rs17573858, rs916896, rs9468, rs5820605, rs1052594, rs17574040, rs16940799, rs7687, and rs7521) and were analyzed in the patients (n = 13) and available family members (7 asymptomatic at risk) and in 60 healthy nonrelated individuals from Barcelona.

Clinical Characterization

We retrospectively reviewed the demographics and clinical features of the mutation carriers. Patients were retrospectively reassessed according to the current diagnostic criteria for behavioral variant frontotemporal dementia [15] or primary progressive aphasia (PPA) [16].

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Neuropathological Evaluation

Brain specimens were processed according to the established protocol at the Neurological Tissue Bank of the IDIBAPS-Hospital Clínic following the consensus recommendations of BrainNetEurope. Sections were stained with hematoxylin and eosin (HE) and immunohistochemistry was performed using a panel of monoclonal (mc) and polyclonal primary antibodies: anti-bA4-amyloid (DAKO, Glostrup, Denmark; mc, clone 6F/3D, dilution 1:400), anti-phosphorylated tau (Thermo Scientific, Rockford, IL, USA; mc, clone AT8, dilution 1:200), anti-tau 3-repeat isoform (RD3) (Millipore, clone 8E6/C11, dilution 1:1,000), anti-tau 4-repeat isoform (RD4) (Millipore, clone 1E1/A6, dilution 1:50), anti-alpha-synuclein (Novocastra, Newcastle, UK; mc, clone KM51, dilution 1:500), anti-TAR DNA-binding protein 43 (TDP-43) (Abnova, Taipei, Taiwan; mc, clone 2E2-D3, dilution 1:500), and anti-alpha-B-crystallin (Newcastle, UK; mc, clone G2JF). Immunoreaction was visualized by EnVision+ system peroxidase procedure (DAKO). In selected cases, a number of further anti-pTau antibodies which recognized Thr181, Ser262, Ser396 and Ser422 (Calbiochem, La Jolla, CA, USA) were applied. Semiquantitative assessment of neuronal loss, gliosis, and spongiosis on HE-stained, pTau and βA4 amyloid pathology was performed in different brain areas. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) score was applied. The presence of TDP43 and alpha-synuclein aggregates was evaluated in all areas.

Statistical Analysis

The analyses were performed using the IBM-SPSS Statistics Version 20.0, and the level of significance was established at $p = 0.05$. Correlations were assessed with Spearman coefficients. The association of key clinical features with the genotype was evaluated with χ^2 test analysis.

Results

Thirteen patients carrying the P301L (c.902C>T) mutation in the *MAPT* gene from 10 different, apparently nonrelated, families were identified. All subjects shared the same geographical origin (Baix Llobregat County). In 9 patients, brain tissue was available.

Demographics and Clinical Features

The main demographic, genetic and clinical features are presented in Table 1. The mean age at disease onset was 51 years (range 43–69 years) and the average duration of the disease was 7 years (range 4–13 years). Twelve patients (92%) showed a pattern of autosomal dominant inheritance. During life, 6 patients received the clinical diagnosis of behavioral variant frontotemporal dementia, 3 received the clinical diagnosis of semantic variant of PPA, and 4 patients were diagnosed with Alzheimer disease. Behavioral changes constituted the presenting symptoms in 7 patients (54%), followed by language impairment in 4 (31%) and memory dysfunction in 2 (15%). One patient had previously been diagnosed with major depression. Of note, most patients developed the whole clinical spectrum along the course of the disease (behavioral changes in 84%, language impairment in 61%, and memory dysfunction in 61%). None presented extrapyramidal signs at onset, but 6 (46%) presented rigid-akinetic parkinsonism during the disease. Four patients (31%) suffered from generalized seizures. Neuroimaging showed bilateral temporofrontal atrophy. SPECT was performed in 8 patients and showed frontotemporal hypoperfusion. There were no clinical differences between subjects based on *MAPT* H1/H2 haplotype or *APOE* genotype.

Neuropathological Features

The average brain weight was 1,086 g (range 935–1,340 g). Total brain weight inversely correlated with disease duration (Spearman rho –0.778, $p < 0.05$). Gross examination revealed atrophy in frontal and temporal lobes with enlargement of the lateral ventricles (Fig. 1). Moderate to severe pallor of substantia nigra was observed in all donors.

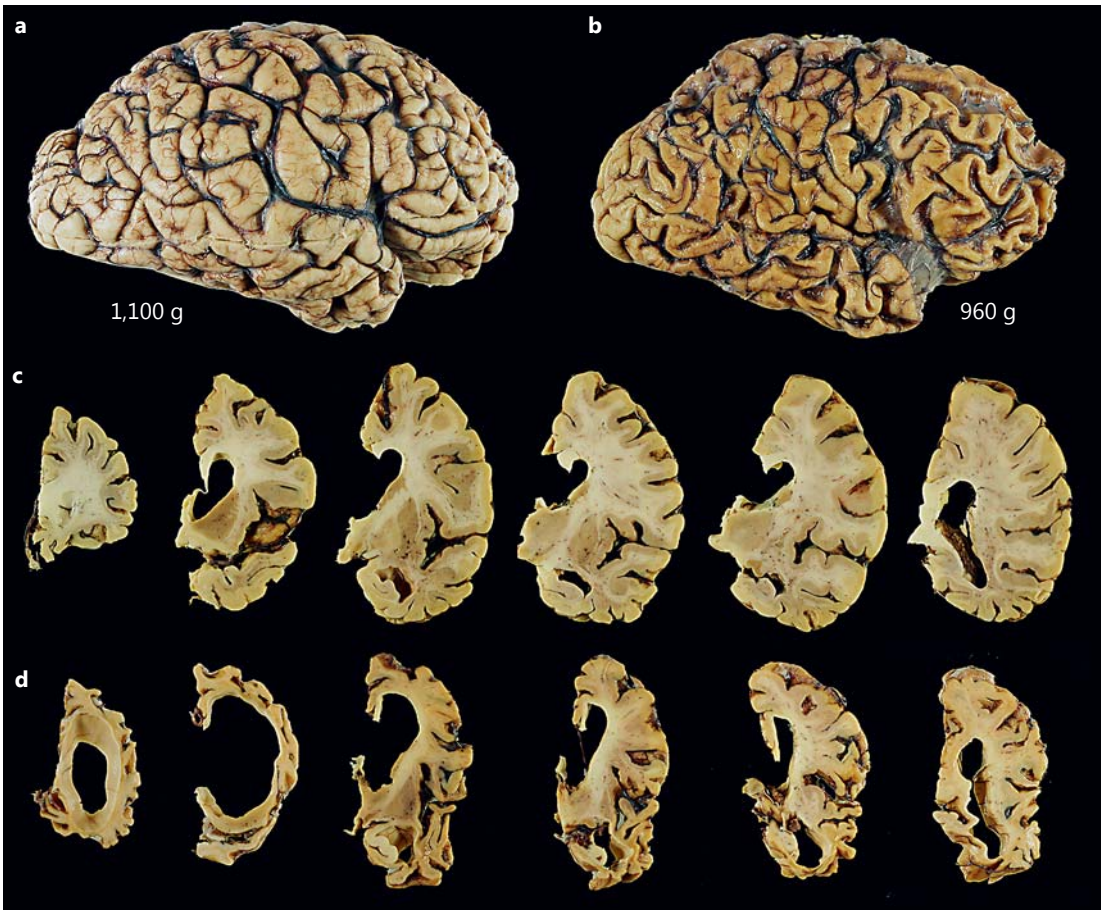

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Fig. 1. Gross neuropathological findings. Lateral (**a, b**) and coronal (**c, d**) sections of two *MAPT* P301 mutation carriers with different degrees of pathological severity and disease duration. **a, c** Case 5. **b, d** Case 4.

Table 1. Demographic and clinical features of P301L *MAPT* mutation carriers

Subject	Gender	Family	Age at onset, years	Age at death, years	Disease duration, years	Clinical onset	Parkinsonism	Clinical diagnosis	*MAPT*	*APOE*
1	M	A	45	52	6	Behavior	No	bvFTD	H1/H1	ε3/ε3
2	M	B	53	58	5	Behavior	Yes	bvFTD	H1/H1	ε3/ε4
3	M	C	46	56	10	Language	No	svPPA	H1/H1	ε3/ε3
4	M	D	59	72	13	Memory	Yes	AD	H1/H1	ε3/ε3
5	F	E	59	63	4	Language	No	AD	H1/H2	ε3/ε3
6	M	F	46	53	7	Memory	Yes	AD	H1/H1	ε3/ε3
7	F	F	57	61	5	Behavior	Yes	bvFTD	H1/H1	ε3/ε3
8	M	G	51	58	7	Behavior	No	bvFTD	H1/H1	ε3/ε4
9	M	G	67	75	7	Behavior	Yes	AD	H1/H2	ε3/ε3
10	M	G	69	alive	2	Language	No	svPPA	H1/H1	ε3/ε3
11	F	H	50	58	8	Behavior	No	bvFTD	H1/H1	ε3/ε4
12	M	I	43	alive	3	Language	No	svPPA	H1/H1	ε3/ε3
13	M	J	50	56	6	Behavior	Yes	bvFTD	H1/H1	ε3/ε4

AD, Alzheimer disease; bvFTD, behavioral variant frontotemporal dementia; svPPA, semantic variant of primary progressive aphasia.

Histology showed a spectrum of pathological changes depending on the disease duration (Fig. 2; Table 2). An extensive cortical neuronal loss and gliosis was evident in all cases. The most affected areas were frontal, temporal and parietal cortices. Occipital cortices and the hippocampus were comparatively better preserved. No cases with hippocampal sclerosis were seen. There was also prominent neuronal loss and gliosis in the striatum and substantia nigra.

Immunohistochemistry showed extensive neuronal and glial pathology dominated by 4R tau isoforms (Table 2). In cortical neurons, there was a predominance of pretangles mainly involving layer II/III and layer V. A characteristic finding was the perinuclear enhancement as frequently seen in pretangles, and particularly, small ubiquitin-negative cytoplasmic aggregates resembling mini-Pick bodies in the granule cells of the dentate gyrus of the hippocampus. These were also immunoreactive for phosphospecific anti-tau antibodies Thr181, Ser396, and Ser422 and were negative for Ser262. Threads were abundant in gray and white matter. In addition, pTau-immunoreactive astrocytes were found in the cerebral cortex in the form of tufted- and granular-fuzzy-like structures. No abnormal alpha-synuclein or TDP43 protein aggregates were detected. Only few diffuse BA4-amyloid deposits were found in 4 cases, without neuritic plaque pathology.

Interestingly, in 1 subject (patient 7) additional Gallyas-positive and 4R tau-immunoreactive globular oligodendroglial inclusions were observed in frontotemporal white matter (Fig. 2). This patient had a more severe degeneration of white matter and showed only mild involvement of the motor cortex and corticospinal tract. No globular astrocytic inclusions were identified.

Haplotype Analysis

The 15 P301L *MAPT* carriers (13 patients and 2 asymptomatic mutation carriers) studied shared the same haplotype linked to the P301L *MAPT* mutation (A, C, G, G, C, T, A, C, T, A, T [P301L], C, T, T, T, del, G, A, T, T, A). This haplotype was not common (frequency of 0.083) in the control population.

Discussion

We described the clinical and neuropathological features of 13 subjects from 10 apparently nonrelated families with FTLD caused by the P301L mutation in the *MAPT* gene from the same area in Barcelona (Baix Llobregat County). To the best of our knowledge, this study represents the largest reported case series of P301L *MAPT* mutation carriers. The P301L mutation was linked to a common haplotype in the mutation carriers, suggesting a founder effect of this mutation in the region.

The clinical features of our patients are relatively homogeneous and consistent with the previously described phenotype [8–13]. The most frequent presentation in our series was behavior disturbances (54%). In some patients (31%), the clinical onset consisted of isolated language impairment with word-finding difficulties and semantic errors, meeting the criteria for the semantic variant of PPA. Although some studies suggest that genetic mutations are not commonly associated with PPA, it is not rare that carriers of mutations in the *MAPT* gene present language impairment with semantic deficits at onset [17, 18], suggesting that family history should be investigated in patients with the semantic variant of PPA. By contrast, *PGRN* mutations have been related to nonfluent or mixed PPA [7, 19].

In our series, 46% showed rigid-akinetic parkinsonism during the disease course. The postmortem detection of a moderate or severe loss of pigmented neurons of the substantia nigra pars compacta in all patients suggests that parkinsonism could have been clinically

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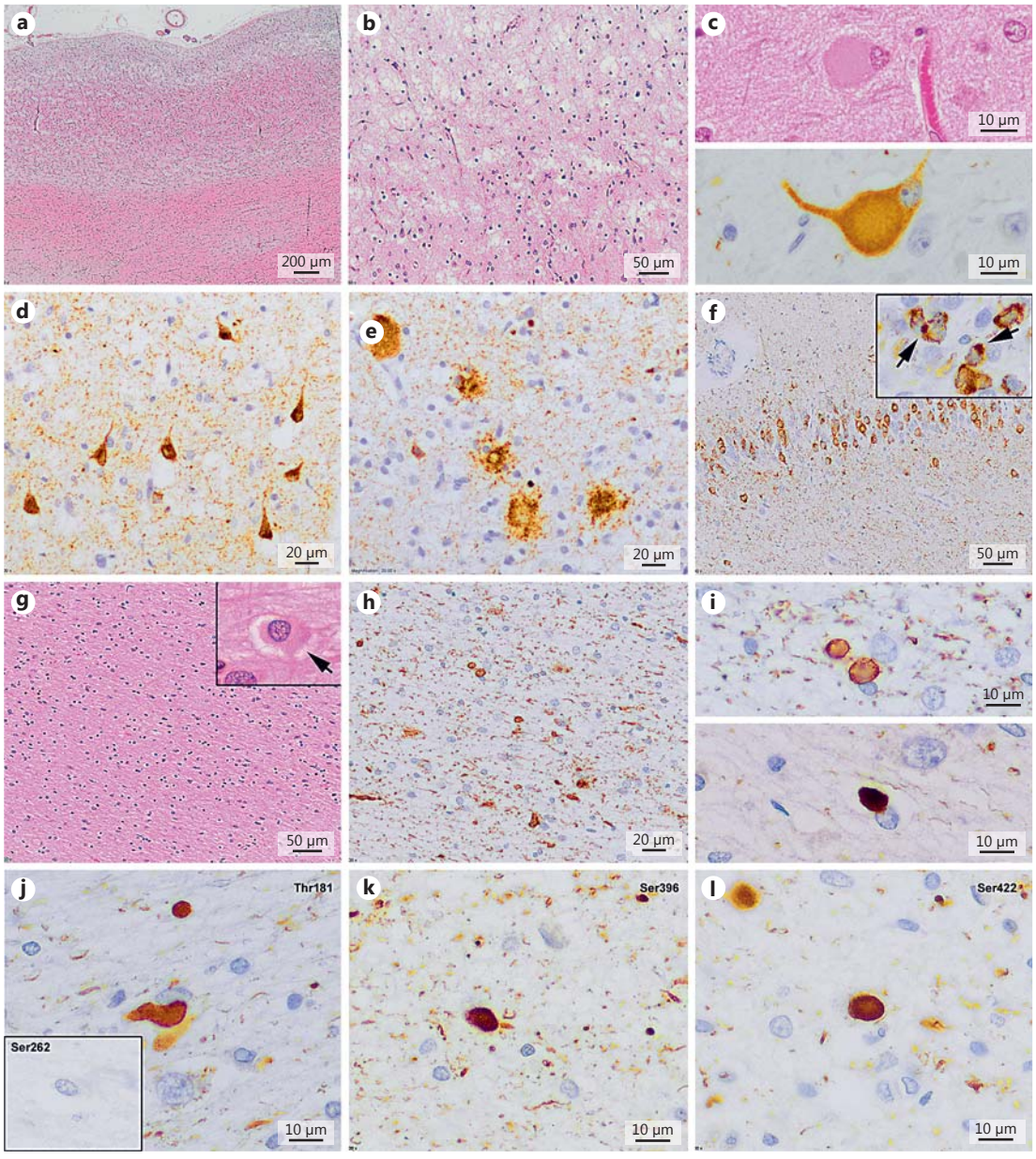

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Fig. 2. Characteristic histological findings. **a**, **b** Pattern of frontotemporal lobar degeneration with prominent neuronal loss, gliosis, and superficial spongiosis (HE). **c** Ballooned neurons are frequently observed in cases with shorter disease duration (upper panel: HE, lower panel: alpha-B-crystallin). **d–f** Hyperphosphorylated tau (AT8) shows abundant pretangles in cortical areas with abundant neuropil threads (**d**), frequent AT8-positive astrocytes with undefined morphology resembling tufted and granular fuzzy astrocytes (**e**), characteristic involvement of granular neurons of the dentate gyrus with pretangles, and small spherical inclusions or "mini-Pick"-like bodies (**f**). GGT-like pathology in case 7 (**g**). White matter appears gliotic and some glial cells contain ill-defined basophilic inclusion in their cytoplasm (**inset**, arrow). **h** Tau immunohistochemistry reveals a moderate amount of globular glial-like inclusions (GGI). **i** GGI appear more frequently in oligodendrocytes and are strongly immunoreactive for AT8 (upper image), 4-repeat tau (lower image), and phospho-specific anti-tau antibodies such as threonine 181 (**j**), serine 396 (**k**), and serine 422 (**l**). They are negative for serine 262 (**inset j**). Scale bars: 10 µm for **c**, **i–l**; 20 µm for **d**, **e**, **h**; 50 µm for **b**, **f**, **g**; 200 µm for **a**.

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Table 2. Neuropathological features of P301L *MAPT* mutation carriers

Subjects[a]	Neuronal loss and gliosis/pTau immunohistochemistry (AT8)											Tau isoforms
	frontal	precentral	postcentral	hippocampus (CA4-CA1; DG)	parahippo-campus	occipital	WM	striatum	thalamus	SN	dentate nucleus	
1	++/+++	+/++	+++/++	–/+++	++/+++	–/+	na/+++	+/++	–/++	++/+++	–/–	4R
2	+++/+++	+++/+++	+++/+++	–/+++	++/+++	–/+	na/++	++/++	–/+	+++/+++	+/–	4R
3	+++/+++	+++/++	+++/++	–/+++	+++/+++	–/+	na/+++	++/+++	++/+	+++/+++	+/–	4R
4	+++/+++	+++/+++	+++/+++	+/+++	+++/+++	++/+	na/+++	+++/+++	++/+++	+++/+++	–/+	4R
5	++/++	–/+	++/+++	–/+++	++/+++	–/–	na/++	++/++	+/+	+++/+++	–/+	4R
6	+++/+++	+/+	+++/+++	–/+++	+++/+++	–/+	na/+++	+++/+++	+/+	+++/+++	–/–	4R
7	++/+++	+/+	+++/+++	–/+++	++/++	–/–	na/+++[b]	++/+++	++/+	+++/+++	–/+	4R
8	+++/++	+/++	+++/+++	–/+++	+++/+++	–/–	na/+++	+++/+++	+/++	+++/+++	–/+	4R
9	++/+++	+/++	+/++	–/+++	++/+++	+/–	na/++	++/++	–/+	+++/+++	–/–	4R

na, not applicable; DG, dentate gyrus; WM, white matter; SN, substantia nigra pars compacta; pTau, phosphorilated tau. 0 = Absent; + = mild; ++ = moderate; +++ = severe. [a] Subject number corresponds to that of Table 1. [b] Globular glial tauopathy.

Table 3. Summary of clinicopathological studies on P301L *MAPT* mutation carriers

Authors	Year	Cases, *n*	Neuropathological features						Ref.
			ballooned neurons	mini-Pick-like bodies in DG	hippocampal involvement (CA4-CA1)	SN degeneration	glial pathology	cases with GGI	
Spillantini et al.	1998	3	yes	yes	mild	severe	glial fibrillary tangles	0	11
Mirra et al.	1999	2	yes	yes	not involved	severe	astrocytic plaque-like	0	12
Bird et al.	1999	3	yes	not mentioned	mild	severe	not mentioned	0	9
Nasreddine et al.	1999	2	yes	not mentioned	not involved / moderate	severe	astro-oligodendroglial	0	10
Adamec et al.	2002	2	yes	yes	moderate	severe	astrocytic plaques tuft-shaped coiled bodies	0	13
Tacik et al.	2016	8	yes	yes	mild except in one case	moderate-severe	granular fuzzy tufted astrocytes coiled bodies	1	8
Borrego et al.	2017	13 (9)	yes	yes	not involved	severe	granular fuzzy-like tufted-like	1	–

DG, dentate gyrus; GGI, globular glial inclusions; SN, substantia nigra.

underestimated. Four patients developed secondary epilepsy in the latest stages of the disease according to previous reports showing that epilepsy is not uncommon in patients with *MAPT* mutations [8, 20]. Four patients were clinically misdiagnosed as having Alzheimer disease. The presence of memory complaints or memory deficits at the examination and the report of bilateral temporal atrophy might account for the misdiagnoses.

Previous clinicopathological studies of P301L patients have been summarized in Table 3. While Tacik et al. [8] report that the P301L mutation is associated with clinical and pathologic heterogeneity based on the variability of the glial inclusions, in our series the neuropathological findings were relatively homogeneous. There was a classic FLTD atrophy pattern with prominent neuronal loss, gliosis and superficial spongiosis in frontotemporal and parietal cortical regions, basal ganglia and substantia nigra with relative preservation of the hippocampus and occipital cortex. In contrast to Tacik et al. [8], in our cases, the parahippocampal gyrus was severely affected. The underlying pTau pathology was relatively uniform and extensive and consisted of 4R isoforms with neuronal and glial involvement. Pretangles and neuropil threads dominated the neuronal pathology, while cortical tufted-like and granular fuzzy-like astrocytes, and small white matter oligodendroglial open rings represented the glial pathology. A characteristic feature was the invariable involvement of the dentate gyrus with pretangles and frequent so-called "mini-Pick"-like bodies [21]. These pathological features were consistent with the previous literature (Table 3). The presence of ballooned neurons with extensive 4R tauopathy involving the white matter might lead to a misdiagnosis of corticobasal degeneration. The presence of distinct glial inclusions and the widespread involvement of the dentate gyrus may help in the differential diagnosis from other tauopathies (e.g., sporadic corticobasal degeneration) and be a morphological hint for genetic testing.

In subject 7, in addition to the P301L features, 4R tau-positive globular glial inclusions (GGI) were found as those described in globular glial tauopathies (GGT). GGT are 4R tauopathies neuropathologically characterized by GGI [22]. Despite GGT having been related to sporadic phenotypes, some cases of GGT caused by *MAPT* mutations, including P301L, have recently been described [8, 23]. Most neuropathological descriptions of *MAPT* mutations were made before the description of GGT pathology, and the presence of GGI might have been underdiagnosed. Interestingly, GGI were not found in the patient's sibling (subject 6), suggesting that environmental factors or associated combined genetic factors may have influenced the presence of GGT.

In summary, our P301L *MAPT* mutation carrier series presented a relative homogeneous clinicopathological phenotype, except for one case with additional GGI. The geographical aggregation of the cases and the haplotype results suggested a founder effect of the P301L mutation in the Baix Llobregat County that might have determined the relative homogeneity of our series.

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Trabajo 2

Novel P397S *MAPT* variant associated with late onset and slow progressive frontotemporal dementia

S Borrego-Écija, A Antonell, JA Puig-Butillé, I Pericot, C Prat-Bravo, MT Abellán-Vidal, J Mallada, J Olives, N Falgàs, R Oliva, A Lladó, R Sánchez-Valle

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BRIEF COMMUNICATION

Novel P397S *MAPT* variant associated with late onset and slow progressive frontotemporal dementia

Sergi Borrego-Écija[1], Anna Antonell[1], Joan Anton Puig-Butillé[2], Inmaculada Pericot[3], Carme Prat-Bravo[4], Maria Teresa Abellan-Vidal[5], Javier Mallada[6], Jaume Olives[1], Neus Falgàs[1], Rafael Oliva[7,8], Albert Lladó[1] & Raquel Sánchez-Valle[1]

[1]Alzheimer's Disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clinic, Institut d'Investigació Biomèdica August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain
[2]Biochemistry and Molecular Genetics Department, Hospital Clinic, Centro de Investigación Biomédica en Red en Enfermedades Raras (CIBERER), Barcelona, Spain
[3]Neurology Department, Hospital Santa Caterina, Girona, Spain
[4]Neurology Department, Hospital Parc Taulí, Sabadell, Spain
[5]Unit of Cognitive Disorders and Psychogeriatrics, Institut de Neuropsiquiatria i Addiccions, Centre Emili Mira, Parc de Salut Mar, Barcelona, Spain
[6]Neurology Department, Hospital General Universitario de Elda, Alicante, Spain
[7]Biochemistry and Molecular Genetics Service, Hospital Clinic, Institut d'Investigació Biomèdica August Pi i Sunyer (IDBAPS), University of Barcelona, Barcelona, Spain
[8]Genetics Unit, Department of Biosciences, University of Barcelona, Barcelona, Spain

Correspondence
Raquel Sanchez-Valle, Alzheimer's Disease and Other Cognitive Disorders Unit, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), University of Barcelona, Villarroel, 170 08036 Barcelona, Spain. Tel: +34932275785; Fax: +34932275783; E-mail: rsanchez@clinic.ub.es

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Abstract

Mutations in the *MAPT* gene cause frontotemporal dementia with tau deposits. We report the novel p.P397S *MAPT* variant in eight subjects from five apparently nonrelated families suffering from frontotemporal dementia with autosomal dominant pattern of inheritance. In silico analysis reported conflicting evidence of pathogenicity. The segregation analysis support that this variant is likely pathogenic. The mean age at onset (61.4 years) and mean disease duration (13.9 years) of these subjects and their affected relatives were significantly higher compared with our series of p.P301L *MAPT* mutation carriers. These findings suggest that p.P397S variant could be a new *MAPT* mutation associated with a less aggressive phenotype than other *MAPT* mutations.

Introduction

Mutations in the microtubule-associated protein tau (*MAPT)* gene in chromosome 17 were described in 1998 as a cause of frontotemporal lobar degeneration (FTLD) with tau pathology.[1–3] To date, nearly 50 pathogenic *MAPT* mutations have been reported.[4] Clinically, FTLD related to *MAPT* mutations may affect behavior, language, memory, and executive functions. Parkinsonism has also been reported in some cases. Most *MAPT* mutations are characterized by early onset (mean 47.9 years) and a rapidly progressive clinical deterioration with a mean age at death of 58.7 years.[5]

We describe the clinical phenotype of a novel exon 13 *MAPT* variant – a Proline to Serine substitution at codon 397 (p.P397S) – in eight subjects with FTLD with an autosomal dominant pattern of inheritance from five apparently nonrelated families. We hypothesize that this variant could be causative of FTLD. In addition, we compare the clinical phenotype of these cases with our series of the most frequent (p.P301L) *MAPT* mutation.[6]

Methods

Subjects were recruited from the Genetic counseling program for familial dementias (PICOGEN program) at the Hospital Clínic de Barcelona.[7] A detailed clinical history and neurological evaluation of all subjects were performed. We reviewed the information available from all the affected cases in the pedigrees. The diagnosis of behavioral variant frontotemporal dementia (bvFTD) was performed following the current diagnostic criteria.[8] Cerebrospinal fluid (CSF) analysis for Alzheimer's disease (AD) biomarkers was measured with INNOTEST ELISAs following manufacturer's instructions (Fujirebio, Ghent, Belgium). Our laboratory normal reference values for amyloid beta 42 (AB42), total tau (t-tau), and phosphorylated tau at threonine 181 (p-tau) are >660, <385, and >65 pg/mL, respectively.

Ethics

This research was performed according to the guidelines of the Declaration of Helsinki. The participants provided written informed consent for genetic testing and publication of relevant findings. The study was approved by the Hospital Clínic Ethics committee.

Genetic analysis

Genetic screening for *MAPT* (exons 1 and 9–13) and *progranulin* (*GRN*) (exons 1–13) or serum progranulin levels was performed in the eight subjects as previously described.[9] The *MAPT* haplotype (H1/H2) was determined studying the SNP rs1800547 and *APOE* genotype with two genotyping assays (rs429358 and rs7412), using TaqMan genotyping technologies (Life Technologies, Carlsbad, CA). *C9ORF72* GGGGCC hexanucleotide repeat expansion was tested with a repeat-primed PCR. Subject II.II.VIII was examined by exome sequencing using MedExome (Roche, Basel, Switzerland) in an Illumina NextSeq500.

We searched for functional information at ENSEMBL database (https://www.ensembl.org), where the variant was described as rs1295855402, and with algorithms that predict whether an amino acid substitution affects the protein function such as SIFT, Polyphen-2, REVEL, Mutation Assessor, CADD, and MetaLR.

Statistical analysis

Mean age at onset, disease duration, and age at death were calculated considering together subjects with proven variant and their affected relatives. Results in p.P397S carriers were compared with our series of p.P301L using Fisher's exact test. Disease duration was analyzed using Kaplan–Meier estimator and compared with Log-rank test. The analyses were performed using the SPSS Statistics Version 20.0 IBM Corp, Chicago, IL), and the level of significance was established at a P level of 0.05 (two-sided).

Results

Clinical phenotype

We include here the description of several representative cases.

Family I

Subject I.III.IV developed behavioral disinhibition, sweet food preference, deficits in executive tasks, and semantic language impairment at the age of 60 meeting criteria for bvFTD. MRI showed severe medial, lateral, and polar bitemporal atrophy. Currently, after 17 years of disease, she is in a stage of moderately severe dementia with severe semantic impairment scoring 5/30 at Boston Naming Test (BNT), but preserved motor functions. She had an autosomal dominant family history of dementia on her mother, aunt, grandmother, and three siblings (Fig. 1). All of them developed cognitive and behavioral dysfunction starting from 50 to 77 years old and showing slow disease progression. Due to this family history of dementia genetic exam was performed in subject I.III.IV and one of her brothers (subject I.III.III) revealing the presence of the p.P397S *MAPT* variant in both.

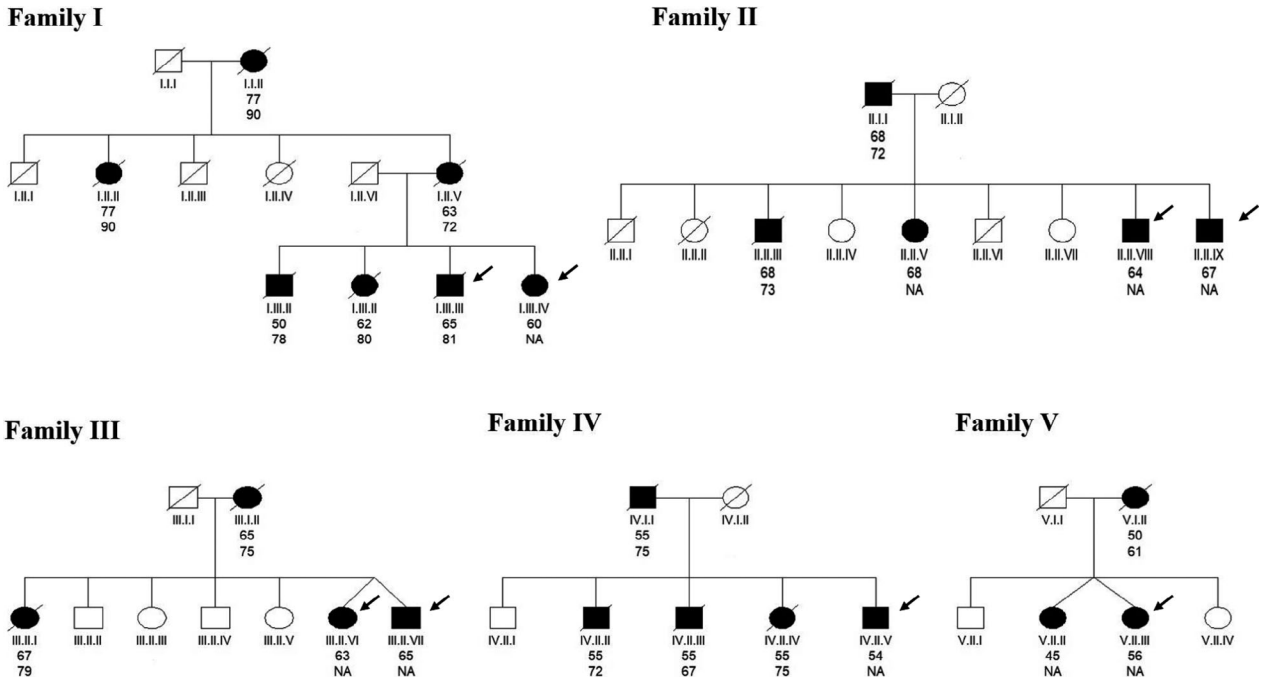

Figure 1. Pedigree of the five reported families. Arrows indicate the probands described in the main text. Upper ages beneath each symbol are age at onset. Lower ages beneath each symbol are age at death.

Family II

Subject II.II.IX developed socially inappropriate behavior at the age of 64. Neuropsychological evaluation revealed short-term memory and semantic language impairment (BNT 35/60). The MRI demonstrated moderate bitemporal atrophy (Fig. 2). The CSF core biomarkers analysis showed normal levels of AB42 (1308 pg/mL), but increased levels of t-tau (414 pg/mL) and p-tau (87 pg/mL). He had a family history of late-onset dementia on

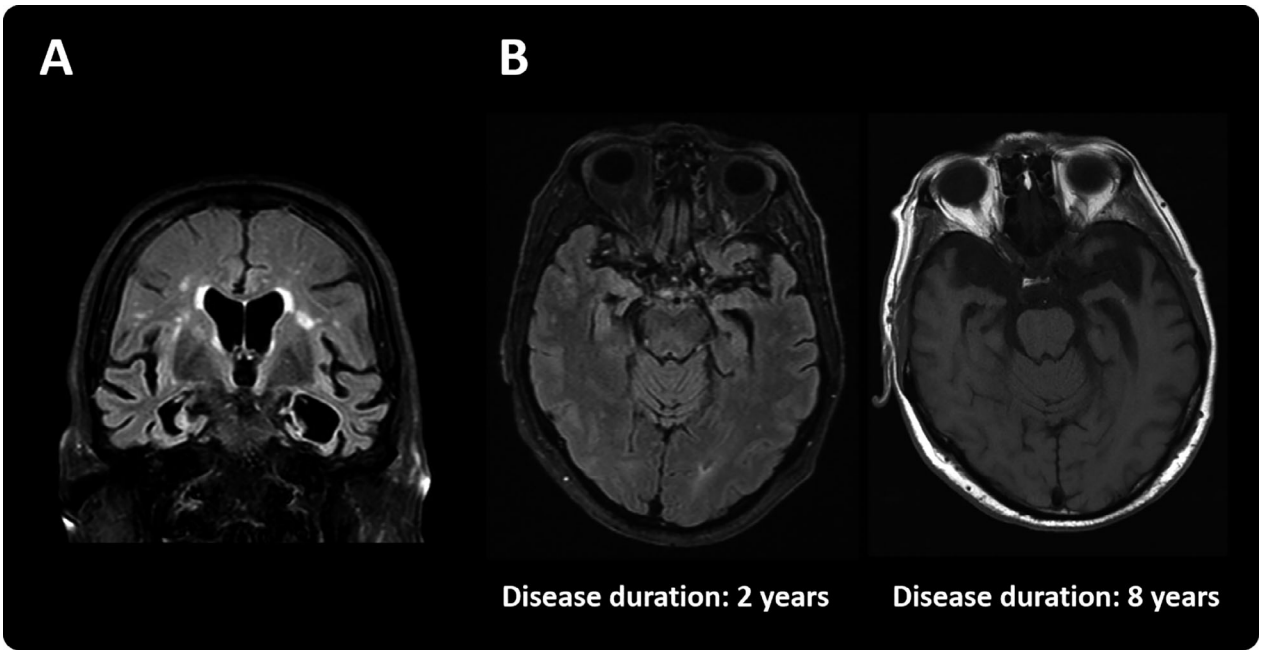

Figure 2. MRI of subject III.II.VI showing important medial and polar bitemporal atrophy (A). Longitudinal MRI examinations of subject II.II.IX (B).

his father, and three siblings (Fig. 1). The genetic study demonstrated the presence of the p.P397S variant in the *MAPT* gene. Currently, after 8 years of disease duration, he still is in a moderate stage of dementia, scoring 24 at the MMSE and 102 at the Revised Cambridge Behavioral Inventory (CBI-R).

Subject II.II.VIII was a sibling of subject II.II.IX. He initially developed episodic and semantic memory loss and behavioral disinhibition at the age of 67. The CT scan showed bitemporal atrophy and the CSF exam presented decreased levels of AB42 (437 pg/mL) and increased t-tau (543 pg/mL) and p-tau (80 pg/mL), so AD diagnosis was established. During the follow-up, the patient developed severe apathy, loss of empathy, hyperorality, and executive dysfunction, highly suggestive of bvFTD. He also presented mild rigid-akinetic parkinsonism. Currently, after 8 years of disease progression, he scores 25 at the Mini Mental State Examination (MMSE) and he is independent for basic activities of daily living and most of the instrumental activities. The genetic test confirmed the presence of the p.P397S variant, consequently the final diagnosis of genetic bvFTD with possible concomitant AD was established.

The p.P397S *MAPT* variant was not found in subject II.II.VII, an asymptomatic 68-year-old sibling.

Family III

Subjects III.II.VI and III.II.VII were dizygotic twins. Their mother developed dementia at the age of 65. Another sibling presented clinically with behavioral impairment dysfunction strongly suggestive of bvFTD (Fig. 1). Subject III.II.VI presented with disinhibition, apathy, and ritualistic behaviors at the age of 63. Neuropsychological evaluation revealed impairment in memory and naming. MMSE score was 19 at the age of 69. The MRI showed severe medial bitemporal atrophy (Fig. 2) and the amyloid PET scan (IMM Flutemetamol-F18) was negative. Subject III.II.VII became apathetic, exhibit diminished social interest, poor hygiene, and significantly increased smoking and alcohol intake at the age of 64. MRI demonstrated severe bitemporal atrophy. The genetic study revealed the p.P397S variant in both twins.

Summary of the clinical phenotype of p.P397S carriers

Table 1 summarizes the demographic and clinical features of the eight subjects who are confirmed carriers of the p.P397S *MAPT* variant.

All of them developed behavior problems and semantic language impairment. Three of them developed mild rigid-akinetic parkinsonism. None of the subjects

Table 1. Demographic and clinical features of subjects with the p.P397S *MAPT* variant.

Subject	Gender	Ethnicity	Age at onset (y)	Age at death (y)	Disease duration (y)	Current dementia stage	Current MMSE	Boston Naming Test	CBI-R	Parkinsonism	Clinical Diagnosis	*MAPT* haplotype	AD biomarkers			
													APOE	AB42	t-tau	p-tau
I.III.IV	Female	Caucasian	60	Alive	16	Moderately severe	9	5/30	94	Yes	bvFTD	1/2	3/3	NA	NA	NA
I.III.III	Male	Caucasian	64	81	17	Dead	–	NA	NA	No	AD	1/2	3/3	NA	NA	NA
II.II.IX	Male	Caucasian	64	Alive	8	Moderate	24	30/60	102	No	bvFTD	1/2	3/3	1308	414	87
II.II.VIII	Male	Caucasian	67	Alive	8	Moderate	25	35/60	NA	Yes	bvFTD	1/2	3/3	437	543	80
III.II.VI	Female	Caucasian	63	Alive	6	Moderate	19	NA	NA	No	bvFTD	1/1	2/3	NA	NA	NA
III.II.VII	Male	Caucasian	65	Alive	3	Moderate	25	NA	NA	No	bvFTD	NA	NA	NA	NA	NA
IV.II.V	Male	Caucasian	54	Alive	18	Severe	0	NA	NA	Yes	bvFTD	NA	NA	NA	NA	NA
V.II.III	Female	Caucasian	56	Alive	3	Mild	27	31/60	22	No	bvFTD	1/2	2/3	1423	850	91

AD, Alzheimer's Disease; bvFTD, behavioral variant frontotemporal dementia; CBI-R, Revised Cambridge Behavioral Inventory; NA, not available; y, years.

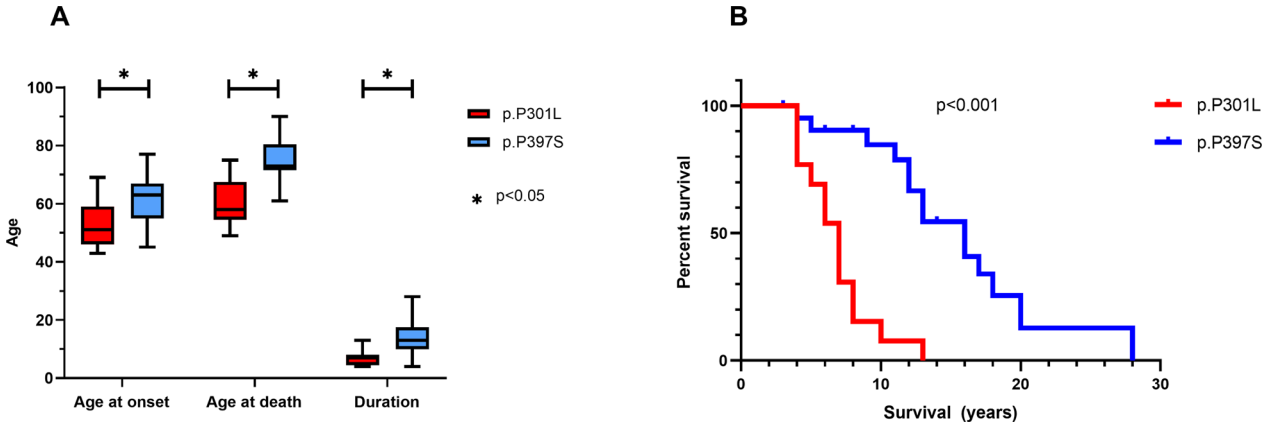

Figure 3. Distribution of onset ages, disease durations, and ages at death associated with p.P397S and P301L *MAPT* carriers (A). Survival curves of p.P397S and P301L *MAPT* carriers (B).

presented oculomotor impairment. All subjects presented bitemporal atrophy with relative frontal lobe preservation (Fig. 2). The frequency of these clinical symptoms does not differ from our published series of p.P301L mutation carriers.[6]

Subjects with the p.P397S *MAPT* variant presented a significantly older age at onset than the patients with the p.P301L *MAPT* mutation (61.3 years vs. 53.5 years; $P = 0.016$). In addition, p.P397S carriers showed a significantly older age at death (76.3 years vs. 60.3 years; $P = 0.009$) and longer disease duration (14.0 years vs. 6.9 years; $P = 0.002$) (Fig. 3).

Genetic results

The eight patients studied carried the exon 13 *MAPT* p.P397S variant. Six of them were probands (subjects I.III.IV, II.II.IX, III.II.VI, III.II.VII, IV.II.V and V.II.III) and the other two (I.III.III and II.II.VIII) were tested after the results found in their relatives. None of them present *c9ORF72* expansion. *GRN* mutations were excluded by direct sequencing in three p.P397S carriers (subjects I.III.IV, II.II.VIII, and IV.II.V) and the rest presented normal serum progranulin levels. In addition, one subject was studied by whole-exome sequencing (II.II.VIII) excluding any other variants in known genes involved in neurodegeneration. This novel variant was predicted as likely pathogenic according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology guidelines and as definitively pathogenic by Guerreiro et. al. algorithm for mutation's pathogenicity.[10,11] In silico analysis produced different results, being predicted pathogenic by SIFT bioinformatics algorithm and probably damaging by Polyphen-2. However, other algorithms scores prediction classified this variant as likely benign (CADD and REVEL algorithms), tolerated (MetaLR), or without a functional consequence (Mutation Assessor). This variant was not described in gnomAD database (http://gnomad.broadinstitute.org/) and it was present at ENSEMBL database as rs1295855402.

Discussion

In this study, we report a new *MAPT* variant (p.P397S) in eight subjects from five families suffering from FTLD with autosomal dominant pattern of inheritance. Even if the eight probands belong to five apparently nonrelated families, we could track a common geographical origin in the southeast of Spain, suggesting a possible founder effect of the p.P397S variant in this area.

We hypothesize that this new *MAPT* variant might be causative of FTLD. According to segregation analysis, their presence in eight affected bvFTD patients and their absence in one unaffected relative is predicted to be likely pathogenic in segregation analysis. In silico analysis produced discrepant results. The p.P397S variant is predicted to destroy a Proline/Serine phosphorylation site at the S396 position. Previous experimental data suggest that phosphorylation at S396 site is necessary to promote the long-term depression at the hippocampus. In this sense, the p.P397S variant potentially will modify the physiological function of tau.[12,13]

All patients with the p.P397S variant presented clinical features consistent with the diagnosis of bvFTD. Most patients also developed semantic language impairment. Three patients developed mild rigid-akinetic parkinsonism. These clinical features do not differ from those of patients with the p.P301L mutation. However, patients with the p.P397S *MAPT* variant showed a

significantly older age at disease onset and slower disease progression compared with p.P301L mutation carriers. Neuroimaging of all patients revealed bitemporal atrophy in concordance with the typical atrophy pattern described in FTLD due to *MAPT* mutations, but with relative preservation of other areas of the brain including frontal areas.[14,15]

Mutations in the *MAPT* gene produce FTLD characterized by early onset dementia, with an age of onset between the third and fifth decade of life and 8–10 years of disease duration.[5] However, previous studies reported that different *MAPT* mutations may show considerable phenotypic variations. This variability might be explained by the microtubule binding properties of the mutant protein.[16] In spite of this, some mutations show an important variability in their age at onset or progression suggesting that other genetic or environmental factors can play an important role in the phenotypic expression.[17] Only a few cases of *MAPT* mutation carriers have been reported to date presenting after the sixth decade.[18–20] Interestingly, other mutations located at the exon 13 are also characterized by a slow rate of disease progression. The p.R406W mutation has a median age of onset of 55 (IQR 51.25–61.75) years and a median disease duration of 14 (IQR 9–26 years).[21] As in our series, memory impairment was a marked symptom in p.R406W carriers. The p.T427M *MAPT* mutation, described only in one family, also seems to have a delayed age at onset (range 60–71 years) and death (67–79 years).[22] However, the p.G389R mutation, also located in exon 13, has been related to an early onset presentation.[23]

The CSF AD biomarkers were tested in three of the p.P397S carriers (subjects II.II.IX, II.II.VIII, and V.II.III). Surprisingly, levels of p-tau ant t-tau were increased in all three, with decreased levels of Aβ42 only in one of them. Several previous reports have described diminished CSF Aβ levels in sporadic or/and genetic FTLD.[24–27] It is discussed whether this finding is related to an increased deposition of Aβ spices or to a reduction of Aβ production, as it is associated with a reduction in soluble APP levels. However, in the absence of neuropathological studies in our patients, we cannot rule out the presence of concomitant AD pathology. Of note, the presence of increased p-tau and t-tau at CSF, in addition with the temporal lobe atrophy, some of these patients could be misdiagnosed as suffering from AD. Of course, it is not possible to discard that the presence of AD concomitant pathology was the cause of these results.

The main limitation of this study is the absence of neuropathological and direct functional data. In the absence of functional evidence, we cannot ultimately rule out the possibility that this *MAPT* variant is just a rare polymorphism. Future functional assays are needed to confirm this variant as a pathogenic mutation.

In conclusion, we report a novel *MAPT* variant, consisting in the substitution of a Proline for a Serine in the codon 397, in eight subjects from five apparently nonrelated families suffering from frontotemporal dementia with autosomal dominant pattern of inheritance. We hypothesize that this new *MAPT* variant might be causative of a less aggressive FTLD than other *MAPT* mutations. In silico analysis using several prediction software produce different results about the potential pathogenicity of this novel variant and segregation analysis predicted it to be likely pathogenic. Thus, in the absence of neuropathology or functional data, we cannot confirm this variant is definitively pathogenic. Future studies are needed in order to determine the pathogenicity of this variant.

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Author Contributions

Conception and design of the study: S. B.-E., A. L., R. S.-V. Acquisition of the data: S. B.-E., I. P., C. P. B., M. T. A.-V., J. O., N. F. Analysis of the data: S. B.-E. Genetic and laboratory analysis: A. A., J. A. P.-B. All authors contributed to the critical revision of the manuscript. Study supervision: R. S.-V.

Conflict of Interest

None of the authors have conflict of interest to be disclosed.

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Trabajo 3

Disease-related cortical thinning in presymptomatic granulin mutation carriers

Sergi Borrego-Écija , Roser Sala-Llonch, John van Swieten, Barbara Borroni, Fermín Moreno, Mario Masellis, Carmela Tartaglia, Caroline Graff, Daniela Galimberti, Robert Laforce Jr, James B Rowe, Elizabeth Finger, Rik Vandenberghe, Fabrizio Tagliavini, Alexandre de Mendonça, Isabel Santana, Matthis Synofzik, Simon Ducharme, Johannes Levin, Adrian Danek, Alex Gerhard, Markus Otto, Chris Butler, Giovanni Frisoni, Sandro Sorbi, Carolin Heller, Martina Bocchetta, David M Cash, Rhian S Convery, Katrina M Moore, Jonathan D Rohrer, Raquel Sanchez-Valle,
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Disease-related cortical thinning in presymptomatic granulin mutation carriers

Sergi Borrego-Écija [a,1], Roser Sala-Llonch [b,1], John van Swieten [c], Barbara Borroni [d], Fermín Moreno [e], Mario Masellis [f], Carmela Tartaglia [g], Caroline Graff [h], Daniela Galimberti [i,j], Robert Laforce Jr [k], James B Rowe [l], Elizabeth Finger [m], Rik Vandenberghe [n], Fabrizio Tagliavini [o], Alexandre de Mendonça [p], Isabel Santana [q], Matthis Synofzik [r,s], Simon Ducharme [t,u], Johannes Levin [v,w,x], Adrian Danek [v], Alex Gerhard [y], Markus Otto [z], Chris Butler [aa], Giovanni Frisoni [bb,cc], Sandro Sorbi [dd,ee], Carolin Heller [ff], Martina Bocchetta [ff], David M Cash [ff], Rhian S Convery [ff], Katrina M Moore [ff], Jonathan D Rohrer [ff], Raquel Sanchez-Valle [a,b,*], on behalf of the Genetic FTD Initiative GENFI

[a] *Alzheimer's disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi I Sunyer, Barcelona, Spain*
[b] *Departament de Biomedicina, Institute of Neuroscience, University of Barcelona, Institute of Biomedical Research August Pi i Sunyer (IDIBAPS), Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain*
[c] *Department of Neurology, Erasmus Medical Centre, Rotterdam, Netherlands*
[d] *Centre for Neurodegenerative Disorders, Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy*
[e] *Cognitive Disorders Unit, Department of Neurology, Donostia University Hospital, San Sebastian, Gipuzkoa, Spain*
[f] *LC Campbell Cognitive Neurology Research Unit, Sunnybrook Research Institute, University of Toronto, Toronto, Ontario, Canada*
[g] *Toronto Western Hospital, Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Ontario, Canada*
[h] *Department of Geriatric Medicine, Karolinska University Hospital-Huddinge, Stockholm, Sweden*
[i] *Biomedical, Surgical and Dental Sciences, University of Milan, Centro Dino Ferrari, Milan, Italy*
[j] *Fondazione IRCCS Ca' Granda, Ospedale Policlinico, Neurodegenerative Diseases Unit, Milan, Italy*
[k] *Clinique Interdisciplinaire de Mémoire, Département des Sciences Neurologiques, Université Laval, Québec, Canada*
[l] *Department of Clinical Neurosciences and Medical Research Council, Cognition and Brain Sciences Unit, University of Cambridge, Cambridge, United Kingdom*
[m] *Department of Clinical Neurological Sciences, University of Western Ontario, London, Ontario, Canada*
[n] *Laboratory for Cognitive Neurology, Department of Neurosciences, KU Leuven, Leuven, Belgium*
[o] *Fondazione Istituto di Ricovero e Cura a Carattere Scientifico, Istituto Neurologica Carlo Besta, Milano, Italy*
[p] *Faculty of Medicine, University of Lisbon, Lisbon, Portugal*
[q] *Faculty of Medicine, University of Coimbra, Coimbra, Portugal*
[r] *Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research and Center of Neurology, University of Tübingen, Tübingen, Germany*
[s] *German Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany*
[t] *Department of Psychiatry, McGill University Health Centre, McGill University, Montreal, Québec, Canada*
[u] *McConnell Brain Imaging Centre, Montreal Neurological Institut, McGill University, Montreal, Québec, Canada*
[v] *Department of Neurology, Ludwig-Maximilians-University, Munich, Germany*
[w] *German Center for Neurodegenerative Diseases (DZNE), Site Munich, Munich, Germany*
[x] *SyNergy, Munich Cluster for Systems Neurology, Munich, Germany*
[y] *Faculty of Medical and Human Sciences, Institute of Brain, Behaviour and Mental Health, University of Manchester, Manchester, UK*
[z] *Department of Neurology, University of Ulm, Ulm, Germany*
[aa] *Department of Clinical Neurology, University of Oxford, Oxford, United Kingdom*
[bb] *Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy*
[cc] *Memory Clinic LANVIE-Laboratory of Neuroimaging of Aging, University Hospitals and University of Geneva, Geneva, Switzerland*
[dd] *Department of Neuroscience, Psychology, Drug Research, and Child Health, University of Florence, Florence, Italy*
[ee] *Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Don Carlo Gnocchi, Florence, Italy*
[ff] *Dementia Research Centre, Department of Neurodegenerative Disease, Queen Square UCL Institute of Neurology, London, UK*

* Corresponding author at: Alzheimer's disease and other cognitive disorders Unit, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), University of Barcelona, Villarroel, 170 08036 Barcelona, Spain.
E-mail address: rsanchez@clinic.cat (R. Sanchez-Valle).
[1] These authors contributed equally.

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ABSTRACT

Mutations in the *granulin* gene (*GRN*) cause familial frontotemporal dementia. Understanding the structural brain changes in presymptomatic *GRN* carriers would enforce the use of neuroimaging biomarkers for early diagnosis and monitoring. We studied 100 presymptomatic *GRN* mutation carriers and 94 noncarriers from the Genetic Frontotemporal dementia initiative (GENFI), with MRI structural images. We analyzed 3T MRI structural images using the FreeSurfer pipeline to calculate the whole brain cortical thickness (CTh) for each subject. We also perform a vertex-wise general linear model to assess differences between groups in the relationship between CTh and diverse covariables as gender, age, the estimated years to onset and education. We also explored differences according to *TMEM106B* genotype, a possible disease modifier. Whole brain CTh did not differ between carriers and noncarriers. Both groups showed age-related cortical thinning. The group-by-age interaction analysis showed that this age-related cortical thinning was significantly greater in *GRN* carriers in the left superior frontal cortex. *TMEM106B* did not significantly influence the age-related cortical thinning. Our results validate and expand previous findings suggesting an increased CTh loss associated with age and estimated proximity to symptoms onset in *GRN* carriers, even before the disease onset.

1. Introduction

Frontotemporal dementia (FTD) is a clinically, genetically and pathologically heterogeneous group of neurodegenerative diseases characterized by behavioral and language impairment. FTD is a highly heritable disorder, with mutations in several genes causing genetic forms of the disease. Mutations in the *progranulin* (*GRN)* gene were identified in 2006 as a cause of familial FTD with TAR-DNA binding protein 43 (TDP-43) inclusions (Baker et al., 2006; Cruts et al., 2006). The prevalence of *GRN* mutations has been estimated at 6% of all FTD patients and 20% of familial FTD (Cruts and Van Broeckhoven, 2008). The majority of FTD due to *GRN* mutations patients present a behavioral variant FTD, non-fluent primary progressive aphasia or corticobasal syndrome (Moore et al., 2019).

In 2010, a genome-wide association study revealed *transmembrane protein 106B* (*TMEM106B)* gene as a risk factor for FTD with TDP-43 inclusions (Van Deerlin et al., 2010). Further studies had replicated these findings, showing an extremely low presence of the *TMEM106B* minor allele in homozygosis in *GRN* patients, indicating that individuals who are homozygous for the minor *TMEM106B* allele are less likely to develop symptoms (Finch et al., 2011; Nicholson and Rademakers, 2016).

Previous work using structural MRI revealed that symptomatic *GRN* mutation carriers typically show a widespread but asymmetric pattern of grey matter (GM) loss, affecting frontal, temporal and parietal lobes (Beck et al., 2008; Fumagalli et al., 2018; Whitwell et al., 2009). Studies in presymptomatic *GRN* mutations carriers have shown divergent results, with many of them reporting no significant brain structural differences compared with noncarriers (Borroni et al., 2012, 2008; Caroppo et al., 2015; Cash et al., 2018; Olm et al., 2018; Panman et al., 2019; Pievani et al., 2014; Rohrer et al., 2015). *TMEM106B* variants have also been studied in the general population using neuroimaging, with the risk allele being related to reduced volume of the left temporal lobe in non-demented subjects (Adams et al., 2014). In this line, and complementing the structural findings, Premi et. al. used functional MRI and found that, in *GRN* carriers, the *TMEM106B* risk haplotype was associated with decreased functional connectivity in the left frontoparietal network (Premi et al., 2014).

In a previous cross-sectional study with a limited number of subjects, we observed that presymptomatic *GRN* mutation carriers presented greater loss of cortical thickness (CTh) by age in temporal areas compared to noncarriers (Moreno et al., 2013). Here, we aimed to expand these previous findings by investigating the change in CTh in a much larger cohort of presymptomatic mutation carriers using data from the Genetic Frontotemporal Dementia Initiative (GENFI). We also aimed to investigate the potential influence of the *TMEM106B* genotype in the grey matter loss in *GRN* carriers.

2. Methods

2.1. Participants

We analyzed cross-sectional data from the GENFI study (Rohrer et al., 2015), Data Freeze 3. The GENFI cohort includes subjects at risk of genetic FTD, from centres across Europe and Canada (https://www.genfi.org/). Subjects in the cohort undergo a standardized clinical and neuropsychological assessment as well as an MRI exam once a year (Rohrer et al., 2013). Our work included the baseline data from 100 presymptomatic mutation carriers and 94 noncarriers from 54 different families. For each subject, sex, age, estimated years to onset (EYO) and education were obtained from the GENFI database. The EYO was computed considering the difference between the subject's age and the average familial age of symptom onset. Asymptomatic status was ascertained based on relative's interview, neurological examination and normality on behavioral scales and neuropsychological tests. Local ethics committees at each site approved the study and all participants provided written informed consent.

2.2. TMEM106B genetic analysis

TMEM106B rs1990622 (C/T) single nucleotide polymorphism was performed according to standard procedures (Premi et al., 2014) in 90 subjects: 46 presymptomatic *GRN* carriers and 44 noncarriers.

2.3. Demographic and clinical statistical analysis

Differences in the clinical and demographic data between carriers and presymptomatic carriers were assessed using *t*-test for continuous variables and chi-squared test was used for dichotomous data. Differences in demographics between *TMEM106B* genotypes were assessed with non-parametric tests (Fisher Test for dichotomous data and Kruskall-Wallis Test for continuous data).

2.4. Image acquisition and processing

Participants underwent a 1.1-mm isotropic resolution volumetric T1 MR imaging on a 3 T using the sequences defined within the GENFI consortium.

MRI images of all subjects were downloaded from GENFI database and processed using FreeSurfer version 6.0 (http://surfer.nmr.mgh.harvard.edu/), with the main goal of computing individual CTh surface maps. Briefly, the FreeSurfer pipeline includes skull stripping (Ségonne et al., 2004), segmentation of the subcortical white matter and deep gray matter volumetric structures (Fischl et al., 2004, 2002), tessellation of boundaries, and definition of the transition between tissue classes. Then, CTh is calculated as the closest distance from the gray/white boundary to the gray/cerebrospinal fluid boundary at each vertex (Dale et al.,

1999; Fischl and Dale, 2000).

Individual CTh maps were visually inspected to detect and correct processing errors. From an initial sample of 114 presymptomatic mutation carriers and 101 noncarriers, 21 subjects were excluded due to bad reconstruction or other FreeSurfer processing errors, resulting in the final sample of 100 presymptomatic carriers and 94 noncarriers. Surface maps were registered to the standard average space and smoothed with a Full Width at Half Maximum (FWHM) of 15 mm.

2.5. Image-based statistics

We first obtained whole brain CTh for each subject, calculated as the average CTh across all vertices (i.e., weighted average between the two hemispheres). This measure was correlated using Pearson's coefficient with age to investigate global age-related trajectories in the two groups. Linear and non-linear regression models were explored in the whole group as showed in the Supplementary material to determine the association between the whole CTh and age (Supplementary material). Due the lack of difference between linear and non-linear models, we used vertex-wise general linear models as implemented in FreeSurfer to test differences between carriers and noncarriers as well as interaction with age at the regional level. We added sex, education and the scanner used as covariates. Homoscedasticity of the samples were assessed by the Non-Constant Variance Test. In addition to chronological age, we also assessed the effect on CTh of the EYO. All maps were corrected for multiple comparisons using precomputed Monte Carlo permutations with a significance threshold of $p < 0.05$ (for both thresholding and cluster significance), as implemented in Freesurfer.

To study whether there were differences in asymptomatic carriers as they approached the predicted symptoms onset, we repeated the group comparison analysis (i.e., carriers vs non-carriers) using only the subgroup of subjects that were closer to the disease onset (i.e. those with EYO > -10 years).

Finally, we repeated the multiple linear regression adding the *TMEM106B* genotype as covariable. For this analysis, we assess the ROIs found significant different in the previous analyses.

3. Results

3.1. Demographic and genetic results

The demographic and genetic data of the 194 subjects are described in Table 1. The mean age at onset of the 54 different families included was 60.1 years (range 43 – 74.5 years). There were no differences in age, EYO, sex or education between groups. On average, presymptomatic mutation carriers presented an EYO of −13.0 years. No significant differences were found in *TMEM106B* haplotypes between groups. In both groups, the homozygosity for the protective genotype (C/C) were rare (6.8% in noncarriers carriers and 6.2% in presymptomatic carriers). No differences in gender, age, EYO or education were found between the different *TMEM106B* genotypes.

Table 1
Demographic characteristics and *TMEM106B* genotype. EYO: Estimated Years to Onset, SD: standard deviation; ns: not significant, *TMEM106B*: *transmembrane protein 106B*.

	Noncarriers n = 94	*GRN* presymptomatic carriers n = 100	Group differences p value
Age, years mean (SD)	47.5 (13.2)	46.8 (12.2)	0.595
EYO, years mean (SD)	−13.2 (14.9)	−13.0 (12.2)	0.967
Sex male/female	51/43	65/35	0.141
Education, years mean (SD)	14.2 (3.8)	14.6 (3.6)	0.658
TMEM106B (rs1990622)	**n = 44**	**n = 46**	
C/C (%)	3 (6.8%)	3 (6.5%)	0.889
C/T (%)	27 (61.4%)	26 (56.5%)	
T/T (%)	14 (31.8%)	17 (37.0%)	

3.2. Group differences in cortical thickness

There were no differences in CTh at the group level when comparing presymptomatic mutation carriers and noncarriers groups, neither with global measures nor with vertex-wise analyses. We hypothesized that this lack of difference might be consequence of the inclusion of subjects far from the predicted age at onset, and we also performed the whole-brain vertex-wise analysis between carriers and noncarriers in the subgroup of subjects nearest to the expected onset (EYO > -10), but no significant differences arose.

3.3. Correlation between cortical thickness and age

When we evaluated the CTh correlation with age at the whole-brain level, both presymptomatic mutation carriers and noncarriers showed a pattern of cortical thinning associated with age ($r = -0.59$ vs $r = -0.53$, both significant with $p < 0.001$), but no significant differences were observed differences between them at the whole brain level ($p = 0.272$) (Fig. 1).

3.4. Vertex-wise general linear models

When comparing carriers and noncarriers at the vertex-wise level with an interaction model, we identified a cluster with significant results (corrected $p < 0.05$) in the left superior frontal cortex (Fig. 2A). Age, gender, education and scanner were included as covariates. When studying the trajectories separately for each group within the significant ROI, we found that presymptomatic carriers showed a significant negative correlation between age and CTh ($r = -0.57$, $p < 0.001$), while noncarriers did not ($r = -0.12$, $p = 0.265$) (Fig. 2B). Additionally, we performed a multiple linear regression model within the ROI to quantify the effect of age education and gender into this result. Only Age and Age × group interaction were significant ($p < 0.001$, see Table 2).

3.5. Correlations between cortical thickness and EYO

Due to the presence of different *GRN* mutations that may present different ages of onset, we repeated the interaction analysis using EYO instead of actual age. We identified a cluster with significant differences between carriers and noncarriers (corrected $p < 0.05$) covering the right temporal cortex, the banks of superior temporal sulcus, the inferiorparietal and the supramarginal gyrus. It is noticeable that these regions also appeared in the analysis with age, however they did not survive multiple comparisons. Again, we performed multiple linear regression models to predict the ROI-CTh of these areas considering EYO (instead the age), we found similar results, with presymptomatic carriers presenting significant higher CTh loss by age than noncarriers ($p < 0.01$). Fig. 3 shows the correlation between CTh and EYO in both groups. ($r = -0.65$ for carriers vs $r = -0.33$ for noncarreirs, $p < 0.01$) (Fig. 3).

3.6. Influence of the TMEM106B genotype in the CTh – Age relationship

We did not find significant results at the vertex-wise level for the *TMEM106B* analyses for any of the comparisons tested. Therefore, we performed a hypothesis-driven study by focusing on the region that resulted significant in the *age* × *group* interaction. We divided the *GRN* carriers in groups according their *TMEM106B* genotype we found a significant negative correlation in the T/C carriers ($r = -0.52$, $p < 0.01$) and the T/T carriers ($r = -0.47$, $p < 0.05$), but not in the three subjects with the C/C genotype ($r = -0.365$, $p = 0.762$; Fig. 4). Only the correlation of the T/C carriers was significant and showed statistical

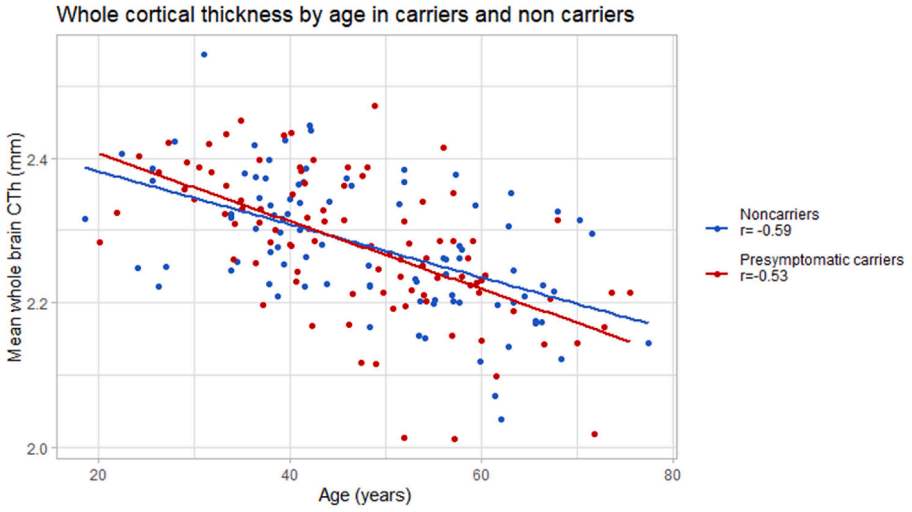

Fig. 1. Scatter plot showing correlation between whole CTh and age in presymptomatic *GRN* carriers (red) and noncarriers (blue). No statistical differences between trajectories were found. CTh: Cortical Thickness. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

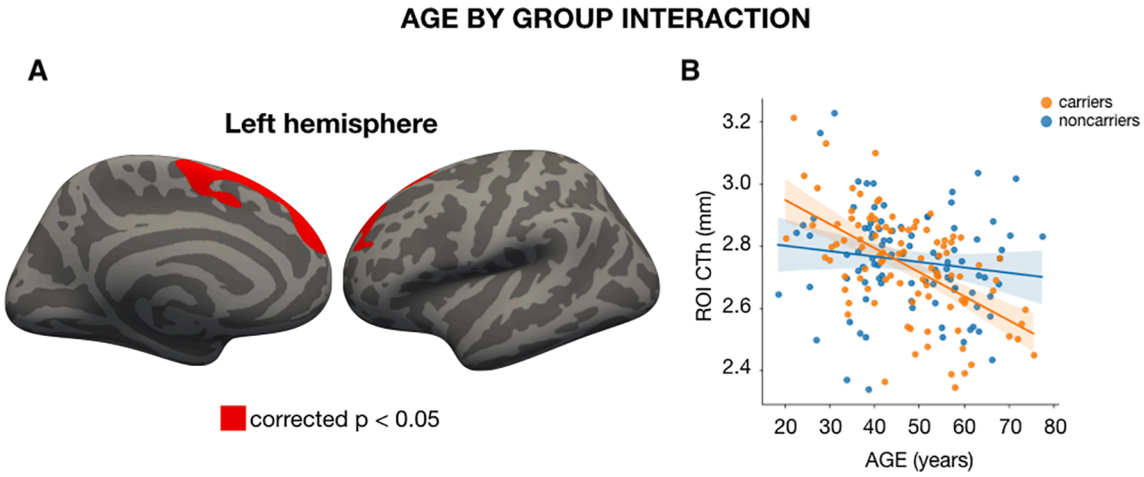

Fig. 2. Relationship between CTh and age in the selected area of the cortex where significant differences between carriers and noncarriers where found: A) Brain maps showing the area with statistical differences between presymptomatic carriers and noncarriers ($p < 0.05$). B) Scatter plot showing relationship between CTh and age in presymptomatic *GRN* carriers (red) and noncarriers (blue) in the selected area. Lines represent estimated linear regression models for both groups. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Multiple linear regression model to predict CTh based on the presence of GRN mutation (presymptomatic carriers vs noncarriers) and age. Sex and education were added as covariates.

	β (95% CI)	t value	p value
Intercept	3.021 (2.857, 3.185)	36.82	<0.001
GRN	0.019 (−0.025, 0.063)	0.85	0.393
Noncarriers vs *carriers*			
Age (years)	−0.008 (−0.010, −0.005	−6.03	**<0.001**
Education (years)	0.050 (−0.001, 0.011)	1.68	0.094
Gender	−0.001 (−0.046, 0.043)	−0–05	0.956
Male vs female			
Age × *GRN*	0.006 (0.003, 0.010)	3.50	**<0.001**

differences with the noncarriers group ($p < 0.05$). When we added the *TMEM106B* genotype as covariate to the multiple linear regression analysis we did not find any influence of this over the CTh, neither for presymptomatic carriers nor the noncarriers.

4. Discussion

In this study, we used data from the GENFI cohort to evaluate CTh in presymptomatic *GRN* mutation carriers. Although we did not find differences between carriers and noncarriers at the group-wise comparison, we found differences in the influence of aging and estimated years to onset in CTh, suggesting a greater cortical loss in presymptomatic carriers as they approach the clinical onset.

Several cross-sectional and longitudinal studies have evaluated GM loss in presymptomatic *GRN* mutation carriers with different methodologies, with partially divergent results. Our study, as most previous cross-sectional studies using structural MRI, did not find gray matter cortical thickness differences between presymtomatic *GRN* mutation carriers and controls (Borroni et al., 2012, 2008; Caroppo et al., 2015; Cash et al., 2018; Dopper et al., 2013; Fumagalli et al., 2018; Moreno et al., 2013; Panman et al., 2019). By contrast, few studies found gray matter atrophy pattern in presymptomatic carriers: Pievani and colleagues found greater GM loss in frontal areas, (Pievani et al., 2014) while Rohrer et. al. found significant differences between carriers and

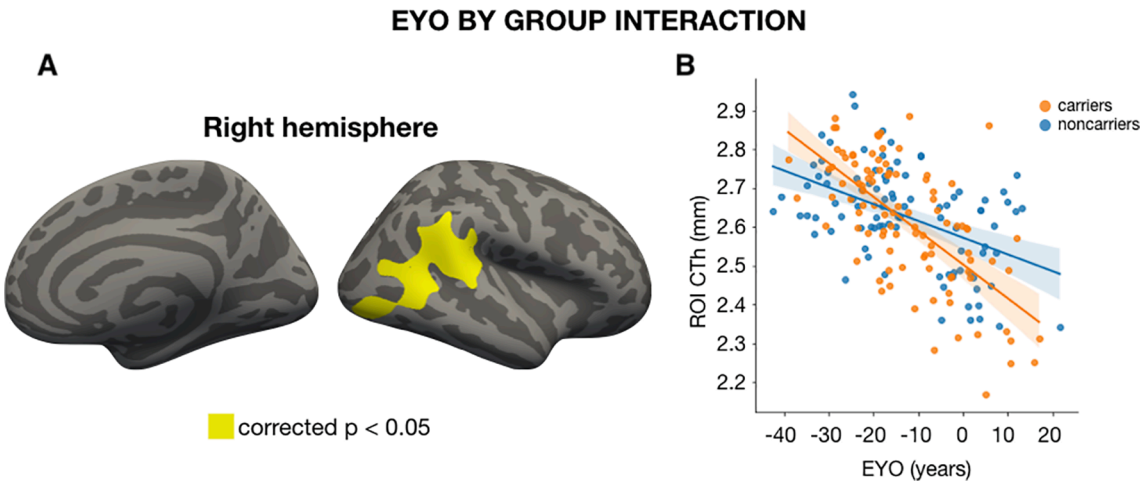

Fig. 3. Relationship between CTh and EYO: (A) Brain maps showing areas with statistical differences between carriers and noncarriers. B) Scatter plot illustrates the relationship between CTh and EYO in carriers and noncarriers. The X-axis represents the EYO. The Y-axis represents the mean CTh of the ROI covering all areas with significant differences between carriers and noncarriers. CTh: Cortical Thickness; EYO: estimated years to onset; ROI: Region of Interest.

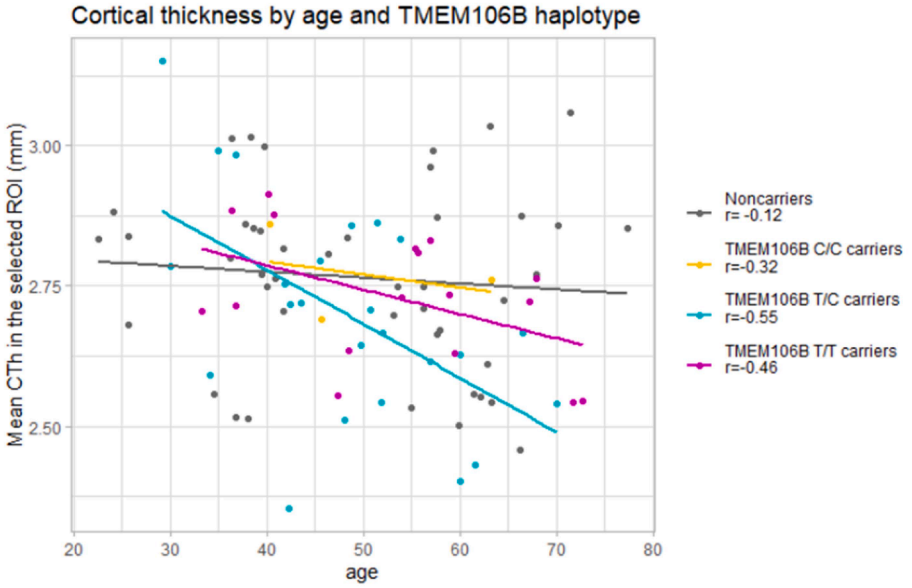

Fig. 4. Scatter plot showing relationship between CTh and age in *GRN* carriers according their *TMEM106B* genotype and noncarriers in the left superior frontal cortex.

noncarriers in the insula 15 years before the expected onset, and in the temporal and parietal lobes 10 years before the expected onset (Rohrer et al., 2015). These discrepancies in the quantification of GM loss in presymptomatic *GRN* mutation carriers differ from the extensive GM atrophy observed in symptomatic mutation carriers, even with a visual inspection. Several explanations have been proposed to explain this divergence of results. First, in a group cross-sectional comparison, subjects far from symptom onset are mixed with subjects close to disease onset; if CTh loss in *GRN* mutation carriers accelerates around the time of symptoms onset, mixing subjects at different intervals from symptom onset could cancel any apparent differences with noncarriers. In addition, the asymmetric pattern of atrophy in *GRN* mutation carriers might limit the differences in group-wise neuroimaging analyses.

In a previous study, in a sample of 13 presymptomatic *GRN* mutation carriers we observed that presymptomatic carriers presented greater age-related cortical thinning in the temporal areas when compared with controls (Moreno et al., 2013). In the present study, we expand these previous results in a much larger cohort of subjects at risk of FTD due to mutations in *GRN*. We found that both, presymptomatic *GRN* carriers and noncarriers showed a negative correlation of their CTh with age, with older subjects presenting lesser CTh. With the interaction analysis, we found a group-by-age effect in the left superior frontal cortex. In addition, the results of the multiple linear model of our study showed that, in this area, the presymptomatic carriers showed significantly greater loss of CTh with age than noncarriers. It might suggest that presymtomatic *GRN* carriers suffer a greater neuronal loss in this area due to neurodegeneration rather than normal aging. This is an area particularly affected in symptomatic patients (Cash et al., 2018) that have also been found to have increased rates of atrophy in longitudinal studies with presymptomatic carriers (Caroppo et al., 2015; Chen et al., 2019).

Recent work suggests that EYO has limited value in *GRN* families, due to a weak correlation between the individual age at onset and family age at onset (Moore et al., 2019) but better predictive markers of the disease age of onset are still lacking. Thus, as the present sample includes different *GRN* mutations, we also investigate the effect of EYO in

CTh in addition to the effect of the chronological age. When we study the correlation between CTh values and EYO, we found significant differences between presymptomatic *GRN* carriers and noncarriers in the right supramarginal gyrus and the banks of the rightsuperior temporal sulcus, similar to the area found in a previous work using only subjects with the same *GRN* mutation (Moreno et al., 2013) and thus, chronological age was interchangeable with EYO. Even if the localization of the significant clusters were not the same when EYO was used in the interaction analysis instead of age, we believe that both the dorsofrontal and the supramarginal/temporal gyrus areas are important in the disease, as they both appear at the uncorrected level. However, the fact that the magnitude of the effects is small the inclusion of a large number of covariates might hide some results when we corrected for multiple comparisons.

Variants in the *TMEM106B* gene have been hypothesized to be a genetic modulator of risk for *GRN* carriers. Previous works suggests that *TMEM106B* minor allele in homozygosis (C/C in rs1990622) is protective or might delay the onset in individuals with pathogenic *GRN* mutations. On this basis, we evaluate the influence of the *TMEM106B* genotype in our results. Despite the fact that we did not find differences between the different *TMEM106B* genotypes, we found a trend suggesting that C/C carriers might present a lower loss of CTh by age than the T/C and T/T carriers in the left superior frontal cortex. The absence of statistical differences in these analyses may be consequence of the small sample of subjects carrying the C/C genotype in our series. This would be in consonance with previous works with functional MRI that found decreased brain connectivity within the middle frontal gyrus and the left frontoparietal network in *GRN* carriers with the risk *TMEM106B* allele in front those with the protective allele (Premi et al., 2014).

The main strength of this study lies in the large sample of presymptomatic subjects carrying mutations in *GRN*. We also acknowledge some limitations. First, our age-related results are based on cross-sectional rather than longitudinal data. Although our analysis suggests a faster atrophy in presymptomatic carriers, further studies with longitudinal data are needed to corroborate this hypothesis. Another limitation is the fact that our study includes different *GRN* mutations which may present different ages at symptom onset, and EYO was used in some of the analysis to overcome this limitation. Finally, the *TMEM106B* haplotype was not available in all subjects. This fact, combined with the low frequency of the C/C haplotype in our series, limit the validity of statistical analysis performed to evaluate the influence of the *TMEM106B* gene in *GRN* carriers.

5. Conclusions

In conclusion, despite no differences in CTh were found at the whole-group comparison, the proposed linear model showed that presymtomatic *GRN* carriers present a significantly greater loss of CTh with age and proximity to expected disease onset. These findings suggest a faster process of neuronal loss in carriers, supporting that structural neuroimaging might be useful to monitor the effect of disease-modifying therapies even in presymptomatic phases of the disease.

Role of the funding source

The funding sources have no role in the design of this study, its execution, analyses, interpretation of the data, or the decision to submit results.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2020.102540.

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GRN 8

Trabajo 4

Cognitive decline in amyotrophic lateral sclerosis: neuropathological substrate and genetic determinants

S Borrego-Écija, J Turón-Sans, T Ximelis, I Aldecoa, L Molina-Porcel, M Povedano, MA Rubio, J Gámez, A Cano, M Paré, L Bajo, J Sotoca, M Balasa, A Lladó, R Sánchez-Valle, R Rojas-García, E Gelpi

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RESEARCH ARTICLE

Cognitive decline in amyotrophic lateral sclerosis: Neuropathological substrate and genetic determinants

Sergi Borrego-Écija[1] | Janina Turon-Sans[2,3] | Teresa Ximelis[4] | Iban Aldecoa[4,5] | Laura Molina-Porcel[1,4] | Mónica Povedano[6] | Miguel Angel Rubio[7] | Josep Gámez[8,9] | Antonio Cano[10] | Martí Paré-Curell[11] | Lorena Bajo[12] | Javier Sotoca[13] | Jordi Clarimón[2,3] | Mircea Balasa[1] | Anna Antonell[1] | Albert Lladó[1] | Raquel Sánchez-Valle[1,4] | Ricard Rojas-García[2,3] | Ellen Gelpi[4,14]

[1]Alzheimer's Disease and Other Cognitive Disorders Unit, Neurology Department, Hospital Clínic, Institut d'Investigacions Biomediques August Pi i Sunyer, University of Barcelona, Barcelona, Spain

[2]Neurology department, Research Institute, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain

[3]Center for Networked Biomedical Research into Neurodegenerative Diseases (CIBERNED), Madrid, Spain

[4]Neurological Tissue Bank, Biobanc-Hospital Clínic-IDIBAPS, Barcelona, Spain

[5]Pathology Department, CDB, Hospital Clinic Barcelona, Barcelona, Spain

[6]Service of Neurology, Motor Neuron Unit, IDIBELL, Bellvitge University Hospital, Hospitalet de Llobregat, Spain

[7]Neuromuscular Unit, Department of Neurology, Hospital del Mar, Barcelona, Spain

[8]ALS Unit, Neurology Department, Vall d'Hebrón University Hospital, Vall d'Hebrón Research Institute (VHIR)

[9]European Reference Network on Rare Neuromuscular Diseases (ERN EURO-NMD), Department of Medicine, UAB, Barcelona, Spain

[10]Neurology Department, Hospital de Mataró, Mataró, Spain

[11]Neurology Department, Hospital Germans Trias i Pujol, Badalona, Spain

[12]Servei de Geriatria, Fundació Hospital de la Santa Creu, Hospital Universitari de la Santa Creu de Vic, Vic, Spain

[13]Neurology Department, Hospital Mutua de Terrassa, Terrassa, Spain

[14]Division of Neuropathology and Neurochemistry, Department of Neurology, Medical University of Vienna, Vienna, Austria

Correspondence
Ellen Gelpi, Division of Neuropathology and Neurochemistry, Department of Neurolgy, Medical University of Vienna Medizinischer Universitaetscampus Wien, Allgemeines Krankenhaus der Stadt Wien, Leitstelle 4J, Waehringer Guertel 18-20, 1090 Vienna, Austria.
Email: ellen.gelpi@idibaps.org, ellen.gelpi@meduniwien.ac.at

Ricard Rojas García, Neurology Department, Institut d'Investigacions Biomèdiques-Hospital de la Santa Creu i Sant Pau, Carrer de Sant Quintí, 89, Barcelona 08041, Spain.
Email: rrojas@stpau.cat

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Abstract

Cognitive impairment and behavioral changes in amyotrophic lateral sclerosis (ALS) are now recognized as part of the disease. Whether it is solely related to the extent of TDP-43 pathology is currently unclear. We aim to evaluate the influence of age, genetics, neuropathological features, and concomitant pathologies on cognitive impairment in ALS patients. We analyzed a postmortem series of 104 ALS patients and retrospectively reviewed clinical and neuropathological data. We assessed the burden and extent of concomitant pathologies, the role of *APOE* ε4 and mutations, and correlated these findings with cognitive status. We performed a logistic regression model to identify which pathologies are related to cognitive impairment. Cognitive decline was recorded in 38.5% of the subjects. Neuropathological features of frontotemporal lobar degeneration

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(FTLD) were found in 32.7%, explaining most, but not all, cases with cognitive impairment. Extent of TDP-43 pathology and the presence of hippocampal sclerosis were associated with cognitive impairment. Mutation carriers presented a higher burden of TDP-43 pathology and FTLD more frequently than sporadic cases. Most cases (89.4%) presented some degree of concomitant pathologies. The presence of concomitant pathologies was associated with older age at death. FTLD, but also Alzheimer's disease, were the predominant underlying pathologies explaining the cognitive impairment in ALS patients. In sum, FTLD explained the presence of cognitive decline in most but not all ALS cases, while other non-FTLD related findings can influence the cognitive status, particularly in older age groups.

KEYWORDS
Alzheimer's disease, amyotrophic lateral sclerosis, ALS-FTD, frontotemporal dementia, neuropathology, TDP-43 protein

1 | INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by progressive weakness and atrophy because of loss of motor neurons. Cognitive decline and behavioral impairment have been recently recognized as part of the disease (1,2). Approximately half of the patients with ALS have cognitive or behavioral impairment and around 10–20% fulfill clinical diagnostic criteria of any of the clinical variants of frontotemporal dementia (FTD), mainly behavioral variant (bvFTD), but also of primary progressive aphasia (PPA) (2–5). The majority of ALS cases and almost half of FTD patients harbor neuronal, and a proportion also glial, inclusions of TAR DNA-binding protein 43 (TDP-43) at neuropathological examination. In addition, both diseases share genetic alterations such as the hexanucleotide expansion in *chromosome 9 open reading frame 72* (*C9orf72*) gene, as well as mutations in the *tank-binding kinase 1* (*TBK1), sequestosome-1 (SQSTM1), TAR DNA-binding protein* (*TARDBP*), or *valosin-containing protein* (*VCP)*. For these reasons, ALS and FTD are now recognized as a disease continuum (6). FTLD is the pathological substrate underlying most FTD cases. The term frontotemporal lobar degeneration (FTLD) is used as an umbrella term for the different clinical variants (bvFTD, semantic, and non-fluent PPA) as well as a neuropathological term that refers to neuronal loss and gliosis of the frontal and temporal lobes. As a matter of clarity, we refer to the neuropathological concept when using the term FTLD throughout the text.

On the other hand, concomitant neurodegenerative disease-related proteinopathies are frequent and have been primarily associated with age at death (7–9). It remains unclear whether this overlap is coincidental or driven by common physiopathological mechanisms leading to global protein misfolding and aggregation. Genetic factors, especially the presence of *apolipoprotein E* (*APOE*) ε4 allele, an extensively studied potential genetic risk in Alzheimer's disease (AD), has been associated with the presence of AD-related co-pathologies (9–11). Previous work suggests that *APOE* haplotype also influences the risk of developing hippocampal sclerosis (HS) with TDP-43 proteinopathy in aging (12,13), as well as FTD in patients with ALS (14,15). Whether cognitive impairment in ALS patients is related to underlying FTLD or because of another concomitant pathology remains unclear.

The purpose of this study was to evaluate the influence of demographic, genetic, and pathologic features, including the presence of concomitant pathologies, on cognitive impairment in a neuropathologically defined cohort of ALS-TDP patients.

2 | METHODS

2.1 | Standard protocol approvals, registrations, and patient consents

All cases meeting criteria for a primary clinicopathological diagnosis of ALS associated with TDP-43 proteinopathy were selected from the Neurological Tissue Bank (NTB), Hospital Clínic - IDIBAPS Biobank in Barcelona from the period 1994 to 2018. In order to get a homogeneous sample, patients without TDP-43 pathology and mimics were excluded. The study was approved by the Ethics Committee of Hospital Clínic de Barcelona. All individuals or relatives had given their informed consent for the use of brain tissue for research.

2.2 | Clinical classification

We retrospectively reviewed the medical records available at the NTB and contacted the neurologists who attended the patients during life. As we included ALS donors since 1994, some cases had not been systematically screened for cognitive impairment. In those subjects, the presence and the grade of cognitive impairment were assessed by the global impression of the treating neurologist. They were asked to fill in a form that included the following clinical information: age at onset, age at death, disease duration, site of onset (bulbar/spinal), presence or absence of cognitive or behavioral impairment, and diagnosis of bvFTD or PPA. The severity of the cognitive/behavioral impairment was retrospectively assessed according the CDR® Dementia Staging Instrument plus National Alzheimer's Coordinating Center Behaviour and Language Domains (CDR plus NACC-FTLD) (16). This rating scale classifies the severity of the dementia according eight domains in: no cognitive impairment (score of 0), mild cognitive impairment (score of 0.5) or mild, moderate or severe dementia (scores of 1, 2, and 3, respectively). The cognitive domains affected were assigned according to the expert opinion of the attending neurologist or the neuropsychological evaluation. For every patient, we considered the last information available before death. Cases with insufficient clinical information to be classified were excluded from the study. With this information, patients were classified into three clinical subtypes: i) ALS cognitively not impaired (ALSni) when cognitive decline was not present or reported, ii) ALS with cognitive impairment (ALSci) when cognitive or behavioral decline were reported but without fulfilling bvFTD or PPA criteria and iii) ALS with frontotemporal dementia (ALS-FTD) when patients fulfilled criteria for bvFTD or PPA (2,17,18).

2.3 | Neuropathological work-up

Neuropathological examination was performed according to standardized protocols at the NTB. Half brain was dissected in the fresh state, frozen and stored at −80°C and the other half was fixed in formaldehyde solution for 3 weeks. At least 25 representative brain areas were embedded in paraffin, cut at 5 μm and stained with hematoxylin & eosin and luxol fast blue in selected brain areas. Immunohistochemistry was performed using various antibodies including anti-βA4, anti-pTau, anti-RD3 and anti-RD4 Tau, anti-α-synuclein, anti-ubiquitin, anti-p62, anti α-internexin, anti-FUS, and anti-TDP-43, and phTDP-43. Immunoreaction was visualized by the EnVision + system peroxidase procedure (DAKO). Details on tissue section pretreatment, antibody dilution, and incubation time are shown in Table S1.

Disease assessment was performed according to international consensus criteria. All cases were staged following the criteria proposed by Brettschneider for ALS (19). Presence of FTLD was established when neuronal loss and gliosis were observed in temporal and/or frontal regions (20). FTLD-TDP subtype classification was performed based on TDP-43 or pTDP-43 immunohistochemistry following current recommendations (21). HS was considered on HE-stained sections when neuronal loss and gliosis at least in the subiculum and/or CA1 sector of the hippocampus were observed (22). Cases that showed only mild gliosis in subiculum were considered incipient HS. In all cases, we evaluated the presence of fine TDP-43 immunoreactive neurites in the CA1 sector. As previous reports stated that the presence of TDP-43 inclusions in the anterior cingulate may identify patients with clinical bvFTD, we specifically assessed this area in cases with available tissue (23).

For the analysis of concomitant pathologies, we considered the following findings: neurofibrillary pathology, staged according to Braak criteria (24), β-amyloid phases, evaluated according to Thal criteria (25), and neuritic plaque score, assessed according to the Consortium to Establish a Registry for Alzheimer Disease criteria (26). The National Institute on Aging-Alzheimer's Association (NIAA) Guidelines for neuropathologic assessment of AD were applied and a final ABC score was assigned (27). Cerebral amyloid angiopathy was recorded as present or absent. Additionally, possible and definite PART pathology was defined following the current pathological criteria (28). In order to assess whether progressive spinal cord degeneration is associated to astroglial tau pathology, we also evaluated the presence of age-related tau astrogliopathy (ARTAG) at the cervical, dorsal, and lumbosacral spinal level when available and defined thorn-shaped astrocytes and granular fuzzy astrocytes as present/absent, and recorded their location (subependymal, subpial, and perivascular) and the affected level (cervical, dorsal, and/or lumbosacral) (29). Assignments of Lewy body pathology were performed following McKeith criteria (30). Argyrophilic grain disease (AGD) was staged according to the Saito criteria (31). Limbic-predominant age-related TDP-43 encephalopathy (LATE) was evaluated according to the recently described criteria, but only in those subjects without FTLD, as both are TDP-43 proteinopathies and may overlap in temporomedial regions (32). In addition, the presence of granulovacuolar degeneration (GVD) was assessed on HE- and ubiquitin-stained sections in the hippocampus and, if present, was extended to further limbic areas and staged according to Thal et al. (33). Vascular pathology including microvascular lesions and territorial infarcts were recorded as present or absent.

2.4 | Genetic analysis

DNA was extracted from fresh-frozen cerebellum using the QIAamp DNA Mini kit for DNA purification from

tissues (QIAGEN Co.) following the manufacturer's instructions. For *APOE* genotyping two single nucleotide polymorphisms (SNPs) in *APOE* gene were determined (rs429358 and rs7412) using TaqMan genotyping technologies (Life technologies, Carlsbad, USA).

Systematic screening for potential *C9orf72* expansion mutation carriers was performed searching for ubiquitin/p62-positive inclusions in the cerebellum and hippocampus as surrogate and as reported previously (34). The *C9orf72* repeat was confirmed in suspected cases by repeat-primed PCR and fragment-length analysis (35). The identification of other mutations was not performed by systematic screening but by previous studies or usual clinical practice according to the criteria of the treating neurologist (36). Information on other affected family members was not available.

2.5 | Statistical analysis

We compared clinical, genetic, and pathology variables between ALS, ALSci, and ALS-FTD groups with χ^2 or Fisher tests for categorical data and Wilcoxon or Kruskal–Wallis test for ordinal and continuous data. The association of the clinical, demographic, and genetic data with the neuropathological findings was assessed by Kruskal–Wallis test and Fisher test. Finally, we performed a logistic multivariate regression to test the association between cognitive status and neuropathological variables. In this logistic multivariate regression, we included the presence of cognitive decline as dependent variable and the presence of the following neuropathological items as independent variables: frontotemporal lobar degeneration, hippocampal sclerosis, Alzheimer's disease, Lewy body disease, CAA, PART, ARTAG, AGD, GVD, LATE, and vascular pathology. As some of these covariates may suffer from relevant collinearity, other reduced models including less covariates were also assessed. Statistical significance was set at $p < 0.05$ for all analyses.

3 | RESULTS

3.1 | Demographic and genetic features of the ALS cohort

Of the 112 donors with a neuropathological diagnosis of ALS, four were excluded because of the absence of TDP-43 pathology (three ALS-FUS cases and one subject without pathological inclusions). Another four cases were excluded because of incomplete neuropathological exam or insufficient clinical information. Finally, a total of 104 cases (50 male and 54 female) were included in the study. The onset was bulbar in 30.9% of cases. Mean age at onset of motor symptoms was 63.8 years (range 29–87) and the mean age at death was 67.7 (range 34–92).

Cognitive decline was recorded in 40 subjects (38.5%). Thirty-one of them (29.8%) fulfilled diagnostic criteria for bvFTD or PPA (29 and 2 cases, respectively), while nine were classified as ALSci. Table 1 shows demographic, neuropathological, and genetic features of ALSni, ALSci, and ALS-FTD patients. No demographic differences in age at onset, age at death or disease duration were found between ALS, ALSci, and ALS-FTD patients. Bulbar onset was more frequent in subjects with ALS-FTD ($p = 0.03$).

Most patients (67.6%) were homozygous for *APOE* ε3/ε3, 5.1% had ε2/ε3, 22.2% ε3/ε4, and 5.1% ε4/ε4. None of them were homozygous for *APOE* ε2. No significant differences in *APOE* genotypes were found between groups. Mutations in genes reported as causative of or at risk for ALS were found in 21 subjects: 14 *C9orf72*, 2 *TARDBP* (p.A90V and p.I383V), 2 *SQSTM1* (both c.1157C > T), 1 *TBK1* (p.T79del), 1 *VCP* (p.I27V), and 1 *TAF15* (p.G462S). Most of these mutation carriers have been previously reported (34,36,37). More than half of ALS-FTD subjects presented an ALS/FTD related mutation (61.3%), this percentage was higher than in ALSci (0%) and ALSni (3.1%) subjects ($p < 0.001$).

3.2 | Neuropathological features and their relationship with cognitive impairment and genetics

Figure 1 summarizes the main neuropathological features of ALS patients.

3.2.1 | Brettschneider staging (Figure 2A)

The distribution of the TDP-43 pathology according to the Brettschneider score for ALS was as follows: 9 subjects were scored as stage 1, 17 as stage 2, 26 as stage 3, and 51 as stage 4. In one subject the Brettschneider staging was not at all applicable because of the presence of TDP-43 pathology in motor cortex and entorhinal region but not in inferior olive, medullary reticular formation, prefrontal cortex or striatum. The Brettschneider stage was strongly associated with the CDR plus NACC FTLD stage of dementia ($p < 0.01$, Figure 2A). ALS-FTD patients showed significant higher Brettschneider stages than ALSni patients ($p < 0.001$; see Table 1). The presence of a genetic mutation was associated with a higher Brettschneider stage ($p < 0.01$, Figure 2A), while APOE was not. No significant differences concerning disease duration were found between the different Brettschneider stages.

3.2.2 | Hippocampal sclerosis (Figure 2B)

HS was detected in 10 subjects (9.6%). Other eleven cases showed signs of incipient HS. Subjects with ALS-FTD or

TABLE 1 Demographic, neuropathologic, and genetic features in patients ALS, ALSci, and ALS-FTD

	ALSni	ALSci	ALS-FTD	
	n = 64 (61.5%)	n = 9 (8.7%)	n = 31 (29.8%)	*p* value
Demographic features				
Male/female	30/34	4/5	16/15	ns
Age at onset	63.0 (14.3)	67.1 (10.9)	64.3 (11.6)	ns
Mean (SD)				
Age at death	66.6 (13.6)	71.2 (10.3)	68.7 (10.7)	ns
Mean (SD)				
Bulbar onset (%)	23.0%	22.2%	54.2%	<0.05*
CDR plus NACC FTLD	64/0/0/0/0	0/9/0/0/0	0/0/6/9/16	<0.001
0/0.5/1/2/3				
Neuropathologic features				
Brettschneider stage (stage 1/2/3/4)	8/15/21/19	0/1/3/5	1/1/2/27	<0.001*
FTLD (%)	4.7%	44.4%	87.1%	<0.001*,**,***
HS (%)	6.2%	33.3%	41.9%	<0.001*, <0.05**
Genetics				
APOE genotype (%)				
ε2/ε3	6.3%	0 %	3.7%	ns
ε3/ε3	69.8%	55.6%	66.7%	
ε3/ε4	20.6%	22.2%	25.9%	
ε4/ε4	3.2%	22.2%	3.7%	
Mutations (%)				
C9orf72	2 (3.1%)	0 (0%)	12 (38.7%)	<0.001*,***
TARDBP	0 (0%)	0 (0%)	2 (6.5%)	
VCP	0 (0%)	0 (0%)	2 (6.5%)	
TBK1	0 (0%)	0 (0%)	1 (3.2%)	
SQSTM1	0 (0%)	0 (0%)	1 (3.2%)	
TAF15	0 (0%)	0 (0%)	1 (3.2%)	

Abbreviations: ALS, amyotrophic lateral sclerosis; ALSci, amyotrophic lateral sclerosis with cognitive impairment; ALS-FTD, amyotrophic lateral sclerosis with frontotemporal dementia; CDR plus NACC FTLD, Clinical Dementia Rating Dementia Staging Instrument PLUS National Alzheimer's Disease Coordinating Center frontotemporal lobar degeneration Behaviour and Language Domains; FTLD, frontotemporal lobar degeneration; HS, Hippocampal Sclerosis; ns, statistically not significant.

*Significant differences between ALSni and ALS-FTD.

**Significant differences between ALSni and ALSci.

***Significant difference between ALSci and ALS-FTD.

ALSci presented HS more frequently than ALSni cases ($p < 0.01$ and $p < 0.05$, respectively). The presence of HS was also significantly associated with higher stages in the CDR plus NACC FTLD ($p < 0.01$). HS was strongly correlated with Brettschneider staging and presence of FTLD: all subjects with HS presented a stage 4 of Brettschneider, and 88.2% presented also with FTLD. TDP-43-positive neurites in CA1 sector were not detected in any of the cases with HS or incipient HS in the ALSni group. In contrast, two cases with additional AD-type pathology showed frequent fine TDP-43-positive neurites in CA1 sector. The presence of a genetic mutation or the *APOE* ε4 were not associated with a higher prevalence of HS.

3.2.3 | Frontotemporal lobar degeneration (Figure 2C)

Neuropathological features consistent with FTLD were found in 34 subjects (32.7%). Three cases were classified as type A, 25 as type B, 5 showed mixed features of type A and B, and 1 subject had FTLD type C. Most cases with neuropathological FTLD fulfilled clinical criteria of ALS-FTD (77.1%) or ALSci (14.3%). FTLD was strongly associated with higher stages in the CDR plus NACC FTLD ($p < 0.001$). It was present in 87.1% of the ALS-FTD cases, in 44.0% of ALSci, and only in 4.7% of ALSni cases. Moreover, FTLD was present in 63.0% cases with a Brettschneider ALS stage 4 and in

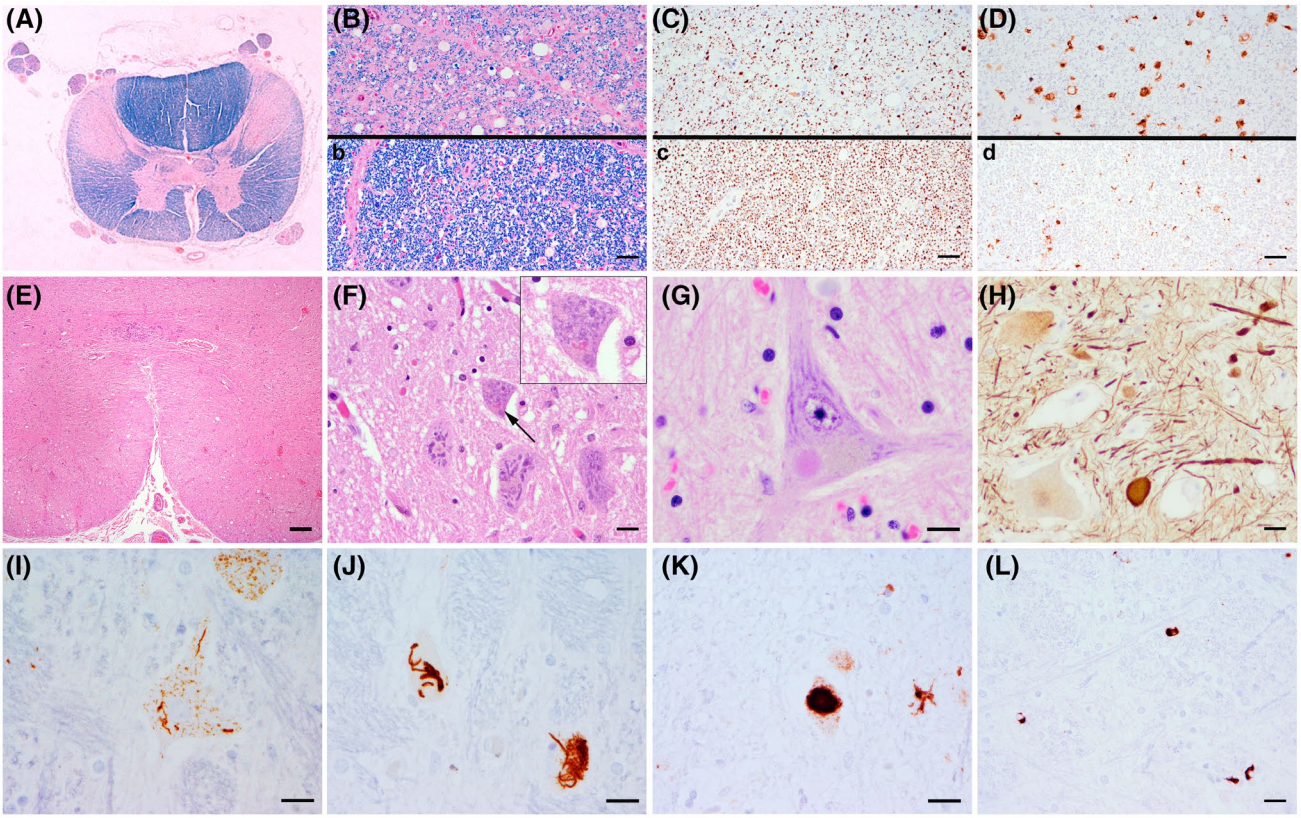

FIGURE 1 **Characteristic neuropathological features of ALS. (A)** Cross section through the thoracic spinal cord shows prominent degeneration of the lateral and anterior corticospinal tract and atrophy of the anterior horns (higher magnification in **E**). There is also myelin pallor in anterior roots compared to posterior roots. The degeneration of the lateral corticospinal tract is characterized by loss of myelin sheaths **(B)**, reduction of axonal profiles **(C),** and increased macrophagic activity **(D)**, as compared with posterior horns **(b, c, d)**. **(F)** some residual motor neurons (here from the n. XII) may contain granular eosinophilic cytoplasmic inclusions or Bunina bodies (arrow and inset). Others may appear as pale spherical inclusions **(G)**. Axonal damage with swellings **(H)** in anterior horns is well depicted by anti-neurofilament immunohistochemistry (large brown structures). **I–L:** Immunohistochemistry for pTDP-43 reveals a spectrum of inclusions including a fine granular pattern in large motor neurons **(I)**, fibrillar and skein like inclusions **(J)**, compact spherical neuronal inclusions **(K)**, and coiled-body like inclusions in oligodendrocytes **(L)**. Scale bars: 20 μm in Bb, Cc, Dd, F; 10 μm in G–L, 100 μm in E

4.3% of cases in stage 3, while it was absent in stages 1 and 2.

ALS subjects with any genetic mutation presented significantly more risk to develop FTLD than ALS without known mutations (80.9% vs. 20.4%, OR 15.92, IC 95% 4.45–73.66, $p < 0.001$). By contrast, *APOE* ε4 was not associated with a higher risk of FTLD or a specific FTLD subtype. Cases carrying the *C9orf72* expansion were neuropathologically classified as subtype B or A/B. We did not find statistically significant differences in the neuropathological subtypes according the APOE genotype.

3.2.4 | Cingulate involvement (Table 2)

Cingulate involvement was present in 70.0% of cases (sparse 16.7%, mild 18.9%, moderate 25.5%, and severe grade in 8.9%). All subjects with FTLD showed, at least, a mild grade of TDP-43 pathology in the anterior cingulate. In contrast, 70% of subjects without FTLD presented none or only sparse pathology in this area.

3.3 | Concomitant pathology

Figure 3 shows some of the representative concomitant pathologies found in ALS patients. Figure 4 shows the proportion of these concomitant pathologies found in our series.

3.3.1 | Presence of concomitant neurodegenerative pathologies (Figure 4A)

Considering all concomitant pathologies together, most (89.4%) ALS patients presented at least one co-pathology at neuropathological examination. Concomitant AD neuropathologic changes, according to "ABC" score, were found in 52 subjects (50.0%) at low, in 8 (7.7%) at intermediate, and in 7 patients (6.7%) at high level. CAA was found in 17 cases (16.3%). Nearly half (48.1%) of the subjects fulfilled criteria for PART pathology (25.0% possible, 23.1% definite). In addition, 38.5% presented ARTAG (18.3% granular

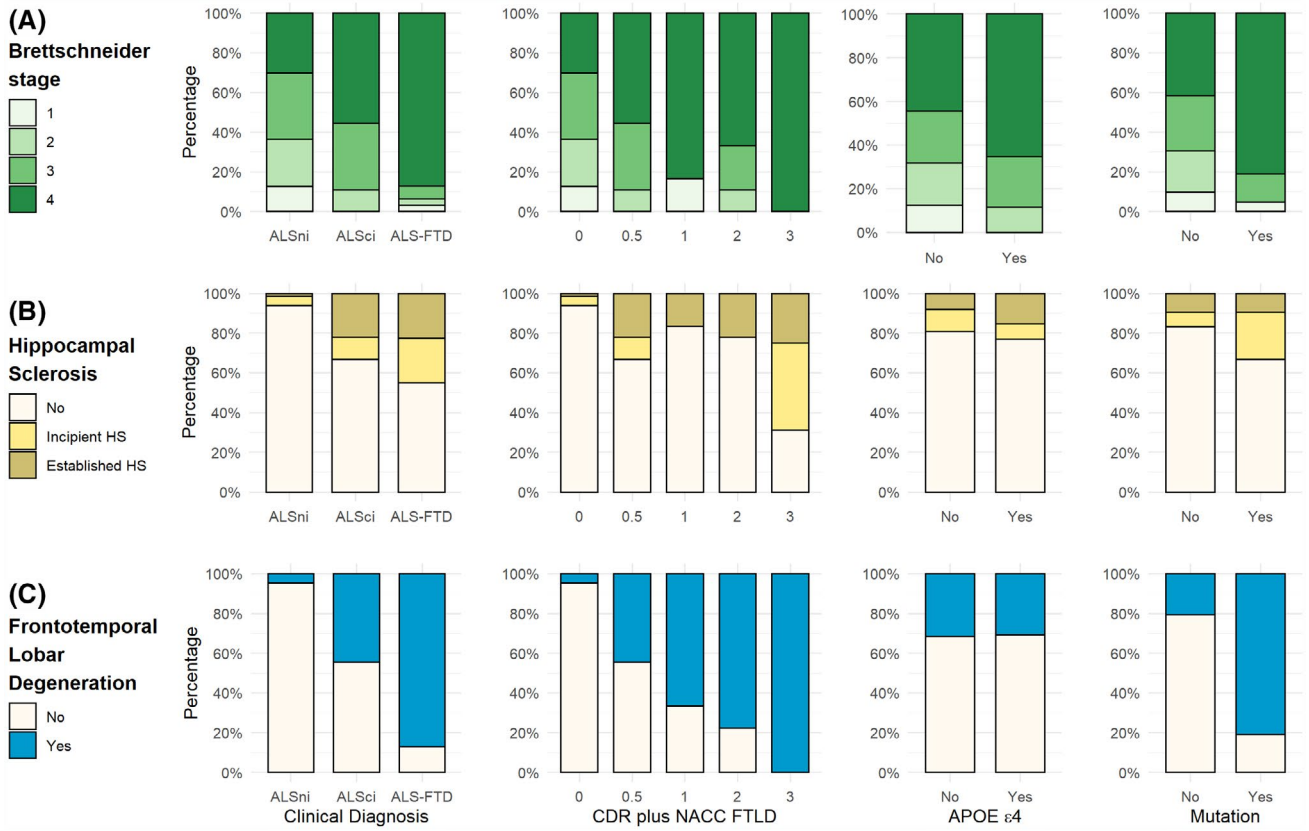

FIGURE 2 Barplots representing the proportion of ALS cases with (A) the different Brettschneider stage, (B) the presence of hippocampal sclerosis and (C) Frontotemporal Lobar degeneration according to their clinical diagnosis, CDR plus NACC FTLD stage, presence of APOE ε4, or presence of mutations in genes reported as causative of or at risk for ALS/FTLD

TABLE 2 Prevalence of TDP-43 pathology in anterior cingulate cortex in ALS spectrum

Presence of TDP-43 in anterior cingulate n (%)	ALSni n = 58	ALSci n = 6	ALS-FTD n = 26
No	25 (43.1%)	1 (16.7%)	1 (3.8%)
Sparse	14 (24.1%)	1 (16.7%)	0 (0%)
Mild	13 (22.4%)	0 (0%)	4 (15.4%)
Moderate	6 (10.3%)	4 (66.7%)	13 (50%)
Frequent	0 (0%)	0 (0%)	8 (30.8%)

Note: n = 90 cases out of 102.

Abbreviations: ALSci, amyotrophic lateral sclerosis with cognitive impairment; ALS-FTD, amyotrophic lateral sclerosis with frontotemporal dementia; ALSni, amyotrophic lateral sclerosis without cognitive impairment.

fuzzy astrocytes, 13.5% thorn-shaped astrocytes, 6.7% both), with 8.3% presenting astroglial pathology also in the spinal cord. ARTAG pathology was found more frequently, but not exclusively, in subjects with AD pathology (44.8% vs. 27.0%, $p < 0.05$). GVD was found in 42.3% of the subjects and was mostly very mild and restricted to the hippocampal subfields CA2/CA1 with only mild entorhinal involvement. GVD was not only strongly related to AD pathology ($p < 0.001$), but also to the presence of FTLD and the *C9orf72* expansion ($p < 0.05$). AGD was detected in 14 subjects (13.5%; 11 subjects at Saito stage 1, 1 subject at stage 2, and 2 subjects at stage 3), while Lewy body pathology was identified in 9.6% (seven subjects restricted to brainstem nuclei, three with limbic involvement, and only one with neocortical involvement). Vascular lesions were identified in 14 subjects (13.5%); 11 of them with small vessel pathology and only 3 of them presenting large vessel infarcts. We observed LATE in 19 (27.1%) of the 70 ALS cases without FTLD.

3.3.2 | Relationship of concomitant pathologies and age at death (Figure 4B)

Concomitant pathologies were more frequent in subjects who died at older age. ABC score for AD was significantly higher in subjects with older age at death ($p < 0.01$). Particularly, Braak and Braak stages for neurofibrillary pathology and amyloid Thal phases were significantly increased in older subjects ($p < 0.01$). The presence of LATE, ARTAG, Lewy body pathology, GVD, and vascular lesions also was significantly higher in older age groups ($p < 0.05$). CAA, PART, and AGD concomitant pathologies did not show significant association with age at death.

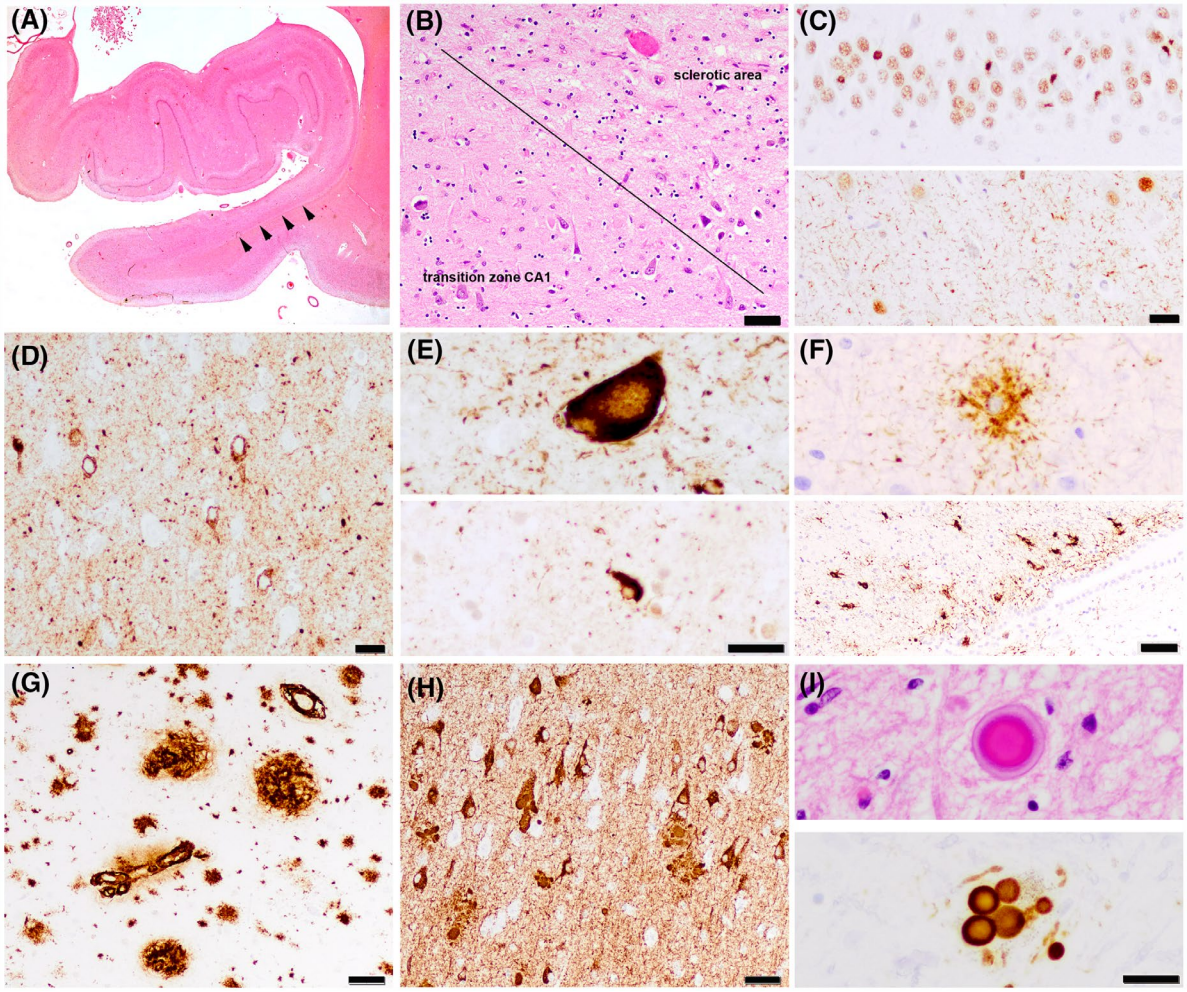

FIGURE 3 **Frequent co-pathologies in ALS. (A–C)** Segmental hippocampal sclerosis is sometimes better identified in more anterior segments of the hippocampus (A, arrowheads). It is characterized by neuronal loss and prominent fibrillar gliosis (B) in the CA1 sector and subiculum. In most cases granule cells of the dentate gyrus harbor TDP-43 protein inclusions in the cytoplasm (C, upper row) and in some cases there are abundant fine threads in the CA1 sector within and adjacent to the sclerotic area (C, lower row). **(D and E)** Argyrophilic grain pathology is well depicted by the AT8 anti-tau antibody (D) and consists of grain-like structures along neuronal processes, abundant threads, and a diffuse cytoplasmic neuronal staining (pretangle type) instead of compact tangles. They are frequently associated with ballooned neurons in the amygdala (E, upper row) and oligodendroglial coiled bodies in temporomedial white matter (E, lower row). Astrocytic tau pathology in form of granular fuzzy astrocytes (F, upper row) and thorn-shaped astrocytes in the glia limitans (F, lower row, here as subependymal and perivascular thorn-shaped astrocyte) are features of age-related tau astrogliopathy (ARTAG). **(G and H)** Alzheimer's disease neuropathologic changes have been also found as co-pathology in a fraction of cases. It is characterized by dense cored amyloid plaques with or without amyloid angiopathy (G) and tau positive neurofibrillary pathology with tangles, neuropil threads, and dystrophic neurites around amyloid deposits (H). **(I)** Lewy body pathology has been detected less frequently and can be encountered in brainstem neurons (HE, upper row, medulla oblongata) and/or limbic system and is well identified with anti-alpha-synuclein antibodies (lower row), where Lewy-neurites are also frequently seen (here in the locus coeruleus). Scale bars: 50 μm in B, F, G, H; 20 μm in C (upper and lower panel), D, E, and I (upper and lower panel)

3.3.3 | Relationship of concomitant pathologies with CDR-NACC FTLD (Figure 4C)

Concomitant pathologies were found more frequently in subjects with reported cognitive impairment (97.5% vs. 84.0%, $p < 0.05$). The CDR-NACC-FTLD score was significantly related to the Braak and Braak stage for neurofibrillary pathology ($p < 0.01$). Subjects with severe stages of dementia also presented more GVD pathology ($p < 0.05$) No significant associations were found between the CDR-NACC-FTLD score and Thal phase of amyloid, CAA, PART, ARTAG, AGD, Lewy body, LATE, or vascular co-pathologies.

3.3.4 | APOE status and concomitant pathologies (Figure 4D)

CAA was found more frequently in subjects with an *APOE* ε4 allele (34.6%, vs. 9.6%; $p < 0.05$). For the rest of the concomitant pathologies, no statistical differences were according to the *APOE* status.

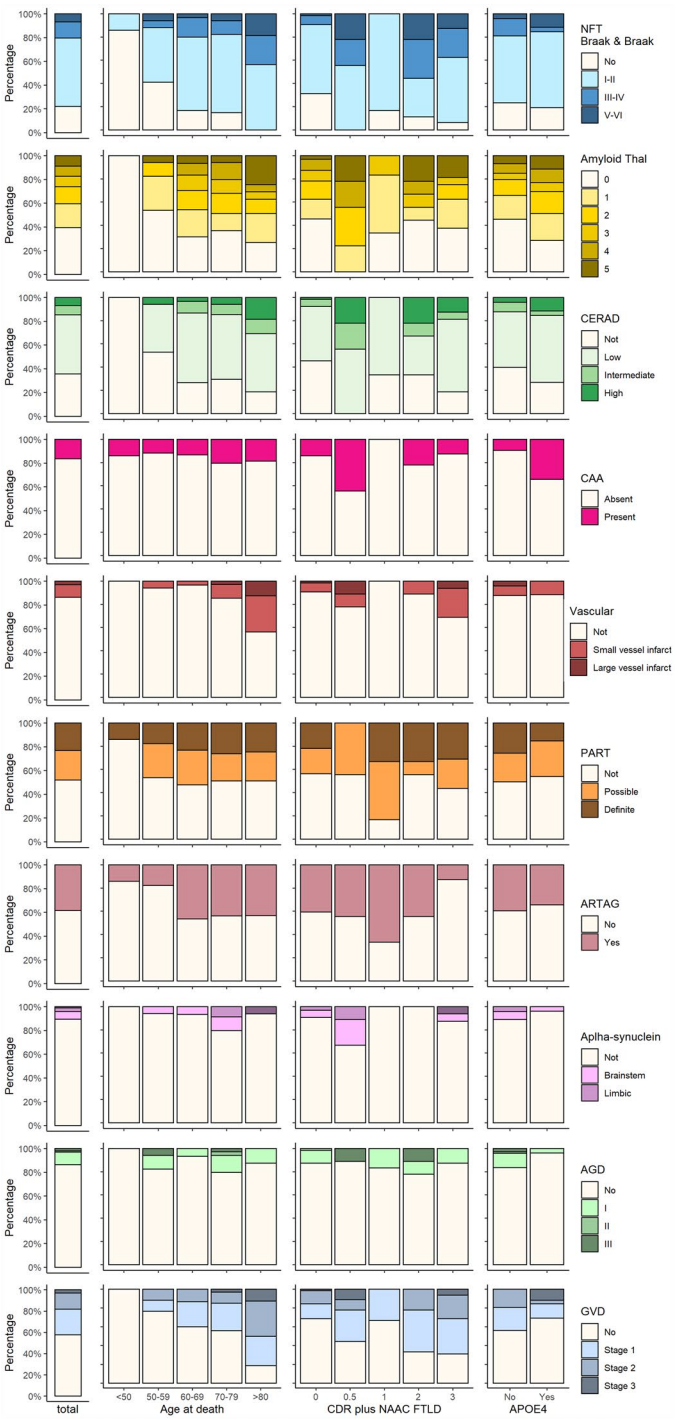

FIGURE 4 **Presence of total concomitant pathologies in ALS cases (A), distribution of pathology according to age at death (B), distribution of pathology according to the CDR NACC plus FTLD staging (C), and distribution of pathology according to the presence of *APOE* ε4 allele (D).** Neurofibrillary tangles were staged following Braak stages (24). Amyloid represents Aβ Thal phases (25). Globally, Alzheimer's disease pathology was staged according the NIA/AA guidelines and an ABC score was assigned (27). Cerebral Amyloid angiopathy (CAA) has been reported as absent or present. Vascular lesions were classified according to small or large vessel pathology. Assignments of Lewy Body pathology were performed following McKeith criteria (30). Possible or definite primary age related tauopathy (PART) was assessed according the current neuropathological criteria (28). Age-related tau astrogliopathy (ARTAG) was evaluated according the current criteria (29). Argyrophilic grain disease (AGD) was staged according to the Saito criteria (31). Granulovacuolar degeneration (GVD) was staged according to Thal et al (33)

3.4 | Determinants of cognitive impairment in ALS

Finally, to assess whether neuropathologic and concomitant pathologies influence the presence of cognitive impairment in ALS patients a logistic regression analysis was performed (Table 3).

Regarding the neuropathological substrate, the presence of FTLD was the strongest determinant for cognitive decline in our model (OR 359.5, 95% CI 20.4–1940.2, $p < 0.001$). We also found that the presence of AD concomitant pathology independently influenced the development of cognitive impairment in ALS patients, especially in those with higher burden of pathology (OR 36.5, 95% CI 3.4–444.2, $p < 0.05$). Other concomitant pathologies did not influence cognitive symptoms in ALS subjects with these analyses. Reduced models considering less covariates in order to avoid collinearity problems showed similar results (Table S2).

Five subjects with reported cognitive impairment (three ALSci and two ALS-FTD) did not present an

TABLE 3 Logistic multivariate regression

Neuropathologic features	Odds ratio (95% CI)	*p* value*
Age at death	0.9 (0.8–1.0)	0.177
Sex (female)	2.7 (0.6–14.5)	0.219
Frontotemporal lobar degeneration	359.5 (20.4–1940.2)	**<0.001**
Hippocampal sclerosis	0.3 (0.1–5.5)	0.459
Alzheimer's disease neuropathological change	36.5 (3.4–444.2)	**<0.05**
Cerebral amyloid angiopathy	2.9 (0.4–20.9)	0.279
α-synuclein	2.3 (0.1–13.7)	0.559
PART	2.3 (0.4–7.1)	0.325
ARTAG	1.9 (0.4–6.5)	0.461
Argyrophilic grain disease	1.0 (0.1–5.5)	0.995
LATE	3.4 (0.3–11.3)	0.315
Granulovacuolar degeneration	1.7 (0.3–8.0)	0.542
Vascular lesions	1.5 (0.1–7.7)	0.748

Note: The dependent (outcome) variable of the model was the presence of cognitive impairment (ALSci and ALS-FTD were considered together). Frontotemporal lobar degeneration was established according to the presence of neuronal loss and gliosis in temporal and/or frontal cortices. Alzheimer's disease neuropathological change was considered as a moderate or high burden of tau and amyloid pathology according the NIA-AA.

Abbreviations: ARTAG, age-related tau astrogliopathy; LATE, limbic-predominant age-related TDP-43 encephalopathy. PART, primary age-related tauopathy.

*p values were obtained from Wald's test, bold indicates statistical significance.

unequivocal neuropathological substrate explaining their symptoms. This situation was found more frequently in the ALSci group than in the ALS-FTD group (33.3% vs. 6.5%; $p < 0.05$). The two subjects fulfilling criteria for ALS-FTD but lacking an obvious neuropathological substrate presented genes reported as pathogenic of or at risk for ALS (1 *TARDBP* and 1 *VCP*). The *TARDBP* carrier presented frequent TDP-43 inclusions in amygdala with focal gliosis and neuronal loss. The *VCP* carrier, by contrast, had very few TDP-43 inclusions restricted to motor neurons (Brettschneider stage I), but presented concomitant AGD pathology (Saito I).

4 | DISCUSSION

In this work, we have analyzed the potential effects of the extent of TDP-43 pathology, concomitant pathologies, and genetic variables on the cognitive-behavioral status in a large series of neuropathologically confirmed ALS patients.

Though the growing evidence of ALS and FTLD overlap, only few neuropathological series have been reported so far (9,38). In our series of neuropathologically confirmed ALS patients, cognitive impairment was reported in more than one-third (38.5%) of cases. Most of them showed a pattern of FTLD at neuropathology. Neither the Brettschneider stage nor the presence of FTLD were associated with sex, age at death or disease duration. Spencer et al. recently reported that patients with long-duration ALS (>10 years) showed less frequent TDP-43 pathology and less severe lower motor neuron involvement. In our series, only six patients had a disease duration of 10 years or more and two of them had no TDP-43 but FUS pathology (39). However, those harboring TDP-43 pathology had no different TDP-43 severity stages (as assessed by Brettschneider staging) compared to the standard duration group in their study (40). This was also observed in our series. Also in accordance with previous studies, the presence of a mutation was the strongest determinant for comorbid ALS and FTLD (14), even in the absence of family history within the ALS/FTD spectrum (36). Bulbar onset was more frequent in subjects with ALS-FTD, a finding that also has been found in other studies (37,41).

We found that an FTLD neuropathological pattern and/or HS are the most frequent neuropathologic substrate of cognitive impairment in ALS subjects. Neither the Brettschneider stage nor the presence of FTLD or HS were associated with disease duration (40). At the same time, the presence of cognitive impairment was associated with a Brettschneider ALS stage 4 and the presence of TDP-43 pathology in anterior cingulate. In such cases, showing extensive ALS and FTLD-related TDP-43 proteinopathy it might be useful either to apply both proposed staging systems in parallel, that is, that for ALS-TDP and that bvFTD with TDP-43 inclusions, or even to combine both and propose a new staging fusing both particularly when one or the other system fails to appropriately classify the pathology. One of the caveats of Brettschneider ALS stages is that the topography alone may not explain cognitive symptoms. Previous studies have suggested that the burden of TDP-43 pathology in the anterior cingulate cortex discriminates cases with clinical bvFTD (23). In our work, almost all subjects diagnosed as ALS-FTD showed at least mild TDP-43 pathology in the anterior cingulate, and this was also present in 32.7% of subjects in the ALSni group. This finding suggests that the cingulate burden of TDP-43 could be more sensitive than specific to discriminate cognitive involvement in ALS patients. However, because of the retrospective methodology of our study, it is difficult to elucidate if subtle cognitive alterations might have been underdiagnosed in these subjects, especially in the last stages of the disease.

Moreover, in HS the presence of TDP-43 proteinopathy is a frequent finding, independently of motor neuron involvement. In ALS cases with HS, the application of TDP-43-stages according to Brettschneider's proposal might result in stage 4 although hippocampal involvement by TDP-43 would be more likely related to HS and not necessarily to a sequential progression of pathology from motor regions. Therefore, the staging systems for ALS, FTLD, and HS, when appearing concomitantly, should be critically rethought. This also applies to the

recently described "LATE," which by itself may also contribute to cognitive decline. It should be considered a separated entity from FTLD, as it manifests with a different phenotype, it occurs particularly in older age groups (the "oldest old," >85 years) and has a relatively restricted neuroanatomical distribution (32). In the context of ALS-TDP, whether the presence of TDP-43 pathology in the limbic system represents LATE pathology, or whether it is a restricted form of FTLD, remains a matter of discussion.

Our study also reveals a high frequency of concomitant pathologies in ALS patients, however, at different severity grades. It is currently well known that the accumulation of brain pathologies is common and that these may influence the cognitive state or lower the threshold for the development of cognitive decline (7,8). The frequencies of concomitant pathologies found in our series are in accordance with those reported in previous works (9,38). Most ALS patients presented any grade of tau pathology in the form of neurofibrillary tangles: PART pathology was found in approximately half of the cases; around one-third to one-half also presented amyloid-β pathology; and about a 10th presented any grade of α-synuclein pathology. Our study also evaluated the presence of ARTAG pathology in the brain and spinal cord of ALS subjects, as it represents the most frequent astrocytic tau pathology found in aging brains. We found that more than a third cases (38.5%) presented ARTAG pathology. In a few subjects, ARTAG pathology was also found in the spinal cord, particularly in older age groups. Whether the spinal astroglial pathology may be elicited by the chronic neurodegenerative process or not remains a matter of further studies.

In addition, we found a quite considerable prevalence of AGD co-pathology in our series (15.5%). AGD is a common sporadic neurodegenerative disease of old age but is rarely seen in young subjects. Of note, some of the subjects with AGD pathology were relatively young (mean age at death 70.9 years, range 58–84). Previous reports point that AGD and TDP-43 are occasionally concurrent in ALS/FTLD cases (42,43). These findings may strengthen the hypothesis of the existence of a link between TDP-43 pathology and argyrophilic grains. However, the pathogenesis of this potential interaction remains unclear.

GVD, a feature observed in cellular stress conditions and some neurodegenerative pathologies including AD was not a frequent feature and, if present, was mostly very mild and restricted to the hippocampal subfields CA2/CA1 with only mild entorhinal involvement (33). GVD was not only more frequently related to AD pathology, including both, neurofibrillary tangles and amyloid plaques, but was also observed more frequently in individuals with FTLD pathology or in those carrying the *C9orf72* expansion. An association between GVD and TDP-43 pathologies has been recently reported. It has been suggested to be potentially triggered by TDP-43 in the hippocampus and has been linked to a cell death mechanism (44,45). GVD could, therefore, represent an additional, TDP-43-related mechanism leading to cognitive impairment in ALS besides its relationship with Alzheimer's disease neuropathological change.

In our series the strongest determinant for the presence of concomitant pathologies was the age at death: while subjects aged 60 or less rarely had co-pathologies, those >60 years had at least 1, and those >70 years had 2 or more co-pathologies. Although it is not possible to further elucidate this observation given the absence of a matched control group in our study, this frequency of co-pathologies seems to appear at relatively early ages in ALS cases. Moreover, subjects with reported cognitive impairment presented with more severe AD pathology.

The presence of the *APOE* ε4 allele is a well-known risk factor to develop AD. For other dementias, this relationship is not well established. Chio et al. found that the presence of the allele ε2 significantly increased the risk of cognitive impairment in ALS patients (14). Other studies, including two meta-analyses, found a relationship between FTLD and the presence of the ε4 allele (15,46). These studies were limited, as they were not performed in a pathologically confirmed cohort or in ALS patients. Recently, Yang et al. found an age-dependent effect of *APOE* ε4 on TDP-43 and HS, (13) and Wennberg et al. on TDP-43 deposition independently of Aß in AD pathology in a large postmortem series (12). In our postmortem study, we also analyzed the effect of *APOE* on ALS and cognition, but we did not find statistically significant associations between *APOE* ε4 allele and the presence of FTLD, FTLD subtypes or HS. Except for CAA, we neither found differences in the presence of concomitant pathologies in *APOE* ε4 carriers. Because of the low frequency of the *APOE* ε2 allele observed in our series, an association with the presence or absence of FTLD cannot be established.

When evaluating the clinical impact of neuropathological findings, we found that the presence of FTLD is, by large, the predominant underlying pathology that better explained the cognitive impairment in ALS patients. However, our analysis reveals that AD also can influence the development of cognitive symptoms in these patients. In addition, we found a few cases with reported cognitive decline but without FTLD or enough AD pathology to justify cognitive impairment. The two subjects with ALS-FTD diagnosis lacking an obvious neuropathological substrate for cognitive impairment were carriers of mutations in genes reported as pathogenic of or at risk for ALS (1 *TARDBP* and 1 *VCP*). The *TARDBP* mutation carrier showed TDP-43 inclusions in amygdala associated with focal gliosis and neuronal loss, while the *VCP* mutation carrier showed mild argyrophilic grain pathology with ballooned neurons. The involvement of the amygdala in behavioral disturbances is well known (47). Therefore, despite not having a classical FTLD-pattern, the presence of focal amygdala

degeneration could have been sufficient explanation for the cognitive impairment. So finally, in three ALS cases in which cognitive impairment was reported, we found no adequate neuropathological explanation. Given the retrospective nature of the study, we cannot draw any conclusion concerning this finding. However, it may be an interesting focus of interest in future studies with precisely phenotyped series.

Our study has several limitations. First, due the retrospective nature of the study, which included cases that had been evaluated years before the acceptance of the concept of the ALS-FTLD continuum, two-thirds of the subjects had not been systematically screened with a formal neuropsychological evaluation, cognition was assessed by the global impression of their neurologist. While this might underestimate the presence of cognitive impairment particularly when it was subtle or in the last stages of the motor neuron disease, we still found a good association between the cognitive information reported by the neurologist and the neuropathological examination. In that sense, further prospective studies with accurate screening tools for cognitive decline are needed to validate the conclusions of this work. Second, as a single Brain Bank series, it is possible that our results do not represent the whole ALS population. Therefore, some clinical or genetic features may be overrepresented because of a selection bias for brain donation. Finally, while a morphological screening for *C9orf72* was performed in all cases, genetic tests for other mutations have not been performed systematically (34).

5 | CONCLUSIONS

Cognitive impairment is a frequent finding in patients with ALS. Most, but not all cases, present with FTLD with or without hippocampal sclerosis as the underlying pathology, have higher ALS-Brettschneider stages and higher burden of TDP-43 pathology in the anterior cingulate. AD can also influence the development of cognitive symptoms in ALS patients. The presence of a mutation, but not the *APOE* genotype, is a strong determinant for the presence of cognitive decline. Moreover, concomitant neurodegenerative pathologies, particularly AD, are more frequent in older ALS subjects with cognitive decline, while AGD may appear earlier than in non-ALS patients.

These findings contribute to broaden the knowledge on the overlap between ALS and FTD and emphasize the importance of an accurate assessment of non-motor features in ALS patients including clinical, neuropsychological, neuroimaging, and biofluid biomarkers through an integrative approach. Well-characterized patients will undoubtedly profit from better directed therapeutic interventions and care.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

JTS, MB, RSV, AL, MP, MAR, JG, AC, MPC, LB, JS, and RRG collected clinical data. SBE, IA, LMP, and EG collected neuropathological data. TX, AA, and JC performed genetic analysis. SBE drafted and prepared the manuscript. SBE and MB performed statistical analysis. RRG and EG conceived the study and its design. All authors read, revised, and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Sergi Borrego-Écija https://orcid.org/0000-0003-2557-0010
Martí Paré-Curell https://orcid.org/0000-0002-0627-5350
Ellen Gelpi https://orcid.org/0000-0003-2948-4187

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.
Supplementary Material

TABLE S1 Antibodies used for immunohistochemistry and their pretreatments
TABLE S2 Reduced model of logistic regression: The dependent (outcome) variable of the model was the presence of cognitive impairment (ALSci and ALS-FTD were considered together). The reduced model avoids covariates with collinearity problems

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Trabajo 5

Does ALS-FUS without FUS mutation represent ALS-FET?

Report of three cases

S Borrego-Écija, E Cortés-Vicente, L Cervera-Carles, J Clarimón, J Gámez, J Batlle,

G Ricken, L Molina-Porcel, I Aldecoa, R Sánchez-Valle, R Rojas-García, E Gelpi

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Scientific Correspondence

Does ALS-FUS without *FUS* mutation represent ALS-FET? Report of three cases

Abnormal cytoplasmic accumulation of fused in sarcoma (FUS) protein is the pathological hallmark of some cases of amyotrophic lateral sclerosis (ALS) with transactive response DNA-binding protein of 43KDa (TDP-43)-negative pathology that lack SOD1 mutations. FUS is an RNA-binding protein located predominantly in the nucleus and is involved in regulation of transcription, alternative splicing, RNA stability, microRNA biogenesis, apoptosis and cell division. FUS, Ewing's sarcoma (EWS) and TATA-binding protein-associated factor 15 (TAF15) proteins constitute the FET (FUS/EWS/TAF15) family, highly conserved and ubiquitously expressed RNA-binding proteins that shuttle between nucleus and cytoplasm assisted by the nuclear import protein Transportin 1 (Trn1) [1].

Accumulation of FUS also occurs in other related neurodegenerative conditions such as atypical frontotemporal lobar degeneration with ubiquitinated inclusions (aFTLD), neuronal intermediate filament inclusion disease (NIFID) and basophilic inclusion body disease (BIBD), the three currently recognized forms of frontotemporal lobar degeneration with FUS pathology (FTLD-FUS) [2].

Recent work suggests different pathological processes underlie ALS-FUS and FTLD-FUS. First, most ALS-FUS cases are caused by *FUS* mutations [3], while most FTLD-FUS cases are not [2,4]. Neumann *et al.* described that in ALS-FUS, the cytoplasmic inclusions consist solely of FUS protein while in FTLD-FUS, the inclusions include other FET family proteins such as TAF15 or EWS [5]. In addition, they observed that Trn1, a protein involved in the nuclear transport, accumulates specifically in FTLD-FUS inclusions but not in ALS-FUS. These findings led the authors to suggest that ALS with *FUS* mutations is more restricted to FUS dysfunction, while in FTLD-FUS, there is a more global and complex dysregulation of all FET proteins. They suggest changing the nomenclature and recommended using the term FTLD-FET for FTLD-FUS but to preserve the term ALS-FUS [5].

We describe three cases of ALS-FUS with TAF15 and Trn1 accumulation in which *FUS* mutations were not detected. Brain donors and/or next of kin had given their written informed consent for the use of brain tissue for research, and the research protocol has been approved by the Ethics Committee of the Hospital Clinic Barcelona.

Patient 1, a 63-year-old man, developed slowly progressive weakness in the distal muscles, dysarthria and dysphagia. Neurological examination revealed symmetrical weakness and hyperreflexia, fulfilling the criteria for ALS. Cognitive and behavioural symptoms were not reported during follow-up. He died of respiratory failure at 69 years. After brain donation, the unfixed brain weight was 1390 g. A prominent atrophy of the medullary pyramids, anterior nerve roots and spinal cord was appreciated on gross examination, but without brain atrophy. Histologically, prominent neuronal loss of motor neurones of the anterior horn was observed at all levels of the spinal cord and was also present in the motor nuclei of the brain stem and the primary motor cortex. Degeneration of the corticospinal tracts was also observed. Several of the remaining spinal and cortical motor neurones showed relatively large cytoplasmic basophilic inclusions. These inclusions were also observed in nonmotor pyramidal neurones and were partly basophilic and partly fibrillar. These inclusions were immunoreactive for FUS protein, p62, TAF15 and Trn1 (Figure 1A1–A5), and partially for ubiquitin, alpha-internexin and phosphorylated neurofilaments. These findings were consistent with ALS with FUS-positive basophilic and fibrillary inclusions.

Patient 2, a 71-year-old woman, presented with progressive weakness of lower extremities, dysarthria and dysphagia. Neurological examination revealed pyramidal signs. No lower motor neurone signs were found on examination, and she was diagnosed with primary lateral sclerosis. During the disease course, she developed an akinetic–rigid syndrome without response to levodopa. DAT-SPECT showed bilaterally reduced putaminal tracer uptake. No cognitive symptoms were reported. The patient died at the age of 83 years after

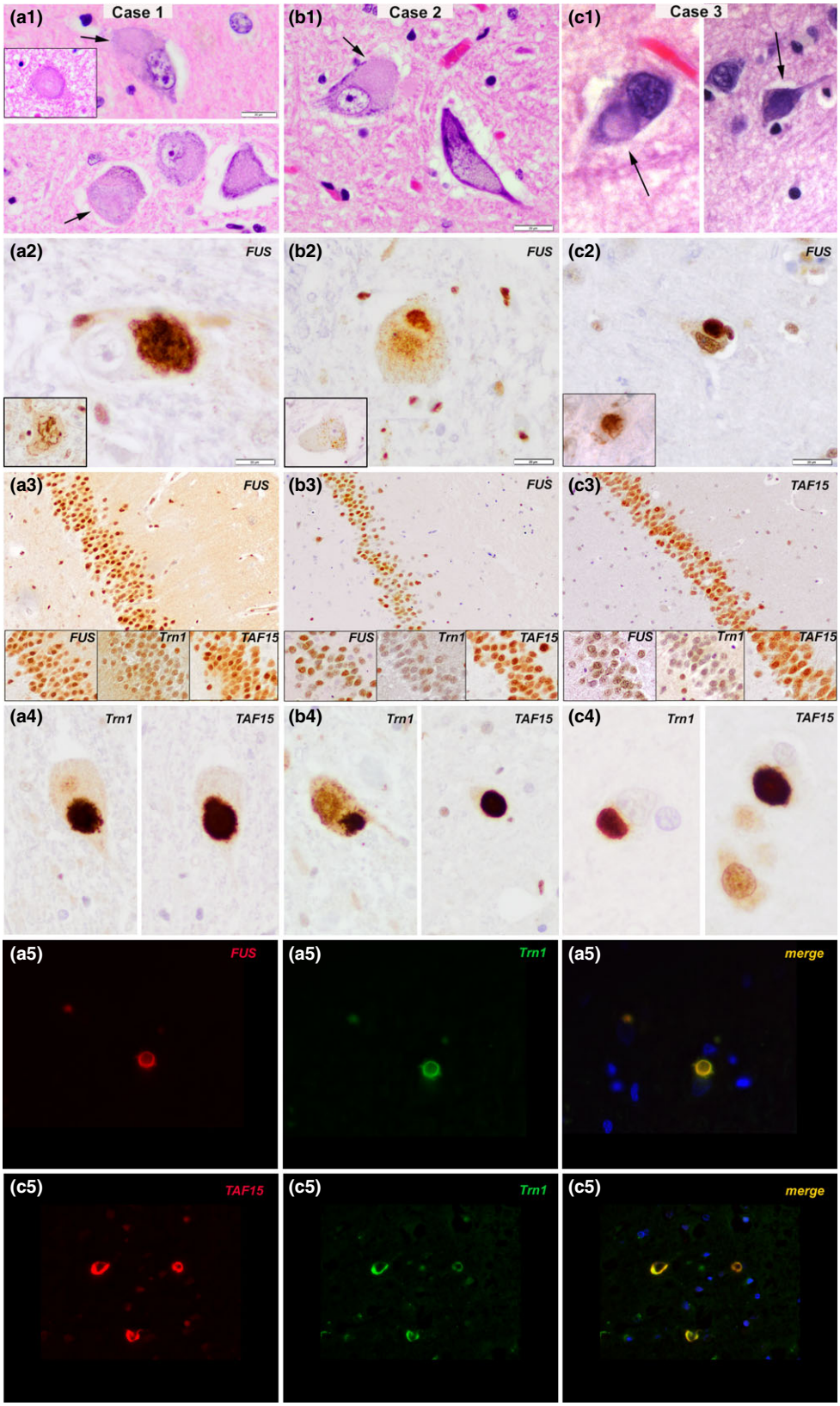

Figure 1. Representative neuropathological findings in the three cases: (**A1**, **B1**, **C1**) HE-stained sections show different types of intraneuronal inclusion bodies in the motor neurones of the frontal cortex, brainstem and spinal cord (arrows) varying in shape and tinctorial properties (basophilic, pale, with a condensed centre or with fibrillar appearance). (**A2, B2, C2**) Inclusions are FUS-positive and appear either compact, more fibrillar or skein-like (inset) (immunohistochemistry for FUS; slightly counterstained with haematoxylin). (**A3, B3, C3**) There is no involvement of the dentate gyrus of the hippocampus, and granule cells are devoid of FUS/TAF15/Trn1 + inclusion bodies (immunohistochemistry for FUS (**A3**, **B3** and insets), TAF15 (**C3** and insets) and Transportin 1 (Trn1)(insets)). (**A4, B4, C4**) Intraneuronal inclusion bodies in motor cortex, brainstem and spinal cord neurones are also strongly immunoreactive for Transportin 1 and TAF15 (immunohistochemistry for Transportin 1 (Trn1) and TAF15 shown in the left and right panel, respectively; slightly counterstained with haematoxylin). **A5**: Double immunofluorescence for FUS (red, left panel), Trn1 (green, middle panel) and merged image (yellow-orange, right panel) shows codistribution of both proteins in the same inclusion body in patient 1. **C5**: Double immunofluorescence for TAF15 (red, left panel), Trn1 (green, middle panel) and merged image (yellow-orange, right panel) shows codistribution of both proteins in the same inclusion body in patient 3. **A1**–**A5** are from patient 1, **B1**–**B4** are from patient 2, and **C1**–**C5** are from patient 3. *Scale bars: A1, B1, C1, A2, B2, C2, A4, B4, C4: 20 μm, A3, B3, C3: 50 μm.*

a total disease duration of 12 years. After brain donation, the unfixed brain weight was 1035 g. Gross examination showed moderate brain atrophy with preferential involvement of the frontotemporal regions. Diffuse nigral pallor was also observed. Histologically, severe loss of motor neurones at all levels of the spinal cord and brain stem nuclei was observed. In contrast, no prominent neuronal loss of primary motor cortex neurones and no unequivocal signs of corticospinal tract degeneration were identified. In addition, there was a depletion of pigmented neurones of the substantia nigra and neuronal loss and gliosis of the subthalamic nucleus and internal pallidum. Residual motor neurones of the spinal cord and hypoglossal nucleus showed relatively large, faintly basophilic inclusions that showed strong FUS immunoreactivity (Figure 1**B**1–**B**4) and were negative for ubiquitin, neurofilaments and TDP-43. Some FUS-positive glial inclusions were also identified. Most of these inclusions showed immunoreactivity for TAF15 and Trn1 antibodies (Figure 1**B**4). The final diagnosis was motor neurone disease with preferential involvement of lower motor neurones with pallidoluysian atrophy and nigral degeneration with abundant neuronal and lesser glial FUS-positive inclusions. Concomitantly, advanced Alzheimer's disease neuropathological change (A3, B3, C3 score according to the NIAA/AA consensus criteria) was found [6].

Patient 3, a 43-year-old man, presented with leg weakness. On neurological examination, there was generalized amyotrophy, fasciculations and hyperreflexia. He developed dysarthria and dysphagia during follow-up and died of pneumonia at the age of 48 years. Cognitive and behavioural symptoms were not reported. The clinical diagnosis was ALS. He had no family history of ALS or dementia. After brain donation, the unfixed brain weight was 1500 g. Gross examination revealed mild brain atrophy with preferential involvement of the precentral and postcentral gyri. Histologically, loss of motor neurones was evident in the primary motor cortex, hypoglossal nuclei and also at all levels of the spinal cord. Moreover, in the pre- and postcentral regions as well as in the temporal cortex, laminar spongiosis and gliosis were evident in superficial cortical layers. While with H&E staining, inclusions were difficult to identify (Figure 1**C**1), immunohistochemistry for FUS showed frequent neuronal cytoplasmic inclusions (Figure 1**C**2), short neurites and few intranuclear inclusions. Inclusions were more abundant in the precentral gyrus, in the brainstem nuclei and in the spinal cord. They were also immunoreactive for TAF15 and Trn1 (Figure 1**C**4–**C**5) and were negative for TDP-43. The final diagnosis was ALS-FUS. In all three cases, granular neurones of the dentate gyrus were devoid of inclusions (Figure 1**A**3, **B**3, **C**3 and insets).

Genetic analysis of the *FUS* gene was performed in the three donors. All 15 *FUS* exons including intron–exon flanking regions, as well as the 3′UTR region of *FUS* gene, were amplified through PCR. Final PCR products were purified and Sanger-sequenced using BigDye terminator chemistry (Applied Biosystems). Sequences were run on an Applied Biosystems® 3130 Genetic Analyzer, and resulting electropherograms were visually inspected using Sequencher (version 4.1, Gene Codes Corp.). Genetic analysis did not disclose any *FUS* mutation in any of these three patients. Since several variants in the 3′ untranslated region (3′UTR) of the *FUS* gene have been described with uncertain pathogenicity (that is c.*48G>A, c.*59G>A, c.*108C>T and c.*110G>A) [7,8], we also screened the genomic region containing these variants. We only

Table 1. Demographic and clinical features of ALS-FUS cases in the literature

	Present study					
	Patient 1	*Patient 2*	*Patient 3*	*Fujita et al.* [10]	*Matsuoka et al.* [11]	*Takeuchi et al.* [12]
Gender	Male	Female	Male	Female	Female	Female
Family history	No	No	No	No	No	No
FUS mutation	No	No	No	No	No	No
Age at onset (y)	63	71	43	73	75	73
Age at death (y)	69	83	48	75	79	75
Motor neurone	Yes	Yes	Yes	Yes	Yes	Yes
Onset	Spinal	Spinal	Spinal	Spinal	Spinal	Spinal
Dementia	No	No	No	No	No	No
Parkinsonism	No	Yes	No	No	No	No
Neuropathology	ALS-FUS	ALS-FUS	ALS-FUS	ALS-FUS	ALS-FUS	ALS-FUS
FUS IHC	+	+	+	+	+	+
TAF15 IHC	+	+	+	NE	NE	+
TRN1 IHC	+	+	+	NE	NE	+

ALS, amyotrophic lateral sclerosis; FUS, fused in sarcoma; NE, not evaluated; y, years; IHC, immunohistochemistry.

found one patient harbouring the c.*41G>A rare heterozygous variant (rs80301724) [9]. Previous studies have reported this polymorphic variant to be equally present in ALS cases and controls, thus showing a lack of genetic association between this particular nucleotide change and ALS [8,10].

Here, we describe the clinicopathological phenotype of three ALS patients with abundant FUS-positive protein aggregates. The inclusion bodies were also immunoreactive for TAF15 and Trn1, and no mutation in the *FUS* gene was detected. Similar cases had been reported in Japan by Matsuoka *et al.*, Fujita *et al.* and Takeuchi *et al.* (Table 1)[11–13]. Other possible genes that could have mutations include *TPN1* and *TAF15*, among others, that were not tested in our cases.

These findings differ from the ALS-FUS cases previously reported by Neumann and behave immunohistochemically similar to FTLD-FET cases. Whether these cases might be specific to certain populations is unresolved. Based on our results, we confirm the concept that the presence of FET and Trn1 proteins within the inclusions is strong indicator of a lack of pathogenic mutations within *FUS*. However, this immunohistochemical profile does not differentiate between an ALS and FTLD phenotype. If we hypothesize that FTLD-FUS with *FUS* mutations will not show Trn1 or any other FET family protein than FUS, a change of the nomenclature in the ALS-FUS and FTLD-FUS with no mutations of *FUS* should be considered, and the use of the terms ALS-FET and FTLD-FET might be more appropriate.

ALS-FUS mutation cases seem to have different morphological phenotypes depending on the age of onset or disease duration; neuronal basophilic inclusions being more frequently detected in early juvenile forms, while fibrillary or tangle-like inclusions and glial inclusions tend to appear in late-onset cases [3]. Similar findings have been described in some sporadic FTLD-FUS cases [4,14]. Interestingly, ALS-FUS cases without *FUS* mutations seem to have an older age of onset and a less aggressive progression than cases with mutations [3].

While some reports have detected *FUS* mutations in 'juvenile ALS with basophilic inclusions' [15], others have not found mutations in the adult-onset group [11,12]. It might be therefore that a subgroup of 'adult-onset ALS with basophilic inclusions' represents the ALS counterpart of basophilic inclusion body disease and may therefore be considered an ALS-FET subtype without *FUS* mutations.

Our study expands the clinicopathological spectrum of nongenetic ALS-FUS cases and reinforces the idea that not all ALS-FUS cases are secondary to *FUS* mutations. It also corroborates the usefulness of TAF-15 and Trn1 immunohistochemistry for the neuropathological diagnosis of nongenetic FTLD-FET and ALS-FET patients. Whether these cases represent a different pathogenetic subgroup among ALS-FUS is unclear and

requires further detailed clinical, neuropathological and molecular studies.

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Authors' Contributions

Study concept and design: SBE, RRG, EG; Acquisition of data: SBE, ECV, RRG, LCC, GR, JG, JB, EG; Analysis and interpretation of data: SB, JC, EG; Drafting of the manuscript: SB, EG; Critical revision of the manuscript and editing: All authors.

Conflict of Interest

The authors do not report conflict of interests related to this article.

S. Borrego-Écija*,†
E. Cortés-Vicente‡
L. Cervera-Carles‡,§
J. Clarimón‡,§
J. Gámez¶
J. Batlle**
G. Ricken††
L. Molina-Porcel†
I. Aldecoa†,‡‡
R. Sánchez-Valle*,†
R. Rojas-García‡,§
E. Gelpi†,††

**Neurology Department, Hospital Clínic-IDIBAPS, †Neurological Tissue Bank of the Biobanc-Hospital Clínic-IDIBAPS, ‡Neurology Department, Institut d'Investigacions Biomèdiques-Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, §Center for Networked Biomedical Research into Neurodegenerative Diseases (CIBERNED), Madrid, ¶Neurology Department, Hospital de la Vall d'Hebron, Barcelona, **Neurology Department, Hospital Sant Pau y Santa Tecla, Tarragona, Spain, ††Institute of Neurology, Medical University of Vienna, Vienna, Austria and ‡‡Pathology Department, CDB, Hospital Clinic Barcelona, Barcelona, Spain*

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Trabajo 6

Integrated strategies for the identification

of genetic frontotemporal dementia

S Borrego-Écija, Ellen Gelpi, Raquel Sánchez – Valle

(en preparación)

Title: **Integrated *s*trategy for the identification of genetic frontotemporal dementia**

Running Title: Genetic screening in frontotemporal dementia

Authors:

Sergi Borrego-Écija [1], Ellen Gelpi, [2,3,*], Raquel Sánchez – Valle [1,*]

* Equal contribution

Affiliations:

1 – Alzheimer's disease and other cognitive disorders unit. Neurology Service, Hospital Clínic de Barcelona, Institut d'Investigacions Biomediques August Pi i Sunyer, University of Barcelona, Barcelona, Spain.

2- Neurological Tissue Bank, Biobanc-Hospital Clínic-IDIBAPS, Barcelona, Spain.

3 - Division of Neuropathology and Neurochemistry, Department of Neurology, Medical University of Vienna, Vienna, Austria

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Corresponding authors:

Raquel Sanchez-Valle, MD

Alzheimer's disease and other cognitive disorders unit. Hospital Clinic de Barcelona, Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), University of Barcelona

Villarroel, 170 08036 Barcelona (Spain)

Phone: +34932275785.

E-mail address: rsanchez@clinic.cat

Ellen Gelpi, MD

Neurological Tissue Bank of the Biobanc-Hospital Clinic-IDIBAPS

Facultad de Medicina

c/Casanova 143, planta 0, ala sur

08036 Barcelona, Spain

Phone/Fax: +3493 4517664

Email: ellen.gelpi@idibaps.org

and

Division of Neuropathology and Neurochemistry, Department of Neurology

Medical University of Vienna

AKH 4J, Waehringer Guertel 18-20

1090 Vienna, Austria

Email: ellen.gelpi@meduniwien.ac.at

ABSTRACT

Background: Frontotemporal lobar degeneration (FTLD) is highly heritable with several genes causing genetic FTLD (gFTLD). Genetic diagnosis of FTLD is needed to provide patients and their relatives accurate genetic counseling, but it is still a challenge for clinicians.

Objective: We aim to investigate different strategies to guide the genetic screening in FTLD patients.

Methods: We reviewed the demographic, clinical, neuropathologic and genetic data of 203 neuropathologically confirmed FTLD cases from the Neurological Tissue Bank of Barcelona. We assembled four possible strategies to guide the genetic screening based on: a) family history (modified Goldman score), b) age at disease onset, c) clinical diagnosis, and d) neuropathological diagnosis.

Results: Forty-four patients (21.7%) carried a pathogenic mutation. The modified Goldman score strongly predicted the likehood of identifying a pathogenic mutation. Genetic cases presented a significantly younger age at onset than sporadic cases. The proportion of pathogenic mutations differed across clinical and neuropathological phenotypes.

Conclusion: Key clinical and neuropathological features might guide genetic testing in FTLD. Neuropathological studies in affected subjects are of particular relevance for familial genetic counselling.

INTRODUCTION

Frontotemporal lobar degeneration (FTLD) represents an umbrella term for several clinical syndromes that merge in a progressive dysfunction of the frontal and temporal lobes. Clinical syndromes include the behavioral variant of frontotemporal dementia (bvFTD), the semantic variant of primary progressive aphasia (svPPA) and the non-fluent/agrammatic variant of primary progressive aphasia (nfvPPA)(Gorno-Tempini *et al.*, 2011; Rascovsky *et al.*, 2011; Convery *et al.*, 2018). Patients with FTLD can also present with or develop motor symptoms either in form of amyotrophic lateral sclerosis (ALS) or atypical parkinsonism such as progressive supranuclear palsy syndrome (PSPS) or corticobasal syndrome (CBS) (Kertesz *et al.*, 2000; Armstrong *et al.*, 2013; Höglinger *et al.*, 2017; Strong *et al.*, 2017).

From a neuropathological point of view, FTLD encompasses a variety of underlying misfolded protein aggregates / proteinopathies that affect different neuronal populations and frequently also glial cells (Cairns *et al.*, 2007). Abnormally hyperphosphorylated tau deposits account for tauopathies or FTLD-tau, and are classified as 3- repeat taupathies (i.e. Pick's disease, PiD), or 4-repeat tauopathies (i.e. corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), argyrophilic grain disease and globular glial tauopathy (GGT)) according to the predominant tau isoform ratio (Kovacs, 2015; Forrest *et al.*, 2019). In 2006, TAR DNA-binding (TDP-43) protein aggregates were identified in most of the tau negative FTLD cases (FTLD-TDP) and almost all ALS cases (ALS-TDP) (Neumann *et al.*, 2006). FTLD-TDP cases are currently subdivided into four neuropathological patterns (A-D) depending on the morphology and distribution of neuronal TDP43 inclusions and neurites (Mackenzie *et al.*, 2011; Mackenzie and Neumann, 2017). A third disease group is represented by FTLD-FET, which is caused by the accumulation of proteins of the FET protein family - an acronym used for Fused in Sarcoma (FUS), Ewing Sarcoma (EWS) and TAF15 (TATA-Box Binding Protein Associated Factor 15) (MacKenzie and Neumann, 2012) proteins. Finally, the term FTLD-UPS (Ubiquitin-proteasome system) is reserved for rare cases with ubiquitin-positive inclusions that are still negative for tau, TDP-43 and FET proteins.

FTLD is a highly heritable disorder: 30 to 50% of the patients have at least one affected relative and 10 to 23% present an autosomal dominant inheritance pattern (Goldman *et al.*, 2005). The great majority of this heritability is accounted by mutations in three genes: *MAPT (microtubule-associated protein tau)*, *GRN (progranulin)* and *C9orf72 (chromosome 9 open reading frame 72)*. However, in the last years, an

increasing number of additional genes have been associated with autosomal dominant FTLD: *VCP (valosin containing protein)*, *CHMP2B (charged multivesicular body protein 2B)*, *TARDBP (transactive response DNA binding protein)*, *FUS (fused in sarcoma)*, *SQSTM1 (sequestosome 1)*, *TBK1 (tank binding kinase 1)*, *OPTN (optineurin)* and *TIA1 (Cytotoxic Granule Associated RNA Binding Protein)*.

A good knowledge of the specific genotype-phenotype correlations of the mutations causing FTD is extremely important to guide the genetic counseling for other family members. The presence of a family history of dementia or motor neuron disease is frequently the main clue indicating the presence of a pathogenic mutation. However, pathogenic mutations have been found in around 10% of apparently sporadic cases of FTD (Turner MR et. al. , 2007). The search for some specific clinical features in those patients could alert on the presence of a particular mutation. Also, some characteristic neuropathologic findings in FTLD subjects can be a hint for the presence of a pathogenic mutation.

In this work, we assess the proportion of genetic cases according to their family history, age at onset, clinical and neuropathological features in a Brain Bank cohort of FTLD cases and discuss which characteristics might guide the genetic testing in these patients.

METHODS

Subjects

All brain donors with a final neuropathologic diagnosis of FTLD or FTLD-ALS were selected from the Neurological Tissue Bank (NTB), Hospital Clínic de Barcelona – Institut d'Investigació Biomèdica August Pi I Sunyer (IDIBAPS) Biobank from 1994 to 2019. The study was approved by the Ethics Committee of Hospital Clínic de Barcelona. All donors and/or next-of-kin gave their written informed consent for the use of their brain tissue and associated clinical data for research purposes.

Clinical classification

We retrospectively reviewed the medical records available at the NTB. The following information was recorded: age and symptoms at onset, age at death, disease duration, presence of behavioural and/or cognitive impairment, parkinsonism or motor neuron disease (MND) signs, clinical diagnosis, and family history of dementia, MND or parkinsonism. All cases were retrospectively re-classified according to the current diagnostic criteria for diseases within the FTLD spectrum in the following diagnostic categories:

bvFTD, nfvPPA, svPPA, ALS, ALS-FTD, CBS and PSPS (Gorno-Tempini *et al.*, 2011; Rascovsky *et al.*, 2011; Armstrong *et al.*, 2013; Höglinger *et al.*, 2017; Strong *et al.*, 2017). For heritability classification, each patient was graded using the modified Goldman heritability classification score (Goldman *et al.*, 2005; Rohrer *et al.*, 2009). A modified Goldman score of 1 corresponds to an autosomal dominant inheritance pattern, with at least three affected members in two generations linked by a first-degree relative. A score of 2 indicates a familial agregation of at least three relatives but without fulfilling criteria for score 1. If only one relative is or was affected, the modified Goldman scores 3 if the age at onset of this relative is <65 years, but 3.5 if >65 years. A modified socre of 4 implies no known family history of neurodegenerative disease.

Neuropathologic work-up

Neuropathologic examination was performed according to standardized protocols at the NTB. Half brain was dissected in the fresh state, frozen and stored at -80°C and the other half was fixed in formaldehyde solution for 4 weeks. At least 25 representative brain areas were embedded in paraffin, cut at 5µm and stained with hematoxylin & eosin and luxol-fast-blue in selected brain areas. Immunohistochemistry was performed using various antibodies including anti-βA4 (DAKO, Glostrup, Denmark, clone 6F/3D), anti-pTau (Thermo Scientific, Rockford, USA, clone AT8), anti-RD3 Tau (Millipore, Temecula, CA, USA; clone 8E6/C11), anti-RD4 Tau (Millipore, clone 1E1/A6), anti-α-synuclein (Novocastra, Newcastle, UK, clone KM51; and Analytics Jena clone 5G4), anti-ubiquitin (DAKO, Glostrup, Denmark, Polyclonal Rabbit), anti-p62 (BD Biosciences, San Jose, USA, Purified Mouse Anti-p62 LCK ligand), anti α-internexin (Novex, Invitrogen, clone 2E3), anti-FUS (Sigma Aldrich HPA008784, St. Louis, MO, USA; pc), anti-transportin 1 (Abcam, Cambridge, UK, clone D45), anti-TAF 15 (Novus Biologicals, Centennial, CO, USA, policlonal), anti-TDP-43 (Abnova, Taipei, Taiwan, clone 2E2-D3), and anti-phTDP-43 (Cosmo Bio, Tokyo, Japan, clone 11-9). Immunoreaction was visualized by the EnVision+ system peroxidase procedure (DAKO) and diaminobenzidine was used as chromogene.

Disease assessment was performed according to international consensus criteria (Cairns *et al.*, 2007; MacKenzie *et al.*, 2010; Ahmed *et al.*, 2013). FTLD-TDP subtype classification was performed based on TDP-43 or pTDP-43 IHC following the current recommendations as type A, B, C or D(Mackenzie and

Neumann, 2017). Cases were assigned to more than one subtype if they had overlapping features of both subtypes.

Genetic testing

Genetic testing was performed in all subjects with a family history of dementia, parkinsonism or motor neuron disease with a Goldman score of 1, 2 or 3. (Goldman *et al.*, 2011; Koriath *et al.*, 2020).

In addition, we performed genetic testing based on the presence of morphological hallmarks, in particular: a) we studied the presence of the *C9orf72* expansion in FTLD-TDP cases with ubiquitin/p62 positive inclusions in cerebellar cortex and hippocampal dentate gyrus (Ramos-Campoy *et al.*, 2018); b) we screened for the presence of *GRN* mutations in all cases with FTLD-TDP subtype A, c) we screened for the presence of *MAPT* mutations in FTLD-tau cases with "mini-Pick bodies" in the dentate gyrus of the hippocampus (Borrego-Écija *et al.*, 2017) and d) we screened for the presence of *FUS* mutations in all FTLD-FET. Finally, other les frequent genes were analysed in gene-specific research studies or by whole-exome sequencing in a subgroup of patients (Van Der Zee *et al.*, 2014; van der Zee *et al.*, 2017; Dols-Icardo *et al.*, 2018; Ahmed *et al.*, 2019).

Genetic analyses were performed as follows: DNA was extracted from fresh-frozen cerebellar tissue using the QIAamp DNA Minikit for DNA purification from tissues (QIAGEN Co.) following the manufacturer's instructions. For *MAPT,* direct sequencing of exons 1 and 9-13 was performed. *GRN* exons 1-13 and the flanking intronic regions were amplified by PCR. The *C9orf72* repeat was typed by repeat-primed PCR and fragment-length analysis. *SQSTM1*, *TARDBP*, VCP, *TBK1* and *FUS* genes were screened by whole-exome sequencing (WES) as previously reported (Dols-Icardo *et al.*, 2018).

Genetic screening strategies considered

We considered four different approaches to guide the genetic screening in FTLD patients: a) the family history according the modified Goldman score, b) the age at onset, c) the clinical symptom at onset and d) the neuropathological features.

Statistical analysis

We used χ2 or Fisher tests to analyse categorical data and Wilcoxon test or ANOVA for continuous data. Sensibility and specificity according to age at onset were plloted on the receiver operating characteristic (ROC) curve. Statistical significance was set at $p < 0.05$. All analyses were performed in R Studio (Version 1.3.1).

RESULTS

Neuropathological diagnoses and clinical syndromes

We identified 203 donors (116 male, 87 female) with neuropathologically confirmed FTLD. The mean age of onset was 63.9 years and the mean age at death was 71.6 years. Most subjects (n=145) were diagnosed during life with one of the clinical syndromes across the FTLD-ALS spectrum: 46 bvFTD, 9 PPAnfv, 9 PPAsv, 21 ALS-FTD, 14 CBS, and 46 PSPS. For the remaining fifty-eight subjects available clinical data did not suffice to be classified among one of these syndromes and they were grouped as "dementia not otherwise specified" (dementia NOS: 53 cases), or "parkinsonism not otherwise specified" (parkinsonism NOS: 5 cases).

Neuropathological diagnostic categories comprised FTLD-TDP in 72 subjects (12 FTLD-TDP type A, 37 type B, 12 mixed type A/B, and 11 type C); FTLD-tau in 123 cases (14 PiD, 61 PSP, 33 CBD, 4 GGT, and 11 FTLDP-17, FTLD-FET in 5 cases (3 NIFID, 1 BIBD, 1 aFTLD), and FTLD-U in 4.

Pathogenic mutations

Pathogenic mutations were identified in 43 subjects (21.7%): 20 subjects carried the *C9orf72* mutation, 12 carried a pathogenic mutation in *MAPT* (10 p.P301L, one p.S320F, one p.V636I), 6 *GRN* (p.A303AfsX57, IVS7+6_9delTGAG, p.C366fsX1, IVS7-1G>A, and 2 Q4545fsX58), 2 *SQSTM1* (c.1175C>T and c.98C>T), 2 *TARDBP* (two p.I383V), 1 *VCP* (p.R159H) and 1 *TBK1* (p.Thr79del). Table 1 shows the distribution of the clinical syndromes and the heritability of each gene in our series.

While most of the mutations have been reported before, the p.Q454fsX58 variant in the *GRN* gene has not been described previously in the literature. We identified this mutation in two unrelated subjects: one was a 60-year-old man with a four-year history of cognitive and behavioural impairment, fulfilling criteria for bvFTD. He had a rapid clinical progression and developed an asymmetric parkinsonism. The

patient died due to aspiration pneumonia in a stage of severe dementia 4 years after disease onset. There was no known family history of dementia or other neurodegenerative condition. The other patient was a 55-year-old man who developed progressive cognitive and behavioural impairment in form of apathy, loss of empathy and increased alcohol consumption, also consistent with a clinical diagnosis of bvFTD. He progressively deteriorated to a point where he required continuous assistance for all activities of daily living. He also developed parkinsonism until getting wheelchair bound. A single-photon emission computed tomography (SPECT) revealed a fronto-temporo-parietal hypometabolism, predominantly involving the right brain hemisphere. The patient died at age 64 in a severe dementia stage. His mother died at the age of 50 years of an undiagnosed neurological disorder. Neuropathological examination of both patients showed a characteristic FTLD atrophy pattern with neuronal loss, gliosis and superficial spongiosis. The hippocampus was relatively well preserved and no signs of hippocampal sclerosis were observed. There was also prominent neuronal loss and gliosis of the caudate nucleus and the substantia nigra pars compacta. These changes were associated with abundant and widespread neuronal TDP43-protein aggregates predominantly in superficial layers of frontal, temporal and parietal cortices, in the limbic system, basal ganglia and brainstem neurons (Figure 1). Abundant cat-eye neuronal intranculear inclusions were observed in the limbic system.

The p.Q454fsX58 *GRN* mutation characterized by a single nucleotide insertion in exon 11 (c.1359_1360insG) and causes a frameshift of the coding sequence at codon 454 and introduces a premature termination codon after a read-through of 58 residues. Sequence analysis of *GRN* messenger RNA (mRNA) confirmed that the mutated mRNA allele was almost absent in brain tissue, supporting a loss of function mutation by haploinsufficiency mechanism. The clinical and neuropathological phenotype of the two patients carrying this p.Q4545fsX58 mutation was similar to other *GRN* mutations and consisted of an asymmetric fronto-temporo-parietal lobar degeneration with predominantly behavioral, language and motor involvement.

Genetic screening based on family history

Figure 2A shows the frequency of pathogenic mutations considering the modified Goldman score in the whole cohort. Fifty-nine subjects (29%) showed no familial history of dementia, parkinsonism or motor neuron disease. Eight subjects had an autosomal dominant family history of disease (Goldman 1). A

genetic cause was identified in all of them (6 *MAPT*, 2 *C9orf72*). Another 8 subjects presented a modified Goldman score of 2, with an identified genetic cause in 62.5% of them (3 *c9orf72*, 1 *MAPT*, 1 *GRN*). A score of 3 was identified in 19 subjects, with a pathogenic mutation in 53% of them (4 *C9orf72*, 2 *MAPT*, 2 *GRN,* 1 *TARDBP* and 1 VCP). Twenty-four subjects had a score of 3.5, with a genetic cause of dementia in 12.5% of them (2 *GRN*, 1 *TARDBP*). Finally, pathogenic mutations were found only in 7 (9.7%) of subjects without a family history of dementia (Goldman score of 4).

Figure 2B shows the cumulative proportion of cases tested and the altered genes found in our cohort according the genetic screening based on the patient's Goldman score. To perform a genetic screening only in cases with a GS of 1 would represent testing only 5.6% of the sample, but would enable to detect 24.2% of the mutation carriers. A genetic screening in subjects with a GS 1 or 2 would represent testing 11.3% of the sample and detect 29.4% of the pathogenic mutations. If the genetic screening is extended to subjects with a GS of 1, 2 or 3, then 24.8% of the sample would be tested and 69.7% of the mutations would be detected. To include subjects with a GS of 3.5 would represent testing 41.8% of the sample and detecting the 78.8% of the mutations.

Genetic sreening based on age at onset

The patients that carried a genetic mutation had a significantly younger age at disease onset (mean 57.8 years, SD 10.6 years) than non-mutation carriers (mean of 65.7 years, SD 10.7; $p<0.001$). When we compared the cases with mutations in the three main causative genes for FTLD (*MAPT*, *GRN* and *C9orf72*) with the sporadic counterparts of their respective neuropathologics groups, we found significant younger ages at onset in *MAPT* mutation carriers than in sporadic FTLD-tau cases and in *C9orf72* expansion carriers than in sporadic FTLD-TDP type B cases. We did not find statistically significant differences between *GRN* mutations carriers and sporadic FTLD-TDP type A cases (Figure 3A).

An age at onset below 61.5 years distinguished genetic cases from sporadic ones with a sensitivity of 67.5% and a specificity of 69.7%, and 0.71 area under the ROC curve (95% CI: 0.6301-0.7982) (Figure 3B).

Genetic screening based on the clinical syndrome

The proportion of mutation carriers and the causative genes varied widely between clinical syndromes (Figure 4A). An underlying genetic cause was found in 38.1% bvFTD cases, being *C9orf72*, *MAPT* and *GRN* the genes most frequently affected. Patients with ALS-FTD also showed a high prevalence of pathogenic mutations (37.5%), most of them harbouring a *C9orf72* expansion. We found a pathogenic mutation in 3 of 8 patients with svPPA (37.5%), all three carrying *MAPT* mutations, and in 2 of 9 patients with nfvPPA (22.2%), both of them carrying *GRN* mutations. Only one pathogenic mutation (7.1%) was found in subjects with CBS. We did not find any pathogenic mutation in subjects diagnosed as PSP-S

Genetic screening based on neuropathological features

The proportion of mutation carriers also varied widely between neuropathological subtipes (Figure 4B). *MAPT* mutations were found in one of 15 3R-tauopathies (6.7%; p.S320F *MAPT* mutation) and in 10 of 108 4R-tauopathies (9.3%; 10 p.P301L *MAPT,* 1 p.V363I. These 10 cases carrying the p.P301L *MAPT* mutation, presented “mini-Pick”-like bodies in the gentade gyrus of the hippocampus, a hallmark that distinguishes those cases from sporadic 4R-tauopathies.

Concerning FTLD-TDP cases, the subtypes A and B, and those with mixed A/B features showed a high proportion of pathogenic mutations (66.7%, 50% and 50%, respectively). No *FUS* mutations where found in the 5 FTLD-FET cases. As previsouly reported, all *C9orf72* expansion carriers showed ubiquitin positive inclusions in granule cells of cerebellum and in the dentate gyrus of the hippocampus (Ramos-Campoy *et al.*, 2018).

DISCUSSION

In this work we summarize demographical, clinical and neuropathological features of a single Brain Brank FTLD cohort with the aim to delineate particular features that might suggest an underlying genetic condition. We identified a pathogenic mutation in 21.2% of FTLD cases in our cohort. This proportion is in consonance with previous reports and underscores the high heritability of these group of diseases and the importance of an adequate genetic screening. However, we cannot exclude a Brain Bank selection bias as patients with FTLD might be more prone to donate their brain for research purposes if there is a family history or early disease onset. Currently, there is still no clear consensus on when a genetic screening should be performed. The clinical, neuropathological and genetic heterogeneity of the

FTLD disorders represents a real challenge for clinicians to decide which genes and when should be tested. Here we integrate four strategies to perform a genetic testing in FTLD patients based on the family history, the age at disease onset, and particular clinical and neuropathological features.

First, we explored the proportion of genetic cases in our cohort according the presence of a family history stratified according the modified Goldman score. This score enables the classification of the family history on grades based on the number of a patient's affected relatives and has been proposed as the main trait to guide genetic testing in FTLD. In consonance with previous studies, we found that this score strongly predicts the likehood of identifying a pathogenic mutation (Goldman *et al.*, 2011; Goldman, 2015; Koriath *et al.*, 2018): 100% in subjects with a Goldman Score of 1; 62.5% with a score 2, and 52.6% with a score of 3. Mutations were less frequent in subjects with a modified score of 3.5 or 4, but in a still relevant proportion (12.5% and 9.7%, respectively). Importantly, 23.3% of known mutation carriers would be undiagnosed according to the family history criteria for genetic screening if only GS 1 would be taken into accound.

Interestingly, the different genes showed different patterns of modified Goldman score (table 1). A modified Goldman score of 1 was found in 55% of the *MAPT* mutation carriers, but only in 18% of the *C9orf72* cases and in none of the *GRN* mutation carriers. This can be due to the different penetrance or age at onset depending on the mutations, with *MAPT* cases presenting younger age at onset than *C9orf72* or *GRN* cases (Moore *et al.*, 2020).

Although FTLD is considered an early-onset type of dementia, we found that patients carrying a disease-causing mutation have a a younger age at onset than sporadic cases. This can also help in the decision-making process for genetic screening in these patients. We found for the age at onset an AUC of 0.71 to differenciate genetic cases. However, the best cut-off that is considered to be at 61.5 years, showed only a sensitivity and specificity of 67.5% and 69.7%, respectively. Certainly, to consider the age at onset as the only criterium for genetic testing might lead to a considerable amount of undiagnosed cases, especially of mutations accuring at older ages, such as *GRN* mutations. On the other hand, some sporadic FTLD diseases such as Pick's disease or FTLD-FET cases frequently present a very young age at onset without carrying any pathogenic mutation. Besides these limitations, it is important to include the age at onset as a criterium, as it may allow to identify some genetic cases as *de novo* mutations.

The third characteristic we analysed were some particular clinical features at disease onset. We found significant differences in the proportion of genetic cases among the different clinical syndromes, with bvFTD and ALS-FTD being the phenotypes most frequently associated with pathogenic mutations. Based on these results, a genetic screening might be considered in all bvFTD and ALS-FTD cases. We observed a surprisingly high proportion of mutation carriers in svPPA cases (37.5%), a syndrome tipically considered to be sporadic. Two of these genetic svPPA cases where related to *MAPT* mutations, and the other one to a *TARDBP* mutation (Gelpi *et al.*, 2014; Borrego-Écija *et al.*, 2017). Although this proportion might have been overestimated due to a relatively high number of *MAPT* mutation carriers in our series, there is increasing evidence that this syndrome could be indeed associated with pathogenic mutations, particularly in *MAPT* or *TARDBP* (Caroppo *et al.*, 2016; Borrego-Écija *et al.*, 2017). Among patients with a nfvPPA we found a *GRN* mutation in 2 (20%). Interestingly, these 2 patients presented mixed features and included those of the logopenic variant of PPA, a characteristic that has been reported to be fairly typical for *GRN* mutation carriers with predominantly left-sided brain atrophy (Rohrer *et al.*, 2010). Some *MAPT* mutations might present in form of CBS or PSPS, so this mutation should be consider in these patients in case of family history (Moore *et al.*, 2019). We did not find any pathogenic mutation in subjects presenting as PSPS, but a *MAPT* variation was identified in one patient with CBD. It is not uncommon that *GRN* carriers also present a CBS (Moore *et al.*, 2019), therefore, progranulin mutation screening has been also suggested in CBS cases (Galimberti *et al.*, 2016).

Finally, we assessed the potential of some neuropathological features to identify genetic cases. We found wide differences in the proportion of genetic cases between the neuropathological diagnoses. A pathogenic mutation (mainly *GRN*) was found in the 66.7% of the FTLD cases with TDP-43 inclusions of the type A, but in none of the TDP-43 type C subjects.

The presence of ubuiquitin/p62 positive, TDP-43 negative inclusions in the granular cells of the cerebellum and hippocampus is highly predictive of a C9orf72 expansion (Al-Sarraj *et al.*, 2011; Simón-Sánchez *et al.*, 2012), and we confirmed that its systematic assessment in all neurodegenerative diseases is a good screening method for the detection of *C9orf72* expansion carriers (Ramos-Campoy *et al.*, 2018). Other possible morphological hallmarks that may indicate the presence of an underlying mutation is the presence of “mini-Pick”-like bodies in the dentate gyrus of the hippocampus in FTLD-tau

cases, a finding frequently related to *MAPT* mutations, particularly to the p.P301L mutation (Ferrer *et al.*, 2003; Borrego-Écija *et al.*, 2017). Finally, co-aggregation of FET proteins and their nuclear import protein, Transportin 1 (Trn1), have been reported in sporadic FTLD-FUS and ALS-FUS cases, but not in genetic ALS-FUS cases with *FUS* mutations (MacKenzie and Neumann, 2012; Borrego-Écija *et al.*, 2018), suggesting that immunohistochemistry for EWS, TAF15 and Trnp1 can be useful in order to guide the the analysis of *FUS* (Neumann 2001, 2012; Borrego-Écija *et al.*, 2018).

Our study has some inherent limitations. First, it is based on a single Brain Bank series and it is likely that it does not represent the whole FTLD population. The proportion of extreme phenotypes or familial cases, for example, might be overrepresented by a selection bias as discussed above. However, the percentage of genetic cases is similar to other clinical and neuropathological cohorts. Second, the clinical assessment and classification have been performed retrospectively according to current diagnostic criteria, and data acquisition might have been inhomogeneous, as some of the cases have been registered previously to more recent diagnostic criteria and modern diagnostic tools. Finally, not all cases have been systematically screened for all mutations, which may have lead to an underrecognition of some mutations, particularly of those that are not associated with a positive family history, that have late disease onset and that do not show particular neuropathological features, which are probably rare. To solve this last limitation we are planning to perform whole-genome sequencing in all the cohort in the future.

In summary, our study supports that a integrated approach that takes into account the family history, the age at disease onset, and particular clinical and neuropathological features might improve the identification of genetic FTLD.

Competing interests

The authors declare that they have no conflict of interest.

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Table 1: Clinical correlates and heritability of each mutation

	C9orf72	*MAPT*	*GRN*	*VCP*	*TARDBP*	*TBK1*	*SQSTM1*	Total
n	20	12	6	1	2	1	2	44
male / female	10/10	9/3	3/3	1/0	2/0	1/0	2/0	28/16
Clinical syndrome, n(%)								
bvFTD	8(40.0%)	6 (50.0%)	3 (50.0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	18 (40.1%)
PPAnfv	0 (0%)	0 (0%)	2 (33.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (4.5%)
PPAsv	0 (0%)	2 (16.6%)	0 (0%)	0 (0%)	1 (50.0%)	0 (0%)	0 (0%)	3 6.8%)
ALS-FTD	6 (30.0%)	0 (0%)	0 (0%)	0 (0%)	1 (50.0%)	1 (100%)	0 (0%)	8 (18.2%)
CBS	0 (0%)	1 (8.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2.3%)
Dementia NOS	5 (25.0%)	3 (25.0%)	1 (16.7%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)	11 (25%)
Parkinsonism NOS	1 (5.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2.3%)
Goldman score, n(%)								
1	2 (18%)	6 (55%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	8 (23.5%)
2	3 (27%)	1 (9%)	1 (17%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (14.7%)
3	4 (37%)	2 (18%)	2 (33%)	1 (100%)	1 (50%)	0 (0%)	0 (0%)	10 (29.4%)
3.5	0 (0%)	0 (0%)	2 (33%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	3 (8.8%)
4	2 (18%)	2 (18%)	1 (17%)	0 (0%)	0 (0%)	1 (100%)	1 (100%)	8 (23.5%)

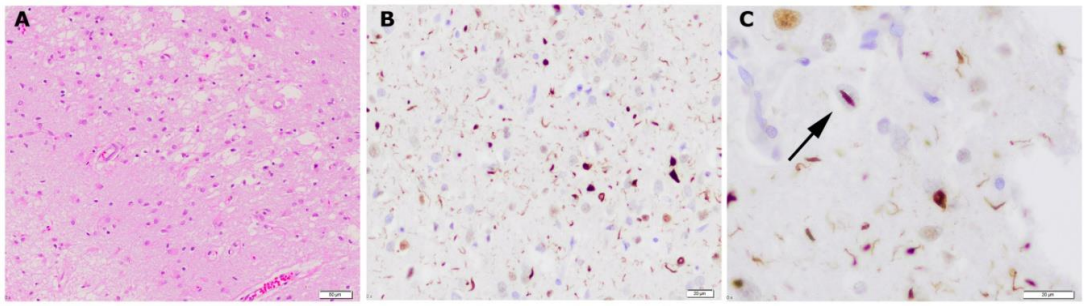
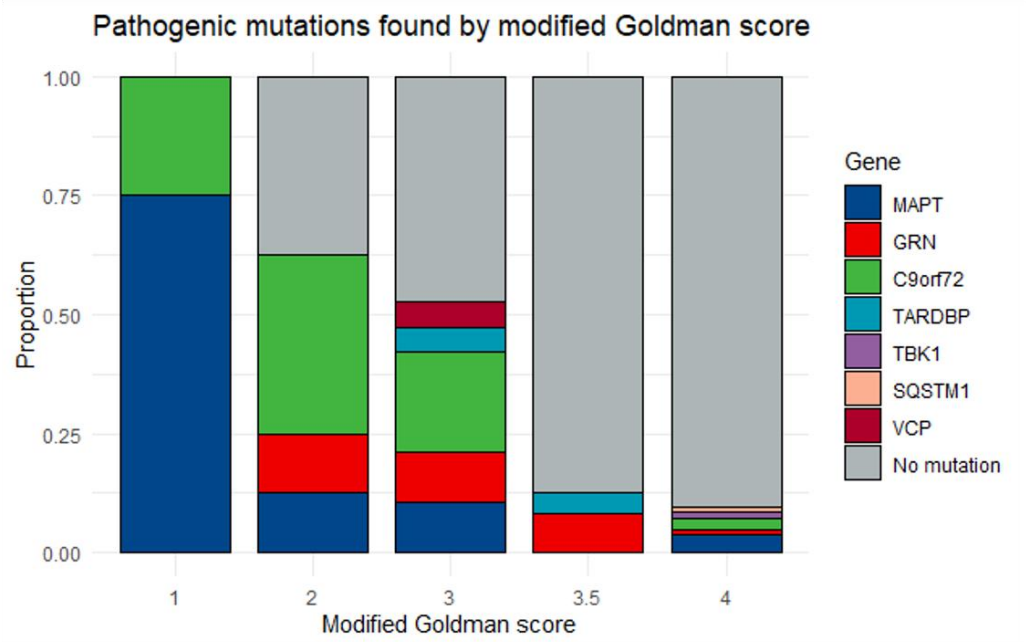


Figure 1: Neuropathology of the p.Q4545fsX58 *GRN* mutation carrier. A: Hematoxylin-eosin staining of frontal cortex shows a diffuse thinning / narrowing of the cortical ribbon, and a prominent laminar microvacuolation / spongiosis of the superficial cortical layers. **B-C**: Immunohistochemistry for TDP-43 protein reveals abundant pathological aggregates with numerous short thin and thicker neurites in the grey matter and also frequent processes in the white matter tracts. In addition, frequent compact and round cytoplasmic neuronal inclusions were detected in superficial neurons. Furthermore, frequent neuronal nuclear inclusions were detected (C, arrow, “cat-eye” type of intranuclear inclusion).

A)



B)

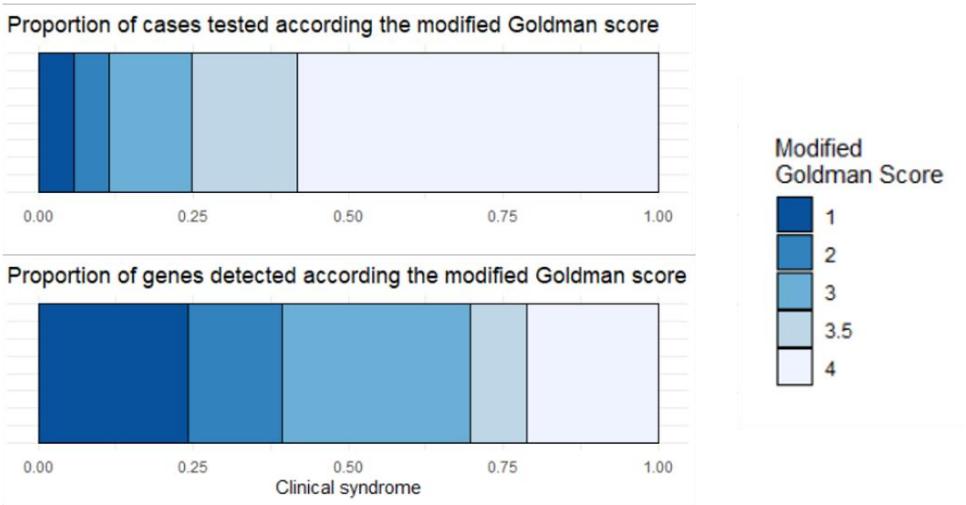


Figure 2: A) Proportion of pathogenic mutations by the modified Goldman score. B) Proportion of cases tested (up) and genes detected (down) by genetic screening according to the modified Goldman Score.

A) B)

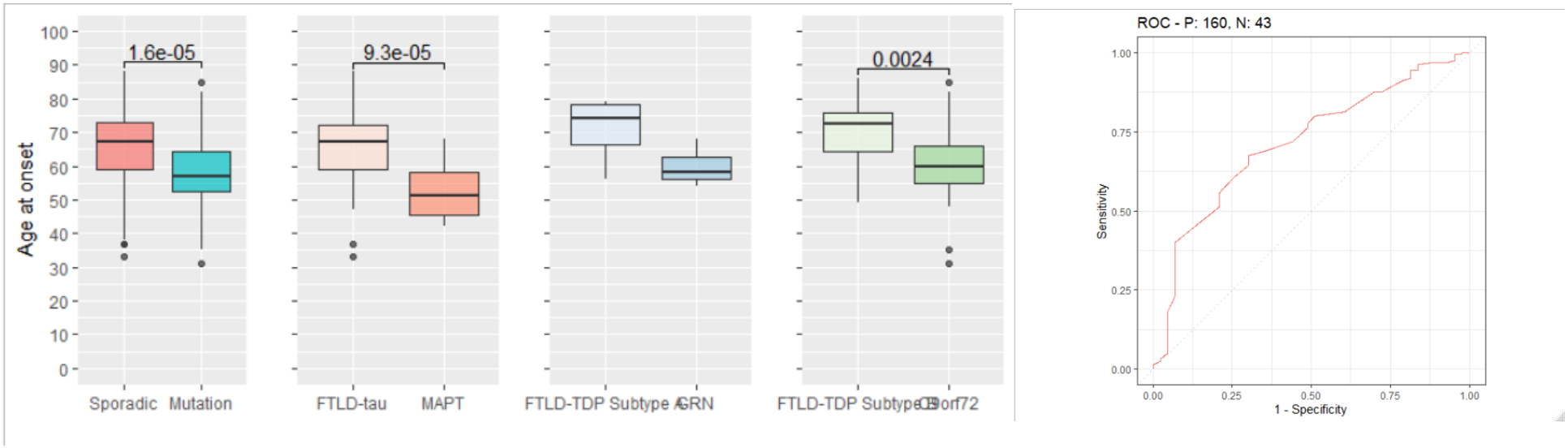


Figure 3: A) Boxplot representing the age at onset of genetic and sporadic cases for all subjects, and for FTLD-tau,FTLD-TDP subtype A and FTLD -TDP type B cases. B) ROC curve for age at onset discriminating between genetic and sporadic cases.

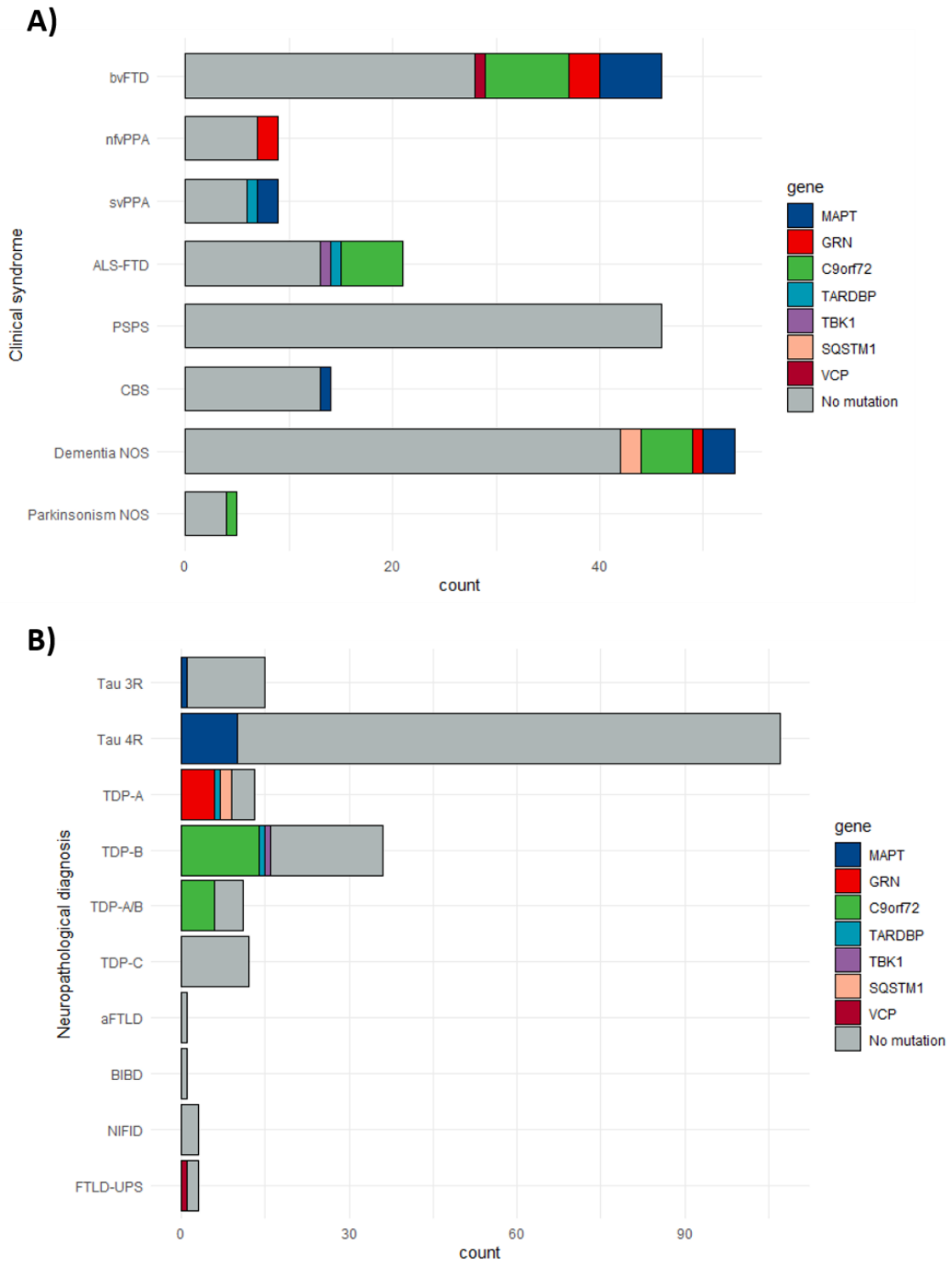


Figure4: Proportion of pathogenic mutations across de different clinical syndromes (A) and neuropathologic diagnoses (B).

VI.

OTROS RESULTADOS

Tauopathy with Hippocampal 4-Repeat Tau Immunoreactive Spherical Inclusions in a Patient with PSP

Borrego-Écija S, Grau-Rivera O, Colom-Cadena M, Molinuevo JL, Tolosa E, Sánchez-Valle R, Gelpi E..

Brain Pathol. 2018;28:284-286.

Distinct Clinical Features and Outcomes in Motor Neuron Disease Associated with Behavioural Variant Frontotemporal Dementia

Cortés-Vicente E, Turon-Sans J, Gelpi E, Clarimón J**, Borrego-Écija S**, Dols-Icardo O, Illán-Gala I, Lleó A, Illa I, Blesa R, Al-Chalabi A, Rojas-García R..

Dement Geriatr Cogn Disord. 2018;45:220-231

Aim: To determine the motor phenotype and outcome in a clinically ascertained group of patients with motor neuron disease (MND) and frontotemporal dementia (FTD).
Methods: This is an observational retrospective clinical study of patients fulfilling the clinical criteria for MND-FTD. A contemporary series of patients with amyotrophic lateral sclerosis (ALS) without dementia were included for comparison. Demographic, clinical, genetic, and neuropathological data were collected. A descriptive and comparative data analysis was performed.

Results: We identified 22 patients with MND-FTD. Selective distal upper limb muscle weakness and atrophy with non-significant lower limb weakness during follow-up was the most frequent motor pattern, present in 18 patients - in 15 of them associated with severe dysphagia. Aspiration pneumonia was the most common cause of death (12/19; 63%) despite gastrostomy. One-third of the patients did not develop upper motor neuron dysfunction. When compared to classic ALS without dementia (n = 162), these features were significantly different. A neuro-pathological examination was performed on 7 patients, and it confirmed the presence of MND with TDP43 protein aggregates in all patients.

Conclusions: The MND-FTD patients frequently displayed a distinctive motor pattern characterized by weakness and atrophy in distal upper limb muscles and dysphagia, with no or little spreading to other regions. These features may help to define specific subgroups of patients, which is important with regard to clinical management, outcome, and research.

Clinical value of cerebrospinal fluid neurofilament light chain in semantic dementia.

Meeter LHH, Steketee RME, Salkovic D, Vos ME, Grossman M, McMillan CT, Irwin DJ, Boxer AL, Rojas JC, Olney NT, Karydas A, Miller BL, Pijnenburg YAL, Barkhof F, Sánchez-Valle R, Lladó A**, Borrego-Ecija S**, Diehl-Schmid J, Grimmer T, Goldhardt O, Santillo AF, Hansson O, Vestberg S, Borroni B, Padovani A, Galimberti D, Scarpini E, Rohrer JD, Woollacott IOC, Synofzik M, Wilke C, de Mendonca A, Vandenberghe R, Benussi L, Ghidoni R, Binetti G, Niessen WJ, Papma JM, Seelaar H, Jiskoot LC, de Jong FJ, Donker Kaat L, Del Campo M, Teunissen CE, Bron EE, Van den Berg E, Van Swieten JC.

J Neurol Neurosurg Psychiatry. 2019;90:997-1004.

Background: Neurofilament light chain (NfL) is a promising blood biomarker in genetic frontotemporal dementia, with elevated concentrations in symptomatic carriers of mutations in GRN, C9orf72, and MAPT. A better understanding of NfL dynamics is essential for upcoming therapeutic trials. We aimed to study longitudinal NfL trajectories in people with presymptomatic and symptomatic genetic frontotemporal dementia.

Methods: We recruited participants from 14 centres collaborating in the Genetic Frontotemporal Dementia Initiative (GENFI), which is a multicentre cohort study of families with genetic frontotemporal dementia done across Europe and Canada. Eligible participants (aged ≥18 years) either had frontotemporal dementia due to a pathogenic mutation in GRN, C9orf72, or MAPT (symptomatic mutation carriers) or were healthy at-risk first-degree relatives (either presymptomatic mutation carriers or non-carriers), and had at least two serum samples with a time interval of 6 months or more. Participants were excluded if they had neurological comorbidities that were likely to affect NfL, including cerebrovascular events. We measured NfL longitudinally in serum samples collected between June 8, 2012, and Dec 8, 2017, through follow-up visits annually or every 2 years, which also included MRI and neuropsychological assessments. Using mixed-effects models, we analysed NfL changes over time and correlated them with longitudinal imaging and clinical parameters, controlling for age, sex, and study site. The primary outcome was the course of NfL over time in the various stages of genetic frontotemporal dementia.

Findings: We included 59 symptomatic carriers and 149 presymptomatic carriers of a mutation in GRN, C9orf72, or MAPT, and 127 non-carriers. Nine presymptomatic carriers became symptomatic during follow-up (so-called converters). Baseline NfL was elevated in symptomatic carriers (median 52 pg/mL [IQR 24-69]) compared with presymptomatic carriers (9 pg/mL [6-13]; p<0·0001) and non-carriers (8 pg/mL [6-11]; p<0·0001), and was higher in converters than in non-converting carriers (19 pg/mL [17-28] vs 8 pg/mL [6-11]; p=0·0007; adjusted for age). During follow-up, NfL increased in converters (b=0·097 [SE 0·018]; p<0·0001). In symptomatic mutation carriers overall, NfL did not change during follow-up (b=0·017 [SE 0·010]; p=0·101) and remained elevated. Rates of NfL change over time were associated

with rate of decline in Mini Mental State Examination (b=-94·7 [SE 33·9]; p=0·003) and atrophy rate in several grey matter regions, but not with change in Frontotemporal Lobar Degeneration-Clinical Dementia Rating scale score (b=-3·46 [SE 46·3]; p=0·941).

Interpretation: Our findings show the value of blood NfL as a disease progression biomarker in genetic frontotemporal dementia and suggest that longitudinal NfL measurements could identify mutation carriers approaching symptom onset and capture rates of brain atrophy. The characterisation of NfL over the course of disease provides valuable information for its use as a treatment effect marker.

No supportive evidence for TIA1 gene mutations in a European cohort of ALS-FTD spectrum patients.

Baradaran-Heravi Y, Dillen L, Nguyen HP, Van Mossevelde S, Baets J, De Jonghe P, Engelborghs S, De Deyn PP, Vandenbulcke M, Vandenberghe R, Van Damme P, Cras P, Salmon E, Synofzik M, Heutink P, Wilke C, Simon-Sanchez J, Rojas-Garcia R, Turon-Sans J, Lleó A, Illán-Gala I, Clarimón J, Borroni B, Padovani A, Pastor P, Diez-Fairen M, Aguilar M, Gelpi E, Sanchez-Valle R, **Borrego-Ecija S**, Matej R, Parobkova E, Nacmias B, Sorbi S, Bagnoli S, de Mendonça A, Ferreira C, Fraidakis MJ, Diehl-Schmid J, Alexopoulos P, Almeida MR, Santana I, Van Broeckhoven C, van der Zee J;
BELNEU Consortium; EU EOD Consortium.

Neurobiol Aging. 2018;69:293.e9-293.e11

We evaluated the genetic contribution of the T cell-restricted intracellular antigen-1 gene (TIA1) in a European cohort of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) patients. Exonic resequencing of TIA1 in 1120 patients (693 FTD, 341 ALS, 86 FTD-ALS) and 1039 controls identified in total 5 rare heterozygous missense variants, affecting the TIA1 low-complexity domain (LCD). Only 1 missense variant, p.Met290Thr, identified in a familial FTD patient with disease onset at 64 years, was absent from controls yet received a combined annotation-dependent depletion score of 11.42. By contrast, 3 of the 4 variants also detected in unaffected controls, p.Val294Glu, p.Gln318Arg, and p.Ala381Thr, had combined annotation-dependent depletion scores greater than 20. Our findings in a large European patient-control series indicate that variants in TIA1 are not a common cause of ALS and FTD. The observation of recurring TIA1 missense variants in unaffected individuals lead us to conclude that the exact genetic contribution of TIA1 to ALS and FTD pathogenesis remains to be further elucidated.

Diverse, evolving conformer populations drive distinct phenotypes in frontotemporal lobar degeneration caused by the same MAPT-P301L mutation.

Daude N, Kim C, Kang SG, Eskandari-Sedighi G, Haldiman T, Yang J, Fleck SC, Gomez-Cardona E, Han ZZ, **Borrego-Ecija S**, Wohlgemuth S, Julien O, Wille H, Molina-Porcel L, Gelpi E, Safar JG, Westaway D.

Acta Neuropathol. 2020;139:1045-1070.

Tau protein accumulation is a common denominator of major dementias, but this process is inhomogeneous, even when triggered by the same germline mutation. We considered stochastic misfolding of human tau conformers followed by templated conversion of native monomers as an underlying mechanism and derived sensitive conformational assays to test this concept. Assessments of brains from aged TgTau^{P301L} transgenic mice revealed a prodromal state and three distinct signatures for misfolded tau. Frontotemporal lobar degeneration (FTLD)-MAPT-P301L patients with different clinical phenotypes also displayed three signatures, two resembling those found in TgTau^{P301L} mice. As physicochemical and cell bioassays confirmed diverse tau strains in the mouse and human brain series, we conclude that evolution of diverse tau conformers is intrinsic to the pathogenesis of this uni-allelic form of tauopathy. In turn, effective therapeutic interventions in FTLD will need to address evolving repertoires of misfolded tau species rather than singular, static molecular targets.

A unique common ancestor introduced P301L mutation in MAPT gene in frontotemporal dementia patients from Barcelona (Baix Llobregat, Spain)

Leire Palencia-Madrid, Raquel Sánchez-Valle, Ierai Fernández de Retana, **Sergi Borrego**, Oriol Grau-Rivera, Ramón Reñé, Isabel Hernández, Consuelo Almenar, Giacomina Rossi, Paola Caroppo, Veronica Redaelli, Isabelle Le Ber, Agnès Camuzat, Alexis Brice, Anna Antonell, Mircea Balasa, Ellen Gelpi, Albert Lladó, Marian M de Pancorbo

Neurobiol Aging. 2019 Dec;84:236.e9-236.e15

The County of Baix Llobregat (Barcelona, Catalonia, Spain) presents a high prevalence of familial frontotemporal dementia (FTD) in the presence of P301L mutation in the MAPT gene. To evaluate a possible unique founder effect of P301L, and its age, the analysis of 20 single-nucleotide polymorphisms covering 50 kb and 12 single-nucleotide polymorphisms located along 30 Mb around the mutation was performed by developing 2 multiplex single-base extension reactions. In addition, families with affected and healthy individuals from France and Italy were analyzed. The FTD-affected individuals from Barcelona carried the same 50-kb haplotype linked to P301L mutation, suggesting a unique common ancestor, as opposed to French patients. Italian patients are also probably descendants of a unique ancestor, which would be different from that of Barcelona. Diversity of 30-Mb haplotypes found in Barcelona and the inference of the mutation age in these populations, among other reasons, suggest that prevalence of FTD linked to P301L MAPT mutation is the result of a locally originated mutation.

Durante la realización de la presente tesis doctoral el doctorando ha participado como coinvestigador de la GENetic Frontotemporal Initiative (GENFI), un consorcio internacional que incluye centros de Europa y Canada. El propósito de GENFI es crear una cohorte de sujetos con o en riesgo de degeneración lobular frontotemporal genéticamente predeterminada para investigar la enfermedad en sus étapas iniciales con el fin último de desarrollar biomarcadores que permitan identificar el inicio de la enermedad o monitorizar futuros ensayos clínicos.

Como resultado de su participación como coinvestigador en el consorcio GENFI, el doctorando ha sido coautor de la siguiente publicación científica:

Age at symptom onset and death and disease duration in genetic frontotemporal dementia: an international retrospective cohort study

Moore KM, Nicholas J, Grossman M, McMillan CT, Irwin DJ, Massimo L, Van Deerlin VM, Warren JD, Fox NC, Rossor MN, Mead S, Bocchetta M, Boeve BF, Knopman DS, Graff-Radford NR, Forsberg LK, Rademakers R, Wszolek ZK, van Swieten JC, Jiskoot LC, Meeter LH, Dopper EG, Papma JM, Snowden JS, Saxon J, Jones M, Pickering-Brown S, Le Ber I, Camuzat A, Brice A, Caroppo P, Ghidoni R, Pievani M, Benussi L, Binetti G, Dickerson BC, Lucente D, Krivensky S, Graff C, Öijerstedt L, Fallström M, Thonberg H, Ghoshal N, Morris JC, Borroni B, Benussi A, Padovani A, Galimberti D, Scarpini E, Fumagalli GG, Mackenzie IR, Hsiung GR, Sengdy P, Boxer AL, Rosen H, Taylor JB, Synofzik M, Wilke C, Sulzer P, Hodges JR, Halliday G, Kwok J, Sanchez-Valle R, Lladó A, **Borrego-Ecija S**, Santana I, Almeida MR, Tábuas-Pereira M, Moreno F, Barandiaran M, Indakoetxea B, Levin J, Danek A, Rowe JB, Cope TE, Otto M, Anderl-Straub S, de Mendonça A, Maruta C, Masellis M, Black SE, Couratier P, Lautrette G, Huey ED, Sorbi S, Nacmias B, Laforce R Jr, Tremblay ML, Vandenberghe R, Damme PV, Rogalski EJ, Weintraub S, Gerhard A, Onyike CU, Ducharme S, Papageorgiou SG, Ng ASL, Brodtmann A, Finger E, Guerreiro R, Bras J, Rohrer JD; FTD Prevention Initiative.

Lancet Neurol. 2020;19:145-156.

Background: Frontotemporal dementia is a heterogenous neurodegenerative disorder, with about a third of cases being genetic. Most of this genetic component is accounted for by mutations in GRN, MAPT, and C9orf72. In this study, we aimed to complement previous phenotypic studies by doing an international study of age at symptom onset, age at death, and disease duration in individuals with mutations in GRN, MAPT, and C9orf72.

Methods: In this international, retrospective cohort study, we collected data on age at symptom onset, age at death, and disease duration for patients with pathogenic mutations in the GRN and MAPT genes and pathological expansions in the C9orf72 gene through the Frontotemporal Dementia Prevention Initiative and from published papers. We used mixed effects models to explore differences in age at onset, age at death, and disease duration between genetic groups and individual mutations. We also

assessed correlations between the age at onset and at death of each individual and the age at onset and at death of their parents and the mean age at onset and at death of their family members. Lastly, we used mixed effects models to investigate the extent to which variability in age at onset and at death could be accounted for by family membership and the specific mutation carried.

Findings: Data were available from 3403 individuals from 1492 families: 1433 with C9orf72 expansions (755 families), 1179 with GRN mutations (483 families, 130 different mutations), and 791 with MAPT mutations (254 families, 67 different mutations). Mean age at symptom onset and at death was 49·5 years (SD 10·0; onset) and 58·5 years (11·3; death) in the MAPT group, 58·2 years (9·8; onset) and 65·3 years (10·9; death) in the C9orf72 group, and 61·3 years (8·8; onset) and 68·8 years (9·7; death) in the GRN group. Mean disease duration was 6·4 years (SD 4·9) in the C9orf72 group, 7·1 years (3·9) in the GRN group, and 9·3 years (6·4) in the MAPT group. Individual age at onset and at death was significantly correlated with both parental age at onset and at death and with mean family age at onset and at death in all three groups, with a stronger correlation observed in the MAPT group (r=0·45 between individual and parental age at onset, r=0·63 between individual and mean family age at onset, r=0·58 between individual and parental age at death, and r=0·69 between individual and mean family age at death) than in either the C9orf72 group (r=0·32 individual and parental age at onset, r=0·36 individual and mean family age at onset, r=0·38 individual and parental age at death, and r=0·40 individual and mean family age at death) or the GRN group (r=0·22 individual and parental age at onset, r=0·18 individual and mean family age at onset, r=0·22 individual and parental age at death, and r=0·32 individual and mean family age at death). Modelling showed that the variability in age at onset and at death in the MAPT group was explained partly by the specific mutation (48%, 95% CI 35-62, for age at onset; 61%, 47-73, for age at death), and even more by family membership (66%, 56-75, for age at onset; 74%, 65-82, for age at death). In the GRN group, only 2% (0-10) of the variability of age at onset and 9% (3-21) of that of age of death was explained by the specific mutation, whereas 14% (9-22) of the variability of age at onset and 20% (12-30) of that of age at death was explained by family membership. In the C9orf72 group, family membership explained 17% (11-26) of the variability of age at onset and 19% (12-29) of that of age at death.

Interpretation: Our study showed that age at symptom onset and at death of people with genetic frontotemporal dementia is influenced by genetic group and, particularly for MAPT mutations, by the specific mutation carried and by family membership. Although estimation of age at onset will be an important factor in future pre-symptomatic therapeutic trials for all three genetic groups, our study suggests that data from other members of the family will be particularly helpful only for individuals with MAPT mutations. Further work in identifying both genetic and environmental factors that modify phenotype in all groups will be important to improve such estimates.

VII.

Discusión

La presente tesis doctoral y los trabajos que la conforman tienen como objetivo profundizar en el conocimiento de las relaciones genotipo-fenotipo en el contínuo DLFT-ELA. Un mejor conocimiento del fenotipo clínico y neuropatológico puede llevar a una mejor comprensión de la fisiopatología de la enfermedad, así como a orientar al clínico en decidir cuándo realizar un estudio genético y qué gen evaluar en primer lugar.

Para conseguir este objetivo, los trabajos de la presente tesis doctoral se centran en dos cohortes no excluyentes de pacientes con DLFT-ELA: a) una cohorte postmortem de pacientes con DLFT y/o ELA diagnosticada neuropatológicamente, y b) una cohorte clínica multicéntrica de pacientes con DLFT genéticamente determinada.

La **cohorte neuropatológica** se obtuvo gracias a la colección de muestras biológicas de tejido nervioso del Banc de Teixits Neurològics del Biobanco del Hospital Clínic de Barcelona – IDIBAPS. Dicho biobanco cuenta con un fondo de más de 2200 muestras de cerebro y médula espinal obtenidas de donantes con enfermedades neurodegenerativas, quienes con la donación del tejido contribuyen de forma altamente generosa y altruista al avance de las neurociencias. Todos disponen de un diagnóstico neuropatológico detallado. Esta cohorte permite el estudio de sujetos con el diagnóstico demostrado neuropatológicamente. El análisis postmortem del cerebro es especialmente relevante para el estudio de la fisiopatología subyacente de la enfermedad. Está ampliamente demostrado que la presencia de una mutación patogénica determina la neuropatología subyacente tanto en la DLFT como en la ELA: las mutaciones en *MAPT*, por ejemplo, se asocian a DLFT-tau, mientras que las de *C9orf72* o *GRN* se asocian a depósitos de proteína TDP-43 (Lashley *et al.*, 2015). No obstante, las relaciones entre el genotipo y el fenotipo neuropatológico subyacente van más allá de el establecimiento del subtipo neuropatológico. Los pacientes con expansiones patológicas en *C9orf72*, por ejemplo, presentan ciertas características neuropatológicas específicas que las

distinguen de otras DLFT-TDP. En estos casos, es posible apreciar además de TDP-43, la presencia de inclusiones ubiquitina y p62 positivas, pero TDP-43 negativas, en múltiples áreas cerebrales incluyendo cerebelo, hipocampo y neocórtex (Mahoney *et al.*, 2012; Simón-Sánchez *et al.*, 2012; Snowden *et al.*, 2012). El conocimiento de estos fenotipos neuropatológicos propios puede ayudar a la identificación de mutaciones genéticas subyacentes, haciendo posible el consejo genético en los familiares del paciente (Ramos-Campoy *et al.*, 2018). En este sentido los trabajos 1, 4, 5 y 6 de la presente tesis doctoral pretenden profundizar en los fenotipos neuropatológicos propios de la DLFT y la ELA genéticamente determinada.

La **segunda cohorte viene conformada por pacientes con DLFT genéticamente predeterminada**. Esta cohorte se reclutó a través del Programa de Información y Consejo Genético para demencias familares (PICOGEN) del Hospital Clínic de Barcelona, y gracias al consorcio GENFI (Genetic Frototemporal Initiative; http://genfi.org.uk/), que recluta pacientes en riesgo de desarrollar una DFT genéticamente predispuesta. Estas cohortes permitieron la identificación de una nueva mutación en el gen *MAPT* que se describe en el trabajo 2 de la presente tesis doctoral. Además, el análisis de cohortes de pacientes presintomáticos como los reclutados en el consorcio GENFI, permite estudiar las fases más iniciales de la enfermedad, incluso los cambios previos a la aparición de los primeros síntomas (Rohrer *et al.*, 2013). Estos estudios constituyen una oportunidad para encontrar biomarcadores propios de las diferentes mutaciones que, a su vez, puedan servir para la monitorización de futuros ensayos clínicos (Rohrer *et al.*, 2015; van der Ende *et al.*, 2019). En este sentido el trabajo número 3 de la presente tesis doctoral pretende estudiar los primeros cambios cerebrales estructurales en sujetos presintomáticos portadores de mutaciones en *GRN*.

El análisis de estas cohortes ha permitido estudiar las características clínicas y neuropatológicas de un número importante de pacientes con DLFT y ELA con un sustrato o

subtipo molecular definido. A continuación se discuten por separado los resultados de cada uno de los artículos de la tesis.

Los trabajos 1, 2 y 6 de la presente tesis doctoral incluyen descripciones del fenotipo clínico y neuropatológico de diferentes mutaciones en *MAPT*. El primer trabajo describe una serie clínica de 13 pacientes con DLFT genéticamente predeterminada por la mutación p.P301L del gen *MAPT*. De 9 de estos 13 pacientes se dispuso de muestras de tejido cerebral postmortem, pudiendo describir también las características neuropatológicas de los mismos. Este trabajo constituye la serie más grande de pacientes con la mutación p.P301L descrita hasta hoy. Los 13 casos descritos procedían de 10 familias aparentemente no emparentadas entre sí, pero que compartían un mismo origen geográfico. El trabajo incluye un estudio genético filogénico en el que se demuestra la coincidencia genómica entre los sujetos en la región circundante a la mutación. Dicha coincidencia sugiere la existencia de un único evento mutacional en un ancestro común a las diferentes familias descritas en nuestro artículo. Ante estos hallazgos, otro trabajo reciente ha estudiado otras familias portadoras de la mutación p.P301L en Francia y en Italia con el fin de analizar si podrían provenir de este mismo ancestro común (Palencia-Madrid *et al.*, 2019). El estudio demuestra que las familias descritas en Francia provienen diferentes antecesores, pudiendo uno de ellos coincidir con el de la familia de Barcelona. Así mismo, uno de los casos descritos en Italia podría compartir el ancestro común a las familias de Barcelona. El resto de los casos italianos, en cambio, provendrían de un ascendiente común diferente del de los casos de Barcelona. El hecho de encontrar diferentes orígenes para una misma mutación en una zona geográficamente tan limitada como el suroeste de Europa parece sugerir que el codón 301 del exón 10 de *MAPT* es una región del genoma especialmente susceptible a sufrir mutaciones.

En nuestra serie, los sujetos con la mutación p.P301L en *MAPT* compartían un fenotipo clínico y neuropatológico relativamente homogéneo. La mayoría de los pacientes (54%) se

presentaron en forma de DFTvc, pero en un 31% de ellos el síntoma inicial fue una alteración del lenguaje en forma de APPvs. Aunque ausente como forma de debut, un 46% de los casos presentaron un parkinsonismo rígido acinético durante el curso de la enfermedad. Esta sintomatología se correspondería con los hallazgos neuropatológicos consistentes en una marcada pérdida neuronal y gliosis en córtex frontotemporal, ganglios basales y sustancia negra mesencefálica. Todos los casos mostraron una taupatía neuronal y glial predominada por isoformas tau de 4R, con el hallazgo característico de "mini-cuerpos de Pick" en el giro dentado del hipocampo, una característica morfológica que ha sido observada en todas las descripciones neuropatológicas de la mutación p.P301L y que podría ser una característica específica de la misma.

Existe cierta discusión en la bibliografía sobre la heterogeneidad fenotípica entre casos con la mutación p.P301L en *MAPT*. En una serie de 8 casos con estudio histológico, Tacik *et al* argumentaron la heterogeneidad del fenotipo patológico a raíz del hallazgo en uno de ellos de una neuropatología compatible con una GGT. Nuestra serie también incluye un caso con neuropatología compatible a una GGT, y recientemente el grupo de Sídney ha reportado nuevos casos de GGT en pacientes portadores de p.P301L en *MAPT* (Forrest *et al.*, 2018). También se han descrito cambios neuropatologicos de GGT en estudios postmortem de pacientes con DLFT-tau por mutaciones en p.P301T (Erro *et al.*, 2019), p.K317N (Tacik *et al.*, 2015) y p.K317M (Zarranz *et al.*, 2005) en el gen *MAPT.* Ante estos hallazgos, Forrest et. al., así como nuestro grupo, han sugerido que, en realidad, el fenotipo neuropatológico de una misma mutación de *MAPT* es relativamente homogéneo y que los distintos fenotipos que pueden encontrarse en diferentes mutaciones serían equivalentes genéticos de los diferentes subtipos de DLFT-tau esporádica (p.ej. GGT, PSP, CBD, Pick). La localización y consecuente alteración funcional de la proteína vendría a ser el factor determinante de la morfología neuropatológica, de forma que mutaciones puntuales en los exones 9 o 11 provocarían una DLFT-tau de 3R y un fenotipo de PiD; mientras que mutaciones en los exones 10 y 13 producirían un fenotipo

neuropatológico de DCB, PSP o GGT. El hallazgo en nuestra serie neuropatológica de un paciente con la mutación p.S320F en el exón 11 y un sustrato neuropatológico compatible con una PiD (ver trabajo número 6) vendría a reforzar esta teoría.

El **segundo trabajo** que conforma la presente tesis doctoral consiste en una descripción clínica de 8 pacientes con DLFT debida a una nueva mutación en el gen *MAPT*: la sustitución del aminoácido prolina por serina en el codón 397 (p.P397S). Los 8 casos descritos provienen de 5 familias aparentemente no emparentadas. No obstante, todas tienen un origen geográfico común en el sureste de le península Ibérica, en las provincias de Málaga, Alicante y Albacete, lo que hace pensar en un posible ancestro común. Aunque no se dispone de confirmación neuropatológica de ninguno de estos casos, los estudios de segregación, con la presencia de la mutación en los 8 individuos afectos y su ausencia en una familiar asintomática, predicen la probable patogenicidad de esta nueva mutación. Su mecanismo de acción podría estar relacionado con la destrucción de un punto de fosforilación Prolina/Serina en el codón 396, lo que comportaría una modificación funcional de la proteína tau resultante (Iqbal *et al.*, 2015; Regan *et al.*, 2015).

Las características clínicas de los pacientes con la mutación en p.P397S consisten en alteraciones conductuales especialmente en forma de desinhibición, falta de decoro e hiperoralidad para dulces. Los sujetos también desarrollaron déficits en las funciones ejecutivas y en la semántica del lenguaje. Tres de ellos desarrollaron un parkinsonismo leve rígido-acinético durante la enfermedad. La neuroimagen mostró una atrofia moderada de predominio temporal anterior con relativa preservación de los lóbulos frontales. Estas características clínicas no difieren de las encontradas en la serie de pacientes con la mutación p.P301L en *MAPT.* Sin embargo, los pacientes con la mutación p.P397S presentaron una edad de inicio significativamente más tardía (media de edad de inicio a los 61,3 años) y una duración de la enfermedad notablemente más larga (media de duración de la enfermedad de 14 años)

que la de los pacientes con la mutación p.P301L. Cabe destacar que las mutaciones p.R406W y p.T427M, también localizadas en el exón 13 del gen *MAPT*, también presentan una edad de presentación y de muerte tardías (Giaccone *et al.*, 2005; Ygland *et al.*, 2018). Esto es especialmente llamativo por el hecho de que las mutaciones en *MAPT* han sido tradicionalmente consideradas como causantes de DLFT en edades especialmente tempranas, entre la tercera y quinta década de la vida (Moore *et al.*, 2019). No obstante, trabajos previos han señalado que existe una importante variabilidad en la edad de presentación de los síntomas y la duración de enfermedad entre diferentes mutaciones en *MAPT* (van Swieten *et al.*, 1999; Moore *et al.*, 2019) (Figura 11). Es probable que estas diferencias clínicas entre mutaciones en *MAPT* sean debidas a diferencias funcionales en la proteína codificada por el gen mutado. En otras palabras, el fenotipo clínico y neuropatológico de los pacientes con mutaciones en *MAPT* dependería del tipo de mutación y de la repercusión funcional de ésta en la codificación de la proteína tau.

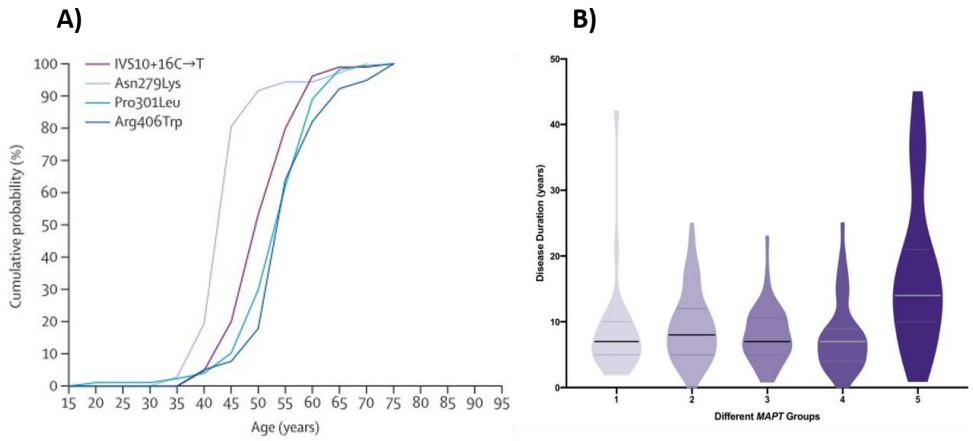


Figura 11: A) Probabilidad acumulada de edad de inicio para diferente mutaciones en *MAPT*. Se puede apreciar como la mutación Asn279Lys presenta una edad de inicio notablemente mas precoz que la de otras mutaciones. B) Duración de la enfermedad en pacientes con mutaciones en *MAPT* agrupados en función del tipo de mutación y patología (Grupo 1: R5H, R5L, G55R, K257T, I260V, L226V, G272V, IVS9-5T>C, IVS9-11G>C, IVS9-10G>C, IVS9-10G>T; Grupo 2: N279K, deltaK280, L284L, L284R, S285R, C291R, N296N, K298E, IVS10+3G>A, IVS10+4A>C, IVS10+11T>C, IVS10+12C>T, IVS10+12C>A, IVS10+13A>G, IVS10+14C>T, IVS10+15A>C, IVS10+16C>T,G303V, G304S, S305N, S305I, S305S; Grupo 3: P301T, P301S, P301L; Grupo 4: L315R, L315L, K317M, S320F, P332S, G335S, G335V, Q336H, E342V, S352L, S356T, V363A, P364S, G336R, K369I, E372G, G389R (2170G>A), G389R (2170G>C), T427M; Grupo 5: V337M, R406W). Imagen adaptada de K Moore et al, 2020

En el **trabajo 3** de la presente tesis doctoral estudiamos los cambios estructurales cerebrales en sujetos presintomáticos portadores de mutaciones en *GRN* de la cohorte GENFI. Para ello se determinó el grosor cortical mediante resonancia magnética nuclear (RMN) cerebral en 100 portadores asintomáticos y 94 controles. Aunque no se apreciaron diferencias estadísticamente significativas del grosor cortical en el análisis global entre portadores y no-portadores, sí se observó que los sujetos portadores de una mutación mostraban una pérdida de grosor cortical significativamente mayor en relación a la edad estimada de presentación que los sujetos controles, sugiriendo una pérdida neuronal más acelerada en estos sujetos. Estos resultados siguen la misma línea que artículos previos que también describen una mayor neurodegeneración asociada a la edad en portadores de mutaciones en *GRN* (Moreno *et al.*, 2013). Las áreas en las que se apreciaron estas diferencias fueron las circunvoluciones superior y medial del lóbulo frontal, áreas típicamente afectadas en los sujetos sintomáticos (Cash *et al.*, 2018; Chen *et al.*, 2019). Estos hallazgos sugieren que éstas serían las primeras áreas cerebrales afectadas en los pacientes con DLFT-TDP por mutación en *GRN*. Estos hallazgos quizás sean extrapolables a las otras formas esporádicas de DLFT con inclusiones de proteína TDP-43 subtipo A que presentan un fenotipo clínico semejante al de los portadores de mutaciones en *GRN* (Rohrer *et al.*, 2010*b*).

El subanálisis por grupos según el genotipo de *TMEM106B,* no mostró diferencias significativas entre grupos. No obstante, dado el escaso número de sujetos con el genotipo propuesto como protector de *TMEM106B* (C/C), cabe considerar que la ausencia de diferencias significativas entre grupos sea debida a una falta de potencia estadística del estudio. En este sentido, son necesarios nuevos estudios con la inclusión de un mayor número de sujetos con el genotipo protector de *TMEM106B* para conocer su influencia biológica en los portadores asintomáticos de mutaciones en *GRN*.

En el **trabajo número 4** analizamos las características genéticas y neuropatológicas que determinan la aparición del deterioro cognitivo en una cohorte neuropatológica de 104 pacientes con ELA. El trabajo concluye que en la gran mayoría de casos de ELA con deterioro cognitivo, la causa subyacente del mismo es la presencia de DLFT en el estudio neuropatológico. En esta cohorte, la presencia de mutaciones en genes causantes o de riesgo para DLFT (especialmente la expansión patológica en *C9orf72*) se asoció significativamente a un mayor riesgo de presentar síntomas cognitivos y cambios neuropatológicos de DLFT. Estos hallazgos están en consonancia con los publicados previamente en la bibliografía, que muestran la presencia de DFT hasta en un 50% de los pacientes con ELA debida a expansiones patológicas en *C9orf72* (Byrne *et al.*, 2012).

Por otro lado, en nuestro trabajo no hemos encontrado diferencias estadísticamente significativas en la presencia de deterioro cognitivo en función del haplotipo de APOE, un conocido factor de riesgo genético para enfermedades neurodegenerativas como la enfermedad de Alzheimer. Trabajos previos han mostrado una asociación entre el haplotipo APOE y la presencia de deterioro cognitivo en la ELA (Rubino *et al.*, 2013; Chiò *et al.*, 2016; Su *et al.*, 2017). No obstante, algunos de estos estudios asocian la presencia de DLFT con el haplotipo ε2 de APOE, mientras que otros los asocian al haplotipo ε4. A diferencia de nuestro trabajo, estos estudios previos no están realizados en cohortes neuropatológicas, lo que podría implicar una asociación del deterioro cognitivo a otras causas, diferentes de la DLFT. Nuestro estudio, en cambio, no muestra ninguna influencia de APOE en el desarrollo de DLFT en pacientes con ELA. No obstante, el tamaño de nuestra cohorte podría ser insuficiente para determinar efectos de menor magnitud.

El trabajo 5 de la presente tesis doctoral explora las características neuropatológicas de tres sujetos afectos de ELA asociada a depósitos de proteína FUS. En los tres casos, los agregados patológicos mostraron también inmunoreactividad para otras proteínas del grupo FET, como

TAF15, y su transportador Trnp1. El estudio genético descartó mutaciones en el gen *FUS* en los tres casos. Estos hallazgos son similares a otros casos reportados en Japón en los últimos años (Fujita *et al.*, 2011; Matsuoka *et al.*, 2011; Takeuchi *et al.*, 2013). Nuestros casos y los descritos en Japón difieren de la teoría planteada por Neumann y colaboradores según la cual los casos de ELA y DLFT por acúmulo de proteína FUS tendrían mecanismos fisiopatológicos distintos. Esta teoría se fundamenta en diferencias tanto neuropatológicas como genéticas entre las dos entidades (Neumann and Mackenzie, 2018). Por un lado, mientras los casos de DLFT-FUS co-agregan otras proteínas FET y Trnp1 (por lo que cabría referirse a ellas como DLFT-FET), los casos de ELA-FUS no presentarían este co-depósito. Por otro lado, las DLFT-FUS con co-depósitos de proteínas FET no suelen tener mutaciones en FUS, mientras que son frecuentes en ELA-FUS, que acumula únicamente la proteína FUS (Kwiatkowski *et al.*, 2009).

La identificación de TAF15 y Trnp1 en los tres casos ELA-FUS sin mutaciones en el gen, como hemos descrito en trabajo 6 iría a favor de un mecanismo fisiopatológico común entre las DLFT y las ELAs con acúmulo de proteínas FET. Por otro lado, sí cabría esperar un mecanismo fisiopatológico distinto en los casos de ELA o DLFT FUS sin co-depósito de proteínas FET, pudiendo estos estar relacionados con mutaciones en FUS. Ello supondría que el estudio neuropatológico, en concreto la inmunohistoquímica para las proteínas FUS, EWS, TAF15 y Trnp1, podría ser de utilidad como método de cribado para la detección de mutaciones en *FUS* en los casos de DLFT o ELA con acúmulo de proteína FUS.

Finalmente, **el último trabajo** de la presente tesis doctoral pretende explorar diferentes estrategias para el cribado genético de la DLFT. Para ello el trabajo compila cuatro estrategias complementarias en la cohorte de DLFT confirmada neuropatológicamente del Banco de Tejido Nervioso de IDIBAPS. Estas cuatro estrategias se basan en a) la historia familiar, b) la edad de incio de la enfermedad, c) el fenotipo clínico y d) el fenotipo neuropatológico.

La primera de estas estrategias, basada en la historia familiar, es probablemente la más ampliamente utilizada en la práctica clínica habitual. Para estratificar la historia familiar, nos hemos basado en la escala modificada de Goldman, que clasifica la historia familiar de los pacientes en diferentes grados en función del número de individuos afectos. Una puntuación de 1 corresponde a una herencia autosómica dominante, con al menos tres casos afectos en dos generaciones. Una puntuación de 2 corresponde a varios individuos afectos en la familia sin llegar a cumplir los criterios de herencia autosómica dominante. Si el individuo tiene un único familiar afecto, se puede clasificar como 3 o 3,5 en función de si este es menor o mayor de 65 años. Finalmente, una puntuación de 4 en la escala modificada de Goldman corresponde a la ausencia de historia familiar conocida.

Nuestro trabajo muestra que la escala modificada de Goldman se asocia fuertemente a un mayor riesgo de encontrar una mutación patogénica como causa de la enfermedad. En nuestra serie hemos encontrado una mutación patogénica en todos los individuos con una puntuación en la escala de Goldman de 1, en el 62.5% de los sujetos con una puntuación de 2, en el 52.6% con una puntuación de 3, en un 12.5% con una puntuación de 3,5 y solo en un 8.5% de aquellos con una puntuación de 4. No obstante, aunque es evidente que la probabilidad de encontrar una mutación es mayor en aquellos casos con una mayor historia familiar (Goldman de 1, 2 o 3), nuestro estudio también pone de manifiesto que si se realizase el estudio genético sólo en estos individuos, hasta un cuarto de los casos genéticos quedarían sin diagnosticar. Por este motivo es importante explorar otras variables que ayuden a predecir la existencia de mutaciones subyacentes.

El segundo de los factores evaluados es la edad de inicio de la enfermedad. La edad de inicio es uno de los factores más considerados a la hora de determinar la necesidad de un estudio genético en otras enfermedades neurodegenerativas como la enfermedad de Alzheimer. La edad de presentación más temprana en las DLFT, hace más dudosa su utilidad como

herramienta de cribado genético. No obstante, nuestro trabajo muestra que los pacientes con DLFT genética muestran una edad de presentación significativamente menor que los casos esporádicos (58,7 contra 65,7 años). A la hora de estudiar el potencial valor de la edad de inicio como cribado genético, observamos que una edad menor de 61,5 años se asoció a una sensibilidad del 67,5% y una especificidad del 69,7% para predecir mutaciones. Por supuesto, utilizar la edad de inicio de manera aislada para decidir el cribado genético puede infradiagnosticar muchos casos debidos a mutaciones, especialmente de aquellas con una mayor edad de inicio de la enfermedad, como puede ser la mutación p.P397S en *MAPT* presentada en el trabajo 2 de la presente tesis doctoral.

El cribado genético puede realizarse también en función del fenotipo clínico. En este sentido los síndromes de DFTvc y de ELA-DFT, son los más frecuentemente asociados a una mutación subyacente. El fenotipo clínico puede también ayudar a determinar qué gen estudiar en primer lugar. En este sentido, tal y como quedó reflejado en los resultados del trabajo 5 de la presente tesis doctoral, los casos que combinan presentación cognitiva y de neurona motora deberían ser cribados sistemáticamente para la expansión patogénica en *C9orf72*.

Finalmente, el fenotipo neuropatológico puede ser de gran ayuda a la hora de orientar el diagnóstico genético de los casos con DLFT, dando así la posibilidad de un consejo genético a sus familiares. Nuestro trabajo muestra que existe una gran variación en la proporción de casos genéticos entre los distintos fenotipos moleculares, pudiendo ser el diagnóstico molecular una guía de cuándo realizar un cribado genético. La presencia de una mutación en *GRN*, por ejemplo, es más frecuente en los casos de DLFT por depósitos de TDP-43 subtipo A. Así mismo, el diagnóstico neuropatológico puede ayudar a determinar qué gen o genes evaluar puesto que existe una importante correlación entre los subtipos moleculares y los genes causantes. Los casos de DLFT-tau, por ejemplo, sólo pueden ser causados por mutaciones en *MAPT*. Finalmente, algunas características neuropatológicas son específicas de determinadas

mutaciones, pudiendo ser, por lo tanto, señales de alarma para su detección. La neuropatología de la expansión patológica en *C9orf72*, por ejemplo, presenta de forma específica inclusiones ubiquitina/p62 positivas en las células granulares del cerebelo y el hipocampo, pudiendo ser este un método de screening para la detección postmortem de esta mutación (Sieben *et al.*, 2012; Ramos-Campoy *et al.*, 2018). La presencia de las inclusiones "mini-Pick" en el giro dentado del hipocampo descritas en los casos de la mutación p.P301L del artículo 1 de la presente tesis doctoral también son altamente sugestivas de esta mutación. Finalmente, tal y como se ha descrito en el trabajo 5 de la presente tesis doctoral, la ausencia de co-acumulación de proteínas FET en los casos de DLFT y ELA-FUS podría ser un marcador de mutación en el gen *FUS*.

Todas las estrategias presentadas en el trabajo 6 de la presente tesis doctoral presentan ciertas limitaciones. Desde nuestro punto de vista, estos diferentes abordajes no deben ser vistos como excluyentes, si no como complementarios.

VIII.

Conclusiones

1. El fenotipo clínico y neuropatológico de los pacientes con degeneración lobular frontotemporal genéticamente predeterminada por mutaciones en *MAPT* viene determinado por el tipo de mutación.

   1a) Portadores de la mutación p.P301L del gen *MAPT* tienen un fenotipo clínico y neuropatológico homogéneo. Desde el punto de vista clínico este fenotipo se caracteriza por alteraciones conductuales, semánticas y la presencia de parkinsonismo. Desde el punto de vista neuropatológico el fenotipo consiste en una degeneración lobular frontotemporal con inclusiones tau isoformas de 4 repeticiones y con la presencia de "mini cuerpos de Pick" en el giro dentado del hipocampo, y ocasionalmente asociada a inclusiones gliales globulares. La presencia de un haplotipo común entre los sujetos estudiados sugiere un origen ancestral común de la mutación.

   1b) Los sujetos con la mutación p.P397S en el gen *MAPT* tienen un fenotipo clínico homogéneo entre sí, pero distinto al de la mutación p.P301L, caracterizado por una mayor edad de presentación de la enfermedad y una progresión clínica más lenta.

2. Los sujetos presintomáticos portadores de mutaciones en el gen *GRN* no muestran diferencias significativas en el volumen de grosor cortical respecto a controles. No obstante, los portadores presintomáticos sí muestran una mayor pérdida de grosor cortical relacionada con la edad y con la distancia estimada del inicio de los síntomas en determinadas áreas cerebrales, lo cual sugiere una mayor pérdida neuronal debida a un mecanismo de neurodegeneración propio de la enfermedad. No se encuentran diferencias significativas del grosor cortical en los portadores de mutaciones en *GRN* según los diferentes genotipos de *TMEM106.*

3. El deterioro cognitivo es frecuente en los pacientes con esclerosis lateral amiotrófica, siendo la degeneración lobular frontotemporal la principal causa subyacente de este deterioro cognitivo. El principal factor de riesgo para el deterioro cognitivo es la presencia de una mutación patogénica.

4. El co-depósito de proteínas FET y su transportador Trnp1, no es exclusivo de los casos de degeneración lobular frontotemporal con acumulo patológico de FUS esporádica, pudiendo encontrarse también en los casos de esclerosis lateral amiotrófica con depósito de FUS sin mutaciones. La ausencia de este co-depósito de proteínas FET y Trnp1 es un potencial biomarcador de mutaciones en el gen *FUS.*

5. El abordaje multivariante que incluya la historia familiar, la edad de presentación y elementos clave de los fenotipos clínico y neuropatológico permite identificar casos genéticamente determinados que no serían identificados si se utilizase cada uno de estos criterios de manera aislada

IX.

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